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Food safety and quality management system performances in Serbian meat industry*

Radovanović Radomir[1], Tomašević Igor[1]

A b s t r a c t: In the last ten years a significant number of domestic meat processing facilities implemented and certified (or are in the process of implementation) different safety management systems (HACCP, ISO 22000, IFS). The largest number of food companies and business have determined for the implementation of the concept of Hazard Analysis and Critical Control Points (HACCP). These activities, at least in most cases, followed the adoption of appropriate regulations - Veterinary Law, and particularly Food safety law. Thanks to many years of work related to various aspects of food safety, including consulting and auditing, and other activities that have enabled the authors of this paper to gain insight into the numerous FSMS systems, in this paper we have decided to share our impressions related to the performance analysis of these systems. Among others, we would like to mentioned top management commitment, selection of consultants, defining the process, grouping and product description, hazard analysis, critical control points (CCPs) and critical limits (CLs), monitoring and verification of CCPs, corrections, corrective and preventive measures, as well as other main elements affecting the performance of FSMS.

Key words: *food safety and quality management, system performances, Serbian meat industry.*

Introduction

In the last decade on numerous occasions we have pointed out that insufficient availability and safety of food (in addition to noticeable climate changes and dramatic environmental pollution) are the most important global issues of the modern world. We took advantage of all opportunities: published scientific papers, lectures in domestic and international congresses, particularly personal contacts with governmental officials to draw attention to the more prominent problems regarding food availability and its safety by communicating and analyzing up-to-date information. Also, we were free to propose specific solutions, fully aware of our limited influence on this matter *(Radovanović,* 2008*; Radovanović,* 2009; *Radovanović et al.,* 2010). Therefore, we present you this paper with a similar „mission“, knowing that problems are not solved but instead have multiplied.

Human-kind has an increasing problem regarding hunger and/or insufficient alimentation: production of basic foodstuff (particularly food grains) is not sufficient and with constant increase of population it led to increase of number of people under threat of starvation from 800 million to 1,2 billion, just in the last 3 to 5 years. Every 30 seconds a person dies from hunger (mostly children under 5 years of age). A situation regarding food safety is not much better. Number of epidemics and food borne incidents related to different biological, chemical and physical hazards as well as hospitalized or even fatal cases is increasing. It is often considered that those incidents and their consequences are mainly connected with the characteristics of poorest and developing countries - however this is not true.

In addition to problems that occurred in EU member states (recent cases of dioxin contamination in Germany, Holland and some other countries in the last quarter of 2010), serious problems have emerg-

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ed in USA, Australia and other highly developed countries. Perhaps the best example is the USA, country with the most rigorous food safety legislations and food industry with the best developed system of preventive measures and official control of production, import and trading of agricultural goods.

In spite the fact that in the last twenty years (since 1990) number of food borne illnesses (76 millions), hospitalizations (325.000) and deaths (5.000) have decreased in 2010 to 47,8 millions illnesses, 128.000 hospitalizations and 3.000 deaths (Table 1.), because of uttered rise of the cases with unknown etiology, the cost of food borne diseases in USA has increased from 13,2 (*Mead et al.* 2000*)* to enormous 152 billion US $ - **(***Georgetown University research, cit. Shire,* 2011) in the mentioned period of time. This situation, especially attempt to improve the control of domestic small and medium sized production facilities producing food of animal origin as well as imported food - regarding fulfilment of working conditions, food safety and quality requirements - have generated putting into force new food safety law - so called Food Safety Modernization Act *(FSMA-S.510,* 2011). This act was adopted by the US Congress on December 19th 2010 and was signed by president Obama on January 4th 2011.

Table 1. Estimated annual number of episodes of domestically acquired food borne illness (FBI), hospitalizations and deaths caused by 31 pathogens and unspecified agents - transmitted through contaminated food, US[1] (*Scallion et al., 2011*)

Tabela 1. Procenjeni godišnji broj epizoda lokalno dobijenih bolesti koje se prenose hranom (BPH), broj hospitalizacija i smrtnih slučajeva, koje su prouzrokovali 31 patogen i nepoznati agensi – koje se prenose preko kontaminirane hrane, SAD[1] (*Scallion et al., 2011*)

FBI Cause/ Uzrok BPH	Illnesses Mean (90% Credible Interval) Bolesti/ Srednja vrednost (90% interval pouzdanosti)	%	Hospitalizations Mean (90% Credible Interval)/ Hospitalizacije Srednja vrednost (90% interval pouzdanosti)	%	Deaths Mean (90% Credible Interval)/ Smrtni slučajevi Srednja vrednost (90% interval pouzdanosti)	%
31 Major known pathogens/31 glavnih poznatih patogena[2]	9.4 million *(6.6–12,7)*	20	55.961 *(39.534–75.741)*	44	1.354 *(712–2.268)*	44
Unspecified agents/ Nepoznati agensi[3]	38,4 million *(19.8–61,2)*	80	71.878 *(9.924–157.340)*	56	1.686 *(369–3.338)*	56
Total/Ukupno	47,8 million *(28,7–71,1)*	100	127.839 *(62.529–215.562)*	100	3.037 *(1.412–4.983)*	100

NOTES/NAPOMENA:

[1] *All estimates were based on US population of 300 million in 2006; CrI, credible interval/Sve procene se baziraju na populaciji SAD od 300 miliona u 2006. godini; CrI, interval verovatnoće*

[2] *The 31 major known pathogens are astrovirus, Bacillus cereus, Brucella spp., Campylobacter spp., Clostridium botulinum, Clostridium perfringens, Cryptosporidium spp., Cyclospora cayetanensis, enterotoxigenic Escherichia coli (ETEC), Shiga toxin–producing E. coli (STEC) O157, STEC non-O157, diarrheagenic E. coli other than STEC and ETEC, Giardia intestinalis, hepatitis A virus, Listeria monocytogenes, Mycobacterium bovis, norovirus, rotavirus, sapovirus, nontyphoidal Salmonella spp., S. enterica serotype Typhi, Shigella spp., Staphylococcus aureus, Streptococcus spp. group A, Toxoplasma gondii, Trichinella spp., Vibrio cholerae, V. vulnificus, V. parahemolyticus, other Vibrio spp., and Yersinia spp./*
31 glavni poznati patogen: astro virus, Bacillus cereus, Brucella spp., Campylobacter spp., Clostridium botulinum, Clostridium perfringens, Cryptosporidium spp., Cyclospora cayetanensis, enterotoxigenic Escherichia coli (ETEC), Shiga toxin–producing E. coli (STEC) O157, STEC non-O157, diarrheagenic E. coli koji osim STEC i ETEC, Giardia intestinalis, hepatitis A virus, Listeria monocytogenes, Mycobacterium bovis, norovirus, rota virus, sapo virus, nontyphoidal Salmonella spp., S. enterica serotype Typhi, Shigella spp., Staphylococcus aureus, Streptococcus spp. group A, Toxoplasma gondii, Trichinella spp., Vibrio cholerae, V. vulnificus, V. parahemolyticus, ostali Vibrio spp., i Yersinia spp.

[3] *Unspecified agents are defined as agents that cause acute gastroenteritis other than the 31 major known pathogens listed above. They include known agents with insufficient data to estimate agent-specific episodes of illness; known agents not yet recognized as causing food borne illness; microbes, chemicals, and other substances known to be in food but whose pathogenicity is unproven and agents not yet describe/ Nepoznati agensi su definisani kao agensi koji izazivaju akutni gastroenteritis osim 31 poznatog patogena navedenog u prethodnom paragrafu.Uključuju poznate agense sa nedovoljno podataka na osnovu kojih bi mogla da se uradi procena epizoda bolesti izazvanih specifičnim agensom; poznati agensi koji još nisu prepoznati kao agensi koji izazivaju bolesti koje se prenose hranom; mikrobi, hemikalije, i ostale supstance koje se pojavljuju u hrani, ali čija je patogenost još uvek nedokazana i agensi još uvek nisu opisani.*

Having in mind all previous remarks and statements, authors are taking advantage of 56th International Meat Industry Conference invited lecture to shred some light on certain important facts and circumstances regarding food safety management system implementation, certification and performances in Serbian meat sector. Our choice was driven by several, in our opinion, very important facts. There is no food borne diseases surveillance network in Serbia and therefore their causes and consequences that had to do with unsafe meat and meat products remain unknown. More-over, we took into account the fact that unsafe food of animal origin, particularly meat and meat products, are the main cause of food borne diseases (cca 75%) worldwide.

The total annual value of international food trade was about 985 billions of US $ with the major influence of the group that includes trade of livestock and products of animal origin - an average of 672 million US $ or about 70%. We would also like to mention that Serbia was, and we are hoping that in the future will become again, significant exporter of this food group. In order to fulfil these hopes, guaranteed food safety represents the key condition or even imperative. On the basis of the results achieved in academic, scientific and professional activities, especially long term experience gained through the food safety, quality management and integrated system implementation and auditing in Serbian and foreign food production facilities, authors believe that the part of their professional duty is to point out all observed deficiencies, direct or indirect, in food safety management performances in domestic meat production facilities. We are doing this hoping that our experience will be of benefit to those who will be working on the introduction of modern food safety requirements for the meat industry products, especially on the systematic improvement of performances in implemented systems.

Management commitment

It is widely acknowledged that full commitment of top management and active support to the development and implementation of any modern meat and meat products (hereinafter: food or product) safety management system represents one of the key requirements for the successful implementation, maintenance, certification and continual improvement of its effectiveness. In situations where the above condition is met, the appropriate stated vision and mission of the company, and clearly defined policies and objectives in terms of food safety are just a consequence of accepting the fact that product safety is imperative, a key demand of the market (customers, end users/consumers) and the most important condition before anyone could discuss any other aspect of quality. This active approach is the basis for the precise definition and the correct implementation of many other activities within the food safety management system (FSMS), as well as for serious and responsible analysis of system performance.

Although anachronistic, our current experience, unfortunately, has shown that the top management of the domestic meat industry facilities, especially general managers, mostly (thankfully not always!) are not fully committed and dedicated to food safety management systems.

As a result, the implementation (certification is not a legal requirement), including activities that are the foundation of FSMS and form its important support, is primarily generated by binding requirements of adequate regulation. In Serbia, these are first of all, the Veterinary Law *(Official Journal of RS,* 91/2005*)* and Food Safety Law *(Official Journal of RS,* 41/2009), and in the EU General Food Law *(General Food Law,* 2002) and appropriate EC Directives 852, 853 and 854 from 2004., whose application has became mandatory in EU since January 1st 2006. The lack of interest and passive attitude of the top management, unfortunately, is very swiftly recognized and widely accepted by other employees, particularly the operating (HACCP/FSMS) team, and the person in charge and responsible for FSMS (management representative and/or HACCP team leader).

The highest number of concrete actions, along with the slow pace of work and extended deadlines, can be determined until the first, and as a rule always successful (!?), certification. After attaining this „major" objective, acquisition and delivery of certification, the motivation is slowly disappearing, defined activities are less frequently managed and are often overlooked - until the time before the first (or next) audit. Only then activities speed up, again. Specifically, various annual plans (e.g. training, preventive maintenance), simulations of withdrawal and recall of products, internal audits, reviewing and analysis of performance in order to improve the FSMS and other important activities are carried out unsystematically, while the supporting documents, particularly records are, while missing in the real-time, formed afterwards *("post festum")*.

We would like to stress out that these situations usually occur in small plants (up to 20 employees), less frequently in medium-sized, and rarely in large companies. Specifically, within the first group of manufacturers, because of the small number of emplo-

yees, it often happens that the operating HACCP team is made of majority or even all employees who are responsible for the execution of the main processes; it is similar with the HACCP team leader who is, almost as a rule, the production manager. Therefore, all activities defined under FSMS are perceived and performed as an additional, imposed and incidental activity, which is particularly evident in circumstances where there is lack of commitment and support of the owner and/or the top management.

In working environment of most mid-sized and almost all large manufacturers, the competence of employees is generally at a higher level. Organizational and functional scheme usually defines distinct food safety and quality management department and its head manager is HACCP team leader. Other members of the HACCP team are appointed by the organizational units whose activities have an important influence on the product safety.

This, sometimes regardless of the attitude and level of support of the top management, provides a slightly more serious approach, defined duties and responsibilities are distributed over a number of immediate operators - which are individually less burdened - and the planned activities are sometimes, though not always, accomplished properly and in a timely manner.

Selection of consultants

Regardless of the fact that the facilities of the meat industry do not need to hire consultants for the implementation and development of the food safety management systems and, in principle, all the planned activities to meet the FSMS requirements can be achieved using their own resources, the practice shows that this, nevertheless, happens very rarely. This is typical for small and medium enterprises, which mostly have modest resources - primarily in terms of necessary knowledge, experience and skills on specific (and current) Prerequisite Programs and requirements of the Hazard Analysis and Critical Control Points (HACCP) concept or any other model system for the management of food safety (e.g., ISO 22000, BRC, IFS). Namely, in this phase of implementation, serious mistakes are committed, especially in the working environments where the lack of commitment and support of the owner and/or top management are present, or when the most responsible employees do not dispose necessary and reliable information. Then, often decisions are made routinely while seeking bids where the key criterion is the price rather than the actual competence of consultants which would have to be proven (knowledge, practice/ experience, skills) and confirmed (insight of the previously successfully completed projects for the same or similar processes of food production). Adding to this, claimed specific expertise, often aggressive attitude of interested parties (consultants), unconfirmed recommendations or offers which are characterized by a conflict of interest (consulting services associated with the certification bodies and auditors), it is clear why in a number of FSMS (fortunately not all) serious deficiencies have been noted . This is especially true in terms of the complete absence or deficiency in the performance analysis of the implemented system, where the results (outputs) should serve as a base (inputs) for further and continuous improvement of efficiency and effectiveness of FSMS - which is one of the main general requirements of modern FSMS models. Experience with audits show that similar (and very often the same) nonconformities arise while measurable indicators of the actual improvement of FSMS are very rare, even after re-certification (after three years of application?) and/or consecutive audits.

Without examining the reasons (although we have in mind a number of everyday responsibilities and problems, while not attending conferences, outdated or insufficient monitoring of the regulations, technical literature, etc.), we would like to point out our practical experience by which the owners and/or top management of the Serbian meat industry generally are not aware and not familiar with the content, and therefore do not use standard ISO 10019:2005 which defines guidelines for the selection of quality management system consultants and use of their services.

The issuance of this international standard sends a clear message and is a confirmation of the importance of consultants and their activities as well as their undeniable responsibility for the quality of services provided (design and realization of optimal management solutions for the specific conditions within business organizations); in the same time standard is a reflection of past experiences in terms of the number and severity of the registered and/or possible consequences arising from the improper consulting services, which is in the process of food production, particularly in terms of food safety and the health of consumers, extremely important.

True, this standard is primarily related to the consultants for the current version of ISO 9001:2008. However, in the first part of ISO 10019, in the explanation of application areas (Scope), as well as in Note 2 of the Annex 1, it is emphasized that the Standard, with appropriate modifications, applies to all other management systems therefore including food safety management system(s) (e.g. ISO 22000).

Annex 1: ISO 10019 - 1 SCOPE

”This International Standard provides guidance for the selection of quality management system consultants and the use of their services……..

***NOTE 2 This International Standard addresses the realization of a quality management system but, at the same time, could be used with appropriate adaptation for the realization of any other management systems.”/*Aneks 1: ISO 10019 – 1 OBIM**

”Ovaj Međunarodni standard obezbeđuje smernice za odabir konsultanata za system upravljanja kvalitetom, kao i za korišćenje njihovih usluga ……..

NAPOMENA 2 Ovaj Međunarodni standard se bavi realizacijom sistema upravljanja kvalitetom, ali, istovremeno, uz adaptaciju, mogao bi da pude prihvatljiv i za realizaciju bilo kog drugog sistema upravljanja.”

Without getting into detailed discussion about the provisions of ISO 10019 (this must be the subject of a separate paper), we want to stress that it strongly emanates ”competence” - both in terms of required education, knowledge, experience and skills related to specific management systems (e.g. quality management - QMS, environmental management - EMS, etc), and in terms of the level of competence related to specific knowledge, relevant experience and skills related to business activities of the organization, which is undoubtedly of great importance for all processes in the whole food chain” - FSMS (Figure 1).

nificant non-conformances have been noticed. One of the main reasons for this, at least in our view, is inadequate competence of a number of consultants like mechanical engineers, electrical engineers and even forestry, mining and geology specialists.

In fact, consultants often do not possess even a basic but indispensable knowledge about food production (the corresponding primary education), specific experience (in food processing plants) and skills in the activities, processes, operations and procedures during the production of specific groups and type of food products (e.g. within the meat industry plants). A particular risk is the lack of know-

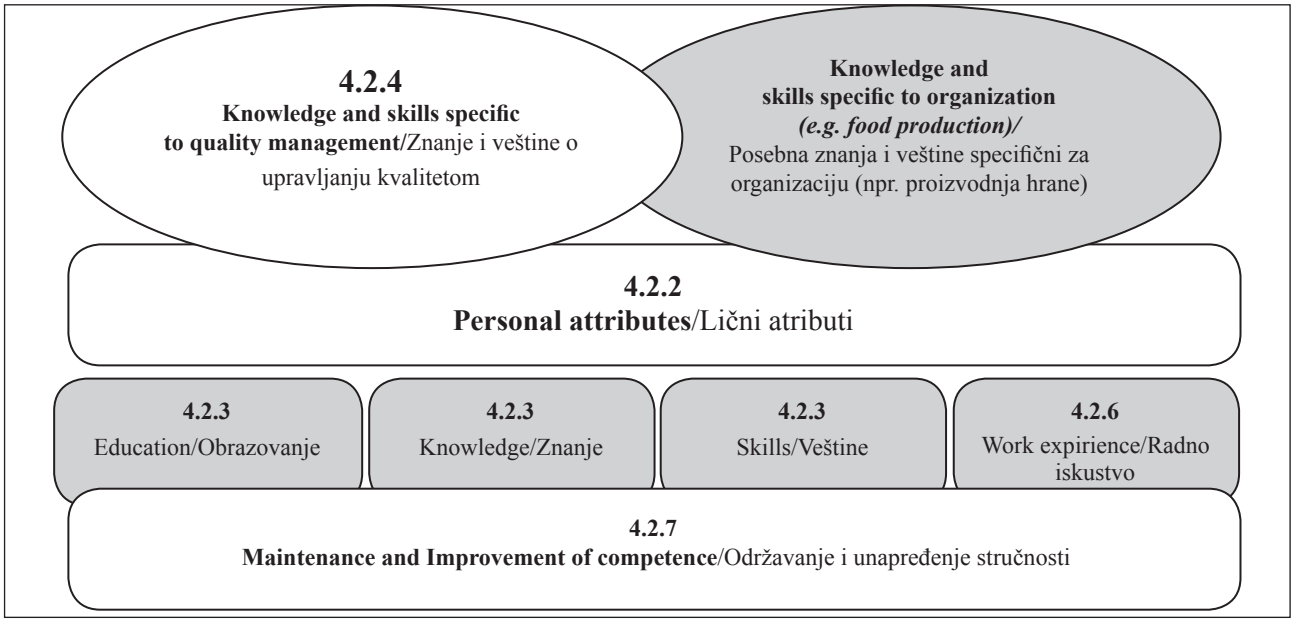

Figure 1. Concept of competence of consultants *(ISO 10019)*
Grafikon 1. Koncept kompetentnosti konsultanata (ISO 10019)

Preliminary remarks have been made mainly due to the fact that, at least according to our experience, in a large number of domestic food processing plants, especially in the meat industry, with implemented concept of HACCP and FSMS according to the requirements of ISO 22000, several sig-

ledge about (re)emerging hazards and their specific characteristics that, in the case of microbiological and some chemical hazards (e.g. mycotoxins), is not constant but is evolving depending on environmental conditions. In this way the risk of unsafe products or production increases or unnecessary time for the

control and funds are spent. In order to provide more comprehensive insight of important requirements of ISO 10019 related to the necessary competence of consultants, they are, because of the precision, given in the original form (Annex 2).

Not knowing the process and/or not understanding the essence of its phases, particularly their mutual relations and interdependence, by the consultants bridged with the universal layman approach is the main cause of nonconformities that arise in terms of not fulfilling Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) - requirements as well as specific system requirements for the management of food safety – particularly HACCP requirements.

Annex 2/ Aneks 2

Annex 2: ISO 10019 - Selected requirements *(Scope)/* Aneks 2: ISO 10019/Izabrani zahtevi *(Obim)*

4.2.3 Education, knowledge and skills/ Obrazovanje, znanje i stručnost/veštine

"*Quality management system consultants should have the appropriate education needed to acquire the knowledge and skills relevant for the consulting services to be provided. A typical example is provided in Annex B./Konsultanti za sistem upravljanja kvalitetom moraju imati adekvatno obrazovanje neophodno za sticanje znanja i veština relevantnih za konsultantske usluge koje će se pružati. Tipičan primer je dat u Aneksu B."*

4.2.5 Knowledge and skills specific to the organization/Posebna znanja i veštine specifična za organizaciju

4.2.5.1 Statutory and regulatory requirements/*Statutarni i regulatorni zahtevi*

"Knowledge of statutory and regulatory requirements relevant to the organization's activities and to the consultant's scope of work is essential for quality management system consulting. …/ Poznavanje statutarnih i regulatornih zahteva relevantnih za aktivnosti organizacije i opseg rada konsultanta je od ključne važnosti za konsalting u oblasti sistema upravljanja kvalitetom…."

4.2.5.2 Product, process and organizational requirements/*Zahtevi koji se odnose na proizvod, proces i organizaciju*

"Quality management system consultants should have a reasonable knowledge of the organization's products, processes and customer expectations prior to initiating their consulting services, and should understand the key factors relevant to the product sector in which the organization operates/ Konsultanti u oblasti sistema upravljanja kvalitetom moraju dovoljno poznavati proizvode organizacije, procese i očekivanja potrošača/klijenata pre početka pružanja usluga, i trebalo bi da imaju puno razumevanje ključnih faktora koji su relevantni u proizvodnom sektoru u kom deluje organizacija.

They should be able to apply this knowledge as follows/ Trebalo bi da budu u stanju da primenjuju ovo znanje na sledeći način:

a) to identify the key characteristics of the organization's processes and related products/identifikuju ključne karakteristike procesa organizacije i relevantne proizvode;

b) to understand the sequence and interaction of the organization's processes and their effect on meeting product requirements/razumeju sekvencu/niz i interakciju procesa organizacijei njihov uticaj na ispunjenje zahteva potrošača;

c) to understand the terminology of the sector in which the organization operates/ da razumeju terminologiju sektora u kojem deluje organizacija;

d) to understand the nature of the structure, functions and relationships within the organization/ da razumeju prirodu strukture, funkcija i odnosa u okviru organizacije;

e) to understand the strategic linkage between business objectives & competence resource needs/ da razumeju strateške veze između ciljeva biznisa/delatnosti & potreba sa stanovišta resursa."

4.2.5.3 Management practices/*Praksa u upravljanju*

"Quality management system consultants should have knowledge of relevant management practices to understand how the quality management system integrates and interacts with the overall management system of the organization, including its human resources, and how it will be deployed to secure the goals and objectives of the organization/ Konsultanti u oblasti sistema upravljanja kvalitetom moraju poznavati relevantne upravljačke prakse kako bi mogli da razumeju na koji način se sistem upravljanja kvalitetom integriše i dovodi u interakciju sa ukupnim menadžment sistemom organizacije, uključujući ljudske resurse, i kako će se koristiti u obezbeđivanju ciljeva organizacije .

In some cases, additional competencies can be required to meet the organization's needs, expectations and overall objectives for its quality management system, such as business and strategic planning, risk management, and business improvement tools and techniques (Annex B)/ U nekim slučajevima, dodatna stručnost može biti potrebna kako bi se odgovorilo na potrebe organizacije, očekivanja i sveukupne ciljeve njenog sistema upravljanja kvalitetom, kao što su biznis planovi, strateški planovi, upravljanje rizikom, i tehnike i instrumenti za poboljšanje i unapređenje delatnosti (Aneks B)."

4.2.6 Work experience/Radno iskustvo
”The quality management system consultant should have relevant work experience in managerial, professional and technical aspects of the consultant services to be provided. This work experience can involve the exercise of judgement, problem solving and communication with all interested parties (Annex B)/ Konsultant u oblasti sistema upravljanja kvalitetom mora imati relevantno radno iskustvo u menadžerskim i tehničkim aspektima konsultantskih usluga koje se pružaju. Ovo radno iskustvo može uključivati donošenje sudova, rešavanje problema i komunikaciju sa svim uključenim stranama (Aneks B) .
Verifiable references to past work experience and achievements are important and should be made available to the organization/ Reference o prethodnom radnom iskustvu i dostignućima, koje se mogu proveriti, su važne i moraju biti dostavljene organizaciji.”…

Given the importance and impact of Pre-requisite programs on food safety, in this paper, however, we will not discuss nonconformities in this regard that arise in the domestic meat industry plants. The flaws that the authors of this paper have witnessed are not only numerous but also very versatile, so that, at least in our opinion, they deserve serious investigation and shall be a part of a separate paper. Therefore we will in the following text first of all comment on important and most frequent deficiencies related to the requirements of the HACCP concept, which are largely a result of complete ignorance or of the essence of food safety requirements, especially from the viewpoint of characteristics of particular groups and categories of food/meat products.

Defining the process, grouping and product description

Since the process approach is one of the most important characteristics of modern management systems, proper definition of the process, particularly within the group of main (core) processes, is one of the most important activities in the development of food safety management systems. This is primarily due to the fact that more specific sub-processes can be often defined, within a class of main processes - e.g. ”Meat Products”; of which, for example, we would like to point out only one - e.g. ”Cooked sausage”. Within this sub-process, we can define more specific groups of products - e.g. ”Fine chopped cooked sausages”, ”Rough chopped cooked sausages” and ”Cooked sausages with chunks of meat”.

Although in each of these groups a number of different products exists - e.g. hot dogs, frankfurters, Parisian sausages, extra sausages etc. (the example for,”Fine chopped cooked sausage”) - all of them have the same production and, consequently, there is no difference in their flow charts and since they share the same conditions of heat treatment (pasteurization), hazard analysis defines same critical control points (CCPs) and same values for the so-called critical limits. Generally speaking, a HACCP plan may encompass *(„de facto” and „de jure”)* multiple products, however, provided that the hazards, critical control points, critical limits and procedures / monitoring frequency of CCPs are essentially the same. This means that all the characteristics of the HACCP plan are unique to a group of products that are clearly and visibly marked as such and followed in practice. So, for these groups of products same activities related to monitoring, corrective actions (in case of deviations from critical limits), and verification must be defined. However, all these products differ significantly, since they have distinct composition or ratio of basic raw materials, they have various types of spices, additives, preservatives, diverse types of casings or different diameters, etc.

That is why all of these and other possible differences are defined within product specifications unique for each product. According to the requirements of the HACCP concept (similar to the ISO 22000 requirements 7.3.3 and 7.3.4), this document consists of information about the product composition, important preservation characteristics, intended use, identification of consumer suitability etc. For the same products, according to local regulations, so-called ”manufacturing specifications” must be prepared with the same information mentioned above, but also with the additional requirements/information’ (short description of the process, composition, physicochemical and sensory properties, type of individual or bulk packaging, labelling instructions, special distribution control), and it is only rational to encompass all the above requirements in a single document that will provide a more complete ”picture” of the product.

Unfortunately, practical experience shows that in a number of certified food safety management systems *(HACCP - CAC/RCP 1-1969; Rev.4.2003; ISO 22000)*

- processes are not optimally defined and grouped
- number of HACCP plans is unnecessarily large, as they relate to individual products and not to groups of products,

- documentation is extensive and inefficient, hence the application of the system - is significantly more difficult in practice, and
- requirements related to the product description and its characteristics are presented incomplete and in several documents, etc.

Hazard analysis, critical control points and critical limits

Listed (first, second and third) of the seven principles of HACCP concept are also requiring activities in the process of developing one or more HACCP plans. Understanding of the essence of certain processes/sub processes, responsible approach to the above mentioned principles (requirements of the concept) and the correct implementation of basic and related activities, have strong impact on the performance (efficiency and effectiveness) of the whole system. According to our opinion, hazard analysis is the first and, fundamentally important, principle of HACCP concept, because realization of all other principles / requirements essentially depends on precise and correct analysis of the possible dangers and appropriate records. However, attitude towards this phase of work is casual and superficial both by the HACCP team members and by consultants which is totally unacceptable. During our field of work we have witnessed that:

- a list of possible biological, chemical and physical hazards is very rarely composed (although it is mandatory) and when it exists it is often generic but not specific to each stage and/or procedure within defined and grouped process/sub process;
- almost always, qualitative assessment and quantitative evaluation of the hazards is absent (their probability and frequency), especially assessment of their impact on the consumers health and potential consequences;
- incompetent and irresponsible consideration of the probability and conditions of survival and/or growth of specific microorganisms in different stages of the defined process;
- incompetent and irresponsible consideration of the optimal control measures for specific identified hazards.

In the identification (positioning) of critical control points (CCPs) for specific process steps, professional and analytical approach is not used, but almost exclusively so-called „Decision tree". Answering routinely to four questions from „the tree" which should guide us to the final decision (wheatear it is or is not CCP) is not sufficient, since it lacks serious analysis of the relationships / interconnections between the phases of the whole process; this often generates insufficient definition of control measures, or a decision that one control measure can cover more hazards, or that one hazard needs multiple control measures.

Casual approach in certain situations can lead to a situation where control point (CP) - because of "fear of errors" and/or attempts to obtain a higher level of security, is wrongly identified as a critical (CCP). The additional effect is unnecessary monitoring and verification. All this, within serious performance analysis, suggests that a significant number of the implemented food safety management systems in the domestic meat industry facilities are not sufficiently effective and efficient.

Critical limits (CL) for the identified critical control points, according to our experience, are usually defined correctly. This is from at least three reasons. Most commonly used values are already defined in the regulations (e.g. +7 ^{0}C as the upper limit for the cold storage of meat), or have been taken from validated literature sources (e.g. water pressure in the shower for the final washing of carcasses of min. 2 bars, which significantly reduces the total number of microorganisms from its surface - so--called "antimicrobial washing"). Finally, because of certitude and/or other reasons and based on their years of experience, manufacturers often determine the regimes that are at a higher or lower level compared to ones defined by relevant regulations. This is, for example, often the case with the cooling process and exactly in such situations may be a particular problem. Specifically, in defining the conditions of cooling often, because of ignorance or the need for some comfort in the work, risky "corrections" are made in terms of reducing the temperature by only 2–3 °C. For example in the case of slicing area of cooked meat products can create conditions in which the most important pathogenic microorganisms (in this case *Listeria monocytogenes*), independently of type of packaging (vacuum or modified atmosphere), can multiply much faster than anticipated by the declared shelf life of the products.

Monitoring and verification of CCPs

If hazard analysis, identification of critical control points and critical limits are a foundation for the development of a HACCP plan, then definition of monitoring activities and verification of CCPs are the core operational activities, through application of HACCP plan and should enable highly controlled production and the full safety of the final products.

Because of this, precise definition, later consistent application of monitoring and verification are very important and responsible activities. During the development of one or more HACCP plans, key attention should be paid not only to the needs (WHAT is the subject of the action?), but also to the conditions that should ensure that the defined activities of monitoring/verification are filled during application. Before all, available measurement equipment (HOW?), which must be recorded, reliable and under constant control (e.g. calibration plan, calibration certificate issued by competent institutions, measuring equipment records, etc.), which in reality is not often the case. Dynamics (frequency) of monitoring and verification (WHEN?) is often not synchronized with the dynamics of the process, or cannot be achieved in real time during the actual events at the appropriate stages of the process that has been identified as a CCP. The consequence of this situation is that both activities are not implemented in the defined time; monitoring is executed less frequently than expected, and even there are cases that all relevant records are ”filled” at the end of the process or end of shifts. In addition, the verification activities usually do not ask for adequate measurements but instead checking of the documents that arise during the application of a HACCP plan. So here, the question of responsible person in charge of these activities, not depending on a defined (WHO?) - is very problematic. These situations are generally the characteristics of the small-sized facilities that, as a rule, have modest resources (people, technology and measuring equipment). In plants which have modern technology and measuring equipment (usually medium-sized and large facilities), these situations are rare, certainly the situation where the HACCP plans monitoring and verification are correctly defined. Certain deficiencies in the monitoring and verification activities are the consequences of incidents, when the process stops and the problems are resolved by applying pre-defined corrective measures, eliminating errors. Moreover, in these facilities, technological apparatus is increasingly equipped with adequate measuring devices that allow critical process stages to product safety to be followed on monitors and/or diagrams (e.g. cooling, freezing, pasteurization, sterilization). In addition, it is important to point out that some (though not all) stages of the process that are subject to monitoring and verification in the appropriate HACCP plans, are also the subject of inspections / official controls of competent inspection services (e.g. veterinary inspections at meat production plants). However, it should be noted that official control is usually achieved by routine inspection of carcasses and products (sensory, by touching or cutting), during which it is not possible to determine all of the possible hazards to product safety. For example, the inspection of carcasses and organs can identify a disease of animals that occurred ”ante mortem” and determine the conditional use of such meat/organs during further processing (e.g. obligatory pasteurization / sterilization, or confiscation). It is similar with the routine examination of the presence of *Trichinella spiralis* in swine carcasses. However, during routine inspection it is simply not possible to determine the level of microbiological contamination of carcass surfaces, eventual residues of heavy metals in the organs and so on. As a consequence, the appropriate professional services within the EU started seriously to think about the changes to the veterinary inspection procedures. Finally, we wish to point out that during the monitoring and verification activities within the related documents (notes, charts/diagrams, etc.) a number of important data and information are noted and registered. Most of them are important indicators, key input elements, and therefore the basis for performance analysis and continuous improvement of the meat / meat products safety management systems. Unfortunately, in terms of the domestic meat industry, as far as we can tell, a large number of measured values (indicators) remain where they are registered, occasionally are archived, but almost never become parts of serious performance analysis of the food safety system and are not used as a tool for continual improvement.

Corrective and preventive measures v / s corrections

Since it is a principle (requirement) of the HACCP concept, corrective actions are pre-defined as an integral part of HACCP plan and they follow monitoring activities. In fact, all non-conformities that are found during monitoring of CCPs, especially deviations from the defined critical limits, ask for **urgent** action(s) that are designed to prevent, eliminate or reduce risks to an acceptable level (so-called **correction**), but also measures to eliminate detected nonconformities and its cause and other undesirable situations affecting the safety of food (so-called **corrective action**). We emphasize that in the process of food safety management in the domestic meat industry plants often a misunderstanding of the essential difference between the terms ”correction” and ”corrective action” exists and therefore improper definitions of appropriate actions/activities that are implicit. The ”corrective actions” in the HACCP plan are usually defined as simple ”corrections” or

measures for immediate resolution of the problem, while a serious analysis of the causes for a deviation (the reason, impact(s) and their relationship, the particular circumstances/ conditions, appropriate tests or simulations, measurements, etc.) - are usually absent. So, there is a lack of the proper definition (in the HACCP plans) and the correct implementation of appropriate corrective actions (through application of HACCP plans in practice). Therefore, analysis of implemented corrective actions is also missing (efficiency, resource availability, responsiveness, the reasons for possible delays, etc.), especially analysis of actual effects. All this leads to the absence of important inputs for performance analysis and systematic improvement of FSMS.

Other elements affecting the performance of FSMS

HACCP concept does not explicitly require documented procedures for the management of documents and records (as opposed to the standard ISO 22000 – 4.2.2 and 4.2.3), but in its seventh principle and requirement 5.7 (CAC / RCP 1-1969, Rev.4-2003) only provides for the obligation to establish appropriate documentation and its archiving. We have witnessed only few HACCP systems that introduced and consistently applied management of documentation and records (?!). This practice has resulted in many gaps in the documentation: documentation levels are not defined, they are wrongly used (for example, interference between the guides / instructions and records), there are differences in the appearance of the documentation within the same level; not defined encryption and other important elements (e.g. version / edition, copy, date of adoption and/or application, the manner, place and time of filing, etc.). Important disadvantage is a lack of record distribution procedure, process changes and withdrawal of documents, the manner, place and time of filing invalid documentation etc.

One of the most important and most responsible actions in the processes of production of meat and meat products, especially from the standpoint of security, is purchasing. This is primarily because the subject of purchasing are the product ingredients (e.g. primary and secondary raw materials, spices, additives, etc.), but also materials that are in direct contact with products (e.g. packaging materials, packaging). To our knowledge, **purchasing control** is infrequently introduced as a part of the HACCP system, although appropriate requirements exists - both within the HACCP concept (Incoming material requirements - CAC / RCP 5.3), and within the requirements of ISO 22000 (Product characteristics - 7.3.3). Thus, within the certified HACCP systems in the Serbian meat industry, existence of documented procedures relating to purchasing requirements, defined within the relevant specifications (the input specifications), is rare, do not exist or are incomplete, criteria for evaluating, ranking and selection of suppliers is not determined, etc. How is it possible to execute full performance analysis of FSMS, if numerous and important data and information relating to purchasing are not available. This is even more important, if other important purchasing outputs important to food safety (like equipment, tools, supplies, hygiene items, etc.) are taken into consideration.

A significant part of the system for managing food safety is, beyond doubt, the management of nonconforming products. Without going into details of this requirement (in the HACCP concept and ISO 22000 - 7.10), in this part we just want to draw attention to the terms of „recall" and „withdrawal" of the products. These requirements are indeed, although not in the same manner, specified in the HACCP concept as well as in ISO 22000 standard. Specifically, the document CAC / RCP 1-1969, Rev.4-2003. (Section 5.8) states the term "recall", while the standard ISO 22000 (Section 7.10.4) states the term „withdrawal"; (though the article 7.3.1 of this standard, within the note, defines that term recall includes withdrawal of the product). Regardless of the note, we wish to emphasize that in most of the HACCP systems to which the authors of this paper had access, withdrawal and recall are more or less correctly defined in the relevant documents (procedures). However, they do not perform mock recalls, since this required activity, as a rule, is not simulated as planned in the real conditions/situations, but only „filling in" of the appropriate forms/ records exists.

HACCP concept does not explicitly requires documented procedures for internal audits (as opposed to the standard ISO 22000 - 8.4.1), although the importance of these activities needs not to be proved. Routine relationship with the introduction of HACCP concept generates situation, at least in systems in which the authors had access, where internal audits are not designed so they have not been implemented. In our opinion, it is the result of the internal audit that provides an effective opportunity to create a realistic insight into the performance of the system (FSMS). Data and information that are acquired during the internal audits represent inputs to analyze the performance, efficiency and effectiveness of food safety management systems, as well as powerful support in the efforts for con-

tinuous improvement. While the HACCP concept also does not explicitly require management reviews (as opposed to the standard ISO 22000 - 5.8), the importance of this activity is undeniable. Unfortunately, to our knowledge, most domestic plants in the meat industry in which HACCP have been introduced, this activity have not been defined and enforced. A disadvantage of this approach, primarily by the consultant and members of the HACCP team, is the fact that the organization has been denied for a number of very useful information (review outputs) that are not only the basis for further analysis and in making judgments about the performance of systems based on the facts (one of management principles), but are the basis for the continuous improvement of FSMS.

Instead of conclusion

In the last ten years a significant number of domestic meat processing facilities (as well as other food producers), implemented and certified (or are in the process of implementation) different safety management systems (HACCP, ISO 22000, BRC, IFS). The largest number of food companies and business have determined for the implementation of the concept of Hazard Analysis and Critical Control Points (HACCP). These activities, at least in most cases, followed the adoption of appropriate regulations - Veterinary Law (*Official Journal of RS,* 91/2005), and particularly Food Safety Law (*Official Journal of RS,* 41/2009). Thanks to many years of work related to various aspects of food safety, including consulting and auditing, and other activities that have enabled the authors of this paper to gain insight into the numerous FSMS systems, in this paper we have decided to share our impressions related to the performance analysis of these systems.

The largest part of the comments relates to the identified deficiencies, can be classified into several groups:

- In most meat industry plants there is no genuine preference, full commitment and active support of the top management for food safety management systems;
- Members and leaders of the operational teams (HACCP teams), in particular lower levels of employees, access binding activity as the imposed additional duties and responsibilities - which are not additionally evaluated and rewarded (lack of motivation);
- Consultants are often incompetent people without proper, specific training and experience, while the available experience and skills are usually acquired in the implementation of other management systems;
- Requirements of the appropriate standards (HACCP concept, ISO 22000, etc.) are not well understood and are superficially implemented and routinely executed.

We wish to point out that serious and responsible analysis of the performance of the implemented FSM system generally do not exists, especially not as a tool for systematic improvement of efficiency and effectiveness of the system and enhancement of product safety. Certified systems with defined key performance indicators of the process (KPI) that should be systematically monitored, precisely measured and seriously analyzed, are almost impossible to find. In this paper we have primarily analyzed and pointed out the major deficiencies of FSMS in domestic meat industry facilities and emphasized the necessity of, in time ahead of us, changes to the existing ”practices”. Selection, application and analysis of the most important performance indicators for the processes of meat production are not given, since it was a topic of our paper that we have already presented at the International Quality Day 2010 (*Đekić et al.,* 2010**).**

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Performanse sistema za upravljanje bezbednošću i kvalitetom u pogonima industrije mesa Srbije

Radovanović Radomir, Tomašević Igor

R e z i m e: Poslednjih desetak godina značajan broj domaćih pogona inudtrije mesa uveo je i certifikovao neki od aktuelnih sistema za upravljanje bezbednošću proizvoda (HACCP, ISO 22000, IFS). Osnova svih tih sistema je koncept analize rizika i kritičnih kontrolnih tačaka (HACCP), a navedene aktivnosti je generisala aktuelna domaća regučativa - Zakon o veterinarstvu (Sl. glasnik RS, 91/2005) i Zakon o bezbednosti hrane (Sl. glasnik RS, 41/2009). Zahvaljujući višegodišnjem iskustvu stečenom tokom rada u oblasti bezbednosti hrane, uključujući istraživanja, konsalting i zvanična ocenjivanja za više domaćih i međunarodnih sertifikacionih tela, autori rada su se opredelili da daju prikaz važnijih performansi sistema za upravljanje bezbednošću i kvalitetom u pogonima industrije mesa Srbije. Posebna pažnja ukazuje se na posvećenost vrhovnog rukovodstva, izbor konultanata, definisanje glavnih procesa, grupisanje i opis proizvoda, postupak analize rizika, definisanje kritičnih kontrolnih tačaka (KKT) i kritičnih granica (KG), definisanje i sprovođenje postupka monitoringa, korekcija/ korektivnih i preventivnih mera i verifikacije kritičnih kontrolnih tačaka. Komentarišu se i drugi važni elementi od uticaja na performanse sistema upravljanja bezbednošću i kvalitetom u procesima proizvodnje i prerade mesa.

Ključne reči: *sistem za upravljanje bezbednošću i kvalitetom, performanse sistema, industrija mesa u Srbiji.*

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Innovative approaches for assuring meat safety, quality and authenticity*

Alewijn Martin[1]

A b s t r a c t: The analysis of meat for the presence of environmental contaminants, bio-contaminants and residues of veterinary drugs is challenging due to the wide range of analyte/matrix combinations and the, at least partly, 'black market' situation, urging the need for constant awareness of the possibility of new, previously unknown, compounds. Additionally, analytical laboratories are facing new questions in relation to specific health or production claims. All of these "old" and "new" questions have in common that the number of compounds to be detected increases. This has consequences for the analytical methods used, but also for the evaluation of the results and the validation of the method.

The validation of such (screening) methods until recently was poorly developed. Most validation strategies focussed on confirmatory methods designed for a small number of analytes. In the field of veterinary drugs, a working group – from EU Reference Laboratories - came up with a practical approach to validate multi-analyte multi-matrix screening methods. It describes the selection of relevant analyte/matrix combinations for validation studies. The validation is preferably at the concentration of 0.5 times the maximum residue limit. A minimum set of 20 different samples has to be tested. The requirement set is that the number of false negatives should be ≤ 5% (β-error: ≤ 5%). This approach is currently also applied to the chemical screening of meat for low levels of antibiotics.

This approach, however, is not applicable to all situations. In specific cases the target is not the level of one or a few analytes, but rather a 'chemical profile' of many analytes. Examples are steroid hormone patterns in urine of meat indicating the illegal use of steroid hormones for growth promotion or carotenoid patterns used for verification of specific production claims. For the evaluation of such multivariate results and the validation of the analytical methods new strategies are being developed. During this presentation some practical examples from recent studies will be discussed.

Key words: *meat safety, quality, authenticity.*

Inovativni pristupi u obezbeđivanju bezbednosti, kvaliteta i autentičnosti mesa

Alewijn Martin[1]

A p s t r a k t: Ispitivanje mesa na prisustvo kontaminanata iz životne sredine, bio-kontaminanata i rezidua veterinarskih lekova, predstavlja izazov zbog širokog spektra kombinacija analita/matriksa, kao i, delimično, „crnog tržišta". Iz tih razloga javlja se potreba za postojanjem svesti o mogućnosti pojave novih, ranije nepoznatih jedinjenja. Takođe, analitičke laboratorije se susreću sa novim pitanjima u vezi sa posebnim proizvodnim ili zdravstvenim aspektima i zahtevima. Sva navedena „stara" i „nova" pitanja imaju zajedničko to što se broj jedinjenja koja treba da se otkrivaju stalno povećava. To ima posedice po analitičke metode koje se koriste, ali i za evaluaciju rezultata i validaciju metode.

Validacija ovakvih (skrining - screening) metoda do skoro nije bila razvijena. Većina strategija validacije se fokusirala na konfirmatorne metode namenjene za mali broj analita. U oblasti veterinarskih lekova, radna grupa – iz EU referentnih laboraotrija - je zauzela praktični pristup validaciji skrining metoda sa višestrukim analitima/matriksima. Opisuje se izbor relevantnih kombinacija analita/matriksa za studije validacije. Poželjno je da se validacija radi pri koncentraciji od 0,5 puta maksimalno dozvoljene količine rezidua. Minimalni set od 20 različitih uzoraka se testira. Zahtev seta je da broj lažnih

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negativnih rezultata bude ≤ 5% (β-greška: ≤ 5%). Ovaj pristup se, takođe, primenjuje za skrining antibiotika prisutnih u mesu u malim količinama, hemijskim metodama.

Ovaj pristup, međutim, nije primenjiv u svim situacijama. U posebnim slučajevima, cilj nije ispitivanje prisustva jednog ili više analita, već „hemijski profil" više analita. Primer za to su steroidni hormoni u urinu što ukazuje na nelegalno korišćenje steoridnih hormona u cilju pospešivanja rasta ili profil karotenoida koji se koriste za verifikaciju posebnih proizvođačkih zahteva. Za evaluaciju ovakvih multivarijantnih rezultata i validaciju analitičkih metoda razvijaju se nove strategije. Tokom ove prezentacije biće razmatrani neki praktični primeri iz najnovijih istraživanja.

Ključne reči: *bezbednost mesa, kvalitet, autentičnost.*

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Modern approaches to enhancing beef quality*

Declan Troy[1]

A b s t r a c t: Consumer focused research into meat eating quality has shown that tenderness, juiciness, flavour and overall palatability remain the most sought after attributes by consumers and that tenderness is deemed most important.

The most important factors known to influence eating quality of beef are established. Production, processing and cooking factors all effect meat quality. Breed, sex, feed, handling, environment, finishing weight and age at slaughter are among the main production factors that affect palatability. While from a processing point of view pH/temperature regime, hanging methods, days of maturation, and whether or not the carcass has been subjected to electrical stimulation, cooking method and 'degree of doneness' all affect beef palatability.

Providing consistently tender beef should be key priority for the beef industry. While there have been many successful efforts at improving the tenderness of beef, research has shown that an unacceptable level of variability still remains in beef tenderness. There are many controls that can be introduced into the beef processing line in order to alleviate meat tenderness inconsistencies such as hanging the carcass by its aitch bone, electrically stimulating muscles and preventing muscles from shortening.

This paper concisely reviews the main determinants of beef tenderness and how this knowledge can be utilised in the industry to produce a more consistent product of high eating quality.

Key words: *beef quality, tenderness, juiciness, flavour, palatability.*

Introduction

A thorough understanding of the most important factors which influence meat eating quality is imperative in order to produce a consistent product in line with consumers' expectations. Furthermore this understanding is necessary in order to enable the meat industry to implement appropriate interventions and controls to produce a consistent high quality product. In general however the meat industry is not highly science driven compared to other sectors like the pharmaceutical or information technology industry and does not invest heavily in research and development. Therefore there is a strong reliance by the industry on the research community to develop outputs which, based on good science, can enhance meat eating quality.

Consumer focused research into meat eating quality has shown that tenderness, juiciness, flavour and overall palatability remain the most sought after attributes by consumers and that tenderness is deemed most important (*Miller et al.,* 2002). Although consumers are willing to pay more for guaranteed tender beef a high variability (up to 20%) still exists in the market place. In research from Norway it was found that consumers were willing to pay 50 % more for very tender beef and 25 % more for tender beef compared with less tender beef (*Alfnes et al.,* 2005). Providing consistently tender beef should be key priority for the beef industry. While there have been many successful efforts at improving the tenderness of beef research has shown that an unacceptable level of variability still remains in beef tenderness (*Maher et al.,* 2004).

There are many controls that can be introduced into the beef processing line in order to alleviate meat tenderness inconsistencies such as hanging the carcass by its aitch bone, electrically stimulating muscles and preventing muscles from shortening.

This paper concisely reviews the main determinants of beef tenderness and how this knowledge can be utilised in the industry to produce a more consistent product of high eating quality.

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Pre-slaughter Factors

Important factors known to influence eating quality of beef are now established and many of these have been reviewed by *Thompson* (2002). Production, processing and cooking factors all effect meat quality. Breed, sex, feed, handling, environment, finishing weight and age at slaughter are among the main production factors that affect palatability. While from a processing point of view pH/temperature regime, hanging methods, days of maturation, and whether or not the carcass has been subjected to electrical stimulation, cooking method and 'degree of doneness' all affect beef palatability.

Breed and Sex

Because of the interfering influences within and between breeds such as feed, growth rates etc., it is difficult to determine the effects of breed on meat quality. *Wulf et al.,* (1996) and *Maher et al.,* (2004) both reported that breed did not have an effect on WBSF, while in a recent study *Dikeman et al.,* (2005) found that considerable variation in WBSF of M. *longissimus* muscle steak exists between breeds, while selecting for marbling to improve tenderness would be expected to result in only subtle improvements in tenderness in most breeds. *Maher* (2003) found heifers to be more variable for tenderness than steers (mixed breed), however this maybe have been somewhat confounded by age. However, *Purchas et al.,* (2002) reported that bull beef was tougher than steer beef.

Feeding regime, finishing weight and age at slaughter

Feeding, finishing weight and the age of the animal at slaughter all have an important role to play in the determination of meat quality. Feeding regime is thought to have an indirect effect on tenderness. Leaner animals require more precise chilling control than fatter carcasses in order to reduce the risk of cold shortening (*Troy*, 1995). A number of studies have examined the effect of growth rate on tenderness by altering the feeding regime before slaughter with mixed results. *Sazili et al.,* (2003) reported that animals placed on a restricted diet for 30 days had lower shear force values than fast growing animals. However, they also reported that after 45 days on a restricted diet the shear values were similar to those for animals on the high plane of nutrition. While *Thompson et al.,* (1999) and *Purchas et al.,* (2002) reported that steers on a faster growing regime were more tender than those finished on a restricted diet.

Age at slaughter is reported to effect beef tenderness. *Wheeler et al.,* (1996) compared the tenderness scores of yearling heifers and 2 year old cows. They reported that there was a very slight difference in the tenderness scores between the two age groups, however, there was greater variation in tenderness within each group that there was between age groups. *Wulf et al.,* (1996) reported that beef from cattle slaughtered at 15 months was more tender than from those slaughtered at 18 months.

Post-slaughter Factors

Post-slaughter factors have higher influence on meat eating quality because the main determinants of meat tenderness are the extent of proteolysis on key structural proteins and the degree of shortening of the muscle fibres (a third factor is due to the connective tissue component and is often referred to as "background toughness", it is little effected by post-slaughter events and its contribution to toughness is linked to the age of an animal and/or the muscle type). Both these events take place at varying rates and extents during the post-mortem period. The most likely causative agents responsible for the breakdown of key structural proteins in post-mortem tenderisation are the calpains (*Dransfield*, 1993) even though their precise mode of action is unclear. There is strong evidence that the proteins of the cyto-skeletal network such as titin, nebulin and desmin are degraded by calpains during the tenderisation process. Furthermore it is now established that ageing beef beyond 10–12 days at 0–2C does not contribute to any greater degree of breakdown of structural myofibbrillar proteins nor detectable increase in eating quality.

It is well known that the shorter the muscle fibres the tougher the meat. This is because the contraction allows the z-discs of the myofibril to be nearer each other thereby increasing the density of filaments. This occurs during the normal onset of rigor. However when early post-mortem variables of pH, temperature and time interact in such a manner as to induce cold-shortening (sarcomere lengths < 1.7 microns) extreme toughness is experienced. Pre-rigor muscle shortens on exposure to temperatures below about 10° C. The faster the temperature decline the slower the rate of glycolysis and therefore the greater the degree of shortening. Cold-shortening occurs as calcium is uncontrollably released into the sarcoplasm which in turn is due to the decrease in temperature and pH resulting in reduced ability of the sarcoplasmic reticulum and mitochondria to retain calcium.

The increase in concentration of free calcium in the presence of sufficient ATP present results in increased shortening of the sarcomeres (*Locker*, 1985). Shortening causes the thick filaments to penetrate the z-discs and may interact with actin filaments in adjacent sarcomeres. This results in the extreme cases of a continuum of myosin throughout (*Marsh and Carse*, 1974).

This dense structure is responsible for the increased toughness experienced by consumers of cold-shortened beef.

In relation to beef carcasses it follows that if pH, temperature and time post-mortem (i.e. the biochemical dynamics) of the early post-mortem period are critical in determining the tenderness/toughness of meat then meat throughout a carcass will experience a variety of biochemical profiles resulting in meat of highly variable eating quality. The rate of pH fall varies from animal to animal (*O'Halloran et al.,* 1997) and the temperature varies considerably throughout a chill, a carcass and a muscle and hence their interaction is quite variable.

Meat science has contributed to providing scientific data that has been employed effectively by the meat industry to reduce the risk of cold-shortening. From understanding of the early post-mortem period specific recommendations have been implemented by meat processors.

Chilling Rates

Although post-mortem chilling processes are primarily aimed at complying with food safety standards and to a lesser extent at extending shelf-life and reducing overall weight drip loss, chilling rates of beef carcasses can influence the rate of pH fall and thereby contribute to cold-shortening especially in lean carcasses. The well known 10/10 rule i.e. no part of the carcass should fall below 10^{o} C within 10 hours of slaughter (*Troy*, 1995) is implemented in many meat processing operations in Ireland and the UK. Various temperature and chilling regimes have been suggested (*Savell et al.,* 2005) to avoid or reduce cold-shortening. Some retailer specifications can go as high as maintaining carcass temperature above 12^{o} C for 12 hours post-slaughter. But it is not simply a matter of controlling the temperature in order to ensure high eating quality beef. Time post-mortem (especially during the first 24hours but often up to 48 hours), pH of the muscle and temperature all synergistically contribute to influencing quality. A more modern approach at factory level is to monitor what is often described as the pH/temperature window as a function of time post mortem. *Thompson* (2002) describes the biochemical basis underlying the recommendations regarding optimum values of the measured parameters. Basically cold shortening can only occur when muscles have a pH value of 6.0 or higher, contains some residual ATP to allow muscle contraction and the temperature is less than 12° C. It is thought that under such conditions calcium is uncontrollably released into the sarcoplasm and severe contraction occurs. When muscles experience pH values < 6.0, temperatures higher than 35° C (particularly during the early post-mortem period of electrically stimulated carcasses) a phenomenon known as heat shortening can occur resulting in severely contracted fibres. Heat-shortening muscle often appears in deep inside hind quarter muscles. In relation to beef carcasses it follows that if pH, temperature and time post-mortem (i.e. the biochemical dynamics) of the early post-mortem period are critical in determining the tenderness/toughness of meat then meat throughout a carcass will experience a variety of biochemical profiles resulting in meat of highly variable eating quality. The rate of pH fall varies from animal to animal (*O'Halloran et al.*, 1997) and the temperature varies considerably throughout a chill, a carcass and a muscle and hence their interaction is quite variable and complex. This partly explains why beef varies so much in eating quality.

Electrical Stimulation

Applying electrical current to a beef carcass shortly after death is widely known as electrical stimulation (ES). ES has a long history of development dating back to the 1950s (*Chrystall et al.*, 1985). The primary reasons for the use of ES in the meat industry is to ensure that the pH of the carcass is less than 6 when temperatures are less than 12° C and thereby can be chilled more rapidly. Many forms of ES applications exist ranging in magnitude of voltage, current, frequency of pulses, and waveforms (*Simmons et al.,* 2008).

Severe muscular contractions are induced throughout a carcass on applying ES. The resulting increased energy expenditure of the muscles due to contractions results in a rapid decline in pH. There is an early development of rigor thereby reducing the risk of cold-shortening should the carcass encounter temperatures below 12° C (*Troy,* 1985). Generally, meat from ES carcasses is more tender than those of non-ES treated carcasses when placed in environmental conditions that would promote cold shortening. ES can increase post-mortem temperatures in carcasses, increase the drip loss and water holding capacity of meat and increase the brightness values of the red colour of beef (*Eikelenboom and*

Smulders, 1985). Three mechanisms of tenderisation associated with ES have been reported. Firstly, the disruption of the lysosomal sac with subsequent release of proteolytic cathepsins at low pH/high temperature environment as described in *Dutson et al.*, (1980). Secondly, the physical disruption of the muscle fibres brought about by severe contractions of the muscles (*Sorinmade et al.*, 1982), especially in the case of high voltage stimulation (HVES see later) and, thirdly, a reduction in collagen cross--linking (*Judge et al.*, 1980). These mechanisms combined would result in major and consistent benefits in meat eating quality which is not the case in reality. *Pommier et al.*, (1987) reported that ES induces a toughening effect or in the absence of cold--shortening, accelerates the tenderisation process through increased initial activity of calpain brought about by inducing a higher temperature during rigor but then creating denaturing conditions which will reduce the effect (*Dransfield*, 1992; *Simmons et al.,* 2008). The application of ES is a highly variable process (voltage, frequency, etc), the type of carcass used, the subsequent chilling conditions and the location of the muscle sample within the carcass.

There are broadly two commercial systems of ES namely high voltage (HVES, 300-1000V) and low voltage (LVES, 50-120V) stimulation. Even though HVES is more expensive and requires greater safety precautions to be implemented the benefits over and above those of LVES are not immediately apparent from the literature. The rate and extent of pH decline is similar (*Eikelenboom and Smulder,* 1985; *Koh et al.*, 1987) for both HVES and LVES although *Simmons et al.*, (2008) has suggested that HVES produces a greater rate of pH decline and one which is more consistent and less variable than LVES treatments.

When ES is applied there is a risk of "excessive stimulation", that is the formation of a pale, soft and exudative (PSE) like meat which can occur through a very rapid pH fall early post-mortem when the carcass temperature is high. A major decrease in meat tenderness (due to heat-shortening), increased water-holding capacity (due to protein denaturation) and detrimental colour stability (due to protein denaturation and the increase in free water resulting in increased reflectance) (*Simmons et al.,* 2008). These effects can be offset by reducing the chilling temperature (*Strydom et al.,* 2005).

ES (high or low voltage) should be seen as just one other tool to adjust a parameter (pH) of a complex system or environment under which muscle is converted to meat. While it can reduce ageing times and increase the consistency of tenderness it can also contribute to the production of poor quality meat especially where cold-shortening is not a risk. A clearer understanding of the optimum pH/temperature environment throughout the carcass would aid greater precision in its application. Great care needs to be taken in avoiding over-stimulating beef carcasses. *O'Halloran et al.,* (1996) have demonstrated that there is a great variability in the rate of pH decline in the first 24 hours post-mortem between animals and the application of ES may not be required in some cases.

Carcase Suspension Methods

Methods to reduce the degree of cold-shortening or increase the degree of stretching of sarcomeres by altering the normal hanging method of carcasses are becoming more commonly used by the industry. Various methods of hanging carcasses have been tried as an alternative to the conventional Achilles tendon method (*Troy,* 1995). Among the different hanging techniques developed to improve meat tenderness are 'tenderstretch', 'tendercut', and a forequarter hanging method (*Filho et al*., 2005).

By far the most popular of these newer techniques in use is the 'tenderstretch' method. For this technique the hanging position is switched from the Achilles tendon to the aitch bone thereby allowing the hind legs to hang freely. Tenderstretch induces a stretching effect on key hindquarter muscles preventing the sarcomeres from shortening and in some cases actually stretching the distances between z lines reducing the density of overlap between the filaments. *Troy* (1999) found that sarcomere lengths increases by 15%, 30%, 33% and 30% on average from tenderstretch suspended carcasses of *m. longissimus dorsi*, *semimembranous*, *biceps femoris* and the *gluteus medius* respectively. Similarly sensory analysis showed that panellists consistently rated all muscles from pelvic suspended carcasses as more tender (average 20%). In another study less cold--shortening occurred in steaks from conventionally hung compared to tenderstretch suspension carcasses in those carcasses chilled faster (*Sorheim et al.,* 2001). The *m. psoas major* or fillet has been found to be slightly toughened in pelvic suspended carcasses because of its particular position in the carcass.

In Ireland and the UK pelvic suspension forms part of numerous retailer specifications often in combination with a slow chilling regime or in combination with electrical stimulation. The industry cites some drawbacks however including the requirement for more chiller space, demands for greater labour input and the distortion in shape of some muscles.

Researchers at Virginia Polytechnic Institute and State University have examined strategic pre-

rigor cutting of the backbone to improve beef tenderness (*Wang et al.,* 1994). This procedure is referred to as 'tendercut' and requires and additional input of making cuts in the skeleton of the pre-rigor carcass shortly after slaughter while maintaining the Achilles tendon suspension. The weight of the carcass below the points of cutting stretches many of the major loin and round muscles. As with 'tenderstretch' the 'tendercut' method does not benefit all muscles. *Shanks et al.,* (2002) reported that the use of the 'tendercut' technique resulted in increased tenderness in some muscles with decreased tenderness in others. A report from Australia suggests that it is not as effective in increasing tenderness as the tenderstretch method. This technique has not been widely adapted by the industry. *Filho et al.,* (2005) examined the effect of forequarter hanging on the *longissimus* and *biceps femoris* muscles. Hanging by the forequarter caused a significant improvement in tenderness of the *longissimus* muscles without any detrimental effect on *the biceps femoris*. This method has not been fully characterised up to now.

Tenderbound System

A novel method to improve the eating quality of meat by reducing the degree of contraction in hot boned beef is in the process of been developed for industry use. As discussed earlier a consistent optimum window of pH and temperature as a function of post-mortem time is very difficult to achieve given that the chilling rate, muscle location, level of fat cover, animal to animal variation in metabolism among other factors are different. The Tenderbound System or a similar type approach overcomes these difficulties.

Hot boning provides a significant advantage in that it enables individual muscles to be processed in a specific tailored fashion. The advantages and disadvantages of hot-boning have been highlighted previously (*Pisula and Tyburch,* 1996). Hot-boning requires the major commercial cuts or muscles to be excised within 90 minutes of slaughter and thereby reduces weight loss during chilling, requires less chiller space, consumes less energy, lowers labour input and increases turnover in productivity. Its major drawbacks are that it needs precise synchronisation of slaughter, boning and processing activities, very strict hygiene control and induces toughness through greater ability of muscle fibres to contract in the absence of skeletal restraint resulting in cold shortening. Hot-boning in combination with a pre-rigor restraint technique using elasticated film constitutes the Tenderbound system.

Pi-Vac Elasto-Pack system (*Maixner and Karnitzschky*, 2001) involves stretching tubes of elastic film to the inside walls of the packaging chamber, after the muscle is inserted into the chamber pressure is released and the elastic film returns to its original dimensions. The elastic film then hinders the diametrical expansion of the muscle, which restricts muscle contraction (*Troy*, 2006). *Troy* (2006) has found that hot boned meat packed in Pi-Vac can be chilled much more rapidly while the beef is tender and of consistent quality. Hot boned muscle without any restraint had higher shear force values (force required to shear through meat) after 14 days ageing compared to muscle packed in Pi-Vac (72 N and 40 N respectively). The sarcomere length, a measure of muscle extension or stretch was increased considerably by use of the Tenderbound method (1.7µm) while the sarcomere length for hot boned muscle without restraint was 1.3µm. The pre-rigor meat forms into the shape of its constraining pack and is not distorted. Using this system variability of tenderness is reduced as individual muscles can be treated optimally. Drip loss is reduced by packing early post-mortem thereby improving flavour and succulence, bacterial growth is reduced and shelf life improved because of the ability to chill at lower temperatures. Other potential benefits may include increased yield, reduced energy costs, quicker turnover of unit product in meat plant, savings in labour and transport costs, although these have yet to be verified at industry level.

Conclusions

In order to produce beef with consistently high eating quality, post-mortem parameters must be taken into account. The two main strategic approaches are: to enhance the degree of proteolytic breakdown of cyto-skeletal proteins as well as decrease the degree of shortening of sarcomeres. Ensuring a high degree of proteolysis can be approached in a number of ways but as long as the beef carcase or muscle is aged for at least 10 to 12 days almost all degradation relevant to the tenderisation process is complete. Reducing contraction can be easily carried out by aitch bone hanging or by the Tenderbound process. Pre-slaughter factors do not impinge on eating quality to a major extent within the normal production systems of Northern Europe unlike other areas where *bos indicus* type cattle are prevalent. The beef industry has the necessary knowledge to avoid producing tough beef but needs to implement more rigorous practices to do so.

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Moderni pristupi poboljšanju kvaliteta goveđeg mesa

Declan Troy

R e z i m e: Istraživanja usmerena na potrošača koja se odnose na senzorski kvalitet mesa, pokazuju da mekoća, sočnost, ukus i ukupna prihvatljivost hrane i dalje su najtraženiji atributi mesa koje zahtevaju potrošači, a da se mekoća mesa smatra najvažnijom osobinom.

Utvrđeni su najvažniji faktori, za koje se zna da utiču na kvalitet goveđeg mesa. Faktori koji utiču na proizvodnju, preradu i kuvanje utiču i na kvalitet mesa. Rasa, pol, hrana, rukovanje, životna sredina, uzrast i masa pre klanja su među faktorima koji utiču na ukus. Sa stanovišta prerade, pH, temperaturni režim, način kačenja, trajanje zrenja, kao i činjenica da li je trup bio podvrgnut električnoj stimulaciji, način kuvanja i „stepen završne obrade", utiču na ukus goveđeg mesa.

Obezbeđivanje konzistentno mekanog goveđeg mesa bi trebalo da bude ključni prioritet u industriji mesa. Iako je bilo mnogo uspešnih pokušaja da se poboljša mekoća mesa, istraživanje je pokazalo da još postoji neprihvatljivi nivo variranja ove osobine. Postoje mnoge kontrole koje se mogu uvesti u proces prerade goveđeg mesa kako bi se povećala mekoća mesa odnosno ublažile nedoslednosti ove osobine, kao što su kačenje trupova, električno stimulisanje mišića i sprečavanje skraćivanja mišića.

Ovaj rad daje revijalni prikaz glavnih odrednica mekoće goveđeg mesa, kao i načina na koji ovo znanje može da se iskoristi u industriji za proizvodnju konzistentnijih proizvoda koji će biti visokog kvaliteta.

Ključna reč: *kvalitet goveđeg mesa, mekoća, sočnost, ukus.*

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Salt reduction in meat products – challenge for meat industry*

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A b s t r a c t: History of salt is ancient as the history of human population. Discovery and use of salt have made the food sustainable for longer period, available regardless of the season of the year and enabled its transport over long distances. For several millions of years, the prehistoric men consumed less than 0.5 g of salt daily. But today, daily salt intake is very high. Due to negative effect of salt (sodium) on the human health, modern nutrition trend is to decrease salt content in processed food. Because of that the meat products are one of the main source of sodium, role of the meat industry in the reduction of salt content in the contribution to the human health will be great. But it is not so common problem, because salt has important contribution to technological and sensory properties as well as to the microbial stability of meat products. Eleven EU countries have entered to the program of salt reduction, 16% in the next 4 years. In the goal to human health protection it is very important for food industry of each country to make decision to permanently decrease salt/sodium content.

***Key words**: salt, sodium, meat products.*

History

History of salt is ancient as the history of human population. Discovery and use of salt have made the food sustainable for longer period, available regardless of the season of the year and enabled its transport over long distances. It was produced from sea water or mined in mines. The oldest mines in the world were located in hills where the salt was mined, packed into leather bags and transported by animals, to be traded for amber, gold and copper. It was one of the first categories of trade exchange; also it has been subject of fees, taxes, caused wares and brought colonial power, created and crushed empires.

It was equally important for Jews, Egyptians, Chinese, Greeks and other ancient peoples. The Roman Empire controlled the price of salt, corrected its price, from the highest price when the earnings were used to conduct wars, to the lowest when the poor could afford it. One part of the pay of Roman soldiers was paid in salt, explaining the name for pay, wages in certain languages, e.g. English „salary" corresponding to Latin word „salarium". Even soldiers in the American civil war were sometimes paid in salt. With the development of Rome, salt roads were built to enable easier transportation of salt from the Adriatic Sea, known for very high salinity. At that time, famous salt roads were created, such as „Via salaria" in Italy, „Salzstraβe" from Lűneburg to Lűbeck, and „Golden road" from Passau to Bőhmen.

Salt was transported over long distances to German tribes or North Africa, to the central part and south of the continent, when 40 thousand camels transported salt on a 400 mile road to central African states where it was traded for the same amount of gold or for slaves.

Salt is even mentioned in the bible. It was used in burnt offerings, and also in metaphoric sense. Jesus said to his apostles „You are the salt of the

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earth“ (*Matthew* 5:13), and sometimes he instructed them „to have salt within them“.

Salt also played important part in determining the power and location of great cities. So, Liverpool, in the 19th century, developed from small English port into primary export port and became warehouse for salt produced in Cheshire mines.

Salt mines in Poland led to a flourishing kingdom in 16th century, which was lost when Germans brought sea salt, at a better price. Venice was at war with Genoa because of salt. Salt pits were source of income for Columbus travels. Christopher Columbus and Giovanni Caboto, who were citizens of Genoa, later destroyed the Mediterranean by introducing the New World into the salt trade. States, cities and duchies imposed heavy taxes and fees on salt roads, and in 1158 the city of Munich was founded, when the Duke of Bavaria, Henry the Lion, decided that the bishop of the city of Freising could not benefit any more from the salt profits. The French had the hated tax „la gabelle“, which was in effect from 1286 to 1790, when the value of salt was high that it caused the exodus, attracted conquerors and caused wars. The abolition of this fee was main goal of the revolution in year 1789, although Napoleon immediately reintroduced the fee to finance his wars.

The oldest data on application of salt in the medicine date from 3000 b.c. and are associated with Egyptian builder and physician Imothepa, who stated that salt dried infected wound and can slow down inflammatory process, and implementation of salt in the human medicine is continued by Hypocrates in ancient Greece. Paracelsus introduces the salt as the third element, in addition to sulphur and mercury, and interrupts the dualist concept of alchemy and states than only well salted food can be digested properly. He is one of the first people to use salt baths in treatment of skin diseases.

The highest consumption of salt was reached in 1870. With the advent of refrigeration and freezing, salt was no longer necessary in food preservation. And this lasted until 20th century when it was realized that higher income can be generated in production of salty food products. Also, salt became the first functional food stuff by adding of iodine in prevention of goiter.

Human requirements in salt

For several millions of years, the prehistoric men consumed less than 0.5 g of salt daily (*Feng et al.,* 2010). Intentional adding of salt to food started around 5000-10000 years ago, at the beginning of the agricultural development and daily intake of salt reached average value of 10g, which is in evolutionary sense relatively recent. Intake of common salt is caused not only by physiological needs, but also habits which are acquired in the early childhood, as well as tradition in nutrition (region, i.e. climatic conditions, preparation of food, livestock resources, etc.). Of total daily amount of common salt introduced into organism by common amounts of food (dishes prepared in the household, bread, bakery products, cheese, etc.), approx. 20% derives from meat products (*Wirth*, 1991).

Sodium from salt is mainly located in the extracellular fluid in the organism and it influences the maintaining of the water balance, nerve function, acid-base balance and muscle contractions. Although even unexpected, reduced intake of sodium can lead to muscle contractions, nausea, vomiting, anorection and coma. Because of this important role of sodium in the organism, requirements of humans in salt are often expressed as sodium requirements. According to some data, daily requirement in sodium for adults, to maintain metabolic processes and needs, is below 1500 mg. In case of athletes, requirements are higher, and even exceed 10000 mg per day, when large amount of sodium is lost through intensive sweating. However, daily intake of sodium is often over 5000 mg (*Benardont*, www.healthline.com/hlbook/nut-sodium). American Heart Association recommends for persons with hyper tension daily intake of not more than 1500 mg, and for persons with congestive heart disorders, daily sodium intake of not more than 1000 mg.

Table 1 presents recommended and tolerable intake of sodium and chloride for infants, children, men and women, pregnant and nursing women.

The effects of excessive intake of salt (sodium)

Excessive intake of sodium can lead to:

- Direct risk of heart attack (*Perry and Beevers*, 1992),
- Hypertrophy of the left heart chamber (*Schmieder and Messerli*, 2000),
- Sodium retention in extracellular fluid, i.e. water retention and clinical and idiopathic edema, especially in women (*MacGregor and de Wardener*, 1997),
- Increased hardness, i.e. decrease of elasticity of blood vessels, especially arteries, independent of the blood pressure (*Avolio et al*., 1986),

Table 1. Recommended and tolerable daily intake of sodium and chloride (*Dietary Reference Intakes for Water, Potassium, Sodium, Chloride and Sulphate,* 2004)
Tabela 1. Preporučeni i tolerišući dnevni unos natrijuma i hlorida (Dietary Reference Intakes for Water, Potassium, Sodium, Chloride and Sulphate, 2004)

	Recommended daily intake/ Preporučeni dnevni unos, g		Tolerable daily intake/ Tolerišući dnevni unos, g	
	Sodium/Natrijum	**Chloride/Hloridi**	**Sodium/Natrijum**	**Chloride/Hloridi**
Infants/Bebe				
0-6 months/meseci	0.12	0.18	/	/
7-12 months/meseci	0.37	0.57	/	/
Children/deca				
1-3 years/godine	1.0	1.5	1.5	2.3
4-8 years/godina	1.2	1.9	1.9	2.9
Males/muškarci				
8-13 years/godina	1.5	2.3	2.2	3.4
14-18 years/godina	1.5	2.3	2.3	3.6
19-30 years/godina	1.5	2.3	2.4	3.6
31-50 years/godina	1.5	2.3	2.4	3.6
51-70 years/godina	1.3	2.0	2.4	3.6
>70 years/godina	1.2	1.8	2.3	3.6
Females/žene				
8-13 years/godina	1.5	2.3	2.2	3.4
14-18 years/godina	1.5	2.3	2.3	3.6
19-30 years/godina	1.5	2.3	2.4	3.6
31-50 years/godina	1.5	2.3	2.4	3.6
51-70 years/godina	1.3	2.0	2.4	3.6
>70 years/godina	1.2	1.8	2.3	3.6
Pregnant women/ Trudnice				
14-18 years/godina	1.5	2.3	2.3	3.6
19-30 years/godina	1.5	2.3	2.3	3.6
31-50 years/godina	1.5	2.3	2.3	3.6
Lactating women/dojilje				
14-18 years/godina	1.5	2.3	2.3	3.6
19-30 years/godina	1.5	2.3	2.3	3.6
31-50 years	1.5	2.3	2.3	3.6

- Proteinuria, primarily to urinary excretion of albumin, resulting in increased risk of heart and kidney diseases (*Du Cailar et al*., 2002),
- Greater possibility of infection by *Helicobacter pylori* and risk of stomach cancer (*Tsugane et al*., 2004),
- Increase of urinary excretion of calcium and risk of forming of kidney calculi (*Capuccio et al.*, 2000),
- Risk of reduced bone density, resulting in osteoporosis and compressive bone fractures, especially in case of women in menopause (*Devine et al.*, 1995),
- Exacerbations (more intensive and longer) of asthmatic seizures (*Mickleborough et al.*, 2005),
- Increase of HOMA (homeostasis model assessment) insulin resistance in patients with essential hypertension, majority of which

have reduced glucose tolerance (*Kuroda et al.*, 1999), and
- Indirect incidence of obesity due to intensive intake of refreshing, non-alcoholic beverages (*Feng et al*., 2010).

In addition to stated harmful/adverse effects, increased intake of sodium is one of the major causes of hyper tension which represents the greatest risk for development of cardio vascular diseases. This topic is in the focus of attention due to continuous increase in consumption of salty food, bad nutritional habits and poor physical activity of people in the modern society.

The earliest record of the effect of salt from food on blood pressure (*Huang Ti Nei Ching Su Wein,* 2698-2598 BC) is following „If too much salt is used for food, the pulse hardens...“ and „Therefore if large amounts of salt are taken, the pulse will stiffen and harden“.

Of all established hypertension conditions, 95% represent essential hypertension. The most common medical advice is to consume less salty food.

The mechanism leading to increase of blood pressure due to excessive intake of salt is inability of kidneys to excrete the excess sodium amount until the arterial blood pressure is elevated and in this way the excretion of fluids through kidneys is increased (*Wirth and Offermanns,* 2008). Studies show that with the increase of intake of salt the body mass, total sodium content in blood, extracellular volume, plasma and blood volume also increase. At the same time, the levels of renin, angiotensin and norepinephrine decrease (*Haddy*, 2006).

For the first time, in year 2000, in the journal Progress in Cardiovascular Nursing, the salt sensitivity is mentioned, representing the elevation of blood pressure as response to increased intake of sodium. In sodium sensitive persons, fluctuations of blood pressure as response to increased or reduced intake of sodium are more dramatic compared to non-sensitive persons. Sodium sensitivity occurs more often than we think, even around 30% in normotensive persons and more than 50% in hypertensive persons. It is more common in black race, elderly and people with kidney insufficiency and diabetes.

Considering that prehistoric man consumed less than 0.5 g of salt daily, intake of salt exceeding this amount, in the evolutionary sense, is more recent occurrence, and it is clear that people are not genetically programmed to consume higher amounts of salt. Epidemiological studies in the 20th century vary from absence of hypertension in population consuming less than 3 g of salt daily to high incidence of hypertension in populations consuming over 20 g of salt per day.

About 40 indigenous tribes from South America, Africa, Pacific and Arctic consume less than 3 g of salt per day and their pressure is not elevated with the age. In South America, members of the Yanomamo tribe, living on the border between Venezuela and Brazil, consume less than 0.5 g of salt daily and average blood pressure in men is 105/70 mmHg and 95/60 mmHg in women.

There is positive correlation between HOMA insulin resistance and sodium sensibility in patients with essential hypertension, and in many of them also glucose tolerance. Increase insulin level in blood leads to sodium sensibility through increase of sodium absorption in kidney channels (*Kuroda et al*., 1999).

In addition, today, according to modern techniques, also the genetic basis of sodium sensitivity is studied. Over 20 gens are responsible for essential hypertension or for rare Mendelian disease with high or low blood pressure (*Lifton et al*., 2001; *Mein et al*., 2004). Majority of these genes encode proteins which are mediators of sodium excretion through kidneys (*Meneton et al*., 2005). Gene mutations can lead to increased sodium absorption resulting in elevation of blood pressure.

Analyses of genomes in human population showed that in persons with hypertension, very important is the role of the renin-angiotensin system. Angiotensin II regulates the blood pressure and salt retention in the organism. In case of hypertensive persons, angiotensin molecules are different from those in healthy persons (substrate for the action of renin) are determined. These mutated angiotensin variants are directly responsible for incidence of essential hypertension. By analysis of the genome of diseased persons the functional mutation of gene in charge of angiotensinogen synthesis (AGT) was detected, which is manifested in substitution of adenine for guanidine in the AGT promoter region. Translation of mutated gene causes the acceleration of the AGT gene transcription and consequently the increase of the angiotensinogen concentration in the systemic circulation, i.e. to increase of blood pressure (*Charles et al*., 2005).

In addition to the effect on elevation of blood pressure, AGT gene also has impact on salt sensibility. Namely, persons with homozygous allele AA and heterozygous allele AG show statistically significant variations in blood pressure depending on the concentration of sodium chloride in food and represent risk group for incidence of essential hypertension, whereas persons with homozygous mutated allele GG (and constantly elevated blood

pressure) had no reaction to the change of sodium chloride concentration (*Melo et al*., 1998; *Watkins et al*., 2010).

Physiological regulation of blood pressure takes place through several protein complexes, including atrial natriuretic peptide (ANP). This protein consists of 28 amino acids, and it is synthesized in heart atriums. When ANP is applied in physiological doses to the organism, blood pressure drops and excretion of salt is increased. Certain researchers have studied if the changes in the gene for ANP synthesis influence changes of blood pressure. It was established that in mice which by genetic manipulation had their ANP gene promoter extracted (homozygous mutants), significant increase of blood pressure occurred in consumption of standard diet (0.5% NaCl) and intermediary diet (5% NaCl), whereas in heterozygous mutants the hypertension occurred in diet with high content of NaCl (8%). It was established that the polymorphism of ANP promoters exists in humans (*Unger et al.*, 1990).

Third known mutation of gene responsible for changes in blood pressure depending on the intake of salt is mutation of gene for synthesis of adducin. Hypertensive persons which have the mutation (Gly 460) of adducin, in nutrition poor on sodium, show drop in arterial blood pressure (*Steaessen and Bianchi*, 2005; *Manunta et al.,* 2007).

Functional properties of salt in meat products

Salt in meat products causes the salinity (*Ruusunen and Puolanne*, 2005) and together with fats contributes to numerous sensory properties. Increase of saltiness is more distinct in products with increased amount of fat, and in products with higher protein content, the sense of saltiness is lower. One of the major functions of salt in meat products is solubilisation of functional myofibril proteins, which activates the proteins to increase the hydration and water holding capacity (WHC) and, accordingly, improve the texture of product. Increase of WHC in meat reduces cooking loss and increases the tenderness and softness of meat products. There are two hypotheses on role of salt in meat WHC. According to *Hamm* (1986), chlorine ions have the tendency to penetrate myofilaments causing their dissolution, whereas *Offer and Trinick* (1983) claim that sodium ions form ion "clod" around filaments. They base hypothesis on selective bonding of chlorine ions to myofibrillar proteins. Dissolved myofibrillar proteins form sticky exudate on the surface of meat pieces which are subsequently connecting in this way during the heat treatment of the product. Matrix of proteins coagulated by heat tie in "trap" the free water. In emulsified meat products such as cooked sausages, dissolved proteins in form of continuous phase, represent the film around fat and water drops.

Salt has antimicrobial effects. Inhibitory effect of salt on bacteria is based on lowering of the activity of water. At a certain concentration of kitchen salt, water exits the cells through osmosis, and this can slow down or completely stop the microbial development/growth. Relatively high concentrations of salt are necessary to inhibit microorganisms. Limit concentrations of sodium chloride for microbial growth are: 5% for *Clostridium botulinum* type E and *Pseudomonas fluorescens*, 6% for *Shigelae* and *Klebsiellae*, 8% for *Escherichia coli*, *Salmonellae*, *Bacillus cereus*, *C. botulinum* type A and *C. perfringens*, 10% for *C. botulinum* type B and *Vibrio parahaemolyticus*, 15% for *Bacillus subtilis* and *Streptococcaceae*, 18% for *Staphylococcus aureus*, 25% for *Penicillium* and *Aspregillus* species and 26% for *Halobacterium halobium*, *Bacterium prodigiosum* and *Spirillium* species (*Prändl, 1988*).

Content of salt in meat products

Content of salt in meat products depends, primarily, on technologically justified amounts, and, of course, on the influence of salt on the saltiness. There are numerous studies on content of salt in different meat products (*Vranic et al*., 2009). The lowest salt content is in the cooked sausages and meat cans. In cooked sausages, content of salt ranges from 1.28 to 2.03 g/100 g, in average 1.66 g/100 g, whereas in meat cans it ranges from 1.35 to 1.84 g/100 g, in average 1.67 g/100 g. In smoked meat products the salt content is slightly higher and it ranges from 1.66 to 3.11 g/100 g, i.e. in average 2.19 g/100 g. In dry fermented sausages, technologically justified amount of salt is considerably higher and therefore 2.5–3.0% is added, since these products are not subject to heat treatment, and salt serves for maintaining of the microbiological stability of the products. Salt content in these sausages is 2.08–3.98 g/100 g, i.e. in average 2.61 g/100 g. The dry meat products have the highest salt content. Due to long production process, i.e. curing, these products are salted or cured using 5–10% of salt or curing salt, in order to reduce, by action of the salt, the water activity in order to prevent growth of undesirable microorganisms. Salt content in dry meat ranges from 3.78 to 7.35 g/100 g, in average 5.09 g/100 g.

Possibilities for reduction of salt in meat products

Present trends in nutrition to reduce the content of sodium in meat products, as reported by *Ruusunen and Puolanne* (2005) and *Desmond* (2006), can be achieved in the following way: (1) by reducing the amount of sodium chloride added (*Sofos*, 1983; *Lilić*, 2000); (2) by substituting part of NaCl with other salts (*Sofos*, 1983; *Terell*, 1983; *Guàrdia et al.,* 2006; *Lilić et al.*, 2008).; (3) by using flavour/aroma enhancers and masking agents (*Desmond*, 2006); (4) combination of mentioned procedures (*Sofos*, 1983; *Terell*, 1983); (5) adding of spice herbs and spice extracts to meat products (*Lilić and Matekalo-Sverak*, 2007; *Matekalo-Sverak et al.*, 2007); (6) optimisation of the physical form of salt (*Angus et al*., 2005); and (7) alternative process techniques (*Claus and Sørheim*, 2006).

Potassium chloride is most common salt replacer, however, complete substitution of salt is not possible since, already in case of 50% substitution the bitter flavour is intensified and saltiness is reduced. Use of potassium salts has often been disputed because of potential sensitivity of one part of human population, such as persons suffering from diabetes type I, chronic renal insufficiency, last stage of kidney diseases, persons with heart and adrenal insufficiency (*FSAI*, 2005). US Dietary Guidelines (2005) indicate that diet rich in potassium weakens the effects of salt on blood pressure and daily potassium intake of 4.7 g is recommended.

Various diet salts as mixtures of sodium chloride and potassium chloride which improve the excretion of sodium from the organism are already on the market.

In cooked hams, sodium chloride can be substituted with potassium chloride and 50% without any effect on sensory properties (*Frye et al*., 1986). In hams, the use of 70% of NaCl and 30% of KCl, i.e. 70% NaCl and 30% $MgCl_2$, has no effect on flavour, tenderness and overall impression compared to hams produced only using NaCl (*Collins*, 1997).

In fermented sausages (*Gou et al*., 1996), researchers have established absence of any difference in texture in substitution, but bitter flavour can be sensed already when 30% of KCl has been added. They also report that substitution of 40% with KCl and potassium lactate in dry meat does not lead to undesirable flavour characteristics.

According to *Ruusunen and Puolanne* (2005), reduction of salt in fermented sausages is not possible below 2% due to inability to reach sufficiently low water activity which provides microbiological stability of these products.

Recent production processes are developed in a way that brine is injected into meat, and it contains KCl in combination with calcium citrate, calcium lactate, lactose, dextrose, potassium phosphate, ascorbic acid and sodium nitrite (*Riera et al*., 1996).

Phosphates are also very successful in reduction of salt in products, although they act in synergy with sodium chloride. They increase the WHC by increasing the ion strenght when free groups of negative charge enable that proteins tie more water (*Trout and Schmidt*, 1984). However, phosphates are also sodium carriers. So, sodium polyphosphate contains 31.24% of sodium, compared to 39.34% in sodium chloride, however its use is limited to approx. 0.5% in the product.

Ruusunen et al. (2002) established that the production of Bologna sausage and cooked ham with less salt (1.0–1.4%) is possible and that reduction of sodium content can be achieved by using potassium salts. It can be claimed that the use of phosphates in relation to sodium chloride is in the equivalent of 0.2% NaCl.

There is another possibility to compensate for technologically desirable properties of NaCl, and that is the use of ingredients such as fibres, hydrocolloids and starches which enable forming of the gel and protein coagulates (*Collins*, 1997).

One of the possibilities for reduction of salt in meat products is the use of flavour enhancers and masking agents. There are many different commercial mixtures which usually contain yeast extracts, lactates, monosodium glutamate and nucleotides. Flavour enhancers activate the receptors in the mouth/oral cavity and they compensate for the reduction of salt in the product (*Brandsma*, 2006).

Certain authors (*Pasin et al*., 1989) have established that it is possible to reduce NaCl to 75% in cooked sausages, by combining KCl, preparation containing ribotide (commercial mixture of 5'–ribonucleotides IMP and GMP). Any addition of monosodium glutaminate leads to drop in the acceptability of the flavour of the product even by 50% due to incidence of bitter flavour caused by potassium salts. Linguagen, company from USA has patented a blocker of the bitter flavour, adenosine 5'-monophosphate, which blocks the activation of the gustducine in flavour receptor cells and accordingly, prevents stimulation of the nerve which is responsible for taste receptors (*McGregor*, 2004). This blocker can be used to improve the flavour when combinations of KCl and NaCl are used.

There are several preparations on the market such as NeutralFres which removes the metallic, bitter flavour of KCl and gives the flavour similar to sodium salt, Magifique Salt-Away and Mimic,

which mask the bitter and metallic character of KCl as well as SaltTrim.

Other combinations such as lysine and succinic acid are used as substitutes (*Turk*, 1993). These substances have salty flavour and some antimicrobial and antioxidative properties and can be used as salt substitutes up to 75%. In regard to technological properties, i.e. WHC, phosphates can be used, as well as starches and rubber.

Gou et al. (1996) studied the effect of glycine and potassium lactate as salt substituent and established that substitution of 40% of NaCl is possible using some of these compounds, which if used in larger amounts give unacceptably sweat flavour. In dry meat, substitution of up to 40% with potassium chloride and potassium lactate is possible without any significant difference in flavour, whereas 30% is maximum allowed amount if glycine is used as substituent.

There are also derivatives of mycoproteins (Mycoscent) which offer possibility to reduce the amount of sodium chloride by 50% in biscuits and snack foods and by 25% in hot, spicy dishes. Mycoscent 400 is natural source of ribonucleotide and glutamic acid, and it has flavour resembling broth and can be used to achieve the flavour of cooked meat in meat applications (*Mycoscent*, 2005). Yeast autolysates are also known to suppress the bitter flavour of KCl, such as Provesta preparations, Aromild and Maxaromeselect. A problem with autolysates is their distinct broth flavour, which is not desirable in some products, and some of them have typical original umami flavour. By using certain technological procedures it is achieved that these preparations are optimized for meat products with neutral flavour and optimal umami effect.

Level of saltiness depends also on physical form of salt. Salt flakes are proven to be functional in terms of binding, increasing pH, increasing protein solubility in emulsion model systems (*Campbell*, 1979). Salt flakes are better and faster soluble compared to granules, and this can be problem when in formulas no water is used, therefore flakes can be used for products where no water is added, for instance dry meat. Leatherhead Food International studied the optimization of the physical form of salt and monitored changes in the physical form of salt which is becoming more available and hence could be used in smaller amounts. This includes increase of its efficiency, change of the structure and modification of the perception of salt (*Angus et al*., 2005).

Alternative process techniques include use of prerigor meat in manufacturing of meat products or use of high pressure technology (*Claus and Sørheim*, 2006, *Dederer*, 2009).

Economical justification of the salt reduction

Several studies have shown that reduced slat intake in population is economically justified (*Asaria et al*., 2007). So, *Murray et al*. (2003) have shown that health interventions, including government actions to stimulate the reduction of salt content in food products were economically justified in sense of decrease of the incidence of cardiovascular diseases. One of the studies showed that reduced intake of salt to 6 g per day in Norwegian population had lead to drop in systolic blood pressure by 2 mm Hg and reduced cost by 4.7 million dollars annually (*Selmer et al*., 2000). Study carried out in Canada showed that reduction of salt intake to 4.6 g daily can save approximately 430 million dollars annually in treatment costs, visits to doctors and laboratory testing of the causes of hypertension (*Joffres et al*., 2007).

In their study, *Asaria et al*. (2007) have evaluated the effects and costs of the strategy to reduce the salt intake and tobacco control for 23 less developed and developing countries, and have proven that with the reduction of salt by 15% in the period 2006 to 2015, the death of 8.5 million people suffering from cardiovascular diseases could be prevented, and by reduction of smoking by 20% death of 3.1 million people. Moderate salt reduction could be achieved by reduction of salt content in food by producers, as well as through continuous media campaign. Cost of implementation of such programs of salt reduction is estimated at the amount of 0.09 dollars *per capita* annually. Cost of tobacco control including free measures and cost amount to 0.26 dollars *per capita* annually. These data clearly indicate that the reduction in salt intake is more or at least to the same extent economically justified compared to tobacco control in reduction of prevalence of cardio vascular diseases.

Role of food industry in salt reduction

Many countries have developed their own guidelines for the programs of salt intake reduction. In developed countries, approx. 80% of salt is added to food through different production stages.

Because of the importance of this topic, many producers initiate salt reduction programs in their production and starting with reformulation of their products. But there are many producers who are not supporters of these programs because of commercial reasons. If very salty food is consumed consistently, receptors become used to saltiness and demand for salty food increases. Salt is main determinant in

sense of thrust and reduction in salt intake would definitely have impact on sale and consumption of refreshing non-alcoholic beverages and mineral water (*He et al*., 2008). However, some of the snacks producing companies in the world are also part of companies manufacturing such beverages; therefore it is understandable that they do not want to participate in programs for reduction of salt in food. World health organisation (WHO) initiated the reduction strategy through regional directorates. Eleven EU countries have agreed to and signed the program of salt content reduction of 16% in the next 4 years. Food industry in Serbia also can be included in this program and meat industry could have one of the main role in this reduction program, contributing to the human health.

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Redukcija soli u proizvodima od mesa – izazov za industriju mesa

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R e z i m e: Istorija soli stara je koliko i istorija ljudskog roda. Otkriće i upotreba soli učinili su hranu dostupnijom u dužem vremenskom periodu, nezavisno od sezone i omogućilo njen transport na veće udaljenosti. Tokom miliona godina, praistorijski čovek unosio je manje od 0,5 g soli dnevno. Ali danas, unos soli je veoma veliki. Usled negativnih efekata soli (natrijuma) po ljudsko zdravlje, moderni nutricionistički trend je da se smanji sadržaj soli u proizvedenoj hrani. Zbog toga što su proizvodi od mesa jedan od osnovnih izvora natrijuma, uloga industrije mesa u redukciji sadržaja soli kao doprinos ljudskom zdravlju, biće velika. Ali to nije tako jednostavan problem zato što so značajno doprinosi tehnološkim i senzorskim karakteristikama, kao i mikrobiološkoj stabilnosti proizvoda od mesa. Jedanaest zemalja Evropske Unije ušle su u program redukcije soli za 16% u naredne četiri godine. U cilju zaštite ljudskog zdravlja, veoma je važno da industrija hrane iz svake zemlje donese odluku da počne sa permanentnim smanjenjem sadržaja soli/natrijuma.

***Ključne reči**: so, natrijum, proizvodi od mesa.*

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Remediation of by-products from slaughtered animals in regard to new regulations*

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A b s t r a c t: The environment is now threatened by the creation and accumulation of waste materials in all branches of industrial production and in the meat industry. The importance of safe animal waste disposal increases with the intensification of animal breeding and with development of meat processing plants.

Solving of the problem of safe animal waste disposal is very important. It is irreplaceable, preventive veterinary and sanitary measure used in control of livestock diseases and zoonosis. Nowadays, it has an important role in environment protection.

In the World, the best way of safe animal waste disposal from meat industry as well as dead animals is their collecting and utilization (for feed production, chemical industry and fuel), depending on the raw material structure and its characterization, their processing in special plants with modern equipment and technology.

It was emphasized that facilities for animal wastes processing should be treated from two aspects: as processing plants aiming at the environment protection and as possible environment polluters.

Key words: *by-products of animal origin, safe disposal, utilization of by-products, environment.*

Introduction

Fundamental task of agriculture is the production of adequate quantities of high quality foods and raw materials of organic origin for the existing World's population and for rapid increase of that population of about 93 million people per year (*Kenedy*, 1993). Even growing demands for food production impose the needs for more efficient managing of economic resources that such production entails. Management of agricultural resources is crucial for survival of mankind, i.e. for the economic, cultural and social development of the society.

Safe disposal of inedible animal wastes was treated differently during its long history until today, when it obtained an exceptional significance. Today, it is considered as irreplaceable veterinarian-sanitary and preventive measure in suppression of spreading of infectious animal and human diseases (*Ristić et al.*, 2003). Even more and more is emphasized its role in rehabilitation and protection of the environment.

With respect to realization of this, arises the necessity of organized collection, storage and disposal of animal by-products from slaughtering, by their technical processing in specialized plants, which produce from this raw material (depending on category foreseen in the Directive EU 1774/2002) high-quality animal feed or raw materials for biofuel production (biogas, biodiesel) with the complete protection of the environment (*Ristić et al.*, 2007; *Okanović et al.*, 2007).

In order to realize the complexity of this problem, we described in this work the contemporary

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position and solutions for this problem, what, in fact, represent the objective of this study.

Importance of safe disposal of animal by-products

There is growing need for finding of the solution for safe disposal of animal by-products by their utilization and processing into animal feeds and biofuels, because of the intensive livestock production and increase of capacities of industrial slaughterhouses, building of new small slaughterhouses, of meat processing plants and increase of the volume of international trade of commercial animal products (*Okanović et al.*, 2006).

Adequate solving of safe disposal of animal by-products can be perceived through three key aspects that should fulfil the technological solutions for solving the disposal of such materials by their processing, namely:

- the epidemiologic-epizoothiological aspect,
- aspect of the environment protection, and
- economic aspect.

a) Epidemiologic-epizoothiological aspect

Keeping in mind that animal by-products (inedible by-products of animal slaughtering, dead animals and other waste generated on livestock farms) must be treated as potential sources of infectious diseases of humans and animals, special significance must be directed to the issue of their sanitary disposal (*Ristić et al.* 2006, *Okanović et al.*, 2008a).

In case of animals that dead due to infectious diseases, before their death bacteraemia, vitaemia (salmonellosis, red wind, swine plough and other carriers) were found. Because of that, each dead animal and inedible slaughterhouse by-product should be regarded as the highest possible source of infection.

Animals and humans can be infected from the environment by direct contact with animal by-products, primarily with unprocessed or inadequately processed animal products, food contamination through air or by mediation of other vectors (insects, rodents, dogs, wild animals, birds), or in some other way. Under distinct conditions, the opposite cases can occur – transferring of diseases form people to animals. Such conditions create some kind of the infectious circle with moving of infection provokers in the environment between humans and animals, with or without distinctive mediator (*Prince i et al.,* 2003).

Exceptionally dangerous are animals that dead from the infectious diseases whose carriers are sporoes, such as anthrax and the gas edema. Sporogenous forms are very resistant to the environmental factors, so their survival life is long-lasting. If the dogs or wild animals are allowed to plunder parts of the infected animals, diseases carriers can be transferred over larger areas and considerably contaminate soils, representing high and long-term danger for animals of that region, as well as for human health (*Okanović et al,* 2010a).

According to *Ristić et al*., (2008), number of the recognized zoonoses in the World is high (about 180), and we are witnesses of the appearing of new ones which, until today, were not registered as zoonoses (lime-boreliose, Ebola hemorrhagic fever, ehrlichiosis and since 2005 officially the bird flu as well).

In recent years, global epizoothiologic and epidemiologic situation with respect to communicable diseases and zoonoses has been considerably changed. That was influenced, of course, by definite number of facts, such as rapid development of communications, accelerated development of technology and raw material exchange, inadequate utilization of animal wastes, wars, economic crises and other.

According to the World Health Organization data, present epizoothiologic – epidemiologic situation in the World indicates the fact that high number of communicable diseases show trends of expansion, so that it can justifiably be said that future of human population belongs to communicable diseases that day by day take their tribute on distinct parts of the World.

Cited data confirm that animal by-products from epizoothiologic and epidemiologic points of view represent high danger with respect to animal and human health, what necessitates the need for rapid and efficient and, at the same time, safe disposal of the mentioned materials.

b) Aspects of the environment protection

Today, more attention is directed to the protection and improvement of human environment, because it is under the threat due to creation and accumulation of waste materials. The country strives to produce the highest possible quantities of products in order to satisfy human needs for the best possible standard of living and to create optimal conditions for maintaining of sanitary conditions. Nevertheless, together with welfare aspects that are necessary to human population, modern technical civilization creates high quantities of waste, which exert negative effects on the environment, degrading it to such degree that it becomes harmful to human and animal health (*Ristić* 2000; *Okanović et al.,* 2008a).

It seems that the aspirations for something better can have adverse consequences. This is fully applicable on agricultural and livestock production, which have survived many changes. Such tendency is enabled with industrial preparation of feeds and with even higher automation and mechanization of livestock production. Dead animals and inedible slaughterhouse by-products, as waste materials created in the production process, must be disposed safely, or, otherwise, they can become a serious obstacle for further development of production, in this case of food and, as such, they are serious polluters of the environment. On the other hand, they can so severely contaminate the environment, in a way that it begins to hinder intellectual and operative capabilities of humans and disables the possibilities for their recreation (*Okanović et al.,* 2007).

Dead animals and inedible slaughter by-products, like all organic substances, are susceptible to very rapid degradation. Such process is accompanied by the creation of products of decay, mainly of gases (ammonia, hydrogen sulphide, mercaptans), but also of other products of decay, such as fatty acids, aromatic acids and other substances (*Okanović et al.*, 2008b).

Animal wastes during putrification contaminate not only the atmosphere, but also the soil, food and water. The greatest part of blood flows into the sewage, i.e. in waste water, and only small share of blood is collected and processed. Water courses are physically polluted, and at the same time, in such environments, blood appears as nutrient for microorganisms, many of which are pathogenic strains to humans and animals. Biological oxygen demand of blood, according *Baras et al.* (2007), is about 100,000 mg O_2/L. In the year 1982, contamination of water courses with waste blood in SFR of Yugoslavia was about $57 \cdot 10^9$, what corresponds to the pollution caused by about 1 million of inhabitants.

Pollution of the environment by animal waste shows other adverse effects as well. Such places are, also, locations with ideal conditions for development of insects and rodents. They enable spreading of infections and substantially contribute to degradation of visual acceptance of environment in which they live. Aesthetic unacceptability of environment threatened is one of the problems that deserve even more space and time.

The environment, especially air and water, can be contaminated even in the process of safe disposal of dead animals and inedible by-products of slaughtered animals and their processing into feed and raw materials for chemical industry. Because of that „the facilities for animal wastes processing“, according to *Ristić et al* (1998; 2008b) should be considered from two points of view – as facilities serving for environment protection, i.e. as manufacturing plants and, at the same time, as environment polluters.

Polluters deriving from the process of safe disposal of by-products are:

- scattered inlet raw materials,
- waste waters,
- waste gases,
- organic dust, and
- contaminated solids not adequate for processing.

Scattered input raw materials in the facility for safe disposal of animal wastes, if they are not removed on time, can be the significant sources of environment pollution and they can complicate normal technological process in the factory.

Waste waters in factories for processing of animal wastes appear in reception part, processing (final) part and in the station for cleaning and disinfection of transportation vehicles. Waste water obtained in reception part must be collected and sterilized using thermal or chemical processes and, together with other waste waters, transferred into separate facility for waste water treatment (*Nježić and Okanović,* 2010).

With respect to the facilities for animal wastes processing, they should fulfil two basic functions – to protect the environment from pollution cause by animal wastes and to generate sanitary safe products and, during the designing of the facility and during its regular operation, as well as during the unwanted incidents, to implement regular measures for protection of the environment. (*Ristić and Okanović*, 2008).

All above-mentioned indicates that the methods of disposal of dead animals and of inedible by-products used so far, in spite of all advancements, suffer from many problems associated with the absence of unique solutions, so that in this domain today, at the beginning of 21st century, we still find a mosaic of procedures known from relatively long history of disposal of this kind of organic materials. It is understandable that such situation does not satisfy contemporary needs, either from the aspect of suppressing of cattle contagion, or in connection with the improvement and intensification of livestock production and the environment protection (*Ristić et al.*, 2008b; *Okanović et al.,* 2008b).

Disregard of the rules on safe disposal of polluters reflects on soil, atmosphere, surface and underground water quality in the neighbourhood, i.e. on climate and, further, on plant and animal life and on health of human population, in other words, on the eco-system as the whole.

c) Economic aspect of disposal of dead animals and inedible by-products obtained from slaughtered animals

Economic side of this problem implies collection and safe disposal of huge quantities of biological materials incurring certain costs which have to be incorporated in the price of obtained products. If waste of animal origin is not processed (recycled), it is lost raw material that could have been incorporated in production of protein - energetic feed, technical fat for chemical industry or of fuels of high calory value (*Okanović et al.*, 20010).

According to the European Union directives included in the Regulation (EC) Nº 1609 (2009), by processing of sanitary safe inedible by-products generated in slaughtering of animals (Category 3 materials), it is possible to obtain:

- protein, protein-mineral and energy products aimed for feeding of animals,
- technical fats,
- feathers for textile industry,
- skins, horns, hoofs and hairs.

and from dead animals (Category 2 materials):

- meat-and bone meal as an energy component,
- technical fat as an energy component, or raw material for further processing in chemical industry or for production of bio-diesel, and
- biogas, compost.

Safe disposal of the described animal wastes (Category 1 material) by combustion at high temperatures (over 850°C) enables obtaining of hot water or steam as energy component for processing plant that uses warm water or steam and ash as the material used for construction of roads. Products (meat- and bone meal and fat) obtained by processing of Category 1 materials are suitable for use as energy fuel, i.e. as fuel for direct combustion in architecturally separated facilities, respecting the corresponding legislative rules.

We shall mention only that with respect to procedures of blood collection and its technological processing, various articles for human use can be obtained, primarily products which are used as functional additives in manufacturing of meat products. Special processing procedures enable their use as raw materials in pharmaceutical industry or for production of functional foods (*Matekalo-Sverak et al.*, 2007; *Okanović et al.,* 2008b).

On the other hand, industrial waste blood can be collected and processed using corresponding technological procedure in a plant for processing of other animal by-products, using special processing unit. Such procedure enables obtaining of feed of high protein content, which mostly contains high quantities of essential amino acids, vitamins and mineral substances, and particularly iron (*Okanović et al.,* 20010c; *Ristić et al.*, 2009).

If all cited aspects were treated correctly, it is clear that organized processing and safe disposal of inedible slaughter by-products obtained from slaughtered or dead animals is of great importance for prevention of spreading of contagious diseases, successful protection and rehabilitation of the environment and for rational use of such wastes.

Methods of rehabilitation of animal wastes

Each period of economic development and of scientific cognition in human and veterinary medicine leaves its contribution in understanding and solving of problems of safe disposal of animal waste. Regardless of historical period, basic aim of the activities in this domain was to achieve rapid degradation of organic substances and to inactivate eventually present infectious organisms, with, at the same time, prevention of contacts of humans with the contagious materials. It was realized in different ways:

- burying on animal graveyards,
- disposal of dead animals in landfill/pits--repositories,
- combustion in special furnaces, and
- technical processing.

Changes in the modes of rehabilitation, according to *Ristić et al.,* (2008a), occurred with the incidence of neurodegenerative diseases of animals and humans that characterize spongiform degeneration of brain – diagnosed as spongiform encephalopathy of bovine animals, i.e. the BSE, whose carrier, as it was found in the year 1986, is feed containing inadequately produced meat and bone meal obtained from ruminants as stated in the Regulative of European Parliament on inedible animal by-products (*Regulative (EC) Nº 1774*/2002).

According to the contemporary regulations in the European Union (*Regulative (EC) Nº 1609*/2009)), animal waste can be safely disposed, depending on the category, in the following way:

1. burying on graveyards for pets,
   - burying on locations where organizing of other methods of safe disposal is hardly practicable because of the inaccessibility or for some other reason,
   - burying at the place of outbreaks of described contagious diseases,
2. incineration of raw waste in special furnaces at high temperatures (850 – 1200° C),

3. combustion or co-combustion, after technical processing in the plant, which fulfils requirements/conditions for such method of safe disposal,
4. processing in production of compost and biogas, and
5. heat treatment and processing into feed.

Prerequisites for choice of some of the available methods of safe disposal that correspond to our region lie in recognizing of basic characteristics of different utilization methods.

Burying is one of the oldest methods of safe disposal of dead animals, which does not fully achieve the goal of the fastest possible extermination of contagious materials, because decay processes of organic substances in the ground are relatively slow, depending also on the ground quality. Burying of dead animals and inedible by-products obtained from slaughtered animals represents insanitary and uneconomical method of their safe disposal. The existing data on resistance of carriers of contagious diseases in decaying materials clearly indicate that burying of corpses of animals which died as consequence of contagious diseases is not safe disposal, and it represents conservation of contagious diseases carriers for the period of several decades. Burying of corpses as a method of disposal can be, according to the cited authors, tolerated only as emergency solution in case when there is no possibility for applying of heat methods because of inapproachability of terrain, as well as for corpses of small animals (pig, lamb, dog, cat, rabbit and similar). There is objective hazard, if dead animals are buried improperly, that the contagious material can be transferred with underground water to the neighbouring region. Because of that, Scientific Committee of European Union prescribed that only dead pet animals can be buried, on orderly arranged/organized graveyards for pets or dead animal not heavier than 50 kg; the owner can bury it on his property, 2.5 meters from the border with neighbouring property and in inhabited settlement 20 meters from the neighbouring building (with exception of spaces where difference of levels of surface and underground water does not exceed maximal value of 1 meter). Well constructed graveyard for pets is satisfactory mode of safe disposal for urban settlements, where significant numbers of pet animals appear.

Combustion of dead animals and inedible raw slaughter by-products is safe, but the most cost--effective method of safe disposal of the infected materials (*Ristić et al.,* 1997).

Co-combustion (co-incineration) of animal by--products (Category 1 materials) and of products obtained from by-products of processing of Category 2 materials – meat and bone meal, is the safest and economically justified method of safe disposal of dangerous animal waste.

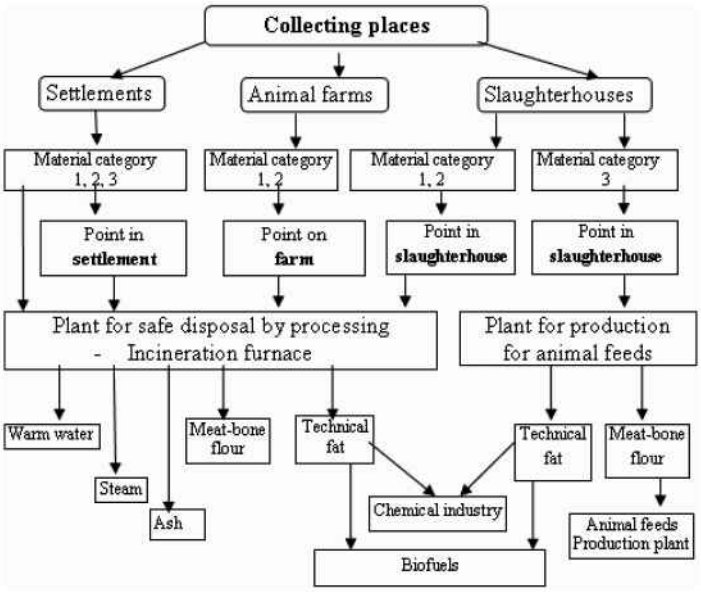
Safe disposal of animal waste by its processing and production of biogas represents one of the alternative methods for safe disposal of category 2 and 3 materials. This method of safe disposal of animal waste is relatively cost-effective, because it needs heat processing of animal waste, i.e. the corresponding infrastructure for biogas production with complicated technological process of bio-fermentation and biogas production.

According to *Ristić et al.,* (2006), without any doubt, the newest and the best method of safe disposal of animal waste is technical processing of separate categories into products for chemical industry, bio-fuels and feed for specific species of animals.

Prerequisite for safe disposal of animal waste using one of the described methods is organized collection and delivery of raw materials. Modern disposal of waste materials requires orderly constructed plants with adequate capacities, which should assure permanent and continuous supply of raw materials. This confirms the importance of recognizing the raw materials fundaments for each facility, i.e. organizing of epizoothiological and economically acceptable region, which should enable obtaining of adequate quantities of animal wastes that should allow designing and construction of modern facility for their safe disposal (*Ristić et al.*, 2008a).

Exceptionally important is to emphasize the need for transferring of animal wastes from the place where they were generated to the storage place as fast as possible, as well as the need for rapid performing of the processing procedure. This is very significant, not only from the epidemiologic-epizoothiologic aspect or from environment protection aspects, but equally from the aspect of the technical processing. Namely, fresh raw materials are processed easier, with generation of lower quantities of waste gases and obtaining of better quality products (*Okanovic*, 2009.)

In such collecting cycle, organizing collection of animal wastes represents very delicate problem; the successful operation of the processing plant depends on the solution of this problem. This problem, in any case, is relevant to both plant that process raw materials of animal origin and livestock farms and slaughterhouses that generate such raw materials. Also, local municipal communities have important role in solving of this problem. They are, according to the existing legislative rules on suppression of contagious diseases, obligated to organize safe disposal of animal waste in their region. In



Scheme 1. Organizing of collection storaging and safe disposal of animal wastes
Shema 1. Organizacija sakupljanja, čuvanja i neškodljivog uklanjanja životinjskog otpada

other words, organization of collecting of mentioned raw materials should be based on contracts between plants for safe disposal and processing of animal wastes and local municipal communities or their corresponding organizations (slaughterhouses, animal farms etc.).

The accepting of safe disposal of animal waste by processing and incineration excludes classical forms of disposal (holes, animal graveyards), except in exceptional occasions, so that locations for such facilities, should be foreseen nevertheless. It is valuable to add that, in the most cases, unsolved issue of training of participants in such activities, their inadequate number or inadequate qualifications, lack of equipment with the corresponding vehicles for transportation of dangerous things, further complicate the problem (*Ristić et al.*, 2010).

Legislation in the EU and Serbia

In the European Union, area of by-products of animal origin (animal carcasses and by-products from the facilities for slaughtering and meat processing and preparation of meat products) is regulated by Regulation (EC) No 1069/2009 and Regulation (EC) No142/2011, which are implemented since March 2011.

On the territory of Serbia, treatment of by-products of animal origin is regulated by Veterinary Law (Official Journal of RS No. 91/2005). In accordance with the Law, local authorities organize zoo-hygienic service on their territories, which remove animal corpses from public areas and run facilities for the cultivation, possession or transport of animals and their transport to the facility for collection, processing or destruction of by-products of animal origin in a manner that does not represent a risk to other animals, humans or the environment. Amendments to the Law stipulate the obligation of the local government to establish a facility for collection of animal carcasses and collection of small quantities of other by-products of animal origin from the animal slaughtering facilities and food of animal origin from households.

It is prohibited to throw corpses into rivers or other waterways or drains or leave them on the roads, open space, in the woods or elsewhere. Animal corpses and other by-products of animal origin must be collected, processed, or destroyed in the facilities for processing, treatment or destruction of animal corpses and other by-products of animal origin and only in exceptional cases, by-products of animal origin can be buried or burned on the animal cemetery or landfill/pit, which meets the requirements.

In dealing with waste of animal origin, producers of secondary products of animal origin are included, legal entities involved in their collection, processing and destruction, as well as the Ministry responsible/competent for veterinary care.

Veterinary and sanitary conditions for the construction of facilities for collecting, processing and destruction of by-products of animal origin are regulated by the Regulation on safe disposal of animal corpses and by-products of animal origin and the conditions to be met by facilities and equipment for collecting, safe disposal and determination of the cause of death and transport means for the transport of animal carcasses and animal waste (Official Gazette of SFRJ No. 53/89).

In order to taking measures to prevent the occurrence, detection, prevention of spreading, prevention and eradication of transmissible Spongioform Encephalopathy (Official Journal of RS No. 17/2006) wastes of animal origin, depending on the hazards are, classified as the Category 1, Category 2 and the Category 3. Category 1 material carries the risk associated with transmissible encephalopathy spongioform diseases (all communicable diseases from spongiform encephalopathy, including „mad cow disease") and Category 2 material carries, the risks associated with other animal diseases and zoonoses.

Category 1 material must be burned at temperature of at least 850°C.

To comply with the conditions regulating the field of waste products of animal origin, a draft regulation is prepared, which shall regulate/issue the manner of classification, treatment of by-products of animal origin, methods of their processing, hygienic conditions, the method of loading, unloading and reloading, veterinary and sanitary conditions for the construction, form and content of the register which is kept in the facilities for collecting, processing and destruction of by-products of animal origin, treatment of by-products in exceptional cases, the manner of implementation of official control and self-control, and the conditions for animal cemeteries and graveyards/pits and way of burying and burning of secondary products of animal origin.

Conclusion

Agricultural and food industry by-products, if without any value, are disposed of on landfills, lagoons, buried in arid terrains or thrown in open water courses, thus contaminating the environment.

Keeping up with laws and regulations, measures on safe disposal of contaminants must be strictly complied, to protect the quality of soil, air, surface and underground waters, plant and animal life, as well as the health of human population.

The most rational solutions of waste disposal are processing into feed, or raw materials for chemical industry and production of biofuels. By manufacturing of feed from sanitary safe raw materials (animal by-products belonging to Category 3 materials) added value is created, with assurance of the rational development of livestock production and of protection of the environment.

Application of biofuels contributes to reduction of oil consumption (i.e. of imports), reduction emissions of detrimental gases, stimulation of sustainable development of rural regions and increasing of available quantities of high-quality animal feed.

If all mentioned ecological and economical aspects are recognized properly, it becomes clear that organized solving of safe disposal of inedible by-products obtained from slaughtered or dead animals, by their technical processing, is a valuable task. This contributes to prevention of spreading of contagious diseases, to prevention and rehabilitation of the environment and to rational use of waste materials.

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Pravilnik o načinu neškodljivog uklanjanja životinjskih leševa i споредних производа životinjskog porekla i o uslovima koje moraju da ispunjavaju objekti i oprema za sabiranje, neškodljivo uklanjanje i utvrđivanje uzroka uginuća i prevozna sredstva za transport životinjskih leševa i otpadaka životinjskog porekla 1989. „Službeni list SFRJ" broj 53/89).

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Sanacija sporednih proizvoda zaklanih životinja u svetlu novih propisa

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R e z i m e: Životna sredina je, danas, ugrožena stvaranjem i gomilanjem otpadnih materija u svim granama industrijske proizvodnje pa i u klaničnoj industriji. Značaj neškodljivog uklanjanja sporedhih proizvoda klanja životinja raste sa intenziviranjem stočarske proizvodnje i razvojem pogona za preradu mesa.

Rešavanje problema neškodljivog uklanjanja otpadaka životinjskog porekla ima izuzetan značaj. Danas je to nezamenjiva veterinarsko sanitarna i preventivna mera u suzbijanju stočnih zaraza i zoonoza, a sve se više naglašava njena značajna uloga u saniranju i očuvanju životne sredine..

Najcelishodniji put za neškodljivo uklanjanje otpadaka iz industrije mesa, kao i uginulih životinja, u svetu je, upravo, njihovo sakupljanje i iskorišćenje (za proizvodnju hrane za životinje, hemijsku industriju ili pogonsko gorivo), u zavisnosti od vrste i strukture sirovina i njihove kategorizacije, tehničkim prerađivanjem u specijalnim fabrikama sa savremenom opremom i tehnologijom.

Istaknuto je da je potrebno da se objekti za preradu animalnih otpadaka tretiraju dvojako: kao proizvodna jedinica u službi zaštite životne sredine i kao mogući zagađivači životne sredine.

Ključne reči: *sporedni proizvodi klanja životinja, neškodljivo uklanjanje, iskorišćenje.*

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The current status of the Montenegrin meat industry*

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A b s t r a c t: Quantitative and qualitative parameters of the meat sector in Montenegro, ddressing both production and processing, have been evaluated. In Montenegro, livestock production is focused on cattle, sheep and goats and to lower extent pig and poultry. In the past decade meat processing industry in Montenegro is in its expansion. There are two medium sized enterprises and about 20 – 30 further small family enterprises. All of the slaughterhouses and all establishments for meat processing are in private ownership. Montenegro has the goal of improving the food safety system within an adequate legislative framework that conforms to European and international standards. The support is needed for the structural adjustment in the livestock sector, increasing of the overall competitiveness and productivity, improving the status of attainment of national and EU standards and improvement of the performance and offer of the service providers.

***Key words**: livestock, meat production, meat processing industry, EU standards.*

1. Introduction

Agriculture is a very important sector in the Montenegrin economy, since it participates in total Gross Agricultural Output (GAO) with 8.3% and plays a multifunctional role (*Bulletins of Central Bank of Montenegro,* 2010). In spite of agricultural resources available (about 0.80 ha of agricultural land per capita), the country is highly dependent on import of food. This goes also for meat and milk as the main products of animal origin.

Livestock production is with around 170,000 tons of milk production (23% of GAO) and 17,000 tons of meat production (28% of GAO) per year the largest contributor to Montenegro's agricultural economy (*Bulletins of Central Bank of Montenegro,* 2010). Most significant farm products are cattle, sheep and goat's milk and the production of lamb with traditional breeds as well as veal and beef meat.

Generally, structural characteristics of the Montenegrin agriculture, particularly of the livestock sector, are not favourable. An insufficient knowledge on modern production techniques/ technologies and standards, low productivity and therefore low international competitiveness of production, insufficient use of potential of natural resources for fodder production (pasture and meadows) and training on effective pasture and meadow management, difficult access to financial resources for investments (particularly for farmers), insufficient implementation of existing food safety legislation and public food inspection, etc. represent the main obstacles for the development of this sector.

On the other hand, there has been overall very dynamic development of the Montenegrin meat processing industry during the last 10 years, with significant investments in modernisation and implementation of improved hygienic standards and modern slaughtering and meat processing equipment. There are 4 big enterprises that are covering about 2/3 of the total domestic meat production and utilize the capacity per enterprise between 1,000 – 6,000 tons meat per year (*Monstat,* 2009). They have integrated system of slaughtering and meat processing as well as the retail activities. Furthermore, 2 medium sized enterprises utilize a capacity between 500 – 1,000 tons meat per year (*Monstat,* 2009). A few enterprises are specialised on slaughtering and processing of poultry meat. Very important are the producers of traditional air-dried and smoked prosciutto - Njeguš ham. The bigger prosciutto producers are active with export to neighbouring countries.

The most important strengths of the meat sector lie in the fact that there are large areas of pasture and

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meadows available for ruminants. There is also high potential of domestic markets for meat products and export of several products. This is supported by the dynamic development of meat industry and strong linkage to retail activities as well as the preferences of consumers for domestic meat products.

Very important for further development of meat sector is the fact that Montenegro currently has the status of potential candidate country to become member of the European Union (EU). Montenegro signed the Stabilization and Association Agreement (SAA) with the EU in October 2007, while Interim Agreement entered into force in January 2008. Furthermore, the harmonization with the World Trade Organization (WTO) principles and the implementation of the regional Central European Free Trade Agreement (CEFTA) are currently important issues for Montenegro. In 2007 and 2008, Montenegro has also joined several other international organisations: Food and Agriculture Organisation (FAO), World Organisation for Animal Health (OIE) and Codex Alimentarius.

2. Overview on Meat Production and Processing

The highest share of total meat production in Montenegro is coming from ruminants; largest quantity of meat is bovine meat (40%), followed by meat of small ruminants (sheep and goats), 24%. The rest is pork (19%) and poultry meat (18%) (*Monstat,* 2010). The total domestic meat production is covering only about 40% of the total meat consumption in the country.

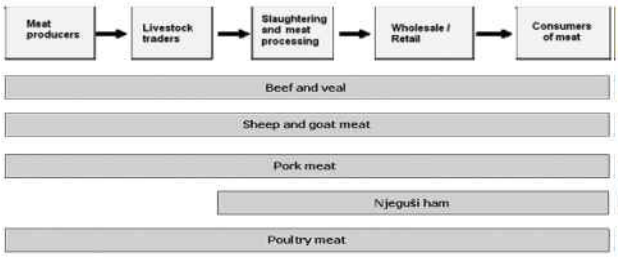

Figure 1. Key actors of the meat supply chain
Slika 1. Ključni akteri u lancu snabdevanja mesom

About 100% self-sufficiency is with sheep and goats meat but significant share of cattle and poultry meat, and particularly, of pork meat consumption is based on imports (Table 1). The production and consumption of traditional air-dried and smoked prosciutto ham (Njeguši ham) has long tradition in the country. It is based mostly on imported pork for ham production.

3. Overview on Livestock Sector

Livestock is a dominant sector in the Montenegrin agriculture due to the fact that meadows and pasture have a very high participation in total agricultural land. Montenegro does not fully use its production potential in the livestock sector. Total number of livestock units (LSU[1]) is about 110.000. If it is compared only to the total area of meadows (127.000 ha), even ratio is bellow 1.

Table 1. Overview on Meat Production and Consumption in Montenegro (2009)
Tabela 1. Pregled proizvodnje i potrošnje mesa u Crnoj Gori (2009)

Meat Product/Meso	Domestic Production, tons/ Domaća proizvodnja, t	Import, tons/ Uvoz, t	Domestic Consumption, tons/Domaća potrošnja, t	Export, tons/ Izvoz, t	Deficit, tons/ Deficit, t	Self-sufficiency/ Samodovoljnost, %
Beef & Veal/ Govođe i teleće meso	6,790	5,355	12,069	76	5,279	56
Sheep & goat meat/ Ovčetije i kozje meso	4,120	100	4,220	0	100	98
Pork/ Svinjsko meso	3,170	21,692	23,672	1,190	20,502	13
Poultry meat/ Živinsko meso	3,030	4,756	7,631	155	4,601	40
Meat total/ Ukupno mesa	**17,110**	**31,903 *)**	**47,592 **)**	**1,421**	**30,482**	36

[1] Livestock unit is equivalent for aggregation of the different livestock species and poultry. One adult cattle is 1 LSU, one animal of cattle young stock is up to 0,6 LSU, 8 adult sheep or 10 adult goats are equivalent to 1 LSU.

Cattle breeding are the largest sub-sector in the livestock production in Montenegro. In total 25.928 agriculture holdings are currently holders of cattle (*Information of Animal I&R unit,* 2010). The total cattle population counts currently about 90.000 heads, while dairy cows thereof are about 62.500 heads.

Sheep breeding sector is mainly characterized by the semi-extensive way of production and is a very important sector of livestock production and economically is just behind cattle production. Annual volume of output is about 3.600 t of meat and 7.000 t of milk (*Annual Report of Livestock Selection Service,* 2009).

Goat breeding is also very important for the karstic regions in Montenegro where natural conditions do not allow for the breeding of other kinds of ruminants. Estimation is that total number of goats is currently around 35,000 to 40,000. The total number of goats has been stable during the last years. Annual production of goat meat (young goats, and meat of the mature and culled animals) is estimated at about 460 tons.

Pig production in Montenegro is a weak sector, primarily due to lack of suitable areas for domestic cereals production for pig feed and appropriate utilisation of the nutrition value of manure/ slurry in the whole production cycle. According to MONSTAT Census, in June 2010 about 43,000 pigs had been identified (*Monstat,* 2010).

Poultry production is characterized by significant changes occurred in the recent years, ranging from creating numerous family farms for eggs production, the expansion of broiler production, to the founding of slaughterhouses and processing capacities for that sector.

4. Meat processing industry

The development of Montenegrin meat processing industry was dynamic during the last 10 years with significant investments in modernisation and implementation of state-of-the art buildings and equipment as well as improved food hygiene standards.

4.1. Overall Structure of Meat Industry

Two medium sized enterprises utilize an annual meat production capacity between 500–1,000 tons. These two have also an integrated system of slaughtering and meat processing with strong regional retail activities (*GTZ,* 2008). They are currently not exporting meat to neighbouring countries. About 20 – 30 further small family enterprises exist, which are specialized on slaughtering, fresh meat production and/or limited number of processed products. Several of them have also their own regional/local retail shops. This sub-group is focused particularly on regional/local markets (e.g. butcheries, restaurants, regional retail shops) in Montenegro. Three specialized enterprises are operating slaughtering and processing of poultry meat.

An estimated of about 221 active meat retail shops exist. Many larger supermarkets have also a butchery section or are directly linked to meat processors. In addition, specialized producers of traditional air-dried and smoked Njegusi ham are very important for the Montenegrin meat industry. Beside some bigger specialised producers, also several small family units are producing traditional air-dried and smoked prosciutto ham.

4.2. Slaughterhouses and approved establishments for meat

According to the Veterinary Law (*O.J. MNE,* No. 27/07), cattle slaughtering is permitted for home consumption, but commercial slaughter may only be done in officially approved or registered slaughterhouses. Rendering plants do not exist.

All the slaughterhouses and all establishments for meat processing are in private ownership. The establishments meet the minimum required veterinary, sanitary and technical conditions (*Rulebook, OJ MNE, No. 56/00,* 2000). However, some of these establishments do not have sufficient cold stores, which are resolved by transportation of meat, immediately after dripping and examination, in a prescribed means of transportation to retail shops, which have cold stores of adequate capacity for cooling and keeping meat till it is sold. Very often slaughterhouses do not have conditions for removal of offal and waste in a prescribed way and the conditions are not provided at the level of the country, either there is not a city pond, burial pit, cattle cemetery, incineration plant or sites or dumps for disposal of animal by-products. The waste is collected into containers and then transported to the city garbage dump, where it is buried.

4.2. Level of attainment of the relevant EU standards

In the area relevant to Food Safety Montenegro is committed to international integration, it is in the final stage of accession to the WTO and it is a member of FAO, (2007), OIE (2008), Codex Alimentarius (2008), and WHO (2007).

The Montenegrin Strategy of food production and rural development (2006) has the goal of im-

proving the food safety system within an adequate legislative framework that conforms to European and international standards. The strategy is drawn up according to the Action Plan for the European Partnership. Framework legal document - the Law on Food Safety (*OJ, MNE, No.* 14/07) lays down the general food safety rules, requirements for placing on the market of food and feed and provides a basis for adoption of secondary legislation in this field.

The rules in the food safety field are laid down, in addition to the law mentioned above, also by a large number of secondary legislation from the former legal system (Federal Republic of Yugoslavia, Serbia and Montenegro). Adoption of new rulebooks, instructions and guides is planned in accordance with the EU legislation in this field by the year 2012. It is also necessary to set up a system for appointing food control laboratories (*EC Regulation* 882/2004) and National Reference Laboratories (whether in Montenegro or externally) to provide a co-coordinated and overarching approach to food safety testing (*GTZ,* 2007).

The Montenegrin Food Law is partially harmonized with the EU Regulation 178/2002, but still further alignment is required. In particular some aspects are still to be clarified, like for instance the separation between risk management and risk assessment that is not fully in compliance with EU principles.

Legislation included in the so called „EU Food Hygiene Package" has been partially transposed in Montenegrin legislation and missing laws are under preparation. At the moment following rulebooks were adopted in 2008:

- Rulebook on Hygiene Requirements for Food of Animal or Plant Origin (OJ of Montenegro, No. 14/09) transposed Regulations 852/2004;
- Rulebook on Special Hygiene Requirements for Food of Animal Origin (OJ of Montenegro, No. 14/09) transposed Regulations 853/2004;

New secondary legislation is being developed, but will not come into full effect until 2012 according to existing plans. A detailed schedule for the preparation of the secondary legislation is outlined in the National Programme for Integration 2008–2012 by the Montenegrin Government (2008).

The integration of data systems for food safety needs to be developed. There is, for example, no beef labelling and tracing system to link with the new animal identification system. There is no unified data information system linking border posts, laboratories, and the register of food processing and marketing establishments.

Due to the investments in recent years there are several advanced meat processing companies with state-of-the art equipment. But, the Montenegrin meat processing industry includes also many small-scale meat processors. Their technology is generally not 'EU compliant'. Those companies are serving only the domestic markets.

Several processing units are not in compliance with EU requirements concerning the premises, facilities, equipments or general management. One of the main reasons can be found in the high costs necessary for the renovation of the establishments, premises and equipment up to EU standards. In addition, the lack of infrastructure, such as systems for the treatment of waste water, is one of the biggest concerns.

A deadline of 1st January 2010 was declared by Government policy in order for food companies to become compliant with food law and HACCP. As not all meat processors were able to respect the deadline, the final term is being extended. However, a system for official control and monitoring on implementation and use of HACCP is not in place. In addition, there are no officially approved or recommended manuals or guides for good hygiene practice, good manufacture practice or HACCP in the country apart from a guideline developed for meat industry under GTZ comprising EU and international requirement and practice on self control of the meat industry (*GTZ,* 2006).

There is a high number of constrains in the meat sector. Some of the most important ones are the lack of technical knowledge and adoption of improved technology and management practices in many meat processing enterprises, deficiencies in plants not having EU standards related to technical design, equipment, operation methods, professional staff and inadequate level of internal control systems, incomplete HACCP implementation, inadequate construction of the buildings, older equipment, etc.

There is little specific depth to the current Montenegrin legislation and it lacks precise definition. Fully approximation of Montenegrin and EU legislation will be a lengthy process as there is little specific depth to the current legislation it lacks precise definition, it is not in line with Codex standards and has multiple missing links.

One of the most critical issues for the primary and secondary processing is the safe disposal of ABPs (animal by-products) for the protection of environment and the health of humans and animals. EC Regulation 1774/2002 deals with ABP management in connection with Regulation 999/2001 on TSE control and eradication.

The area of animal welfare is governed by the Law on Animal Welfare (*OJ, MNE, 17/08,* 2008). This Law regulates rights, obligations and responsibilities of physical and legal persons for animal welfare protection in relation to animal protection from torture, during keeping and breeding, killing and slaughter, performing of surgeries on animals, transportation and carrying out of experiments, rules of animal treatment, as well as other issues relevant for animal welfare protection. The Law sets forth general provisions for transposition of the EU legislation into secondary legislation for the purpose of full harmonization of this field with the EU legislation.

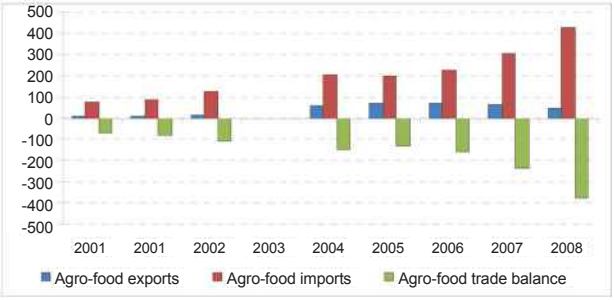

Figure 2. Agri-food trade (in EUR million); 2000–2008;
Slika 2. Trgovina poljoprivrednim proizvodima (u milionima Eura); 2000–2008

5. Import and Export of Meat and Live Animals

Montenegro is a net importer of food products. The high dependency on food imports is shown in the share of agricultural products in total import which exceeds two times their share in export. Agro-food trade is growing continually, import is significantly increased in the last few years (see figure below), while export was declining in the last three years. Thus, as consequence of those trends, the import deficit is growing.

The export value of agro-food products in 2008 amounted to EUR 46.7 million. More than half of this value (EUR 25.9 million, or 55.5%) goes to one group of products – beverages. Amongst beverages, the most important is wine (EUR 17.2 million, or 66%). Other relevant export products are meat (EUR 2.9 million); vegetables (EUR 3.99 million); fruits (EUR 3.22 million); meat preparations (EUR 2.30 million) and tobacco (EUR 4.30 million). Taken together, meat and meat preparations are second ranked in total food export (EUR 5.1 million or 11.1%) (*Monstat,* 2009).

The breakdown of the total import valued at EUR 426 million in 2008, shows that major tariff groups are meat (EUR 58.9 million or 13.8%); beverages (EUR 56.3 million); dairy produce (EUR 38.7 million); preparations of cereals (EUR 27.6 million); processed meat (EUR 24.9 million) and preparations of cereals (EUR 23.6 million) (*Monstat,* 2010).

The development of import and export of live animals (Chapter One of Customs Tariff), meat of all species (Chapter 2 of the CT) and meat preparations (Chapter 16 of the CT) over the last five years is presented in the next two tables.

Data presented in the above table show constant increases of import until 2008. In 2009, total import was similar to that of the year before, for poultry meat and meat preparations it was even lower than the year before. Pig meat is the main import commodity; it has a share of around 70% in the total import.

Export of meat and meat preparations is very modest, but the trend is positive. The main export commodities are smoked products and meat preparations. It is realistic to expect that the positive trend will continue, especially for smoked ham and meat preparations. The export is still only to the CEFTA countries, Serbia, BandH, Macedonia, small quantity to Albania. Once technical barriers are removed for export to EU countries, lamb meat will also be exported.

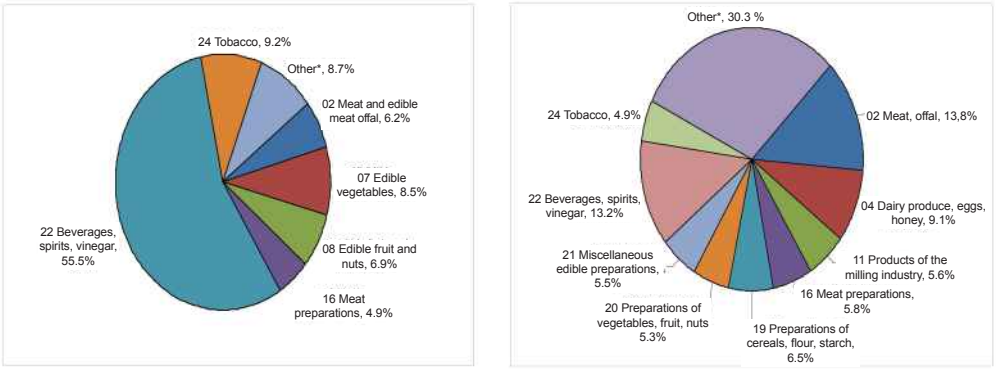

Figure 3. Composition of agri-food exports (left) and imports (right) by main commodity group
Slika 3. Profil izvoza poljoprivrednih proizvoda (levo) i uvoza (desno) prema glavnim grupama proizvoda

Table 2. Data on import of live animals, meat and meat preparations
Tabela 2. Podaci o uvozu živih životinja, mesa i proizvoda od mesa

Product/ Proizvod	**2005**		**2006**		**2007**		**2008**		**2009**	
	Qty, tons/ Količ. t	**Value/ Vred., 000 €**	**Qty, tons/ Količ., t**	**Value/ Vred., 000 €**	**Qty, tons/ Količ., t**	**Value/ Vred., 000 €**	**Qty, tons/ Količ., t**	**Value/ Vred., 000 €**	**Qty, tons/ Količ., t**	**Value/ Vred., 000 €**
Live animals/ Žive životinje	**1,704**	**3,027**	**2,781**	**4,726**	**3,410**	**4,683**	**10,681**	**15,965**	**9,690**	**16,174**
Beef & Veal/ Goveđe i teleće meso	474	2,480	922	3,315	2,260	5,935	3,375	11,223	3,193	11,177
Sheep & Goat/ meat Ovčije i kozije meso	0	3	0	26	58	239	126	545	97	481
Pork/Svinjsko meso	4,907	20,527	6,484	26,241	12,860	26,316	18,028	38,262	17,878	39,232
Poultry meat/Živinsko meso	1,557	2,214	1,213	1,754	1,973	3,267	3,740	7,286	3,666	6,728
Other (incl. dried & smoked prod.)/ Ostalo (uklj. Sušene i dimljene proizvode)	154	735	169	1,028	799	1,336	663	1,546	944	2,234
Total meat (Chapter2)/ Ukupno mesa (pogl. 2)	**7,092**	**25,959**	**8,788**	**32,364**	**17,950**	**37,093**	**25,932**	**58,862**	**25,778**	**59,852**
Meat preparations/ Proizvodi od mesa	1,891	6,163	1,489	5,956	4,456	11,802	6,750	20,781	5,448	17,237
Total meat & preparations / Ukupno proizvoda od mesa	**8,983**	**32,122**	**10,277**	**38,320**	**22,406**	**48,895**	**32,682**	**79,643**	**31,226**	**77,089**

Source: Ministry of Agriculture and Rural Development – MARD / Izvor: Ministarstvo poljoprivrede i ruralnog razvoja

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Live animals/Žive životinje	**128**	**36**	**6**	**6**	**36**	**14**	**12**	**4**	**69**	**13**
Beef & Veal/Goveđe i teleće meso	19	43	20	49	7	32	36	112	0	2
Sheep & goat meat/ Ovčije i kozje meso	0	0	0	0	0	1	0	0	0	0
Pork/Svinjsko meso	117	225	47	72	128	31	37	43	49	126
Poultry meat/Živinsko meso	1	1	0	1	10	19	21	34	4	1
Other (incl. dried & smoked prod.)/ Ostalo (uklj. Sušene i dimljene proizvode)	307	1,341	213	1,594	421	1,668	745	2,713	607	2,816
Total meat/ Ukupno mesa	**444**	**1610**	**280**	**1716**	**566**	**1751**	**839**	**2902**	**660**	**2945**
Meat preparations/Proizvodi od mesa	87	389	45	203	290	1054	617	2203	755	2692
Total meat & preparations / Ukupno proizvoda od mesa	**531**	**1999**	**325**	**1919**	**856**	**2805**	**1456**	**5105**	**1415**	**5637**

Source: Ministry of Agriculture and Rural Development – MARD / Izvor: Ministarstvo poljoprivrede i ruralnog razvoja

6. SWOT analysis of meat processing sector

The main strengths and weaknesses, as well as opportunities and threats of the meat industry, with specific focus on processing side, can be summarised in the following table.

7. Future Meat Sector Developments

Since the average size of livestock farms in Montenegro is currently too small it is important to facilitate the structural adjustment during the next years through support programmes focused on economic viable farm structures, including the support

Table 4. SWOT analysis of the meat processing sector
Tabela 4. SWOT analiza sektora za preradu mesa

Strengths
– Competitive processing of meat and meat products
– Traditional production and consumption of processed and Smoked Products
– The tradition in production and well known technology
– Consumer preference for local branded products
– Readily available labour force at affordable prices
– Quality Products with export potential to the lucrative EU market
– Strong Local Market and tourism related consumption
– Recognizable branded products and products origin
– Affordable product at retail level
Weaknesses
– Raw material is mainly imported
– Insufficient level of compliance with environmental and hygiene standards
– Lack of food safety training within the sector
– Small fragmented agricultural farms to produce continuous supply of raw material
– Lack of Market infrastructure
– High Market prices of domestic production due to high production costs
– Lack of added value in fresh meat products
– Insufficient product diversification
– Insufficient knowledge of sanitary standards
– Insufficient awareness of environmental protection
Opportunities
– Market potential (EU Export and Domestic)
– Domestic supply, a vast unutilized area of pasture land is available for veal and lamb production
– Higher value products as national and international markets are developed
– Potential for the increase of competitiveness and safety of meat products
– Greater variety of value added cuts for the export and retail market
– Ongoing restructuring of the sector by establishing new production units in line with EU requirements
– Existence of internationally competitive processing with EU export licenses
– Increased market opportunities if Community standards are met
– Regionally recognised branded processed meat products
– Increase of meat consumption
Threats
– Non-compliance with relevant European community standards
– Imported products will saturate the market with Quality /Safe products at lower prices
– Non–compliance to ecological conditions and situation on animal welfare and husbandry
– Costly investment in restructuring and upgrading
– Lower level of quality and safe products due to outdated processing equipment
– No competitiveness of meat processing industry on global markets
– Capital investment
o High interest rates from Bank Loans
o Insufficient Bank Support
– Insufficient skill and food safety training absorbed by personnel
– Insufficient co-operation among competent state bodies

for the establishment of producer groups (machinery rings etc). Besides competiveness also overall compliance of livestock farming with national and EU standards regarding animal health and welfare, environmental protection, food safety and occupational safety has to be improved considerably.

The meat processing industry should be supported in their dynamic development targeting their competitiveness in the CEFTA countries and the future EU market. Important strength for the domestic market is the strong link to the consumer through own retail activities.

With regard to food safety, environment protection and animal welfare standards, facilities at many meat enterprises require upgrading to comply with EU requirements, especially in small enterprises. The industry needs support to raise standards for hygiene and environmental protection. Areas for intervention are in addition to further modernizing the production processes, production hygiene as a prerequisite for EU approval and increasingly as a prerequisite to access export markets.

Montenegro will also have to introduce carcass classification system, fully compliant with the EU regulations, in order to better fit production to market demand.

Conclusion

Taking in consideration the overall situation, the future interventions in the sector should be oriented mainly to the support of the structural adjustment in the livestock sector, increasing of the overall competitiveness and productivity through investments for application of modern production techniques and technologies and better management, improving the status of attainment of national and EU standards, improvement of the awareness, knowledge and skills in the sector by vocational training and improvement of the performance and offer of the service providers.

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Status industrije mesa u Crnoj Gori

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R e z i m e: Kvantitativni i kvalitativni parametri sektora proizvodnje mesa u Crnoj Gori, sa stanovišta proizvodnje i potrošnje, su predmet analize ovog rada. U Crnoj Gori, stočarstvo je fokusirano na uzgoj goveda, ovaca i koza, a u manjoj meri svinja i živine. U prethodnoj deceniji, počela je ekspanzija industrije mesa u Crnoj Gori. Postoje dva preduzeća, srednje veličine, i 20 do 30 manjih porodičnih preduzeća, koja posluju u ovom sektoru. Sve klanice i objekti za preradu mesa su u privatnom vlasništvu. Cilj Crne Gore je poboljšanje i unapređenje sistema bezbednosti hrane u zakonskim okvirima koji su usklađeni sa Evropskim i međunarodnim standardima. Podrška je neophodna za strukturna usklađivanja u stočarstvu, povećanje ukupne konkurentnosti i produktivnosti, poboljšanje statusa u ispunjavanju nacionalnih i EU standarda i poboljšanje proizvodnih rezultata i ponude usluga.

***Ključne reči**: životinje za klanje, proizvodnja mesa, industrija prerade mesa, EU standardi.*

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Food safety and microbiological criteria*

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A b s t r a c t: The major cause of food borne diseases according to epidemiological data and risk analysis, are microorganisms and toxins of microorganisms. Uniform approach to ensure food safety is essential to achieve the same level of protection of human health and facilitate food trade between countries. Therefore with the new approach to food safety it was necessary to set up adequate microbiological criteria for foods. Microbiological criteria may be applied at various points in the food chain. They may be used during production and/or to asses the final product. Microbiological criteria may, however, play a supplementary role in the verification of HACCP.
Key words: *Microbiological criteria, limits, food safety, HACCP, FSOs, POS.*

Introduction

Economic importance and ubiquity of food in everyday life suggests that most attention in this area must be devoted to food safety by the society as a whole, public authorities, especially from direct producers (*Bunčić* et al., 2006). In recent decades there has been an enormous development in food manufacturing, processes and procedures necessary to ensure the achievement of acceptable standards of food safety. The situation in the field of food safety in the last decades of the twentieth century and the major issues that were being reported (BSE, emerging and re-emerging pathogens, polychlorinated biphenyls, dioxins, GM food ...) moved step closer to general conclusion that there were serious deficiencies in food safety, which has caused the need to change the policy of food safety. Uniform approach to ensuring food safety is essential to achieve the same level of protection of human health and facilitate food trade between countries. Today, the approach to food safety is based on an objective, rational scientific approach, on hazard analysis and risk assessment (*Katić*, 1997; *Bunčić i dr.,* 1996). The major cause of food borne disease according to epidemiological data and risk analysis, are microorganisms and toxins of microorganisms. Therefore with the new approach to food safety it was necessary to set up adequate microbiological criteria for foods. Principles for the development of risk assessment of microbiological hazards have been developed by the Codex Alimentarius (CAC) and EU Scientific Committee for Food. Codex Alimentarius has published principles for the development of microbiological criteria (*CAC*, 1997). In its development CAC used opinions of the International Commission on Microbiological Specification for Foods (*ICMSF,* 1986). Taking in account the principles of the CAC, and opinions of the Scientific Committee on Veterinary Measures relating to Public Health, the Commission of the European Union laid down the microbiological criteria for food in Regulation (EC) No 2073/2005. The main objective of this Regulation was to harmonize the microbiological criteria in the European Union countries. Harmonization of regulations in the field of food safety in Serbia with the European Union includes also the harmonization of legislation on microbiological criteria for foods. Ministry of Agriculture, Forestry and Water Management is October 2010, adopted „Regulation on general and special conditions of food hygiene at any stage of production, processing and transport (72/10)", which is in correspond dance in line with Regulation (EC) No. 2073/2005. Food safety is ensured through the application of food safety systems (HACCP), which starts from raw materials through the manufacturing

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process with the GHP, processing (including labeling), handling, distribution, storage, sale and consumption of food. Microbiological criteria used for verification of the applied control system. The limit values for certain microorganisms and their toxins were selected based on their importance to food safety, as well as for evaluation of hygiene in the production.

Regulation on microbiological criteria for food

The main causes of foodborne illnesses are microorganisms or their toxins.

Determination of microbiological criteria for foods should contribute to a certain degree of confidence that food is safe and appropriate quality throughout shelf-life, if it is handled properly with (*Regulation (EC) No.* 178/2002). Application of microbiological criteria should ensure that products are obtained under good hygienic conditions and that they are from a microbiological standpoint, safe for consumption, and to separate acceptable from unacceptable manufacturing products, to separate acceptable from unacceptable manufacturing practices and should be an integral part of the procedure for ensuring food safety, applied by food business operators. Also they should help in the validation and verification procedures that were established on the principles of HACCP (*Montville et al*., 2005).

Microorganisms in food are distributed heterogeneously, so testing a single unit sample is not sufficient to detect pathogenic microorganisms in the batch of food. Microbiological testing of final products can never ensure food safety even when examining a large number of samples (eg n = 60), and that when it does not get any positive result.

Microbiological criteria defined in the Regulation (72/10) are based on a scientific risk assessment. Regulation introduced some new criteria which apply to certain categories of food, and microorganisms that have not previously been an integral part of the Regulation on microbiological safety of food (e.g. criteria for cut fruits and vegetables, *Salmonella* species in carcasses of slaughtered animals, and *Listeria monocytogenes* in certain foods ready for consumption). Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs and Regulation (72/10) introduced two types of criteria: the criterion for food safety and process hygiene criteria. Food safety criterion is the criterion by which the acceptability of a product or series of products applicable to products placed on the market. These criteria are not applicable to products intended for further processing by the food business operator. Food safety criteria include pathogenic microorganisms, their toxins and metabolites. The advantage of establishing criteria for the safety of food pathogens is that the harmonized standards on eligibility for food authorities and food industry. The process hygiene criterion indicating the acceptable functioning of the production process and does not apply to products that are put on the market. It sets an indicative contamination values above corrective actions which are required to maintain the hygiene of the process in compliance with the food law Offical Journal of Republic of Serbia no (41/09).

Microbiological criteria in food safety system

Microbiological criteria in the Regulation (72/10) intend to be used by food business operator at various points in the food chain. They may be used during production and / or to asses the final product. However, microbiological criteria may be involved in primary production, the customer requires that the primary product meets certain microbiological conditions that are considered to affect the safety and quality of its final product (e.g. improve process hygiene and selection of raw materials). Food business operator must ensure that food is fully compliant with the requirements of the Regulations (72/10) also has to take necessary actions if it determines that the food does not meet any of the criteria.

The Regulations (72/10) doen not specify the minimum requirements for testing (except for the carcasses of cattle, pigs, sheep's, goats, horses and poultry and also for minced meat, meat and semi-mechanically separated meat. If microbiological testing are required, with the exception of the above mentioned cases, Regulation (72/10) allows food business operators to use their own food safety management procedures to establish an appropriate sampling regime. The number of required samplings will depend on the applied procedures of food safety risks associated with the product, as well as the nature and extent of production.

Food business operator has to define in what circumstances it is appropriate to use microbiological testing to prove compliance with the criteria, however, the microbiological examination (with the exceptions mentioned above) is not always necessary. For example, when the established food safety management procedures based on the principles of HACCP and good hygiene practice, routine monitoring of physical parameters (such as monitoring the temperature and time, pH, levels of preservatives and water activity) can provide adequate security to the criteria met (*Bunčić et al.,* 2009).

Sampling and microbiological testing are not the only measures that are applied to ensure food safety and do not replace the application of measures to prevent the occurrence of hazards and risk

management by the food business operator shall carry out. Mandatory microbiological criterion is defined only for food and / or stages in the production process for which there is no other effective mean of monitoring measures applied to prove the usefulness of such criteria to ensure greater consumer protection.

Sampling plan and limit values for microorganisms are selected on the basis of the risk to human health, the conditions in which certain foods are stored and how it is consumed. The sustainability and growing of microorganisms in food depend on the characteristics of microorganisms, factors related to food (nutrients, aw, pH, redox potential, antimicrobial compounds) and environmental factors (humidity, concentration of gases, temperature) (*Mossel et al*., 1991, *Katić*, 1995, *Katić et al.,* 1998). In determining the limits all the factors are taken into account that determine microbial growth and/or formation of toxins.

For microbiological testing of food, used 2--class or plan of 3-class plan are most commonly plan. Pathogenic microorganisms may not be present in a given quantity of food. To prove it the "2-class plan" used. The results obtained by such sampling are classified as "presence" and "absence".

When a firm 2-class plan is not necessary, 3--class plan frequently used to examine for hygiene indicators where enumeration of microbes units--volume or mass is possible. 3-class plan allows the proportion of the units of the sample between acceptance and non-acceptance.

The sample was composed of one or several units or portion of matter selected by different means in a population or in an important, quantity of matter which is intended to provide information on a given characteristic of the studied population or matter and to provide a basses for a decision concerning the population or matter in question or concerning the process which has produced it. An integral part of microbiological criteria, the number of sample units that represent the sample (n), the threshold number of bacteria (m), the maximum value of the number of bacteria (M) and number of samples (c) where the number of bacteria may exceed the limits (m) and less than the maximum value (M). In the food safety criterion m = M, and c = 0, except for histamine, the result is considered satisfactory if the number of bacteria in all units of the sample below the threshold value, the result is considered not acceptable if the bacteria in one or more of the same sample unit or greater than M.

Salmonella spp. most frequent causes alimentary toxic infections, and and for the emergence of the disease is required a small number of microorganisms, and for *Salmonella* spp. within the food safety criteria stipulated in the absence of 25 g in all five sample units.

Limit for staphylococcal enterotoxin is prescribed for cheese, milk powder and whey powder if the number of coagulase-positive staphylococci greater than 10^5 / g of cheese made from raw milk or 1000 / g of cheese made from pasteurized milk. In the process hygiene criteria provided for the enumeration of coagulase-positive staphylococci during cheese making process when it is expected that the number of coagulase-positive staphylococci to be the biggest. In determining the threshold values for coagulase-positive staphylococci the conditions for the growing and production of enterotoxins in cheese were taken into account.

When it comes to inspections there is no specified sampling and testing frequency. In cases where the inspection decides on the basis of results of applied measures of self-control in such conditions there is no guarantee to obtain safe products. For the purposes of the monitoring plan adopted by the competent national authority sampling frequency and types of tests depend on the aim.

The results of microbiological analysis of food depend on the method used to isolate and identify microorganisms or detection of their metabolites, so the test must use the reference methods laid down in the microbiological criteria. Using reference methods food testing results are obtained after several days. In order to obtain data on the microbiological safety of food in shorter time alternative rapid methods can be used to test. The application of alternative methods is acceptable if the validation of the method is given in relation to the reference method specified in the Regulations (72/10) or if the food business operator has applied a method that is certified by a third party in accordance with the protocol established EN / ISO standard 16140 or other internationally accepted protocol, and their usage approved by the Ministry in charge of agriculture. Application procedures of sampling and testing methods other than those defined in the regulations, are permitted provided that the food business operator can demonstrate that the application of those procedures and practices provided at least the same level of reliability and the criterion prescribed in the Regulations (72/10). Testing for the presence of other microorganisms, as compared to the corresponding microbiological limits that apply to them, and to examine other parameters, except for microbiological, can be performed only in terms of process hygiene criteria. If a food business operator in the food business examines other microorganisms, they must be included in a plan of self-control (e.g. at the request of the manufacturing specifications, the recommended parameters, etc..), as well as all the components that make up the microbiological criteria (food to which relations, a microorganism, the sampling plan, the limit values, meth-

ods, the phase in which the criterion applies, the corrective measures in case of unsatisfactory results).

Besides sampling the food, the Regulations (72/10) stipulates that food business operators must be required to take samples from the production areas and equipment (swabs) in facilities that manufacture ready-to-eat food able to support the growth and development of *L. monocytogenes*, in order to test the presence of these bacteria. Food business operator must determine the frequency of sampling with production areas and equipment (swabs) and is included in the plan of self-control. When determining the frequency of sampling and determining the production areas from which samples are taken, the determination of eligibility criteria and corrective measures, food business operators must take into account all information is available about the potential dangers and ways of managing, and that can occur during all phases of production.

In general, how should the results of microbiological tests could take into account when assessing the assurance provided by the food business operator, methods and frequency of sampling must be described and explained in detail in the plan of self-control food business operator.

In the Regulations (72/10) there are clearly defined phases in which the microbiological criteria appliy. Food safety criterion is applied only to food placed on the market during their shelf-life, a process hygiene criterion points to the proper functioning of the production process, is used in various stages of production and can not be applied to products on the market.

Application of microbiological criteria in HACCP system and the development of HACCP should run in parallel. For the successful management of food safety, the manufacturer shall: establish microbiological hazards significant for food safety; establishes the procedures to be applied to prevent, eliminate or reduced the hazard to an acceptable level; to have information on the degree of variability and the factors that influence on it; determine the parameters process taking into account the variability to ensure that critical limits are met; to establish procedures for monitoring applied measures; collects and interprets data obtained during the process.

Based on data collected through the time of the finding of micro-organisms, the producers sets the baseline process for the level of self-control that is achievable when the procedure GHP and HACCP in control. Data from the microbiological tests are used to obtain the baseline process, and are collected from at least five sources that vary by location or time of sampling within the process (e.g. raw materials, samples during production, samples from equipment/manufacturing area, and samples of end of shelf life). Once they establish a baseline of the process the next test results that differ from this line indicate deviations from normal due to changes in production conditions. Also, the baseline can be used for the establishment of microbiological specifications.

Control chart is made from data collected over time from one lot or from multiple lots (Ryan, 1989). On the X axis control chart is applied to the time at which the samples were taken during the process and the Y axis values obtained during testing. The control chart has three parallel lines: lower control limit, central line and upper control limit. In some instances where the lower control limit is below the lower limit detection (e.g. absence) assumed to be zero or predesignated value below lower limit of detection (*Jay et al*., 2005).

Each step in the production process has a certain degree of variability. When combined variability of each step in the system, we get the overall variability of the system. In well-controlled systems, the data show a tendency to claster around the baseline. In the case of propagation of bacteria in an environment where growth has been uneven, as in food, increase population density is exponential. Declines under adverse conditions are also roughly exponentially. It has long been observed that the concentration of microorganisms has a log - normal distribution. Control chart generally assumes that the distribution of data collected during the process is log-normal or approximately normal. Based on the normal distribution, approximately 68% of the value will fall between plus or minus one standard deviation of the mean, approximately 95% within 2 standard deviations and 99.7% within 3 standard deviations from the mean. Control limits are most commonly set at plus or minus 3 standard deviations. When the limit of 3 standard deviations set the probability that any data will be beyond the control is 0.3% if the process is truly under control. If the frequency at which the values fall above or below three standard deviations is greater than 0.3% then it is considered that the process is not under control (ICMSF, 2002).

Based on the collecting data on the control chart producer can evaluate any future results obtained and to determine whether the obtained result is within one, two or three standard deviations.

With improvement of food safety policy change and microbiological criteria for food makes use Food safety objectives (FSOs) and Performance objectives (POS). Food safety objectives (FSOs) and Performance objectives (POS) industry provide quantitative targets to be achieved in terms of species, in production, taking into account the hazard at the time of consumption (FSOs). As defined by Codex Alimentarius FSO is the maximum frequency and

/ or concentration of hazards in food, which at the time of consumption provides or contributes to a satisfactory level of health protection, and PO is the maximum frequency and / or concentration of hazards in food at the certain step of food production that contributes to a defined FSO or Appropriate Lovell off Protection (ALOP-in) in the case.

Microbiological criteria are not immutable, and future changes can be expected in relation to emergence of new microbial hazards in food, changes in industrial processes which consequently lead to changes in microbial population, and other factors important for food safety.

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Bezbednost hrane i mikrobiološki kriterijumi

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R e z i m e: Ujednačen prilaz osiguranju bezbednosti hrane je neophodan da bi se postigao isti nivo zaštite zdravlja ljudi i olakšao promet hrane između različitih zemalja. Danas se prilaz bezbednosti hrane zasniva na objektivnoj, racionalnoj i na naučnim osnovama zasnovanoj analizi hazarda i proceni rizika. Glavni uzrok bolesti prenosivih hrane, prema epidemiološkim podacima i analizi rizika, su mikroorganizmi i njihovi toksini. S toga je bilo potrebno da se, u skladu sa novim prilazom bezbednosti hrane, izvrše adekvatne promene mikrobioloških kriterijuma za hranu. Mikrobiološki kriterijumi se mogu primeniti na različitim mestima u lancu ishrane. Mogu se koristiti tokom proizvodnje hrane i / ili da se oceni gotov proizvod. Pored toga, mikrobiološki kriterijumi mogu imati ulogu u verifikaciji sistema HACCP.

Ključne reči: *mikrobiološki kriterijumi, limiti, bezbednosti hrane, HACCP, FSOs, POS.*

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Recent advances in understanding the virulence of enterohemorrhagic *Escherichia coli* in food*

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A b s t r a c t: Most of the genes recently found in pathogenic E. coli encode various factors which directly determine their virulence and pathotype. Two main virulence factors characteristic for enterohemorrhagicE. coli(EHEC) are attaching/effacing lesions and Stx1/Stx2 toxins. Genes responsible for expression of aforementioned virulence factors are heavily regulated by environmental conditions. Low iron concentration induces massive expression of stx1 gene and subsequent toxin synthesis. Stress response of EHEC to starvation, acid challenge, cold shock and osmotic changes which damage DNA, induce"SOS" response. This response mediated by RecA protein not only repairs damaged DNA fragments but also induces conversion of lysogenic bacteriophage lifecycle to lytic phase followed by intensive expression of stx2 genes. Bacterial stress adaptation of E. coli to novel technologies and the potential for stress-associated enhanced virulence need to be addressed in more detail to prevent potential risk of disease. An increased understanding of expression of virulence-associated genes will provide information for control of pathogens and increase microbial safety of foods.

***Key words**: EHEC, virulence, gene expression, food.*

Introduction

The scientific history of *Escherichia coli* started with its first description in 1885 by Theodor Escherich. He identified a bacterium he called *Bacterium colicommune* as the cause of infantile diarrhea. Its present name *Escherichia coli*was officially accepted in 1958 in honor of its originator. Although in research on basic genetics and molecular biology *E. coli* was generally known as a non-pathogenic bacterium, in medicine *E. coli* is known as an important pathogen infecting worldwide millions of humans each year both in industrialized and in developing countries. Therefore, during the last few decades molecular biologists have started to work on the mechanisms of bacterial pathogenicity of *E. coli*(*Neidhardt,* 1996; *Gyles,* 1994; *Nataro*, 1998,; *Kaper,* 1998). The huge amount of new knowledge and genetic data on pathogenic *E. coli* indicates that up to 10–20% of the genomic information found in highly pathogenic *E. coli* is not present in *E. coli* K–12. Most of the additional genes found in pathogenic *E. coli* encode various virulence factors which directly determine their virulence and pathotype. Diagnostic methods used nowadays focus on the detection of either specific toxins and their virulence attributes or specific target genes which permit he identification of the corresponding pathotype.

E. coli is a main component of the normal intestinal flora of humans and other mammals. A great diversity of commensal non-pathogenic *E. coli* strains belonging to many different serotypes can be isolated from the feces of healthy persons. These strains are massively shed in the environment and may contaminate food of animal origin or other foods like vegetables, fruits and their derivatives. They may also contaminate surface and underground water, generally without any adverse effects on human health.

E. coli from the normal intestinal flora are usually harmless to the host and represent opportunistic pathogens. Only in very rare cases can they become a threat to healthypersons. This is mainly the case in patients with impaired immune defenses not able to

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contain these commensals in their natural habitat or after a traumatic break in the natural barriers between the gut and other normally sterile sites of the body or after surgical interventions. They can also be part of mixed infections when primary pathogens break down the local defenses of a host.

Enteric pathogenic *E. coli* (*Nataro,* 1998; *Levine,* 1987) have been broadly divided into enterotoxigenic*E. coli* (ETEC), enteropathogenic*E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enteroinvasive*E. coli* (EIEC), enteroaggregative*E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC).

STEC (previously called Shiga-like toxin-producing *E. coli* or verotoxin-producing *E. coli*) cause a broad range of symptoms in humans (*Griffin,* 1999) including uncomplicated diarrhea, but also more severe diseases like hemorrhagic colitis and the often deadly hemorrhagic uremic syndrome. STEC infections represent a typical disease of industrialized countries and severe forms of infection are observed mainly in young children and the elderly. Cattle form the main reservoir of STEC and fecal contamination of food represents the usual source of infection for humans but due to an apparently low infectious dose, human to human transmission has also been observed in outbreaks. STEC have also been shown to be responsible for diarrhea in calves (*Butler,* 1994) and some specific serotypes are responsible for edema disease in pigs (*Bertschinger,* 1994) STEC belong to a very large variety of serotypes, but the majority of clinical infections registered in humans and particularly in food-borne outbreaks are associated with the serotypes and serogroups O157:H7, O157:H-, O26, O103, O111, O113, and O145. Those serotypes and strains associated with severe disease and outbreaks are also called enterohemorrhagic*E. coli* (EHEC). STEC colonize the colon where they cause necrosis of villus tips but they do not invade the intestinal mucosa. The majority of EHEC have been shown to present the typical pattern of localized adherence on cell cultures and to be able to cause attaching and effacing lesions also associated with the latter pathogens (*Nataro,* 1998).

Escherichia coli O157:H7, the prototype and most virulent enterohemorrhagic*E. coli* (EHEC), was isolated in 1982 from outbreaks of hemorrhagic colitis associated with eating undercooked meat in fast-food restaurants (*Riley et al,.* 1983). EHEC O157 was also isolated from sporadic cases of hemorrhagic colitis (*Uyeyama et al.,* 1982).

The detection of toxin production by EHEC O157 led to the discovery of its causative role in the development of a previously idiopathic condition known as hemolytic uremic syndrome (HUS), a clinical pathological triad consisting of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (*Johnson et al.,* 1983; *Karmali et al.,* 1985; *O'Brien et al*., 1983). Although EHEC O157 is the most common serotype isolated from humans, over 100 other serotypes, characterized collectively as non-O157 EHEC, are recognized by the World Health Organization as zoonotic emerging pathogens (*Nastasijevic,* 2009). Non-O157 EHEC have pathogenic and outbreak potential and are associated with diarrhea, hemorrhagic colitis, and HUS in humans (*Brooks et al*. 2005; *Hedican et al*. 2009). Genomic comparison of EHEC O157 and three clinically important non-O157 EHEC (O26, O111, and O103) revealed that all share very similar virulence gene sets, providing insight into EHEC parallel evolution (*Ogura et al*., 2009).

Main virulence factors

A/E Lessions

The key figure of EHEC pathogenicity is the formation of attaching and effacing (A/E) lesions on intestinal epithelial cells (*Nataro,* 1998). A/E lesions are characterized by the localized destruction of brush border microvilli and the intimate attachment of bacteria to the membrane of host cells. The formation of A/E lesions is mediated by the type III secretion system (T3SS), which translocates virulence factors called effectors into host cells, and the outer-membrane protein intimin, which is necessary for intimate attachment. These genes are encoded in the LEE pathogenicity island, a chromosomal locus in enteropathogenic strains of *E. coli.* The LEE genes consist of five operons and several cistrons, and their expression is coordinately regulated at the level of transcription (*Mellies et al.,* 2007). The expression of the LEE genes is regulated by a variety of environmental factors through a cascade controlled by two O157-specific virulence regulators. The LEE-encoded protein Ler activates the transcription of the LEE genes, except for the LEE1 operon genes, including the ler gene, whose transcription is activated by the Pch regulators, which are encoded by extra-LEE chromosomal loci (*Iyoda*, 2004). The combined actions of the Ler and Pch regulators control the expression of many virulence-associated genes, along with the LEE genes (*Abe et al.,* 2008).

Toxins

Toxins are the most obvious virulence factors found in almost all pathogenic *E. coli*. Some toxins show a strong association with specific pathotypes (Table 1). The Shiga toxins (formerly designated

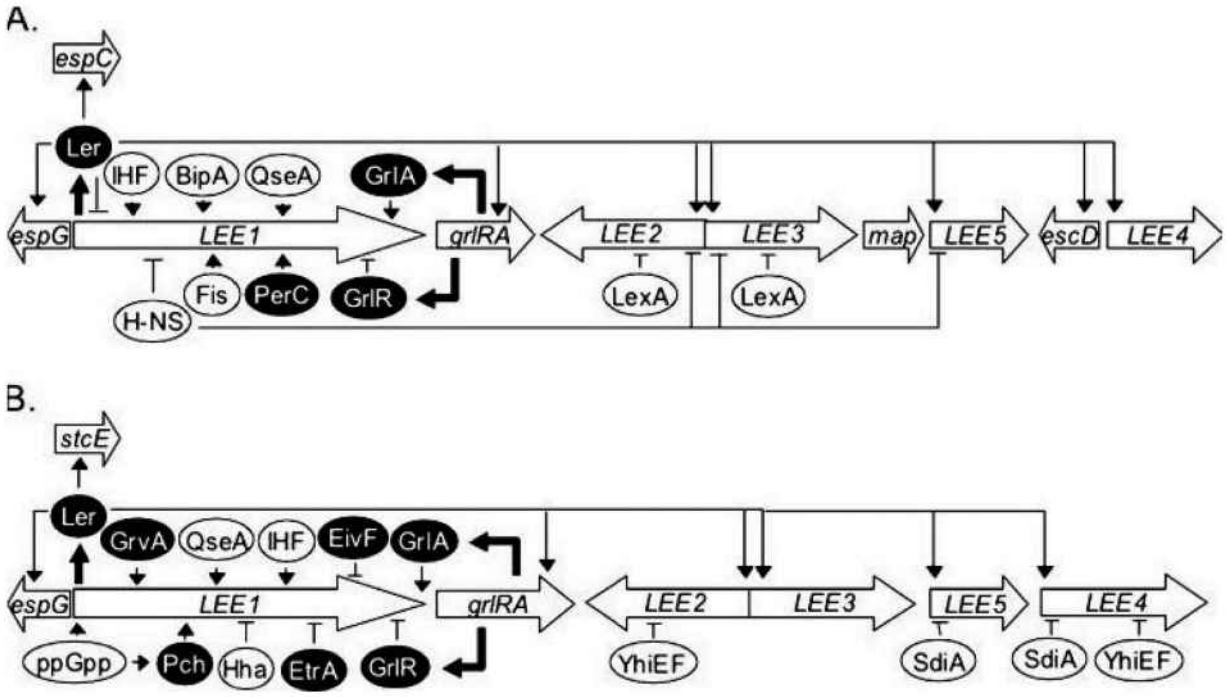

Figure 1. Regulation of the LEE pathogenicity island in EPEC (A) and EHEC (B) (by *Iyoda,* 2004).
Slika 1. Regulacija LEE ostrvapatogenostikod EPEC (A) i EHEC (B), (prema *Iyoda,* 2004)

Shiga-like toxins or verotoxins) Stx1 and Stx2, encoded by the genes *stx1* and *stx2*, respectively, are noticeable toxins and gave the name to the Shiga toxin-producing *E. coli* pathotype STEC. The stream of pathogenesis shows that after internalization (*Melton-Celsa et al.,* 1998) Stx1 and Stx2 depurinate specific residues of the host cell ribosomes (*Endo et al,*. 1988, *Saxena et al.,*1989) thereby blocking the binding of aminoacyl-tRNA to the ribosomes and inhibiting the protein synthesis.Stx, produced by EHEC during colonization of the intestinal tract, gains entry to the host through epithelial cells and acts on submucosal immune cells that release cytokines; these in turn induce inflammation and increase the expression of the Stx receptor globotriaosylceramide (Gb3) (*O'Loughlin,* 2001).Stx then targets the endothelium of organs in which the Gb3 receptor is expressed (e.g., the intestine, kidneys, and brain; *Boyd,* 1989). Because the Gb3 receptor is a glycosphingolipid, variations in the lipid moieties of its structure may influence Stx binding (*Kiarash et al.,* 1994). Stx-mediated endothelial injury activates coagulation, and inhibition of fibrinolysis leads to accumulation of fibrin and thrombosis (*Tarr et al.,* 2005). The combination of Stx and O157 lipopolysaccharide (LPS) induces platelet-leukocyte aggregates and tissue factor release and thus contributes to a prothrombotic state (*Stahl et al,*. 2009). Upon induction, Stx-encoding bacteriophages increase toxin production and play a role in horizontal transfer of stx genes by infecting other bacteria, as demonstrated in in vivo and in vitro experiments (*Acheson et al.,* 1998; *Herold et al.,* 2004; *Wagner et al.,* 2001)

Gene expression and regulation

Pathogenic bacteria must respond properly to their surrounding environment to coordinate virulence gene expression and to survive within a specific niche. Clarification of the kinetics of AE lesion formation demonstrated that this complex phenotype is tightly regulated in response to temperature and growth phase.

Activation of EHEC at 37°C in tissue culture medium enhanced the formation of AE lesions on human epithelial cells in culture (*Rosenshine,* 1996). AE lesions do not form if the bacteria are incubated at 28° C prior to infecting host cells at 37° C, and they are formed more readily by cultures in the early exponential phase of growth. Consistent with the temperature control of AE lesion formation, protein secretion via the EPEC TTSS occurs maximally at host body temperature in tissue culture medium such as Dulbecco's modified Eagle's medium (DMEM), at pH 7, and at physiological osmolarity (*Kenny,* 1995; *Kenny,* 1997). Secretion of EspA, EspB, EspC, and Tir proteins is also stimulated in the presence of iron and sodium bicarbonate, whereas it was inhibited by ammonium chloride or by omission of calcium from the growth medium (*Ide,* 2003).

Table 1. Some *E. coli* virulence factors
Tabela 1. Neki faktori virulencije kod *E. coli*

FACTOR	PATHOTYPE	TOXIN CLASS	TARGET MOLECULE	ACTIVITY/EFFECT
Thermolabile enterotoxin (LT)	ETEC	AB subunit, effector type II	G_s	Activation of adenylatecyclase, ion secretion
Shiga toxin (Stx)	EHEC	AB subunit	rRNA	Depurination of rRNA, no protein synthesis, inducing apoptosis
Cytolethal distending toxin (CDT)	Different	ABC subunit	DNA	DNA-se I activity, mitosis blocked in G2/M phase
*Shigella*enterotoxin 1 (ShET1)	EAEC, EIEC	AB subunit	-	Ion secretion
Urease	EHEC	ABC subunit	?	Urea decay NH_3 i CO_2
EspC	EPEC	Autotransporter	?	Serine protease, Ion secretion
EspP	EHEC	Autotransporter	?	Serine protease
Haemoglobine-binding protease (Tsh)	EPEC	Autotransporter	Hem	Haemoglobin decay, release ofiron
Pet	EAEC	Autotransporter	Spectrin	Serine protease, Ion secretion
Pic	EAEC, EIEC	Autotransporter	?	Protease, mucinase
Sat	EPEC	Autotransporter	?	Vacuolization
SepA	EIEC	Autotransporter	?	Serine protease
SigA	EIEC	Autotransporter	?	Ion secretion
Cycle inhibiting factor (Cif)	EPEC, EHEC	Effector type III	?	Mitosis blocked in G2/M phase, Cdk1 inactivation
EspF	EPEC, EHEC	Effector type III	?	Opens tight junctions, induces apoptosis
EspH	EPEC, EHEC	Effector type III	?	Phyllopodia modulation
Map	EPEC, EHEC	Effector type III	Mitochondria	Breaks membrane potential of mitochondria
Tir	EPEC, EHEC	Effector type III	Nck	Loss of microvilli
IpaA	EIEC	Effector type III	Vinculin	Actin depolymerization
IpaB	EIEC	Effector type III	Caspase 1	Apoptosis, release of IL-1
IpaC	EIEC	Effector type III	Actin	Actin polymerization, activation of Cdc42 i Rac
IpaH	EIEC	Effector type III	Nucleus	Modulation of inflammation

An additional two-component system controlling the EHEC AE phenotype has been recently described. The QseEF proteins are a sensor kinase and response regulator, respectively, and are transcribed from a single operon QseF activates transcription of the EspFu effector protein secreted into the host cell by the TTSS, and a *qseF* deletion mutant fails to form AE lesions. The QseEF system is activated by epinephrine through the QseC sensor kinase. The precise mechanism of the sensor kinase/response regulator control of quorum-sensing signaling in EHEC continues to be under intense investigation (*Sircili,* 2004).

An elegant study by *Deng et al.,* 2004 revealed that SepD and SepL, encoded by the *LEE2* and *LEE4* operons, respectively, constitute a molecular switch controlling secretion of translocators and effector molecules, and they began to clarify the role of calcium in these processes. Beginning with *C. rodentium*, but expanding their studies to EHEC and EPEC, they found that low-calcium conditions inhibit the secretion of translocators, such as EspA, EspD, and EspB, but enhance the secretion of effectors, such as Tir and NleA.

Genes responsible for synthesis of shiga-like toxins (stx1 and stx2) are not encoded by bacterial chromosome rather they are encoded by λ-like bacteriophage. It has been adopted that during the evolution this lambdoid bacteriophage participated in horizontal transfer of stx genes from *Shigelladysenteriae* to *E. coli.* This also explains high degree of homology between toxins produced by these bacteria. Bacteriophage can exist in one out of two possible forms: lysogenic or lytic phase. Being in lysogenic phase, bacteriophage has been incorporated into bacterial circular chromosome and it synchronously replicates along with bacterial DNA. When it comes to lytic phase, bacteriophage inserts its genetic material into a bacterium, replicates independently, reproduces and rearranges its ori-

ginal structure. At some moment, host cell bursts and release a new progeny of phages.

Considering that genes encoding Stx subunits in the genome of prophages are located next to the lytic genes in lambdoid family of bacteriophages there are two main signals from environment which induce switching from lysogenic to lytic lifecycle: **low iron concentration** and **DNA damaging** caused by acid stress, cold stress, presence of antibacterial substances etc. (*Velebit,* 2010).

Iron is the most important micronutrient used by bacteria. This metal is essential for cellular metabolism, since it is required as a cofactor for a large number of enzymes, except for *Lactobacillae*. However, this element is not easily available to microorganisms in aerobic environments. While in anaerobic conditions Fe^{2+} is soluble at physiological pH and cells easily obtain iron from the external medium, Fe^{2+} becomes quickly converted to Fe^{3+} upon exposure to oxygen and forms insoluble hydroxides at neutral pH, making the available metal very scarce. In order to acquire iron from the extracellular medium, virtually all aerobic bacteria produce and secrete low-molecular-weight compounds termed siderophores (siderosphoros, iron carriers). These compounds chelate Fe^{3+} with high affinity and specificity. Subsequently, the cell recovers the ferrisiderophore complexes through specific outer membrane receptors. Some of these high-affinity systems of iron uptake are important virulence factors in bacteria infecting animal fluids and tissues because they can chelate the metal bound to host proteins. Furthermore, because iron availability is generally growth limiting for bacteria thriving in an animal milieu, the lack of the metal is a major environmental signal to trigger expression of virulence determinants. However, an excess of iron is toxic because of its ability to catalyze Fenton reactions and formation of active forms of oxygen. Iron uptake has to be, therefore, exquisitely regulated to maintain the intracellular concentration of the metal between desirable limits. Considering that excretion mechanisms for iron are not known in bacteria, microorganisms appear to control iron homeostasis, regulating its transport through the membrane (*Escolar et al.,* 1999).

The Fur protein of *E. coli* is a 17-kDa polypeptide which acts as a transcriptional repressor of iron-regulated promoters by virtue of its Fe^{2+}-dependent DNA binding activity.Under iron-rich conditions Fur binds the divalent ion, acquires a configuration able to bind target DNA sequences (generally known as Fur boxes or iron boxes), and inhibits transcription from virtually all the genes and operons repressed by the metal. On the contrary, when iron is scarce, the equilibrium is displaced to release Fe^{2+}, the RNA polymerase accesses cognate promoters, and the genes for the biosynthesis of siderophores and other iron-related functions are expressed. In reality functional promoter pStx1, up streams from stx1 regulated by iron concentration through the iron-dependent Fur transcriptional repressor. Subsequently iron binding confers Fur metalloprotein a conformational change, and then high iron concentration Fur-regulated promoters become repressed. If the iron concentration is low, Fur does not bind close to pStx1 and toxin transcription can occur. Since bacteria need iron to grow could this be the reason why bacteria express and release toxins?

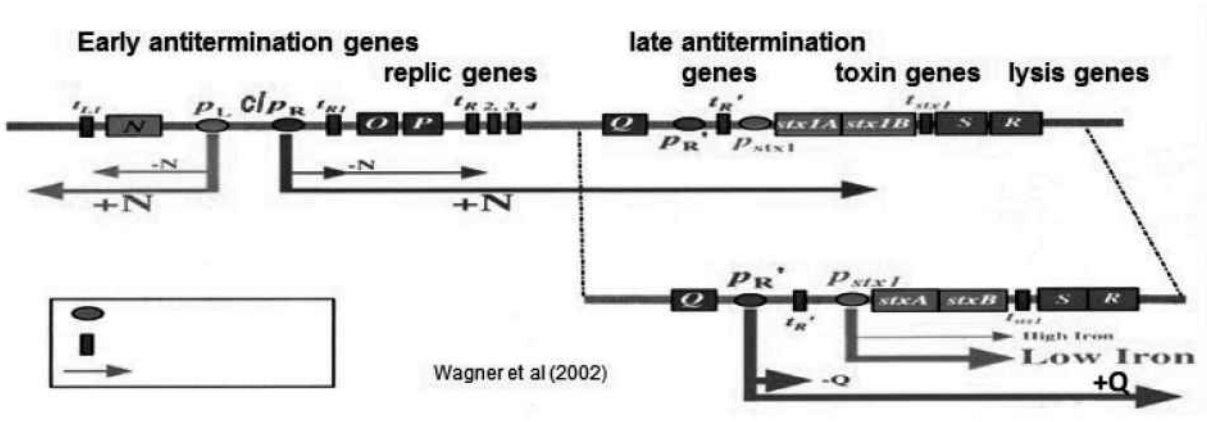

Figure 2. „Visual prints“ of pork, beef, lamb and venison made with the „E-nose“
Figura 2. „Vizuelni otisak“ svinjskog, junećeg, jagnjećeg mesa i divljači pomoću „Elektronskog nosa“

Damaged DNA sensors induce DNA repair mechanism known as “SOS” response. There are two SOS-induced genes:Lex A transcriptional re-

pressor protein and Rec A co-protease. This protease has several roles, of which most important are participation in re combinational DNA repair, assembly ofss DNA gains a co-protease activity Rec A, it is being involved with the self-cleavage of Lex A.de-repressionof SOS-regulated genes and finallyand the most important, cleaves the cI repressor of λ lambda phage, allowing the conversion of lysogenic form to a lytic form (*Wagner et al.,* 2001).

Stress response and changes of virulence

Under stress conditions microorganisms have developed signal transduction systems (movement of signals from outside the cell to inside) to sense environmental stresses and to controlthe coordinated expression of genes involved in cellular defense mechanisms (*Kennelly,*1996). These evolved protective or adaptive networks assist microorganisms to modify their environments and/or to survive the stress condition. A common regulatory mechanism involves sigma factors. Sigma factors are small proteins that bind to RNA polymerase (RNAP). The core RNAP has 5 components or subunits (α1, α2, β, β, and ω). In orderto bind promoter-specific regions, the core enzyme requires another subunit, a sigma factor (σ). The sigma factor greatly reduces the affinity of RNAP for nonspecific DNA, while increasing specificity for certain promoter regions, depending on the sigma factor. A sigma factor is normally present as part of the RNA polymeraseholoenzyme complex and the complete holoenzyme, therefore, has 6 subunits. The presence of the sigma factor allows theRNAP enzyme to bind at specific promoter sequences on thechromosome, and initiate transcription of particular genes (*Abee,* 1999). In this manner, genetic expression is controlled at the transcription level. In E. coli, the housekeeping σfactor, σ70, is responsible for transcription from many of the genepromoters under normal nonstress conditions.Under stress conditions, alternative σ factors to σ70 with different promoter specificities are induced, resulting in the expressionof specialty regulons (a system in which two or more structuralgenes are subject to coordinated regulation by a common regulator molecule) in response to a variety of stresses. In this manner, gene expression is modified by different sigma factors. In E. coliand other enteric bacteria, σS(RpoS) is the master regulator of thegeneral stress response.

In a recent study, cytotoxic genes (stx1, stx2, eaeA) expression of *Escherichia coli* O157:H7 in foodstuffs of animal origin was studied (*Velebit,* 2010). Experiment was set up in a way it simulated the most common storage conditions. Minced beef, UHT milk and soft cheese were inoculated by an overnight broth culture of reference strain ATCC 35150 E. coli O157:H7. Samples were incubated at the temperatures of 4°C and 12°C during 24 hours and 48 hours, respectively.Genetic expression profile was frozen at determined time points. In order to measure relative expression of cytotoxic genes, prokaryotic mRNA has been purified and converted to cDNA to be used in a RealTime PCR. Simultaneously, changes in growth kinetics were recorded.

Results indicated that growth of *E. coli* O157:H7 in experimentally contaminated minced beef kept at the temperature of 12°C during 48 hours and expression of stx1 and stx2 genes were considerably higher rather than in foodstuffs subjected to other experimental conditions.Regarding UHT milk growth kinetics and gene expression remained as similar as in minced beef, however at a somewhat lower intensity.Growth kinetics in soft cheese appeared to be not affected. Interestingly, stx1 gene was down--regulated during initial phase of incubation; while later up-regulation to a base level occurred by the end of experiment.Expression of eaeA gene in case of experimentally contaminated beef, milk and cheese and expression of stx2 and eaeA genes in milk and cheese was negligible i.e. experimental conditions didn't provoke up-regulation of respective genes. It seemed that foodstuffs with higher fat content down-regulated stx2 genes. A strong correlation has been established between growth kinetics and gene expression in minced beef and partially UHT milk while no correlation has been established between growth kinetics and gene expression in soft cheese.

Conclusion

Food is composite environment in which varieties of microorganisms are present and compete strongly for uptake of limiting nutrients to initiate and maintain growth. Survival of pathogens in foods takes place under stress conditions such as limited nutrient availability, adverse pH osmolarity, oxidation, temperature, chemical residues, as well as competition by other microorganisms. Spoilage and pathogenic bacteria coexist and compete in foods. In this respect, prediction of *E. coli* response to stress should take into account all microecological factors involved in the processing of a specific food.

The primary objective of food scientist is to control occurrence and evolution of stress-resistant pathogens in real foods and, consequently, to impro-

ve food safety. The stress response of *E. coli*is complex, robust, and versatile. Bacterial stress adaptation of *E. coli* to novel technologies and the potential for stress-associated enhanced virulence need to be addressedin more detail to prevent potential risk of disease. An increased understanding of expression of virulence-associated genes will provide information for control of pathogens and increase microbial safety of foods. It will also encourage development of new efficient methods for reducing the virulence of this pathogen in contaminated foods.

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Nova istraživanja virulentnosti enterohemoragičnih *Esherichia coli* u hrani

Velebit Branko, Teodorović Vlado, Borović Branka

R e z i m e: Većina gena koji su poslednjih godina utvrđeni kod patogenih E. coli kodiraju različite faktore koji direktno utiču na njihovu virulentnost i pripadnost određenom patotipu. Dva glavna faktora virulencije kod enterohemoragičnih E. coli (EHEC)su attaching/effacing lezije Stx1/Stx2 toksini. Geni odgovorni za ekspresiju pomenutih faktora virulencije pod jakim su uticajem uslova sredine. Niska koncentracija gvožđa indukuje jaku ekspresiju stx1 gena i posledičnu sintezu velike količine Stx1 toksina. Stresni odgovor EHEC na manjak hranljivih supstanci, povećanje kiselosti sredine, izloženost hladnoći kao i na promene osmolarnosti, koji oštećuju DNK, indukuje „SOS" odgovor. „SOS" reakcija posredovana RecA proteinom ne samo da popravlja oštećene fragmente DNK molekula, već i pokreće mehanizam konverzije bakteriofaga integrisanog u hromozom E. coli iz lizogene faze u litički ciklus tokom koga dolazi do intenzivne ekspresije stx2 gena. Da bi se prevenirao potencijani rizik nastanka bolesti, neophodno je detaljno proučavati prilagođavanje E. coli novim tehnologijama pripreme i konzervacije hrane i potencijal za stres-indukovanu virulenciju. Dobro poznavanje ekspresije gena odgovornih za virulenciju obezbediće informacije neophodne za kontrolu patogena i povećaće mikrobiološku bezbednost hrane.

Ključne reči:*EHEC, virulentnost, genskaekpresija, hrana.*

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Methicillin susceptible and resistant *Staphylococcus aureus* from farm to fork impact on food safety*

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A b s t r a c t: Methicillin susceptible Staphylococcus aureus (MSSA) are well known as one of the leading cause of food poisoning worldwide for decades. Their food safety importance is due to their ability to form staphylococcal enterotoxins (SE) referred to as superantigens and enterotoxin-like superantigens (SEl) in foodstuffs. The five classical staphylococcal enterotoxin types (A to E) are the most important with regard to food poisoning. Recently, also Methicillin resistant Staphylococcus aureus (MRSA) have been detected in food animals and food with a remarkable prevalence. The majority of these MRSA strains belong to the clonal complex (CC) 398 and were not described in humans prior the detection in food animals. A major concern of public health authorities is the potential spread of these strains into the human population. Meat from poultry displayed the highest prevalence of MRSA whereas the prevalence of MRSA on poultry farms does not seem to be higher than the prevalence in pigs. Moreover, MRSA strains that are not associated with the CC398 (Non-CC398) contribute to the contamination of food items, thus posing an alternate threat to public health. Up to present, the likelihood of MRSA strains isolated from farm to fork to harbour staphylococcal enterotoxine genes seems to be low. However, more research is needed to quantify the risk of colonization of consumers with MRSA via the food chain. Hence, continuous surveillance is needed in order to study whether the presence of enterotoxins or other virulence factors will increase.

Key words: *MSSA, MRSA, enterotoxins, antibiotic resistance, food chain, food safety.*

Introduction

From a food safety perspective, coagulase positive staphylococci and in particular *Staphylococcus (S.) aureus* is by far one of the most important foodborne pathogen due to its ability to produce a wide variety of toxins including heat-stable staphylococcal enterotoxins (SEs). Up to present, more than 21 SEs were described, including SEs with a demonstrated emetic activity (SEA-SEE, SEG-SEI, SER-SET) and the so called staphylococcal-like (SE*l*) SEs, which are not emetic in a primate model (SE*l*L, SE*l*Q) or have yet not been tested (SE*l*J, SE*l*K, SE*l*M-SE*l*P, SE*l*U, SE*l*V) (*Argudin et al.*, 2010).

SEs are a major cause of food poisoning worldwide, e.g. around 5.3 % (n = 293) of all reported foodborne outbreaks in 2009, were caused by staphylococcal enterotoxins (EFSA, 2011). As a prerequisite, enterotoxigenic strains of *S. aureus* (naturally or artificially) contaminating varying food matrices have to produce SEs during their exponential growth. After ingestion of food containing preformed SEs symptoms do rapidly occur, including nausea, violent vomiting, abdominal cramps and diarrhea (*Seo and Bohach*, 2007). Usually, the illness is self-limiting and warrants hospitalization in occasional cases, only. Food-stuffs typically involved in foodborne outbreaks due to SE intoxication are (raw) milk and dairy products, meat and products, salads and bakery products, particularly cream-filled pastries and cakes. In most cases food handlers carrying enterotoxin-producing *S. aureus* in their noses or hands are regarded as the main source of food contamination due to improper handling and subsequent storage at elevated temperatures (*Argudin et al.*, 2010). However, *S. aureus* is also present

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in food animals, and dairy cattle sheep and goats, particularly if affected by subclinical mastitis, are likely contaminants of milk (*Stewart,* 2005).

In the EU, Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs lays down certain food safety criteria with regard to SEs to be applicable to products placed on the market during their shelf-life. Accordingly, staphylococcal enterotoxins may have to be undetectable in 25 g of certain cheeses (i.e. cheeses made from raw milk, resp. milk that has undergone a lower heat treatment than pasteurization, resp. ripened or unripened cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment), milk and whey powder. Hence, process hygiene criteria as layed down in Regulation (EC) No 2073/2005 necessitates the analyses of certain foodstuffs on their amount of coagulase positive staphylococci at the time during the manufacturing process when the number of staphylococci are expected to be highest. Foodstuffs of which this criterion applies for are certain cheeses (as mentioned above), milk and whey powder as well as shelled and shucked products of cooked crustacaens and molluscan shellfish. In case of unsatisfactory results, actions to be taken by the food business operator include improvements in production hygiene and selection of raw materials. Further, the batch has to be tested for the presence of SEs in the case that values > 10^5 Colony forming units (CFU) per gram are detected.

Methicillin resistant *Staphylococcus aureus* (MRSA) has been described as an important nosocomial pathogen in hospital settings for a long time. Specific strains of MRSA have also been shown to spread in the community. More recently, MRSA were also detected in livestock and companion animals. It has been shown that MRSA from livestock colonize professionals (farmers, veterinarians, etc.) dealing with live farm animals (*Voss et al.,* 2005). The overwhelming majority of the isolates from livestock in Europe have been assigned to clonal complex CC398. As this clonal complex was not described as MRSA in humans prior to the detection in animals it has been named 'livestock-associated (la-) MRSA'.

La-MRSA has also been detected in food, i.e. meat and milk (*de Boer et al.*, 2009; *Käsbohrer et al.,* 2010; *Spohr et al*., 2010) causing concern over a potential spread of MRSA to consumers via the food chain. Contamination of meat with MRSA is likely to occur at various stages of the processing chain by carry over from colonized body sites of the animal to the carcass, by cross contamination, through the environment of processing facilities or by people involved in the handling of carcasses or meat. Data from The Netherlands and from our laboratory show that MRSA of the same clonal lines can be detected in farm animals and food. Among meat samples, the prevalence is highest for meat from turkey and broilers and substantially higher than in pork *(de Boer et al.,* 2009; *Käsbohrer et al.,* 2010*)* while currently there is no evidence that the prevalence of MRSA in poultry is higher than the very high prevalence observed in pigs. However, data on MRSA in poultry is still scarce.

Moreover, data from the Netherlands and preliminary data from Germany indicate that MRSA strains that have not been described in livestock and are not associated with the CC398 (non-CC398) also contribute to the contamination of food items, thus posing an alternate threat to public health (*de Boer et al*., 2009; *Fetsch et al.,* 2009). The origin of this contamination is not clear. While it is likely that colonized people involved in the processing of food contaminated the product it cannot be excluded that the presence of these non-CC398 strains in primary production has been overlooked so far. MRSA ST398 was not noticed in farm animals in Germany before 2007, because *S. aureus* was not a relevant pathogen in the host species and therefore not investigated. According to German data, MRSA CC398 has been present in pigs since at least 2004 (*Meemken et al*., 2009).

Up to present, MRSA isolates of the CC398 usually lack important virulence determinants typical for other community and hospital isolates. Also staphylococcal enterotoxin genes carrying laMRSA isolates have been reported in a small number of cases, only (*Kadlec et al*., 2009; *Laurent et al.*, 2009).

Previous food safety research focussed on Methicillin susceptible *Staphylococcus aureus* and its ability to form staphylococcal enterotoxins in foodstuffs. However, the detection of Methicillin resistant *S. aureus* in livestock and food in recent years raised additional food safety concern. Therefore, this paper aims to gives an overview on studies carried out in Germany on the prevalence, molecular epidemiology and staphylococcal enterotoxin gene content of MRSA isolated from farm to fork and to discuss their possible impact on food safety.

Materials and Methods

The strain collection of the National Reference Laboratory for coagulase positive staphylococci incl. *Staphylococcus aureus* (NRL-Staph) at the BfR contains MRSA strains isolated in the course of various national and international monitoring programs or research projects. Around 2200 MRSA strains isolated from production holdings (breeding (n =

106) and fattening (n = 145) pigs, turkeys (n = 34), dairy cows (n = 24)), at slaughter (pigs (n = 636), turkeys (n = 202), veal calves (n = 115)) and at retail (raw meat, raw meat preparations and minced meat of pigs (n = 198), veal calves (n = 64), broilers (n = 233) and turkeys (n = 283)) are available.

All genotypically confirmed MRSA isolates were typed using SCC*mec*- and *spa* typing protocols as described elsewhere (*Shopsin et al.*, 1999; *Zhang et al.,* 2005). In addition, of one isolate per identified *spa* type the Multi locus sequence type (MLST) was determined (*Enright et al.*, 2000). *Spa*- and Multi--locus sequence type (MLST) were determined using the software Ridom Staphtype and the *S. aureus* MLST database.

A subset of MRSA isolates (n = 100), all of CC398, was further investigated for the presence of staphylococcal enterotoxine genes (sea, seb, sec, sed, see, seg, she, sei, sej, sek, sel, sem, sen, seo, seq, ser, seu) by PCR as described by *Argudin et al.* (2011).

Susceptibility testing and evaluation of resistance were performed with all isolates in accordance with Clinical and Laboratory Standards Institute guidelines by broth dilution or disc diffusion method, respectively.

Results

Typing and antimicrobial resistance testing results of MRSA isolated from farm to fork

Pigs: Among the isolates from holdings of breeding and fattening pigs, *spa* types t011, t034 and t108 and SCC*mec* type V predominate and the proportion of Non-CC398 MRSA (ST9, ST39, ST97) was less then five percent. CC398 MRSA do also predominate in pigs at slaughter, however the proportion of *spa* types t011, t034 and t108 varied greatly per abattoir (*Tenhagen et al.*, 2009). In raw meat at retail, the proportion of Non-CC398 MRSA among the isolates was highest (up to 13 %) as was the percentage of SCC*mec* types other than V, with 11 % of the isolates harbouring SCC*mec* type IVa. Resistance patterns of isolates from pigs along the food chain differed slightly, overall showing high frequencies of resistance against beta-lactames and tetracycline (both up to 100 %), lincosamides and macrolides (clindamycin and erythromycin, both up to 80 %) and aminoglycosides (gentamicin and kanamycin, ~ 20 %).

Cattle: At farm level, samples from dairy cows resp. bulk milk harboured CC398 MRSA with *spa* types t034 and t011 and SCC*mec* types V resp. III, only. Moreover, all MRSA isolates from veal calves at slaughter belonged to CC398. The proportion of Non-CC398 MRSA among the isolates from raw veal meat at retail was low (< 2 %). As among isolates with porcine origin, resistance to beta-lactames and tetracycline (~100 %), lincosamides (up to 72 %) and macrolides (up to 61%) were highest. Remarkably, isolates from veal calves at slaughter showed a significantly higher proportion of resistance against streptogramin antibiotics (quinupristin/dalfopristin 27%) and folate synthesis inhibitors (sulfamethoxazole/trimethoprim (42 %) then pig isolates.

Poultry: As among CC398 MRSA from porcine and cattle origin, t011 and t034 with SCC*mec* type V were also the most often *spa* types among poultry isolates. MRSA from poultry at farm level are scarce up to present. Only isolates from turkey flocks were available for analysis, displaying a high proportion of Non-CC398 MRSA (21 %, mainly *spa* type t002 (ST5)). Interestingly, the high percentage of SCC*mec* types IVa was exclusively detected in MRSA of *spa* type t011. At slaughter, the proportion of Non-CC398 MRSA among turkey isolates was lower than at farm level (< 8 %) whereas around 15 % of the isolates from raw turkey meat at retail level were associated with Non-CC398 *spa* types (mainly t002).

Raw meat from broilers showed a high proportion of Non-CC398 MRSA (~ 30 %), with *spa* type t1430 (ST9) predominating. Interestingly, this *spa* type was not detected in raw meat of other origin and was most often associated with SCC*mec* type IVa. Overall, MRSA isolates from raw meat from poultry (broiler and turkey) harboured significantly more often type IVa SCC*mec* than those from other origin. Resistance to beta-lactames, tetracycline, lincosamides and macrolides among poultry isolates were equally frequent as among pig and cattle isolates. However, the percentage of MRSA strains from poultry in particular from raw meat and raw meat preparations being resistant to ciprofloxacin (~ 30% of the isolates) was higher than in isolates from other origins.

Staphylococcal enterotoxin gene content of MRSA isolated from farm to fork

None of the subset of 100 CC398 MRSA isolates derived from various sources from farm to fork in Germany carried any of the staphylococcal enterotoxin genes tested (*Argudin et al*., 2011).

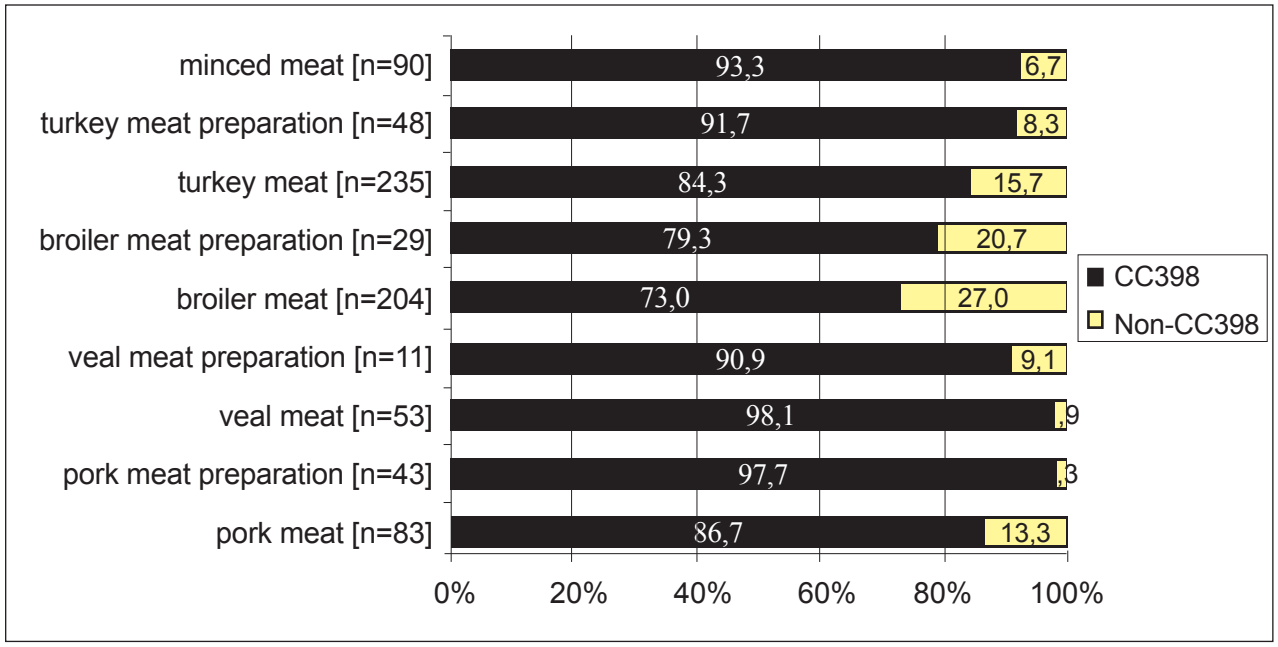

Figure 1. Percentage of Non-CC398 MRSA found in isolates from food at retail
Slika 1. Procenat Ne- CC398 MRSA utvrdjenih u izolatima hrane u maloprodaji

Legenda/Legend:
Minced meat/Mleveno meso
Turkey meat preparation/Proizvodi od ćurećeg mesa
Turkey meat/Ćureće meso
Broiler meat preparation/Proizvodi od pilećeg mesa
Broiler meat/Pileće meso
Veal meat preparation/Proizvodi od teletine
Veal meat/Teleće meso
Pork meat preparatio/Proizvodi od svinjskog mesa
Pork meat/Svinjsko meso

Discussion

For a long period of time MRSA were seen as a problem of human medicine and hospital hygiene alone. However, the MRSA complex has a zoonotic component, which is closely linked to strains of the clonal complex (CC) 398 and to food animal populations (*Meemken et al.,* 2009). People dealing with livestock are at higher risk of colonization than those without direct contact to the animals (*Cuny et al.,* 2009; *Mulders et al.*, 2010).

Currently, the majority of laMRSA isolates from farm to fork belong to *spa* types which can be assigned to clonal complex CC398. As shown in Fig.1, MRSA from food at retail showed the highest diversity among the isolates. In particular, MRSA from poultry showed the highest proportion of Non-CC398 MRSA. Additionally, prevalences from farm to fork varied greatly between and within the different species. Whereas meat from poultry displayed a substantially higher prevalence of MRSA than red meat, the prevalence of MRSA on poultry farms does not seem to be higher than the prevalence in pigs. Specific clonal lineages seem to predominate in specific origins, e.g. MRSA of *spa* type t1430 (belonging to ST9) harbouring SCC*mec* type IVa and with a high ciprofloxacin resistance rate isolated from raw broiler meat respectively, MRSA of *spa* type t002 (ST5) isolated from raw turkey meat. These findings call for strain and clonal lineage specific risk characterization to assess the consumers risk associated with MRSA in food.

None of the subset of 100 MRSA isolates of the CC398 ST398 harboured any of the staphylococcal enterotoxin genes tested. This is in line with previous studies, in which a low occurence of either seb or sed and seg was reported for MRSA CC398 isolates from Germany and France (*Kadlec et al.,* 2009; *Laurent et al.,* 2009). It has been speculated that the environment contributes to the low presence of virulence determinants in this clone (*Argudin et al.*, 2011). However, the presence of enterotoxin genes among Methicillin susceptible *S. aureus* (of undetermined CCs) can be high (*Nitzsche et al.,* 2007). Hence, MRSA of Non-CC398 and isolated from farm to fork seem to harbour staphylococcal entero-

toxin genes at a much higher percentage than CC398 MRSA (Fetsch et al., personal communication). Thus, the horizontal gene transfer between strains of different clonal lineages harbouring staphylococcal enterotoxin genes is likely to occur and calls for further investigations.

While the presence of MRSA in food is well established there is a substantial lack of knowledge on the quantity of MRSA in meat and commodities and on dose-response relationship. Therefore, a valid quantitative assessment of the consumers risk is currently not feasible. Up to now, there is no evidence that MRSA ST398 is spread via the food chain. However, animal products remain a potential source of MRSA. More research is needed to quantify the risk of colonization of consumers with MRSA via the food chain and to monitor a possible evolve of isolates with a potential higher pathogenic potential, i.e. staphylococcal enterotoxin gene content.

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Meticilin-rezistentni sojevi *Staphylococcus aureus*, od farme do trpeze – uticaj na bezbednost hrane

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R e z i m e: Sojevi Staphylococcus aureus (MSSA - Methicillin susceptible) osetljivi na meticilin su dobro poznati kao vodeći uzroci trovanja hranom širom sveta. Njihov značaj sa stanovišta bezbednosti hrane proizilazi iz njihove sposobnosti da formiraju u prehrambenim proizvodima stafilokokne enterotoksine (SE) koji se još nazivaju superantigenima i superantigene koji su slični enterotoksinima (SEl). Pet klasičnih tipova stafilokoknih enterotoksina (od A do E) su najvažniji sa stanovišta trovanja hranom. Nedavno su otkriveni i meticilin-rezistenti sojevi Staphylococcus aureus (MRSA) u korisnim domaćim životinjama i hrani sa značajnom prevalencom. Većina ovih MRSA sojeva pripada klonalnom kompleksu (CC) 398 i nisu registrovani kod ljudi pre nego što su otkriveni kod domaćih životinja. Najveći razlog za zabrinutost nadležnih organa za javno zdravlje je potencijalno širenje ovih sojeva na ljudsku populaciju. Meso šivine je pokazalo najvišu prevalencu MRSA, dok na živinarskim farmama prevalencija MRSA nije veća nego kod svinja. Štaviše, MRSA sojevi koji nisu povezani sa CC398 (Ne-CC398) doprinose kontaminaciji prehrambenih proizvoda, i na taj način predstavljaju dodatnu opasnost po javno zdravlje. Do sada, verovatnoća da će MRSA sojevi izolovani od farme do trpeze imati gene stafilokoknih enterotokcina se čini da je mala. Međutim, potrebna su istraživanja kako bi se kvantifikovao rizik od kolonizacije potrošača sa MRSA preko lanca ishrane. Prema tome, stalni nadzor je potreban kako bi se ispitalo prisustvo enterotoksina ili drugih virulentnih faktora.

Ključne reči: *MSSA, MRSA, enterotoksini, rezistentnost na antibiotike, lanac ishrane, bezbednost hrane.*

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Pre-slaughter intervention by vaccination of cattle – new approach to reduce risk of meat contamination by foodborne pathogens, and consequently reduce incidence of human disease and economical burden*

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A b s t r a c t: Enterohaemorrhagic Escherichia coli (EHEC) is an important zoonotic pathogen of humans. It is a commonly found fecal coliform bacterium present in the intestine of ruminants, from where it is frequently shed into the environment. Cattle are the main reservoir of E. coli O157. Whilst causing no disease in cattle, exposure of humans to very few bacteria (10 or fewer) is responsible for major outbreaks of hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) throughout the world. The most common mode of transmission of E. coli O157 to the human population is by consumption of contaminated food, which accounts for 61% of human illness. The United States Centres for Disease Control and Prevention (CDC; 2008) reported that consumption of beef products and produce (including fruits and vegetables) was a significant mode of transmission, accounting for 45% and 34% of food borne illnesses respectively. In addition to the foodborne mode of transmission, drinking contaminated water accounted for 15% of all cases of human illness associated with E. coli O 157. In Canada approximately 26 000 people become ill each year because of exposure to E. coli O157.

Traditionally, work has focused on improving cattle meat safety through the implementation of post-harvest antimicrobial treatments. However for effective disease control in human populations, which are susceptible to E. coli O157 arising from not only contaminated beef, but contaminated produce, drinking water and direct contact, it becomes apparent that a comprehensive 'One Health' initiative is probably the most suitable approach to control or eliminate the amount of pathogens that enter our environment. This approach identifies and evaluates the connections between animals, human and environmental domains. The value of this approach is demonstrated by the data from CDC which estimates that three-quarters of all new and emerging diseases are zoonotic. Presently, E. coli O157, and several other enteric pathogens found in livestock including Salmonella, and Campylobacter jejuni, found in sheep, cattle swine and poultry, and Yershinia enterocolitica found in swine contribute to the estimated 79 million people in the United States that become ill after consuming food contaminated with pathogens.

An on-farm vaccination strategy is one of the best methods to reduce the prevalence of E. coli O157 both within in its primary host and the general environment. The focus of this manuscript is to describe E. coli O157, the central role of cattle in disseminating E. coli O157 in the environment, and the rational for the development and implementation of an efficacious pre-harvest vaccination strategy using a fully licensed vaccine.

Key words: *vacctioantion of cattle, meat contamination, foodborne pathogens.*

Introduction

Enterohaemorrhagic *Escherichia coli O157* (EHEC)

EHEC consists of a subset of *E. coli* strains that have acquired virulence factors and are pathogenic to humans. By definition *E. coli* O157 is used to describe the subgroup of shiga toxin-producing *E. coli* that have the potential to cause haemorrhagic colitis (HC) in humans.

In cattle, the terminal rectal mucosa in the colon, located at the recto-anal junction (RAJ), has been identified as the principal site of *E. coli* O157 colonization (*Naylor et al.*, 2003). The RAJ con-

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tains the highest concentration of this organism and contributes more to the fecal load of bacteria than the rest of the gut combined. Other sites of *E. coli* O157 colonization include the rumen, small intestine, and the proximal colon. An important aspect of colonization is the formation of attaching and effacing lesions (A/E lesions) which involves host cell cytoskeletal rearrangements, and pedestal formation at the sites of bacterial attachment. Central to these mechanisms is the Type III secretion system, described in the next section.

The critical virulence factor for *E. coli* O157 is their ability to produce shiga toxins (Stx). These toxins are synonymously called vero toxins (VT) due to their specific cytotoxicity to Vero cells (African green monkey kidney cells). Consequently the *E. coli* bacteria that produce VT are called verotoxin producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC). These designations, VT/Stx and VTEC/STEC, are used interchangeably.

Major virulence factors

Shiga toxins (stx)

Shiga toxins are synthesised by gram negative bacteria, including *E. coli* O157. In the human, cell surface Stx glycolipid receptors, Gb3 (globotriaosylceramide) and Gb4 (globotetraosylceramide) are present on glomerular endothelial and other vascular endothelial and epithelial cells and play a major role in the pathogenesis of human disease including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Based on toxin-neutralization and nucleotide sequence analyses shiga toxins are classified in two major groups, Stx1 and Stx2, showing approximately 60% nucleotide sequence identity. Generally, Stx2 has been more closely associated with severe disease and HUS, and has been shown to be 1000 times more toxic to human renal endothelial cells, than VT1 (*Moxley*, 2004 for review).

In mature cattle, after *E. coli* O157 challenge the degree of colonization is dependent on age of cattle, challenge duration and magnitude, however the mechanisms behind these different colonization rates are not fully understood. Research by *Lowe et al*., 2009, determined that *E. coli* O157 strain origin, from either human or bovine, lineage type and stx2 expression all affect the amount of *E. coli* O157 colonization in the intestines of cattle. Stx2 promotes the intestinal colonization of *E. coli* O157 in cattle through an increased expression of non Tir colonization sites and toxicity to the absorptive epithelial cells. In cattle although stx2 increases colonization, it is not cytotoxic to the cells of the jejuna and descending colon of cattle (*Bains et al*., 2008).

Type III Secretion System

The type III secretory system (TTSS) is the mechanism for translocation of bacterial proteins into host cells. The TTSS enable *E. coli* O157 to adhere to enterocytes causing histopathological lesions, called ‘attaching and effacing lesions’ (A/E lesions) which are characterized by localized destruction of the brush border microvilli, enabling *E. coli* O157 to attach intimately to the plasma membrane of host epithelial cells. Once attached the bacteria stimulate host cell actin polymerization and rearrangement of the cytoskeletal architecture, forming an attachment pedestal which secures it to the host cell.

The genes that encode the A/E lesions are located in a region of the genome called the ‘locus of enterocyte effacement’ (LEE). The LEE of *E. coli* O157:H7 is 43 kb and an additional region of 7.5kb prophage sequence, not found in other EHEC. The arrangement of the 41 genes is in three distinct regions. The first region encodes TTSS, the second region encodes an adhesion molecule and its translocated receptor, and finally the third region encodes several translocated molecules.

The bacterial TTSS is an essential component of the host-pathogen interaction, a major feature of TTSS is the translocation of a variety of virulence factors from within the bacterium into the host cell via a filamentous needle complex spanning the bacterial and host cell membranes, whereby the bacteria are able to inject their proteins, called effectors into the host cell. Once injected, the proteins can modify the function of the host cell.

The proteins that comprise the TTSS apparatus comprise the structural proteins. Other proteins called ‘translocators’ serve the function of translocating another set of proteins into the host cell, for example an adhesion molecule called intimin and its translocated receptor, called Tir, which is a bacterial outer membrane protein encoded by *eaeA*. Collectively, translocated proteins are called ‘effectors’ since they are the virulence factors that effect the changes in the host cells, allowing the invading pathogens to colonize and multiply in the host.

A subset of TTSS are the translocated secretory proteins, *E. coli* secretory proteins (Esp). These effector proteins including, Map, EspF, EspG, EspH, EspB and sepZ, modify the host cell signal transduction mechanisms during the formation of A/E lesions. When translocated into the host cell, they elicit a variety of reactions resulting in diarrhoea and

transmigration of acute inflammatory cells to the infection site (*Coburn et al.*, 2007).

Of particular interest is the secretory protein, EspA. Its function is to form the filamentous, hollow needle-like structure that assembles as a physical bridge between bacteria and host cell surfaces, which functions as a physical conduit for the translocation of bacterial effectors into host cells. After translocation of effector proteins, the filamentous complex is removed from the bacterial cell surface. This is necessary to make room for the intimate bacterial attachment between intimin and Tir that is essential for the A/E lesions (*Lim et al.*, 2010).

E. coli O157 has been regarded as causing no clinical or subclinical signs of infection in cattle. However, recent evidence indicates that *E. coli* O157 is not strictly a commensal bacterium in the bovine host. Experimental infection with high doses of *E. coli* O157:H7 causes diarrhoea, with A/E lesions in neonatal calves and transient watery diarrhoea in weaned calves. Histopathological damages**,** A/E lesion formation and enterocyte remodeling associated with removal of the epithelial layer at the site of colonization have been recorded in cattle that are persistent shedders *(Nart et al.,* 2008). Generally the relative resistance of cattle to the systemic effects of Stx may be explained by the observation of *Hoey et al.*, 2003, who found that unlike in the human, Stx Gb3 receptors only present in the proliferating crypt cells of bovine intestines, and not in the vascular cells.

Source of Human Infection
{TC "*Source of Human Infection*" \f C \l "2"}

Recent data and outbreaks have shown that *E. coli* O157 is capable of contaminating a variety of foods, and this knowledge has increased our understanding that multiple interventions are needed to reduce the risk of infection (*LeJeune* 2007; *Khanna*, 2008). According to the CDC (2008), 61% of human illnesses from *E. coli* O157 are foodborne and 15% are caused by consuming contaminated drinking water. Ground beef (33%) and produce (34%) are the two most frequent causes of foodborne illnesses (Figure 1).

Direct human/animal contact accounts for a significant number of *E. coli* O157 infections. A survey of thirty-two U.S. agricultural fairs demonstrated that *E. coli* O157 is widespread in animals at these exhibits (*LeJeune,* 2004): *E. coli* O157 was isolated from livestock at 97% of the fairs tested, with 11% of cattle testing positive.

The distribution of VTEC cases in Ontario, Canada, were studied to evaluate geographic relationships between livestock density and human VTEC incidence. Study results show a seasonal pattern of illnesses with higher rates from April to October than in winter (July is the peak). The data also showed a positive relationship between the incidence rate of VTEC illness in a community and cattle density. Data from Scotland found a similar relationship between bovine shedding of *E. coli* O157 and human illnesses (*Pearce,* 2009; *Ferens and Hovde*, 2011).

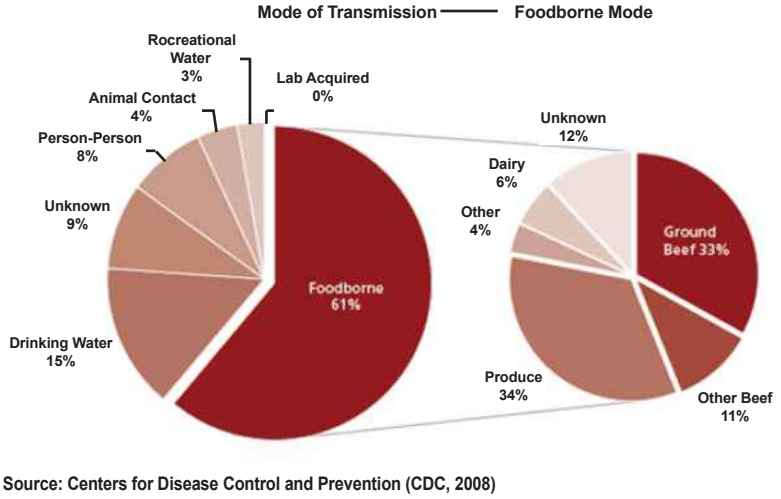

Figure 1. Sources of *E. coli* O157 Illness
Slika 1. Izvori oboljenja *E. coli* O157

Prevalence and estimated cost of human illness in Canada

{TC "*Human Illness – Primary Outcomes*" \f C \l "2"}

HC is characterized by abdominal cramping and watery diarrhea, which may turn into bloody diarrhea and fever. On average, 2% to 7% of these cases progress, becoming HUS, however, numbers have reached as high as 15% (*Center for Foodborne Illness Research and Prevention*, 2009). A portion of patients with HUS develop End Stage Renal Disease (ESRD) and die.

In Canada, VTEC infections confirmed through laboratory analysis must be reported to the Public Health Agency of Canada (PHAC). The rate of confirmed infections in Canada was 3.36 per 100,000 population. Using the model developed by *Frenzen* (2005) and the PHAC data, we estimate that each year Canada has 26,209 cases of E. coli O157 infection. Estimates for the actual annual number of illnesses resulting from *E. coli* O157, indicate that between 10 and 47 cases exist within the community for every positive case reported (*Thomas*, 2006). Based on the number of infections annually and Canadian health care cost information, the total cost of VTEC illness in Canada is estimated to be $29.6M(*Foodborne Illness cost calculator, United States Department of Agriculture*, 2006; Table 1).

Table 1. Cost of VTEC-related Illness to Canada
Tabela 1. Troškovi VTEC- u odnosu na bolest u Kanadi

Medical Costs/ Medicinski troškovi	Lost Productivity and Premature Death/ Gubitak produktivnosti i prevremena smrt	Total/ Ukupno
$3,627,956	$26,014,184	$29,687,139

On-Farm Prevalence

{TC "*On-Farm Prevalence*" \f C \l "2"}

A review of existing studies demonstrates prevalence rates of animals testing positive for *E. coli* O157 ranging from 9% to 88% (*Karmali* 2010). This wide range exists both within and between herds. A study conducted on Ontario beef cow-calf farms found *E. coli* O157 at 52% of farms surveyed (*Cernicchiaro* 2009). Based on that study, the author concluded in an interview with Canadian Cattlemen magazine (2010) that "*farmers need to assume that the organism [i.e., E. coli O157] is present on their farm".*

The concentration at which *E. coli* O157 is shed in feces varies from animal to animal and ranges from 10^2 to 10^5 cfu/g. Within a herd of cattle is a sub group referred to as 'super shedders', shedding $>10^4$ cfu/g. These animals contribute disproportionally to the *E. coli* O157 burden in a herd. Models of transmission dynamics have demonstrated that more than 80% of *E. coli* O157 spread arises from less than 20% of the most infectious individual cattle.

Presence of a 'super-shedder' on a farm has been associated with a high proportion of low--level shedding by other herd members. *Matthews* (2006) report that cattle exposed to a super-shedder increase shedding of *E. coli* O157 six fold in other cattle housed in the same pen. DNA evidence shows that these super-shedders transmit *E. coli* O157 to animals housed in the same pen. Super-shedder cattle represent the greatest risks of contaminating the food chain and maintaining high prevalence of *E. coli* O157 within cattle populations.

Rational for pre-harvest intervention

Interventions currently employed to minimize or eliminate the risk of human illness from *E. coli* O157 are focused on the harvest and processing segments within the beef industry (*Rogan et al.*, 2008). Hides are the key source of carcass microbial contamination (*Keen et al.*, 2002), and lairage at the processing plant are a major source of hide contamination, rates of hide contamination increase from 50.3% to 94.4% between the time cattle were loaded onto tractor-trailers at the feedlot and the time hides were removed in the processing plant. *E. coli* O157 prevalence on hides is associated with prevalence on pre-evisceration carcasses (*Elder et al.,* 2000).

Effective vaccination would presumably have its greatest impact on super-shedders, which, although low in proportion, are the most important transmission vector. Blocking colonization on super--shedders not only reduces shedding and hide contamination in those animals, but simultaneously reduces risk of contaminating co-penned animals. Likewise, a vaccination strategy aimed at reducing super-shedder numbers can simultaneously reduce the risk of carcass contamination associated with presence of super-shedders in truckloads of animals being transported to slaughter.

Together these data suggest a role for on-farm control of *E. coli* O157 to reduce the pathogen load entering the plant, our environment and ultimately to reduce human exposure to the pathogen.

The United States Department of Agriculture's Food Safety Inspection Service modeling paper demonstrated that vaccinating cattle was a cost effective intervention strategy for reducing EHEC associated illness in humans *(Withee et al.,* 2009) using vaccine efficacies ranging from 50% to 100%. Although data generated through this risk assessment and economic analysis showed a different break-even point at each efficacy level, there was a consistent reduction in human illness.

Econiche™ Vaccine Efficacy

Bioniche, in collaboration with Dr. Brett Finlay, University of British Columbia, and the Vaccine and Infectious Disease Organization developed and fully licensed a vaccine (*Econiche™)* for use in healthy cattle. The vaccine has been shown to reduce the shedding of *E. coli* O157 in cattle. The vaccine targets the type III secretory system (TTSS), which is essential for *E. coli* O157 attachment in the recto anal junction of cattle.

The studies of *Potter,* 2004, *Rogan,* 2007, *Rogan* 2009, and *Allen*, 2011 have shown this approach to be efficacious in reducing the amount of shedding of *E. coli* O157. Controlled challenge studies demonstrate that vaccinating cattle with *Econiche™* results in:

1. reduction in number of days the bacterium is shed in the feces
2. 64% reduction in duration of shedding
3. 71% reduction in the proportion of animals shedding
4. 2.28 $\log_{10}$ reduction in number of bacteria shed in the feces.

Although calves usually do not have high titers to TTSS proteins, vaccination with Econiche does result in the production of serum antibodies against EspA, Tir and total Type III secretory proteins after vaccination at day 0, 21 and 42, see Figure 2. Overall the challenge studies clearly demonstrate that that vaccination of cattle is an effective approach to reduce pre-harvest *E. coli* O157 burden in cattle. The value of this approach is to reduce the quantity of E. coli in the environment and reduce hide and carcass contamination, allowing the benefits of HACCP to be fully realized.

The efficacy of *Econiche™* has been evaluated under field conditions where cattle are naturally exposed to *E. coli* O157 during summer months. A 2004 feedlot study demonstrates that animals vaccinated under field conditions are 98% less likely to be colonized by *E. coli* O157 in the terminal rectum mucosa, resulting in a vaccine efficacy of 92.3% (*Peterson*, 2007).

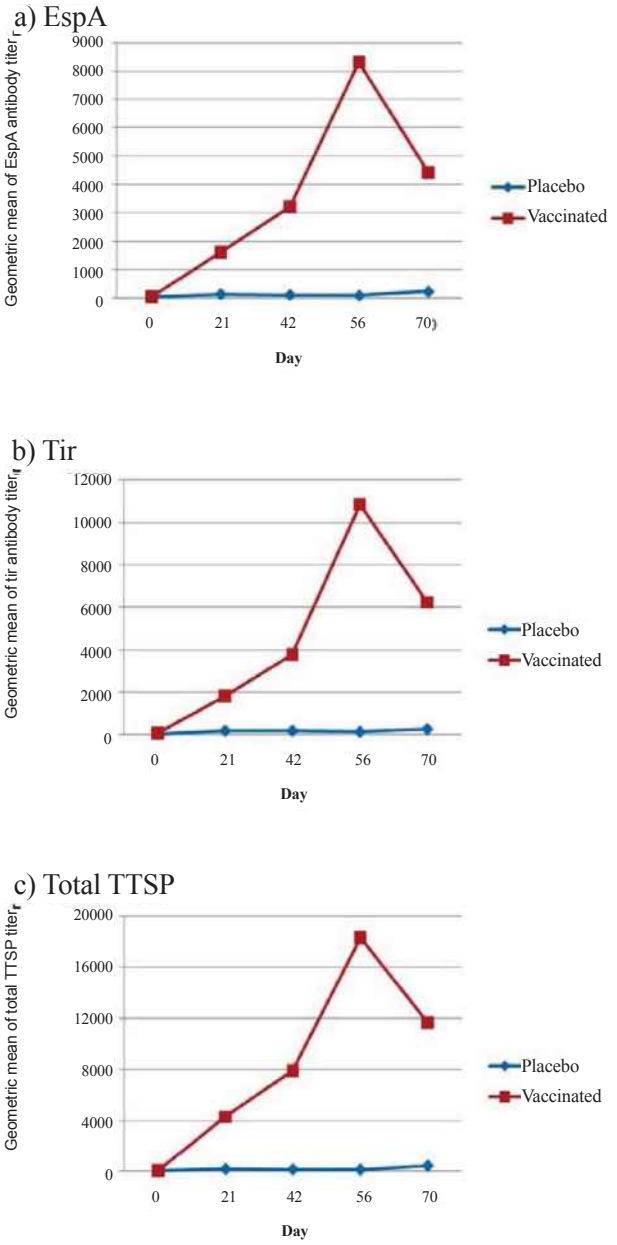

Figure 2. Serum titres of antibodies against EspA, Tir, and total type III secreted protein (TTSP) for 30 placebo-treated calves and 30 calves vaccinated with 3 doses of a TSP vaccine, calculated from the geometric mean with the use of $\log_{10}$(titre + 1) values.

Slika 2. U serumu antitela protiv titre Espa, TIR, a ukupan tip III luči protein (TTSP) za 30 lečenih placebom teladi i 30 teladi vakcinisanih sa 3 doze vakcine TSP, računajući od geometrijska sredina uz korišćenje LOG10 (Titre + 1) vrednosti

A large-scale study across nineteen commercial feedlots was also conducted in 2004 to evaluate efficacy of 2 vaccinations, one upon entry to the feedlot and a second at re-implantation of hormones. This study demonstrates that, compared to unvaccinated

cattle on a per pen basis, vaccinated cattle have a 92% lower probability of being colonized by *E. coli* O157 and are less likely to test positive, indicating that the vaccine reduces environmental exposure of cattle to *E. coli* O157 (*Smith,* 2008).

While it is clear that reducing *E. coli* O157 in the feedlot and environment is best achieved by reducing shedding of the organism in the feces, there is a strong correlation between hide removal and subsequent prevalence on the carcass. In order to evaluate the effect of vaccination on hide contamination, a feedlot study was conducted in 2005. The hides of cattle vaccinated with Econiche™ were found to be 58% less contaminated than control animals (*Smith*, 2009).

A large-scale clinical vaccine trial (*Smith,* 2009) of commercially fed cattle in 2005 tested the efficacy of vaccinating all cattle in a feed yard using a two-dose regimen of Econiche™. Five hundred and four cattle were randomly assigned to 63 pens within three treatment regions in the feed yard. The researchers concluded that the two-dose regimen effectively reduces *E. coli* O157 fecal shedding and hide contamination. This same study also demonstrates that vaccinating at least some cattle within each feed yard region provides greater protection against hide contamination than commingling vaccinates and non-vaccinates.

Conclusion

While vaccination has beneficial short-term effects at the packing plant, in reducing carcass contamination, it has a longer term effect at the feedlot or farm level. By dramatically reducing colonization at the terminal rectal mucosa, vaccination also reduces the probability for environmental transmission of *E. coli* O157 within commercial farm operations (*Smith et al.*, 2008), thus gradually limiting the re-infection cycle within the herd.

The reduction of on-farm *E. coli* O157 through vaccination could also reduce the risk of contaminating produce or wells via run-off of water contaminated by manure or slurry, thereby limiting the potential of transmitting the pathogen to people. On-farm vaccination against *E. coli* O157 is an important component of the comprehensive 'One Health' initiative designed to keep our environment healthy for all to prosper in.

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Vakcinacija goveda pre klanja kao preventivna mera – novi pristup smanjenju rizika od kontaminacije mesa patogenima koji se prenose hranom, i smanjenje pojave bolesti kod ljudi i ekonomske štete

Rogan Dragan, Goebel Susan, Zak Louisa, Medellin-Pena Maira

R e z i m e: Enterohemoragična Escherichia coli (EHEC) je važan zoonotska bakterija kod ljudi. To je fekalna kolformna bakterija koja je obično prisutna u crevima preživara, odakle dospeva u spoljašnju sredinu. Goveda su glavni rezervoar E. coli O157. Dok kod goveda ne izaziva nikakva oboljenja, izlaganje ljudi veoma malom broju bakterija (10 ili manje) izaziva pojavu hemoragičnog kolitisa (HC) i hemolitičkog uremičnog sindroma (HUS) širom sveta. Najčešći način prenošenja E. coli O157 na ljudsku populaciju je konzumiranjem hrane, što čini 61% oboljevanja kod ljudi. Centar za kontrolu i prevenciju bolesti SAD (CDC; 2008) izveštava o tome da je konzum od goveđeg mesa kao i drugih prehrambenih proizvoda (uključujući voće i povrće) predstavljalo značajan način prenošenja bolesti, i to 45% i 34% od svih bolesti koje se prenose hranom, respektivno. Pored prenošenja bolesti hranom, konzumiranje kontaminirane vode je činilo 15% svih slučajeva oboljevanja ljudi od E. coli O 157. U Kanadi, približno 26.000 ljudi oboljeva svake godine zbog dolaska u dodir sa E. coli O157.

Tradicionalno, aktivnosti su usmerene ka poboljšanju bezbednosti mesa goveda kroz implementaciju antibakterijskog tretmana pre klanja. Međutim, za efikasnu kontrolu bolesti u ljudskoj populaciji koja je podložna oboljevanju od E. coli O157 i to ne samo od kontaminiranog goveđeg mesa, već i kontaminiranih prehrambenih proizvoda, postaje očigledno da sveobuhvatna inicijativa "Jedno zdravlje" je verovatno najpogodniji pristup kontroli i uklanjanju patogena koji dospevaju u našu sredinu. Ovaj pristup identifikuje i ocenjuje veze između domena životinja, ljudi i životne sredine. Vrednost ovog pristupa demonstriraju podaci CDC koji ocenjuju da tri četvrtine svih nobih bolesti koje se pojavljuju su zoonoze. Trenutno, E. coli O157, kao i nekoliko entero bakterija koje se mogu naći u domaćim životinjama, uključujući Salmonella, i Campylobacter

jejuni, koje se mogu naći u ovcama, govedima, svinjama i živini, kao i Yershinia enterocolitica koja se nalazi kod svinja, doprinose oboljevanju 79 miliona ljudi u SAD nakon konzumiranja hrane koja je kontaminirana patogenima.

Strategija vakcinacije na farmi je jedna od najboljih metoda za smanjenje preovladavanja E. coli O157 u njenom primarnom domaćinu, kao i u životnoj sredini. Fokus ovog rada je bio opis E. coli O157, centralne uloge goveda u širenju E. coli O157 u prirodi, i razlog za razvoj i implementaciju strategije efikasne vakcinacije pre klanja, korišćenjem potpuno licencirane vakcine.

Ključne reči: *vakcinacija goveda, kontaminacija mesa, patogeni prenosivi hranom.*

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The significance of rational use of drugs in veterinary medicine for food safety*

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A b s t r a c t: Rational use of drugs in veterinary medicine has manifold significance. By using each drug, only when it is really necessary (indicated), in right dose and route of administration, the potential damage of their use is reduced, efficiency increased, and the risk of microorganisms resistance development (in case of antimicrobials) significantly decreased. All of this becomes more important when these drugs are used in food producing animals.

Simultaneously with the intensifying of cattle breeding and exceptional increase of animal productivity, the number of used drugs is unavoidably increased. There are almost no animals today in intensive as well as in extensive production/rearing system, which haven't received at least one drug during their life.

In poultry, cattle and pig production, which are main sectors for production of food stuffs of animal origin, the use of drugs has increased and reached the limits which are considered alarming for health of people.

On the first place are antibiotics, or antimicrobial drugs, which are used very often in therapy or prevention of bacteria diseases, and also, very often, as growth stimulator. In addition to antibiotics, many other drugs are used, with proven numerous harmful effects, even with carcinogenic and teratogenic features, whose residues in food intended for human consumption can very seriously endanger the health of people, as potential consumers of this kind of food. Therefore, the control of use of antimicrobial, and other drugs in livestock production is exceptionally significant.

***Key words**: non-rational use of drugs, antimicrobials, side effects, food producing animals.*

Introduction

Non-rational use of drugs is a problem, which already existed for a long time in the clinical practice in human and veterinary medicine. Although this issue was on many occasions subject of discussion on numerous Symposiums and Congresses, it is still very current, maybe even more than before. First of all, such use of drugs can easier cause certain harmful effects and damages in treated animals. Bearing this in mind, and also the fact that there aren't any significant improvements in the clinical practice, we must devote much more time and attention to follow and control use of drugs on animals (*Ćupić and Živanov*, 1990).

Non-rational use of drugs in veterinary medicine, as well as the need for control of their use becomes even bigger problem when used on food producing animals. In that case, there is the possibility that minimal quantities of drugs and their metabolites (residues) which remain in edible tissues, i.e. in animal products (meat, milk, eggs, honey) induce certain harmful effects in people as potential consumers of such food (*Ćupić,* 1997).

We are witnesses that productivity in food producing animals has manifold increased during the last 50 years, first of all due to improvement of selection, veterinary-medical care, as well as improvement of diet and good organization of production. With the help of these measures, which are permanently corrected and amended, even in the countries where they were brought to the perfection), animals became "real small factories" for production of food. Simultaneously with attaining of higher productivity of animals, the need for the decrease of their number occurred. Actually, only animals whose keeping is economic profitable in production are retained.

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Evidence of increase of animal productivity over time can be illustrated in the following example: in 1928 year, in poultry rearing (broilers), in order to reach the commercial weight of 1,7 kg per unit, 112 days were necessary and birds needed to consume about 22 kg of food, in average; in 1990, broiler reach the weight of 2 kg in 42 days by consuming only 4 kg of food per one unit/animal. In the 1930-ties, hens laid 93 eggs per year, in the 1950-ties 174, and in 1993 252 eggs yearly.

Similar example exists in production of cow milk. In USA, for example, with the decrease of dairy cows, there was the increase of milk production, due to the increase of milk yield per cow. In comparison to year 1983, when milk yield in USA amounted to 5.598 kg/cow in average, in 1995, milk yield increased to 7.478 kg/cow, etc.

One of measures, which besides others, no doubt, contributed to higher the productivity of animals was veterinary-medical care in animal production. First of all, it is reflected in the efficiency of prevention of different animal diseases, and understanding of the use of different vaccines and others prophylactic remedies. Also, not less significant was the role of antibiotics, i.e. antimicrobial drugs and anti-parasitic drugs, which are used mainly in therapy, but also for prevention of diseases, as well as drugs which are used as growth stimulators, among which the most frequent are hormones and some antimicrobials (*Adams, 2001; Brander et al.,* 1991).

The use of antimicrobial drugs in animals attracts special attention. In therapy of diseases of bacterial etiology they are certainly indicated and in those kinds of cases their use is justified, it is better to say rational, regardless of all hazards due to residues of these drugs in food stuffs consumed by humans (*Giguere et al.,* 2006).

Namely, it is considered that damage in untreated animals could be much higher, than short-term inability to use food stuffs (obtained from such animals) in determined period subsequent to therapy, depending on the withdrawal period. Prophylactic use of antimicrobial drugs or use of these drugs for the purpose of growth stimulation in animals understands giving of smaller doses than therapeutic of antimicrobial drugs. Thereby, with this kind of drug use, the risk from incidence of diseases bacteria etiology is decreased and utilization of food improved and growth stimulated. However, subsequent to such use of antimicrobial drugs simultaneously the risk of incidence of undesirable effects increases considerably. First of all, rate of development of resistance of bacteria to used antimicrobial drugs is increased (*Ašanin et al.,* 2005) and certainly risk from residues in animal food stuffs intended for human consumption is higher than when antimicrobials are used only in therapeutic purposes (*Sanders,* 2007*; Spirić et al.,* 2007). Because of this reason, in our country, maximum care is taken so that antimicrobials are not used in prophylactic purposes, but only in therapeutic, as well as that they are not used for stimulation of growth in animals (*Ćupić et al.,* 1997).

Rational use of antimicrobial drugs

Nowadays, in the clinical practice of human and veterinary medicine throughout the world a large number of antimicrobial drugs are used. Likewise many scientists intensively work on discovery and synthesis of new drugs with broader antimicrobial spectrum, stronger action and more satisfactory safety profile. Unfortunately we are witnesses of rather non-rational use of these drugs. Despite constant indicating of all failures and harmful effects of such use, it is present in every-day clinical practice.

Most mistakes during antimicrobial therapy may occur when pathogenic microorganism is unknown and therapy starts empirically. Most often combinations of two or more antibiotic drugs are used. To avoid these mistakes, clinically confirmed, effective antibiotic combinations should be used. These combinations are useful in treating serious infections, mixed bacterial infections, when resistance occurs enzymatic destruction of a drug, and in order to reduce toxicity (*Giguere et al.,* 2006*)*.

Unwanted effects of antimicrobial drugs

Unwanted effects of antimicrobial drugs can be quite mild and pass in a form of quite slight disturbances, then also unexpected reactions may appear, (such as idiosyncratic or allergic reactions, even anaphylactic shock), as well as different damages to the function of organs. Sometimes, non-rational use of drugs, can provoke the most serious disturbances, as are mutagenesis, carcinogenesis and teratogenesis. Because of all of this, today, in clinical practice we must be very careful and consider frequency of application and dose of these drugs, and also possible side effects, which some of these drugs can provoke, and especially in case of those drugs which are banned (because of proved toxicity), (*Ćupić and Živanov,* 1990*; Giguere et al.,* 2006*; Ćupić and Dobrić,* 2003).

Forbidden antimicrobial and other drugs for use in food producing animals

Because of their toxicity, for animals alone (to whom they are applied), and also for people, potential consumers of products, which derived from

such animals, Food and Drug Administration (FDA) banned the use of some antimicrobials, as well as other drugs in food animals. Those are: *chloramphenicol, nitroimidazoles, nitrofurans*, *quinoxalines, fluoroquinolones, sulfonamides, glycopeptides, ionophors, cephalosporins, diethylstilbestrol, dypirone, phenylbutazone, clenbuterol and some antiviral drugs in poultry* (*Payne et al.,* 1999*; Davis et al.,* 2009).

Some of the mentioned drugs (majority) FDA prohibited completely, and some of them are prohibited as extra label drugs, which means that when a drug is used in a manner that is not in accordance with the FDA approved label, or Instruction. This includes use in a species or for a disease or condition not listed on the label; use at dosages, frequencies or routes of administration that differ from those stated on the label; or deviation from the labeled withdrawal time. According to AMDUCA (Animal Medicinal Drug Clarification Act) from the year 2010, all of the aforementioned drugs now are completely prohibited for use in food animals (*AMDUCA,* 2010).

Chloramphenicol - This drug is forbidden since the year 1984, because it can cause (although rarely) idiosyncratic (non dose dependent, irreversible) aplastic anemia in people. Likewise, the use of all preparations including ophthalmic ointments, or spray for wounds in food producing animals is forbidden. However, the newer members of amphenicols like florfenicol or tiamfenicol are allowed. Florfenicol is available for use in cattle, swine, and some aquatic species (*Davis et al,* 2009; *Payne et al.,* 1999).

Nitroimidazoles - All members of this group (including dimetridazole, metronidazole, ronidazole, tnidazole and ipronidazole) are forbidden because of their *in vitro* and *in vivo* carcinogenic properties. Although they are used in humans and companion animals, the use of any drug from this group in food producing animals is illegal (*Payne et al.,* 1999*)*.

Nitrofurans - These drugs are also forbidden because of carcinogenicity and mutagenicity (*Payne et al.,* 1999; *Batas et al.,* 2007). The use of these drugs for therapy of systemic infections in human medicine was withdrawn in 1974 and for treatment of systemic infection in veterinary medicine was banned in 1991. Later studies have documented that topical application of these drugs for therapy of eye or surface wounds in cattle, sheep and goats, also results in milk and meat residues. Because of that, FDA prohibited the use of topical nitrofurans intended for human and veterinary medicine in food producing animals (*Smith et al.,* 1998). Since year 2002, all systemic and topical use of nitrofuran products has been prohibited (*US FDA Web site,* 2009).

Quinoxalines - It has been known for a long time that quinoxalines (carbadox, olaquindox and cydox) are carcinogenic compounds. Because of that, many countries have forbidden or withdrawn these drugs from use (*Payne et al.,* 1999*)*.

Fluoroquinolones - These drugs became interesting, because it was shown that they stimulate development of bacterial resistance. The best instance are resistant salmonellosis infections in humans. Therefore, the use of fluoroquinolones intended for humans is banned since 1997, as well as their use in food producing animals. So, the use of enrofloxacin is banned in all food producing animals, except in calves and heifers. Precisely this drug may not be used in cows in period of lactation or dry period, heifers, dairy calves, ewes, goats and deer. Also, because of that, enrofloxacin may not be stored in dairy farm drug cabinets (*Payne et al.,* 1999*)*.

Sulfonamides - Sulfonamides have been banned in adult dairy cows. Adult dairy cows are defined as any dairy cow over 20 months of age, regardless of milking status. This ban was instituted because of the concern over carcinogenic effects detected in laboratory animals, which coincided with reports of sulfonamide residues detected in up to 73% of commercial milk samples. Only 1 of the 3 sulfonamides that have label indications for lactating cows, sulfadimethoxine (SDM) is currently being marketed. Currently, use of any sulfonamide other than SDM in dairy cattle older than 20 months is illegal. Additionally, extralabel use of SDM in lactating dairy cattle is prohibited (for example use of a higher dose or slow-release SDM boluses in dairy cattle is not permitted), (*Payne et al.,* 1999*; Davis et al,* 2009*)*.

Glycopeptides - The only glycopeptide antibiotic available in the United States is the human product vancomycin. Vancomycin is often used for the treatment methicillin-resistant *Staphylococcus aureus* infections in humans. Avoparcin, a compound chemically similar to vancomycin, has been used in European animal feeds as a growth promoter since the mid 1970s. FDA in 1977 issued an order prohibiting the extra label use of all glycopeptides in food producing animals. The restriction of fluoroquinolone and glycopeptide use represents a novel exercise of FDA discretionary authority: restriction based not on the drugs direct toxicity, but on its potential for increasing human pathogen resistance (*Jung et al.,* 2007*; Klare et al.,* 2003*; Song et al.,* 2005).

Ionophors- The use of ionophore antibiotics such as monensin and lasalocid is banned in lactating dairy. This is valid also for ewes and goats in period of lactation (*Payne et al.,* 1999).

Cephalosporins - In July 2008, FDA proposed prohibition on extra label use of cephalosporins in food producing animals, because of the increased incidence of cephalosporin-resistant food-borne pathogens, particularly *Salmonella spp*. Monitoring system revealed an increase in resistance of *Salmonella* isolates from both humans and food-producing animals to ceftiofur, a member of third-generation cephalosporin drug marketed for use in cattle, sheep, dairy goats, and swine as multiple injectable formulations as well as intramammary preparations for lactating and non-lactating cows (*US FDA Web site,* 2009).

Diethylstilbestrol - This drug is forbidden in food producing animals since 1979, because of its carcinogenic potential. Namely, it was recorded that in pregnant women it provokes development of reproductive tract abnormalities and tumors in female offspring of diethylstilbestrol treated patients (*Newbold,* 2008).

Dipyrone- It is known that dipyrone is drug which belongs to the large group of Nesteroidal-anti-inflammatory drugs. However, it was recorded that this drug, in addition to the anti-inflammatory, antipyretic and analgesic action, also induces very toxic effects in humans (non-dose dependent teratogenic effects, prolonged bleeding times and agranulocytosis). Because of that FDA abolished its use and withdrew this drug from the market in 1977, while its use in the veterinary medicine was first abolished in small non-food-producing animals in 1995, with suggestion that this must be instituted in all food-producing animals also. Today, use of dypirone in any food producing animal is considered illegal (*Center for food Safety and Applied Nutrition Web Site,* 2009).

Phenylbutazone - Phenylbutazone (as sulfonamides) in dairy cattle over 20 months of age was prohibited in 2003. This was based on detection of phenylbutazone residues in culled dairy cattle and the discovery of phenylbutazone products on dairy farms. It is considered that phenylbutazone in humans can induce blood dyscrasis (such as aplastic anemia, leucopenia, agranulocytosis and thrombocytopenia) and cause death. It is also considered a carcinogen. Because of that, currently phenylbutazone use is strictly prohibited only in dairy cattle over 20 months of age (*New animal drugs*, 2003).

Clenbuterol - This drug is known bronchodilatator which acts on the β-adrenergic receptors in bronchial tree. As that kind of drug, its use is allowed in horses, but in cattle and sheep it can provoke relaxation of uteri miometrium. Also, it has secondary anabolic effects. Because of this anabolic effect it was used illegally for stimulation of growth in food animals intended for humans, and for increase of lean body mass and weight gain in humans. However, in order to attain these effects, high doses of this drug are needed. High doses usually provoke adverse effects in humans. There are reports from Spain, France, Italy, Portugal and other countries in humans who consumed liver of treated cattle and lambs, and many people were hospitalized, and some of them died (*Payne et al.,* 1999; *Salleras et al,* 1995*; Barbosa et al.,* 2005*; Brambilla et al.,* 2000).

Antiviral drugs in poultry - Two classes of antiviral drugs currently marketed for use in humans have been added to the list of prohibited drugs in poultry (*US FDA Web Site*, 2009). These are the adamantine inhibitors, rimantadine and amantadine, as well as the neuraminidase inhibitors, oseltamivir and zanamivir. These antiviral drugs have been used in countries outside the United States to treat or prevent the development and spread of avian influenza in poultry. The prohibition extends specifically to chickens, turkeys, and ducks. The prohibition order is based on the potential for the development of resistance to these compounds (*Parry,* 2005*; He et al.,* 2008*; Cyranoski,* 2005).

Forbidden antimicrobial drugs for use in food animals (present situation in Serbia)

According to the proposal of the Medicines and Medical Devices Agency, (published in *Official Journal,* No. 96/09), the following antimicrobial drugs are prohibited in Republic of Seriba: quinaxalines, nitrofurans, nitroimidazoles, glycopeptides, sulfonamides in adult dairy cows, fluoroquinolones) (*Official Journal of RS*, 2009).

Conclusions

When drugs are used in animals which are intended for humans, then there is possibility for producing adverse effects in humans as potential consuments of food, orginating from treated animals. To prevent this possibility it is necessary to rationally use drugs, i.e. to use them only when they are really indicated, in the right way, right time and in the right dose. Also, it should respect withdrawal period, permanently follow, register and announce all about adverse effects after use of any drug, regularly control sensibility of bacteria to antimicrobial agents, and regularly control residues of antimicrobials common used in clinical practice.

Finally, having in mind that our country is in transition, and aspiring to become EU member, as soon as possible justifies further discussions and studies of this topic.

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Značaj racionalne primene lekova u veterinarskoj medicini za bezbednost hrane

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R e z i m e: Racionalna primena lekova u veterinarskoj medicini ima višestruk značaj. Korišćenjem lekova samo kada su stvarno neophodni (indikovani), u pravoj dozi i na pravi način, smanjuje se potencijalna šteta od njihovog korišćenja, a ujedno se povećava delotvornost, i smanjuje rizik od nastanka rezistentnosti mikroorganizama (odnosi se na antimikrobne lekovei). Sve navedeno postaje još važnije kada se veterinarski lekovi koriste u lečenju farmskih životinja, odnosno životinja čiji se proizvodi koriste za ishranu ljudi.

Istovremeno sa intenziviranjem stočarske proizvodnje i povećanjem produktivnosti životinja, povećava se i broj lekova koji se koriste. Danas ne postoji skoro nijedna životinja, u intenzivnom, kao i ekstenzivnom uzgoju, koja nije primila barem jedan lek tokom svog života.

U živinarstvu, govedarstvu i svinjarstvu, kao glavnim sektorima gde se proizvode prehrambeni proizvodi životinjskog porekla, upotreba lekova je dostigla granice koje se mogu smatrati alarmantnim za zdravlje ljudi.

Na prvom mestu su antibiotici, ili antimikrobni lekovi, koji se veoma često koriste u terapiji ili prevenciji bakterijskih bolesti, a ne tako retko i kao promotori rasta. Osim antibiotika, koriste se i mnogi drugi lekovi, koji imaju dokazano štetno dejstvo, pa čak i kancerogene ili teratogene osobine/svojstva. Njihove rezidue u proizodima životinjskog porekla koji se koriste u ljudskoj ishran, mogu veoma ozbiljno da ugroze zdravlje ljudi, koji su potencijalni konzumenti ove vrste hrane. Zbog toga je kontrola primene antimikrobnih lekova, kao i ostalih lekova u stočarstvu, od izuzetne važnosti.

***Ključne reči**: neracionalna primena lekova, antimikrobni lekovi, neželjeni efekti, životinje čiji se proizvodi koriste za ishranu ljudi.*

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Chemical safety in meat industry*

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A b s t r a c t: Since the Second World War the consumer behaviour in developed countries changed drastically. Primarily, there was the demand for sufficient food after a period of starvation, afterwards the desire for higher quality was arising, whereas today most people ask for safe and healthy food of high quality. Therefore a united approach comprising consistent standards, sound science and robust controls is required to ensure consumers' health and maintain consumers' confidence and satisfaction. Chemical analysis along the whole food chain downstream (tracking) from primary production to the consumer and upstream (tracing) from the consumer to primary production is an important prerequisite to ensure food safety and quality. In this frame the focus of the following paper is the "chemical safety of meat and meat products" taking into account inorganic as well as organic residues and contaminants, the use of nitrite in meat products, the incidence of veterinary drugs, as well as a Failure Mode and Effect Analysis (FMEA) system assessing (prioritizing) vulnerable food chain steps to decrease or eliminate vulnerability.

Key words: *Feed; Meat; Meat products; Inorganic residues; Organic residues; Nitrite; Veterinary drugs; FMEA.*

1. Introduction

Taking consumer behaviour into consideration since the time after the Second World War in developed countries, there was primarily the demand for sufficient food, afterwards the desire for better quality in the food area and nowadays almost everybody asks for safe and healthy food of high quality. With increasing global distribution of feed, food and ingredients the different countries in our world have never been before more dependent on each other with respect to their food supply (*Wall*, 2009). A united approach with consistent standards based on sound science and robust controls is necessary to ensure consumers' health and maintain consumers' confidence.

Caused by increasing skills of analytical chemistry and forensic microbiology more and more incidents of contamination will be revealed in the food area. Some of these can be major health threats, others may be technical breaches of the legislation that are unlikely to lead to adverse health effects. Aforementioned occurrences and the spectrum of incidences between these two extremes require various approaches of risk management. Appropriate process controls, biosecurity, adequate traceability and good hygiene and manufacturing practices are the indispensable requirements for every food business.

An important role within these prerequisites to ensure food safety and quality is to be assigned to chemical analysis along the whole food chain downstream (tracking) from primary production to the consumer and upstream (tracing) from the consumer to primary production (*Schwägele*, 2005).

The following contribution is dealing with "chemical safety of meat and meat products" taking into account inorganic as well as organic residues and contaminants, the use of nitrite in meat products, veterinary drugs and an extra chapter is addressed to a Failure Mode and Effect Analysis (FMEA) system assessing (prioritizing) vulnerable food chain steps to decrease or eliminate vulnerability.

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2. Inorganic residues and contaminants

2.1. Toxic heavy metals in domestic animals

2.1.1. Arsenic and mercury

These toxic elements are found mostly in seafood. In meat and offal they are present only in marginal concentrations, often below the limit of detection. Since the contribution of these foodstuffs to the total intake of arsenic and mercury is low they will not be dealt with in the following considerations.

2.1.2. Lead

Over the past decades, the lead (Pb) level in food has decreased significantly owing to source related efforts to reduce the emission of Pb and improvements in quality assurance of chemical analysis. Pb is present at low concentrations in most foods. Offal and molluscs may contain higher levels. Contaminations of food during processing or food production in contaminated areas are the main reasons for enhanced Pb intake via foodstuffs.

Absorption of ingested Pb may constitute a serious risk to public health. Some chronic effects of Pb poisoning are colic, constipation and anaemia. It may also induce increased blood pressure and cardiovascular disease in adults. Fetal neuro--developmental effects and reduced learning capability in children are among the most serious effects.

The Codex Alimentarius system and the EC regulations (*EC*, 2008) set the same maximum residue levels (MLs) for Pb in meat of bovine animals, sheep, pig, and poultry (0.1 mg/kg) and for edible offals of these animals (0.5 mg/kg).

2.1.3. Cadmium

Cadmium (Cd) is a heavy metal found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources. Foodstuffs are the main source of Cd exposure for the non-smoking general population. Cd absorption after dietary exposure in humans is relatively low (3–5%), but Cd is efficiently retained in the kidney and liver in the human body, with a very long biological half-life ranging from 10 to 30 years. Cd is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction. Cd can also cause bone demineralisation, either through direct bone damage or indirectly as a result of renal dysfunction. After prolonged and/or high exposure the tubular damage may progress to decrease glomerular filtration rate, and eventually to renal failure. The International Agency for Research on Cancer has classified Cd as a human carcinogen (Group 1) on the basis of occupational studies. Newer data on human exposure to Cd in the general population have been statistically associated with increased risk of cancer such as in the lung, endometrium, bladder, and breast (*EFSA,* 2009).

Cd bioavailability, retention, and consequently toxicity are affected by several factors such as nutritional status (low body iron stores) and multiple pregnancies, preexisting health conditions or diseases (*EFSA*, 2009).

The EC regulations (*EC*, 2008) set maximum levels for Cd in meat of bovine animals, sheep, pig, and poultry as 0.05 mg/kg wet weight and for edible offal of these animals as 0.5 mg/kg for liver, and 1.0 mg/kg for kidney, respectively. In 2004, the Codex Committee on Food Additives and Contaminants decided to discontinue work on establishing maximum residue levels for Cd in livestock and poultry because the foods from these production classes were not significant contributors to Cd intake.

The Scientific Panel on Contaminants in the Food Chain (CONTAM) was asked by the European Commission to assess the risks to human health related to the presence of Cd in foodstuffs (*EFSA*, 2009). To provide an updated assessment of exposure from foodstuffs, about 140,000 data covering the period from 2003 to 2007 on Cd occurrence in various food commodities were received from 20 member states and considered by the CONTAM Panel. High Cd concentrations were detected in the following food commodities: seaweed, fish and seafood, chocolate, and foods for special dietary uses.

In the food category "meat and meat products, and offal" the fractions of samples exceeding the maximum levels (MLs) are: bovine, sheep, and goat meat 3.6%; poultry and rabbit meat-none; pork – 1.6%, liver (bovine, sheep, pig, poultry, and horse) – 3.7%, kidney (bovine, sheep, pig, poultry, and horse) – 1.0%. The corresponding median values are: 0.0050, 0.0030, 0.0050, 0.0430, 0.1520 mg/kg.

In a German food monitoring, a total of 4955 samples of domestic and foreign origins were analysed in 2007 (*Bundesamt für Verbraucherschutz und Lebensmittelsicherheit*, 2008). Beef, wild boars meat, and air cured ham were selected from the market basket among the food of animal origin. Contaminations with Cd were all below the MLs (90th percentile in mg Cd/kg: beef 0.004, wild boar 0.01, and ham 0.03). There was only one case of noncompliance (0.16 mg/kg) with the ML for Pb in beef (90th percentile in mg Pb/kg: beef 0.057, wild boar 2.56, and ham 0.029). Regarding wild boars, Pb concentrations along the bullet channel were very

high (288 mg/kg). Some projectile particles were penetrating deeply into the neighbouring tissue, so that they could not always be gotten by an ample excision. The contamination levels of heavy metals generally had decreased since a similar monitoring in 2002 (*Bundesamt für Verbraucherschutz und Lebensmittelsicherheit*, 2004).

In 2003–2004, the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) conducted an exploratory assessment to determine the occurrence and levels of Cd and Pb in randomly collected samples of kidney, liver, and muscle tissues of mature chickens, boars/stags, dairy cows, and heifers (*Pagan-Rodriguez et al.,* 2007). The study found that in each production class tested, levels of Cd and Pb were higher in kidney and liver samples than in the muscle samples. None of the muscle samples contained Cd or Pb exceeding the MLs established by other countries or international organizations. There are sporadic cases in which liver samples from mature chickens and boars/stags contained elevated Cd or Pb levels; however, the 95th percentile and the mean residue levels for liver samples were below the MLs established by other countries or international organizations. In boars/stags, mature chickens, and dairy cows, the Cd levels for the 95th percentile (not for the mean) of kidney samples were above internationally accepted levels, exceeding the ML (1000 ppb) established by the EU. The results of the current and previous FSIS studies showed that the incidence (percent of positive samples) and levels of Cd in kidney, liver, and muscle did not increase between 1985 and 2004.

Waegeneers et al. (2009) investigated the effect of animal age on concentrations of Cd, Pb, As, Cu and Zn in bovine tissues (meat, kidney, and liver) sampled from animals reared in contaminated areas or reference regions in Belgium. Cd concentrations in meat samples had an increasing trend with age. In addition, a significant positive linear relation was found between animal age and renal or hepatic Cd levels. Pb concentrations in kidneys and liver also increased with age. Renal Cd concentrations were predicted for bovines at different ages by using the slope of the linear regression equation. Calculations for 2-year-old animals from reference areas showed that in this group the European maximum level of 1 mg/kg for Cd in kidneys would be exceeded in zero to 5% of cases.

The aims of a study of *Lopez-Alonso et al.* (2007) were to evaluate toxic and essential metal concentrations in meat and offal from pigs in northwest Spain to compare these with reported metal concentrations in pigs in other countries and in cattle in this region, and to relate the observed concentrations to maximum acceptable concentrations. The concentrations of toxic metals can be considered low as the maximum admissible concentrations established by the EU were not exceeded in any sample. The 90th percentiles for muscle, liver, and kidney regarding Cd are 0.015, 0.100, 0.446, regarding Pb 0.006, 0.007, and 0.011 (mg/kg).

Due to the growing interest in organic products, *Ghidini et al.* (2005) undertook a comparison between the chemical safety of organic and conventional products. Milk and meat were the products chosen for the study. The parameters evaluated to assess chemical safety were organochlorine pesticides, polychlorinated biphenyls (PCBs), Pb, Cd, and mycotoxin contamination. Pb and Cd residues were very low (all within the EU ML) and did not differ between organic and conventional products.

2.2. Pb contamination from ammunition residues in game meat

Human consumption of wildlife killed with Pb ammunition may result in health risks associated with Pb ingestion. This hypothesis is based on published studies showing elevated blood Pb concentrations in subsistence hunter populations, retention of ammunition residues in the tissues of hunter-killed animals, and systemic, cognitive, and behavioural disorders associated with human Pb body burdens once considered safe.

The use of Pb isotope ratios has definitively identified Pb ammunition as a source of Pb exposure for First Nations people in Canada (*Tsuji et al.*, 2009), but the isotope ratios for Pb pellets and bullets were indistinguishable. Thus, Pb contaminated meat from game harvested with Pb bullets may also be contributing to the Pb body burden. There were elevated tissue Pb concentrations (up to 5726 mg/kg) in liver and muscle samples of big game harvested with Pb bullets and radiographic evidence of Pb fragments. Accordingly the tissue surrounding the wound channel should be removed and discarded, as this tissue may be contaminated by Pb bullet fragments.

The objective of *Hunt et al.* (2009) was to determine the incidence and bioavailability of Pb bullet fragments in hunter-killed venison, a widely-eaten food among hunters and their families. They radio graphed 30 eviscerated carcasses of white-tailed deer shot by hunters with standard Pb-core, copper-jacketed bullets under normal hunting conditions. All carcasses showed metal fragments and widespread fragment dispersion. They took each carcass to a separate meat processor and fluoroscopically scanned the resulting meat packages, flu-

oroscopy revealed metal fragments in the ground meat packages of 80% of the deer and 32% of the ground meat packages contained at least one fragment. Fragments were identified as Pb in 93% of samples. Isotope ratios of Pb in meat matched the ratios of bullets, and differed from background Pb in bone. They fed fragment-containing venison to pigs to test bioavailability; controls received venison without fragments from the same deer. Mean blood Pb concentrations in pigs peaked at a significantly higher level after 2 days following ingestion of fragment-containing venison than the controls. They concluded that people risk exposure to bioavailable Pb from bullet fragments when they eat venison from deer killed with standard Pb-based rifle bullets and processed under normal procedures.

It has also been shown that the practice of marinading game meat (quails) in vinegar increases the concentration of Pb in the edible tissues, when Pb pellets are present (*Mateo et al.,* 2006). There are trials to substitute Pb in bullets with non-toxic metals, e.g. Cu (*Knott et al*., 2009). A global review of legislation controlling the use of Pb ammunitions can be found in *Avery and Watson* (2009).

3. Organic residues and contaminants

3.1. Status survey of PCDD/Fs and PCBs in German feedstuffs, meat and meat products

The term „dioxins and dioxin-like PCBs" summarizes 29 toxicologically relevant single compounds or congeners of three classes of chlorinated compounds (polychlorinated dibenzo-*p*-dioxins = PCDDs; polychlorinated dibenzofurans = PCDFs; polychlorinated biphenyls = PCBs), which include 419 congeners in total. These undesirable 29 congeners show similarities in toxicological and chemical behaviour and include a toxic potential which can be estimated by the so called TEQ-value (*Van den Berg et al.*, 1998).

Dioxins (PCDD/Fs) and dioxin-like PCBs (dl-PCBs), inclusive six marker PCBs (PCBs 28, 52, 101, 138, 153, and 180) have different sources. The formation of the component class of PCDD/Fs for example takes place in any combustion process and results in very different levels, depending on the physical and chemical conditions, in which the combustion process occurs. Other sources for the formation of dioxins are certain industrial processes (e.g. metallurgical industry, production of chemicals) or natural processes (e.g. volcanic eruptions, forest fires). PCDD/Fs are formed as undesirable by-products from a number of human activities like accidents at chemical factories as 1976 in Seveso, Italy (*Homberger et al.,* 1979). Thereof high emissions resulted mainly with the most toxic congener 2, 3, 7, 8-TCDD as well as the contamination of a large local area. Other dioxin sources include, for example, domestic heaters, agricultural and backyard burning of household wastes.

In contrast to PCDD/Fs, the substance class of PCBs has been produced industrially between the 1930s and 1970s for a wide range of applications. Nowadays in a great number of states, inclusive the European Union PCBs are banned, but they are still in use in closed systems like electrical capacitors and are contained in paintings and sealing materials, which were produced before the 1970s. Today the release of PCBs occurs from leakages, accidental spills and illegal disposals (*Ballschmiter and Bacher*, 1996). When released into the air PCDD/Fs and PCBs can deposit locally on plants and on soil contaminating both food and feed. They can also be widely distributed by a long range transport (*Lorber et al.,* 1998). Because of their stability they are highly persistent in the environment for a long time. Dioxins and PCBs are highly lipophilic and poor soluble in water. Therefore, an intake of these compounds by the roots of feed plants is generally negligible. But a contamination of feed plants is possible with particles of dust or soil at the surface of the feed plants. In this way PCDD/Fs and PCBs can carry over from feed plants to the tissues of farmed animals where both undesirable compounds can accumulate in the fat to a greater or lesser extent.

Therefore, the Max Rubner-Institute (MRI) carried out a representative status survey programme on behalf of the German Federal Ministry of Food, Agriculture and Consumer Protection, which was coordinated by the MRI Analysis Division, located in Kulmbach (*Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz,* 2009).

The analytical work started with sample acquisition in 1994 and lasted up to 2008. In 2009 the analytical data from more than 1100 samples of animal feedstuffs, meat and meat products, hen eggs and some products thereof, dairy products, fish and fishery products were statistically evaluated.

3.1.1. PCDD/Fs and PCBs in feedstuffs

In Germany, dioxin exposure of the population ascribable to foods of animal origin is about 90% and feedstuffs are the main input source of PCDD/Fs and PCBs. Due to the so called "carry-over effects" these substances turn over from feedstuffs into foods of animal origin and accumulate. For prevention and reduction of these undesirable substances in food their reduction in feedstuff is already necessary.

Therefore, the complete status survey project started with a representative assessment of the initial situation in feedstuffs. Within this survey the levels of PCDD/Fs, dl-PCBs and marker PCBs in 206 German feed samples were analysed in the years 2004/2005 (*Schwind et al*., 2009). The sampling plan included compound feed ($N = 115$) and roughage and succulent feed ($N = 91$) reflecting the representative feeding situation in Germany. The median content of WHO-PCB-TEQ in analysed feed samples was 0.017 ng/kg, 88% dry matter (d.m.), and consequently more than ten times 10 below the action level of 0.35 ng/kg, 88% d.m. (EC, 2006b). A differentiation between compound feed, roughage and succulent feed showed that compound feed (median 0.007 ng/kg 88% d.m.) were significantly lower contaminated with dioxin-like PCBs than roughage and succulent feed (median 0.058 ng/kg 88% d.m.). The median sum contents of the six marker PCBs were 0.16 µg/kg (88% d.m.) for compound feed and 0.56 µg/kg (88% d.m.) for roughage and succulent feed. The median of the WHO-PCDD/F-TEQ was 0.03 ng/kg (88% d.m.), the maximum level of 0.75 ng/kg (88% d.m.) was not exceeded. The median of the WHO-PCDD/F-PCB-TEQ was 0.05 ng/kg (88% d.m.) and consequently by factor of 25 below the maximum level of 1.25 ng/kg (EC, 2006a). In addition, samples of roughage and succulent feed were analysed according to their contents of ash insoluble in HCl, representing the degree of the proportion of earthy components in feed. A slight correlation was found between ash insoluble in HCl and WHO-PCDD/F-TEQ ($R^2 = 0.59$), whereas no correlation was found between ash insoluble in HCl and WHO-PCB-TEQ ($R^2 = 0.06$), (*Schwind et al*., 2009).

3.1.2. PCDD/Fs and PCBs in meat and meat products

In a second step more than 300 representative German samples of meat and meat products were analysed for their levels of PCDD/Fs, dl-PCBs and marker PCBs. The sampling plan included different types of meat (pork, poultry meat, beef and sheep) and meat products (Bologna type sausage, raw ham, cooked liver sausage and raw sausage). For sampling the German National Nutrition Survey of the year 2004, the actual consumer behaviour and the population of the different states in Germany were taken into consideration. To get highly representative data the Federal Institute for Risk Assessment in Germany (BfR) was additionally consulted. Therefore, about 300 samples of meat and meat products were collected, which ensured a preferably high level of representativeness. (*Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz*, 2009).

3.1.2.1. dl-PCBs in meat and meat products

A total of 161 meat samples (55 pork, 49 poultry meat and 57 beef) (*Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz,* 2009) were analysed for levels of dl-PCBs (12 WHO-PCB congeners). TEQs were calculated using toxic equivalency factors (TEFs) laid down by the WHO (*Van den Berg et al.,* 1998). For non-detected congeners the upper-bound level has been used. Correspondent maximum residue levels for dl-PCBs and PCDD/Fs in meat and products thereof are given in Commission Regulation (EC) No 1881/2006 of 19 December 2006 (*EC*, 2006c).

The median content of WHO-PCB-TEQ in beef samples was 0.9 ng/kg fat and consequently in the range of the action level of 1.0 ng/kg fat. Subdividing the analysed beef samples in beef ($N = 44$) and veal ($N = 13$), it was shown that the contents of dl-PCBs in veal (median: 0.23 ng WHO--PCB-TEQ/kg fat) were significantly lower than in beef (median: 1.08 ng WHO-PCB-TEQ/kg fat) (*Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz*, 2009). For veal, with the exception of one extreme value, all samples were below the action level of 1 ng/kg fat. For beef about 50% of the analysed samples exceeded the action level (*EC*, 2006b). An explanation for this fact could be the different age of slaughtering for calves and cattle. Calves (in Germany) were slaughtered at the age of about 6 months; cattle at the age of about 20 months. The uptake and deposition of these undesirable compounds in ruminants and their tissues seem to be age-related. In poultry meat a median content of WHO-PCB-TEQ was determined, which was more than by a factor of 10 below the action level of 1.5 ng/kg fat. For pork the determined results for the median content of WHO-PCB-TEQ were in a similar manner more than six fold below the action level in force.

In meat products the WHO-PCB-TEQ ranged from 0.06 ng/kg fat for raw ham to 0.13 ng/kg fat for raw sausages (salami). The WHO-PCB-TEQ in meat and meat products was dominated by PCB 118, PCB 126 and PCB 156, which together contributed in a range between 87% (for pork) and 96% (for beef) to the WHO-PCB-TEQ. The congeners PCB 114, PCB 123, PCB 157, and PCB 189 were only detected in very small amounts compared to the other mono-ortho PCBs.

3.1.2.2. PCDD/Fs in meat and meat products

In total, 169 samples of different types of meat (pork, poultry meat, beef and sheep) were analysed according to their contents of the 17 WHO--PCDD/Fs (*Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz,* 2009). The median contents of WHO-PCDD/F-TEQ ranged from 0.09 ng/kg fat (pork), 0.11 ng/kg fat (poultry), 0.19 ng/kg fat (lamb) up to 0.24 ng/kg fat (beef) and were significantly below their maximum levels. Meat of the ruminants beef and sheep (lamb) showed significant higher median PCDD/F levels than meat of poultry or pork. This might be again attributed to the different ages of slaughtering for pork (about 6 months), poultry (about 3 months), lamb (about 6 months), and beef (about 20 months). Maximum contents (without outliers and extreme values) were in the range of 0.2 ng WHO-PCDD/F-TEQ/kg fat for pork and poultry meat and about 1 ng/kg fat. The EU maximum residue levels (MRLs) for pork (1 ng WHO-PCDD/F-TEQ/kg fat), poultry meat (2 ng WHO-PCDD/F-TEQ/kg fat) and beef (3 ng WHO-PCDD/F-TEQ/kg fat) were not exceeded in all three types of meat (EC, 2006c). The 5 analysed sheep (lamb) samples showed a median of 0.2 ng WHO-PCDD/F-TEQ/kg fat which were in the range of beef. The WHO-PCDD/F-TEQ of veal (median 0.1 ng/kg fat) was significantly lower than that of beef (median 0.35 ng/kg fat). In comparison to an earlier survey conducted 10 years ago, especially for beef and poultry meat significant decreases of the PCDD/F-contents were observed.

In the investigated meat products (Bologna type sausage, raw ham, raw sausage, cooked liver sausage) the median WHO-PCDD/F-TEQ levels varied from 0.05 ng/kg fat (Bologna type sausage) to 0.09 ng/kg fat (cooked liver sausage). The maximum contents (without outliers and extreme values) were in the range of 0.2 ng/kg fat. Because the analysed meat products were mainly produced from pork and consequently a maximum residue WHO-PCDD/F--TEQ level of 1 ng/kg fat has to be used, the median contents were at least by a factor of 10 below the maximum residue level (MRL). No exceeding of the MRL for the WHO-PCDD/F-TEQ was observed.

3.1.3. Marker PCBs in meat and meat products

The EU intends to regulate marker PCBs in food on the basis of sum contents of the six marker PCBs (*DG Sanco*, 2008). Actually for meat and meat products the following MRLs for the sum contents of six marker PCBs are discussed: 50 µg/kg fat for bovine animals and sheep, 30 µg/kg fat for poultry and 15 µg/kg fat for pork.

The sum contents of the 6 marker PCBs on median basis in meat increased from pork (1.41 µg/kg fat), poultry meat (1.73 µg/kg fat) to beef (5.33 µg/kg fat). These levels are from 3 to 10 times below the actual discussed MRLs by the Commission of the European Union (http://www.bmu.de/files/english/pdf/application/pdf/non_dioxin_like_pcbs_090728_bf.pdf). In the analysed samples for pork, five extreme values were above the proposed maximum level of 15 µg/kg fat. For poultry meat only one exceeded the disputed MRL of 30 µg/kg fat and for beef one of them exceeded the discussed MRL of 30 µg/kg fat (*Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz*, 2009). In meat products medians of sum contents of six marker PCBs for Bologna type sausage, raw ham, raw sausage (salami) and cooked liver sausage were in the range of 1 to 3 µg/kg fat. Maximum levels (without outliers and extreme values) for Bologna type sausage and raw ham were in the range of 3 to 4 µg/kg fat, for raw sausage and cooked liver sausage from 7 to 8 µg/kg fat. Because the analysed meat products were produced mainly with pork, a MRL of 15 µg/kg fat for the sum of the six marker PCBs has to be applied. Consequently, the proposed MRLs would be exceeded in the bologna type sausage, raw ham and cooked liver sausage.

3.1.4. Uptake of PCDD/Fs and dl-PCBs from meat and meat products

On the basis of the determined data for PCDD/Fs and PCBs in German meat and meat, products the Analysis Division of the MRI assessed that an adult consumer with 70 kg body weight with the consumption of meat and meat products takes up about 3% of the tolerable weekly intake (TWI), which was set to 14 pg WHO-PCDD/F-PCB-TEQ/kg body weight by the Scientific Committee on Food (*SCF*, 2001).

3.2. Polycyclic aromatic hydrocarbons (PAH) in smoked meat products

Smoking is one of the oldest technologies for conservation of meat and meat products and is defined as the process of penetration in meat products of volatiles resulting from thermal destruction of wood (*Toth*, 1983). It is assumed that in Germany 60% of meat products are smoked (*Frede*, 2006). As a non-desired consequence of smoking, polycyclic aromatic hydrocarbons (PAH) are generated during the incomplete combustion of wood. About 660 different compounds belong to PAH group (*Sanders and Wise*, 1997). Some representatives show carcinogenic properties (*IARC*, 2009). The best known

carcinogenic PAH compound is benzo[a]pyrene (BaP), which has been used as a leading substance until now.

In the European Union, a maximum level of 5 µg/kg benzo[a]pyrene (BaP) in smoked meats and smoked meat products exists (*EC*, 2006c). Furthermore, the European Commission (EC, 2005a) recommended that the member states should investigate not only the contents of BaP in smoked meat products, but also other PAH seen as carcinogenic by SCF. These 15 PAH compounds are: benzo[a]anthracene (BaA), chrysene (CHR), cyclopenta[c,d]pyrene (CPP), 5-methylchrysene (5-MC), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[j]fluoranthene (BjF), BaP, dibenzo[a,h]anthracene (DhA), indeno[c,d]pyrene (IcP), benzo[g,h,i]perylene (BgP), dibenzo[a,l]pyrene (DlP), dibenzo[a,e]pyrene (DeP), dibenzo[a,i]pyrene (DiP) and dibenzo[a,h]pyrene (DhP). In particular, DlP has been in the spotlight of scientific interest recently, because toxicological investigations indicated that DlP probably has a much stronger carcinogenic potential than BaP (*Higginbotham et al*., 1993, (*Luch et al*., 1994) and (*Schober et al.,* 2006). The European Food Safety Authority (EFSA) additionally recommends the analysis of benzo[c]fluorene (BcL), which was assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (*JECFA*, 2005), consequently all in all 15 + 1 PAH are classified as priority in the EU.

In order to analyse these 15 + 1 EU priority PAH in smoked meat products at the MRI Kulmbach an analytical method was developed (*Jira,* 2004a; *Jira et al*., 2008), which included accelerated solvent extraction (ASE), gel permeation chromatography, solid phase extraction (SPE) with silica gel and a quantification by gas chromatography/high resolution mass spectrometry (GC/HRMS). Therefore, a semipolar GC column with 50% polyphenylsiloxane was used, which enables a chromatographic separation and consequently an identification and quantification of all 15 + 1 EU priority PAH with the exception of a separation of CHR and triphenylene (TP). Consequently with the help of this method only the sum of contents of CHR + TP can be determined. A disadvantage of this method is the long runtime of 72 min. Therefore a Fast-GC/HRMS method for the quantification of the 15 + 1 EU priority PAH with a runtime of only 25 min, using a TR-50 ms column (10 m × 0.1 mm × 0.1 µm) was developed (*Ziegenhals et al.,* 2008).

In order to investigate the contents of the 15 + 1 EU priority PAH in representative samples of smoked meat products in Germany, a total of 113 samples of smoked meat products (raw sausages $N = 25$; raw ham $N = 23$; cooked ham $N = 17$; frankfurter-type sausages, $N = 23$ and liver sausages, $N = 25$ were analysed (*Jira,* 2010). These samples originated from different states in Germany, considering populations in the single states. The median BaP contents of the analysed sampled was 0.03 µg/kg and consequently more by a factor of 100 below the maximum level of 5 µg/kg. The P 95 was 0.14 µg/kg and maximum value was 0.43 µg/kg, which was still more than a factor of 10 below the maximum level. The highest PAH contents were observed for BcL and CHR + TP which were the only PAH compounds with median contents above 0.1 µg/kg. Dibenzpyrenes (DeP, DhP, DiP and DlP) were observed only in a few samples. In the most samples contents of dibenzpyrenes were below the limit of detection (LOD) of 0.01 µg/kg. EFSA concluded that BaP is not a suitable indicator for the occurrence of PAH in food and assessed that the sum content of the four PAH compounds BaP, CHR, BaA and BbF („PAH4") is the most suitable indicator of PAH in food (EFSA, 2008). The median contents of „PAH4" were 0.28 µg/kg, P 95 was 1.19 µg/kg and maximum value was 2.46 µg/kg. Because of the above mentioned coelution of CHR and TP also "PAH4" includes contents of TP. The median of the sum content of 15 + 1 EU priority PAH was 0.64 µg/kg, P 95 was 2.58 µg/kg and the maximum value was 5.47 µg/kg. The highest BaP levels were detected in raw ham and frankfurter-type sausages with median concentrations of about 0.05 µg/kg. The highest content of BaP was detected in a frankfurter-type sausage (about 0.4 µg/kg). The lowest BaP content was detected in cooked ham (median: 0.01 µg/kg). The median content of BaP was 0.02 µg/kg for raw sausages and 0.03 µg/kg for liver sausages. The highest „PAH4" levels were observed in frankfurter-type sausages. Within this group of hot smoked meat products median "PAH4" contents of 0.6 µg/kg were observed. The median "PAH4" contents of raw ham and liver sausages were both in the range of 0.3 µg/kg. Raw sausages had a median of 0.2 µg/kg. The lowest „PAH4" levels were observed in cooked ham (median: 0.1 µg/kg). The results of this study analysing representative samples of German smoked meat products clearly demonstrated that the production of smoked meat products with BaP levels below 1 µg/kg is possible without any problems. These findings are in accordance with other studies, which were performed in other European countries like Spain (*Falcon et al*., 1999; *Falco et al*., 2003; *Fontcuberta et al*., 2006), Italy (*Roda et al*., 1999; *Purcaro et al*., 2009), Denmark (*Duedahl-Olesen et al.,* 2006), Ireland (*FSAI, Food Safety Authority of Ireland*, 2006) and Estonia (*Rei-*

nik et al., 2007) in the last 10 years. BaP contents in smoked meat and meat products reported in these studies were less than 0.5 µg/kg.

Considering the genotoxic and carcinogenic properties of several PAH compounds SCF recommended that the PAH contents in smoked meat products should be as low as reasonably achievable (ALARA), (*SCF*, 2002). Actually, the Codex Alimentarius Commission (*CAC,* 2008) works on a draft for a "Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) from Smoking and Direct Drying Processes" with the objective of lowering PAH contents in foods (e.g. smoked meat products). The unreasonably high BaP maximum level of 5 µg/kg is in conflict with efforts to reduce PAH contents in smoked meat products. Therefore lowering the maximum level for BaP from 5 µg/kg to 1 µg/kg seems to be advisable. The observed correlation coefficient between the sum content of the 15 + 1 EU priority PAH and BaP of $R = 0.90$ is an indicator for the suitability of BaP as a marker substance for PAH in smoked meat products. A better correlation coefficient of $R = 0.99$ for the sum content („PAH4") is based on the relatively high contribution of "PAH4" to the sum content of the 15 + 1 EU priority PAH of 42% (median). A substantial disadvantage of using „PAH4" instead of BaP as a marker substance for PAH in food surveillance is the insufficient chromatographic separation of CHR and TP, which is only feasible with a time-consuming GC temperature programme running more than one hour (*Jira et al*., 2008), which is not suitable for routine measurements. In contrast to a sufficient gaschromatographic separation of BaP, also the separation of CHR and CPP respectively BbF, BjF and BkF appears problematic. Furthermore an important disadvantage of using „PAH4" as an indicator of PAH in food is that PAH compounds with very different carcinogenic potential are summed up to a total content without weighting. A very different carcinogenic potential of these four PAH compounds was not only established by the International Agency for Research on Cancer (IARC, 2009), but also by other researchers (*Bostrom et al.*, 2002; *Nisbet and LaGoy*, 1992), who assessed a toxicological potential for BbF, BaA and CHR, which was more than by a factor of 10 lower as observed for BaP. Because within the presented study a median contribution of only 10% of BaP to "PAH4" was determined, this sum content is dominated in PAH compounds with lower toxicological relevance. In order to evaluate the suitability of BaP as a marker in addition to representative samples also suspicious samples of smoked meat products should be analysed for the contents of 15 + 1 EU priority PAH compounds.

In spite of relatively low contents of PAH in smoked meat products in Europe there are still possibilities to lower PAH contents by improving smoking technologies. By analysing cold smoked meat products from Serbia (traditional and industrial smoking) a dependency of PAH contents and smoking time was found. On the other hand, lower PAH contents were observed for industrial smoked meat products in comparison to conventionally smoked products (*Djinovic et al*., 2008a., 2008b., 2008c). Because PAH are adsorbed by the surface of meat and do not penetrate significantly into the inside of smoked meat products (*Jira et al*., 2006) the surface/mass ratio is significantly influencing PAH contents in smoked meat products. Nevertheless, within the mentioned study differences in PAH contents in different types of meat (beef and pork ham) were found for samples with similar surface/mass ratio, indicating different adsorption capacities of the surface for different types of meat products (*Djinovic et al*., 2008a, 2008b, 2008c). In a research project at the MRI Kulmbach starting in the year 2010 the influences of different parameters of smoking, like smoke generation temperature, oxygen content, smoking time, type of casing and wood and fat content on the PAH contents for emulsified sausages and raw sausages will be systematically investigated. The results of this study will be an important tool in order to achieve a further reduction of PAH in smoked meat products.

3.3 Mycotoxins

Application of modern agricultural practices and the presence of a legislatively regulated food processing and marketing system have reduced the mycotoxin exposure in the populations of the developed world very effectively. As *Milićević* (2009) states in a review, the health risks from mycotoxins for populations in developing regions however are higher orders of magnitude.

4. The use of nitrite in meat products

In the European Union the use of nitrite and nitrate in meat products is regulated (*EC*, 2006a, 2006b , 2006c, 2006d). Within this directive the use of nitrates is limited in non-heated meat products to 150 mg (ingoing amount must be calculated as sodium nitrite)/kg, but with several exemptions, and nitrite up to 100 mg, respectively, 150 mg nitrite (ingoing amount)/kg in all meat products, again with a number of exemptions (*Honikel*, 2008). In contrast

to a former regulation in Germany (Nitrit-Pökelsalz--Gesetz; nitrite pickling salt (NPS) law), which only allowed the use of nitrite in meat products in premixes with table salt and was limiting the nitrite content to 0.6%, the percentage of nitrite in NPS is not limited in the EU since 1995.

At MRI Kulmbach in the time between 2000 and 2006 a total of 336 meat products (189 emulsified sausages, 41 cooked sausages, 51 raw sausages, 29 raw hams, 8 cooked hams and 18 cooked cured products) were analysed with respect to their contents of nitrite and nitrate (*Dederer*, 2007) by an enzymatic methodology (*Arneth and Herold*, 1988). Limits of detection (LOD) of this analytical method were 0.2 mg/kg for nitrite and 0.1 mg/kg for nitrate. Median contents in the analysed meat products were 27 mg/kg for nitrate and 11 mg/kg for nitrite. The highest observed levels (without a few outliers and extreme values) were below 100 mg/kg for nitrate and in the range of 50 mg/kg for nitrite. Assuming an estimated addition of 80 to 100 mg nitrite/kg only about 11 to 14% of the added nitrite will be found in the cured meat product.

Nitrite shows both positive and negative effects. Positive effects of the addition of nitrite curing salt in meat products are reddening (*Wirth*, 1991), formation of a curing flavour (Fischer et al., 2005), antioxidative effects (*Arneth*, 2001) and antimicrobial effects (*Lücke*, 2003; *Kabisch et al*., 2008), whereas the latter is not to be discussed within this paper.

An important aspect of the addition of nitrite curing salt to meat products is the formation of stable red colour, which is developed in a number of complicated reaction steps until NO-myoglobin (Fe^{2+}) is formed (*Honikel*, 2008). By heating the NO-myoglobin the protein moiety is denatured, but the red NO-porphyrin ring system still exists and is found in meat products heated to 120° C. An advantage for the consumer is that this heat stable red colour will change on bacterial spoilage, consequently the consumers recognize spoilage by a change of the colour. A second advantage is the formation of a curing flavour. The role of nitrite in the formation of this characteristic flavour is not completely understood until now. There are several evidences that the curing flavour is not caused by a single chemical compound or substance classes, but rather by a combination of many different compounds. It is assumed that compounds which are formed by binding nitrite with proteins or fats, have valuable contribution to the formation of a curing flavour (*Jira*, 2004b). The third and probably the most relevant advantage is the antioxidative effect of nitrite. This effect consists in an oxidation to nitrate, on the other hand a stable complex between heme-bond iron and nitrite is formed, which inhibits the release of iron ions. Consequently, free iron ions (Fe^{2+}) are not available for the initiation of lipid peroxidation (LPO). In addition nitrite is able to form a complex with free iron ions. It is also assumed that nitrite is able to stabilize polyunsaturated fatty acids forming nitro–nitroso derivatives (*Freybler et al.*, 1993). As a consequence cell membranes are protected against lipid peroxidation and the stability of the cured meat product during storage is raised. The antioxidative effect of nitrite is not only limited to an inhibition of LPO. The addition of nitrite to meat products also leads to lower contents of harmful cholesterol oxides (*Arneth and Münch*, 2002). Furthermore nitrite can form different compounds like S-nitrosocysteine (*Shahidi*, 1992), which show antioxidative properties.

As an undesirable consequence of curing with nitrite, the formation of N-nitrosamines (NA) is discussed. This discussion started in the 1970s in USA after the detection of NA in fried bacon (*Fiddler et al*., 1978). N-Nitrosamines are formed by a nitrosation of secondary amines. Primary amines can be nitrosated too, but these products are not stable and decompose to the corresponding alcohols. A nitrosation of tertiary amines is not possible. The chemistry of nitrosation is very complicated and shows a dependency on the pH, the basicity of the secondary amine and temperature (*Mirvish*, 1975; *Ward and Coates*, 1987). At low pH the formation of nitrosating agents like protonated nitrous acid ($H_2NO_2^+$), nitrogen trioxide (N_2O_3) or nitrosyl halogenides (NOX) is stimulated, whereas the concentration of non protonated amine is decreasing. Therefore lightly basic amines like morpholine (pKa = 8.7) are nitrosated more rapidly than strongly basic amines like dimethylamine (pKa = 10.7). The commonly occurring N-nitrosamines in food are the volatile substances N-nitrosodimethylamine (NDMA), -piperidine (NPIP), -pyrrolidine (NPYR), -thiazolidine (NTHZ) and the non-volatile compounds N-nitrososarcosine (NSAR), -hydroxyproline (NHPRO), -proline (NPRO) and -thiazolidine-4-carboxylic acid (NTCA) (*Tricker*, 1997). Within this group of NA the International Agency for Research on Cancer (*IARC*, 1987) classified NDMA in group 2A and NPIP, NPYR and NSAR in group 2B. NDMA is the most frequently detected carcinogen in meat products. For the formation of NDMA dimethylamine is necessary, which can be formed by decomposition of lecithine, sarcosine, creatine and creatinine. The formation of NPIP requires piperidine, which can result from an alkaline hydrolysis of piperine, an important ingredient of pepper. The detection of

NPYR is primarily restricted to bacon, a roasted and cured meat product. During the production of bacon temperatures in meat products are often higher than 150° C, which leads to an anhydrous product and a decarboxylation of the amino acid proline forming pyrrolidine and finally to high NPYR concentrations. In a recent study (*Drabik-Markiewicz et al.,* 2009), investigating the role of proline in NA formation during heating of cured meat, it was demonstrated that the influence of proline on the NPYR contents was larger compared to the added amounts of sodium nitrite. Other investigations showed an increase of NPYR contents in meat products in correlation with an increase of biogenic amines (*Bulushi et al.*, 2009; *Warthesen et al*., 1975). Therefore, an alternative formation of pyrrolidine originating from ring closure of the biogenic amines putrescine, spermin or spermidin was assumed. Besides the formation of NA by curing meat products with nitrite also NA containing elastic rubber nettings can contaminate the edible parts of meat products (*Fiddler et al*., 1998; *Helmick and Fiddler*, 1994).

In meat products the most relevant NA are NDMA, NPIP and NPYR. A formation of these NA is only possible under following conditions:

1) Secondary amines must be present. In fresh meat no or only very low amounts of secondary amines are present. Potential precursors of secondary amines, like creatine and creatinine and the free amino acids proline and hydroxyproline and some decarboxylation products, are present, which can lead to a formation of secondary amines during ageing and fermentation of meat products.

2) pH must be low enough (< 5.5) to form nitrosating agents. This only applies for fermented sausages.

3) At high temperatures (> 130° C; formation of NPYR) or long storage at room temperature (NDMA, NPYR). This only applies for grilling, roasting and the production of raw sausages.

There are no really alternatives to nitrite until now and especially the antioxidative and curing flavour forming effects of nitrite is not possible to be substituted by other additives (*Lücke*, 2003). The negative aspects of the use of nitrite in meat products can be relativised as follows (*Drabik-Markiewicz et al*., 2009): processing technology involving good manufacturing practices and the widespread use of ascorbate which will lower the NA contents in meat products (*Tannenbaum et al*., 1991). Furthermore only 7% of the ingested food derives from food and thirdly the nitrate/nitrite content of vegetables far exceeds that of meat products (*Honikel*, 2008).

Recently vegetal-based extracts were used instead of NPS for curing meat products (*Nochemfood*, 2010). This procedure possibly contains the risk of using higher amounts of nitrite extracted by the vegetables in comparison to the amounts of nitrite added to the meat product if NPS is used. Furthermore an extraction of further residues and contaminants from the vegetables and, as a consequence, higher contamination levels of the meat product can not be excluded.

5. Veterinary Drugs

Exceedingly relevant with respect to safety of food of animal origin are residues of veterinary drugs. The use of veterinary drugs within the European Union is regulated by means of the Council Regulation (*EEC*, 1990) No. 2377/90 describing a procedure for the establishment of MRLs for veterinary medicinal products in foodstuff of animal origin including meat, fish, eggs and honey. Its annexes present substances, for which MRLs have been established (Annex I), substances, for which it is not considered necessary to establish MRLs (Annex II), substances with provisional, temporary MRLs (Annex III) and substances, which are not allowed to be used in food producing species (Annex IV). While Council Directive (*EC*, 1996b) No. 96/23/EC defines measures to monitor certain substances and residues thereof in live animals and animal products it divides veterinary drugs into two groups: group A covering prohibited substances in compliance with the Annex IV of the Council Regulation (EEC) No. 2377/90 and group B containing agents, in compliance with Annexes I and III of the Council Regulation (EEC) No. 2377/90 (Table 1).

Commission Decision 2002/657/EC (EC, 2002a) establishes criteria and procedures for the validation of analytical methods for detection of residues. For substances according to Annex IV of the Commission Regulation (EEC) No. 2377/90 Commission Decision 2003/181/EC (*EC*, 2003a) defines minimum required performance limits (MRPLs) for the determination of their residues in food of animal origin.

The prohibition of the use of growth promoting substances, such as hormones or ß-agonists, is regulated by Council Directives No. 96/22/EC (*EC*, 1996a) and 2003/74/EC (*EC,* 2003b). Since January, 1st 2006, according to Regulation (EC) No. 1831/2003 (*EC*, 2003c), the use of antibiotic growth promoting substances as additives for use in animal nutrition is forbidden. However, coccidiostats and histomonostats, antibiotics intended to kill or inhibit protozoa, are still authorised for use as feed

Table 1. EU legislation on veterinary drugs
Tabela 1. EU zakoni o veterinarskim lekovima

(EEC) No. 2377/90			
Annex II/*Aneks II*	**Annex I/*Aneks I***	**Annex III/*Aneks III***	**Annex IV/*Aneks IV***
Includes substances, for which it is not considered necessary to establish MRLs/ *Uključuje supstance za koje se smatra da nije potrebno utvrđivati MDK*	Includes substances, for which MRLs have been established/ *Uključuje supstance za koje su utvrđene MDK*	Includes substances, for which provisional MRLs have been established/ *Uključuje supstance za koje su utvrđene privremeni MDK*	Includes substances, for which no MRLs could be established; administration prohibited; „zero tolerance"/ *Uključuje supstance za koje se ne utvrđuju MDK; njihova primena je zabranjena "nula tolerancija"*
	Group B/*Grupa B*		Group A/*Grupa A*
	96/23/EC		

additives in accordance with Regulation (EC) No. 1831/2003. Despite the requirements set for feed business operators in regulation No (EC) 183/2005 (*EC*, 2005b), it is generally acknowledged that under practical conditions during the production of mixed feeds, a certain percentage of a feed batch remains in the production circuit and these unavoidable residual amounts can contaminate subsequent feed batches. Therefore MRLs for these substances in animal feed for non-target animals are established by the Commission Directive 2009/8/EC (*EC*, 2009a) amending Annex I to Directive 2002/32/EC (*EC*, 2002b). The occurrence of carry-over of coccidiostats and histomonostats in non-target feed may result in the presence of residues of these substances in food products of animal origin. Consequently the European Commission set MRLs for the presence of coccidiostats or histomonostats in the respective foods of animal origin by means of Commission Regulation (EC) No. 124/2009 (*EC*, 2009b).

The generic term „veterinary drugs" comprises a broad variety of classes of chemical compounds. Among them are antibiotics such as aminoglycosides, ß-lactams, macrolides and lincosamides, quinolones, sulfonamides and tetracyclines, antiparasitic agents like antihelmintics or coccidiostats, stilbens, ß-agonists, amphenicols, nitrofurans, nitroimidazoles, carbamates, pyrethroids and sedatives etc. This list is certainly not exhaustive. Nevertheless it gives an impression how manifold this area and consequently how enormously complex the analytical challenge is.

There is a need for sensitive, selective and reliable analytical methods to detect and monitor veterinary drugs. The scientific literature provides an overwhelming amount of information (*Sanders*, 2007). Even in the field of sample preparation a multitude of methods is available and applicable depending on sample selection or matrix and the target residue (*Kinsella et al*., 2009). Antimicrobial residues and compounds with hormonal activity can be screened by using rapid immunochemical methods, such as radio immunoassays (RIA), enzyme-linked immunosorbent assays (ELISA) or microbial growth inhibition assays (*Bovee and Pikkemaat*, 2009). The recent developments in ultra performance liquid chromatography (UPLC) with fast switching MS/MS and UPLC coupled with full-scan high resolution accurate mass analysers based on time-of--flight (TOF) or orbital trap technologies triggered the development of selective targeted approaches as well as multi-analyte and even multi-class detection methods (*Le Bizec et al*., 2009). Even the „omic" technologies such as transcriptomics, proteomics and metabolomics are used for the screening for veterinary drug-treated or non-treated situations (*Riedmaier et al*., 2009). Given the enormous amount of information acquired, the data handling and analysis becomes more and more important.

A reliable estimation of the contamination of meat and especially meat products with veterinary drugs is difficult. The Annual Report of The Rapid Alert System for Food and Feed (*Rapid Alert System for Food and Feed, RASFF*, 2007) specifies notifications due to the presence of metabolites of the prohibited nitrofurans for meat other than poultry. Also chloramphenicol was found in this category as well as the presence of unauthorised substances, namely the presence of phenbutazone and oxyphenylbutazone. For poultry, one notification on the presence of chloramphenicol and one for sulphachloropyrazine was recorded. According to the RASFF there is a downward trend for notifications on residues in poultry meat. This proved to be true also in the Annual Report of The Rapid Alert System for Food and Feed (*Rapid Alert System for Food and Feed, RASFF*, 2008). However, it seems to be obvious that nitrofurane metabolites are still the most

notified hazards even if the majority appears with regard to crustaceans. Looking at the Annual Report of the *Federal Office of Consumer Protection Food Safety, Germany* (2008) with regard to the National Residue Control Plan it stands out that coccidiostats, namely lasalocid was found in beef and pork liver as well as in broiler meat with contents well above the MRLs. Despite the approval of „unavoidable carry-over of coccidiostats or histomonostats in non-target feed" (Commission Directive 2009/8/EC) and the therefore established MRLs in food resulting from the unavoidable carry-over of these substances there still seems to be a need for a certain amount of action.

6. Failure Mode and Effect Analysis (FMEA)

Risk assessment according to the Codex Alimentarius Commission is a scientific evaluation of known or potential adverse health effects resulting from exposure to food borne hazardous agents. The process consists of four steps: (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment and (iv) risk characterisation. (CAC/GL 62, 2007, Working Principles for risk analysis for food safety for application by governments). Risk assessment is mostly directed towards the safety of the end product and consumer protection. During hazard identification the most significant hazards for the end product are identified and addressed within the scope of risk assessment or using a HACCP-plan. In most HACCP-plans a qualitative approach is used. By using a quantitative approach to risk assessment the hazard analysis can result in a very powerful tool for managing risks. Control measures can be validated and resources can be allocated to minimize the occurrence of hazards, i.e. contaminants at single production steps as well as in the end product.

One of the methods applicable for quantitative risk assessment is the Failure Mode and Effect Analysis (FMEA). FMEA is a systematic process meant for reliability analysis. It is a tool to assure product quality. It improves operational performance of the production cycles and reduces their overall risk level. The FMEA methodology was developed and implemented for the first time in 1949 by the United States Army. In the 1970s its application field extended to general manufacturing. Today the FMEA method is mainly applied in industrial production of machinery and electronic components, but also in food industry (*Scipioni et al*, 2002). Recently it has been used within the industrial processing of snails (*Arvanitoyannis and Varzakas*, 2009a), common octopus (*Arvanitoyannis and Varzakas*, 2009b) and ready to eat vegetables (*Varzakas and Arvanitoyannis*, 2009). The EU-Project *ΣChain* (2006) developed a modified FMEA procedure to identify, assess and address vulnerabilities in food production chains such as poultry meat. A clear and specific understanding and description of the products and processes is a mandatory prerequisite for any FMEA application. Thus the poultry meat production chain was exemplarily mapped. Flow charts were designed to identify the single steps in the chain. Following the consideration that a substantial number of contaminants may enter the poultry meat production chain via the feed chain, the latter was mapped, too.

Vulnerability within the EU-Project ΣChain was defined as a weakness in the system that can result in harm to the system or its operations, especially when this weakness is exploited by a hostile person or organisation or when it is present in conjunction with particular events or circumstances. This definition was applied to the poultry meat production chain, in relation to contamination with agents, hazardous to human health. Vulnerability was understood as lack of traceability whereas the implementation of this traceability was understood as a combination of:

- The documentation accompanying the product
- Appropriate physical and electronic tags including the information about their application
- Identification of relevant contaminants
- Occurrence and dynamics of contaminants
- Analytical methods to detect relevant contaminants including information about appropriateness and application

Vulnerabilities identified were rated according to three criteria, severity (Sev), likelihood (Lik) (of occurrence) and detectability (D). Severity is the rating of the hazard associated with the vulnerability, in the sense of damage to public health and is rated from 1 (no effect) to 10 (immediate effects and/or serious effect on health). The likelihood of occurrence indicates the frequency of a vulnerability event happening. Likelihood of occurrence is rated from 1 (will not occur) to 5 (occurs on a frequent basis). Detectability or likelihood of detection/recognition refers to whether the vulnerability or event happening will be noticed or detected giving the current control measures whereas a rating of 1 was understood as „likely" and 3 as „unlikely" to be detected.

For each potentially vulnerable chain step a Vulnerability Priority Number (VPN) was calculated: VPN = Severity × Likelihood × Detectability.

Thus a prioritisation of vulnerabilities or vulnerable chain steps, respectively, was achieved. The

higher the VPN the higher the priority for addressing the vulnerability.

The identified and prioritized potential vulnerable chain steps were addressed by identifying a set of control measures to reduce or even eliminate the vulnerability (reduce the VPN).

Likelihood of occurrence and detectability are understood to be possibly influenced by control measures. Namely the likelihood of occurrence can be decreased as the detectability can be increased by suitable measures.

It must be kept in mind that vulnerable chains steps and their ranking must be identified and estimated respectively for each individual food business operator and product and a given time. The ranking needs revision and update regularly. It is not possible to create a generic ranking of vulnerabilities for the production of poultry meat. The [Table 2] and [Table 3] illustrate the process of calculation for a few steps in a given part of the poultry meat production chain. These examples have been selected for illustration purposes only.

Table 2. Example for vulnerability assessment in the poultry feed chain
Tabela 2. Primeri ocene ugroženosti u lancu ishrane živine

No. of potentially vulnerable chain step/ ***Broj koraka u lancu koji potencijalno su ugroženi***	**Description of chain step/** ***Opis koraka u lancu***	**Potential failure (contaminant name or tag/documentation failure)/** ***Potencijalna greška (naziv kontaminenta ili greška u oznakama/ dokumentaciji)***	**Cause of failure/** ***Uzrok greške***	**Current control measures in place/** ***Trenutno postojeće kontrolne mere***	**Sev[a]**	**Lik[b]**	**D[c]**	**VPN[d]**
9.01	Delivery of raw materials (feed chain)/ *Isporuka sirovina (lanac hrane)*	PCDD/DF	Contaminated feed additives/ *Kontaminirani aditivi*	In-house testing; 1–6 samples/ year; end product Official control – residues/ *Interna kontrola; 1-6 uzoraka godišnje; krajnji proizvod, Službena kontrola – rezidue*	8	3	2	48
9.09–9.19	Feed chain from "dosing minor components" to "bulk storage"/ *Lanac hrane od „doziranja manjih komponenti" do „skladišta većih količina"*	Coccidiostats/ *Kokcidiostatici*	Cross contamination in subsequent feed batches/ *Unakrsna kontaminacija u uzastopnim šaržama hrane*	In house: determination of degree of carry-over/biennial Official feed control- residues/ *Interno: kontrola nivoa prenosa/dvaput godišnje; Službena kontrola hraniva - rezidue*	1	5	2	10

[a] Severity/*jačina*
[b] Likelihood/*verovatnost*
[c] Detectability/*detektibilnost*
[d] Vulnerability Priority Number/*prioritetni broj sa aspekta ugroženosti*

Table 3. Example for addressing vulnerabilities in the poultry feed chain putting into place new control measures
Tabela 3. Primer rešavanja pitanja ugroženosti u lancu hrane za živinu, uspostavljanjem kontrolnih mera

No. of potentially vulnerable chain step/ ***Broj koraka u lancu koji su potencijalno ugroženi***	**Description of chain step/** ***Opis koraka u lancu***	**Potential failure (contaminant)/** ***Potencijalna greška (kontaminent)***	**Cause of failure/** ***Uzrok greške***	**New control measures that could be put into place/** ***Nove konrolne mere koje mogu biti uvedene***	**Sev[a]**	**Lik[b]**	**D[c]**	**VPN[d]**
9.01	Delivery of raw materials (feed chain)/ *Isporuka sirovina (lanac hrane*)	PCDD/DF	Contaminated feed additives/ *Kontaminirani aditivi*	Test of each batch of feed additives at delivery/ *Testiranje svake šarže aditiva pri isporuci*	8	1	1	8
9.09–9.19	Feed chain from "dosing minor components" to "bulk storage"/ *Lanac hrane od „doziranja manjih komponenti" do „skladišta većih količina"*	Coccidiostats/ *Kokcidiostatici*	Cross contamination in subsequent feed batches/ *Unakrsna kontaminacija u uzastopnim šaržama hrane*	Separate production lines for medicated feed/feed for non-target animals/ *Odvojene linije za proizvodnju medicinirane hrane/hrane za ne-ciljne životinje*	1	1	2	2

[a] Severity/*jačina*
[b] Likelihood/*verovatnost*
[c] Detectability/*detektabilnost*
[d] Vulnerability Priority Number/*prioritetni broj sa aspekta ugroženosti*

7. Conclusion

With changing consumer behaviour over a period of more than six decades since the Second World War and constantly increasing consumer demands with respect to quality and safety food analytical chemical methods experienced simultaneously a wide ranging improvement with respect to sensibility, accuracy, rapidness and reliability. In consideration of this expeditious development in analytical chemistry especially meat and meat products could benefit a lot concerning quality and safety. Nevertheless, in terms of risk assessment there exists the liability of chemical analysts in interaction with toxicologists to decide, if possible, on reasonable MRLs for legislation. In addition, the presented Failure Mode and Effect Analysis (FMEA) system can be an effective means assessing (prioritizing) vulnerable chain steps in the production of meat products to decrease or eliminate vulnerability.

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Hemijska bezbednost u industriji mesa

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R e z i m e: Od Drugog svetskog rata, ponašanje i stavovi potrošača u razvijenim zemljama su se drastično promenili. Prvo je postajala potražnja za dovoljnim količinama hrane nakon ratnih godina gladovanja, zatim se pojavila želja za boljim kvalitetom, a danas, većina potrošača traži bezbednu i zdravu hranu visokog kvaliteta. Prema tome, jedinstven pristup, koji će sačinjavati dosledni standardi, naučni principi i stroge kontrole, neophodan je kako bi se obezbedilo zdravlje potrošača i održalo njihovo poverenje i zadovoljstvo. Hemijska analiza duž celog lanca (praćenje), od primarne proizvodnje do potrošača i od potrošača do primarne proizvodnje (sledljivost), predstavlja veoma važan preduslov za osiguranje bezbednosti i kvaliteta hrane. Predmet pažnje ovog rada je "hemijska bezbednost mesa i proizvoda od mesa" uzimajući u obzir neorganske, kao i organske rezidue i kontaminante, korišćenje nitrita u proizvodima, pojavu veterinarskih lekova, kao i tzv. Failure Mode and Effect Analysis (FMEA) - Analiza neuspeha i efekata, kao sistema kojim se ocenjuju (određuju prioriteti) slaba mesta/koraci u okviru lanca ishrane kako bi se eliminisala odnosno smanjila njegova ranjivost.

Ključne reči: *hrana, meso, proizvodi od mesa, neorganske rezidue, organske rezidue, nitriti, veterinarski lekovi, FMEA.*

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Fish meat quality and safety*

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A b s t r a c t: Fish constitute a major part of animal protein consumption in many parts of the world. Fish is consumed as fresh fish or as frozen, salted, dried, smoked, or canned products.

Fish represents a valuable source of proteins and other nutrients in the diet of many countries. As with many animal products, fish and fishery products contain water, proteins and other nitrogenous compounds, lipids, carbohydrates, minerals and vitamins. However, the chemical composition of fish varies greatly from one species and one individual fish to another depending on age, sex, environment and season. Proteins and lipids are the major components whereas carbohydrates are detected at very limited levels (less than 0.5 percent).

Different effects of several properties like lipid contents of tissues, water temperature and feeding habits have been found responsible for the bioaccumulation and bioconcentration of the odorous compounds in fish. Increasing quantities of organic pollutants are released into the aquatic environment by humans due to industrial production, modern plant protection, more intensive use of medicines, cosmetics, household detergents etc. These compounds are present in the environment as persistent compounds because they might accumulate in the different organisms through bioaccumulation and biomagnifications. Bioaccumulation of certain heavy metals along the food chain is a well-recognized process. Essentiality and toxicity of trace metals in organisms depend on the concentration of the metal; below a certain level they could be considered as essential for biochemical processes, but in the case of a high accumulation in organisms, intoxication may occur.

Fish meat is well suitable for human nutrition, because it contains easy digestible proteins and vitamins and minerals. Among the benefits of fish meat consumption the polyunsaturated omega-3 fatty acid contents can be mentioned again. The role of the omega-3 fatty acids in human metabolism in building cell membranes and receptor structures is unequivocally proved.

Besides these, fish meat is highly nutritious, tasty and several kinds of delicious products and dishes can be prepared form the meat of nearly 1000 fish species.

Key words: *fish, meat quality, safety.*

1. Introduction

With more than 30,000 known species, fish form the biggest group in the animal kingdom that is used for the production of animal-based foods. Only about 1000 of these species are commercially fished and used for food production. Further, some 100 crustacean and 100 molluscan species (for example mussels, snails and cephalopods) are used as food for humans. Fish constitute a major part of animal protein consumption in many parts of the world. Fish is consumed as fresh fish or as frozen, salted, dried, smoked, or canned products.

Most fish and other marine species give rise to products of great economic importance in many countries. The demand for such products has been increasing steadily during the last century and shows no sign of lessening. At the beginning of this decade captures in fisheries have shown a regular production per year of approximately 90-95 million metric tonnes. Fish products are known to provide significant amounts of important dietary factors such as nutritional and digestive proteins including high levels of essential amino acids (lysine, methionine); lipid soluble vitamins (e.g. A and D); microelements (I, F, Ca, Cu, Zn, Fe and others) and highly unsaturated fatty acids. The lipid fraction has a special interest due to its high ω-3 polyunsaturated fatty acids (PUFA) content, which has shown positive role in preveneting certain human diseases.

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2. Basic characteristics of fish flesh

2.1 Structure and organoleptic properties

Compared to terrestrial animals construction of fish muscle is very simply (*Erple*, 2008). That consists of the large generally white to off-white colour lateral muscle located on the both sides of the body. However there are some coloured fish like salmonides (red colour fish). Muscles of these species are coloured due to decomposition of the carotenoids. Fish muscle differs from the higher vertebrates. Basically two forms can be differentiated: the anaerobic white muscle tissue and the aerobe red muscle tissue. All muscle tissues constitute about 60% of the fish body (*Houlihan et al.,* 1995). Both types can be differentiated by chemical composition, physiological importance and nutritional value. Most species have more light than dark muscle.

Fish muscle also differs from the other vertebrates that several species have hundreds of teeny bones and usually they are unloved by the consumers. These fishes can't really be filleted however they are often tasty.

Other important problem of fish meat quality are off-flavours (*Tucker*, 2000).). Off-flavour is the presence of objectionable tastes and/or odorous in food. Unfortunately farmed fish tainted by different flavours perceived as unacceptable by the consumers. The off-flavour in farmed fish either, of salt or freshwater origin, might be caused by feed ingredients or natural foods, post mortem oxidative rancidity or odorous compounds absorbed from the environment (*Whitfield*, 1999). However, muddy-musty taste and odour problems are caused mainly by two natural origin isoprenoid compounds, 2-methylisoborneol (MIB) and geosmin (GSM) synthesised in the pond water and sediment by different micro-organisms, like cyanobacteria and actinomycetes (*Tucker and Martin*, 1991). These two isoprenoids can be found mostly in the sediment of the natural water bodies or fish culture systems.

Description of off-flavours might difficult because several causes:

- there are many chemical compounds responsible for disagreeable flavours;
- the same compound can be described differently by different people;
- the same compound might cause different flavours in different species;
- several flavours may be produced by more then one chemical compounds;
- variation in the concentration of an odorant may cause changes in the flavour characteristic rather than in flavour intensity (*van der Ploeg*, 1991).

2.2 Nutritional composition

Fish represents a valuable source of proteins and other nutrients in the diet of many countries. As with many animal products, fish and fishery products contain water, proteins and other nitrogenous compounds, lipids, carbohydrates, minerals and vitamins. However, the chemical composition of fish varies greatly from one species and one individual fish to another depending on age, sex, environment and season. Proteins and lipids are the major components whereas carbohydrates are detected at very limited levels (less than 0.5 percent).

Depending on their lipid content, which varies greatly from 0.2 percent to 25 percent, fish are classified as lean, semi-fatty or fatty. Fish lipids differ greatly from mammalian lipids in that they include up to 40 percent of long-chain fatty acids of highly unsaturated containing five or six double bonds. This difference entails both health (anti-thrombotic activity of polyunsaturated fatty acids) and technological (rapid development of rancidity) implications. In human nutrition, fatty acids such as linoleic and linolenic acid are considered essential as they cannot be synthesised by the organism. In marine fish, these fatty acids constitute only around two percent of the total lipids - a small percentage compared with many vegetable oils. However, fish oils contain other „essential" polyunsaturated fatty acids which act in the same way as linoleic and arachidonic acids. As members of the linolenic acid family (first double bond in the third position, n-3 counted from the terminal methyl group), they also have neurological benefits in growing children. One of these fatty acids, eicosapentaenoic (C20:5n-3), has attracted considerable attention since Danish scientists found a significant presence of it in the diet of a group of Greenland Eskimos, who proved virtually free from arteriosclerosis. Convincing evidence exists now for the significant role of fish and fish oils in decreasing the risk of developing cardiovascular diseases and in improving foetal brain development.

Proteins of fish comprise structural proteins (actin, myosin, tropomyosin and actomyosin), sarcoplasmic proteins (myoalbumin, globulin and enzymes) and connective tissue proteins (collagen). Fish proteins contain all the essential amino acids and, like milk, eggs and mammalian meat proteins, have a very high biological value. In addition, fish proteins are an excellent source of lysine, methionine and cysteine, and can significantly raise the value of

cereal-based diets, which are poor in these essential amino acids.

Also, fish meat is generally a good source of the B vitamins and, in the case of fatty species, of A and D vitamins. Vitamins can be divided into two groups, those that are soluble in fat, such as vitamins A, D, E and K, and those that are soluble in water, such as vitamins B and C. All the vitamins necessary for good health in humans and domestic animals are present to some extent in fish, but the amounts vary widely from species to species, and throughout the year. Some freshwater species such as carp have high thiaminase activity so the thiamine content in these species is usually low.

As for minerals, fish meat is a particularly valuable source of calcium and phosphorus as well as iron, copper and selenium. Saltwater fish have a high content of iodine. In addition to essential amino acids and proteins, fish nutritional attributes relate to the quality of lipids and vitamin and mineral content (FAO, Fisheries and Aquaculture Department publications).

cumulation and bioconcentration of the odorous compounds in fish. For example accumulated off-flavour contents are correlated to tissue lipid content (crude fat % in the total body) of fish (*Tucker*, 2000). In nature off-flavours may develop within a matter of hours if the level of odorous metabolites in the water rises suddenly, for example during sudden die-off odour-producing algae (*van der Ploeg*, 1991). The rate of off-flavour removal is much slower than uptake. Purge of geosmin and 2-methylisoborneol in clean water are not expected to be presented less than about five days and elimination of these compounds are relatively slow from the lipid rich tissues (*Tucker and Martin*, 1991).

Uptake and depuration of MIB in experiments carried out by *Johnsen et al.* (1996) were increased by time interactions at increasing temperatures. Differences in rates uptake and elimination of off-flavours will differ among species in the same raring system (*Gy. Papp et al.*, 2007) and size of fish too (*Howgate*, 2004).

Table 1. Polyunsaturated fatty acids in the flesh of some of fish species found in Hungary (from *Csengeri*, 2008)
Tabela 1. Polunezasicene masne kiseline u mesu nekih vrsta riba u Mađarskoj (*Csengeri*, 2008)

species (*scientific name*)/ Vrste (naučni nazivi)	Body weight/ masa	Total fatty acids/ Ukupne masne kiseline	AA 20:4 (n-6)	EPA 20:5 (n-3)	Total (n-6)	Total (n-3)
	(g)	(%)	(g/kg)	(g/kg)	(g/kg)	(g/kg)
Asp (*Aspius aspius*)	210	3.38	1.32	1.58	5.5	7.5
Roach (*Rutilus rutilus*)	270	4.45	0.87	0.62	5.3	3.4
Common bream (*Abramis brama*)	639	3.18	1.43	1.07	5.7	4.0
Silver Carp (*Hypophthalmichthys molitrix*)	2780	3.51	1.70	2.37	4.9	10.2
Bighead carp (*Aristichthys nobilis*)	6 kg	16.4	3.66	6.56	14.5	29.6
Common carp (*Cyprinus carpio*)	577	7.84	1.00	0.56	10.4	3.7
Brown bullhead (*Ictalurus nebulosus*)	168	2.96	0.92	0.88	4.2	4.5
Pike (*Esox lucius*)	470	1.40	0.59	0.63	2.5	3.9
Perch (*Perca fluviatilis*)	287	1.47	0.61	0.57	2.2	3.4
Pike perch (*Sandra lucioperca*)	420	1.14	0.56	0.61	2.0	3.0

3. Possible environmental effects on fish flesh quality

3.1 Effects of natural originated odorous contaminants

Different effects of several properties like lipid contents of tissues, water temperature and feeding habits have been found responsible for the bioac-

3.2 Effects of contaminants originated from the industry (pesticides, medicines, cosmetics etc.)

The organic and inorganic compounds present in fish can be divided into three major groups:

Inorganic chemicals: arsenic, cadmium, lead, mercury, selenium, copper, zinc and iron.

Organic compounds: polychlorinated biphenyls (PCBs), dioxins and pesticides (e.g. chlorinated hydrocarbons).

Processing-related compounds: sulphites (used in shrimp processing), polyphosphates, nitrosamines and residues of drugs used in aquaculture (e.g. antibiotics or hormones).

Many of the inorganic chemicals are essential for life at low concentration but become toxic at high concentration. While minerals such as copper, selenium, iron and zinc are essential micronutrients for fish, other elements such as mercury, cadmium and lead show no known essential function in life and are toxic even at low concentrations when ingested over a long period. These elements are present in the aquatic environment as a result of natural phenomena such as marine volcanism and geological and geothermal events, but are also caused by anthropogenic pollution arising from intensive metallurgy and mining, waste disposal and incineration, and acidic rain caused by industrial pollution.

Increasing quantities of organic pollutants are released into the aquatic environment by humans due to industrial production, modern plant protection, more intensive use of medicines, cosmetics, household detergents etc. These compounds are present in the environment as persistent compounds because they might accumulate in different organisms through bioaccumulation and biomagnifications. In predatory fish species increasing amounts of chemicals may also be found in such way. Similarly, higher values could be present as a result of bioaccumulation, when chemicals in the body tissues accumulate over the life span of the individual. In this case, a large (i.e. older) fish will have a higher content of the chemical concerned than a small (younger) fish of the same species. The presence of chemical contaminants in fish is therefore highly dependent on geographic location, species and fish size, feeding patterns, solubility of chemicals and their persistence in the environment. (FAO Fisheries and Aquaculture Department publications)

The typical undesirables derived from marine feed ingredients are persistent organic pollutants (POPs), which are associated mainly with fish oil. In aquaculture the fat-soluble polychlorinated dioxins and furans (PCDD/F) and dioxin-like PCBs (DLPCB), commonly known as 'dioxins', are among one of the greatest challenges to food safety. Some studies has been found out that the dioxin uptake of the human population in different countries is over the WHO guided lowest value level (1-4 pg TEQ/ kg body weight/day). For example in Japan the dioxin uptake is estimated to be 3,22 pg TEQ/ kg body weight/day (*Tsutsumi et a*l., 2001) and about half of this quantity is taken from fish products. Nevertheless, the expected life time in this country is the longest over the world.

In the last decade in monitoring studies of aquatic environment around hundred of pharmaceuticals and their metabolites have been detected (*Jones et al*., 2002). The most dangerous environmental pollutants of the medicines are the antibiotics, which may contribute to the evolution of resistant pathogen bacteria infections. During the last decades tetracycline were the most important antibacterial agents used in fish farming, widely used for treatment of systemic bacterial infections. Antibiotics are permitted for treatment of fish under the responsibility of a veterinarian even in organic fish farming (e.g. EEC 2004; IFOAM, 2000; NATURLAND, 2000; BIOKONTROLL, 2001). In spite of that, several studies have shown that oxytetracycline (OTC) persist for a long time in fish tissues and long withdrawal times are necessary, moreover the OTC residues have immunosuppressive effects and cause liver damage.

3.3 Storage effects on the quality of fish flesh

Estimated results are shown that between 10 and 50 % of all produced foods have to be rejected due to post-harvest or post-slaughter spoilage (*Lunestad* 2008). Storage might cause flesh quality reduction, diseases or off-flavours as results of microbial growth and oxidation effect to the degradation of the fish tissues.

After death of the fish, a series of biochemical reactions starts, which is of paramount importance for the quality and shelf life of the products. These reactions depend on several different factors: the type of fish species, physiological condition of the fish, as well as environmental influences (water temperature, salinity). In addition, catching and harvesting methods, killing procedures have a great effect on the biochemical relations related to disintegration of the fish fillet (*Oehlenschlager and Rehbein*, 2009).

As it is well known, degradation of fish starts with enzymatic and chemical reactions of autolysis. Freezing of fish fillet directly after catch, more or less, stop most of the enzymatic reactions, depending on the temperature of the frozen fish. However, during later thawing chilled storage or further processing of the fish all biochemical reactions continue and may result in quality losses. Directly after catch, de muscle tissue of healthy fish is free from bacteria, but not he gills, skin and intestines. The bacteria penetrate into the fillet during the storage and processing, accompanied by changes in the composition of the bacterial flora. Gram-negative psychotropic

rods (*Shewanella* spp., *Pseudomonas* spp., *Vibrio* spp., *Aeromonas* spp.) are important spoilage bacteria. Bacteria are also responsible for formation of the biogenic amines from precursor amino acids in spoiling fish decarboxylation. Histamine is produced from histidine, cadaverine from lysine, putrescine from ornithine, tryptamine from tryptophane, tyramine from tyrosine and agmatine from arginine (*Lehane and Olley,* 2000; *Shalaby*, 1996).

The degradation level should be characterized by determining the total volatile basic nitrogen (TVBN) content, which is in strong relations with spoilage of fish. For example short chain alcohols, carbonyls and esters, trimethylamine, hydrogen sulphide, methylmercaptan, dimethyl disulfide and dimethyltrisulfide are among the most volatile compounds being produced in degrading tissues and might caused spoilage odours in fish fillets and fish products (Olafsdottir et al., 2004). In the Codex Alimentarius Hungaricus (art.3-1-95/194) could be found MRL levels for TVBN in marine fish between 25-35 mg/100g, but around 50 mg/100 g should be acceptable. For the freshwater fish species 12 mg TVBN/100g was determined by Lengyel et al, 2000.

Fish lipids are not stable during storage at any temperature. Lipolysis and lipid oxidation may occur in chilled or frozen fish, leading to unpleasant flavours and tastes caused by carbonyl compounds and short-chain carbonic acids. Binding of free fatty acids to fish muscle proteins may results in texture deterioration Speed and extent of lipolysis and of oxidation of unsaturated fatty acids are higher in dark muscle than in white muscle. During frozen storage, lipid degradation is not completely stopped, but continues at lower rate, leading to increased concentrations of free fatty acids, which can be used as quality indicators.

4. Demonstration of some studies on fish meat quality in the HAKI

4.1 Fish meat quality and the microelements

Bioaccumulation of certain heavy metals along the food chain is a well-recognized process. Essentiality and toxicity of trace metals in organisms depend on the concentration of the metal; below a certain level they could be considered as essential for biochemical processes, but in the case of a high accumulation in organisms, intoxication may occur. Freshwater fishes can regulate the levels of essential elements: copper, chromium, molybdenum, and zinc over a range of ambient concentrations, and some regulation of nonessential metals such as cadmium and mercury may also occur. Some of our results demonstrated that differences in micro and macroelement concentrations exist among fish collected from different areas, depending on the feeding, water quality and geographical properties of the area. Our investigation also revealed that trace metal levels in fish depend also on age, body size of the fish, feeding habits, season and temperature of the water, and other factors that influence the metabolic rate of fish (*Sandor et. al,* 2001; *Sandor et al.*, 2000; *Zubcova et al.,* 2001). This work was partially supported by the European Union EU FP5 INCO-COPERNICUS project.

4.2 Studies on xenobiotics and toxic metals in fish meat

In the EU FP6 framework (FOOD –CT-2006-16249 Project Aquamax) our institute had evaluated some fish meat quality determinations to assess the transfer of toxicants and xenobiotics from feed to consumable fish meat. Concentration levels of heavy metals and organochlorine pesticide compounds in fish samples from organic farming fish ponds and transition ponds to organic technology were examined in these monitoring assays.

Toxic metals and food safety of fish meat

The sampled fish species were: Common carp, Gibel carp, Black bullhead, Perch, Pike-perch and Pike, that showed low levels of toxic elements in the bony meat portions. Comparison concentrations of toxic elements, Cr, Cd, Ni, Pb (d.m.) in fish samples from different ponds we have found that fish from organic pond showed somewhat lower levels than those from the transition one (*Hegedűs et. al,* 2009, *Oncsik et al.,* 2009). Levels of toxic elements determined in these samples were lower in the fillet of Common carp than in eviscerated whole fish. We have compared the predator fish heavy metal content with some of bottom feeders, where differences could be observed. The sampled predator fish were young specimens and showed low levels of contamination with elements. In conclusion, levels of cadmium and lead measured in this monitoring study were well below to the maximum levels as defined by the COMMISSION REGULATION (EC) No 1881/2006.

Organochlorine contaminants in fish meat and food safety

In the fish meat samples mentioned above some hexachloro-cyclohexane (BBHC – β-HCH and Lindane - γ-HCH), aldrin, low levels DDT residues,

endosulfan and endrin-ketone were observed (*Fazekas et al,* 2009). In samples from transition pond both HCH compounds (BBHC and Lindane) were present. Also the measurements revealed that fatty fish meat (Common carp and Gibel carp) contain generally higher levels of pesticide residues. Finally we conclude that the levels were lower than those defined as maximum residue limits (MRL) for pork meat in the Commission Regulation (EC) No 149/2008. (No MRLs were found for fish in the regulations.)

Antibiotic residue levels in Common carp samples after drug administration

Antibiotics are permitted for treatment of fish under the responsibility of a veterinarian even in organic fish farming (e.g. EEC 2004; IFOAM, 2000; NATURLAND, 2000; BIOKONTROLL, 2001). In connection to the possible emergency use of veterinary drugs laboratory studies were planned in the EU FP7 framework mentioned above to determine the residue depletion of selected antibiotics: Oxytetracycline (OTC) and Flumequine (FLU). Results in the feeding experiments with OTC medicated feed showed a continuous accumulation of OTC level in muscle skin during the treatment period and a very long elimination period. The calculated withdrawal period for maximum residue limit (MRL for quantity at the muscle in EU COUNCIL REGULATION (EEC) No 2377/90) was much longer than the prescribed one in Directive 2004/28/EC. According to our results, this 500 degree-days withdrawal time would not be sufficient enough for decreasing the OTC concentration to the allowed residue level (MRL) at temperature of around 20°C. This is in correspondance with the results reported by *Zhang and Li*, (2007) for grass carp in OTC feeding examinations. The elimination half-lives measured in muscle and liver after oral administration of OTC in common carp are also longer than values of other fish species reared nearly in the same conditions. After some months of the treatments residues of OTC can be detected in liver of common carp and also active OTC molecules in the muscle with skin. According to these observations, we did not recommended administration of OTC treatment for food fish in the Hungarian common carp farming (*Sándor et al.,* 2010), because our investigations confirm the accumulation of OTC and its metabolites in drug-treated animals.

Results of the feeding experiments with flumequine medication present shorter withdrawal periods for muscle with skin and liver than values found in the literature for other fish species, sometimes at similar conditions. Total depletion of FLU could not be detected, but the remaining concentrations are under maximum residue level recommended by the EU regulation (EEC No. 2377/90). In conclusion, our experimental data for flumequine administration in feed of fish suggest that FLU might be used safely in common carp farming.

4.3 Studies on off-flavours in different fish species farmed in Hungary

Accumulation of MIB and geosmin in tissues of five important fish species was studied in the flesh of herbivorous grass carp (*Ctenopharyngodon idella*), the bottom feeding omnivorous common carp (*Cyprinus carpio*), the plankton feeding silver carp (*Hypophthalmichthys molitrix*), the omnivorous tilapia (*Oreochromis niloticus*), and the carnivorous African catfish (*Clarias gariepinus*), (*Gy. Papp et al.,* 2007). Fish were collected from four different experimental aquatic ecosystems of our institute, including a traditional fishpond, and different pilot-scale experimental aquaculture systems like a pond recycling system, effluent-fed fishponds and a combined aquaculture-algae (CAA) system, during 2002-2005. MIB and GSM contents were analysed with an improved GC-MS method of *Zhu et al.* (1999).

According to our published results negligible MIB contents were found in the fish fillets from all of the studied aquatic ecosystems. However geosmin was detected in various concentrations, from trace levels to tens of micrograms per kg in the fillet of analysed fish species during the study period. Off-flavour caused mainly by geosmin and it was always lower in fillets of carnivorous African catfish (*Clarias gariepinus*) and herbivorous grass carp (*Ctenopharyngodon idella*) than in species with other feeding habits in the same aquatic system. GSM concentration was significantly higher in the fillet of carp reared in traditional fish pond than that of fish from the other studied systems. Geosmin concentrations were usually higher in the fillet of bottom feeding omnivorous carp than those we found in the all studied species with other feeding habits in the same aquatic ecosystem at the same day of sampling. Overall, geosmin tainting of fish fillets showed a general relationship with the feeding habits of these species.

5. Benefits and hazards of fish consumption

Fish meat is well suitable for human nutrition because it contains easy digestible proteins and vitamins and minerals.

Among the benefits of fish meat consumption the polyunsaturated omega-3 fatty acid contents can be mentioned again. The role of the omega-3 fatty acids in human metabolism in building cell membranes and receptor structures is unequivocally proved. Function of these fatty acids in vision, in reproduction, in pre-natal and post-natal development of human brain is also well documented (*Lauritzen et al.*, 2001; *Curtis et al.,* 2004; *SanGiovanni and Chew*, 2005).

Besides these, fish meat is highly nutritious and tasty. Several kinds of delicious products and dishes can be prepared form the meat of nearly 1000 fish species.

Contamination, generally, has a negative impact on the quality of food and may imply a risk to human health. Several studies have concluded that levels of different inorganic and organic chemicals in fish intended for human consumption are low and probably below levels likely to affect human health. Nevertheless, they can be of potential concern for populations for whom fish constitutes a major part of the diet and for pregnant and nursing women and young children who consume substantial quantities of oily fish.

The EU has taken measures to minimize contaminants in foodstuffs. Community measures have been taken for the following contaminants of relevance to fish and seafood: metals (cadmium, lead, mercury, inorganic tin), dioxins and PCBs and polycyclic aromatic hydrocarbons (PAH). For heavy metals: (cadmium, lead and mercury), maximum levels have been established by Commission Regulation 466/2001/EC of 8 March 2001, setting maximum residues levels for certain contaminants in foodstuffs. For dioxins and PCB there is separate legislation for food and feed. In the case of food, maximum limit values were set in Council Regulation (EC) No 2375/2001 of 29 November 2001. In the case of feed, maximum limit values were set in Council Directive 2001/102/EC13. The EU regulation (EEC No. 2377/90) limits the drug residues in all animal species which are used for food production. The European Food Safety Authority has published an opinion on the health risks related to the consumption of wild and farmed fish on 22 June 2005 with reference to these contaminants. This provides advice on the safety and nutritional contribution of wild and farmed fish. (See: http://www.efsa.europa.eu/en/science/contam/contam_opinions/1007.html

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Kvalitet i bezbednost ribe

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R e z i m e: Riba u mnogim zemljama predstavlja značajan izvor životinjskih proteina. Konzumira se kao sveža riba ili zamrznuta, soljena, dimljena ili kao konzerva.

Riba predstavlja važan izvor proteina životinjskog porekla, kao i mnogih drugih hranljivih materija, u mnogim zemljama. Kao i sa mnogim proizvodima životinjskog porekla, riba i proizvodi od ribe sadrže vodu, proteine i druga azotna jedinjenja, lipide, ugljene hidrate, minerale i vitamine. Međutim, hemijski sastav ribe varira u velikoj meri zavisno od vrste, kao i pojedinačnih riba, i takođe zavisi od starosti, pola, sredine i sezone. Proteini i lipidi su glavne komponente, dok su ugljeni hidrati otkriveni u veoma ograničenom sadržaju (manje od 0,5 procenata).

Meso ribe je pogodno za ishranu ljudi, jer sadrži lako svarljive proteine, vitamine i minerale. Prednost konzumiranja mesa ribe je i u sadržaju polinezasićenih omega-3 masnih kiselina. Uloga omega-3 masnih kiselina u ljudskom metabolizmu, u izgradnji ćelijskih membrana i strukturi receptora, je neopozivo dokazana.

Različiti uticaji nekoliko odlika kao što su sadržaj lipida u tkivima, temperatura vode i navike u ishrani se smatraju odgovornim za bioakumulaciju i biokoncentraciju mnogih jedinjenja u ribljem mesu. Količine organskih zagađivača koje ljudi kroz industrijsku proizvodnju, modernu biljnu proizvodnju, intenzivnije korišćenje lekova, kozmetičkih sredstava, deterdženata u domaćinstvu, i sl., ispuštaju u vodenu sredinu, su u stalnom porastu. Ova jedinjenja su prisutna u životnoj sredini

kao postojana jedinjenja, jer se mogu akumulirati u različitim organizmima kroz biološku akumulaciju i biološko uvećavanje. Bioakumulacija određenih teških metala duž lanca ishrane je dobro poznat proces. Bitnost i toksičnost metala u tragovima u organizmu zavise od koncentracije metal. Ispod određenog nivoa, mogu se smatrati bitnim/ključnim za biohemijske procese, ali u slučaju visoke akumulacije u organizmu, može doći do intoksikacije.

Pored toga, meso ribe je izrazito hranljivo, ukusno i nekoliko različitih vrsta ukusnih proizvoda i jela se mogu pripremati of mesa koje potiče od skoro 1000 vrsta riba.

Ključne reči: *riba, kvalitet mesa ribe, bezbednost.*

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Meat quality of fish farmed in polyculture in carp ponds in Republic of Serbia*

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A b s t r a c t: Meat quality of one-year, two-years and three-years old carp, two year old silver carp and grass carp, and two years old catfish and zander, which were farmed in different conditions and in different feeding regimes was analyzed in this study. Twelve samples of each type and category of fish were taken from three different fish ponds in December. Chemical analysis, fatty acid and cholesterol content determinations were carried out in the Institute of Hygiene and Meat Technology, Belgrade. Statistical analysis was performed using the Statistica 10 program. The established n-3/n-6 ratios in different categories of common carp were in the range from 0.1 to 0.26. The most favourable ratio was observed in two years old carp fed pelleted food and the least favourable in three-year old carp fed corn as dominant component in food. The dependence of n-3/n-6 ratio with age and diet was established in our work, too. This ratio also widely varies between different species of fish, which is also confirmed. Nutritive value of examinated freshwater fish is high since their fatty acid composition is characterized by satisfactory proportion of n-3 polyunsaturated fatty acids and by high proportion of n-6 polyunsaturated fatty acids, especially linoleic and arachidonic acids.

Key word: *fresh-water fish, polyculture, age, nutrition, fat, proteins, cholesterol, fatty acid profile*

Introduction

The high nutritional value of fish meat is reflected in favourable content of proteins, carbohydrates, minerals and vitamins (*Ćirković et al.*, 2002). It represents the most important dietary source of n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), that have particularly important roles in human nutrition, reflecting their roles in critical physiological processes *(Calder and Grimble*, 2002; *Zhenga et al.*, 2004).

These acids (EPA and DHA) appear to play a key role in neutral development, functioning of the cardiovascular and immune systems (*Lauritzen et al.*, 2001), besides the prevention of some types of cancer, including colon, breast and prostate (*Connor,* 2000), brain aging and Alzheimer disease (*Kyle,* 1999). It is necessary to take into account the nutritional quality of meat because fish is also one of the best sources of animal protein (*Ozogul et al.*, 2006). Composition of fish proteins is better than the composition of proteins of other animals, which is mainly due to more favorable amino acid composition and lots of free amino acids (*Tope et al.*, 2007; *Buchtová et al.,* 2010). High biological value of fish proteins results from the presence of small content of connective tissue and lack of fascia and aponeurosis. Good digestibility of fish meat comes from the content of short muscle fibers, lacks of sclerproteins, collagen and elastin (*Ćirković et al.*, 2002). Fish proteins contain all the essential amino-

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acids for the human organism and they can be used as the sole source of protein in the diet (*Vladau et al.,* 2009). Mammals and fish have a similar percentage of proteins, which in fish is usually in the range of 14-20% (*Spirić et al.,* 2009; *Trbović et al.,* 2009; *Ćirković et al.,* 2010), although some authors state that this range is slightly higher and amounts from 13 to 25% (*Vladau et al.*, 2008), which accounts for 80 to 90% of the energy content of the fish. In terms of fat, the meat of mammals contains much higher percentage of fat (*Saičić et al.*, 2010). The lipid content of fish varies depending on the type of fish, the time of year and what the fish feeds on (*Guler et al.*, 2008; *Ćirković et al*., 2011). Meat of fish contains insignificant amounts of carbohydrates in the form of glycogen and high percentage of water (60–86%) (*Ćirković et al.*, 2002). The content of vitamins and minerals in meat of freshwater fish is very favourable (*Özurt et al.*, 2009). The energy value of fish meat is directly proportional to fat content. It was found that fish fats vary greatly in regard to the percentage of saturated and unsaturated fatty acids and usually contain 15–36% saturated fatty acids (*Ackman,* 2000; *Buchtova et al.,* 2007; *Zakes et al*., 2010) and 58–85% unsaturated fatty acids (*Caballero et al.*, 2002; *Domaizon*, 2000). The most important unsaturated fatty acids are linoleic and linolenic acid, which are essential and should be ingested in the body by food. Results referring to meat quality of carp are different in communications by various authors, with differences mostly caused due to the analysis of fish of different age, breeding systems and food and because of that, there are wide ranges of fat content in carp, from 2.3 to 16.8%, while varying slightly less in case of protein and protein content in range from 14 to 18% (*Vladau et al.,* 2008; *Trbović et al.,* 2009, *Ćirković et al.*, 2010). Beside polyunsaturated fatty acids, fish fats contain cholesterol. Fish meat contains similar amount of cholesterol (49–92 mg/100 g) as pork or beef (45–84 mg/100 g) and cholesterol content is not correlated with fat content (*Piironen et al.*, 2002). Content of cholesterol in freshwater fish from a free-catch and fish from aquaculture is different and depends on the species of fish (*Moreira et al.*, 2001). The amount of cholesterol in freshwater fish is lower in comparison with sea fish *(Luzia et al.,* 2003) and, therefore, the consumption of freshwater fish is more favourable for human health.

Recent research suggests that freshwater fish are capable of producing DHA from α-Linolenic n-3 (*Buzzi et al.,* 1996; *Bell et al.,* 2001) and they express all the desaturase and elongase activities necessary for this biosynthetic pathway (*Sargent et al.*, 2002).). In contrast, sea fish are unable to produce DHA from 18:3n-3 at a physiologically significant rate (*Owen et al.,* 1975; *Sargent et al.*, 2002) due to apparent deficiencies in one or more steps in the pathway (*Ghioni et al.,* 1999; *Tocher and Ghioni,* 1999).

Meat quality of one-year, two-years and three-years old carp (*Cyprinus carpio L.*), two-years old silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*), and two-yearsold catfish (*Silurus glanis*) and zander (*Stizostedion lucioperca*), which were farmed in different conditions and with different feeding regimes was analyzed in this study.

Literature data

The basic reference related to fish that are farmed in polyculture in carp ponds in our country

Fish farming in our region is mostly conducted like polyculture rearing of carp with silver carp, bighead carp, grass carp, catfish and zander (*Ćirković et al.,* 2007). Common carp is the most common fish species in our country, and the cyprinid fish are the predominant fish in world aquaculture with 54% of total production (*Ćirković et al.*, 2002; *FAO,* 2006). According to many international authors (*Andrade et al*., 1995; *Arts et al.*, 2001; *Rasoarahona et al*., 2004), common carp is a symbol of strength, fertility and longevity. It is omnivore fish and very effectively uses food. Fertility of carp is high and it ranges up to 1 500 000 eggs per female (*Ćirković et al*., 2002; *FAO,* 2006). Carp is tolerant to large variations of quality of ambient conditions. This species is not susceptible to disease and is tolerant to handling. Opinion on the culinary quality of carp in our country is not the same as the belief in Anglo-Saxon states, but the fact is that there is not a single fish from which a number of fish speciality can be made. According to *Steffens and Wirth* (2005) the fatty acid composition reflects, to a large extent, the diet, so the n-3/n-6 ratio ranges between 0.08 and 2.4; while in paper of *Ćirković et al.* (2010) established the ratio of these fatty acids of 0.54. A similar ratio (0.5) was found by *Fajmonova et al.* (2003). Carp fed exclusively natural food from fish-pond shows a significant level of total n-3 and n-6 fatty acids (*Ćirković et al.*, 2010). Supplementary feeding with grains leads to reduced amounts of these essential fatty acids and this is due to the lower proportion of natural food in the diet of the carp, which received additional grain.

The so-called “Chinese carps” (grass carp, silver carp, bighead carp) were introduced into the European waters (Danube Basin) in 1960s to produce their polyculture in carp fishponds and stock open waters in order to increase total ichthyoproduction at the expense of food resources available in plankton form (*Lenhardt et al.*, 2010). Silver carp, bighead carp and grass carp were introduced from

the Asian continent. They use the natural feed in fish ponds very well and their percentage ranges from 20–30%, compared to carp (*Ćirković et al.*, 2002; *Ćirković et al.*, 2005). These herbivore fish exploit ecological potential of fish ponds very well and make production more economical. Chinese carps have been present in our country for about 40 years and they are well accepted on the market because of low price and good quality of meat. Culinary quality of these fish is somewhat lower than that of carp, but their biological quality is very good, what was demonstrated in our study. Catfish and zander are carnivore fishes, whose participation in polyculture has a task to significantly reduce the number of less valuable fish, as well as to select fish of poor growth and adverse health conditions. Catfish is reared successfully and effective artificial reproduction have already been developed, including out-of-season spawning (*Brzuska,* 2001). Zander is a carnivorous fish; it generally feeds on other fish species rich in fatty acid (*Celik et al.*, 2005). After fertilization, perch eggs are introduced into the pond, in the form of „nests" from the open water. Perch is very sensitive to handling during introducing and harvesting, and is usually farmed as a one-year perch and rarely as two-years old fish. Production of zander and catfish is possible in monoculture, but in our conditions, due to high feed prices, high costs of balancing environmental conditions and relatively low prices of these species on the market, there is no economic justification for this type of production. In the European Union countries, these fish achieve high price, but there are significant problems in their transport as live fish. Carnivorous fish eat per kilogram of gain up to 15 kg of other fish (*Ćirković et al.*, 2002), so their production can be based on a small number of individuals that control the number of less valuable fish. Some European authors also recomanded farming the catfish species as a component in polyculture with carp, tench, and herbivorous fish (*Duda,* 1994). Growth rate and feed utilization effectiveness obtained by European catfish cultivated in polyculture were more advantageous than those in monoculture *(Ulikowski et al.*, 2003).

Material and methods

Samples of two and three years old carp, two years old silver carp, grass carp, catfish and zander were taken in the winter time from a pond, where the production is organized in the semi-intensive system with the addition of corn (80%) and wheat (20%). The three-year old carp was sampled from two ponds. In one case, feeding was performed using the combinations of barley, maize and wheat, in proportion 40:30:30, while in the second case feeding , it was done with a full feed diet mixtures. Also, the sample of two-years old carp were taken from ponds where the feeding was done with complete feed mixture. Twelve samples of each type and category of fish were taken. Before analysis, samples were stored at the temperature of –18ºC. Before examination fish were left one hour at room temperature, in order to partially defrost and enable easy skin removal, taking the head and tail and remove the viscera. Fish fillets were blended in Braun CombiMax 600 (*Spirić et al.*, 2009; *Trbović et al.*, 2009). For purposes of the examination of fatty acide profiles and cholesterol content, samples were stored in dark plastic bags at temperature of –18ºC, until examination. Meat from dorsal muscles was used for chemical analysis.

Chemical analysis

Chemical composition of fish muscle tissue was determined by standard SRPS ISO methods. Protein content was determined by Kjeldahl (N x 6.25), (Kjeltec Auto 1030 Analyzer, Tecator, Sweden). Water content was determined by drying at 103 ± 2ºC to constant weight (SRPS ISO methods). For determination of total fat, sample was hydrolyzed with 4M hydrochloric acid and extracted with petroleum ether by Soxhlet apparatus. Ash was determined by combustion at 550 ± 25º C. (*Spirić et al*., 2009; *Trbović et al.*, 2009).

Extraction of lipids by ASE

Total lipids for fatty acids determination were extracted from fish muscle tissues by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA). Homogenate of sample mixed with diatomaceous earth, was extracted with a mixture of n-hexane and iso-propanol (60:40 v/v) in 33 ml extraction cell at 100°C and nitrogen pressure of 10.3 MPa (*Spirić et al*., 2009; *Trbović et al.*, 2009). The extracts were collected and the solvent was removed under stream of nitrogen in Dionex Solvent Evaporator 500, at 50°C until dryness. Fat extract was further used for fatty acids determination.

FA analysis by capillary gas chromatography (CGC)

Fatty acid methyl esters (FAMEs) were prepared by transesterification by using trimethylsulfonium hydroxide, according to SRPS EN ISO 5509:2007 procedure. The GC instrument Shimadzu 2010 (Kyoto, Japan), used for FAMEs determination, was equipped with a split/splitless injector, fused silica cianopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, J&W Scientific, USA) and flame ionization detector. The column

temperature was programmed. Injector temperature was 250° C and detector temperature was 280° C. The carrier gas was nitrogen at a flow rate of 1.33 ml/min and injector split ratio of 1:50. Injected volume was 1 µl and total analysis time 50.5 min. Chromatographic peaks in the samples were identified by comparing relative retention times of FAMEs peaks with peaks in a Supelco 37 Component FAMEs mix standard (Supelco, Bellefonte, USA), (*Spirić et al*., 2009).

Cholesterol determination

Cholesterol determination in carp fillets (direct saponification) was performed by using HPLC/PDA system (Waters 2695 Separation module/Waters photodiode array detector, USA) on a Phenomenex Luna C18 (2) reverse/phase column, 150 mm x 3.0 mm, 5µm particle size, with C18 analytical guard column, 4.0 x 2.0 mm, according to *Maraschiello et al*. (1996). The injected volume was 10 µL. The mobile phase was isopropanol-acetonitrile (20:80, v/v) at a flow rate of 1.2 mL/min, isocraticaly. Detection was performed at 210 nm. Total analysis time lasted 10 min. Quantification of cholesterol was done by external standardization. Empower Pro software was used to control the HPLC system as well as for data acquisition and data processing, as described by *Spirić et al.*, 2009. Analyses were done at the Institute of Hygiene and Meat Technology, Belgrade.

Statistical analysis

The average results are presented as means ± SD. The differences between the mean values of the studied parameters were calculated using one-way analysis of variance (ANOVA), at 0,01 significance. When significant inter-group differences were determined ($p \leq 0,01$) further statistical analysis was performed using Tukey HSD test. Calculations were performed by the Statistica 10 program (StatSoft Inc.).

Results and discussion

Results of chemical composition and cholesterol content in fillets of two-years old carp, silver carp, grass carp, catfish and zander, which were farmed in polyculture in semi-intensive system, where feeding was done by adding corn and wheat in ratio 80:20 are shown in Table 1. Water content was the highest in catfish (78.69 ± 0.12), followed by zander (77.58 ± 0.11), silver carp (77.00 ± 0.36), grass carp (76.22 ± 1.03), and the lowest was in common carp (75.02 ± 0.29). The amount of protein was the highest in zander fillets (19.21 ± 0.03), followed by silver carp fillets (18.02 ± 0.15), catfish (17.27 ± 0.10), carp (15.59 ± 0.21) and the lowest percentage of protein was found in grass carp fillets (14.8 ± 0.12). Percentage of fat ranged from 1.74 ± 0.10, in the muscles of zander, to 6.85 ± 0.14 in the meat of carp. Fat percentage in the fillets of catfish, carp and grass carp was 3.43 ± 0.08; 4.07 ± 0.05 and 6.39 ± 0.24, respectively. Ash content was 0.84 ± 0.03 for grass carp, 0.89 ± 0.035 for carp, 0.89 ± 0.03 for catfish, 1.04 ± 0.02 for zander and 1.18 ± 0.01 for silver carp. The total cholesterol content was the highest in silver carp fillets (65.90 ± 0.29), followed by grass carp (65.07 ± 0.13), common carp (57.8 ± 0.11), zander (42.45 ± 0. 17) and the lowest amount of cholesterol was found in catfish (33.00 ± 0.56).

The obtained fat percentage in silver carp muscle was lower compared to the results obtained by

Table 1. Chemical composition of two years old fish reared in polyculture
Tabela 1. Hemijski sastav dvogodišnje ribe gajene u polikulturi

Parameters/ Parametri	**Common carp/ Šaran *Cyprinus carpio***	**Silver carp/ beli tolstolobik *Hypophthalmichthys molitrix***	**Grass carp/ Amur *Ctenopharyngodon idella***	**Wels catfish/ Som *Silurus glanis***	**Zander/ Smuđ zander *Stizostedion lucioperca***
Moisture content/ Sadržaj vlage, %	75.02 ± 0.29a	77.00 ± 0.36b	76.22 ± 1.03c	78.69 ± 0.12d	77.58 ± 0.11b
Protein content/ Sadržaj proteina, %	15.59 ± 0.21a	18.02 ± 0.15b	14.68 ± 0.12c	17.27 ± 0.10d	19.21 ± 0.03e
Fat content/Sadržaj masti, %	6.85 ± 0.14a	4.07 ± 0.05b	6.39 ± 0.24c	3.43 ± 0.08d	1.74 ± 0.10e
Ash content/ Sadržaj pepela, %	0.89 ± 0.035a	1.18 ± 0.01b	0.84 ± 0.03c	0.89 ± 0.03a	1.04 ± 0.02d
Total cholesterol / Ukupni holesterol, mg/100g	57.8 ± 0.11a	65.90 ± 0.29b	65.07 ± 0.13c	33.00 ± 0.56d	42.45 ± 0.17e

Legend/Legenda: Values are means ± SD (n = 12); Values in the same row with different letter notation statistically significantly differ at $p < 0.01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12);Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$

Domaizon et al. (2000), who examined one-year and three-years old silver carp and measured the lipid content in fillets in the range of 4.51 to 6.7%. According to the obtained results, fat content in catfish was 3.43, slightly higher compared to value of 2,33 obtained by *Jankovsaka et al.* (2004) for catfish farmed in ponds with natural food. The obtained values for proteins in zander are higher compared to studies of *Celik et al.* (2005) by which the percentage of proteins was in the range of 18,1 in the cold, to 18.8 in the warm lake. Fat content in our examination was, also, higher than values obtained by the mentioned authors for zander.

Table 2 presents results of chemical analysis and total cholesterol content in one, two and three years old carp, which are sampled from the same pond, where the production took place in semi-intensive conditions with corn and wheat added into diet (80:20). The percentage of water ranged from 77.78 ± 0.07 for one-year old, 75.01 ± 0.29 for two year old to 71.04 ± 0.20 in three year old carp. Protein content was the highest in the meat of one-year old carp (16.86 ± 0.19), followed by two-years old (15.59 ± 0.21), while the lowest value was detected in meat of three-years old carp (14.44 ± 0.16). Fat percentage was the lowest value in one-year old carp (4.41 ± 0.11) and the highest in fillets of three year old carp (11.73 ± 0.11). Fat content in meat of two years old carp was 6.85 ± 0.14%. Ash content, expressed as a percentage, was 0.84 ± 0.01 in three--years old fish, 0.89 ± 0.04 in two year old and 0.94 ± 0.01 in the yearling carp. The amount of total cholesterol was the lowest in one-year carp (37.94 ± 0.02). in the fat of biannual carp it was 57.8 ± 0.11 mg/100 g, while the largest amount of cholesterol measured in the fat of three-years old carp (59.75 ± 09).

Trbović et al. (2009) determined the amount of cholesterol in lipids of one year old carp in April and it was 48.87 ± 2.18 mg/100 g and in samples that were collected in June it was 54.31±1.13 mg/100g. In our studies, the amount of total cholesterol in yearlings is lower, the sampling was done in December. And, according to results published by *Vasha and Tvrzicka* (1995), the amount of cholesterol in the meat of carp was lower during the winter months. Determined cholesterol content in lipids of carp varies considerably in the works of different authors and it is in the range of 47 to 120 mg/100 g, which is consistent with our results for amount of cholesterol in fat of two and three years old carp (*Vacha and Tvrzicka*, 1995; *Bieniarz et al.*, 2001; Kopica and Vavreinova, 2007), but it must be taken into account that tests were carried out in different seasons and at different age categories.

Chemical composition and total cholesterol content in samples of two-years old carp fillets which were sampled from the pond where the diet consisted of added pelleted complete feed in fish farmed ponds where feeding was done by addintion corn and wheat in proportion 80:20, are shown in Table 3. The amount of water, proteins and ash was higher in carp fed diet with added pelleted food. and amounted 78.36 ± 0.04, 17.17 ± 0.05 and 1.03 ± 0.01 respectively, while values of the same parameters in carp whose diet consisted of corn and wheat were 75.01 ± 0.29, 15.59 ± 0.21 and 0.89 ± 0.04 rspectively. The percentage of fat in carp from more intensive production was 3.19 ± 0.05, and for two year old fish from semi-intensive production it was 6.85 ± 0.14. Cholesterol content was higher in carp fed grain (57.8 ± 0.11), compared to carp fed pelleted food (51.31 ± 0.12).

Table 2. Chemical composition of one, two and three years of carp reared in the same conditions
Tabela 2. Hemisjki sastav jednogodišnjih, dvogodišnjih i trogodišnjih šarana gajenih u istim uslovima

Parameters/ Parametri	Carp, one year old/ Jednogodišnji šaran	Carp, two years old/ Dvogodišnji šaran	Carp, three years old/ Trogodišnji šaran
Moisture content/ Sadržaj vlage, %	77.78 ± 0.07a	75.01 ± 0.29b	71.04 ± 0.20c
Protein content/ Sadržaj proteina, %	16.86 ± 0.19a	15.59 ± 0.21b	14.44 ± 0.16c
Fat content/ Sadržaj masti, %	4.41 ± 0.11a	6.85 ± 0.14b	11.73 ± 0.11c
Ash content/ Sadržaj pepela, %	0.94 ± 0.01a	0.89 ± 0.04b	0.84 ± 0.01c
Total cholesterol / Ukupni holesterol, mg/100g	37.94 ± 0.02a	57.8 ± 0.11b	59.75 ± 09c

Legend/Legenda: Values are means ± SD (n = 12); Values in the same row with different letter notation differ significantly statistically at $p < 0.01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12); Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$.

Table 3. Chemical composition of two-year old carp fed with different food
Tabela 3. Hemijski sastav mesa dvogodišnjeg šarana hranjenog različitom hranom

Parameters/ Parametri	Carp, two years old. pelleted feed/ Dvogodišnji šaran, peletirna hrana	Carp, two years old, corn and wheat/ Dvogodišnji šaran, kukuruz i pšenica
Moisture content/Sadržaj vlage, %	78.36 ± 0.04a	75.01 ± 0.29b
Protein content/Sadržaj proteina %	17.17 ± 0.05a	15.59 ± 0.21b
Fat content/Sadržaj masti %	3.19 ± 0.05a	6.85 ± 0.14b
Ash content/Sadržaj pepela, %	1.03 ± 0.01a	0.89 ± 0.04b
Total cholesterol / Ukupni holesterol, mg/100g	51.31 ± 0.12a	57.8 ± 0.11b

Legend/Legenda: Values are means ± SD (n = 12); Values in the same row with different letter notation statistically significantly differ at $p < 0.01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12); Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$

Table 4 presents the results for chemical analysis and total cholesterol content in three-years old carps, which were grown in different ponds. Carp, which was grown in semi intensive production conditions and was fed corn and wheat. in the ratio of 80:20, had a moisture content of 71.04 ± 0.20%, protein 14.44 ± 0.16%, fat 11.73 ± 0.11%, ash 0.84 ± 0.01% and the amount of total cholesterol was 59.75 ± 0.09 mg/100g. Values in percentages of water, protein, fat and ash measured in fillets of three-year old carp, grown in semi-intensive conditions. which was fed barley. maize and wheat (40:30:30) amounted to, 70.67 ± 0.06, 15.81 ± 0.18, 11.73 ± 0.11, 0.93 ± 0.02 respectively, and the total cholesterol content was 66.07±0.04 mg/100g. Moisture content, protein, fat and ash percentage in the three-year old carp, fed complete feed mixture were 70.94 ± 0.06, 17.68 ± 0.12, 10.41 ± 0.06 and 0.94 ± 0.02, and the amount of cholesterol was 36.14 ± 0.04 mg/100g.

(SFA) was the highest in silver carp (34.05 ± 0.08) and lowest in common carp (24.23 ± 0.06). Dominant saturated fatty acids were: palmitic fatty acid (C16:0), which ranged from 17.33% in common carp to 23.04% in grass carp, stearic acid (C18:0) in the amount of 3.37% (grass carp) to 7.04% (catfish), myristic acid (C14:0) with the lowest content common carp (0.72%) and the highest in silver carp (3.82%). In low concentrations, in all species, the following acids were present: lauric (C12:0), in the amount of 0.12% in grass carp to 0.44% in silver carp; pentadecylic (C15:0), 0.01% in common carp up to 1.02% in silver carp; margaric (C17:0). whose content was also the highest in silver carp (1.37%); and arachidonic (C20:0). The most abundant monounsaturated fatty acid was oleic (C18:1. n9), in the amount of 22.56% in silver carp to 51.35% in common carp, followed by palmitooleic (C16:1. n7) and 11-eicosenic (C20:1). Silver carp contained the

Table 4. Chemical composition of three-year old carp grown in different fish ponds
Tabela 4. Hemijski sastav mesa trogodišnjeg šarana iz različitih ribnjaka

Parameters/ Parametri	Carp, three years old. corn and wheat/ Šaran trogodišnji, kukuruz i pšenica	Carp, three years old, barley wheat and corn/ Šaran trogodišnji, ječam, pšenica i kukuruz	Carp, three years old, complete feed/ Šaran trogodišnji, kompletna smeša
Moisture content/ Sadržaj vlage, %	71.04 ± 0.20a	70.67 ± 0.06b	70.94 ± 0.06a
Protein content/Sadržaj proteina, %	14.44 ± 0.16a	15.81 ± 0.18b	17.68 ± 0.12c
Fat content/Sadržaj masti, %	11.73 ± 0.11a	11.73 ± 0.11a	10.41 ± 0.06b
Ash content/Sadržaj pepela, %	0.84 ± 0.01a	0.93 ± 0.02b	0.94 ± 0.02b
Total cholesterol / Ukupni holesterol, mg/100g	59.75 ± 0.09a	66.07 ± 0.04b	36.14 ± 0.04c

Legend/Legenda: Values are means ± SD (n = 12); Values in the same row with different letter notation statistically significantly differ at $p < 0.01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12); Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$

Fatty acid composition of the two-years old carp, silver carp, grass carp, catfish and zander is hown in Table 5. The amount of saturated fatty acids

least amount of monounsaturated fatty acids (MUFA) (39.04%), but the largest percentage was measured in carp (64.34%). Silver carp contained the higest

percentage of polyunsaturated fatty acids (PUFA) 24.23%, of which 18.17% were n-3 and 6.07% n-6. The lowest percentage of PUFA was detected in common carp, which contained 10.95% and the n-3/ n-6 ratio was 0.14. PUFA/SFA, which is an indicator of the quality of lipids in the examined fish was 0.45 (common carp), 0.51 (catfish), 0.53 (zander), 0.63 (grass carp) and the most favourable was in silver carp 0.71 ± 0.01. Also, significant is the ratio of unsaturated (UFA) to saturated (SFA) fatty acids in fish lipids. For studied the species ratio was the best in the fat of common carp 3.14 ± 0.01, then 2.27 in zander, 2.44 in grass carp; 2.03 in catfish and 1.94 in fat of silver carp.

Table 5. Fatty acid composition of two years old fish farmed in the same conditions
Tabela 5. Sastav masnih kiselina u mesu dvogodišnjeg šarana odgajanog u istim uslovima

Fatty acids/ Masne kiseline, %	Common carparp/ Šaran *Cyprinus carpio*	Silver carp/ Beli tolstolobik *Hypophthalmichthys molitrix*	Grass carp/ Amur *Ctenopharyngodon idella*	Wels catfish/ Som *Silurus glanis*	Zander/ Smuđ zander *Stizostedion lucioperca*
Lauric acid/ Laurinska kiselina, C12:0	0.14 ± 0.01a	0.44 ± 0.02b	0.12 ± 0.01c	0.23 ± 0.02d	0.14 ± 0.01a
Myristic acid/Miristoleinska kiselina. C14:0	0.72 ± 0.01a	3.82 ± 0.02b	1.62 ± 0.01c	2.32 ± 0.01d	0.94 ± 0.01e
Pentadecanoic acid/ Pentadekanska kiselina, C15:0	0.01 ± 0.01a	1.02 ± 0.01b	0.32 ± 0.00c	0.85 ± 0.01d	0.32 ± 0.02c
Palmitic acid/ Palmitinska kiselina, C16:0	17.33 ± 0.06a	22.12 ± 0.05b	23.04 ± 0.01c	21.04 ± 0.14d	22.07 ± 0.16b
Palmitoleic acid/ Palmitoleinska kiselina, C16:1	6.23 ± 0.01a	10.32 ± 0.02b	10.73 ± 0.01c	11.34 ± 0.05d	6.16 ± 0.03e
Margaric acid/ Margarinska kiselina, C17:0	0.12 ± 0.01a	1.37 ± 0.01b	0.41 ± 0.00c	1.26 ± 0.05d	0.45 ± 0.00e
Stearic acid/ Stearinska kiselina, C18:0	5.79 ± 0.02a	5.02 ± 0.03b	3.37 ± 0.10c	7.04 ± 0.05d	6.50 ± 0.06e
Oleic acid/ oleinska kiselina, C18:1cis-9	51.35 ± 0.04a	22.56 ± 0.01b	34.90 ± 0.06c	26.23 ± 0.10d	38.34 ± 0.12e
Vaccenic acid/ Vakcenska kiselina,C18:1cis-11	4.54 ± 0.04a	4.89 ± 0.07b	4.60 ± 0.05a	8.24 ± 0.10c	4.56 ± 0.04a
Linoleic acid/ Linolna kiselina, C18:2. ω-6	8.75 ± 0.06a	5.00 ± 0.01b	11.28 ± 0.04c	6.16 ± 0.03d	7.05 ± 0.05e
Linolenic(GLA)/ Linolenska kiselina C18:3.ω-6	0.12 ± 0.01a	0.24 ± 0.01b	0.12 ± 0.01a	0.15 ± 0.01c	0.12 ± 0.00a
α-Linolenic/ α-Linolenska kiselina, C18:3. ω-3	0.64 ± 0.00a	5.24 ± 0.01b	3.27 ± 0.01c	3.06 ± 0.04d	0.97 ± 0.02e
Arachidic acid/ Arahidska kiselina, C20:0	0.12±0.01a	0.26±0.01b	0.15±0.00c	0.22±0.00d	0.18 ± 0.01e
Eicosenoic acid/ Eikosenska kiselina, C20:1	2.22±0.01a	1.27 ± 0.01b	1.06 ± 0.01c	2.20 ± 0.06a	1.67 ± 0.01d
Behenic acid/ Behenska kiselina, C20:2	0.3 ± 0.04a	0.36 ± 0.01b	0.46 ± 0.01c	0.61 ±0 .02d	0.28 ± 0.00a
Dihomo-gamma-linolenic acid/ Di-homo-gama-linolenska kiselina, C20:3. ω-6	0.46 ± 0.02a	0.46 ± 0.01a	0.74 ± 0.01b	0.47 ± 0.02a	0.34 ± 0.02c
Eicosatrienoic acid/ Eikosatrienoična kiselina, C20:3, ω-3	0.06 ± 0.00a	0.60 ± 0.01b	0.38 ± 0.01c	0.58 ± 0.02d	0.27 ± 0.02e
Erucic acid + Arachidonic acid/ Eruična kiselina + arahidonska kiselina. C22:1+20:4	0.74 ± 0.01a	2.75 ± 0.01b	1.44 ± .01c	2.01 ± 0.05d	2.58 ± 0.08e
Eicosapentaenoic acid/ Eikosapentaenska kisleina, C20:5. ω-3	0.19 ± 0.02a	4.46 ± 0.05b	0.49 ± 0.01c	1.16 ± 0.03d	1.24 ± 0.03e
Docosapentaenoic acid/ Dokosapentaenska kiselina, C22:5. ω-3	0.18 ± 0.01a	1.14 ± 0.01b	0.50 ± 0.00c	1.47 ± 0.06d	0.68 ± 0.01e

Docosahexaenoic acid/ Dokosaheksaenska kiselina, C22:6. ω-3	0.25 ± 0.01a	6.73 ± 0.11b	1.01 ± 0.00c	3.28 ± 0.18d	5.16 ± 0.14e
SFA/ZMK	24.23 ± 0.06a	34.05 ± 0.08b	29.03 ± 0.09c	32.96 ± 0.20d	30.62 ± 0.17e
MUFA/MNMK	64.34 ± 0.06a	39.04 ± 0.08b	51.29 ± 0.08c	48.01 ± 0.17d	50.72 ± 0.13e
PUFA/PNMK	10.95 ± 0.09a	24.23 ± 0.18b	18.26 ± 0.04c	16.94 ± 0.31d	16.11 ± 0.19e
ω-6	9.63 ± 0.08a	6.07 ± 0.03b	12.61 ± 0.04c	7.39 ± 0.06d	7.78 ± 0.06e
ω-3	1.32 ± 0.02a	18.17 ± 0.17b	5.65 ± 0.02c	9.55 ± 0.26d	8.33 ± 0.16e
ω-3/ω-6	0.14 ± 0.00a	2.99 ± 0.02b	0.45 ± 0.00c	1.29 ± 0.03d	1.07 ± 0.02e
ω-6/ω-3	7.28 ± 0.08a	0.33 ± 0.00b	2.23 ± 0.01c	0.77 ± 0.02d	0.93 ± 0.02e
PUFA/SFA PNMK/ZMK	0.45 ± 0.00a	0.71 ± 0.01b	0.63 ± 0.00c	0.51 ± 0.01d	0.53 ± 0.01e
UFA/SFA NMK/ZMK	3.14 ± 0.01a	1.94 ± 0.01b	2.44 ± 0.01c	2.03 ± 0.02d	2.27 ± 0.02e

Legend/Legenda: SFA-saturated fatty acids/zasićene masne kiselina. MUFA-monounsaturated fatty acids/mono nezasićene masne kiseline/USFA unsaturated fatty acids/ nezasićene masne kiseline, PUFA-polyunsaturated fatty acids from the n-3 (n-3 PUFA) and n-6 (n-6 PUFA) families/poli nezasićene masne kiseline iz n-3 (n-3 PNMK) i n-6 (n-6 PNMK) grupa

Values are means ± SD (n = 12); Values in the same row with different letter notation statistically significantly differ at $p < 0.01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12); Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$.

The total value of saturated fatty acids in research of *Jankowska et al.* (2004), in catfish farmed traditionally, was 25. 41. In our experiment it was higher (32.96), probably because of the amount of the dominant palmitic acid that was 15. 89% in the research of *Jankowska et al.* (2004) and in our trial it was 21.04%. Also, we determined higher amount of C18:0, which was 7.04%, but *Jankowska et al.* (2004) detected 5.85%. The amount of C14:0 acid (mirystic), C15:0 and C20:0 was similar in both trials. The unsaturated fatty acids were the largest group 74.59% (*Jankowska et al.,* 2004) and in our trial 66.96 %. We established the amount of MUFA of 48.01%. PUFA 16.94 and n3/n6 ratio was 1.29. In research of *Jankowska et al.* (2004). these values were 39.86%, 34.73% and 2.31 respectively. According to *Bieniarz et al.* (2000), the meat of catfish cultivated in a polyculture with common carp has 21.85% PUFA, and the n-3/n-6 ratio of 2.39, but *Fullner and Wirth*. (1996) reported that value of n-3/n-6 was 1.7. The total saturated fatty acid content in lipids was in the range from 30.5 to 32.9% (*Celik et al.,* 2005) in zander caught from two lakes, which is the same value like in our experiments (30.62%). Thus, the fatty acids found in both species (about 70%) were mono and polyunsaturated fatty acids (MUFA + PUFA). The major fatty acids identified in zander were 16:0, 18:0, 18:1 n-9, 18:2 n-6, 20:5 n-3 (EPA) and 22:6 n-3 (DHA). Palmitic acid was the primary saturated fatty acid in lipids of zander. contributing approximately with 66% to the total saturated fatty acid content of the lipids. Similar results were noted for wild zander (*Jankowska et al*., 2003) and for zander caught from two lakes (*Celik et al.,* 2005). Oleic acid was identified as the primary monounsaturated fatty acid. Among the n-3 series, zander is good sources of EPA (1.24%) and DHA (5.16%).

It has been reported that the types and amounts of fatty acids in fish tissues vary with the geographic location, size, age, what the fish eat, reproductive status and seasons (*Leger et al.,* 1977; *Bandarra et al.,* 1997) silver carp and grass carp fed on phytoplankton, zooplankton, macrophytes and are rich in n-3 polyunsaturated fatty acids, especially eicosapentaenoic and docosahexaenoic acids (*Steffans and Wirth*, 2005). The proportion of total n-3 fatty acids varies between 20 and 30% and the n-3/n-6 ratio is about 2 to 3. According to our results, n3/n6 in silver carp was 2.99, which is in agreement with the results of *Steffans and Wirth* (2005), while this ratio in grass carp was lower - 0.45. This can be attributed to the changes in natural food for grass carp, in the pond the amount of macrophyte vegetation was decreased. and grass carp were predominantly fed additional nutrients. Presented by *Domaizona et al.* (2000). the n3/n6 ratio in fillets of silver carp ranged from 1.18 for one-year carp to 1.9 in three-years old carp, while it should be noted that in our study the two-years old silver carp fillets were tested. Silver carp contained significantly higher amount of docosahexaenoic acid in relation to other studied species, which is in agreement with *Domaizon et al.* (2000). He, also, found a high content of n-3 fatty acids in silver carp fillets and showed that the content of these fatty acids in silver carp increased with age of this species due to changes in diet with age. Zooplankton appears as the major contributor to the diet of the one year old silver carp (90.3% of ingested biomass), whereas three year old silver carp exhibited a more evenly

balanced food spectrum between zooplankton (44.8% of ingested biomass) and phytoplankton (55.2% of ingested biomass), (*Domaizon et al.,* 2000). The amount of zooplankton in the nutrition of silver carp decreased with age, while the content of phytoplankton increased (*Shapiro*, 1985). Thus, the content of docosahexaenoic acid in their research of one-year old carp was 2.56% and 7.76% in three--years old silver carp, while in our studies, in fillets of yearlings 6.73% of this fatty acid was measured.

Table 6 shows the percentage ratio of fatty acids in common carp of different age sampled from the same pond, where the production was semi- intensive and diet was supplemented using corn and wheat, as the energy component of food, in relation 80:20. The most favourable UFA/SFA ratio was observed in three-years old carp (3.18), then in two year old 3.14 and in one-year old carps 3.12 ± 0.01. P/S ratio was 0.53 in one-year old carp, 0.45 in two years old carp and 0.42 in the three-years old carp. In all three age groups the most common were monounsaturated fatty acid (61.4% in one-year and 64. 9% in three--year old carp), followed by saturated fatty acids (SFA), (from 23.93% in three-years old to 24.98% in the lipids of yearlings). PUFA content ranged from 10.17% in fat of three-years old to 12.89% in one-year old carp. N3/n6 ratio was between 0.1 (three--years old) and 0.16 (yearling).

Table 6. fatty acid composition of one, two and three years old common carp reared in the same conditions
Tabela 6. Sastav masnih kiselina u mesu jednogodišnjih, dvogodišnjih i trogodišnjih šarana gajenih u istim uslovima

Fatty acids/ Masne kiseline, %	Carp, one years old/ Jednogodišnji šaran	Carp, two years old/ Dvogodišnji šaran	Carp, three years old/ Trogodišnji šaran
Lauric acid/ Laurinska kiselina, C12:0	0.14 ± 0.01a	0.14 ± 0.01ab	0.13 ± 0.01ac
Myristic acid/ Miristoleinska kiselina, C14:0	0.59 ± 0.02a	0.72 ± 0.01b	0.75 ± 0.01c
Pentadecanoic acid/ Pentadekanska kiselina, C15:0	0.1 ± 0.02a	0.01 ± 0.01b	0.02 ± 0.02b
Palmitic acid/ Palmitinska kiselina, C16:0	17.11 ± 0.08a	17.33 ± 0.06b	16.93 ± 0.03c
Palmitoleic acid/ Palmitoleinska kiselina, C16:1	5.78 ± 0.02a	6.23 ± 0.01b	6.01 ± 0.02c
Margaric acid/ Margarinska kiselina, C17:0	0.18 ± 0.01a	0.12 ± 0.01b	0.14 ± 0.01c
Stearic acid/Stearinska kiselina, C18:0	6.02 ± 0.01a	5.79 ± 0.02b	5.84 ± 0.01c
Oleic acid/ oleinska kiselina, C18:1cis-9	54.00 ± 0.04a	51.35 ± 0.04b	51.76 ± 0.13c
Vaccenic acid/ Vakcenska kiselina,C18:1c is-11	0 ± 0.00a	4.54 ± 0.04b	4.71 ± 0.02c
Linoleic acid/ Linolna kiselina. C18:2, ω-6	9.74 ± 0.05a	8.75 ± 0.06b	8.17 ± 0.02c
Linolenic(GLA)/ Linolenska kiselina C18:3,ω-6	0.24 ± 0.01a	0.12 ± 0.01b	0.12 ± 0.00b
α-Linolenic/ α-Linolenska kiselina, C18:3, ω-3	0.74 ± 0.01a	0.64 ± 0.00b	0.28 ± 0.01c
Arachidic acid/ Arahidska kiselina, C20:0	0.14 ± 0.01a	0.12 ± 0.01b	0.12 ± 0.01b
Eicosenoic acid/Eikosenska kiselina, C20:1	1.63 ± 0.01a	2.22 ± 0.01b	2.44 ± 0.01c
Behenic acid/Behenska kiselina, C20:2	0.34 ± 0.01a	0.3 ± 0.04b	0.28 ± 0.01b
Dihomo-gamma-linolenic acid/ Di-homo-gama-linolenska kiselina. C20:3, ω-6	0.84 ± 0.08a	0.46 ± 0.02b	0.70 ± 0.04c
Eicosatrienoic acid/ Eikosatrienoična kiselina, C20:3, ω-3	0.07 ± 0.01a	0.06 ± 0.00b	0.02 ± 0.02c
Erucic acid + Arachidonic acid/ Eruična kiselina + arahidonska kiselina, C22:1+20:4	1.46 ± 0.02a	0.74 ± 0.01b	0.99 ± 0.04c
Eicosapentaenoic acid/ Eikosapentaenska kisleina, C20:5, ω-3	0.23 ± 0.01a	0.19 ± 0.02b	0.14 ± 0.01c
Docosapentaenoic acid/ Dokosapentaenska kiselina, C22:5, ω-3	0.28 ± 0.01a	0.18 ± 0.01b	0.16 ± 0.01c

Docosahexaenoic acid/ Dokosaheksaenska kiselina. C22:6, ω-3	0.42 ± 0.02a	0.25 ± 0.01b	0.30 ± 0.02c
SFA/ZMK	24.28 ± 0.09a	24.23 ± 0.06a	23.93 ± 0.05b
MUFA/MNMK	61.41 ± 0.05a	64.34 ± 0.06b	64.92 ± 0.12c
PUFA/PNMK	12.89 ± 0.09a	10.95 ± 0.09b	10.17 ± 0.06
ω-6	11.15 ± 0.09a	9.63 ± 0.08b	9.27 ± 0.04c
ω-3	1.74 ± 0.03a	1.32 ± 0.02b	0.90 ± 0.03c
ω-3/ω-6	0.16 ± 0.00a	0.14 ± 0.00b	0.1 ± 0.00c
ω-6/ω-3	6.41 ± 0.14a	7.28 ± 0.08b	10.28 ± 0.37c
PUFA/SFA PNMK/ZMK	0.53 ± 0.00a	0.45 ± 0.00b	0.42 ± 0.00c
UFA/SFA NMK/ZMK	3.12 ± 0.01a	3.14 ± 0.01b	3.18 ± 0.01c

Legend/Legenda: SFA-saturated fatty acids/zasićene masne kiselina, MUFA monounsaturated fatty acids/mono nezasićene masne kiseline. USFA-unsaturated fatty acids/ nezasićene masne kiseline. PUFA- polyunsaturated fatty acids from the n-3 (n-3 PUFA) and n-6 (n-6 PUFA) families/poli nezasićene masne kiseline iz n-3 (n-3 PNMK) i n-6 (n-6 PNMK) grupa

Values are means ± SD (n=12); Values in the same row with different letter notation differ significantly statistically at $p < 0.01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12); Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$.

Fatty acid profile of two-years old carp, one of which was fed with corn and wheat (80:20) and another group was sampled from fish ponds where feeding was done by adding food pellets. is shown in Table 7. In two years old carp fed pelleted feed better ratio UFA/SFA was observed (3.46. compared with 3.14 in carp fed grain); the obtainedPUFA/SFA was 1.39 compared to 0.45, n3/n6 was 0.26 versus 0.14. Higher content of PUFA (31.04 compared with 10.95) and less SFA (22.4 versus 24.23) was obtained, too. Lipids of carp in more intensive production contained less MUFA (45.12%) compared to carp from the semi-intensive production (64.34%).

Table 7. Fatty acid composition of two-years old carp fed with different food
Tabela 7. Sastav masnih kiselina u mesu dvogodišnjih šarana hranjenih različitiom hranom

Fatty acids/ Masne kiseline, %	Carp, two years old, corn and wheat/ Dvogodišnji šaran, kukuruz i pšenica	Carp, two years old, pelleted feed/ Dvogodišnji šaran, peletirana hrana
Lauric acid/Laurinska kiselina, C12:0	0.14 ± 0.01a	0.10 ± 0.00b
Myristic acid/ Miristoleinska kiselina, C14:0	0.72 ± 0.01a	0.73 ± 0.01b
Pentadecanoic acid/ Pentadekanska kiselina, C15:0	0.01 ± 0.01a	0.23 ± 0.01b
Palmitic acid/Palmitinska kiselina, C16:0	17.33 ± 0.06a	16.89 ± 0.03b
Palmitoleic acid/ Palmitoleinska kiselina, C16:1	6.23 ± 0.01a	5.20 ± 0.04b
Margaric acid/ Margarinska kiselina, C17:0	0.12 ± 0.01a	0.18 ± 0.01b
Stearic acid/Stearinska kiselina, C18:0	5.79 ± 0.02a	4.16 ± 0.01b
Oleic acid/ oleinska kiselina, C18:1cis-9	51.35 ± 0.04a	34.45 ± 0.01b
Vaccenic acid/ Vakcenska kiselina, C18:1cis-11	4.54 ± 0.04a	2.93 ± 0.01b
Linoleic acid/ Linolna kiselina, C18:2. ω-6	8.75 ± 0.06a	22.56 ± 0.01b
Linolenic(GLA)/ Linolenska kiselina C18:3, ω-6	0.12 ± 0.01a	0.25 ± 0.01b

α-Linolenic/ α-Linolenska kiselina, C18:3, ω-3	0.64 ± 0.00a	2.12 ± 0.01b
Arachidic acid/ Arahidska kiselina, C20:0	0.12 ± 0.01a	0.10 ± 0.01b
Eicosenoic acid/ Eikosenska kiselina, C20:1	2.22 ± 0.01a	2.54 ± 0.01b
Behenic acid/Behenska kiselina, C20:2	0.3 ± 0.04a	0.73 ± 0.01b
Dihomo-gamma-linolenic acid/ Di-homo-gama-linolenska kiselina, C20:3. ω-6	0.46 ± 0.02a	1.02 ± 0.01b
Eicosatrienoic acid/ Eikosatrienoična kiselina, C20:3. ω-3	0.06 ± 0.00a	0.71 ± 0.01b
Erucic acid + Arachidonic acid/ Eruična kiselina + arahidonska kiselina, C22:1+20:4	0.74 ± 0.01a	1.43 ± 0.01b
Eicosapentaenoic acid/ Eikosapentaenska kisleina, C20:5. ω-3	0.19 ± 0.02a	0.93 ± 0.01b
Docosapentaenoic acid/ Dokosapentaenska kiselina, C22:5. ω-3	0.18 ± 0.01a	0.85 ± 0.02b
Docosahexaenoic acid/ Dokosaheksaenska kiselina, C22:6. ω-3	0.25 ± 0.01a	1.86 ± 0.04b
SFA/ZMK	24.23 ± 0.06a	22.40 ± 0.03b
MUFA/MNMK	64.34 ± 0.06a	45.12 ± 0.03b
PUFA/PNMK	10.95 ± 0.09a	31.04 ± 0.03b
ω-6	9.63 ± 0.08a	24.57 ± 0.03b
ω-3	1.32 ± 0.02a	6.48 ± 0.04b
ω-3/ω-6	0.14 ± 0.00a	0.26 ± 0.00b
ω-6/ω-3	7.28 ± 0.08a	3.79 ± 0.02b
PUFA/SFA PNMK/ZMK	0.45 ± 0.00a	1.39 ± 0.00b
UFA/SFA NMK/ZMK	3.14 ± 0.01a	3.46 ± 0.01b

Legend/Legenda: SFA saturated fatty acids/zasićene masne kiselina. MUFA monounsaturated fatty acids/mono nezasićene masne kiseline. USFA unsaturated fatty acids/ nezasićene masne kiseline. PUFA polyunsaturated fatty acids from the n-3 (n-3 PUFA) and n-6 (n-6 PUFA) families/poli nezasićene masne kiseline iz n-3 (n-3 PNMK) i n-6 (n-6 PNMK) grupa
Values are means ± SD (n = 12); Values in the same row with different letter notation statistically significantly differ at $p < 0.01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12); Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$

The content of n-3 in two-years old carp in this study was lower than in the two-years carp fed only natural food from the pond, which was recorded by *Ćirković et al.* (2010). The amount of n-3 in naturally fed carp was 9.85%, compared to the two year old carp fed pelleted feed (6.48%), and in relation to two years old carp in which the dominant feed was corn and the content of n-3 was 1.32%. In carp fed pelleted food a higher content of n-6 fatty acids was established. compared to the data presented by *Ćirković et al.* (2010) for carp fed only natural food (24.57% versus 17.63%), so that the total content of PUFA was higher in carp fed with pelleted food.

Percentages of fatty acids in lipids in three-year old carp sampled from three ponds with different feeding regimes are shown in Table 8. PUFA/SFA ratio was the most favourable in carp fed complete food (1.32), and less in carp fed with maize and wheat (0.42). UFA/SFA ratio was also the best in carp fed a complete feed (3.51), while in carp fed maize and wheat it was 3.18 and for three year old carp fed barley, maize and wheat the ratio was 3.00. due to high content of MUFA in lipids of carp fed corn and wheat (64.78%), which was lower in the carp fed with barley, maize and wheat (57.98%) and the lowest in common carp fed a complete food (47.98%).

Table 8. Fatty acid composition of three-year old carp grown in different conditions
Tabela 8. Sastav masnih kiselina u trogodišnjim šaranima gajenim u različitim uslovima

Fatty acids/ Masne kiseline, %	Carp, three years old, corn and wheat/ Trogodišnji šaran. kukuruz i pšenica	Carp, three years old, barley, wheat and corn/ Trogodišnji šaran, ječam, kukuruz i pšenica	Carp, three years old, complete feed/ Trogodišnji šaran, kompletna smeša
Lauric acid/Laurinska kiselina, C12:0	0.13 ± 0.01a	0.16 ± 0.02b	0.15 ± 0.02b
Myristic acid/Miristoleinska kiselina, C14:0	0.75 ± 0.01a	0.76 ± 0.01a	0.74 ± 0.01b
Pentadecanoic acid/Pentadekanska kiselina, C15:0	0.02 ± 0.02a	0.11 ± 0.00b	0.1 ± 0.00b
Palmitic acid/Palmitinska kiselina, C16:0	16.93 ± 0.03a	18.38 ± 0.15b	16.04 ± 0.05c
Palmitoleic acid/ Palmitoleinska kiselina, C16:1	6.01 ± 0.02a	7.28 ± 0.05b	4.32 ± 0.01c
Margaric acid/ Margarinska kiselina, C17:0	0.14 ± 0.01a	0.20 ± 0.01b	0.18 ± 0.00c
Stearic acid/Stearinska kiselina, C18:0	5.84±0.01a	5.21±0.05b	4.88±0.00c
Oleic acid/Oleinska kiselina, C18:1cis-9	51.76 ± 0.13a	44.63 ± 0.11b	41.96 ± 0.07c
Vaccenic acid/Vakcenska kiselina,C18:1cis-11	4.71 ± 0.02a	4.24 ± 0.08b	0 ± 0.00c
Linoleic acid/ Linolna kiselina, C18:2. ω-6	8.17 ± 0.02a	12.32 ± 0.11b	24.06 ± 0.02c
Linolenic(GLA)/Linolenska kiselina, C18:3.ω-6	0.12 ± 0.00a	0.16 ± 0.02b	0.18 ± 0.01c
α-Linolenic/α-Linolenska kiselina, C18:3. ω-3	0.28 ± 0.01a	1.54 ± 0.02b	2.25 ± 0.02c
Arachidic acid/Arahidska kiselina, C20:0	0.12 ± 0.01a	0.11 ± 0.01b	0.10 ± 0.01b
Eicosenoic acid/Eikosenska kiselina, C20:1	2.44 ± 0.01a	2.02 ± 0.01b	1.7 ± 0.00c
Behenic acid/ Behenska kiselina, C20:2	0.28 ± 0.01a	0.40 ± 0.01b	0.64 ± 0.04c
Dihomo-gamma-linolenic acid/ Di-homo-gama-linolenska kiselina, C20:3. ω-6	0.70 ± 0.04a	0.60 ± 0.01b	0.79 ± 0.08c
Eicosatrienoic acid/ Eikosatrienoična kiselina, C20:3. ω-3	0.02 ± 0.02a	0.12 ± 0.01b	0.26 ± 0.01c
Erucic acid + Arachidonic acid/ Eruična kiselina + arahidonska kiselina, C22:1+20:4	0.99 ± 0.04a	0.69 ± 0.01b	0.59 ± 0.01c
Eicosapentaenoic acid/ Eikosapentaenska kisleina, C20:5. ω-3	0.14 ± 0.01a	0.40 ± 0.01b	0.28 ± 0.00c
Docosapentaenoic acid/ Dokosapentaenska kiselina, C22:5. ω-3	0.16 ± 0.01a	0.19 ± 0.01b	0.18 ± 0.01c
Docosahexaenoic acid/ Dokosaheksaenska kiselina, C22:6. ω-3	0.30 ± 0.02a	0.33 ± 0.02b	0.66 ± 0.02c
SFA/ZMK	23.93 ± 0.05a	24.93 ± 0.15b	22.19 ± 0.05c
MUFA/MNMK	64.78 ± 0.12a	57.98 ± 0.14b	47.98 ± 0.07c

PUFA/PNMK	10.17 ± 0.06a	16.06 ± 0.11b	29.30 ± 0.11c
ω-6	9.27 ± 0.04a	13.48 ± 0.10b	25.67 ± 0.09c
ω-3	0.90 ± 0.03a	2.58 ± 0.04b	3.64 ± 0.04c
ω-3/ω-6	0.10 ± 0.00a	0.19 ± 0.00b	0.14 ± 0.00c
ω-6/ω-3	10.28 ± 0.37a	5.22 ± 0.08b	7.06 ± 0.07c
PUFA/SFA NMK/ZMK	0.42 ± 0.00a	0.64 ± 0.01b	1.32 ± 0.01c
UFA/SFA NMK/ZMK	3.18 ± 0.01a	3.00 ± 0.02b	3.51 ± 0.01c

Legend/Legenda: SFA-saturated fatty acids/zasićene masne kiselina, MUFA-monounsaturated fatty acids/mono nezasićene masne kiseline, USFA-unsaturated fatty acids/nezasićene masne kiseline, PUFA-polyunsaturated fatty acids from the n-3 (n-3 PUFA) and n-6 (n-6 PUFA) families/poli nezasićene masne kiseline iz n-3 (n-3 PNMK) i n-6 (n-6 PNMK) grupa

Values are means ± SD (n = 12); Values in the same row with different letter notation statistically significantly differ at $p < .01$/Vrednosti u tabeli su srednje vrednosti ± SD (n = 12); Vrednosti u istom redu sa različitim slovnim oznakama se razlikuju signifikantno na nivou $p < 0.01$.

According to research conduced by *Buchtová et al.* (2010) and *Ćirković et al.* (2010), carp grown on natural food had a high content of both n-6 and n-3 fatty acids. while carp fed grains, which are characterized by low levels of n-3 PUFA (*Buchtová et al.*, 2010; *Ćirković et al*., 2011), contained lower concentrations of these fatty acids, becouse of a higher concentration of oleic acid *(Steffens et al*. 1998). The above statements are in agreement with our results, where higher content of oleic acid (51.76%) was observed in the carp fed corn, as dominant energy source. than in the carp fed with barley as dominant grain (44.63%). The lowest percentage of oleic acid has been reported in carp fed a complete mixture (41.96%). This difference is much more drastic in two-years old carp whose diet had the largest share of maize (51.35%), while the carp of the same age fed pelleted food contained 34.45% oleic acid. It is known that the application of formulated feed impact the values of many zootechnical coefficients, including, among others, the slaughter yield, proximate composition, and fatty acids profile (*Shearer*, 1994; *Jobling,* 2001).

All sampling was performed in the winter months, when the water temperature was low. *Cordier et al*. (2002) and *Tocher et al*. (2004) demonstrated the importance of temperature on fatty acid composition in lipids of fish. The most important effect of temperature is reflected in desaturation of fatty acids and their betatoxidation. so that proportion of unsaturated fatty acids decreases with the increase of temperatutre. The established n-3/n-6 ratios in different categories were in the range 0.1 to 0.26. The most favourable ratio was observed in two year old carp fed pelleted food and the least favourable in three-years old carp fed corn as dominant component in food. The obtained results are consistent with studies conducted by *Trbović et al.,* (2009) on the yearling carp, but the ratio is lower than the results obtained by *Ćirković et al*. (2010) for two years old carp fish fed with natural food. The dependence of n-3/n-6 relationship and the age and diet was established in our work. This ratio, also, varies widely between different species of fish, which is also confirmed. According to Steffans and *Wirth* (2005) the n-3/n-6 ratio in common carp varies to a large extent, between 0.08 and 2.4 and is most influenced by diet.

Freshwater fish contain high levels of n-3 polyunsaturated fatty acids, which are very important in human nutrition. Essential fatty acids affect the fluidity, flexibility, and permeability of membranes. They are precursors of the eicosanoids and are necessary for maintaining the impermeability barrier of the skin. They are also involved in cholesterol transport and metabolism *(Steffens and Wirth*, 2005). Components of fish are also important in the development and maintenance of the eyes. skin and nervous system (*Vladau et al.,* 2008). Since there are several biochemical interactions between n-6 and n-3 series, a balanced proportion of these fatty acids in the diet is important for the functioning of human and animal life.

Nutritive quality of freshwater fish is even better than quality of sea fish since fatty acid composition of freshwater fish is also characterized by high proportions of n-6 polyunsaturated fatty acids, especially linoleic and arachidonic acids. The ratio of total n-3 to n-6 fatty acids is much lower for freshwater fish than for sea fish (*Malović et al.*. 2010) and ranges from about 0.5 to 3. Unlike sea fish, freshwater fish are able to desaturate and elongate larger quantities of dietary C18 n-6 and C18 n-3 fatty acids to C20 and C22 desaturates (*Steffens and Wirth*, 2005) In addition to nutritional quality of fish from aquaculture in our country, due to the growing technologies in fish ponds, residues of antibiotic in meat of fish were not found (*Đorđević et al,* 2009).

Conclusion

Dependence of n-3/n-6 ratio with age and diet was established in our work. This ratio also varies widely between different species of fish, which is also confirmed. The class of PUFAs and HUFAs are crucial in terms of human feeding physiology. Fish provides not only n-3 fats, but the abundance of vitamins, minerals and nutrients. Fish proteins contain all essential amino-acids for the human organism, with a high biological value. Chemical composition of fish varies greatly from one species and one individual to another, depending on age, feed, environment and season. Lipid content of fish varies depending on type of fish, age, the time of the year and what the fish feeds on. Lipid content of farmed fish can vary widely depending on the feed used. Quantity of n-3 fatty acids varies largely in dependence on the fish species (herbivorous. omnivorous or carnivorous), on the age of fish and on origin of diets (natural food or cereal supplement) and its composition (rich primarily in PUFA n-3 or saccharides). Nutritive value of the examined freshwater fish is high, since their fatty acid composition is characterized by satisfactory proportion of n-3 polyunsaturated fatty acids and by high proportion of n-6 polyunsaturated fatty acids. especially linoleic and arachidonic acids.

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Kvalitet mesa riba gajenih u polikulturi u ribnjacima u Republici Srbiji

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R e z i m e: Kvalitet mesa jednogodišnjeg. dvogodišnjeg i trogodišnjeg šarana. dvogodišnjeg tolstolobika i amura, kao i dvogodišnjeg soma i smuđa, koji su gajeni u različitim sistemima proizvodnje i sa različitim načinima ishrane analiziran je u ovom radu. Po dvanaest uzoraka od svake vrste, kao i navedene starosne kategorije ribe uzeto je sa tri različita ribnjaka u decembru. Hemijske analize, određivanje sadržaja masnih kiselina i ukupnog holesterola sprovedene su u Institutu za higijenu i tehnologiju mesa, Beograd. Statističke analize su urađene u programu Statistica. Odnos n-3/n-6 masnih kiselina kod različitih kategorija šarana kretao se u opsegu od 0.1 do 0.26, pri čemu je najpovoljniji odnos ustanovljen kod dvogodišnjaka, koji su hranjeni peletiranom hranom, a najnepovoljniji kod trogodišnjaka kod kojih je kukuruz predstavljao dominantnu komponentu u ishrani. Zavisnost n-3/n-6 u odnosu na starost i način ishrane je ustanovljena u našem radu. Takođe, ovaj odnos veoma varira između različitih vrsta riba, što je takođe potvrđeno. Nutritivna vrednost ispitivanih slatkovodnih riba je visoka, pošto se njihov masnokiselinski sastav karakteriše zadovoljavajućom količinom n-3 polinezasićenih masnih kiselina, a i sa visokim sadržajem n-6 polinezasićenih masnih kiselina, od koji su posebno značajne linolna i arahidonska.

Ključne reči: *ribe, polikultura, starost, ishrana, masti, protein, ukupni holesterol, masnokiselinski sastav.*

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Rainbow trout (*Oncorhynchus Mykiss*) from aquaculture – meat quality and importance in the diet*

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A b s t r a c t: Rainbow trout (Onchorhynchys mykiss) is one of the most popular fish species in nature, but in many countries it is also recognized and accepted as cultivated/farmed fish species, due to its fast growth and excellent nutritional quality. In the farming technology for rainbow trout, the following elementary conditions must be fulfilled: clear water with sufficient oxygen content (10 mg/l), adequate temperature (8–12 ºC) and flow of water, systematic nutrition using different types of industrial or natural food, etc. In Serbia, most of the produced marketable size rainbow trout is marketed as fresh, cooled (90%), and, in less extent, as scaled and gutted and packaged.

Of all fresh water salmonid species, rainbow trout is mainly farmed for consumption. In addition to the fact that the farming of this fish species is very attractive for a large number of producers, considering the potential for high yields per unit of water volume, it is also characterized by high tolerance to temperature fluctuations and aggravation of the water quality, as well as fast growth rate.

The nutritive value of fish meat is determined by the amount of protein, fat, minerals and vitamins contained and it depends on the fish species and age, farming method, composition of food and season of the year. Rainbow trout farmed in our country contains approx. 18% proteins, 3.3% of fat, 76.3% of water, 1.3% of ash and 48.5 mg/100g of cholesterol. Its energy value is approx. 440 kJ/100g.

The amounts of n–3 and n–6 fatty acids in lipids of marketable size rainbow trout from aquaculture in Serbia give a very favourable n-3/n-6 ratio of approx. 1.60. P/S index for studied fish species is 1.54, and ratio between unsaturated (UFA) and saturated fatty acids (SFA) is 3.51.

***Key words**: Rainbow trout, proximate chemical composition, cholesterol, fatty acid composition.*

Introduction

Fishing is an ancient art. Fishing, for people was easy and simple way to get food, because hunting of other animal species, mammals, required more skill, experience, agility and guile, and also it was considerably more dangerous. Consequently, fish meat, through history of mankind, has represented significant source of food, important for the survival as well as for human development. Fish as the source of food has always been particularly appreciated in countries that had access to the sea. The real economic development of fishing started from the second half of the 19th century, and it peaked in the 20th century. Main cause is in the fact that it was then that method for artificial spawning of fish was invented. Artificial spawning of fish was discovered in the early 18th century by the scientist *Jacobi*, but this discovery was forgotten, before it was introduced into practice 100 years later by two Frenchmen, *Remy* and *Gehin*, who successfully spawned trout *(Drecun et al., 1984)*.

Rainbow trout was imported to Europe around 1882, for the purpose of farming for human consumption. The fish was named "Californian trout" based on its origin, it comes from tributaries of the great Sacramento river, California, USA. Rainbow trout was imported for the first time to fish hatchery Studenac, near Maribor, in 1890, and in 1894 one of the biggest fish hatcheries at the source of the river Bosnia near Sarajevo was constructed. First facilities

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for farming of salmonid species on the territory of Serbia and Montenegro were erected after the Second World War. In Serbia, 16 trout farms were built with total area of 10,38 ha and annual production of 2,333 t. In the mean time, this production dropped to 1.145,33 t per year (*Plavša, 1998*).

Our country has high potentials for development and improvement of trout production, primarily because of very rich natural springs in mountain areas, where trout farming facilities of different purposes and capacities can be erected. Despite a relatively short period of existence, fish farms today have excellent results in fertilization of fish eggs, production of rainbow trout used in human consumption, production of fish spawn for stocking of open waters. However, in Serbia, the most popular fresh water fish from aquaculture is carp species (carp, grass carp, bighead carp), (*Baltić et al.,* 2009). Production of trout makes only 15% of total fish production and it takes place in fish farming facilities with cold water, in hilly-mountainous regions of the country.

Rainbow trout (*Onchorhynchys mykiss*), as one of the most popular fish species in nature, in many countries is also recognized and accepted as farmed fish species, because of its fast growth and exceptional nutritive quality (*Tikeiogly*, 2000). This kind of fish is farmed intensively for consumption, as species exceptionally tolerant to environmental conditions *(Simonović, 2001)*. In the farming technology the following elementary conditions must be fulfilled: clear water with sufficient oxygen content (10 mg/l), adequate temperature (8-12 ºC) and flow of water, systematic nutrition using different types of industrial or natural food, etc. *(Soldatović and Zimonjić, 1988)***.** Fresh fish is, due to its chemical composition, susceptible to spoilage which obligate producers to develop new technologies for its processing and conservation (*Babić et al., 2009*).

In our country, most of produced marketable size rainbow trout is marketed as fresh, cooled fish (90%), and less as scaled and gutted, frozen and packaged into dishes in portions of 4 to 5 pieces, than as smoked and fillets. Chosen technology for processing of fish, as well as complying with necessary technical and technological as well as hygienic conditions and requirements in trout fish farms enables expanding of the range of fish products and improvement of its quality. The rainbow trout product range on the market of Western countries is significantly more diverse and adjusted to the standard, way of living and nutrition of their consumers. Live fish is offered on the market, also cooled, cleaned, frozen, smoked, dried, deboned and breaded.

Considering its nutritional value, fish has always been very important part of the eating habits of our population. Although, today, considerable part of this food originates, either from warm water fish farms situated on flat areas, or cold water, trout fish farms, fishing of trout in their natural habitats in Serbia still represents significant source of fish intended to market *(Simonović, 2001)*. Fish in Serbia is mainly consumed during religious holidays and in the days of fasting. In urban environments consumption of fish has less pronounced osciallations than in rural areas where it is mainly associated with religious holidays. However, in the period from 2001 to 2007, consumption of fish increased from approx. 3 kg to approx. 7kg per capita (*Marković et al., 2009*). It is considered that non-agricultural households consume 4.1 kg of fish, mixed households 3 kg, and agricultureal households 2.9 kg of fish per year *(Milanović, 2000)*. Despite of moderate increase of domestic production, lately, the import of fish and fish products showed drastic increase (*Marković et al., 2009*).

Reasons for such low consumption of fish meat is lack of habit of consuming fish, high price, undeveloped trade network, lack of continuous supply to the market, insufficient supply of different fish species and fish products prepared ready for consumption or quick cooking, according to needs of modern consumers today.

Of all fresh water salmonid species in Serbia, rainbow trout is mainly farmed for consumption. In addition to the fact that the farming of this fish species is very attractive for large number of producers, considering the potential for high yields per unit of water volume, it is also characterized by high tolerance to temperature fluctuations and aggravation of the water quality, as well as fast growth rate (*Marković and Poleksić*, *2008)*. It feeds the fauna from the river bottom, flying insects and other smaller fish, bit it adopts and adjust very easily to additional food (fresh or concentrated) used on fish farms. Because of the quality of meat and poor bone development, they are considered as the most valuable fish species. Farming of fresh water trout species can be divided into two directions: farming of "wild" fish species for the purpose of stocking of open cold waters, flowing and standing, and farming of fish for human consumption.

Proximate composition and cholesterol content

Nutritional and health benefits achieved by consumption of fish is one of the reason of increased demand for these products on the market *(Burger*

and Gochfeld, 2009). Fish represents necessary supplement in total animal protein balance, considering that in regard to its biological value do not differed from proteins of other meat types. Fish meat is important and, in many countries, dominant protein source. It is estimated that close to 15% of demand for animal proteins in the world is covered by the consumption of fish *(Anon, 1999)*. Protein content in fish ranges from 12 to 24%, or in average around 18% *(Cvrtila and Kozačinski, 2006)* and it is very similar to protein content of meat from mammals. Daily needs in proteins for humans can be met by 400 g of fish meat. Fish muscles contain less connective tissue compared to farm animals (less collagen and insignificant amounts of elastin), and, therefore, fish meat is digested faster and easier. Resorption availability of fish proteins and fats is 95% and 91%, respectively *(Baltić and Teodorović, 1997; Baltić and Tadić, 2001)*. Fish meat contains higher quantities of minerals, particularly calcium, phosphorus, magnesium and potassium. In fish fats, vitamins A and E are dissolved (especially in trout) as well as considerable quantities of vitamin D (*Ćirković et al., 2002; Anon, 2003*). Fish meat has high water content (60 to 80%) and negligible content of carbohydrates in form of glycogen (*Šoša, 1989; Ćirković et al., 2002*). However, due to high water content, this type of meat is more susceptible to spoilage compared to meat from warm blooded animals.

Low fat content and relatively low cholesterol content, as well as significant content of minerals, vitamins and essential fatty acids, make fish one of the nutritionally most valuable food stuffs in human nutrition (*Conor*, 2000; *Sidhu*, 2003). Energy value of fish meat is directly dependant on the content of fat, and depending on the type of fish it ranges from 400 kJ/ 100g (trout, codfish) to approx. 900 kJ/ 100g (mackerel, catfish). Fatty fish (mackerel, catfish), in regard to the energy value, can be compared with pork, contrary to lean fish (codfish, trout) which can be compared to chicken meat.

Chemical composition of fish meat, in addition to the genetic factors, is also influenced by quality of water, its pH and temperature, feeding, type of food used, i.e. season of the year, oxygen content, motor activity, age and size of the fish (*Fauconneau et al.,* 1995; *Buchtova et al.,* 2007; *Menoyo et al.,* 2007).

Content of fat increases with the increase of fish size, as well as growth rate and is largely affected by nutrition, and inversely associated with water content (*Kaushik*, 1995; *Vranić et al., 2010*). Protein content is stable during growth period (*Shimeno et al.,* 1990), except in case of insufficient and unbalanced food (*Zeitler et al.,* 1984). It was found that protein content increases if the growth is stimulated by using steroids (*Lone and Matty,* 1984; *Basavaraja et al.,* 1989). Other factors (temperature, mobility, adding of steroids) indirectly stimulate nutrition and also increase the fat content (*Lone and Matty,* 1984; *Viola et al.,* 1992).

Chemical composition and energy value of carp, trout, mackerel, catfish and codfish meat and meat from other types of slaughter animals is presented in *Table 1.*

Table 1. Content of nutrients and energy value of some fish species and certain meat categories (**Bogut et al.*, 1996; ***Ćirković et al.*, 2002; ****Cvrtila and Kozačinski*, 2006)
Tabela 1. Sadržaj hranljivih materija i energetska vrednost u nekim vrstama riba i pojedinim kategorijama mesa (**Bogut i dr.,* 1996; ***Ćirković i dr.*, 2002; ****Cvrtila i Kozačinski,* 2006)

Type of meat/ Vrsta mesa	Water/Voda (%)	Proteins/Proteini (%)	Fats/Masti (%)	Ash/Pepeo* (%)	Energy value/ Energetska vrednost (kJ/ 100g)
Mackerel/Skuša*	61.4	22.5	14.5	1.6	920
Catfish/Som*	71.1	16.5	11.3	1.0	729
Codfish/Bakalar *	81.3	17.0	0.7	1.0	317
Trout/Pastrmka**	76.3	19-20	0.8	1.2	351
Pork/Svinjsko meso**	56.8	17-19	25.3	0.8	1238
Beef/Govođe meso**	74.3	20	3.5		485
Poultry/Piletina**	74.6	21.5	2.5	1.2	460
Mutton/Jagnjetina**	66.4	19.7	12.7		812
Carp/Šaran ***	75.8	18.0	4.8	1.17	522

It is known that increased intake of fish meat is very important for human health because it enables normal development and functioning of the organism

and reduces cardiovascular diseases (*Kris-Etherton et al.,* 2002). In addition to being easy digestible, it is important to mention that fish meat is less burdened by different additives used in modern production during technological procedure of processing of meat from livestock and poultry (*Ćirković et al., 2002)***.**

In case of rainbow trout, average protein content is 20%, content of fat 3% and content of mineral substances 1.2 %, which makes it lean fish meat, recommended as ideal food for children, old and sick persons. Also, trout meat is particularly appreciated because of its softness, juiciness and taste (*Ćirković et al.,* 2002.).

Phillips and Brockwey, 1956, established certain differences in chemical composition of fillets from wild (stream) trout and trout farmed in fish pond. Content of proteins and minerals was lower, and content of fat in meat from farmed trout was higher compared to meat from the wild creek trout. Different literature data (Table 2) show that protein content in marketable size trout fillets ranges from 17.13 to approx. 21%, and of fat from 2.7 to approx. 9%. Content of ash was in the range from 1 to 2%. It is noticeable that contents of main nutrients (proteins, fat and water) range in wide limits, depending on the age, physiological condition (spawning), time of catching and individual differences (*Brkić, 1966*). As a consequence of differences in the content of fat (from 2,7 % to 9 %), the energy value of studied rainbow trout fillets in the mentioned studies varied from 102 to 151 kJ/100g and from 424 to 635 kJ/ 100g, respectively.

Fish reared in aquaculture can show certain variations in chemical composition, but these changes are more constant and can be predicted. Controlled farming conditions, composition of food, content of proteins and fat in the food, environmental conditions, size of the fish and genetic potential affect the composition and quality of farmed fish. The greatest influence considered is composition of food. Most of fish species will use proteins from the food as source of energy rather than lipids. When the content of lipids in food exceeds the maximum that fish can metabolize, fat will be stored in muscle tissue. The higher content of fat will influence the overall quality of fish meat, and, since the excess fat is stored in the belly region which is thrown away in the process of filleting, the utilization of fish is reduced. In controlled farming conditions, with uniform quality of food, it is possible to produce fish of constant quality, without variations in content of proteins, water, fat and ash.

In Table 3, own research results of the chemical composition of most often consumed fish species in Serbia are presented.

The highest content of proteins (18.09%) was determined in rainbow trout fillets, and the lowest in pangasius fillets (11.67%). In addition to the highest water content (85.78%), pangasius also showed the lowest content of fat (0.94%). The highest content of fat and the highest energy value were determined in grass carp (11.59% and 168.75 kcal/ 100g, respectively). The lowest energy value, beside pangasius (56.14 kcal/ 100g) was established in rainbow trout (105.76 kcal/ 100 g, 3.24% of fat).

Fish fats, very rich in polyunsaturated fatty acids, also contain cholesterol. Previous researches showed that most of the studied fish had similar cholesterol content (49- 92 mg/ 100g), as pork or beef (45- 84 mg/ 100g), (*Piironen et al.,* 2002). In the mentioned study, it is stated that cholesterol content is not in correlation with fat content, and that, same as in case of meat from livestock, consuming of fish with reduced fat content doesn't imply that

Table 2. Average chemical composition of rainbow trout fillet – literature data (g/100g)
Tabela 2. Prosečan hemijski sastav fileta konzumne pastrmke – literaturni podaci (g/100g)

Nutrients/Hranjive materije	Phillips and *Brockwey,* 1956		*Bud et al*., 2008	*Celik et al.,* 2008	*Grujić*, 2000	*Plavša et al.,* 2000	*Savić et al.,* 2004	*Ćirković et al.,* 2002
	Rainbow t. kalifor.	Stream t. potočna.						
Proteins/Proteini **(%)**	13.70	21.20	18.88	19.60	20.00	18.33	17.13	19-20
Fat/Mast **(%)**	5.50	3.40	2.94	4.43	3.80	7.64	9.07	2.70
Water/Voda **(%)**	77.20	71.50	77.03	71.65	75.00	73.52	71.95	76.30
Ash/Pepeo **(%)**	2.00	3.30	1.15	1.36	1.20	1.28	1.45	1.50
Energy value/Energetska vrednost (kcal/100g)	110.70	117.80	102.00	130.10	105.20	142.10	151.80	102.30
Energy value/Energetska vrednost (kJ /100g)	452.00	488.34	422.96	540.34	435.70	593.60	635.12	423.80

Table 3. Average chemical composition of fillet of marketable size trout, carp, bighead carp, grass carp and farmed Vietnamese catfish (g/100g)- own data (*Vranić et a*l., 2010, *Đinović et al.,* 2011)
Tabela 3. Prosečan hemijski sastav fileta konzumne pastrmke, konzumnog šarana, tolstobika, amura i gajenog vijetnamskog soma (g/100g)-vlastiti podaci (*Vranić i dr.*, 2010, *Đinović i dr.*, 2011)

Nutrients/Hranjive materije (%)	Rainbow trout/ Kalifornijska pastrmka (*Oncorhynchus mykiss)*	Carp/Šaran (Cyprinus carpio)	Bighead carp/ Tolstobik (Hypophthalmichtys molitrix)	Grass carp/Amur (Ctenopharyngodon idella)	*Vietnamese catfish* /Pangasijus (Pangasius hypophthalmus)
Proteins/Proteini	18.09	15.92	18.69	16.41	11.67
Fat/Mast	3.24	6.99	4.39	11.59	0.94
Water/Voda	76.30	75.59	75.04	71.22	85.78
Ash/Pepeo	1.31	0.96	1.16	0.95	1.36
Energy value/ Energetska vrednost (kcal/100g)	105.76	127.07	117.40	168.75	56.14
Energy value/ Energetska vrednost (kJ /100g)	438.74	531.53	493.33	702.78	237.50

the intake of cholesterol is also reduced. Results obtained by *Cahu et al.,* (2004) indicate that fish from aquaculture, although with higher content of fat, have the same cholesterol content (expressed as g/ 100g of sample) as the same fish species caught in free-catch. However, *Moreira et al., 2001*, indicate that cholesterol content in fresh water fish that had been caught and from aquaculture differs and that it depends on the type of fish. In some fish species there are no significant differences, but in others cholesterol content differs even by 10 mg/ 100g. *Mathew et al.* (1999) and *Luzia et al.* (2003) concluded that, for human health, nutrition which included fresh water fish is more adequate, compared to sea fish, and that cholesterol content in river fish is lower compared to sea fish. Considering clinical and epidemiological studies which point out to the connection between cholesterol introduced by food, cholesterol in blood plasma and atherosclerosis (*Orban et al.,* 2006) relatively low cholesterol content, in addition to composition of PUFA, make the trout very suitable type of fish for human nutrition. However, it is important that the level of cholesterol in blood, in addition to increased alimentary intake of cholesterol and excessive energy intake, is also under influence of increased intake of certain long chain saturated fatty acids (SFA) and increased intake of trans-isomers of unsaturated fatty acids (*Hornstra,* 1999; *Lepšanović,* 2003; *Kris-Etherton et al.,* 2001).

Table 4 shows cholesterol content of meat from different slaughter animals ranging from 44 to 85 mg/ 100g, and in different fish species ranging from 41 to 50 mg/ 100g. Different literature data show considerable variability in the same fish species, which is a consequence primarily of different approaches in sample selection and implementation of different analytical methods (*Žlender and Gašperlin, 2005)*.

Fatty acid profile

Quality of fish meat, in addition to other factors, is valued according to the fatty acid profile. There is growing number of literature studies carried out in order to investigate fatty acid profile of fish from aquaculture which indicate its nutritional significance, compared to same fish species caught in the nature (*Weaver et al.,* 2008). It was concluded that of 30 fish species from aquaculture and free-catching, the highest amounts of n-3 PUFA were determined in farmed salmon and farmed trout (above 4g/100g). The highest variations in content of n-3 fatty acids were established in trout, as a consequence of different rearing methods and feeding systems, in different aquaculture conditions.

It was established that fish fats contain 17-21% of saturated and 79-83% of unsaturated fatty acids (*Bogut et al., 1996*). Of total saturated fatty acids, palmitic (C16:0), stearic (C18:0), myristic (C14:0) and, only in some fish species and in low concentration, also lauric (C12:0) acids were at the most present. Most present monounsaturated fatty acids were oleic (C18:1, n9) and palmitoleic (C16:1n7) acids (Table 5). In addition to mentioned acids, of monounsaturated fatty acids C14:1, C20:1 and C22:1 were present, too. Most of unsaturated fatty acids can be synthesized in the organism during the process of elongation and desaturation of fatty acids, but few of fatty acids are considered essential because they can not be synthesized in the organism. There are two essential fatty acids, *cis n-6*

Table 4. Cholesterol content (mg/ 100g) in different meat types and fish meat
Tablela 4. Sadržaj holesterola (mg/ 100g)u različitim vrstama mesa i mesu ribe

Meat type/Vrsta mesa	Piironen et al., 2002	Emadfa et al., 2001	Kopicova and Vavreinova, 2007	Žlender and Geršperlin, 2005 2000-2004	Celik et al., 2008	Vranić et al., 2010	Đinović et al., 2011
Beef shoulder/ goveđa plećka	55			80			
Back/ leđa		44		64			
Leg/but	52			68			
Pork loin/svinjski kare (lean meat/krto meso)	45	60					
Pork leg/svinjski but (lean meat/krto meso)	47	70					
Poultry breast, no skin/ pileće grudi bez kože	56						
Poultry thigh with skin/ pileći batak sa kožom	84	85					
Farmed table trout/ pastrmka-gajena, konzum	60	55	41		35.04	48.55	
Tuna, can/tuna-konzerva	49						
Shrimp/škampi	142	100					
Table carp/šaran, konzum			49.50			50.86	50.55
Bighead carp/tolstolobik							42.27
Grass carp/amur							40.12
Pangasius/pangasijus							47.14

Table 5. Fatty acid content (% of total fats in certain food stuffs), (*Kulier,* 1990; *Vacha and Tvrzička,* 1994; *Valfre et al.*, 2003)
Tabela 5. Sadržaj masnih kiselina (% od ukupnih masti u pojedinim životnim namirnicama) (*Kulier,* 1990; *Vacha i Tvrzička,* 1994; *Valfre i dr*., 2003)

Fatty acid/ Masna kiselina	Poultry meat with skin/Pileće meso sa kožom	Pork steak/ Svinjski biftek	Eggs/ Jaja	Carp/ Šaran	Catfish/ Som	**Rainbow trout/ Kalifornijska pastrmka**	Mackerel/ Skuša	Salmon/ Losos	Anchovy/ Sardela
14:0	1.1	1.5	0.03	0.97	3.39	3.57	7.2	3.6	5.5
16:0	25.2	25.1	2.10	20.01	19.97	23.67	13.4	17.0	39.0
16:1	8.0	3.6	0.37	8.33	8.47	7.72	4.8	9.6	4.0
18:0	7.0	12.1	0.36	5.97	5.17	6.61	2.2	3.5	5.1
18:1 n9	41.4	44.0	4.08	48.29	19.04	34.79	11.9	21.2	6.7
18:2 n6	12.2	8.1	0.78	9.68	6.51	7.30	2.1	3.7	2.8
18:3 n3	0.90	0.5	0.05	0.73	2.97	0.89	2.0	2.5	0.4
20:1	0.80	1.2	0.02	1.53	4.73	2.20	11.4	1.6	0.4
20:4 n3	0.50	0.50	0.12	0.02	1.01	0.25	0.5	2.9	
20:5 n3	**0.30**	/	/	**0.38**	**3.24**	**1.17**	**6.2**	**7.5**	**10.7**
22:5 n3	0.30	0.30	0.01	0.12	1.78	0.58	0.9	2.5	
22:6 n3	**0.60**	**0.40**	**0.12**	**0.48**	**9.24**	**3.86**	**10.8**	**13.1**	**20.6**

polyunsaturated fatty acid (linoleic acid, 18:2*n-6*) and *cis n-3* polyunsaturated fatty acid (α-linolenic acid, 18:3*n-3*). From these two types of "parent" essential fatty acids, *n-3 (*the most important are EPA, eicosapentaenoic, C20:5 and DHA, docosahexaenoic fatty acid, C22:6) and *n-6* „families" (the most important is arachidonic acid, C20:4) are created, through series of enzyme -catalyzed reactions of desaturation and elongation *(Hunter and Roberts, 2000;. Lunn and Theobald, 2006)*.

Favourable influence of n-3 PUFA from fish meat on human health has been proven in numerous studies (*Mozafarian et al., 2004; Sahena et al., 2009; Barcelo-Coblijn and Murphy, 2009)*, confirming the

link between the consumption of fish and prevention of coronary disease, especially myocardial infarction, artherosclerosis, hypertension and other cardio--vascular diseases (*Kris-Etherton et al., 2002; Mayneris-Perxachs et al., 2010)*. Mechanism responsible for favourable effect of n-3 PUFA on human organism is multiple and its perception exceeds the framework of this study. In addition to prevention of coronary diseases (*Mozafarian et al.,* 2005) and reduction of incidence of hypertension (*Calder*, 2001), favourable influence of n-3 PUFA reflects also in prevention of inflammatory (*Moreno and Mitjavila*, 2003), autoimmune (*Zamaria*, 2004) and malignant diseases (*Terry et al.*, 2004), diabetes (*Nettleton and Katz,* 2005) and other diseases. It was established that lack of polyunsaturated fatty acids plays important role in the ethiology of depression, dyslexia, schizophrenia and Alzheimer's disease *(Lunn and Theobald, 2006)***.** Studies carried out by *Schiepers* et al. *(2010)* indicate the positive effect of fish consumption on quality of life, in sense of improvement of general physical condition, but with less significant impact on improvement of mental health.

of n-3 PUFA, too, due to the fact that this type of fish has better ability of fatty acid desaturation and their transformation in long-chain PUFA (EPA and DHA) compared to sea fish. Table 6. presents data on the content of polyunsaturated fatty acids (n-3; EPA and DHA) in most often consumed fresh water and sea fish (g/ kg*)*. It is known fact that fresh water fish, caught in the nature, contains less fat and higher amounts of EPA and DHA, compared to farmed fish of same species, when these values are expressed as percentage of total fatty acids. However, it should be considered that fish from aquaculture contains higher percentage of total fat and, when values for PUFA are expressed per 100g of fish, intake of EPA and DHA into human organism is higher when farmed fish is consumed, compared to same fish species caught in the nature.

Fatty acid profile in fish varies within and between species (*Haliloglu and Aras,* 2002; *Celik and Ali Gocke,* 2003), and numerous factors, such as temperature, water quality, type and availability of food, season, age, genus, reproduction status, geographical location and individual differences are considered

Table 6. Content of polyunsaturated fatty acids (n-3; EPA and DHA) in most often consumed fresh water and sea fish (g/kg)- comparison of literature data with own studies (*Farkas and Csengeri,* 1990; **Luzia et al.*, 2003; ***Vranić et al.,* 2010; ****Trbović et al.,* 2011)

Tabela 6. Sadržaj polinezasićenih masnih kiselina (n-3; EPK i DHK) u najčešće konzumiranim slatkovodnim i morskim ribama (g/kg)-poređenje literaturnih sa vlastitim podacima (*Farkas i Csengeri,* 1990; **Luzia i dr*., 2003; ***Vranić i dr.,* 2010; ****Trbović i dr.*, 2011)

Fresh water fish/ Slatkovodana riba	20:5 EPA	22:6 DHA	Sea fish/Morska riba	20:5 EPA	22:6 DHA
Sterlet/Kečiga	13.0	9,1	Meckerel/Skuša	14.5	24.6
Grey carp/Sivi glavaš	8.9	6,5	Herring/Haringa	10.5	12.9
White carp/Beli glavaš	8.5	4,5	Flat fish/List	3.4	2.8
Eel/Jegulja	2.5	5,8	Eel/Jegulja	3.0	6.6
Tench/Linjak	1.7	0,7	Salmon/A.losos	2.5	7.3
Carps/Šarani	0.7	0,7	Halibut/A.iverak	1.6	2.2
Pike/Štuka	0.5	1,7	Codfish/Bakalar	1.2	1.9
Grass carp/Amur	0.4	0,5	Grouper/Škarpina	1.4	0.7
Perch/Smuđ	0.4	0,9	Hake/Oslić	0.8	2.3
Trout/Pastrmka**	1.2	3,4	Anchovy/Sardina*	3.0	10.1
Carp/Šaran***	0.01	0,1	Croaker*	6.7	5.9
Pangasius/Pangasijus***	0.001	0,04	Shrimp*	4.2	8.2

Since n-3 fatty acids are necessary for development of central nervous system, brain, growth and development, forming of blood vessels of the foetus, it is necessary to provide them in sufficient amounts during pregnancy *(Innis, 2007; Sidhu, 2003)*. Also, studies have shown that *n-3* fatty acids are essential for growth and development of children.

Study by *Cahu et al.*, 2004 and *Lichtenstein et al., 2006* indicates that fresh water fish can be source

as significant factors which additionally contribute to these variations *(Skalli et al.,* 2006*; Valente et al.,* 2007*; Rubin and Skalli,* 2007*)*. Fatty acid profile of fish feed has significant impact on fatty acid composition of fish meat *(Steffens and Wirth,* 2007*; Valente et al.,* 2007*)*. Food rich in n-3 fatty acids, in same rearing conditions, significantly influences the increase of n-3/ n-6 PUFA ratio in fish tissues (*Bell et al.,* 2001*; Grisdale-Helland et al.,* 2002*; Skalli et al.,* 2006*)*.

Nutrition with balanced n-3/ n-6 ratio is important for farming of healthy fish and for production of high quality food for human nutrition (*Steffens,* 1997).

Quality of fish lipids is determined by PUFA/ SFA and n-3/ n-6 ratios (*Ahlgren et al.,* 1996). Beside optimal quantities of essential fatty acids, the intake ratio is important, too. According to *Baltić et al., 2003* the optimal n-3/ n-6 FA ratio is from 1:4 to 1:5. *Henderson and Tocher* (1987) reported n-3/ n-6 value of 0.5-3.8 for freshwater and 4.7-14.4 for marine fish. Higher amounts of n-3 in marketable size trout fillets and lower amounts of n-6 PUFA give very favourable n-3/ n-6 ratio (1.60) compared to, for instance, carp, where this ratio is less favourable (0.08), due to higher amount of n-6 and lower content of n-3 fatty acids.

In regard to nutritional value, ratio between polyunsaturated and saturated fatty acids is very important, as well as P/S index, which should be above 0,5 (*Žlender and Geršperlin,* 2005). P/S index below 0.45 is considered inadequate (*Santos-Silva et al.,* 2002) because it can lead to hypercholesterolemia. Based on our studies, the obtained P/S value in marketable size rainbow trout was 1.55. Ratio between unsaturated (UFA) and saturated fatty acids (SFA) in fish fat is very important and preferably it should be above 3 (*AFSSA,* 2003). The highest values were determined in rainbow trout (3.51, 2.12, 2.54) and carp (2.68 and 2.68), (Table 7).

\Trout reared in extensive conditions had higher content of PUFA and n-3 fatty acids, as well as n-3 / n-6 ratio, whereas the content of SFA showed no differences in regard to the value registered in conditions of intensive farming, which is probably associated with very strict role of SFA in fish physiology, also confirmed by numerous studies where fish was fed diets of different composition (*Turchini et al.,* 2003a, 2003b). Such fatty acid profile is a consequence, primarily of nutrition of studied fish, but also of of the species, size, age, water quality, region, season, etc.

Table 7. Contents of SFA, MUFA, PUFA (% of total fatty acids), n–3, n–6, n–3/ n–6, P/S , UFA/ SFA ratios in fillets from various fish species, literature and own research data

Tabela 7. Sadržaj *ZMK, MNMK, PNMK* (% od ukupnih masnih kiselina), n–3, n–6, n–3/ n–6, P/S, UFA/ SFA odnosi u filetima različitih vrsta riba, literaturni i vlastiti podaci

Nutrients/Hranjive materije	SFA/ ZMK	MUFA/ MNMK	PUFA/ PNMK	n–3	n–6	n–3/n–6	P/S	UFA/ SFA
Sea bob shrimp[2]				12.50	2.35	5.32		
Croaker[2]				12.60	8.52	1.47		
Mackerel/Sardina[1]	39.4	18.0	42.6	37.8	4.8	7.88	7.57	
Mackerel/Sardina[2]				13.40	2.59	5.17		
Marketable size carp feed peletted food//Konzumni šaran,hranjen peletiranom hranom[3]	27.21	50.40	21.67	1.43	20.12	0.07	0.80	2.65
Marketable size carp Carp feed food based on cereals/ Konzumni šaran, hranjen hranom na bazi žitarica[3]	27.02	63.50	8.91	0.63	8.28	0.08	0.33	2.68
Bighead carp/Tolstolobik[3]	29.40	54.87	16.52	1.14	15.39	0.07	0.56	2.43
Grass carp/Amur[3]	34.48	54.90	9.83	6.70	3.12	2.15	0.28	1.88
Pangasius (farmed Vietnamese catfish)/ Pangasius (gajeni vijetnamski som)[3]	41.36	42.36	15.77	1.31	14.46	0.09	0.38	1.40
Marketable rainbow trout, int. production/ konzum. kalifornijska pastr., int. proizvodnja[4]	22.17	43.50	34.33	21.12	13.21	1.60	1.54	3.51
Lake trout/Pastrmka, jezerska[5]	27.65	35.56	23.09	15.64	7.45	2.10	0.83	2.12
Marketable rainbow trout,/Pastrmka, kalifornijska, konzum[6]	31.92	30.81	36.88	22.41	14.47	1.58	1.55	2.12
Table carp/Šaran, konzum[7]	26.41	59.50	12.07			0.74	0.46	2.70
Trout, extensive production/Pastrmka, ekstenzivna proizvodnja[8]	28.00	17.17	54.20	46.70	7.50	6.6	1.93	2.54

[1]Marin, 2005
[2] Luzia et al., 2003
[3]Trbović et al., 2011
[4]Vranić et al., 2010
[5]Celik et al., 2008
[6]Haliloglu and Aras, 2002
[7]Bieniarz et al., 2001
[8]Turchini et al., 2004

Recommendations

American Heart Association recommends consumption of fish at least twice per week. For persons with cardio vascular problems, daily consumption of 1g EPA + DHA is recommended, but for patients with elevated content of triglycerides in blood 2–4 g EPA + DHA is recommended *(Domingo,* 2007*; Zatsick and Mayket,* 2007*)*.

Our research showed that the content of EPA+DHA in total fatty acids in rainbow trout was 14.12% (*Vranić et al.,* 2010), in carp 0.22% and in pangasius 0.50% (*Trbović et al.,* 2011). When obtained values are calculated per portion, by consumption 200g of fish, intake of desirable fatty acids for trout is 0.91g, for carp 0.03 g, and for pangasius only 0.01 g.

Recommendations for daily intake of fats and essential fatty acids are given in Table 8 (*Lunn and Theobald,* 2006*)*.

and trout, and farming of other fresh water fish species is in very low extent. Our country has the necessary infrastructure and human resources for improvement of production in the sector of fishery, as well as experienced personnel for realization of all activities aimed at harmonization of national regulations relevant to the fishery sector with EU legislation.

Future research in aquaculture should be directed to the study of required amount of energy components, as well as fatty acids in fish food, which will contribute to reaching of optimal production performances, as well as the amount of n-3 fatty acids in fish meat which are essential for preservation of the health of consumers.

Bearing in mind considerable nutritional value of trout (high protein content, relatively low cholesterol content and significant content of n-3 fatty acids) and insufficient presence of fish in the diet of dome stic population, one of the ways to increase its market

Table 8. Recommendations for daily intake of fat (% of energy) *Lunn and Theobald* (2006)
Tabela 8. Preporuke o dnevnom unosu masti (% od energije) *Lunn i Theobald* (2006)

	USA and Canada/ SAD i Kanada	Europe/Evropa (EURODIET project)	FAO/WHO	UK
Fats/masti	20–35	< 0	35	< 35
n–3	0.6–1.2	200 mg DHK/EPK; 2 g	LK:ALK = 5:1–10:1	> 0.2 (450 mg DHK/EPK)
n–6	5–10	4–8	4–10	> 1

General conclusions

Serbia disposes with considerable surface of fish ponds, which, mainly, have small extensive or semi-intensive production. Main production activities in aquaculture in Serbia are farming of carp

value in total supply of farmed fish is the improved supply of trout and trout products adapted to needs of modern consumer. In addition to enriching the product assortment, it is necessary to promote the benefits of fish in the diet, in order to increase its consumption and use in human nutrition.

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Kalifornijska pastrmka (*Oncorhynchus Mykiss*) iz akvakulture – kvalitet mesa i značaj u ishrani

Vranić Danijela, Đinović-Stojanović Jasna, Spirić Aurelija

R e z i m e: Kalifornijska pastrmka (Onchorhynchys mukiss) je jedna od od najpoznatijih vrsta ribe u prirodi, ali je, u mnogim zemljama, poznata i prihvaćena kao uzgajana vrsta, zbog brzog rasta i odličnog nutritivnog kvaliteta. U tehnologiji gajenja kalifornijske pastrmke neophodno je da se obezbede elementarni uslovi: čista voda sa ispunjenim zahtevima za sadržaj kiseonika (10 mg/ l), odgovarajuća temperatura (8–12 ºC) i protok vode, sistematska ishrana različitim vrstama industrijske i prirodne hrane, i dr. U Srbiji, najveći deo kalifornijske pastrmke proizvedene za konzum plasira se na tržište kao sveža, ohlađena riba (90%), a u manjoj meri kao očišćena, zamrznuta i upakovana.

Od slatkovodnih salmonida, za konzum se u Srbiji najviše gaji kalifornijska pastrmka.Osim što je gajenje ove vrste ribe privlačno za veliki broj proizvođača, s obzirom na mogućnost postizanja visokih prinosa po jedinici zapremine vode, nju odlikuje i visoka tolerantnost na temperaturna kolebanja i pogoršanje kvaliteta vode, kao i brz rast.

Hranljiva vrednost mesa riba uslovljena je količinom proteina, masti, minerala i vitamina u njemu i zavisi od vrste i starosti ribe, načina uzgoja, sastava hrane i godišnjeg doba Kalifornijska pastrmka gajena na našem području sadrži oko 18% proteina, 3,3% masti, 76,3% vode, 1,3% pepela i 48.5 mg/ 100g holesterola. Njena energetska vrednost iznosi oko oko 440 kJ/ 100g.

Količine n–3 i n–6 masnih kiselina u lipidima konzumne kalifornijske pastrmke iz akvakulture Srbije daju veoma povoljan n-3/ n-6 odnos, koji iznosi oko 1,60. P/S indeks, za ispitanu vrstu ribe je 1,54, a odnos nezasićenih (NMK) prema zasićenim masnim (ZMK) kiselinama iznosi 3,51.

***Ključne reči**:Kalifornijska pastrmka, hemijski sastav, holesterol, masnokiselinski sastav.*

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Contribution to knowledge of major quality parameters of traditional (domestic) kulen*

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S u m m a r y: In this paper, results of the study of sensory properties, pH value and chemical composition of traditional or domestic kulen are presented. Results show certain disharmony between sensory and chemical and nutritional quality parameters, i.e. sensory quality of kulen is poorer than the chemical composition of the product. The most common quality shortages/deficits of domestic kulen is different smoking level, inadequate colour of the section/slice, presence of dry edge under casing, insufficient connection of the filling, bitter or sour taste. Most of studied samples of domestic kulen had pH value of 5.22 to 5.51, which shows that the ripening process was not finished, but also that the fermentation had been stimulated in certain way, not characteristic of domestic kulen. Change of the micro flora of domestic kulen is slow and typical for natural ripening which occurs during production of kulen in winter period. In the micro flora of kulen, bacteria which ferment sugars to lactic acid are dominant, Pseudomonadaceae and Enterobacteriaceae dye gradually, number of Micrococacea decreases, and enterococci survive ripening. Results of the chemical analysis show that kulen contains slightly over 35% of moisture, that the protein content of meat is above 25%, that the ratio between fat and protein contents was approximately equal and that the share of collagen in meat proteins is below 10%. The amount of sodium chloride in kulen (3.4–3.8%) is adequate to the amount of added table salt. Nitrate residues were detected in kulen (10.5 to 12.1 mg/kg) which orginated from spices added to product (pepper, garlic). Acid number of kulen (7.5–16.6 mg KOH/g) showed value common for fermented dry sausages. TBARS-value was in the range 0.19–0.29 mg MDA/kg, in which the oxidation of fats cannot be perceived by sensory analysis.

***Key words**: traditional or domestic kulen, quality, chemical composition, sensory properties, pH value.*

Introduction

Kulen is traditional fermented dry sausage which has been manufactured for centuries, during winter time, in the North Serbia (Srem, Bačka), Croatia (Slavonia, Baranja) and Hungary (*Vuković* et al. 1988; *Incze*, 2003; *Vuković*, 2006). Depending on the region where it is produced, *kulen* has adequate name, for instance S*remski kulen*, *Ludoški kulen*, *Petrovački kulen*, *Kisački kulen*, *Slavonski kule* etc., but the most common and wide spread name used is *domestic kulen*. Some types of *kulen* today are produced as Products of protected designation of origin (PDO)/Products of protected geographical indication (PGI) (S*remski kulen*, *Slavonski kulen,* etc). Also, according to the Etimology dictionary of Croatian or Serbian language (*Skok*, 1971–1974), name *kulen* is derived from Greek word *kolon*, meaning large intestine. In the folk tradition, however, there are different explanations, for instance that name *kulen* is derived from the word *kula* (tower), designating a sausage that is dried for long time hung high like in a tower. According to Vuk St. Karadžiću (1851), *kulen, kulijen* or *kuljen* would be sausage made of stomachs and compared to some types of cooked sausages (so called *švargla*).

For all variants of basically same product, high quality meat from mature pigs containing less water,

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of more intensive red colour and firmer consistency is used as raw material. In production of *kulen* meat is used, always, which is poor in fat and connective tissue, primarily meat from the leg, shoulder and possibly some parts of neck from which fat tissue, tendons, veins and connective tissue membranes have been removed. There is still tradition, in some regions, to produce *kulen* from non-chilled meat (after slaughtering), although most of producers today use chilled meat in production of *kulen*. It can be made only from meat, but somewhere also smaller amount of firm fat tissue is used, usually pig jowl. Additives used in production of *domestic kulen* are table salt, red pepper spice and garlic. Table salt is added in the amount of 2.1 to 2.3%, red ground spice peppers from 0.9 to 1.4%, whereas the amount of garlic added varies from 0.3 even to 0.8%. Traditionally, adequate mix of sweet and hot peppers is used; sweet peppers are significant for the colour, and hot peppers for savoury taste of the *kulen*. In traditional production of *kulen* curing salt is not used (*Vuković et al.,* 1988).

Meat intended for *kulen* is generally chopped into more coarse fragments, mixed with table salt, peppers and other spices, and only when the filling becomes sticky and well connected, it is filled into natural casings, processed pig colons. Filling is placed into casings firmly, making sure that there is no left over air. *Kulen* filling is most commonly put in appendix, but it can also be used to fill straight intestine or rectum (final straight portion of the large intestine) of pigs. Straight intestines or rectums have somewhat fuller and thicker intestine walls compared to appendix, so products in this intestine are generally without dry edge and always somewhat juicer. After filling, a mesh of rope is placed around *kulen* in appendix, and it is usually placed between natural intestine creases giving the *kulen* distinctive appearance, and also enables safer hanging of *kulen*, especially in case of larger products. *Kulen* in straight intestine/rectum is tied with rope at both ends, with one or two loops in the middle of the sausage. After brief drying, *kulen* is cold smoked using smoke obtained from beech wood and on open furnace, and it should have grey-white colour without admixture of soot. Some producers use also cherry wood, in addition to beech wood, and this smoke gives the surface colour of the product a reddish hue. *Kulen* is smoked occasionally in duration of two to three, even up to four weeks, until it acquires copper red colour. Some producers dip *kulen*, before smoking, into solution of table salt and spices, for some time, where water by osmosis passes from *kulen* into solution, and salt from the solution into sausage filling. Drying of *kulen*, including smoking, and depending on the diameter of sausage, can last three to six months (*Vuković et al.,* 1988; *Vuković*, 2006).

Contrary to other products, such as *Sremska kobasica* and *zimska salama* (winter salami), *kulen* was not studied very often. It is important to say that the first scientific contribution on traditionally fermented sausages in our country was a doctoral thesis, done and defended in the fifties of the last century, at the Veterinary faculty in Belgrade, and in this thesis the ripening processes in *Sremska kobasica* were studied (*Rašeta*, 1958). First scientific papers analyzing major changes which occur during ripening of *kulen* were published after three and five decades (*Vuković et al.,* 1988; *Vuković et al.,* 2004). Significant contribution to study of traditional products, such as *Sremska kobasica* and *Petrovská klobása*, was made by *Vesković-Moračanin* (2007) and *Ikonić et al.* (2010).

Results of investigations so far show that there is difference between kulen sausage produced industrially and traditional *kulen* (*Vuković et al.,* 2004). The fact that in industrial production the hygiene conditions are significantly better, also that brining salt is used, pepper extracts, sugars, starter cultures and artificial casings, is undoubtedly of importance for safety of the product. However, this *kulen*, in its sensory and other quality indicators, differs significantly from domestic *kulen*, and could not be considered as originally traditional product. According to our knowledge, traditional production procedure of domestic *kulen* remained only in smaller producers (crafts, households). In this paper, results of the study of major quality properties of *kulen* produced by small producers in traditional manufacturing, are presented. Results presented in the paper were obtained within the scientific-research project, with aim to improve the technology of production of traditional fermented sausages with Protected designation of origin (PDO) or Protected geographical indication (PGI) and to obtain safe products of standard and distinguishing quality.

Material and Methods

Samples of *kulen* from traditional production, obtained from small producers, were studied in the paper. By using standard assay methods the following parameters were determined: (1) pH value (apparatus WTW 340i, SRPS ISO 2917); (2) a_w-value (apparatus GBX Scientific Instruments, Fa-St/1); (3) moisture content (SRPS ISO 442); (4) protein content (SRPS ISO 937); (5) total fat content (SRPS ISO 1443); (6) ash content (SRPS ISO 936); (7) relative content of collagen in meat proteins – collagen

content was calculated by multiplying with factor 8 of the hidroxyproline content (SRPS ISO 3496); (8) chloride content (Volhard method, SRPS ISO 1841-1); (9) nitrite content (SRPS ISO 2918) and nitrate content (SRPS ISO 3091); (10) acid value (SRPS ISO 660); (11) TBARS (Thiobarbituric Acid Reactive Substances)-value (combined method according to *Tarlagdis et al.*, 1964, and *Holland,* 1971); (12) lactic acid bacteria (MRS-agar, Merck, at 32° C/72 hours anaerobic); (13) *Micrococcaceae* (Baird-Parker-agar, at 37° C/48 hours); (14) *Enterobacteriaceae* (Brilliant-Green-agar, Merck, at 37° C/48 hours); (15) *Enterococcus* spp. (Kanamycin-Esculin-Azide-agar, Merck, 37° C/24–48 hours); (16) *Salmonella*-species (ISO 6579, 2002); *Listeria monocytogenes* (ISO 11290-2, 1998, Amendment 1, 2004); (17) sensory properties, by method applied in assessment of the quality at Novi Sad Agriculture Fair.

Results and Discussion

Based on previous findings and studies, *traditional* or *domestic kulen* can be described as product made of high quality meat from mature pigs. Meat is coarsely ground and mixed well with table salt, ground red pepper and possibly garlic. Filling is then stuffed into natural casings, primarily pig appendix, and subsequent to smoking it is subjected to natural ripening, during which it acquires typical and distinguishing quality properties (*Vuković et al.,* 1988; *Vuković*, 2006). Same as the results of previous studies (*Vuković et al.,* 2004), results obtained in this research show that there are differences in quality of *kulen* samples taken from various producers. In Table 1, results of the sensory analysis of 9 samples of traditional *kulen* (in pig appendix), carried out by nine member expert panel/committee of the Novi Sad Agricultural Fair, are presented as well as results of determination of pH value of *kulen*, done immediately after sensory evaluation. Results show that no *kulen* samples were given the highest score (5.00); most of investigated samples were awarded silver and bronze medal, based on mean evaluation, or were unrewarded (score below 3.5), and only one sample of *kulen* received somewhat higher score, over 4.5 (gold medal). On this basis, it can be stated that *kulen* is product of average quality and not product of exceptional/superior quality, as it is rightly expected of products made from high quality raw materials.

The most common deficits of the quality of domestic *kulen* are different smoking levels, inadequate colour of cross section/slice, presence of dry

Table 1. Sensory assessment and pH value of traditional kulen sausage
Tabela 1. Senzorna ocena i pH vrednost tradicionalnog kulena

Value/Vrednost	Sensory score/ Senzorna ocena	pH value/ pH vrednost
Medium/Srednja	3,87	5,42
Highest/Najveća	4,56	6,28
Lowest/Najmanja	3,07	4,86

edge underneath the casing, insufficient connection of the filling, bitter and sour taste/aroma, more or less expressed. The fact that traditional *kulen* is stuffed into pig appendix gives distinguishing and appealing appearance to this product, but the size of the sausage - diameter requires adequate knowledge and skills. On the cross section/slice of the product there cannot be dry edge which prevents proper drying and makes the inside of the sausage soft, unconnected and enables incidence of untypical aroma, and even product spoilage. Underneath the casing, there can only be darker ring which derives from the smoke. Colour of the cross section/slice of *kulen* should be red to dark red, stable, and forming of the colour is mainly under the influence of carotenoids – natural pepper pigments, but also nitrates which are orginating from peppers and garlic. Consistency of *kulen* should be firm and not disintegrating during cutting, which is not the case in sausages containing less salt, and more peppers. Taste and smell/odour of *kulen* should be mature/ripe and savoury. The hot taste of *kulen* should not be stronger than the natural taste of ripe product, and it should only come across as after taste. Sour taste is not typical for *kulen* and indicates either that the product is insufficiently ripe, or that the natural fermentation is stimulated.

If the lowest (4.86) and the highest (6.28) measured pH values are disregarded, other analyzed samples of domestic *kulen* had pH value from 5.22 to 5.51. It can be concluded that the ripening of *kulen* was not finished and that the products was not stabilized. According to results of previous studies (*Vuković et al.,* 1988), stabilization of *kulen* occurs only after 120 days of ripening, when pH value increases to 5.6. Two analyzed samples of *kulen* had pH value of approx. 4.9, and on one of them the use of starter cultures had been declared; both products had extremely sour taste which is typical for a fermented, semi-dry sausage, but not for domestic *kulen*. One sample of *kulen*, which was produced two years before the sensory analysis, according to declaration, had pH value over 6.2, but the colour and appearance of the cross section/slice, taste,

smell/odour and texture have changed greatly and product was not fit for consumption.

At the beginning of ripening process of domestic *kulen*, pH value ranges from 5.6 to 5.8 and corresponds to pH value of cooled pig meat, and subsequently it starts to decline (*Vuković et al.,* 1988). Certainly, the sugars (fructose, glucose, sucrose) which are natural ingredients of spice peppers, have the major impact on decline of pH value of *kulen*. According to literature data (*Oberdick*, 1988), spice pepper contains approx 15% of sugars, whereas the content of total sugars in domestic spice peppers is higher and amounts to approx. 25% (*Petrović*, 2011). During ripening, bacteria ferment sugars from the peppers to lactic acid, resulting in decrease of pH value of *kulen*. In the first stage of natural ripening of domestic *kulen*, which last approx. 60 days, pH value decreases to the range of 5.2 to 5.3, and in the second stage of ripening, as a consequence of more distinguished proteolysis, pH value gradually increases to reach value of 5.6 at the end of the ripening process (*Vuković et al.,* 1988). Obviously, change of pH value of domestic *kulen* in natural ripening is moderate, but nevertheless very important for sustainability and forming of the colour, consistency and aroma of the product (*Coretti*, 1971). In cases when change in pH value of *kulen* is more distinct, then it is no longer case of natural ripening, but fermentation which was stimulated in some way, for instance by using sugars and starter cultures, higher ripening temperature, or even using glucono delta--lactones; subsequently pH value of *kulen* is low, approx. 5.2 (*Vuković et al.,* 2004).

Present studies, as well as previous (*Vuković et al.*, 1988), show that during the process of natural ripening of *kulen*, microflora typical for fermented dry sausages is developed (Figure 1). In microflora of *kulen*, dominant are bacteria which ferment sugars into lactic acid. Number of *Micrococacea* decreases, *Pseudomonadaceae* and *Enterobacteriaceae* die out, and enterococci survive the ripening process. In industrially produced *kulen*, when curing salt is used, as well as sugars and starter cultures, *Pseudomonadaceae* and *Enterobacteriaceae* die faster, and *Micrococacea* are found very rarely (*Vuković et al.,* 2004). Figure 1 clearly shows that typical micro flora of *kulen* develops slowly, which is typical for ripening in traditional production of *kulen*, at lower temperatures and in natural conditions (*Coretti*, 1971). Pathogen bacteria of *Salmonella*-species and *Listeria monocytogenes* were not detected in any of the *kulen* samples.

Chemical composition and other parameters of interest for the quality of domestic *kulen* are presented in Table 2. Results show that *kulen* contains more than 35% of moisture, that the protein content and content of fats were almost equal, and that the share of collagen in meat proteins was below 10%. Higher moisture content undoubtedly indicates that the ripening process was not finished, but it is

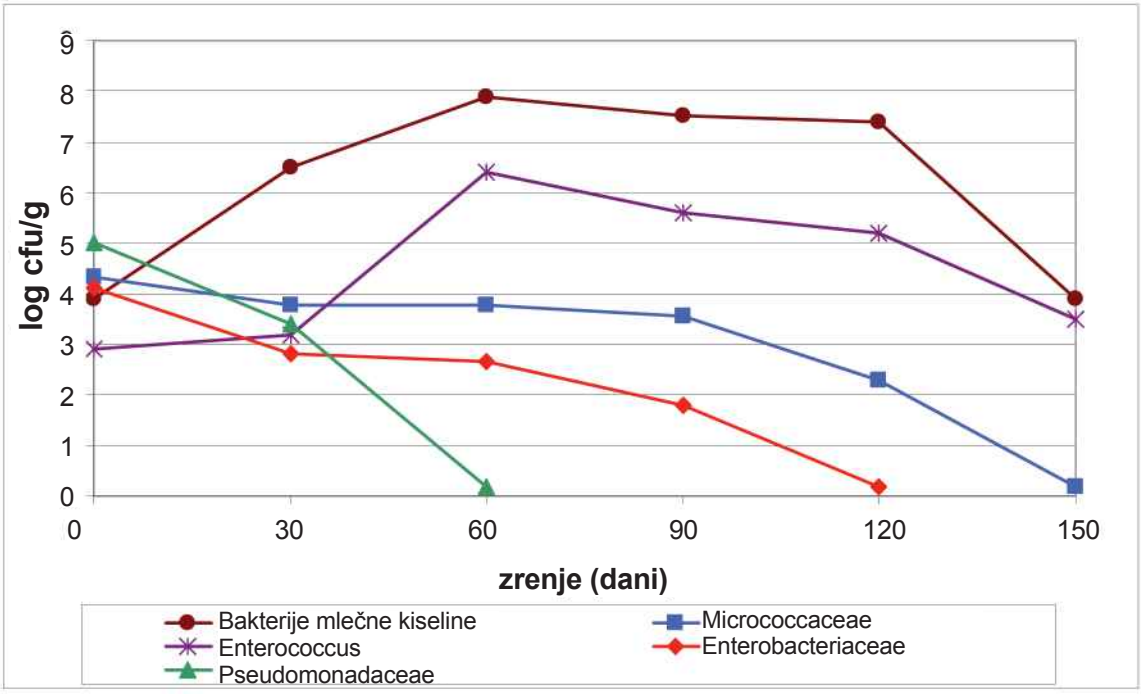

Figure 1. Changes of the microflora of domestic kulen sausage during natural ripening
Slika 1. Promena mikroflore domaćeg kulena za vreme prirodnog zrenja

also consequence of lower fat content of traditional *kulen*. Our previous studies show that the content of fats in so called industrial *kulen* in average is 38.6%, which is by ten percents more than in traditional *kulen*, but, at the same time, industrial *kulen* contains less moisture, below 30% (*Vuković et al.,* 2004). Results of previous studies (*Vuković et al.,* 1988), also show that the content of fat and meat proteins in domestic *kulen* was even. All stated facts confirm that domestic *kulen*, from the standpoint of chemical quality parameters, as well as nutritional aspect, is extremely valuable product.

Table 2. Major chemical components and quality parameters of domestic kulen sausage
Tabela 2. Važniji hemijski sastojci i parametri kvaliteta domaćeg kulena

Ingredient/parameter Sastojak/parametar	**Values/ Vrednosti**
Moisture/Vlaga (%)	36,3 – 37,7
Meat proteins/Proteini mesa (%)	25,5 – 28,7
Share of collagens in meat proteins/ Udeo kolagena u proteinima mesa (%)	6,4 – 8,6
Fats/Masti (%)	27,9 – 28,3
Fat:protein ratio/ Odnos masti:proteini	1,0 – 1,1
Ash/Pepeo (%)	4,8 – 5,4
NaCl content/Sadržaj NaCl (%)	3,4 – 3,8
Nitrates/Nitrati (mg/kg)	10,5 – 12,1
Nitrites/Nitriti (mg/kg)	2,35 - 2,47
Acid number/Kiselinski broj (mg KOH/g)	7,5 – 16,3
TBARS-value/vrednost (mg MDA/kg)	0,19 – 0,29

The amount of sodium chloride in *kulen* (3.4–3.8%) corresponds to added amount of table salt. Traditionally, table salt is added to *kulen* in the amount of 2.1 to 2.3%, which is less than optimum. For efficient inhibition of pathogen and other harmful bacteria, it is necessary to add into fermented sausages 2.5% of table salt (*Leistner,* 1985). In this amount, table salt more efficiently inhibits psychrotolerance pseudomonades which have negative effect on taste (they degrade proteins resulting in bitter taste of *kulen*), and, on the other hand, salt creates more favourable conditions for development of useful/beneficial species such as reducing bacteria (*Micrococacea* and non-pathogen *Staphylococci*) and lactic acid micro flora (lactobacillus, etc.). Table salt, also, dissolves proteins on the surface of meat pieces of the ground meat and enables first their coming together/sticking, and subsequently, when pH value decreases to 5.3, connecting of the filling (*Coretti,* 1971). Contrary, peppers added in excess amount can influence more difficult connecting of the filling. Therefore it happens that *kulen* containing les salt, and more peppers, although seemingly with firmer consistency, disintegrates more or less during cutting.

Even though in production of domestic *kulen* curing salt is not used, in analyzed *kulen* samples certain amount of nitrates and nitrites was determined. In our opinion, nitrate comes from spices peppers and garlic, with which it was added to *kulen*. Nitrate is not natural ingredient of peppers, but it is absorbed by peppers during growing, from the soil (fertilizers) so it appears as contaminant in peppers. According to published data (*EFSA Journal*, 2008), nitrate content in peppers in very wide ranges from 1 to 476 mg/kg, in average 108 mg/kg, which means that in dry peppers as nitrate content should be significantly higher. Content of nitrates in garlic doesn't vary so much, in average it is 70 mg/kg. By adding mentioned spices into *kulen*, especially peppers used in the amount of 0.9 to 1.4%, also certain amount of nitrates are added to *kulen*, and their residues were detected in the product (10 to 12 mg/kg). During ripening of *kulen*, part of these nitrates was reduced by the activity of bacteria to nitrites, which, in addition to carotenoid from peppers, have major role in forming of stable red colour of *kulen*. Acid value of *kulen* (7.5–11.2 mg KOH/g) shows value common for fermented dry sausages. TBARS--number is below the value (0.1–0.3 mg MDA/kg) above which it is possible to perceive, by sensory evaluation, oxidation of fats (*Fernandez et al.,* 1997).

Conclusion and recommendation

Traditional or domestic *kulen* is product made using high quality meat from mature pigs, table salt, ground spice peppers and garlic, stuffed in natural casing – pig appendix, obtained in traditional way by natural ripening, during which typical and distinguishing quality properties are formed. Protein content of meat should be higher than 25%, share of collagen in meat proteins less than 10%, contents of fat and proteins in meat should be equal, and pH value of 5.6.

Sensory properties of domestic *kulen* vary depending on the producer, and aren't always in accordance with chemical parameters of quality. In order for domestic *kulen* to be a product of

superior quality, in our opinion, there are two ways. One way is for meat industry, which disposes with necessary hygiene and other conditions, in addition to mass production of so called industrial *kulen*, to produce also domestic *kulen*, but fully respecting requirements which apply for traditional production (positive example – production of winter salami in one of the neighbouring countries).

The second way is to support the production of domestic *kulen* in small production facilities. Main pre-condition for this is adoption of necessary regulation/rulebook, which is not the case presently, enabling small production facilities and producers of traditional products to register their production, and fully respecting all requirements pertaining to conditions and under control, produce domestic *kulen* in their households/holdings. Regional associations of small producers of domestic *kulen* would be very beneficial in regard to establishing of their own standards, defining of production and quality of *kulen* in all its segments. In this way production of traditional *kulen* of typical, distinguishing and more or less equal quality would be achieved, and it would be legally marketed in the same way as industrial products. Positive examples exist in many countries where the production of various food products, based on traditional technologies, is developed, and in some of neighbouring countries even the production of domestic *kulen*.

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Prilog poznavanju važnijih parametara kvaliteta tradicionalnog (domaćeg) kulena

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R e z i m e: U radu su prikazani rezultati ispitivanja senzornih osobina, pH-vrednosti i hemijskog sastava tradicionalnog ili domaćeg kulena. Iz rezultata proizilazi da između senzornih i hemijskih i nutritivnih parametara kvaliteta postoji izvestan nesklad, odnosno da je senzorni kvalitet kulena slabiji od sastava proizvoda. Najčešći nedostaci kvaliteta domaćeg kulena jesu različit stepen dimljenja, neodgovarajuća boja preseka, postojanje suvog ruba ispod omotača, nedovoljna povezanost nadeva, ukus koji gorči ili kiselkasta ukus. Većina ispitivanih uzoraka domaćeg kulena imala je pH vrednost od 5,22 do 5,51, što pokazuje da proces zrenja još nije dovršen, ali i da je fermentacija stimulisana na neki način, što nije karakteristika domaćeg

kulena. Promena mikroflore domaćeg kulena je spora i tipična je za prirodno zrenje koje se odvija za vreme proizvodnje kulena u zimskom periodu. U mikroflori kulena dominiraju bakterije koje fermentišu šećere do mlečne kiseline, Pseudomonadaceae i Enterobacteriaceae postepeno odumiru, broj Micrococacea se smanjuje, a enterokoke preživljavaju zrenje. Rezultati hemijskog ispitivanja pokazuju da kulen sadrži nešto više od 35% vlage, da je sadržaj proteina mesa veći od 25%, da je odnos između sadržaja masti i proteina približno jednak i da je udeo kolagena u proteinima mesa manji od 10%. Količina natrijum hlorida u kulenu (3,4-3,8%) odgovara dodatoj količini kuhinjske soli. U kulenu su utvrđeni ostaci nitrata (10,5 do 12,1 mg/kg) koji su sa začinima (paprika, beli luk) dodati u proizvod. Kiselinski broj kulena (7,5 - 16,6 mg KOH/g) ima vrednost uobičajenu za fermentisane suve kobasice. TBARS-vrednost je u oblasti (0,19–0,29 mg MDA/kg) pri kojoj oksidacija masti ne može da se percipira senzornim ispitivanjem.

***Ključne reči**: tradicionalni ili domaći kulen, kvalitet, hemijski sastav, senzorne osobine, pH vrednost.*

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Some quality parameters of functional fermented, cooked and liver sausages*

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A b s t r a c t: The most important quality parameters of functional fermented, cooked and liver sausages are presented. Experimental fermented sausages included: a) conventional fermented sausage, b) functional fermented sausage with 2% inulin powder, c) functional fermented sausage with 4% inulin suspension and d) functional fermented sausage with 8% inulin suspension. All fermented sausages contained probiotic strain Lactobacillus casei LC 01. Experimental cooked sausages included: a) conventional cooked sausage, b) functional cooked sausage with inulin suspension, c) functional cooked sausage with flaxseed/rapeseed oil and d) functional cooked sausage with inulin suspension and flaxseed/rapeseed oil. Experimental liver sausages were: a) conventional liver sausage and b) functional liver sausage with inulin suspension. The obtained results show that functional fermented sausages have lower pH (4.77 – 4.93) and a_w (0.89 – 0.90) values then the conventional sausage (pH = 5.18; a_w = 0.93). Functional fermented sausages contain more proteins (24.2–24.5%) and less fat (24.4 – 29.7%) then conventional sausage (proteins = 20.9%; fat = 32.4%). The content of functional ingredients such as inulin and pea fiber (4.1–5.7%), in functional fermented sausages as well as higher number of probiotic strain Lactobacillus casei 01 (8.3–8.5 log cfu/g) than in conventional cooked sausage (8.1 log sfu/g) supports their functional potential. Addition of inulin (both in powder and suspension) and pea fiber improves the appearance, cut surface appearance and texture, but affects the colour of fermented sausages. Addition of 8% inulin suspension affects the odour and taste of fermented sausages. Cooked sausages with inulin suspension contain more water (63%) and less fat (22–23%) than conventional sausage (water – 60%; fat – 25.3%) and sausage containing flaxseed/rapeseed oil (water – 59%; fat – 26%). Protein content is similar in all experimental cooked sausages (10.2 –10.4%). Cooked sausages produced with inulin suspension contain 3.0–3,1% inulin. Sausages produced with flaxseed/rapeseed oil contain more polyunsaturated fatty acids (PUFA), (18.4 – 20.9% of total fatty acid content) and much more favourable omega-6/omega-3 fatty acid ratio (1,6 –2,5) then sausages produced without these oils (PUFA – 11.9–12.1%; omega-6/omega-3 – 22.3 –22.9). Despite the high content of polyunsaturated fatty acids,(TBARS) value in all experimental cooked sausages was 0.00 mg MAL/kg. Addition of flaxseed/rapeseed oil in the amount of 6% affects colour, odour and taste and texture of cooked sausages, but not the appearance and cut surface appearance of the product. Functional liver sausage contains less water (42.5%) and fat (43.6%) than the conventional cooked sausage (water = 44.3%; fat = 44.0%), but protein content is similar in both products (9.0–9.1%). Functional liver sausage contains 3.1% dietary fibre (inulin and pea fiber) which supports its functional potential. Sensory properties of functional liver sausage are rated excellent at the scale from 1 to 5 (average rating 5.0). On the contrary, some sensory properties of the conventional liver sausage were rated somewhat lower (odour and taste 4.8 and texture/juiciness 4.6).

Key words: *functional food, quality, fermented sausages, cooked sausages, liver sausages.*

Introduction

The term functional food refers to food that, in addition to basic nutritional components, contains ingredients which is positively impact on human's health (*Nitsch,* 2006/a). Meat industry and other branches of food industry, in accordance with contemporary scientific knowledge and the market re-

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quirements, begin to manufacture products with properties of functional food. Creating a meat product as functional food is based on knowledge of its nutritional and other characteristics, and includes enrichment of the product with ingredients that have positive effect on human's health and reduction the content of potentially harmful ingredients. The most important ingredients which are added to functional food are probiotics, prebiotics, antioxidants, omega-3 fatty acids, vegetable oils and fats, dietary fibre, micronutrients, vitamins and other (*Jimenez-Colmenero et al.,* 2001*; Arihara,* 2006; *Nitsch,* 2006/a). Because of its technological characteristics, fermented, cooked and liver sausages have a certain potential to be produced as functional food.

The major part in fermented sausage composition is meat (70–80%), which is well known as a source of nutritionally high valuable proteins, B group vitamins, minerals and bioactive substances that are very important in human's diet. Since the fermented sausages are not heat treated during production, these valuable nutrients remain essentially unchanged. In addition, among bacteria of the genus *Lactobacillus* which are involved in fermentation there is the greatest number of strains with probiotic properties, so the use of probiotics as functional ingredients in fermented sausages is of particular importance. Other functional ingredients such as prebiotics (dietary fibre, inulin and oligofructose) and omega-3 fatty acids could be added to fermented sausages too (*Müller,* 2006; *Vuković et al.,* 2007; *Vasilev,* 2010). In this way the functional fermented sausages could be produced and compared to conventional products, have not only greater nutritional value, but also significant potential to have a positive influence on human's health.

Cooked sausages are meat products that in stuffing contain meat batter and are conserved by heat treatment at temperatures of pasteurization. Stuffing of cooked sausages is a complex system made up of true and colloidal solution, swollen protein gels, emulsions, foams and suspensions (*Vuković,* 2006). By production of cooked sausages as functional food, a certain problem could be a reduction of fat, as well as enrichment of products with omega-3 fatty acids, which are mostly found in fish oil and some vegetable oils, such as rapeseed and linseed oil. The use of these oils could influence the aroma, colour and texture of cooked sausages (*Nitsch,* 2006/a) and in addition, polyunsaturated fatty acids easily oxidize, which leads to chemical spoilage of products (*Valencia et al.,* 2007). Functional cooked sausages can be produced by replacing a part of fat tissue with the prebiotic inulin in the form of suspension. Inulin suspension up to 5% of the stuffing has no negative influence on sensory properties of the products (*Nitsch,* 2006/b).

Liver sausages are produced from liver, cooked or boiled meat, fat and connective tissue, salt, spices and additives. Technological base of the stuffing consists of liver, fat and broth. The central role in the sausage stuffing stability belongs to liver protein gelation during heat treatment, which implies pasteurization, cooking or sterilization (*Vuković,* 2006). Liver sausages also have the potential to be a functional food. These products contain at least 10% liver, and it is known that the liver is an important source of vitamin A, B complex vitamins, iron and microelements, whose content in the liver is several times higher than in pork and beef meat (*Vuković,* 2006). In liver sausages, a certain problem could be a reduction of fat tissue content and the addition of oils rich in polyunsaturated fatty acids, due to adverse effects on sensory properties as well as increased susceptibility to oxidation (*Valencia et al.,* 2007). In liver sausages fat tissue can be substituted with up to 20% inulin suspension, without any adverse influence on sensory properties of the products (*Nitsch*, 2006/b).

In this paper some important quality parameters of functional fermented, cooked and liver sausages, which would possess, on the one hand appropriate functional properties and on the other hand acceptable sensory properties are studied.

Material and methods

In this paper were functional fermented, cooked and liver sausages produced and tested.

Functional fermented sausages

a) Conventional fermented sausage – 75% lean beef and pork meat and 25% pork back fat.
b) Functional fermented sausage with 2% inulin powder – 75% lean beef and pork meat, 22% pork back fat, 2% inulin powder (Fibruline instant, Cosucra S.A., Belgium), 1% pea fiber (Swelite, Cosucra S.A., Belgium).
c) Functional fermented sausage with 4% inulin suspension – 75% lean beef and pork meat, 20% pork back fat, 4% inulin suspension (Fibruline XL, Cosucra S.A., Belgium, inulin and water ration 1:1), 1% pea fiber (Swelite, Cosucra S.A., Belgium).
d) Functional fermented sausage with 8% inulin suspension–75% lean beef and pork meat, 16% pork back fat, 8% inulin suspension (Fibruline XL, Cosucra S.A., Bel-

gium, inulin and water ratio 1:1), 1% pea fibre (Swelite, Cosucra S.A., Belgium).

Additions to 1 kg stuffing: 28.0 g curing salt with nitrite, 0.625 g preparation *Lactobaciluus casei* LC 01 strain (Chr. Hansen, Denmark), 1.5 g dextrose, 4.0 g sucrose and 4.0 g mixture of spices.

Inulin suspension was prepared by homogenization of inulin and water (75° C) in a cutter in 1:1 ratio, followed by cooling and freezing.

The stuffing for fermented sausages was prepared by coarse chopping of frozen lean pork meat and pork back fat (including frozen inulin suspension under „c" and „d" in aforementioned products) at the beginning, followed by probiotic starter culture addition. After that, the chilled beef meat, sugars (including inulin powder in „b" sausages), pea fibre and spices were added. The stuffing was comminuted to 2 mm particle size, and at the and of the process the ungrounded pepper and curing salt were added.

The stuffing was filled in the collagen casings 60 mm in diameter, and submitted to fermentation and smoking at a temperature which was decreasing from 26 to 22° C, and then drying and ripening at the temperature which decreased to 15° C. Relative humidity gradually decreased from 91% at the beginning of ripening, to 85% at the end. The total production process lasted 21 days.

Functional cooked sausages:

a) Conventional cooked sausage: 25% pork meat II, 27.5% beef meat II, 22.5% water/ice, 25% pork back fat
b) Functional cooked sausage with inulin suspension: 25% pork meat II, 27.5% beef meat II, 22.5% water/ice, 17% pork back fat, 8% inulin suspension (Fibruline XL, Cosucra S.A., Belgium, inulin and water ratio 1:3),
c) Functional cooked sausage with flax seed/rapeseed oil: 25% pork meat II, 27,5% beef meat II, 22.5% water/ice, 19% pork back fat, 6% flax seed/rapeseed oil (1:1 ratio)
d) Functional cooked sausage with inulin suspension and flax seed/rapeseed oil: 25% pork meat II, 27.5% beef meat II, 22.5% water/ice, 11% pork back fat, 8% inulin suspension (Fibruline XL, Cosucra S.A., Belgium, inulin and water ratio 1:3), 6% flax seed/rapeseed oil (1:1 ratio).

Additions to 1 kg stuffing: 16 g curing salt with nitrite, 3 g phosphate, 4 g spices, 0.3 g ascorbate.

Inulin suspension was prepared by homogenization of inulin and water (75° C) in a cutter in a 1:3 ratio, followed by chilling to +2° C.

The batter was prepared in the usual way for fine grounded cooked sausages and stuffed into polyamide casings 60 mm diameter. Sausages were heat treated at a temperature of 80 ° C, until reaching 72° C in the thermal centre of the sausage, and then chilled to a temperature of +4 ° C.

Functional liver sausages:

a) Conventional liver sausage – 22.5% pork liver, 50% pork fat, 20% pork head meat, 12.5% broth.
b) Functional liver sausage with inulin suspension: 22.5% pork liver, 45% pork fat, 20% pork head meat, 12,5% broth, 4% inulin suspension (Fibruline XL, Cosucra S.A., Belgium, inulin and water ratio 1:3), 1% pea fibre (Swelite, Cosucra S.A., Belgium).

Additions to 1 kg stuffing: 14 g curing salt with nitrite, 3 g phosphate, 4 g spices, 0,3 g ascorbate.

Inulin suspension was prepared by homogenization of inulin and water (75° C) in a cutter in a 1:3 ratio, followed by chilling to + 2°C.

The batter was prepared in the usual way for liver sausages and stuffed into polyamide casings 40 mm diameter. Sausages were heat treated at a temperature of 85°C, until reaching 80°C in the thermal centre of the sausage, and then chilled to a temperature of +4°C.

Experimental sausages were examined using standard physico-chemical, chemical, bacteriological and sensory methods.

pH value was measured by means of digital pH-meter WTW, model 521, with combined electrode (WTW-Wissenschaftlich-Technische Werkstäten GmbH, Weilheim, Germany). Water activity (a_w) was measured by means of a_w-meter (a_w-Wert-Messer) brand Lufft (Durotherm, Stuttgart).

For chemical analysis of experimental sausages the following standard methods were applied: 1) protein was determinated content by method SRPS ISO 937 (1992); 2) relative collagen content in meat proteins was calculated by dividing collagen content and meat protein content. Collagen content was calculated by multiplying hydroxyproline content (%) with factor 8 (hydroxyproline content was determined by method SRPS ISO 3496, 2002); 3) total fat content was determinated by method SRPS ISO 1443 (1992); 4) Acid value by lipid extraction from the sample and further determination by method SRPS ISO 660 (2000); 5) TBARS value (Thiobarbituric Acid Reactive Substances) – according to *Tarladgis et al. (1964) and Holland (1971);* 6) moisture content – by method SRPS ISO 1442 (1998); 7) Sodium chloride content – by method

according to *Volhard* SRPS ISO 1841–1 (1999); 8) Nitrite content – by method SRPS ISO 2918 (1999); 9) Ash content by method SRPS ISO 936 (1999). Total lipids for determination of fatty acids were extracted by Accelerated solvent extraction method with solvents (ASE 200, Dionex, Germany). Fatty acid methyl esters were prepared by transesterification with trimethylsulfonium - hydroxide, according to the method SRPS EN ISO 5509:2007. Methyl esters were determined by capillary gas chromatography with flame-ionizing detector on the device Shimadzu 2010 (Kyoto, Japan) and identified by comparing relative retention times with relative retention times of individual methyl esters in standard Supelco 37 Component FAME Mix.

The number of probiotic strain *Lb. casei* LC 01 in fermented sausages was examined on MRS agar (Merck) supplemented with moxalactam (Sigma M-8158) in the amount of 112 mg / L, at 37°C/72 hrs in anaerobic environment (*Kröckel,* 2006). Confirmation of *Lb. casei* was performed by examining of biochemical characteristics of grown colonies using API 50 CH system (bioMérieux).

Overall sensory quality was investigated by the method of adjusted five-point scale system. Sensory characteristics of fermented sausages were evaluated by scores from 5 (excellent) to 1 (unacceptable), and the obtained scores are multiplied with corresponding coefficients of importance. For fermented sausages the following coefficients are used: appearance 2, cut surface appearance 4, colour and sustainability of colour 4, odour and taste 6 and texture 4. For cooked sausages following coefficients were used: appearance 3, cut surface appearance 3, colour and sustainability of colour 4, odour and taste 6 and texture/juiciness 4. For liver sausage the following coefficients were used: appearance 2, cut surface appearance 3, colour and sustainability of colour 3, odour and taste 7 and texture/juiciness 5. The final rating was given by values from 1 to 5, and the adjusted rating by values from 10 to 100. In order to determine the difference between the sensory properties of the experimental products the triangle test was applied (*Radovanović and Popov-Raljić,* 2001).

The results were statistically analyzed by determining the average value, measure of variation and statistical significance.

Results and discussion

Fermented sausages

The most important functional ingredients, when it comes to fermented sausages, are probiotics and prebiotics. As a probiotic in our experiments was used *Lactobacillus casei* LC 01 strain, which according to the literature data, survives well in fermented sausages and shows no negative influence on sensory properties of these products (*Bactoferm Bulletin,* 2003*; Vasilev,* 2010). The results show that the number of probiotic strain *Lb. casei* LC 01 in functional fermented sausages is from 8.3 to 8.5 log cfu/g, whereas in conventional sausage is 8.1 log cfu / g. This difference was not statistically significant (p-value ranged from 0.07 to 0.15) due to the variations of results. Significantly higher numbers of probiotic bacteria *Lactobacillus casei* LC 01 in functional fermented sausages than in conventional ($p < 0.05$) was determined after 7th and 14th day of production, as shown in Figure 1. The higher number of probiotic bacteria in functional fermented sausages is in accordance with results of *Pennacchia et al.* (2006) and *Vasilev* (2010), by which probiotic bacteria grow better in the fermented sausage containing fructooligosaccharides and inulin. In addition, it should be noted that in all experimental fermented sausages the number of probiotic strain *Lactobacillus casei* LC 01 is more than 2 logarithmic units higher than 6 log cfu/g, which, based on the data from the literature, is defined as the minimum number of probiotic bacteria in the product to be characterized as probiotic food (*Kröckel,* 2006).

As prebiotics in the production of functional fermented sausages were used inulin (soluble fibre) and dietary pea fibre (insoluble fibre). Inulin is indigestible oligosaccharide composed of β-D--fructofuranoses connected with β-(2-1) bonds that are resistant to digestive enzymes (*Nitsch,* 2006/ b). Inulin has the technological characteristics that make it suitable as a replacement for fat in meat products. In water systems inulin forms gel that has a structure similar to fat, has a neutral taste and odour, so in limited quantities does not affect adversely the sensory properties of products (*Janvary,* 2005). The results of our study indicate that the addition of prebiotics such as inulin and pea fibre have some influence on physicochemical properties (pH and a_w) of fermented sausages, chemical composition and on some sensory properties, depending primarily on the amount and form in which inulin was applied.

Functional fermented sausages have significantly lower pH value ($p < 0.001$) than conventional fermented sausages, as shown in Figure 2. Lower pH values in fermented sausages containing oligofructose are found according to *Vasilev et al.* (2009 and 2010) too. Our results show that the pH value of fermented sausages is affected by whether the inulin is added to the stuffing in the form of powder or suspension. pH value at the end of ripening is

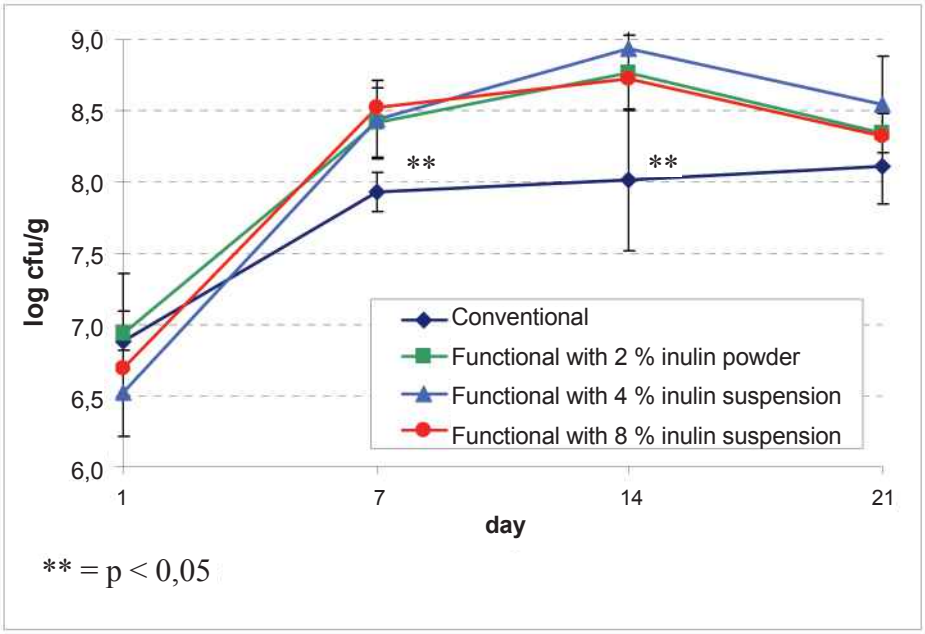

Figure 1. Number of probiotic strain *Lactobacillus casei* LC 01 in fermented sausages
Slika 1. Broj probiotičke bakterije *Lactobacillus casei* LC 01 u fermentisanim kobasicama

the lowest in functional fermented sausage with 2% inulin powder (4.77). In functional fermented sausage with inulin suspension (4 and 8%) pH value was 0.1 and 0.16 units, respectively higher than in sausages in which inulin was added in powder form, and this difference was statistically significant ($p < 0.001$). The reason is probably that the inulin powder, which is thoroughly mixed with the stuffing during preparation, could be more accessible to lactobacilli which ferment inulin (*Pennachia et al.,* 2006) then the inulin which is present in stuffing in the form of peaces of inulin suspension. Although there are differences in pH values of experimental fermented sausages, the measured pH values are typical for this type of meat product and are similar to the values reported by other authors (*Garcia et al.,* 2002; *Muguerza et al.,* 2004).

Water activity at the end of functional sausages ripening in our experiments was lower for about 0.03 a_w-units than in conventional sausage (Figure

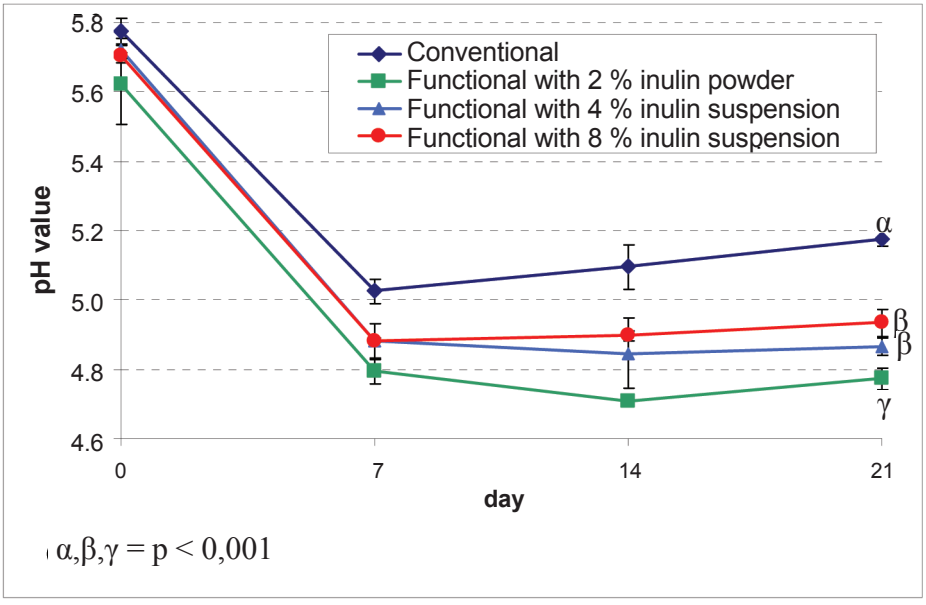

Figure 2. Variations of pH-value in fermented sausages during ripening
Slika 2. Promena pH vrednosti fermentisanih kobasica za vreme zrenja

3), and this difference was statistically confirmed ($p < 0.05$). Other author's reports show that the addition of various dietary fibre decreases water activity of fermented sausages, which at the end of ripening is 0.84 to 0.88 (*Mendoza et al..,* 2001; *Garcia et al.,* 2002; *Sieg,* 2005*; Eim et al.,* 2008; *Vasilev,* 2010). The explanation is in the fact that dietary fibre support drying of fermented sausages by the formation of three-dimensional net, which contributes to more balanced distribution of moisture in the stuffing and its diffusion from the sausage centre to the surface (*Sieg,* 2005).

31.15% (*Saičić et al.,* 2006). Functional fermented sausages from our experiments contain less fat than conventional sausages, in proportion to the amount of fatty tissue that is replaced by prebiotics. Fermented sausage with 8% inulin suspension contains about 8% less fat, fermented sausage with 4% inulin suspension contains 3.86% less fat and fermented sausage with 2% inulin powder contains 2.71% less fat than the conventional sausages (Figure 4). Lower fat content in fermented sausages in which the fatty tissue was replaced with prebiotics were reported by other authors too (*Valencia et al.,* 2007*, Vasilev,*

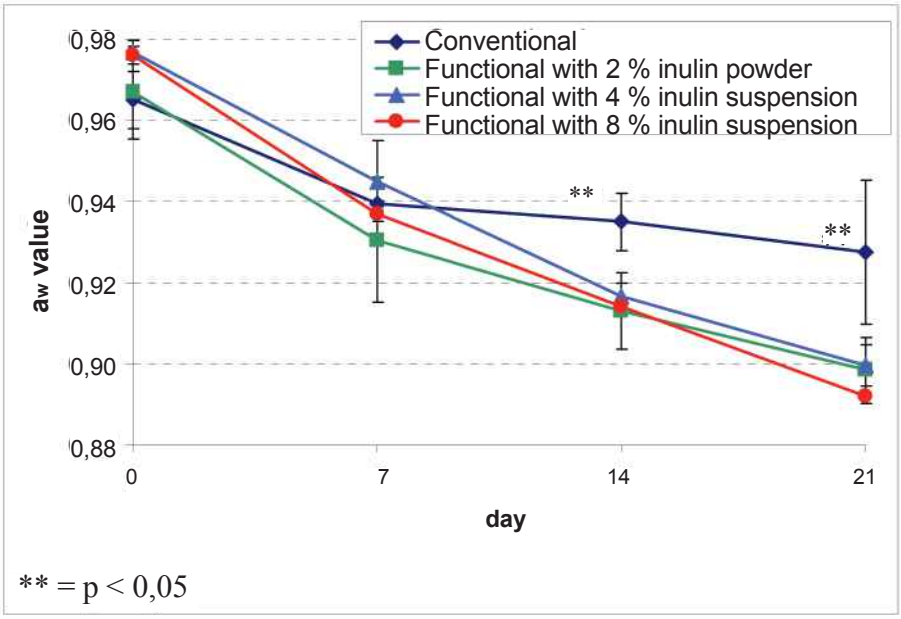

Figure 3. Variations of a_w value in fermented sausages during ripening
Slika 3. Promena a_w vrednosti fermentisanih kobasica za vreme zrenja

Beside the addition of functional ingredients, the concept of functional meat product's manufacture creation includes fat reduction and a higher biological value in terms higher meat protein content, lower connective tissue protein content (*Trumbo et al.,* 2002) and higher content of bioactive substances originating from meat (*Schmid,* 2009). Functional fermented sausages from our experiments contain about 3.3 to 3.6% more meat proteins than conventional fermented sausage (Figure 4). The relative collagen content in meat proteins is similar in all experimental products, ranging from 4.63 to 6.11%, which is significantly less than the norms prescribed by the *Regulation on quality and other requirements for meat products* (2004), in which the relative collagen content in meat proteins in semi-dry fermented sausages can not be higher than 20%. Fermented sausages from domestic market contain 17.52 to 32.96% meat proteins, and the relative collagen content in meat proteins is 6.22 up to

2010). Functional fermented sausage with 8% inulin suspension contains more moisture than the other functional fermented sausages (Figure 4), which can be explained by the fact that the moisture content in the product increases with the amount of added emulsion containing a certain amount of water, which is described by *Muguerza et al.,* (2001) and *Yuldiz-Turp et al.,* (2008). Carbohydrate rest in experimental fermented sausages that represents added prebiotics - inulin and pea fibre, is proportional to the amount of added prebiotics. The highest carbohydrate content was calculated in functional fermented sausage with 8% inulin suspension (5.7%). In the sausage with 2% inulin powder and sausage with 4% inulin suspension (the ratio of inulin and water in the suspension was 1:1) the carbohydrate content is very similar, is 4.07 and 4.10% respectively, which is understandable because in these products the same amount of prebiotics was added.

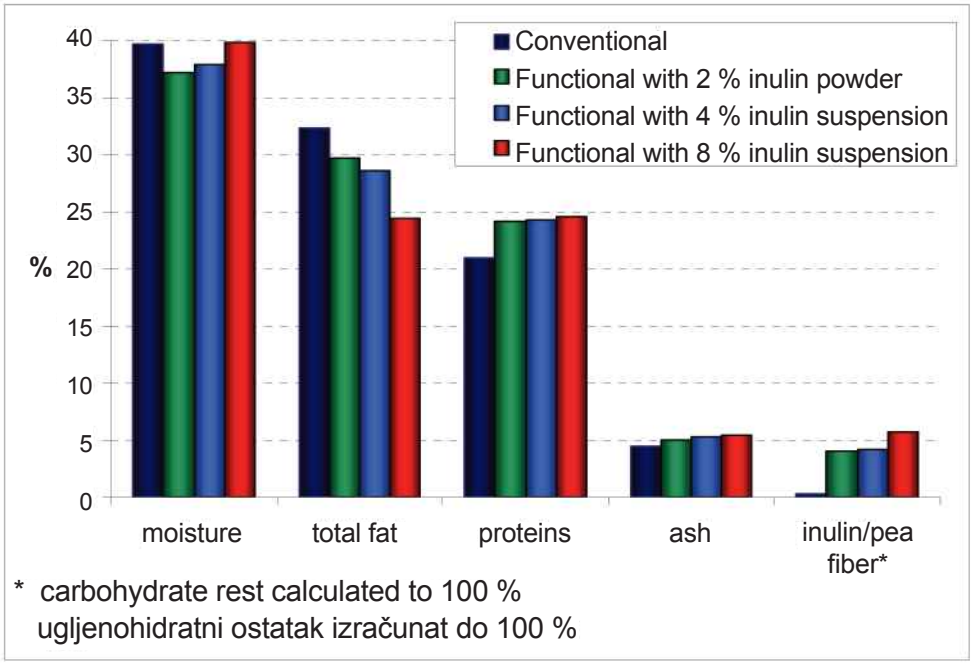

Figure 4. Chemical composition of fermented sausages
Slika 4. Hemijski sastav fermentisanih kobasica

The results of sensory properties examination of the experimental fermented sausages showed that all products are highly evaluated (Figure 5). Functional fermented sausage with 2% inulin powder and functional fermented sausage with 4% inulin suspension were given the best scores for the analyzed sensory properties. The average score for overall sensory quality of both products was 4.80. When taking into account the adjusted ratings, functional fermented sausage with 4% inulin suspension was slightly better rated (96.5) than functional fermented sausage with 2% inulin powder (95.7) because of better aroma and taste, properties which have the highest coefficient of importance for fermented sausages (coefficient 6). Conventional fermented sausage received a score for overall sensory quality of 4.63 (adjusted rating 93.4), because of lower scores for appearance (slightly wrinkled), cut surface appearance and texture. Fermented sausage with 8% inulin suspension got the lowest score for the overall sensory quality, which was 4.58 (adjusted rating 91.0) because of lower scores for odour and taste, which was somewhat different from the typical odour and taste of fermented sausages. Despite the existing differences in individual sensory properties of experimental fermented sausages, the triangle test showed no statistically significant differences between these products, because in 10 repetitions there were between 2 and 4 correct answers obtained. For the statistical significance level of $p < 0.05$ there should be at least 7 correct answers (*Radovanović and Popov-Raljić,* 2001).

Cooked sausages

Results of experimental sausage's chemical composition examination (Figure 6) showed that cooked sausages with inulin suspension contained 2.6 to 3.7% less fat than conventional one, which corresponds to the amount of pork back fat replaced with inulin suspension. Sausages with linseed / rapeseed oil contained about 1% more fat than conventional sausages. The reason for this lies in the fact that the vegetable oil contains almost 100% of total fat (*Swern,* 1972) and adipose tissue of pigs up to 95% total fat (*Vuković,* 2006). Because of that, by the replacement of 6% pork back fat with the same amount of vegetable oil, a slightly higher amount of fat was added in the product. Cooked sausages with inulin suspension contained about 3% more moisture than conventional sausages and sausages with linseed/rapeseed oil, because inulin suspension added into these sausages contained 75% water. The protein content in experimental sausages was similar and ranged from 10.2 to 10.4%, which corresponds to the norms for fine grounded cooked sausages prescribed by *Regulation on quality and other requirements for meat products* (2004). However, collagen content in meat proteins was 13.3 to 16.1% which is significantly less than the norms prescribed by the *Regulation* (25%), so the proteins from the experimental products have a high biological value. Fine grounded cooked sausages from the domestic market contain from 10.06 to 14.85% meat proteins and 8.90 to 14.28% total proteins. The relative

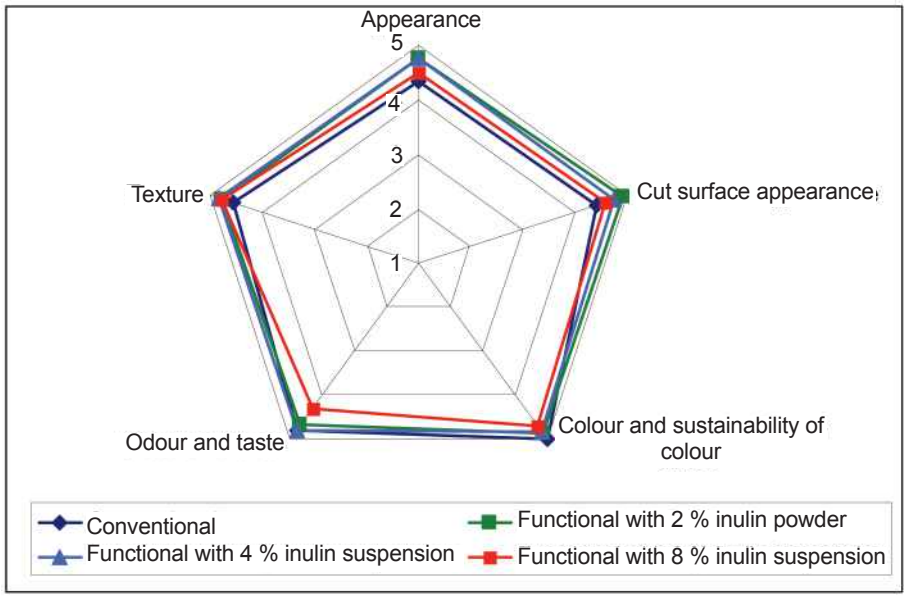

Figure 5. Scores of some sensory properties of fermented sausages
Slika 5. Ocene senzorskih karakterisitka fermentisanih kobasica

content of collagen in meat proteins is between 5.42 and 19.28%, and in total proteins content from 12.18 up to 38.91% (*Saičić et al.,* 2006). Carbohydrate rest in cooked sausages with inulin suspension is 3.0 to 3.1%, corresponding to the amount of inulin added in these products.

Despite the fact that cooked sausages with linseed/rapeseed oil contain more fat, these products have higher nutritional value than the sausages without these oils, because they have a more favourable fatty acid composition, as shown in Table 1. These sausages contain more polyunsaturated fatty acids (18.36 to 20.87%) and more favourable omega-6/omega-3 fatty acids ratio (1.75 to 2.50) than conventional sausage and sausage with inulin, in which the content of polyunsaturated fatty acids was 12.07 and 11.88%, respectively. The N-6/N-3 ratio ranged from 22.29 to 22.94. According to *Jimenez-Colmenero* (2007) the omega-6/omega-3 fatty acids ratio in the diet of man should not be greater than 4.

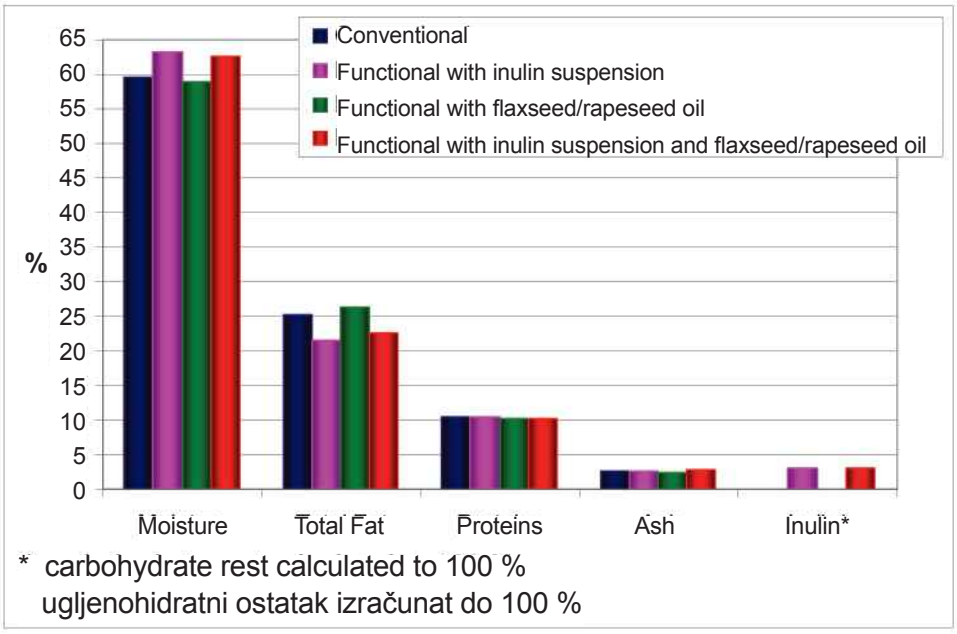

Figure 6. Chemical composition of cooked sausages
Slika 6. Hemijski sastav barenih kobasica

Table 1. Fatty acid composition of cooked sausages (% of total fatty acids content)
Tabela 1. Masnokiselinski sastav barenih kobasica (% od ukupnog sadržaja masnih kiselina)

Fatty acids/ Masne kiseline	Conventional/ Konvencionalna	Functional I/ Funkcionalna I	Functional II/ Funkcionalna II	Functional III/ Funkcionalna III
C14	1,35	1,4	1,12	1,10
C16	25,28	25,53	21,74	20,47
C16:1	2,36	2,42	1,89	1,84
C17	0,35	0,36	0,33	0,33
C18	12,71	12,75	11,38	10,65
C18:1cis-9	42,08	41,80	41,38	41,09
C18:1cis-11	2,98	3,06	2,84	2,73
C18:2n-6	10,40	10,17	11,95	12,11
C18:3n-3	0,43	0,42	5,11	7,46
C20	0,17	0,16	0,20	0,21
C20:1	0,65	0,62	0,70	0,70
C20:2n-6	0,55	0,58	0,56	0,53
C20:3n-6	0,42	0,49	0,48	0,54
C20:3n-3	0,08	0,07	0,08	0,06
C22:1+20:4	0,19	0,15	0,18	0,17
SFA	**39,86**	**40,20**	**34,77**	**32,76**
MUFA	**48,07**	**47,90**	**46,86**	**46,36**
PUFA	**12,07**	**11,88**	**18,36**	**20,87**
N-6	**11,37**	**11,24**	**12,99**	**13,18**
N-3	**0,51**	**0,49**	**5,19**	**7,52**
N6/N3	**22,29**	**22,94**	**2,50**	**1,75**

Since polyunsaturated fatty acids easily oxidize, which can lead to chemical spoilage of sausages (*Valencia et al.,* 2007), the parameters that indicate the degree of fat hydrolysis and rancidity in experimental cooked sausages were investigated. The acid number of sausages that contain linseed/ rapeseed oil is slightly higher (0.91 to 0.93 mg KOH / g) than in conventional (0.72 mg KOH / g) and functional cooked sausage with inulin suspension (0.65 mg KOH / g). However, TBARS-value, which shows the degree of rancidity of fat, in all experimental sausages, was 0.00 mg MAL / kg.

The results of sensory properties investigation of experimental cooked sausages show that functional cooked sausages have poorer sensory quality than conventional sausage which got a total score of 4.90 (Figure 7). The lowest rating for overall sensory quality have cooked sausages with linseed/rapeseed oil (overall rating of 4.36. adjusted rating 84.6. and 85.2) due to the adverse effect of these oils on the colour. texture. odour and taste of these products. The colour was paler than usual for this type of sausage. the texture was softer. and vegetable oil aroma dominated in odour and taste. The adverse effects of vegetable oils on the texture and colour of cooked sausages were determined by *Youssef* and *Barbut* too (2011). Functional cooked sausage with the suspension of inulin is much better rated (overall rating 4.82. adjusted rating 95.9) than functional cooked sausages containing linseed/ rapeseed oil. Literature data also show that functional cooked sausages can be produced with the addition of inulin suspension with no adverse influence on sensory properties of products (*Nitsch,* 2006).

Liver sausages

Results of chemical composition investigation of the experimental liver sausages (Figure 8) show that functional liver sausage contains slightly less moisture but much higher carbohydrate content (3.1%) derived from inulin and dietary pea fibre which represent a functional component of the product. The protein content is very similar and counts 9.0 and 9.1%, which corresponds to norms for liver sausage prescribed by *Regulation on quality and other requirements for meat products* (2004). However, it should be noted that the relative content of collagen in meat proteins in experimental liver sausages (conventional 16.2% and functional 16.6%) is significantly lower than the limits prescribed by *Regulation* (25%). This data support the high

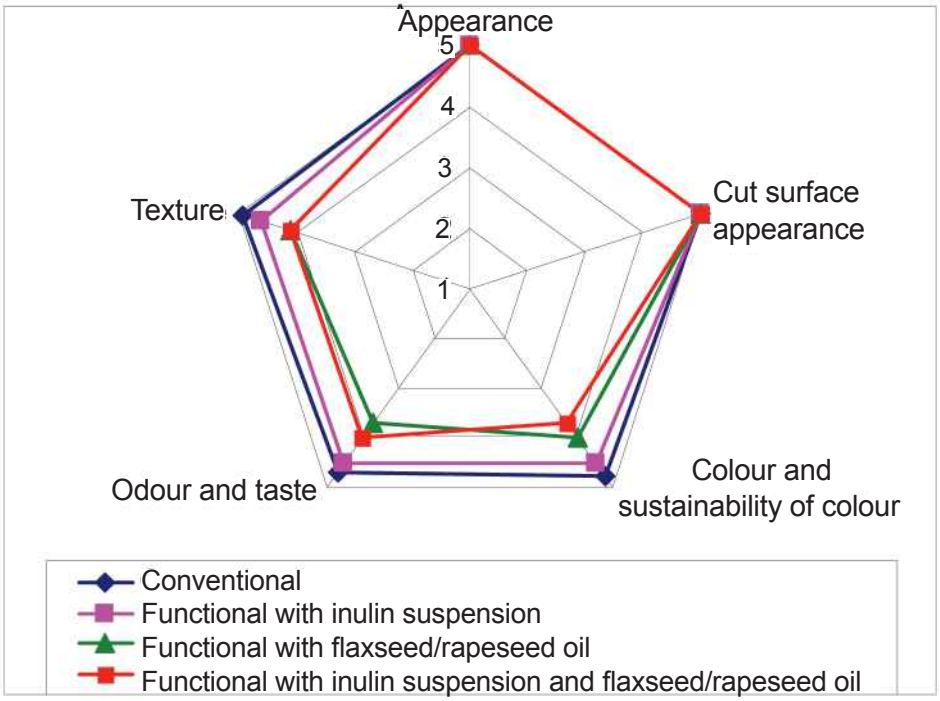

Figure 7. Scores of some sensory properties of cooked sausages
Slika 7. Ocene senzorskih karakterisitka barenih kobasica

biological value of experimental liver sausages. as it is known that proteins of connective tissue contain nearly twice less essential amino acids than muscle tissue proteins (*Prändl,* 1988). Cooked sausages, which include liver sausages too, from the domestic market contain between 8.19 and 12.22% meat proteins and 6.79 to 9.18% total proteins, while the relative collagen content in meat proteins is between 10.78 and 23.26% and in total proteins from 19.87 up to 30.00% (*Saičić et al.,* 2006).

Functional liver sausages have an excellent sensory quality, which is supported by the results of the sensory analysis shown in Figure 9. All sensory properties of functional liver sausage were

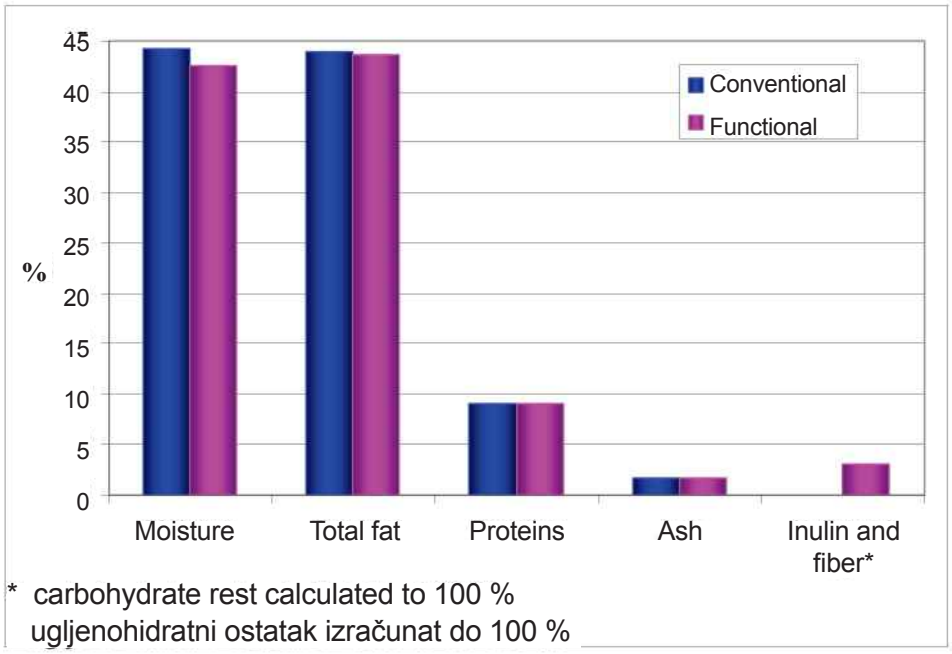

Figure 8. Chemical composition of liver sausages
Slika 8. Hemijski sastav jetrenih kobascia

estimated by a maximum score (overall rating 5.0, adjusted rating 100). On the contrary, odour and taste, and texture and juiciness of conventional liver sausage were slightly lower rated (4.8 and 4.6). The overall rating of sensory properties of conventional liver sausage was 4.88, and adjusted rating 96.6. Literature data show that changing the composition of liver sausage to gain characteristics of functional food by adding vegetable oil emulsions in order to increase content of polyunsaturated fatty acids, adversely affect the colour and texture of these products (*Delgado-Pando,* 2011). On the contrary, functional liver sausages can be produced with the addition of prebiotics in the form of inulin suspension without adverse influence on sensory properties of the products (*Nitsch,* 2006). Results of sensory examination of liver sausage from our experiments show that the addition of 4% inulin suspension and 1% of dietary pea fibre have a positive influence on the colour and texture of liver sausage.

as higher number of probiotic strain *Lactobacillus casei* 01 (8.3–8.5 log cfu/g) than in conventional sausage (8.1 log sfu/g) supports their functional potential. Addition of inulin (both in powder and suspension) and pea fibre improves the appearance, cut surface appearance and texture but affects the colour of fermented sausages. Addition of 8% inulin suspension affects the odour and taste of fermented sausages.

Cooked sausages with inulin suspension contain more water (63%) and less fat (22–23%) than conventional sausage (water – 60%; fat –25.3%) and sausage containing flaxseed/rapeseed oil (water = 59%; fat = 26%). Protein content is similar by all experimental cooked sausages (10.2 – 10.4%). Cooked sausages produced with inulin suspension contain 3.0–3.1% inulin. Sausages produced with flaxseed/rapeseed oil contain more polyunsaturated fatty acids (PUFA) (18.4–20.9% of total fatty acid content) and much more favourable omega-6/omega-

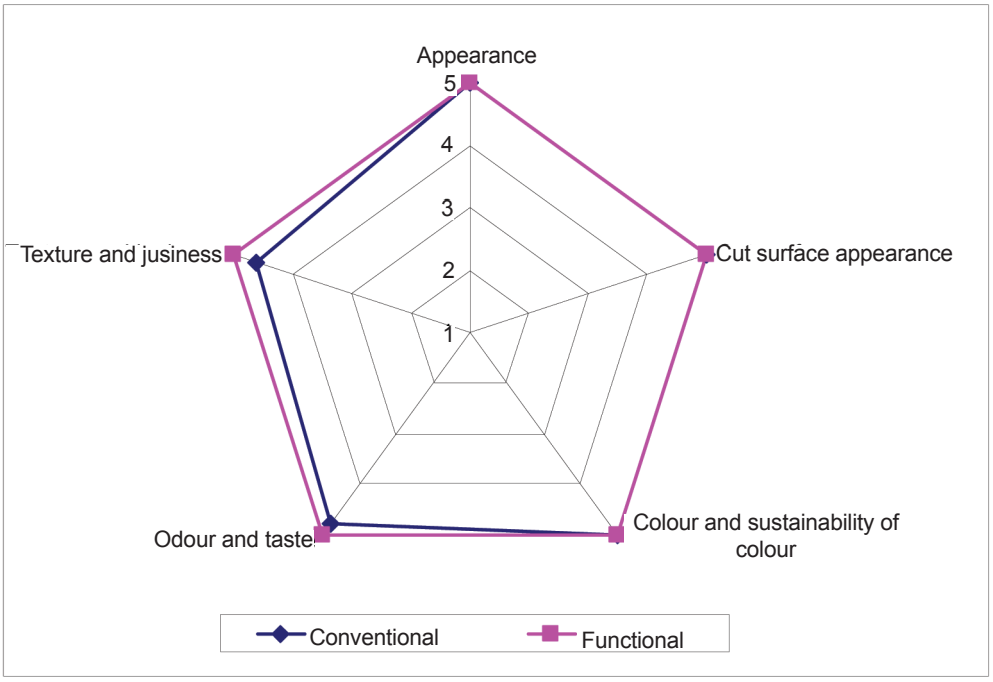

Figure 9. Scores of some sensory properties of liver sausages
Slika 9. Ocene senzoskih karakterisitka jetrenih kobasica

Conclusions

Functional fermented sausages have lower pH (4.77–4.93) and a_w (0.89–0.90) values then the conventional sausage (pH = 5,18; a_w = 0,93). Functional fermented sausages contain more proteins (24.2–24.5%) and less fat (24.4–29.7%) then conventional sausage (proteins = 20.9%; fat = 32.4%). The content of functional ingredients such as inulin and pea fibre (4.1– 5.7%) in functional fermented sausages as well

3 fatty acid ratio (1.6–2.5) then sausages produced without these oils (PUFA –11.9–12.1%; omega-6/omega-3 = 22.3–22.9). Despite the high content of polyunsaturated fatty acids, TBARS value by all experimental cooked sausages was 0,00 mg MAL/kg. Addition of flaxseed/rapeseed oil in the amount of 6% affects the colour, odour and taste and texture of cooked sausages, but not the appearance and cut surface appearance of the product.

Functional liver sausage contains less water (42.5%) and fat (43.6%) than the conventional sausage (water 44.3%; fat 44.0%) but the protein content is similar in both products (9.0–9.1%). Functional liver sausage contains 3.1% dietary fibre (inulin and pea fibre) which supports its functional potential. Sensory properties of functional liver sausage are rated excellent at the scale from 1 to 5 (average rating 5.0). On the contrary, some sensory properties of the conventional liver sausage were rated somewhat lower (odour and taste 4.8 and texture/juiciness 4.6).

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Važniji parametri kvaliteta funkcionalnih fermentisanih, barenih i jetrenih kobasica

Vasilev Dragan, Vuković Ilija, Saičić Snežana

R e z i m e: U radu su prikazani važniji parametri kvaliteta funkcionalnih fermentisanih, kuvanih i jetrenih kobasica. Proizvedene su sledeće eksperimentalne fermentisane kobasice: a) konvencionalna fermentisana kobasica, b) funkcionalna fermentisana kobasica sa 2% inulina u prahu, c) funkcionalna fermentisana kobasica sa 4% suspenzije inulina i d) funkcionalna fermentisana kobasica sa 8% suspenzije inulina. Sve fermentisane kobasice sadržale su probiotski soj bakterije Lactobacillus casei LC 01. Eksperimentalne barene kobasice uključivale su sledeće proizvode: a) konvencionalna barena kobasica, b) funkcionalna barena kobasica sa suspenzijom inulina, c) funkcionalna barena kobasica sa lanenim/repičinim uljem i d) funkcionalna barena kobasica sa suspenzijom inulina i lanenim/repičinim uljem. Iz grupe jetrenih kobasica proizvedene su: a) konvencionalna jetrena kobasica i b) jetrena kobasica sa suspenzijom inulina. Rezultati pokazuju da funkcionalne fermentisane kobasice imaju nižu pH (4,77–4,93) i a_w (0,89–0,90) vrednost nego konvencionalna kobasica (pH = 5,18; a_w = 0,93). Funkcionalne fermentisane kobasice sadrže više proteina (24,2–24,5%) i manje ukupne masti (24,4– 29,7%) nego konvencionalna kobasica (proteini = 20,9%; ukupna mast = 32,4%). Sadržaj funkcionalnih sastojaka kao što su inulin i vlakna graška u količini 4,1–5,7% u funkcionalnim fermentisanim kobasicama kao i veći broj probiotske bakterije Lactobacillus casei 01 (8,3–8,5 log cfu/g) nego u konvencionalnoj kobasici (8,1 log sfu/g) ide u prilog njihovom funkcionalnom potencijalu. Dodatak inulina (kako u obliku praška tako i u obliku suspenzije) i vlakana graška utiče pozitivno na spoljašnji izgled, izgled preseka i teksturu ali nepovoljno utiče na boju fermentisanih kobasica. Dodatak suspenzije inulina u količini od 8% nepovoljno utiče na miris i ukus fermentisanih kobasica. Barene kobasice sa suspenzijom inulina sadrže više vode (63%) i manje ukupne masti (22–23%) nego konvencionalna barena kobasica (voda = 60%; ukupna mast = 25,3%) i kobasice koja sadrži laneno/repičino ulje (voda = 59%; ukupna mast = 26%). Sadržaj proteina je sličan kod svih eksperimentalnih barenih kobasica i iznosi od 10,2 do 10,4%. Barene kobasice proizvedene sa suspenzijom inulina sadrže 3,0 – 3,1% inulina. Kobasice koje su proizvedene sa lanenim/repičinim uljem sadrže više polinezasićenih masnih kiselina (PUFA) (18,4 – 20,9% od ukupnog sadržaja masnih kiselina) i znatno povoljniji odnos omega-6 i omega-3 masnih kiselina (1,6–2,5) nego kobasice koje su proizvedene bez dodatka ovih ulja (PUFA = 11,9 – 12,1%; omega-6/omega-3 = 22,3 – 22,9). Uprkos visokom sadržaju polinezasićenih masnih kiselina, TBARS-vrednost kod svih eksperimentalnih barenih kobasica iznosi 0,00 mg MAL/kg. Dodatak lanenog/repičinog ulja u količini od 6% nepovoljno utiče na boju, aromu i teksturu barenih kobasica, ali ne i na spoljašnji izgled i izgled preseka proizvoda. Funkcionalne jetrene kobasice sadrže manje vode (42,5%) i ukupne masti (43,6%) nego konvencionalna barena kobasica (voda = 44,3%; ukupna mast = 44,0%) ali je sadržaj proteina sličan kod oba proizvoda (9,0–9,1%). Funkcionalne jetrene kobasice sadrže 3,1% dijetnih vlakana (inulin i vlakna graška) što ide u prilog njihovom funkcionalnom potencijalu. Senzorna svojstva funkcionalnih jetrenih kobasica su ocenjena maksimalnom ocenom na skali od 1 do 5 (prosečna ocena 5,00). Na suprot tome, neka senzorna svojstva konvencionalne jetrene kobasice su nešto lošija (miris i ukus 4,8 i tekstura/sočnost 4,6).

Ključne reči: *funkcionalna hrana, kvalitet, fermentisane kobasice, barene kobasice, jetrene kobasice.*

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Nutrition and meat quality*

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S u m m a r y: There are numerous factors which influence the quality of pork meat (genetic basis, rearing conditions, pre-slaughter procedures, carcass treatment, cooling). It is understandable that nutrition is one of the major factors which influence the quantity and quality of meat. Main objective of proper nutrition is the best utilization of the genetic potential of pigs, and obtaining of meat which will satisfy the consumer or be used as raw material in manufacturing of meat products. Quality of pig meat is most often associated with pH value, colour succulence, tenderness, content of intramuscular fat (marbling), sustainability and, in general, acceptability by consumers. In this paper, the effect of certain modifications in pig nutrition on post mortal changes, selected technological properties of meat (pH value, water binding capacity, and colour), content of intramuscular fat, quality of fat and acceptability of meat is presented.

Key words: *pig meat, nutrition, quality.*

Introduction

Consumption of pig meat globally is constantly increasing and is superior to consumption of all other types of meat (beef, poultry). In nutrition of population in Serbia, consumption of pig meat is far greater than consumption of other meat types, so share of pork in total meat consumption is approx. 60%. Consumption of pig meat varies significantly in different regions of the World. It is known that certain religions do not allow use of pig meat in human nutrition. On the other hand, e.g. annual consumption of pork per capita in Denmark is over 60 kg (*Baltić et al.*, 2002; *Baltić et al.*, 2009).

Nutritive value of meat in human nutrition is well known, so the position that the meat constitutes the basis of human nutrition is understandable. In regard to pig meat, there are 14 key reasons why it is considered as nutritionally valuable. Namely, pig meat is exceptional source of proteins, vitamin B12, vitamin B6, thiamine, niacin, selenium, zinc and phosphorus. It is very good source of riboflavin and potassium, also of iron, magnesium and pantothenic acid. Meat is very good energy source, which mainly depends on content of fat in the meat. Pig meat can contain small amounts of sodium (55 mg/100g which is only 2% of daily requirement). This meat type doesn't contain, naturally, trans- or hydrogenized fats, which can be found in ruminants. Value of pig meat is also in the fact that it is used in production of various and highly valued meat products, some of them with very long tradition (*Baltić et al.,* 2010, *Jovanović et al*., 2009a).

Efforts are constantly made to produce more and more meat, including pork. This is achieved in different ways, primarily through genetic selection, nutrition, rearing conditions, etc. Efforts of meat producers, however, are not related only to increase of the production volume, but also are directed towards obtaining of meat of high quality, meat which will meet the requirements of more demanding consumers (*Baltić et al.,* 2010).

There are many factors which influence the quality of pig meat, starting with genetics associated

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factors, followed by nutrition, rearing conditions, handling of animals during loading into vehicles, transportation, depot, stunning, slaughtering and cooling of the carcass. Quality of pig meat is traditionally associated with pH value (45 minutes and 24 hours after slaughtering), colour of meat, texture, content of intramuscular fat, sustainability and acceptability for the consumer subsequent to heat treatment. The consumer, however, recently also in our country as well as in the World, is associating the quality of meat with rearing conditions (housing) for animals, their welfare and ethical issues. From the aspect of meat industry and their desire to satisfy the consumer, quality of meat is associated with safety, chemical composition, nutritional value and sensory properties of meat (*Jovanović et al.,* 2009b).

Nutrition of pigs is one of the major factors influencing the quality of meat. It has to be, first of all, balanced in such way to enable maximum use of the genetic potential of the animal during fattening. Nutrition of pigs and its impact on the quality of meat was always considered to be exceptionally significant. This influence relates to numerous meat quality parameters such as: meat safety (biological, chemical and physiological hazards); nutritional value of meat; post mortal changes in meat and its technological properties (pH, colour, water binding capacity); content of intramuscular fat; meat colour; fatty acid composition and stability of fat during cooling (freezing) and distribution; acceptability of meat to consumers subsequent to heat treatment (*Marković et al.,* 2010;).

Selection of feeds and their impact on meat odour and taste

One of the main tasks of animal nutrition is providing sufficient quantities of food for human population, food of exceptional value as excellent source of proteins, fats, vitamins and minerals which are necessary in human nutrition. Nutrition, among other things, is also way to influence the quality of meat, as well as sensory properties of meat which are of special importance to the consumer.

Reduced amount of energy in fattening mixtures for pigs does not affect the acceptability (taste) of pig meat. However, pork steaks obtained from pigs fed ad libitum had higher scores for tenderness and lower Warner Bratzler value of shear force (WBSF) compared to pigs fed 75% or 80% ad libitum, even though the content of total and soluble muscle collagen and myofibril fragmentation index (indicator of post mortal proteolysis) have not changed. In addition, numerous studies have shown that meat from pigs fed ad libitum, subsequent to sensory analysis, was given higher scores for odour and taste, succulence and general acceptability (*Daza et al.,* 2007).

Selection of cereal/grain, as a component in pig nutrition, can also have impact on acceptability of pork. Cooked pork steaks/cuts from pigs fed wheat had higher scores for taste compared to pork cuts from pigs fed sorghum, whereas pork steaks/cuts from pigs fed mixture of yellow and white corn in ratio 33%:67% or 67%:33% had higher scores for succulence and taste compared to pork steaks/cuts from pigs fed mixture of barley and yellow, or white corn. Also, it is known that meat from pigs fed wheat has lower WBSF value and higher scores for tenderness compared to pigs fed sorghum. It is also observed that pork steaks/cuts from pigs fed barley were scored in sensory analysis as more tender than pork steaks/cuts form pigs fed corn or mixture of barley and triticale. Contrary to this, WBSF value and score for acceptability of pig meat were similar in pigs fed yellow, white corn, wheat, sorghum or triticale (*Lampe et al.*, 2006; *Sullivan et al.*, 2007).

Value of shear force necessary for cutting of cooked pork steaks/cuts increases by almost 23% with the increase of crude protein content in the fattening mixture from 10 to 22%. Also, *Goodband et al.* (1993) established the linear increase of WBSF values in cooked pork steaks/cuts with the increase of content of lysine in food from 0,6 to 1,4%, whereas *Apple et al.* (2008) observed linear increase of WBSF values with the increase of lysine ratio to energy from 1,7 (0,56 to 0,59% of lysine) to 3,1 g/Mcal (1,02 to 1,08% of lysine) in mixtures for fattening. *Goodband et al.* (1993) also recorded decrease in sensory scoring of meat tenderness with the increase of lysine level in feeds. In general, increased level of lysine in pig nutrition has no impact on degree of succulence and taste of cooked pork.

Use of rape seed or/and fish oil influences incidence of unpleasant odour of roasted meat, and has impact on its general acceptability. Use of animal sources of fat has no considerable effect on fatty acid profile and sensory properties of meat, such as tenderness, succulence and intensity of odour and taste. Use of dried distillers grain (DDGS) in nutrition of pigs or addition of glycerol influences the decrease of WBSF values. It has not been proven that addition of conjugated linoleic acid (CLA) has effect on WBSF values or assessment of the acceptability of meat or fat/bacon (*Apple et al.,* 2008; *Engel et al.*, 2001).

It is known that there is a link between calcium content and meat tenderness. It is generally accepted that increase of calcium content leads to post mortal degradation of cytoskeleton due to the effect of calpain which contributes to meat tenderness. Vitamin D

is included in the intercellular mobilization of calcium and addition of considerable amounts of vitamin D in the nutrition for instance of cattle leads to the increase of its content in blood and meat, and most important, leads to enhancement of meat tenderness. Although the calcium concentration in plasma and muscles was increased by 125% in pigs fed finisher diet supplemented with vitamin D3, WBSF value, sensory evaluation of meat tenderness, as attributes which contribute to the acceptability of meat, were not changed. Interestingly, there are literature data presenting that adding of increased amounts of vitamin D3 in pig nutrition can have impact on meat freshness, including increased initial (45 minutes) and final pH value (24 hours), better subjective score of colour, as well as on „a" value of meat colour, reduction of „L" values of meat colour, and decrease of weight loss (*Swigert et al.,* 2004; *Wilborn et al.*, 2004).

Content of intramuscular fat in pigs is of special importance for acceptability of pig meat. It is known that content of intramuscular fat from 2.5 to 3% is necessary in order for consumers to be satisfied with heat treated pork. In some countries, meat with minimum 4% of fat is more acceptable (*Baltić et al.,* 2010; *Lonergan et al.,* 2007).

Meat pig breeds today usually contain in lean meat less than 1% of fat, and one of the main contributing factors is the genetic basis. For increase of content of fat in muscle tissue, certain pig breeds are crossed in order to increase the content of intramuscular fat. Also, intramuscular fat can be influenced by nutrition. One of the ways to contribute to the increase of intramuscular fat is to decrease the content of protein and/or lysine in pig nutrition. Namely, with the decrease of protein content in grower and finisher diet, the content of intramuscular fat can increase from 13.7 to 176.5%. Similar effect, however more expressed, can be achieved by reduction of lysine content in food, in which case the content of intramuscular fat increases from 66.7 to 136.8% (*Apple,* 2010).

It is obvious that long exposure of pigs to lysine and crude protein deficits has damaging economical effect in fattening and feed conversion, but nutrition with lower content of lysine during the final five to six weeks of fattening in practice has no impact on production performance, and content of fat in meat is still increased. On the other hand, increase of level of crude proteins and/or lysine in diets has always caused the decreased level of fat in pig meat. It was observed that marbling of meat decreased linearly with the increase of lysine content from 0.54 to 1.4% (*Apple,* 2010).

Studies have shown that adding of 2% of leucine into finisher diet for pigs leads to increase of marbling of meat (20 to 30%), without any changes in production results in pig fattening. However, it could be stated that increase of fat content of meat is an indirect response to reduced utilization of lysine due to adding of significant quantities of leucine causing the amino acids misbalance.

Limited intake of food in final stage of fattening has no impact on pH value in muscles, freshness and colour of meat. It was presented in the past that food restriction by 75 to 80% of ad libitum feeding lead to decrease of fat content by 8 to 28%. Interestingly, reduction of the amount of energy in finisher diets causes no changes in fat content of meat, or has impact on any other properties of fresh meat. There is no evidence that choice of cereals/grains used in pig nutrition has any effect on marbling of meat (*Sullivan et al.*, 2007).

Fats and oils have been used for decades to increase the energy value of food for pigs, but the results of their effect on fat content of meat are not consistent. Nutrition based on sunflower or rape seed leads to decrease of content of fat in meat. However, *Apple et al.* (2008) observed that content of fat in meat increased with the increase of share of corn oil in nutrition. Content of fat in meat increased by approx. 25% in pigs fed diets containing 5% of beef tallow. In most studies, importance of the selection of fat and/or oil on marbling of meat has been proven (*Sullivan et al.,* 2007).

Conjugated linoleic acid (CLA) is a mixture of position and geometrical conjugation of linoleic acid isomers. Most of the sources of synthetic CLA contain approx. 65% CLA isomers. From July 2009, CLA is sold in USA under commercial name Lutalin® (BASF SE, Ludwigshafen, Germany). It is used in pig and broiler nutrition. There is reliable data showing that addition of CLA in pig nutrition can influence the increase in meat marbling (*Apple,* 2010).

Derivative of vitamin A – retinoic acid is included in regulation of differentiation and proliferation of adipocytes and in theory its deficit can directly influence the increase of fat content of meat. It is a fact that vitamin A deficit in livestock nutrition can lead to increase of meat marbling, without any influence on production results and carcass quality parameters. *D'Souza et al.* (2000) have also shown that vitamin A deficit during growing stage and final fattening stage leads to increase of fat content in meat by almost 54%. *Olivares et al.* (2009) have recorded that addition of 100,000 IU of vitamin A in nutrition leads to increase of fat content in pork meat from pigs with greater genetic inclination towards higher fat content of meat, but not in case of exceptionally meaty pig genotypes. It was proven that both vitamin A deficit and nutrition rich in vitamin A can con-

tribute to higher content of fat in meat, and consequently better meat marbling. In regard to addition of vitamin A into feeds used in pig nutrition, it should be stated that there are still a lot of uncertainties in regard to the effect of vitamin A on marbling of meat, which is understandable considering the possibility to choose feed, mutual interaction of main components and additives, duration of fattening, etc. (*Dikeman,* 2007).

Nutrition and colour stability of meat and fat

It is expected that change in nutrition which influences the increase of polyunsaturated fatty acids (PUFA) in pig meat also leads to inclination of meat towards the oxidation of fats. Accordingly, great number of researches is directed towards use of antioxidants in nutrition, especially vitamin E, or stimulation of endogenous antioxidant enzymes by adding of minerals (selenium, manganese, magnesium) into feeds (*Marković et al.,* 2008; *Marković et al.,* 2009).

Vitamin E (α-tocopherol) is antioxidant which protects the integrity of the cell membrane and slows done the oxidation of fats, especially during cold storage and/or distribution of meat. Therefore, adding of greater amounts of vitamin E into diets for fatteners maybe is the most studied change in nutrition which can improve the quality of pig meat.

Studies have shown many times that pig nutrition with additional 100 to 200 mg/kg of dl- α-tocopherol acetate can efficiently delay the beginning of the oxidation of fats in fresh pork steaks/cuts and minced meat, as well as partially heat treated or canned pork products. Addition of vitamin E into diets for fattening cattle not only slows down the degree of discoloration, but also improves the colour stability of fresh beef. The effect of increased amounts of vitamin E in pig nutrition, in form of dl-α-tocopherol acetate or natural stereo isomer of d-α-tocopherol acetate, on colour of fresh pig meat or colour stability during cold storage, has not yet been established in numerous studies (*Guo et al.*, 2006; *Marković et al.,* 2008).

Vitamin C has antioxidant properties and usually pigs receive sufficient amounts of this water soluble vitamin from D-glucose in liver. However, addition of ascorbic acid four hours prior to slaughtering leads to forming of dark coloured and intensive red pork meat. However, short term or long term addition of vitamin C has no effect on colour or water binding capacity of pork meat. Also, there is no evidence that addition of vitamin C improves the oxidation stability of meat lipids during storage or distribution, and that in fact pig nutrition which contains large amounts of vitamin C leads to increase of TBARS values of meat during cold storage (*Gebert et al.,* 2006).

Selenium (Se) is a component of endogenous antioxidant enzyme glutathione peroxidase and studies have shown that adding of sodium selenite or selenium from yeasts to pig diet enhanced the activity of glutathione peroxidase in serum. However, increased activity of glutathione peroxidase induced by addition of selenium can be associated with more acceptable colour of fresh meat and better water binding capacity, as well as higher lipid stability during meat storage (*Marković et al.,* 2010). There are, however, different opinions (*Han and Thacker,* 2006).

Manganese (Mn) and magnesium (Mg) are divalent, metal cat ions that can replace each other in several biological functions. However, manganese is necessary for activation of superoxide dismutase, which is included in degradation of superoxide free radicals, so TBARS value in fresh meat steaks/cuts was lower in pigs fed diet containing manganese additive. Also, meat from pigs fed diet supplemented with 350 mg/kg manganese had fewer discolorations from the second to seventh day of storage, compared to meat from pigs fed diets without manganese supplement. Also, addition of manganese in pig nutrition has other advantages because it leads to increase of pH of meat and visual scoring of colour and reduction of L* value of fresh meat colour (*Apple et al.,* 2007).

Sources of fats in pig nutrition and quality of pig fat

Fatty acids in fatty tissue of pigs can be obtained by synthesis from non-lipid substrates or directly by absorption of fatty acids from feeds. So, for instance, glucose obtained by digesting corn and barley increases the share of saturated fatty acids (SFA) in relation to polyunsaturated fatty acids (PUFA), which is not the case when so called oil fractions from concentrated feeds are used in pig nutrition. However, as already mentioned, fat is usually used in pig nutrition, because in this way the energy value of the feed is increased and quantity of cereals/grains (especially corn) used in diets for pigs is reduced (*Lampe et al.,* 2006).

Quality of fats used in pig nutrition depends on many factors, including iodine number, firming point and melting point. Sources of saturated fats, such as tallow and fat, have iodine number from 30 to 70, firming point from 32 to 47°C, and melting point is from 45 to 50°C. Contrary, unsaturated oils obtained from soy bean seed, rape seed, corn, sunflower, safflower, usually have higher iodine number from

100, firming point at temperatures below 30°C and melting point at temperatures of 20°C or below. So, fatty acids composition and quality of fat tissue of pigs depends on the selection of fats, i.e. oils used in pig nutrition (*Apple,* 2010).

Even though there is apparent nutritional value of PUFA in human nutrition, increase of polyunsaturated fatty acids in fatty tissue leads to increase of softness of fat tissue, which can consequently lead to poorer usability in processing and shorter shelf life of products (*Baltić,* 1993). Pig fat with content of linolenic acid (C18:2n-6) over 15% is categorized as soft fat. High content of linolenic acid is consequence of its high content in feeds used in pig nutrition. Soft belly fat leads to difficulties in processing of carcass, decrease of share of fat/bacon, changes in colour and appearance, as well as shorter shelf life. However, most important is that such product is not acceptable for consumers. It has been proven that the thickness and firmness of pig belly increase with the decrease of the iodine number of fats used in their nutrition. Nutrition using animal fats does not lead to incidence of soft pig belly, as in case of fats of plant origin used in pig nutrition. It is interesting that the fat/bacon of pigs fed sunflower, safflower and rape seed were scored significantly lower in sensory analysis of freshness, tenderness, amount of salt, odour and taste and general acceptability, compared to fat/bacon from pigs fed diets containing tallow. It was observed that fat/bacon from pigs fed diets containing soy bean oil is softer and of lower quality (*Teye et al.*, 2006).

There is growing evidence that between 50 and 60% of changes of fatty acid composition of pig fat is induced by type of fat used in nutrition. Changes of the fatty acid composition occur during first 14 to 35 days of nutrition with specific fats, while later the changes decrease. It has been proven that fatty acid composition of meat fat and subcutaneous fat tissue in pigs changes significantly to 17,4 kg of body weight, so during the first stage of pig nutrition with 5% of soy bean oil, iodine number of pig fat increases by 12 (*Apple et al.*, 2007). It is known that the time of semi-degradation of linoleic acid (C18:3n-3) of subcutaneous fatty tissue is 300 days. So, economic benefits when traditional food contains high level of fats, associated with increased level of utilization during growing stage of pigs, can cause low quality of pig fat. It is uncertain if removal of all fats from the final stage of pig nutrition or replacement of unsaturated fatty acids with tallow or hydrogenized fats will have consequences on quality of pig fat (*Apple et al.*, 2007).

Conclusion

The quality of meat depends on numerous factors. The attention is most often focused on the effect on nutrition on meat quality, i.e. pH value, tenderness, marbling, quality of fat, sustainability, sensory properties. Of many nutrition factors, mainly commented are the effect of pre-slaughter starvation on meat quality, restricted feeding, compensatory feeding, content of protein and content of lysine in feeds and adding of vitamin E. Studies in this field are numerous and complex, considering that in addition to feeding/nutrition they include the effect of other factors (genetic basis, pre-slaughter handling of animals, etc.) which have considerable impact on meat quality. Also, inevitable economic effects, as well as interest of all stakeholders that consumers are satisfied with the quality of meat, should also be considered.

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Ishrana i kvalitet mesa

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R e z i m e: Brojni su činioci koji utiču na kvalitet mesa svinja (genetska osnova, uslovi gajenja, postupci pre klanja, klanje, obrada trupa, hlađenje). Razume se da je ishrana jedan od veoma značajnih činilaca koji utiče i na količinu i na kvalitet mesa. Osnovni cilj pravilne ishrane je što je moguće više iskorišćavanje genetskog potencijala svinja, i dobijanje mesa koje će zadovoljiti potrošača ili naći svoju namenu u izradi proizvoda od mesa. Kvalitet svinjskog mesa se najčešće vezuje za pH vrednost, boju, sočnost, mekoću, sadržaj intramuskularne masti (mramoriranost), održivost i, uopšte, za prihvatljivost od strane potrošača. U ovom radu prikazan je uticaj određenih modifikacija u ishrani svinja na postmortalne promene, odabrane tehnološke osobine mesa (pH vrednost, sposobnost vezivanja vode, boja), sadržaj intramuskularne masti, kvalitet masti i prihvatljivost mesa.

Ključne reči: *svinjsko meso, ishrana, kvalitet.*

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Free systems of rearing of chickens and layer hens: quality of meat and eggs*

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A b s t r a c t: Conventional broiler and egg production are basis of modern production of poultry meat and table eggs in the World and in our country. Standing requirements and demands for more and better in poultry production have induced continuous work on improvement of technologies of broiler rearing and production of table eggs. Improvements in technologies of production of meat and eggs depend on country's natural resources, environment conditions and, certainly, on consumer opinion and demands. In addition to the quantity of products, considerable attention will be focused on poultry welfare, application of new rearing systems and, accordingly, on quality of the product. Positive effects of the free system of rearing of broilers and layer hens on quality of meat and eggs, which have been established in our many years of research, will be presented in this paper.

Key words: *free system of rearing, quality, poultry meat, table eggs.*

Introduction

Quality properties of poultry meat and table eggs are of very dynamic character and changeable depending on numerous factors. The quality of poultry products, among biological factors, is mostly influenced by genotype, gender and age, and the most important factors of zootechnical nature are housing system, nutrition and health condition of poultry. Presently, market offers plenty of poultry products (meat and eggs) and they are all competing to win, i.e. to realize the best marketing resulting in higher profit. For better marketing of product, in addition to competitive price, also special and guaranteed quality of the product for which consumers are prepared to pay higher price is important (*Pavlovski et al.,* 2001). Alternative, non-conventional poultry housing systems are introduced into production, on one hand because of the poultry welfare, and on the other because of the product quality, i.e. the connection between the quality of life of poultry and quality of product is established in this way (*Pavlovski et al.,* 2010).

Only few researchers in Serbia have studied the rearing programs and quality of poultry products (meat and eggs) in special non-conventional housing systems as well as new methods in production of poultry products (*Bogosavljavić Bošković et al., 2005; Milošević et al.,* 2003; *Milošević et al.,* 2005.; *Pavlovski and Mašić,* 1994; *Pavlovski et al.,*1992; *Pavlovski et al.,* 2002; *Pavlovski and Mašić,* 1986*; Pavlovski et al.,* 2009; *Pavlovski et al.,*2010; *Blagojević et al.,* 2009).

Production of poultry meat and table eggs, due to intensive and interesting development in the last twenty years of the 20th century, has managed to provide to consumers products in large amounts throughout the year and at relatively low prices. However, this has had negative impact on opinion of consumers on quality of poultry products. Eggs were unjustly accused as products with high cholesterol content to be harmful to human health. Also, relative-

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ly poorer quality of poultry meat from intensive broiler production (watery meat, insufficiently expressed/distinct flavour and aroma, high percentage of subcutaneous fat and abdominal fat, weak and breakable bones, etc.), as well as development of consumer awareness of animal welfare and food safety, had induced the perception of poultry meat as unhealthy and unnatural. Poultry meat and eggs, as food products, are unjustly accused as harmful to human health and in, last few years, this injustice is slowly being corrected.

The fact that a new organism is created from the egg – chicken, is evidence confirming that the egg is food rich in nutrients. Egg is source of highly valuable proteins and it contains unique combination of fatty acids necessary in human diet and this fact is main argument in fight/struggle against cholesterol phobia, which has been present in the last 20 years and contributed significantly to drop in the egg consumption.

New housing systems for layer hens (enriched cages, extensive, semi-intensive system, deep litter, organic production) as well as new systems in broiler production (extensive, semi-intensive, rearing of broilers separated according to gender, extended fattening, new light regimes) have not only improved the poultry welfare, but have also provided to consumers new product range (table eggs and poultry meat) of different, better, guaranteed quality.

Increase of production costs in these alternative housing systems and at the same time increase of the price of the product on the market, surely has impact on the decision of consumer in purchasing of such products. The question is raised if the consumers are ready to pay higher price for product because of ensured welfare or because these products have special and guaranteed quality. Majority of buyers appreciate mainly the freshness and safety of eggs when purchasing this product (*Pavlovski and Mašić,*1986; *Pavlovski and Mašić,* 1993*)*. Production system is also the factor influencing the choice of consumers when purchasing this product, i.e. poultry housing system which includes farm conditions, nutrition, welfare, health condition of poultry, etc. Today, there are two categories of consumers: *global consumers,* who are willing to pay higher price for products of special and guaranteed quality, and the other group, so called *consumers* who are aware of the production system and take it into account, but at the same time are not willing to pay higher price for such product.

In this paper, significantly improved technologies for production of table eggs and poultry meat from poultry reared in free system will be presented, as well as the opinion and attitude of consumers towards this type of products .

Rearing of chickens in free system

System features

Problem solved by introduction into practice of the technological procedure in production of poultry meat in rearing system with free range is organization of poultry meat production of special quality, according to principles of rationalized system and, in this way, added value for the poultry carcass is generated, as well as new market for poultry products. Considering specificities of the technological procedure, concerning provision of grazing/pasture surface, this would enable development of rural areas in our country.

After three weeks of fattening in intensive system, chickens are fattened in extensive system. Chickens can remain for further fattening on the holding of the same farmer who fattened them to mentioned age, or they can be transported to other producer who will rear the min extensive system to the slaughtering age (12 to 14 weeks).

Selection of chickens for fattening

Production of chickens in extensive systems is based on slow growing hybrids of coloured plumage, of domestic or imported origin. Chickens used in industrial production (broilers) are not used for this production, or white plumage chickens. In our conditions, domestic, native/autochthonous hens of more fattening type can be used, naked neck hens (with coloured plumage), New Hampshire, Amrock, Grey Plymouth Rock and similar chickens with no white plumage. Researches with chickens of New Hampshire and Amrock breeds were carried out by *Pavlovski et al.* (1992).

Domestic naked neck chickens, reared on our territory for long time are considered as domestic hens. They originate from primitive hens crossed with various foreign breeds of which the influence of the Transylvanian naked neck is the most obvious, since this naked neck trait is transferred as dominant trait. Naked neck hen as autochthonous is present in all neighbouring countries, and differences among them are very small *(Grujić,* 1928). The most distinct exterior trait is naked neck, and plumage is of different colour. Quality of meat is good and the hen is very resistant.

Pavlovski et al. (2009) have initiated investigations which could contribute to better understanding and consideration of possibilities for production

of chicken meat based on mentioned Program, where naked neck chicken of different varieties would be used.

Facilities and equipment

In this production smaller facilities are used, different constructions, different types, stable (fixed) or mobile. Considering that they are used for housing of chickens over night and for protection from bad weather conditions during day, the construction of these buildings is very simple and made of cheap material, such as light eaves or shed. Within the facility feeders and waterers are placed and chickens are housed on natural light, so the facilities can be without electricity. Stable facility is place in the centre of the free range - pasture which is divided into rotations.

Free range – Pasture

Chickens for fattening are reared and kept on unlimited free range (pasture) and as protection from birds of prey and thieves, it is necessary to fence the free range and divide it into small parts – rotations. On free range feeders and waterers are placed. It is desirable to have within the pasture or at least on the edges, some trees, as protection from strong sun light. If there are not trees, it is necessary to provide some type of eaves. Pasture is for poultry the source of proteins, minerals and vitamins A, B, E and C. With additional nutrition using concentrated feeds, especially alfalfa meal supplemented with vitamin D, poultry can utilize the pasture extremely well.

Nutrition

Nutrition is process of digestion, adoption and transformation of food into tissues and energy in chickens' organisms. Therefore it is considered that nutrition as well as housing has decisive effect on production and quality of poultry meat. For the purpose of production of chicken meat of special and guaranteed quality it is necessary to provide special food and nutrition of poultry. Main nutrition principles are:

- grain/cereals as diet basis (minimal share 70% in complete mixtures, except in starter - min. 50%);
- without animal feeds, feeds produced from GMO and growth promoters, and
- with limited number of additives.

System of nutrition of chickens is realized in two phases: 1. nutrition in the facility (first 6 weeks) in general is not different from nutrition of chickens in conventional fattening, except in regard to selection of feeds and less concentrated mixtures; 2. nutrition on the free range/pasture with three separate feeds (energy, protein, mineral) in system of free choice. This system allows for chickens to balance their own diet.

Change of the housing system requires adequate nutrition based on grain mixture and mixture of concentrates with usual mineral and vitamin additives. In compliance with principles of production of natural food and certain demands of European market, these diets are supplemented with natural additives in order to improve production results and quality of chicken meat. The following are considered as new additives: enzymes, probiotics, prebiotics, phytobiotics (medicinal herbs) and mycotoxin adsorbents.

With application of *enzymes* in poultry nutrition better utilization of certain nutritious substances is achieved for the purpose of obtaining better production results (gain, feed conversion), better quality of meat with lower mortality. Special importance has enzyme phytase which improves utilization of phytine phosphorus (to 30%) and reduces pollution of environment.

Probiotics help development of useful to the detriment of harmful (pathogen) microorganisms in digestive tract. With application of probiotics the health condition is improved, also better production results and better quality of meat are realized.

Prebiotics are indigestible feed components (carbon hydrates, certain peptides or lipids) which have favourable effect on host through stimulation/promotion of development of desirable bacteria with simultaneous limiting of development of undesirable bacteria in digestive tract. With application of prebiotics optimal production results are achieved, utilization of food is improved as well as vitality of chickens.

Phytobiotics and their extracts have wide range of effect: they stimulate consumption of food and endogenous secretion of enzymes, they have anti-microbial and coccidiostatic effect, improve production results, poultry health and product quality. Medicinal herbs which can be used as additives are: camomile, lemon balm, mint, fennel, yarrow, thyme, sweet basil, etc.

Presence of mycotoxins in chicken food causes numerous problems, starting with the negative effect on production results to various health problems which often end in death. They can be generated in contaminated food during production process or storage. In the strategy for solving of the problem of mycotoxins in nutrition the best results were realized by different adsorbents. For all adsorbents it is important that they have high adsorbing specificity,

high affinity and adsorbance capacity and that they protect poultry from negative effect of mycotoxins. It is necessary to identify the mycotoxin and accordingly determine the most effective adsorbent which will eliminate or alleviate the negative effect of mycotoxin on production results in poultry.

Water is essential nutritious substance, main component of chicken organism and necessary for all functions in organism. Therefore, microbiologically and chemically proper water must at all times be available for chickens.

Environment and production conditions

In order to realize satisfactory production results in extensive poultry rearing system, certain requirements have to be fulfilled.

Temperature: Optimal and acceptable temperatures, depending on the age of chickens, are: 1. week 30–32^0 C; 2. week 28–30^0 C; 3. week 25–27^0 C; 4. week 23–24^0 C; 5. week 20– 22^0 C. After the age of 5 weeks, chickens are reared in normal conditions without special heating on room temperature of 18–20^0 C.

Ventilation and air humidity: Relative humidity in the facility where chickens are kept in the initial period, when the facility is heated, should be at least 70%, and later 50%. Excessively dry air can lead to respiratory diseases, incidence of cannibalism, and litter gets dusty.

Light program: Extensive system of poultry rearing includes natural light and facility with windows, i.e. open sides. If there is need for it, the facility can have electric light of 5 W/m^2.

Floor surface and free range surface: Program anticipates not more than 11 chickens per m^2 of floor surface, max. 1.000 chickens in box and starting from 6. week of age to the end of fattening (min. 84 days) access to grass free range (surface of min. 2-10 m^2 per chicken).

Feeding surface: Total surface from which chickens are feeding should be 20% of the surface that is heated. From 2. to 5. week of age, chickens take food from cylindrical feeders (50 chickens per feeder). During the period of free range utilization, feeders are moved around free range in order to equally utilize pasture.

Watering surface: In the initial period of several days chickens are watered from plastic waterers of siphon type, and from the 10. days of age from automatic hanging waterers. One hanging waterer is sufficient for 125 chickens. Several waterers in the form of troughs of drip-waterers are placed also on free range neat feeders and also moved all around the free range.

Health protection

Chickens in extensive system, considering the open space where they spend most of the time, must have very solid and efficient protection. Health protection is provided through application of several protective measures and control of health condition of chickens, and it is divided into general and specific. *General protection* includes those measures carried out during the process of development of the farm project and construction on the farm; they contribute that infectants are not introduced to the farm or spread within the farm or from farm to the environment. *Specific protection* relates to protection from diseases which occur in our geographical origin and it is realized through vaccination program. *Control of the health condition* is realized through clinical monitoring of the chickens and laboratory analysis of deceased chickens, or of their organs, in order to determine the diagnosis and successfully treat affected flock.

Slaughtering of chickens

Minimal allowed age of chickens which are slaughtered in extensive rearing system is from 84 to 91 days. Packaged carcass must have adequate label containing following data: duration of fattening, share of grain in food, maximum period from slaughtering to sale, origin of product, producer name, rearing system, organization responsible for quality control, type of slaughter processing and storage conditions.

Quality control includes: control on the location of production, sale and in distribution chain. On fresh carcass the following is examined: conformation (*Pavlovski and Mašić,* 1983; *Pavlovski et al.,*2006*)*, slaughtering quality, condition and colour of skin, processing quality and on roasted carcass: quality of carcass when it is cut, aroma, skin condition, general impression, tenderness and succulence of meat.

Rearing of layer hens in free system

In production of table eggs numerous strategies are implemented aimed at creation of product of distinct and guaranteed quality (brand egg). New production systems were first developed in European Union countries, and later in developing countries, to improve poultry welfare and meet the consumers' demand for eggs of distinct, known and guaranteed quality. This primarily applies to eggs produced in free systems (extensive), eggs from deep litter and from enriched cages, which provide minimum of poultry welfare.

In this paper, main principles of production of table eggs in free housing systems of layer hens will be presented.

Building and equipment

Segments of the grid floor are placed in the hen house and subsequently 2/3 of the floor is covered with dry litter in a layer of 30 cm. Then, hanging feeders and nests with clean litter are placed and functioning of the light and watering equipment is checked, in order to eliminate or rectify potential mistakes and omissions in operation of the equipment.

Selection of layer hens

For production of table eggs in this Program hybrids are used which are not common or used in industrial production. In our conditions domestic indigenous hens can be used, naked neck hen, domestic populations of Rhode Island, New Hampshire, Amrok, Plimuth rock hens and crosses obtained from crossing of these breeds.

Housing of hens into hen house is done at the age of 18 weeks. First, the distant parts of the hen house are housed and then parts closer to the door and finally, food and water consumption of birds is monitored. Two to three days after housing hens are released to free range – pasture. In the morning they are given food in feeders on the free range, and feeders are moved gradually away from the building and closer to the fence of free range.

Free range – pasture

At the age of 18-20 weeks, laying hens are placed on free feeders and drinkers until end of laying (not longer than 72 week of life). It is desirable to have trees within pasture or on its edges which would be used as shade for chickens to hide from hot Sun. If there are no trees it is necessary to make some kind of eaves. Pasture for poultry is source of protein, minerals and vitamins A, B, E and C. With additional nutrition using concentrated feeds, especially alfalfa meal with addition of vitamin D poultry can utilize free range exceptionally well.

Nutrition

Nutrition is process of digestion, adoption/absorption and conversion of food into tissues and energy in the hen organism. Therefore it is considered that nutrition, as well as housing, has decisive role and effect on production and quality of table eggs and poultry meat. For the purpose of production of table eggs of special and guaranteed quality it is necessary to provide special nutrition of poultry. Main nutrition principles are:

- Cereals are basis of the diet (minimum share of 70% in full mixtures except in starter mixture - min. 50%);
- No animal additives, feeds produced from GMO ingredients;
- Limited number of additives;
- Green and juicy (root-tuberous) feeds.

Change of the housing system requires adequate nutrition based on mixture of cereals and concentrate mixture, with usual mineral and vitamin additives. In accordance to principles of production of natural food and some requirements of European market, natural additives are introduced to these diets which are used to improve production results and quality of table eggs. These new additives are enzymes, probiotics, prebiotics, phytobiotics (medicinal herbs) and mycotoxin adsorbents.

By application of *enzymes* in poultry nutrition better utilization of certain nutritious substances is achieved for the purpose of obtaining better production results (laying ability, number and mass of eggs), better quality of eggs and lower mortality. Special importance has enzyme phytase, which improves utilization of phytine phosphorus (up to 30%) and reduced environment pollution.

Probiotics help development of useful to contrary to harmful (pathogen) microorganisms in digestive tract. By application of probiotics the health condition is improved, better production results are realized as well as better quality of eggs.

Prebiotics are non-digestible feed components (carbon hydrates, some peptides or lipids) which have beneficial effect on host through stimulation of development of desirable bacteria with simultaneous limiting of development of undesirable bacteria in digestive tract of the poultry. By application of prebiotics optimal production results are achieved, the food utilization is improved as well as the vitality of poultry.

Phytobiotics and their extracts have wide spectrum of utilization: they stimulate the food consumption and endogenous enzyme secretion, they have antimicrobial and coccidiostatic effect, they improve production results, they have beneficial impact on health of poultry and quality of products. Medicinal herbs which can be used as additives are camomile, lemon balm, mint, anise, yarrow, thyme, basil, etc.

Presence of mycotoxins in food for layer hens causes numerous problems, from negative effect on production results to different health problems which often end in death. They can be generated in contaminated food during process of production or storage. In strategy against mycotixins in poul-

try nutrition the best results were established when adsorbents were used. It is important for all adsorbents to have high specificity of absorbance, high affinity and adsorbing capacity and to protect as much as possible poultry from negative effect of mycotoxins. It is necessary to identify the mycotoxin and determine the most efficient adsorbent which would eliminate, or at least alleviate, the detrimental effect of mycotoxins on production results in poultry.

Light program

Extensive system of housing of layer hens anticipates for prolonging of natural day light with artificial light, but total duration can not exceed 16 hours. Natural light is provided through open sides of the hen house, i.e. windows made of plastic foil. Artificial light is provided by electric bulbs of power of 4.5 W per m².

Health protection

Layer hens in extensive system, considering the open space available to them, must have very thorough and efficient protection. Health care is provided by application of series of protective measures and control of the health condition of hens and it is divided into general and specific. *General protection* includes those measures which are considered during planning of the building and production facility, which contribute to safety and prevent introduction of infectants to the farm and their spreading within the farm or from farm to the surrounding environment. *Specific protection* relates to protection from diseases which occur on our geographical territory and it is realized through vaccination program. *Control of the health condition* is realized by clinical observation of hens and laboratory examination of died birds or their organs for the purpose of establishing of diagnosis and successful treatment of diseased flock.

Procedure with eggs

Eggs should be collected as often as possible, at least 4 times during laying period (from early morning to early afternoon). After collecting, eggs are placed in special storage room (chamber) with temperature of 10-15º C and relative air humidity of 70–75 %. Eggs with dirty or cracked shell, as well as eggs which are not for the market (bellow 45 g or above 75 g, of irregular shape, etc.) are consumed on the household or sold to local buyers. This Program determines minimum conditions in regard to quality of eggs. Eggs of guaranteed and special quality are exclusively non-fertilized fresh eggs in egg shell that hasn't been washed or mechanically cleaned and categorized/sorted one day after production. In order for these eggs to have recognizable image, special packaging is designed in form of boxes or baskets. Beside adequate label, registered commercial name and all data issued by the Regulation, packaging should also contain information about the housing system of layer hens, composition of used food, max. period of time from laying to supply to the store, origin of eggs, minimum quality requirements, organization controlling the quality, storage conditions and shelf life at given conditions. In this Program it is determined that eggs have at the moment of delivery to the place of sale (average for entire package of eggs): minimum 70 Haugh units, egg yolk colour minimum 12 Roche units and egg shell thickness of at least 0.35 mm.

Marketing strategy and consumer attitudes

It is well known that in every production chain, the most important link is the final one – consumer. For each production, even production of table eggs, it is very important to know why consumers are purchasing specific product and what preferences consumers have in relation to that product. It is paradox that poultry production in many countries of the world, even in Serbia, over the period of several decades, has developed suddenly and become industrialized – revolution in livestock production, but almost no attention was directed to researches of consumer demands and marketing strategies. Even in countries with developed poultry production, first among few researches of the consumer relations to poultry products appeared in late sixties. However, in eighties these researches became very up-to-date and intensive. Today on poultry scientific meetings researches of consumer attitudes and marketing strategies are given outstanding position. Favourable circumstance is that in our country considerable attention was directed to researches of the consumer relation to poultry products. Numerous researches directed in various directions have been carried out (*Pavlovski and Mašić,* 1986; *Pavlovski et al.,* 1981*; Pavlovski and Mašić,* 1993; *Pavlovski et al.,* 2002; *Pingel and Jeroch,* 1997; *Rossi;* 2007). It is interesting to mention that in year 1981 70,6% of questioned consumers thought that battery system was acceptable as production method. One decade later this percentage was reduced to 54,6%, and two decades later to 35,6%. During this period the percentage of consumers in favour of banning of cage system almost doubled from 6,4% to 10,3% and 13,2%, respectively. In mentioned research

years number of consumers which were willing to pay higher price by 10% for eggs of guaranteed and controlled quality or from free-range system increased from 46% to 63% and 71,5%. Obviously, number of consumers adherent to the free-range and banning of battery system on Belgrade market increased considerably, which is in accordance with similar trends and new directives of EU.

At least, there is a need for traceability system that gives information on origin, production, retailing and final destination of products. Such system shall enhance consumer confidence in food, enable the regulatory authorities and to withdraw health hazardous and non- consumable products from the markets. An animal product is an element in this "food-to-farm" approach to public health (*Schwägeke and Adrée,* 2009).

Conclusion

Proposed programs for production of table eggs and poultry meat will generate products of slightly lower cholesterol content and ideal ratio of unsaturated and saturated fatty acids, i.e. products of distinct, known and guaranteed quality, which will meet the demand of domestic and foreign consumers, fans of natural food.

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Slobodni sistemi gajenja pilića i kokoši: kvalitet mesa i jaja

Pavlovski Zlatica, Škrbić Zdenka, Lukić Miloš

R e z i m e: Konvencionalna brojlerska proizvodnja i proizvodnja jaja je osnova današnje proizvodnje pilećeg mesa i konzumnih jaja u svetu i u našoj zemlji. Stalni zahtevi u živinarskoj proizvodnji za bolje i više doveli su do toga da se kontinuirano radi na poboljšanju tehnologija gajenja brojlera i proizvodnje konzumnih jaja. Poboljšanja u tehnologijama proizvodnje mesa i jaja zavise od prirodnih resursa određene zemlje, ambijetalnih uslova i svakako mišljenja i zahteva potrošača. Pored količine proizvoda značajna pažnja će se pridavati dobrobiti živine, primeni novih sistema gajenja i shodno tome kvalitetu proizvoda. U radu će biti prikazani pozitivni uticaji slobodnog sistema gajenja brojlera i kokoši nosilja na kvalitet mesa i jaja, koje smo ustanovili u našem dugogodišnjem radu.

Ključne reči: *slobodan sistem gajenja, kvalitet, pileće meso, jaja za konzum.*

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Comparative study of meat composition from various animal species*

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A b s t r a c t: The meat of various species of animals has specific composition, structure and odor, however, the identification of meat raw materials is a difficult problem.

Fatty and amino acid composition of meat components being a part of recipes of meat products has been investigated. The factors influencing the results of analytical determination of individual fatty and amino acids are shown. The main ratios for sheep, pigs, boars, cows, turkeys, horses, chicken, pheasants and wild boars, allowing their use in production of modern meat products with variable nutrition value are presented, too.

Calculation of specific ratios of fatty acids in fats from various species of animals allows revealing the characteristic features. Thus, horsemeat has a high share of fatty acids with 18 atoms of carbon; these are mainly; oleinic, linoleic and linolenic acids. The C18:C12 ratio in horsemeat is 6 times higer than in pork, 18 times higher than in beef, 8 and 2 times higher than in mutton and turkey meat, respectively.

The ratios of amino acids – arginine, histidine and lysine for the investigated species of animals have been obtained. These ratios do not depend on age or weight of the animal. For example, for mutton it is 2:3:1 and for pork 1.5:2.0 – 2.2:1.

Comparative analysis of the readings of the sensors of VOCmeter instrument, as obtained during investigations of volatile components of beef, pork, mutton, chicken meat, ostrich and turkey meat, and their processing by principal components methods, allowed revelation of meat from different species of animals and poultry with a high degree of reliability.

The data obtained can be used for production of advanced meat products.

Key words: *animal species, meat composition, fatty acids, amino acids.*

Introduction

Meat from animals of different species is characterized by specific composition, structure and odour, but in most cases it is difficult to identify species origin of meat raw material by simple methods of physical-chemical and sensor analysis (*Demirel et al.*, 2006; *Lisitsyn et al.,* 2004; *Saadoun and Cabrera*, 2008).

The aim of this paper is to present the results and show the possibility to identify meat from different wild and domestic animals using amino acids (AA) and fatty acids (FA) analysis followed by the E-nose comparison.

Material and methods

M. longissimus dorsi with the weight of 5.0 ± 0.5 g taken from the adult wild and domestic animals including female sheep, pigs, cows, turkeys, horses, chickens seals, walrus and others were analyzed.

As analytical methods, extraction of lipids from muscle tissues and GC/FID fatty acids methyl esters' determination were applied.

For fat content determination lipids were extracted from muscle tissues by the method described by *Folch, Lees,* and *Stanley* (1957).

Fatty acid content: after isolation lipids were subjected to methylation according to official methods (*ISO 5509,* 1978; *AOAC,* 1990). Fatty acid methyl esters (FAMEs) were analyzed by using a HP6890 Hewlett-Packard (USA) gas chromatograph equipped with flame ionization detector (FID) and silica capillary column HP-Innowax 30m x, 32mm x 0,5mkm. Nitrogen was used as a carrier gas as well as automatic data processing Winpeak by Bruker-Franzen Analytic SCPA;

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Amino acid content was determinate with amino acid analyser, PMA GmbH Aracus;

Censor analysis was performed by using multisensory analyser VOCmeter.

Results and discussion

Up to 34 essential fatty acids were determined in the studied samples (fig.1).

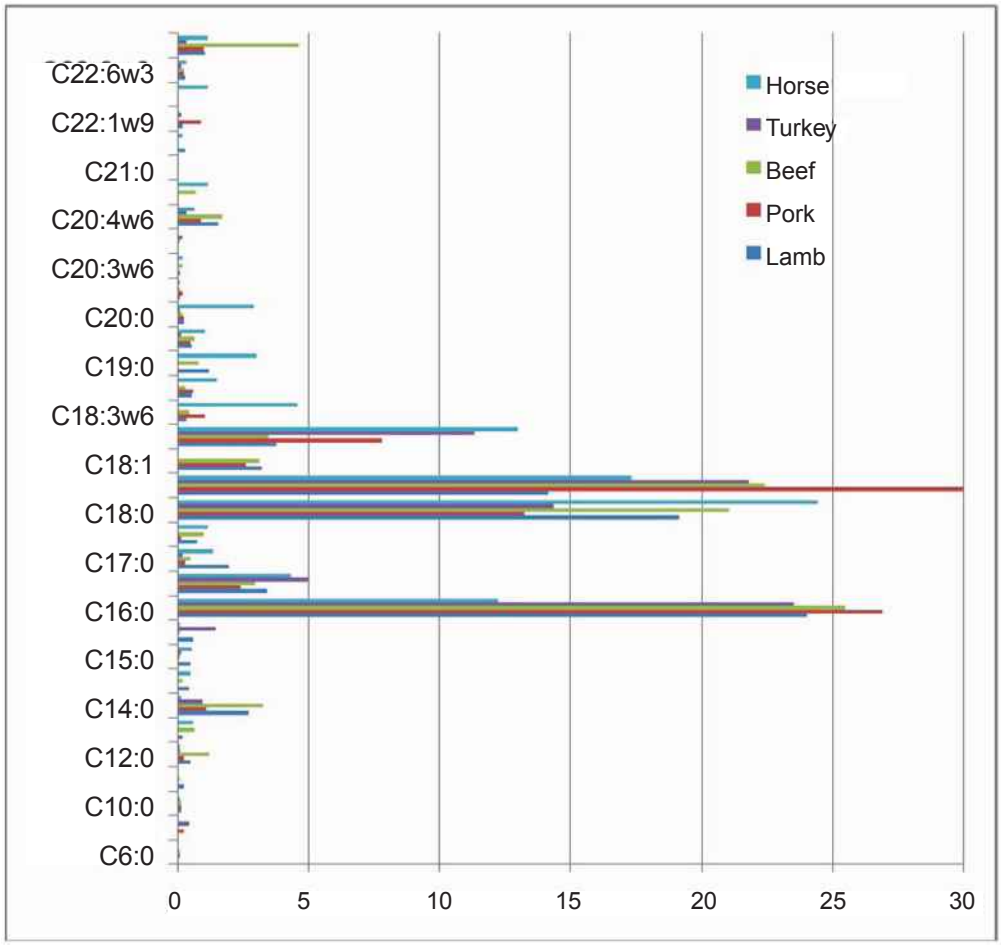

Figure 1. Chromatograms of fatty acids in different type of meats
Figura 1. Masne kiseline u različitim vrstama mesa

From figure 1 it can be distinguished the presence of tridecanoic acid [$CH_3(CH_2)_{11}COOH$] in lipids of sheep and cattle. Its content in beef is 3.7 times higher than in ovine and 15 times higher than in pork and turkey meat.

For ovine presence of pentadecanoic acid with a branched skeleton is typical (0.6% of the total lipids), while in pork only trace amounts of C15:0 are found occasionally.

Horse meat has much higher (1.2%) content of pentaenoic fatty acid than other meat samples. In pork, ovine and turkey meat, these acid range from 0.05% to 0.07%. In beef quantity of this fatty acid is ten times higher (0.7%).

High content of lauric acid (C 12:0) is typical for beef (1, 7%). In pork, ovine, horse, and turkey lauric acid content is 5.5; 2.5 and 1.7 times less than in beef, respectively.

In general, beef is rich in fatty acids of medium molecular weight (from C 8 to C 13). This value is two times higher than that in horse, two and a half times higher than in ovine (1.06%), three times higher than in pork or turkey (0.68 – 0.77%).

Turkey meat reveals almost complete absence of fatty acids with 17 carbon atoms (heptadecanoic acid, heptadecenoic acid, and branched heptadecanoic acid), which can be a specific indicator for turkey meat.

C 19 and C 21 fatty acids were not found in the lipids of pork (*Chernuha et al*., 2009; *Lisitsyn and Shumkova*, 2002)

The main fatty acids ratios are presented for meat from various animal species allowing their use in production of modern meat products with variable nutrition value.

Calculation of specific fatty acid ratios in fats from different animal species allows revealing the distinctive features (*Alfaia et al.,* 2007). For example, the high proportion of fatty acids with 18 carbon atoms (mostly oleic, linoleic and linolenic acids) is characteristic for horse meat. The C18/C12 ratio in horse meat is 325.60, and it is 6 times higher than in pork, 18 times higher than in beef, and 8 times higher than in lamb and two times higher than in turkey meat.

For turkey meat the specific fatty acid ratio can be the C16/C12 ratio. In turkey meat this ratio is 276.47, which is 5.5 times higher than in lamb and 3, 13 and 2 times higher than in pork, beef and horse meat, respectively. The distinctive characteristics of beef and lamb are minimal values of C18:1/C12 and C18:2/C14 ratio.

Table 1. Some fatty acid ratios in lipids from different animal species
Tabela 1. Odnosi nekih masnih kiselina u lipidima različitih vrsta životinja

	C16/C12	C18/C12	C18:1/C14	C18:2/C14
lamb	50.03	39.89	5.22	1.38
pork	105.41	51.82	27.22	7.09
beef	21.49	17.78	6.84	1.056
turkey	276.47	168.94	22.95	11.93
horse	163.47	325.6	111.74	83.94

On the basis of the above mentioned data on the fatty acid composition of meat/raw material from different animal species it appears to be possible to distinguish between these investigated types of meat with high confidence. It would be possible to distinguish beef and lamb from other types of meat by detection their fatty acid composition. The most difficult is to distinguish lamb from beef on the basis of their fatty acid profile.

Data for fatty acid ratios correspond to the results obtained with the electronic nose (fig.2)

The comparative analysis of the visual fingerprints of meat raw material used in meat products manufacture showed that the highest odour intensity was characteristic for samples from fresh venison while the samples of lamb were characterized by the least odour intensity. Differences in the odour visual fingerprint patterns of the tested samples can be explained by differences in the quantitaties and profiles of volatile compounds, including those that are formed during the process of autolytic changes in meat tissue.

Unlike the currently available methods for meat species detection (polymerase chain reaction. enzyme immunoassay. etc.). The use of multisensor systems (E-nose) does not require significant material costs. As well as durable and labour consumption for preparation of samples.

In order to distinguish more clearly between beef and lamb we have made attempts by comparative analysis of their amino acid composition. There is scientific opinion that myoglobin and myogen can

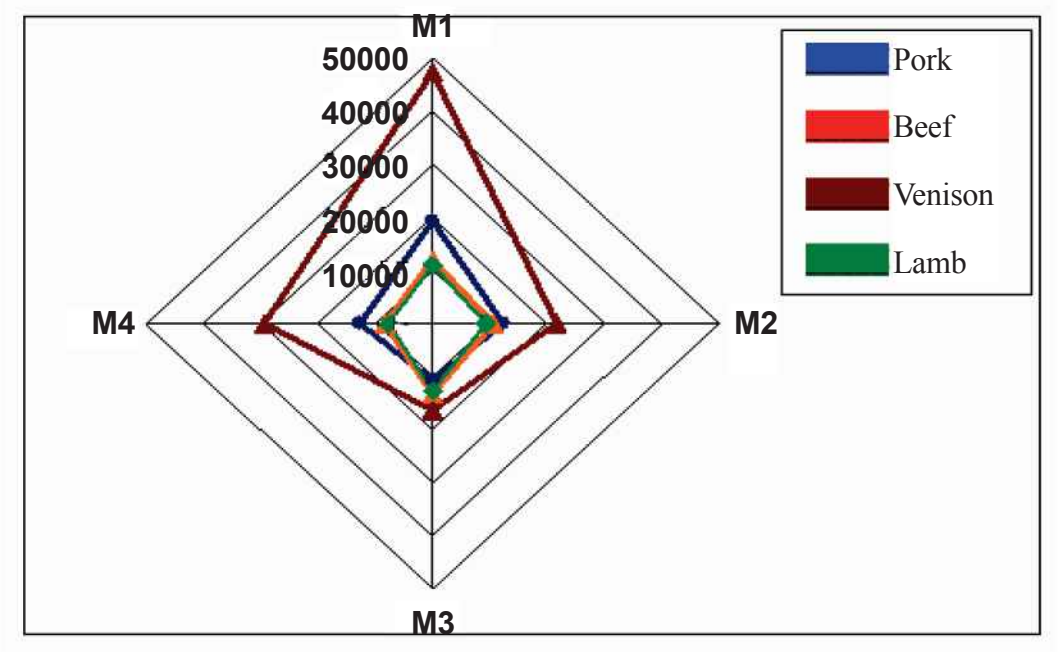

Figure 2. „Visual prints“ of pork, beef, lamb and venison made with the „E-nose“
Figura 2. „Vizuelni otisak“ svinjskog, junećeg, jagnjećeg mesa i divljači pomoću „Elektronskog nosa“

be regarded as biomarkers of meat tissue (*Demirel et al.,* 2006). Our interest was focused on myogen. Myogen accounts for 20 to 30% of all meat tissue proteins. In this study we attempted to calculate species-specific changes in proteins by investigation dynamics of the changes in the ratio of arginine, histidine and lysine. Using the pair correlation method we found that the ratio arginine: histidine (0.42) is the weightiest.

In four experiments the ratios of arginine, histidine and lysine for examined animal species were investigated, because these ratios do not depend on the animal age or fatness. For instance, the ratios we obtained are 2:3:1 and 1.5:2.0–2.2:1 for lamb and pork respectively (fig.3).

At the same time E-nose shows positive results when we compared meat samples of wild and domestic poultry (chicken, ostrich, duck, turkey).

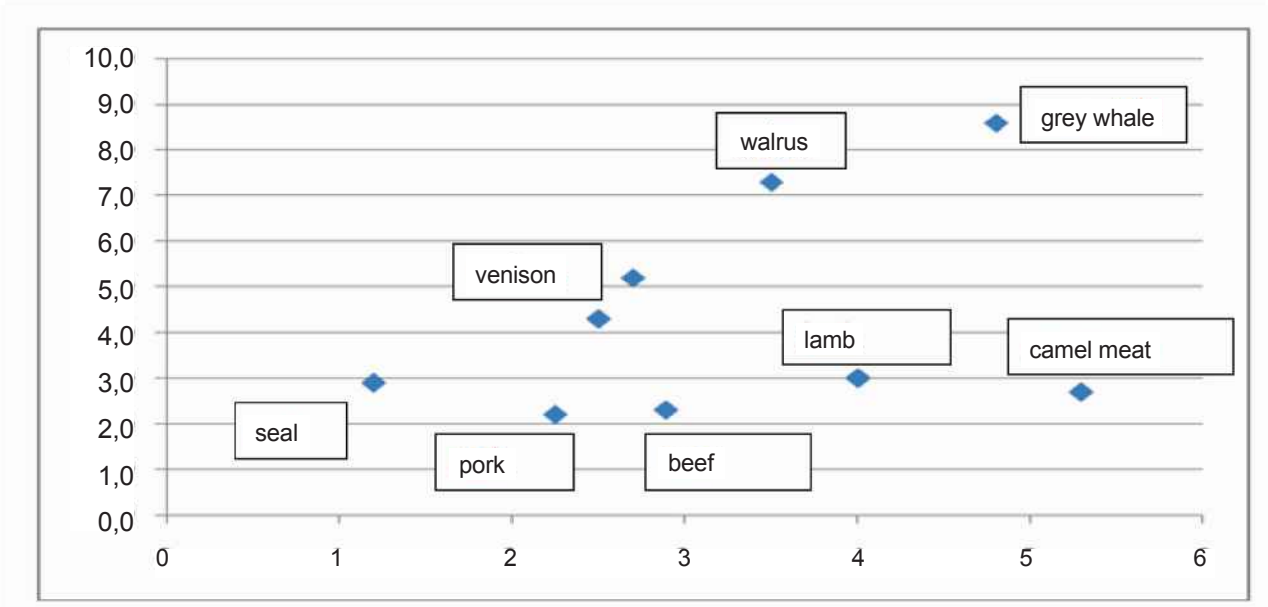

Figure 3. Differentiation of animal species by arginin/histidin ratio
Figura 3. Diferencijacija različitih vrsta životinja pomoću odnosa ariginin/histidin

The areas of points that characterize each meat species are situated close to each other, which allow identifying the areas (clusters) inherent to each meat species. It should be noted that the cluster determinative for venison is situated at the significant distance from the cluster of rabbit meat and has the significantly higher coordinates of the first principal component (PC1). It should be noted that in some cases clusters are situated near each to other in some cases, but they lie at different angle in the space which enable to carry on the analysis of species origin of meat raw material with high confidence.

Meat raw material as different morphological parts of carcasses do not influence the objectivity of the results obtained for determination of the origin of species by multisensor method.

Conclusion

Fatty and amino acid composition of meat components as being a part of meat products has been investigated. Factors influencing the results of analytical determination of individual fatty and amino acids in various animal species were evaluated.

Possibility of the use of multisensor instrumental systems for determination of species origin of meat raw material was established.

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Komparativna studija sastava mesa različitih životinjskih vrsta

Chernukha Irina

R e z i m e: Meso različitih vrsta životinja ima specifičan sastav, strukturu i miris, Međutim, identifikacija mesa kao sirovine predstavlja veliki problem.

Ispitivan je sastav masnih i amino kiselina u mesu odnosno. delovima mesa koje se prema recepturi koristi u proizvodnji proizvoda od mesa. Faktori koji utiču na rezultate analitičkog određivanja pojedinih masnih i aminokiselina su prikazani u radu. Glavni odnosi utvrđeni kod ovaca, svinja, nerastova, krava, ćuraka, konja, pilića, fazana i divljih nerastova su predstavljeni u radu. čime je omogućena njihova upotreba u proizvodnji savremenih proizvoda od mesa različite hranljive vrednosti.

Izračunavanje specifičnih odnosa masnih kiselina u masnom tkivu različitih vrsta životinja omogućava otkrivanje karakterističnih svojstava. Prema tome, meso konja ima visok udeo masnih kiselina sa 18 atoma ugljenika; a to su uglavnom oleinska, linolna i linolenska kiselina. Odnos C18:C12 u mesu konja je 6 puta veći od svinjskog mesa, 18 puta od goveđeg i 8 odnosno 2 puta nego u mesu ovaca i ćuraka.

Odnosi aminokiselina – arginin, histidin i lizin u ispitivanim vrstama životinja su takođe utvrđeni. Ovi odnosi nisu u zavisnosti od uzrasta ili mase životinje. Npr. za ovčije meso 2:3:1 a za svinjsko meso – 1.5:2.0 – 2.2:1.

Uporedna analiza očitavanja senzora na instrumentu VOCmeter koji su dobijeni u ispitivanju isparljivih komponenti goveđeg, svinjskog, ovčijeg, pilećeg, nojevog i ćurećeg mesa. kao i njihova obrada korišćenjem osnovnih metoda. omogućila je otkrivanje mesa različitih vrsta životinja sa visokim stepenom pouzdanosti.

Dobijeni podaci mogu se koristiti za izradu naprednih proizvoda od mesa.

Ključne reči: *životinjske vrste, sastav mesa, masne kiseline, aminokiseline.*

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Über die zartheit des fleisches – ein vergleich zwischen rind, schwein, geflügel und kaninchen*

Ristic Milan[1]

Z u s a m m e n f a s s u n g: Der Verbraucher schätzt die Qualität des Fleisches in der Zartheit und im Aroma/Geschmack. Jedoch besteht auch der Wunsch, diese Eigenschaften rein objektiv bestimmen zu können. Die ersten Einsätze von solchen Messgeräten fanden in Deutschland schon Anfang der 50er Jahre beim Rind-, Kalb- und später auch beim Geflügelfleisch statt (Grünewald, 1957). Mit dieser Thematik haben sich mehrere Dissertationen an Tierzuchtinstituten beschäftigt. Im Laufe der Zeit wurde die Messtechnik wesentlich verbessert. Zurzeit werden verschiedene Messgeräte auf dem Markt angeboten. Teurere Geräte bieten mehr Information zur Erfassung der Druck- und Scherfestigkeit. Die Messwerte werden in lbs, kg oder N angegeben; die Höhe der Messwerte ist Ausdruck für die objektive Zartheit des Fleisches. Die Reifung des Fleisches kann somit sehr gut demonstriert werden. Insgesamt ergeben sich 4 Messabstufungen für das Fleisch folgender Tierarten:

Rind	*40-80 N*	*als Scherkraftwert*
Schwein	*30-60 N*	*als Scherkraftwert*
Geflügel	*10-30 N*	*als objektive Zartheit*
Kaninchen	*10-40 N*	*als objektive Zartheit*

Beim Rindfleisch nahmen die Scherkraftwerte innerhalb einer Reifungsdauer von 2 Wochen um ca. 40% vom Ausgangswert ab.

Abschließend kann gesagt werden, dass die Erfassung der objektiven Zartheit eine gute Ergänzung zur sensorischen Bewertung des Fleisches darstellt. Hierbei wurde eine Abhängigkeit von r = -0,72 ermittelt und zwischen den Messkriterien eines Gerätes von r = 0,86.

Schlüsselwörter: *Zartheit – subjektiv – objektiv – Rind – Schwein – Geflügel – Kaninchen Vergleich.*

Einleitung

Für die Verbraucher ist in erster Linie die Zartheit des Fleisches von Bedeutung. Beim Verzehr wird dadurch der Genusswert gesteigert. *Hoffmann* (1973, 1995) versteht unter Fleischqualität „die Summe aller sensorischen, ernährungsphysiologischen, hygienisch-toxikologischen und verarbeitungstechnologischen Eigenschaften des Fleisches“. Hierbei sind u.a. auch die physikalischen Kriterien nach der Schlachtung wichtig (*Ristic* et al., 2011). Die Zartheit des Fleisches ist eine physikalische Eigenschaft, die durch verschiedene Faktoren beeinflusst werden kann, wie z.B. Züchtung, Haltung, Fütterung, Mastalter, Transport, Schlachttechnologie, Kühlung, Lagerung und nicht zuletzt durch die thermische Behandlung. Sie umfasst mehrere Materialeigenschaften, wie Kaubarkeit, Langwässrigkeit, Derbheit. Die Zartheit kann vielseitig untersucht werden: biochemisch-physikalisch, histologisch, mechanisch und sensorisch (*Riedl* und *Obermowe*, 2010; *Ristic,* 1984; *Ristic*, 2010).

Ziel dieser Arbeit war, die objektive Zartheit des Fleisches verschiedener Tierarten mit Hilfe unterschiedlicher Messgeräte zu erfassen und gleichzeitig mit den Daten der sensorischen Analyse zu vergleichen.

Messgeräte

Eines der ältesten Festigkeitsprüfgeräte für Lebensmittel ist das **Wolodkewitsch-Gerät** (*Grüne-*

**Plenary paper on International 56th Meat Industry Conference held from June 12-15th 2011. on Tara mountain;*
**Plenarno predavanje na Međunarodnom 56. savetovanju industrije mesa, održanom od 12-15. juna 2011. godine na Tari.*

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wald, 1957; *Werhahn*, 1964; *Weipert* et al., 1993). Bei der Messung werden die Proben entweder geschnitten oder gequetscht. Die größte Belastbarkeit beträgt 25 kg. Für die Auswertung der Weg-Kraft-Diagramme werden der Maximaldruck und die beim Quetschen des Fleisches geleistete Arbeit als Hilfsmerkmal für die Zartheit herangezogen. Als Maß für den Maximaldruck ist die Höhe der Kurve, und für die geleistete Arbeit die Fläche unter der Kurve des Diagramms zu betrachten. Die Flächenauswertung erfolgt planimetrisch (*Tawfik,* 1969).

Weiterhin wurde ein **Fleischwolf mit Druckmesseinrichtung** eingesetzt. Dieses Gerät misst und registriert den beim Verarbeiten von Fleisch mit dem Fleischwolf auf die Lochscheibe wirkenden Axialdruck. Zur Druckmessung dient eine Kraftmessanlage der Firma Siemens, bestehend aus Kraftmessdose, Messhalterung und Linienschreiber. Die Kraftmessdose ist durch eine mechanische Halterung mit dem Fleischwolf gekoppelt, so dass eine direkte Kraftübertragung von der Lochscheibe auf die Messdose stattfindet. Der Linienschreiber ist mit einer Öl-Dämpfung ausgestattet. Es wird ein Weg-Kraft-Diagramm registriert, wobei das Maximum der Kurve und die geleistete Arbeit als Kriterien für die Zartheit betrachtet werden (*Hausmann*, 1967).

Ebenfalls wird ein **Warner-Bratzler-Gerät (Modell 2000)** eingesetzt. Der Widerstand der gescherten Probe wird auf der Skala aufgezeichnet, entweder in lbs oder in kg-Einheiten (1 lb. = 453,59 g).

Für die Messung der Festigkeit von Fleisch und Fleischerzeugnissen wird ein **Penetrometer** (Sommer & Runge, Berlin; Stable Micro Systems, England) verwendet. Diese wird vertikal ausgerichtet, damit der Penetrationskörper senkrecht in die Probe eindringt (*Klettner*, 1983; *Kühne* et al., 2005).

Zunehmend kommt das **Instron-Gerät (Modell 1140, 5564)** der Firma Instron Deutschland GmbH zum Einsatz. Mit verschiedenen Prüfvorrichtungen ist dieses Gerät für alle Lebensmittel geeignet. Hierbei handelt es sich um eine sog. Kraft-Weg--Maschine, bei der das Messergebnis als die Kraft dargestellt wird, die über einen vorgegebenen Weg des Scherblattes aufgewendet werden muss. Das Scherblatt schert auf diesem Weg die Probe durch einen Spalt, der der Scherblattdicke entspricht. Bei der Scherkraft- bzw. Zartheitsmessung von Rindersteakproben wird eine Kraftdose von 1 kN (Kilo--Newton) mit einer Geschwindigkeit von 200mm/min eingesetzt. Hierbei können 2 Prüfvorrichtungen verwendet werden: Warner-Bratzler-Schere und Kramer-Scherzelle. Nach der Messung wird die üblicherweise auftretende Kraft-Weg-Energie aufgezeichnet und als Maximum der Druckkraft (N), Energie bei maximaler Kraft (mJ), sowie Energie bei Bruch (mJ) in Form der Weg-Kraft-Kurve ausgewiesen. In der Regel wird nur die maximale Druckkraft (N) dargestellt (*Freudenreich* und *Augustini*, 2001; *Hecht*, 1986).

Die objektive Zartheit des Fleisches kann auch mit Hilfe von biochemisch-physikalischen Kriterien, sowie histologisch und sensorisch untersucht werden. Die biochemisch-physikalischen Methoden basieren auf Untersuchungen des Nukleotidabbaus, wie ATP, ADP, AMP, Glykogen, Glukose, Veränderungen des pH-Wertes, Anteil an Bindegewebe. Bei den histologischen Untersuchungen werden die Dicke und Breite der Muskelfaser, sowie die Sarcomerenlänge gemessen.

Methoden und Messstellen

Die meisten Hersteller von Messgeräten empfehlen, die Messungen bei den Proben nach der thermischen Behandlung durchzuführen. In Abhängigkeit von der Versuchsdurchführung wird das Fleisch verschiedener Tierarten zuerst gekühlt und nach unterschiedlicher Reifung untersucht. So werden z.B. beim Rindfleisch Scheiben mit einer Stärke von 2,5 cm geschnitten und bei bestimmter Lagertemperatur und -dauer gereift. Diese Proben werden in einem platten Kontaktgrill bis zum Erreichen der Kerntemperatur von 70°C gegrillt. Die Temperatur der Platten beträgt ca. 230°C, die Grilldauer etwa 7 Minuten. Im Anschluss werden die gegrillten Proben 24 Std. bei +4°C bis zur M4essung aufbewahrt. Die Scherkraftmessung erfolgt im rechten Winkel zur Muskelfaser. Manche Autoren geben nur eine Abkühlungszeit von 5 Minuten (*Westphal* und *Golze*, 2007). Beim Schweinefleisch empfiehlt sich statt Grillen, die Proben in einem Polyäthylenbeutel im Wasser zu erhitzen (ca. 1 h), bis eine Kerntemperatur von 75°C erreicht wird. Allerdings findet man in der Literatur auch Scherkraftwerte, die an rohem Fleisch durchgeführt wurden (*Kühne et al*., 2005). Ebenso werden Prüfsystem für die Texturanalyse bei Wurst-, Fleisch- und Fischprodukten angeboten (Fa. Zwick Roell AG, Ulm). Dabei werden z.B. bei rohem Fleisch die Scherfestigkeit, Zähigkeit, Zartheit und das Bissverhalten ermittelt, und bei Wurst die Festigkeit und Härte.

Pro Probe werden am gleichen Muskel 6 bis 10 Messungen durchgeführt. Die statistische Auswertung kann zuerst mittels des arithmetischen Mittelwertes oder des Zentralwertes (Median) erfolgen. Für die weitere Berechnung kann die Varianzanalyse herangezogen werden. Der multiple Mittelwertvergleich erfolgt meistens durch den Tukey-Test, oder mit der Prüfung der Grenzdiffe-

renz. Signifikante Unterschiede ($p \leq 0,05$) werden mit unterschiedlichen Buchstaben gekennzeichnet. Für Scherkraftwerte einer Probe werden verschiedene Synonyme verwendet, wie Konsistenz, Textur, Struktur. Die Textur im Vergleich zur Konsistenz bedeutet Gewebe, Gefüge. Sie ist eine Zusammensetzung derjenigen Eigenschaften, deren Ursprung in den physikalisch-strukturellen Elementen liegt, und der Art, wie diese durch die physiologischen Sinne nachgewiesen werden können. Für die Messungen werden verschiedene Muskeln verwendet, wie z.B. M. longissimus dorsi, M. semimembranosus, M. triceps brachii, sowie Brust- und Schenkelfleisch beim Geflügel, und bei Kaninchen Keulen- und Rückenmuskulatur.

Tabelle 1. Scherkraftwerte, Grillverlust und Zartheit nach der Reifung von 7 und 21 Tagen (Färsen, n = 31; *Augustini* und *Spindler*, 2000, mod.)

Merkmal	$\bar{x}$	s	min.	max.
Scherkraft[1] 7 Tg.	7,6	2,87	2,7	13,4
Scherkraft[1] 21 Tg.	5,2	2,05	2,4	9,6
Grillverlust (%) 7 Tg.	20,2	3,01	14,8	28,9
Grillverlust (%) 21 Tg.	19,7	3,29	12,1	27,4
Zartheit[2] 21 Tg.	3,8	0,88	1,8	5,5

[1]in kg/cm^2 - M. long. dorsi

[2]Punktzahl von 1 (sehr zäh) bis 6 (sehr zart)

Tabelle 2. Scherkraftwerte und Garverlust in Abhängigkeit von der Reifungsdauer (n = 20 je Behandlung; *Augustini* und *Freudenreich*, 1998, mod.)

Reifung/ Tage	Scherkraft/N[1]		Garverlust/%	
	$\bar{x}$	s	$\bar{x}$	s
1	82,5[2]	22,4	23,7	2,0
2	77,7	22,5	24,6	2,4
3	71,4	26,9	23,6	2,9
5	67,6	25,6	23,2	2,7
7	69,9	20,2	24,4	3,1
10	57,5	19,3	23,7	2,9
14	52,0	14,0	22,3	2,9
21	46,5	13,4	22,0	2,4
28	43,0	11,6	22,7	2,4
35	38,1	8,5	22,9	2,8

[1]Newton(N/9,81=kg) - M. long. dorsi

[2]$GD_{0,05}$ 15 bzw. 26 N

Versuchsergebnisse

Rindfleisch

Die Zartheit des Rindfleisches ist in erster Linie von der Reifungsdauer und Reifungstemperatur abhängig (*Schwägele*, 1998; *Augustini* und *Freudenreich*, 1998).Die Tab. 1 gibt einen Überblick über die Scherkraftwerte - als Druck angegeben - in Abhängigkeit von der Dauer der Reifung des Fleisches von Färsen. Die ermittelten Scherkraftwerte nach einer Reifung von 21 Tagen haben abgenommen, d.h. das Fleisch war zarter. Der Grillverlust wurde von der Reifungsdauer nicht beeinflusst. Inwieweit eine noch längere Reifungsdauer einen positiven Effekt erzielen kann, zeigt die Untersuchung nach *Augustini* und *Freudenreich* (1998) bei Jungbullen der Rasse Fleckvieh in der Tab. 2. Die Scherkraftwerte wurden während der Reifung bis zu 35 Tagen deutlich geringer. Der Rückgang der Messwerte war bis zum 10. Tag besonders stark, er dauerte bis zum 35. Tag an. Die Streuung ist innerhalb der einzelnen Reifungstage erheblich, sie nahm in der 2. Reifungsphase ab. Der Grillverlust war nach längerer Reifung der Proben tendenziell geringer. Die Mastbullenschlachtkörper wurden mit einem Alter zwischen 16,8 und 23 Monaten in 4 Altersgruppen mit je 14 bis 16 Tieren aufgeteilt (Tab. 3). Die Scherkraftwerte waren in der ersten Gruppe am höchsten, gingen dann bis zur Gruppe 3 linear zurück und stiegen bis zur Gruppe 4 wieder etwas an. Dieser Verlauf deckt sich weitgehend mit der sensorisch ermittelten Zartheit. Es liegt die Vermutung nah, dass ein höherer intramuskulärer Fettgehalt der Gruppe 3 eine positive Wirkung auf die Zartheit hatte.

Tabelle 3. Merkmale der Fleischqualität bei Jungbullen, nach Schlachtaltersklassen (M. long. dorsi, n = 60; *Kögel et al*., 2002, mod.)

Schlachtalter (Monate)	n	Scherkraft (kg)	Zartheit (Punkte)	intramuskulärer Fettgehalt (%)
16,8	15	6,0	3,6	2,85
18,8	15	5,8	3,8	2,58
20,7	14	5,3	4,0	2,89
23,0	16	5,4	3,9	2,30

Schweinefleisch

Die Scherkraftwerte nach unterschiedlichen Zubereitungsvarianten lagen beim gesamten Material um 50 N (Tab. 4). Die günstigsten Werte ergaben die Proben nach der Gefrierlagerung. Die Versuchsgruppe A („rot und trocken“) bei den frisch

gegrillten bzw. gekochten proben erbrachten tendenziell die höchsten Werte. Vergleichsweise günstig schnitt die Gruppe B („blass und trocken“), insbesondere bei den nach Gefrierlagerung gegrilltem proben. Die Scherkraftwerte bei verschiedenen Muskeln in Abhängigkeit vom Mastendgewicht blieben nach dem Kochen konstant, wobei die weiblichen Tiere jeweils zu etwas höheren Werten tendierten (Tab. 5). Im M. semimembranosus kam es zwischen den Gewichtsstufen 110 und 135 kg zu einer signifikanten Verminderung der Scherkraft. Zwischen den Gewichtsgruppen 135 und 160 kg ergaben sich keine Unterschiede. Die Scherkraftwerte nach dem Grillen des M. longissimus dorsi ließen keine gewichtsbedingten Veränderungen erkennen.

Geflügelfleisch

Die Messwerte der objektiven Zartheit (Warner-Bratzler-Gerät) des Brustfleisches von Broilern aus verschiedenen europäischen Produktionen sind in der Tab. 6 ersichtlich. Der Einfluss der Herkunft war hoch signifikant. Die günstigste objektive Zartheit ergaben die Herkünfte B und G mit 1,4 bzw. 1,6 lb. Dagegen wurden die höchsten Messwerte bei den Versuchsgruppen A und E bzw. F gefunden. Die sensorisch ermittelte Zartheit lag bei den Versuchsgruppen D, B und G am günstigsten. Aus der Tab. 7 lässt sich entnehmen, inwieweit sich die objektive Zartheit von Perlhuhn, Fasan und Broiler unterscheidet. Die Perlhühner kamen mit 12, die Fasanen mit 14 und die Broiler mit 5 Wochen zur

Tabelle 4. Scherkraftwerte in Abhängigkeit von End-pH-Werten[1] (*Fischer et al*., 2002, mod.)

Gruppe Charakterisierung n	A rot + trocken 105	B blass + trocken 56	C blass + wässrig 103	D rot + wässrig 54	Total 318	
Merkmal	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	s
maximale Kraft[2]/N	50,5	44,3	45,6	46,4	47,1	14,9
maximale Kraft[3]/N	40,1^{ab}	34,7^{a}	42,2^{b}	42,9^{b}	40,3	12,4
maximale Kraft[4]/N	56,8^{a}	49,2^{b}	46,9^{b}	49,5^{b}	51,0	14,2

[1]72 Std. p.m.

[2]Grillproben (Erhitzung bis 73 °C Kerntemp., 8 Messungen/Scheibe, nach 3-tägiger Kühllagerung bei 2-3 °C, M. long. dorsi)

[3]wie 2, jedoch Proben 3 d p.m. vakuumverpackt und eingefroren, Grillen nach ca. 3-monatiger Gefrierlagerung

[4]Kochproben (Erhitzung bis 75 °C Kerntemp., 8 Messungen/Scheibe)

Tabelle 5. Scherkraftwerte in Abhängigkeit vom Mastendgewicht (FISCHER et al., 2006, mod.)

Merkmal	Muskel[1]		Mastendgewicht/kg		
		n-Zahl	110	135	160
		Kastraten/Sauen	19/17	27/27	17/16
Scherkraft/N	LD	Kastraten	47,2	47,9	48,7
nach Kochen		Sauen	50,2	52,2	50,1
Scherkraft/N	SM	Kastraten	50,8^{a}	42,2^{b}	39,0^{b}
nach Kochen		Sauen	50,1^{a}	41,3^{b}	40,5^{b}
Scherkraft/N	TB	Kastraten	45,9	47,1	47,6
nach Kochen		Sauen	49,6	49,3	50,9
Scherkraft/N	LD	Kastraten	32,2	29,1	33,6
nach Grillen		Sauen	33,3	30,5	30,8

[1]LD=M. long. dorsi, SM=M. semimembranosus, TB= M. triceps brachii

a,b kennzeichnen signifikante Unterschiede bei $p \leq 0,05$

Schlachtung. Die Werte lagen bei Fasanen und Broilern günstiger gegenüber den Messwerten von Perlhühnern. Bei der subjektiv gemessenen Zartheit erreichten die Broiler und Fasanen eine bessere Bewertung als die Perlhühner. Die objektive Zartheit des Brustfleisches, gemessen mit dem Instron-Gerät (Modell 5564) und der Messvorrichtung Warner--Bratzler von Broilern verschiedener Herkünfte, ist in Tab. 8 dargestellt. Die niedrigsten Messwerte, und somit die günstigste Zartheit, ergaben die Cobb 800-Broiler gegenüber 2 anderen Herkünften. An demselben Versuchsmaterial wurde ebenfalls die sensorische Analyse durchgeführt. Die höchste Bewertung der Zartheit erreichten wiederum die Cobb 800-Broiler. Die Cobb 500- und Ross 308-Broiler schnitten in der Bewertung schlechter ab, jedoch wurden, insgesamt gesehen, relativ hohe Bewertungen erreicht. Tab. 9 gibt die Ergebnisse der Scherkraftmessungen an gereiftem. Tiefgefrorenem (bei -18°C), aufgetautem und erhitztem (Wasserbad 85°C) Brustfleisch wieder. Höhere Scherkraftwerte wurden bei einer Reifung bis zu 3 Stunden festgestellt. Im Laufe einer weiteren Reifungsdauer nahmen die Scherkraftwerte ab. Zum Teil ergaben sich Schwankungen bezüglich der Reifungsdauer. Für die objektive Zartheit der Proben des Brustfleisches von Enten und Gänsen kam wieder das Instron-Gerät (Modell 5564) mit der Messvorrichtung Warner-Bratzler zum Einsatz (Tab. 10). Alle 3 Messkriterien ergaben, dass die objektive Zartheit bei Pekingenten günstiger war, d.h., dass das Fleisch etwas fester war. Eine deutliche Abstufung fand sich bei der sensorischen Bewertung der Zartheit. Die Zartheit von Pekingenten (54 Tage) erreichte die höchste Note von 5,3. Die Flugenten schnitten etwas schlechter ab. Die Brustmuskulatur von Gänsen führte zu schlechterer Bewertung der Zartheit.

Tabelle 6. Objektive Zartheit des Brustfleisches von Broilern aus verschiedenen europäischen Ländern (*Ristic*, 1991, mod.)

Merkmal	A[1]	B	C	D	E	F	G	Total
Warner-Bratzler/lb.[2]	3,3	1,4	2,0	2,0	2,9	3,0	1,6	2,3
Zartheit/Pkt.[3]	5,2	5,4	5,3	5,1	5,4	5,6	5,3	5,3

[1]A=D, 37 Tg., herkömml. Mast
B=D, 70 Tg., „Mamsell's Roaster"
C=D, 50 Tg., „Mamsell's Roaster"
D=H, 52 Tg., Tetra
E=NL, 40 Tg., herkömml. Mast
F=I, 40 Tg., „Polo-Arena"
G=F, 90 Tg., „Label Rouge"

[2]1 lb=453,59 g
[3]1=sehr zäh, 6=sehr zart

Tabelle 7. Objektive Zartheit von Perlhuhn-, Fasan- und Broiler-Brustfleisch (*Ristic et al*., 2001, mod.)

Merkmal	Perlhuhn	Fasan	Broiler	Total
Warner-Bratzler/lb.[1]	1,9	1,5	1,6	1,7
Zartheit/Pkt.[2]	4,6	5,2	5,3	5,0

[1]$GD_{0,05}$ 0,4
[2]$GD_{0,05}$ 0,6

Kaninchenfleisch

Der „Weg" – als Merkmal der objektiven Zartheit (Instron-Gerät, Modell 1140) – unterlag dem Einfluss der Herkunft und des Alters (Tab. 11). Diese Daten decken sich mit den Daten der subjektiven

Tabelle 8. Objektive Zartheit des Brustfleisches von Broilern verschiedener Herkünfte (*Ristic* und *Steiner*, 2005, mod.)

Herkunft[1]	max. Kraft/N[2]	Energie bei max. Kraft (mJ)	Energie bei Bruch (mJ)	Zartheit (Pkt.)
Ross 308	12,0	36,9	83,8	4,6
Cobb 500	12,0	34,1	84,1	5,0
Cobb 800	10,9	25,6	75,1	5,4

[1]Mastalter 35 Tage bei Ross 308 und Cobb 500
Mastalter 38 Tage bei Cobb 800
[2]Instron-Gerät (Modell 5564) - Messvorrichtung Warner-Bratzler

Tabelle 9. Objektive Zartheit des Brustfleisches im Reifungsverlauf (*Kühne et al.*, 2005, mod.)

Reifungsdauer/h[1]	Scherkraft/kg[2] $\bar{x}$	s	min.	max.
0	4,8	1,0	3,3	7,5
1	4,9	1,4	2,8	8,1
2	4,9	1,1	2,9	8,7
3	5,2	1,1	3,6	7,9
4	4,1[a]	1,2	2,8	8,9
5	4,2	1,1	2,5	7,8
6	3,2[b]	0,7	2,2	4,9
7	3,1	0,8	1,9	4,6
8	3,0	0,5	2,2	4,5
9	2,9[c]	0,6	1,9	4,8
10	2,9	0,8	1,7	4,6
11	3,3[d]	0,5	1,9	4,7
12	2,9[e]	0,7	1,6	4,3
16	2,8	0,6	2,0	4,2
24	2,4[f]	0,5	1,4	3,6

[1]Zeit post mortem plus 1 h 51 min

[2]Texture Analyser (Stable Micro Systems) - Messvorrichtung Warner-Bratzler

a, b, c…signifikanter Unterschied zu vorherigem Messzeitpunkt bei $p \leq 0,05$

Bewertung der Zartheit. Eine günstigere Zartheit wurde bei Hyla-Kaninchen in der Keulenmuskulatur, sowie nach längerem Mastalter erzielt. Inwieweit die unterschiedlichen Mastendgewichte von Kaninchen verschiedener Rassen einen Einfluss auf die objektive Zartheit hatten, ist in Tab. 12 ersichtlich; bei der Rasse Weiße Neuseeländer zeigte sich ein negativer Einfluss, bei Deutschen Riesen x (Russen x WN) ein positiver. Die männlichen Kaninchen der Rasse DR x (Russen x WN) hatten eine günstigere Bewertung im Hinblick auf die Zartheit, als die weiblichen. Die sensorisch ermittelte Zartheit unterlag dem Einfluss des Mastendgewichts. Diese war bei DR x (Russen x WN) stärker ausgeprägt. In einem Vergleich zwischen Masthybriden und Reinzuchttieren bei Kaninchen wurde festgestellt, dass die objektive Zartheit bei Zika- und Hyla-Hybriden günstigere Messwerte erbrachte (Tab. 13). Die Reinzuchttiere hatten signifikant höhere Messwerte und somit eine ungünstigere objektive Zartheit. Eine günstigere Bewertung der sensorisch ermittelten Zartheit der Keulenmuskulatur ergaben die Hyla-Hybriden und Zika-Reinzuchttiere. Die Information in der Tab. 14 über die Scherkraftwerte verschiedener Kaninchenrassen und -kreuzungen kam in den Jahren 1995 bis 2006 zustande. Die Angora--Kaninchen (n = 10) hatten gegenüber den anderen Rassen bzw. Kreuzungen deutlich niedrigere Scherkraftwerte. Hierbei soll erwähnt werden, dass diese Kaninchen nach unterschiedlichem Mastalter und

Tabelle 10. Objektive Zartheit des Brustfleisches von Enten und Gänsen (*Ristic et* al., 2006, mod.)

Herkunft (n=122)	max. Energie[1] (N)	Energie bei max. Kraft (mJ)	Energie bei Bruch (mJ)	Zartheit (Pkt.)[2]	Grillverlust (%)
Pekingente, 42 Tg.	18,7	55,8	120,0	5,0[3]	21,4
Pekingente, 47 Tg.	17,4	63,4	117,4	4,8	23,5
Pekingente, 54 Tg.	19,2	63,2	124,5	5,3	22,6
Stockente, 120 Tg.	22,1	78,3	144,7	-	17,0
Flugente, 84 Tg.	29,2	93,4	185,9	4,0	22,7
Gänse, 230 Tg.	46,9	202,6	316,2	3,2	30,7
Signifikanz	***	***	***	***	***

[1]Instron-Gerät (Modell 5564) - Messvorrichtung Warner-Bratzler

[2]1=sehr zäh, 6=sehr zart

[3]Grenzdifferenz bei $p \leq 0,05$ mit 0,3

Tabelle 11. Objektive Zartheit der Keulenmuskulatur von Kaninchen (n = 72; *Ristic*, 1986, mod.)

Merkmal	Herkunft		Geschlecht		Alter/Wochen		
	Zika	Hyla	weiblich	männlich	10	12	14
Weg, mm[1]	17,5[a]	16,3[b]	17,1	16,8	17,8[a]	16,8[b]	16,2[b]
Zartheit, Pkt.[2]	3,8[a]	4,2[b]	4,0	4,0	3,8[a]	3,9[a]	4,2[b]

[1]Instron-Gerät (Modell 1140)
[2]1=sehr zäh, 6=sehr zart
a, b kennzeichnen signifikante Unterschiede bei $p \leq 0,05$

Tabelle 12. Objektive Zartheit der Keulenmuskulatur von Kaninchen unterschiedlicher Mastendgewichte (n = 72; *Ristic et al*., 1990, mod.)

Merkmal	Weiße Neuseeländer		DR x (Russen x WN)	
	weiblich	männlich	weiblich	männlich
Warner-Bratzler[1] in lb.	A[3] 2,7	2,2	3,1	2,5
	B 2,7	2,4	2,0	1,7
	C 3,3	3,2	1,9	1,7
Zartheit/Pkt.[2]	A 4,0	3,9	3,5	3,3
	B 3,7	4,0	4,2	4,0
	C 3,4	3,3	4,0	4,0

[1]1 lb.=453,59 g
[2]1=sehr zäh, 6=sehr zart
[3]A=Lebendgweicht 2,6kg, B=2,8 kg, C=3,0 kg

Tabelle 13. Objektive Zartheit der Keulenmuskulatur der Kaninchen von Masthybriden und Reinzuchttieren (n = 60; *Ristic* und *Zimmermann*, 1992, mod.)

Merkmal	Zika[1]	Hyla[1]	Zika[2]
Warner-Bratzler/N	11,6[a]	11,0[a]	14,1[b]
Zartheit/Pkt.	3,6[a]	4,2[b]	4,2[b]

[1]Masthybriden
[2]Reinzuchttiere
a, b kennzeichnen signifikante Unterschiede bei $p \leq 0,05$

Tabelle 14. Objektive Zartheit[1] des Rückenmuskels[2] von Kaninchen verschiedener Rassen und Kreuzungen (n = 232; *Westphal* und *Golze*, 2007, mod.)

Genotyp/Rasse	Alter	$\bar{x}$	s	min.	max.
Zika Hybrid	89	3,9	1,0	2,0	7,6
Holländer	141	3,7	0,8	2,4	5,3
Blaue Wiener	142	3,1	0,9	1,8	4,3
Castor Rex	100	3,9	1,0	2,5	4,9
Angora	100	2,2	0,5	1,5	3,1

[1]Warner-Bratzler/kg
[2]M. long. dorsi 24 Std. p.m.

unterschiedlicher n-Zahl zur Schlachtung kamen. Die höchsten Scherkraftwerte hatten dagegen die Zika-Hybriden und Castor Rex.

Diskussion

Nach *Augustini* und *Spindler* (2000) wurden die Scherkraftwerte nach 7-tägiger Lagerung in 3 Klassen unterteilt: ≤5,8 kg/cm² (Scherkraftwert niedrig), >5,8 und ≤8,7 kg/cm² (Scherkraftwert mittel) und >8,7 kg/cm² (Scherkraftwert hoch). Dabei zeigte sich, dass Proben, bei denen bereits nach 7-tägiger Reifung niedrige Scherkraftwerte ermittelt wurden, zu 80% auch in die Qualitätsklassen mit guter Qualität eingestuft wurden. Inwieweit eine solche Unterteilung in Scherkraftklassen tatsächlich für die Verbraucher relevant ist, ist meiner Meinung nach fragwürdig. Unter entsprechenden hygienischen Bedingungen und bei niedrigen Temperaturen wird das Rindfleisch noch zarter bei einer Lagerung bis zu 8 Wochen. Im zeitlichen Ablauf führt allerdings die Phase kurz nach der Schlachtung zur schnellsten Reduzierung der Zähigkeit. Hat die Zartheit Priorität, so sollte länger als 2 Wochen gereift werden. Die Versuchsergebnisse aus Tab. 2 zeigen, dass die Reifung des Rindfleisches von 10 bis 14 Tagen eine akzeptable Lösung darstellt, für den Fall, dass das Fleisch nicht länger als 28 oder 35 Tage gereift werden kann. Wenn die Scherkraftwerte nach einer Reifung von 10, 14 und 21 Tagen abnahmen, waren jedoch diese Unterschiede nicht statistisch signifikant nachweisbar. *Troeger* (1998) beschreibt moderne Kühlverfahren (Schnellst-/Schockkühlung, Ultra-Schnellstkühlung, Nebelkühlung) für verschiedene Schlachtkörper.

Nach *Fischer et al.* (2002) zeigte sich, dass gerade die Idealgruppe „rot und trocken“ (A) bei den frisch gegrillten bzw. gekochten Proben von Schweinefleisch tendenziell bzw. sogar signifikant die höchsten Scherkraftwerte hatten (Tab. 4). Vergleichsweise günstigere Messwerte ergab die Gruppe „blass und trocken“ (B), insbesondere bei den nach Gefrierlagerung gegrillten Proben. Eine

aufschlussreiche Erklärung für diese Versuchsergebnisse wurde nicht gefunden. Eine mögliche Ursache dafür könnte sein, dass Proben mit starkem PSE-Charakter kaum vertreten waren. Obwohl sich der Kollagengehalt und die Kollagenlöslichkeit zwischen den Versuchsgruppen von 110 gegenüber 160 kg Lebendgewicht verminderte, hatte dies auf die Scherkraftwerte keinen Einfluss. In der sensorischen Prüfung (M. longissimus dorsi) schnitt die 135 kg-Gruppe am besten ab und die 160 kg-Gruppe am schlechtesten (3,8 : 3,5). Man könnte vermuten, dass die Verringerung der Kollagenlöslichkeit auch eine höhere Zähigkeit des erhitzten Muskelgewebes verursacht.

Es ist bekannt, dass mit zunehmender Dauer der Mastperiode sowohl die sensorischen Eigenschaften, als auch die objektive Zartheit des Brustfleisches von Broilern positiv beeinflusst werden (*Ristic et. al.,* 2006) Mit einer Verlängerung der Mastperiode um 3 Tage erzielten die Cobb 800-Broiler die beste Bewertung der sensorisch erfassten Zartheit (siehe Tab. 8). Ebenfalls gibt es bei den o.g. Kriterien auch Unterschiede zwischen verschiedenen Herkünften. Die Zartheit des frischen Geflügelfleisches hängt in erster Linie von der Art der Kühlung (Luft-, Wasser- bzw. Luft-Sprüh-Kühlung) ab. Im Anschluss daran sind noch 3 bis 4 Stunden p.m. notwendig, um eine ausreichende Zartheit zu erreichen. Allerdings mit Anwendung des Rapid Rigor-Systems mit einer Kombination der Elektrostimulation wird die Reifungsdauer auf 1 Stunde verkürzt (*Kranen*, 2003). Bei der Herstellung der gefrorenen bzw. tiefgefrorenen Ware wird der Reifungsprozess durch das Einfrieren unterbrochen. Nach dem Auftauen solcher Ware findet ein Auftau-Rigor statt, in dem das Fleisch noch nachgereift wird. Nach *Kühne et al.* (2005)ist eine Reifung von mindestens 6 Stunden erforderlich, um eine zartes Produkt zu erhalten. Bei der Erfassung der Geflügelfleischqualität wird neben den chemischen und physikalischen Kriterien den Textureigenschaften des Fleisches große Aufmerksamkeit geschenkt, die für die Verbraucher sehr wichtig sind (*Petracci* und *Baeza*, 2007). Bezüglich der sensorischen Kriterien des Entenfleisches wurde nach *Golze* und *Schröder* (2003) festgestellt, dass die beste Bewertung die Stockenten und männliche Flugenten erzielten.

Die schweren Kaninchen der Kreuzung DR x (Russen x WN) zeigten eine günstigere Bewertung der objektiven Zartheit. Die gleiche Tendenz wurde bei der subjektiven Zartheit mit der Erhöhung des Mastendgewichtes besonders bei den Kreuzungstieren festgestellt. Für die Vermarktung von Kaninchenfleisch wird eine Reifungsphase von bis zu 7 Tagen bei 0°C empfohlen (*Westphal* und *Golze*, 2007). Bei einer Reifungsdauer bei +4°C stiegen die Scherkraftwerte bei Kaninchen zunächst mit der Totenstarre an und nahmen danach aufgrund der weiteren Reifung bis zu 4 Tagen ab. Die Autoren stellten fest, dass für die Qualität des Kaninchenfleisches neben der Reifung noch die Herkunft und das Alter einen nicht zu unterschätzenden Einfluss auf die Scherkraft haben.

Nach *Hausmann* (1967) lag der Korrelationskoeffizient zwischen den objektiv und subjektiv ermittelten Werten der Zartheit von Jungbullen (M. longissimus dorsi) bei -0,72. Die Merkmale Wasser- und Fettgehalt zeigten eine starke gegenseitige Abhängigkeit
(r = -0,95***). *Augustini* und *Spindler* (2000) fanden eine Abhängigkeit zwischen der Scherkraft und der sensorisch ermittelten Zartheit von r = -0,6 bzw. r = -0,7 (*Augustini* et al., 1998). Zwischen den Messkriterien geleistete Arbeit und maximaler Druck beim Wolodkewitsch-Gerät lag die Abhängigkeit bei r = 0,86***, sowie beim Fleischwolf-Gerät bei 0,84*** (*Tawfik*, 1969).

Schlussfolgerung

Für die Erfassung der Zartheit des Fleisches stehen subjektive und objektive Methoden zur Verfügung. Die sensorische Analyse ist umfangreich und zeitaufwändig. Demgegenüber wird die Zartheit auch mit Hilfe verschiedener Geräte gemessen, bei denen die auftretende Kraft-Weg-Energie angezeigt wird. Dies ist für den Verbraucher von Vorteil, als Ergänzung zur sensorischen Bewertung. Jedoch sollte besonders die Reifung vom Rindfleisch nicht außer Acht gelassen werden.

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Meat tenderness - a comparison of beef, pork, poultry and common rabit meat

Ristic Milan

S u m m a r y: Consumers appreciate the quality of meat with regard to tenderness and aroma/taste. However, there is a desire to determine these properties objectively. In Germany, measuring devices were applied for the first time in the 50s for beef, veal and later on for poultry (GRÜNEWALD, 1957). Several theses dealt with this issue. In the course of time measurement technique has been improved. At present, several measurement devices are available on the market. Appliances which are more expensive provide more information on compressive strength and shearing strength. Measurements are given in lbs, kg and N, resp., and their value expresses the objective tenderness, thus demonstrating the ripening of the meat. There are 4 levels for the meat of the species as follows:

beef	*40-80 N*	*shear value*
pork	*30-60 N*	*shear value*
poultry	*10-30 N*	*objective tenderness*
rabbit	*10-40 N*	*objective tenderness*

For beef, shear values declined for about 40% within a ripening period of 2 weeks.

Concluding, it can be stated that the recording of the objective tenderness is a useful supplement to the sensory evaluation of meat. In this connection, the dependence was r = -0.72, and r = 0.86 for the measuring criteria of a device.

Key words: *tenderness, subjective, objective, beef, pork, poultry, rabbit, comparison.*

Mekoća mesa – uporedni prikaz goveđeg, svinjskog i živinskog mesa i mesa kunića

Ristić Milan

R e z i m e: Potrošači vrednuju kvalitet mesa na osnovu mekoće i ukusa/arome. Međutim, postoji želja da se ove osobine mesa odrede na objektivan način. U Nemačkoj, merni instrumenti su primenjeni po prvi put pedesetih godina prošlog veka na govođem mesu, teletini i kasnije živinskom mesu (Grünewald, 1957). Nekoliko teza se bavilo ovim pitanjem. Tokom vremena tehnika merenja je stalno unapređivana. Trenutno, nekoliko mernih instrumenata je dostupno na tržištu. Skuplji aparati obezbeđuju više informacija o kompresivnoj snazi i sili sečenja mesa. Mere se daju u lbs, kg and N, respektivno, a njihove vrednosti izražavaju objektivnu mekoću, i na taj način pokazuju zrenje mesa. Postoje 4 nivoa za meso različitih vrsta životinja, i to:

govođe meso	*40-80 N*	*Sila sečenja*
svinjsko meso	*30-60 N*	*Sila sečenja*
živinsko meso	*10-30 N*	*Objektivna mekoća*
meso kunića	*10-40 N*	*Objektivna mekoća*

U slučaju goveđeg mesa, vrednosti sile sečenja su smanjenje za oko 40% u periodu zrenja od 2 nedelje.

Može da se zaključi da je objektivno merenje mekoće mesa korisna dopuna u senzorskoj oceni mesa. S tim u vezi, zavisnost je bila r = – 0.72, i r = 0.86 za merni kriterijum aparata.

Ključne reči: *mekoća, subjektivno, objektivno, govođe meso, svinjsko meso, živinsko meso, meso kunića, poređenje.*

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Welfare of slaughter animals and impact on meat quality*

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A b s t r a c t: Animal welfare is very important in the meat production chain. From the production on the farm to slaughtering, it is the main task of experts and other stakeholders included in the meat production, as well as their obligation, to respect the animal welfare, to treat animals in a humane manner, to spear them fear, suffering and pain. Since slaughterhouses represent unfamiliar environment for the animals, and therefore stressful environment, negative effects can be diminished by adequate handling of animals. Guarantee of proper animal treatment and subsequent meat quality is respecting of adequate regulations and continuous education of persons included in the chain of animal food.

Key words: *welfare, quality, meat.*

Introduction

Production of food is still one of the main human activities without which there is no survival of the human kind. In recent years, 40 % of human population is employed in agriculture, which is more than any other occupation. All cultivated plants and domestic animals are product of human technology. It is indisputable that modern agriculture has its drawbacks, some of them were severely criticized and cause fear in people. In addition to all shortcomings, agriculture is basis/foundation of the civilization as we know it. Cultivated plants and domesticated animals are foundation of the modern world.

One of the reasons influencing the decrease in meat consumption is concern of the consumers in regard to zoonoses, residues, contaminants, etc. So, incidence of bovine encefalopathy had significant impact on consumption of beef in countries where this disease was recorded (*Gregory*, 2000). Recently, there are discussions about other reasons for negative consumer attitude towards the meat consumption, associated with modern animal farming methods. Therefore, ethical dilemma and sense of resentment occur in part of consumers. Producers and suppliers of meat respond to the negative reactions of consumers by marketing of meat prepared for use (heat treated, ready dishes). Reason for this is to disassociate meat for consumption and animal from which the meat comes from, to make this link less distinct and as distant possible (*Baltić et al.*, 2002; *Baltić et al.*, 2010; *Fiddes,* 1997). One of the measures undertaken by producers and suppliers is introduction of modern systems of production process control and control of the final products, as well as informing of consumers about those measures. In this way the level of consumer concern about the quality of foodstuffs is reduced (*Gregory,* 2000).

Standards and legislative regulations in the field of animal welfare

Consumer attitude towards the meat quality represents very important information for producers, because it has direct impact on profit. Quality is very difficult to define, but with numerous researches conducted in this field and adequate public awareness and information campaigns, the consumer opinion and attitude in regard to the meat quality and farming of animals and management of animals, can be improved over time (*Baltić et al.*, 2002; *Baltić*

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et al., 2010a). In recent years, animal welfare has increasingly been in the sphere of interest of experts as well as the general public. Veterinarians are especially important since animal health is one of themost important animal welfare aspects (*Baltić et al.*, 2005). In addition to veterinarians, production managers, workers in the meat production chain, need to acquire knowledge and skills in the field of animal welfare, and special attention must be directed to the procedure of assessment of welfare conditions in regular production.

As the consequence of increasing concerns of consumers, but also state authorities, for the animal welfare, activities followed which lead to tightening of the legislation and introduction of standards which, in addition to legislation, should improve the execution of adequate conduct towards the animals. All standards are based on the fact that food business operators operate within the local legislation, however respecting internationally adopted rules. So, there are three implementation levels of different rules: a) Global - OIE (The World Organization for Animal Health) published animal welfare standards pertaining to slaughtering, transportation and killing of animals in disease control (*OIE*, 2010). These standards represent the minimum requirement for developed countries as well as developing countries; b) Regional – directive/regulation of European Union relating to animal welfare on the farm, in transportation and slaughtering (*Council Directive* 2001/88/EC; *Council Regulatio*n (EC) No 1099/2009); c) National legislation – each state has adopted own legislation in the field of animal welfare (*Defra*, 2010; „*Official journal of RS"*, No. 41/2009; *USDA*, 2010; *MAF*, 1996); and d) Internal standards of large corporations/associations/scientific societies (*FASS*, 2010; *National Pork Board,* 2008), which in some segments are even more severe in requirements than valid/applicable national regulations and/or OIE standards.

Science and scientific information which we obtain as the result of different experimental investigations, measure/quantify the effect of different situations and environment conditions on animals, from the aspect of the animals, and ethics shows us how to treat the animals. Corresponding standards in this field help us to realize good production practice, and legislative regulations show us how to treat the animals. Fighters for animal rights often pose a dilemma that the idea itself of animals being reared only to be killed for our purposes, is cruel/brutal. However, death relates to the quantity of life (duration) more than to the quality. Welfare primarily concerns the quality of life. Even though in certain situations death can have impact on welfare as indicator of previously poor condition of the anima, it is not an aspect of the welfare. The question is raised when is the death relevant from the welfare aspect? - In all situations when death of an animal occurs as the outcome of our poor practice, housing conditions, neglect, etc., such as high mortality of animals due to poor housing conditions, way of dying and applied slaughtering procedure, etc. In general, people want to avoid the poor quality of living and to have as long possible life span (quantity). This aspect (quantity of life) in the issue of animal welfare, from the ethical point differs from the demand relating to quality of life.

Assessment of animal welfare is aimed at evaluation of the condition of the animal as consequence of our actions (housing conditions, neglect, poor construction of the equipment and facilities where animals are housed, etc.). In the chain of meat production there are many steps in the process, and main steps in production of animals farmed for meat production, as following: rearing of animals on farms, transportation from farm to livestock market or slaughterhouse, transportation from the livestock market to slaughterhouse, stay of animals in slaughterhouse and slaughtering operations. In addition, there are numerous between steps which additionally complicate the welfare conditions, so it is necessary to realize the essence of the problem and it is preferable to have as few possible handling steps with animals. On the other hand, if you don't want to endanger animal welfare, then you should not keep it, rear it, transport it, stun or kill it, because every link in the production chain leads to some kind of threat to animals. There are always problems or they occur from the aspect of our relation, conditions which we provide for the animals, however, what we can do is to reduce the negative effects in the process of production of meat.

Good and poor manufacturing practice

Outcomes of poor production/manufacturing practice are numerous and create problems in production chain, and some of the examples are: percentage of tortured animals, injured animals, animals with bruises and wounds, percentage of animals which have not been successfully stunned in the first attempt, etc. All of these examples are consequence of applied procedure and poor manufacturing practice in handling of animals (*Grandin*, 2010). One of the examples of poor practice can also be when large number of animals slip/fall during handling. Reason can be poor construction and quality of floor, if it is slippery and not adequate for animals, or in

excessive use of electric stick causing the panic in animals (*Gregory,* 2007). If after applied stunning procedure animal still doesn't lose consciousness, this can be consequence of several factors such as poor maintaining of the equipment, also in cases when animal is upset, it is more difficult to place the stunning equipment on correct position, untrained staff, as well as poor construction of the stunning pen (*Grandin*, 1998).

In order to ensure the minimum requirement, some of the procedures such as use of force, striking and pulling of animals, especially tied and consciousness animals, are prohibited, whether by reference standards or applicable legislation. In OIE standard, relating to animal slaughtering, it is stated that such practice cannot be used under any conditions (*OIE*, 2010). Therefore, they are described and discussed for a reason, and unfortunately it is the possibility that some of these practices can be implemented by staff which lacks knowledge and training in the field of animal behaviour and sense of pain, as well as inadequate management support. In order to avoid misunderstandings and different interpretations of procedures which refer to fulfilling of animal welfare requirements, it is necessary to emphasize situations and procedures which are stressful and painful to the animals, and therefore are not appropriate to be applied in practice.

When speaking of space or equipment requirements, one should be cautious, because if we define how the equipment should look like, then we will prevent development of new methods and equipment from the scientific side (*Troeger*, 2009.). Majority of problems which relate to equipment can be assessed through outcome of the operation for which it is used. In case of stunning, efficiency of the equipment used can, among other things, be monitored through percentage of animals stunned from the first attempt. However, for some details it is necessary to have some specifications, such as minimum amper voltage value necessary for electric stunning, speed of the stunning wedge used in mechanical stunning and of course the minimum space that has to be provided for the animal in stunning pen (*OIE,* 2010.; *Gregory*, 2007). The fact that animals in transport must be provided adequate area/space in the transportation vehicle is often forgotten, and this is one of the major elements which are considered when welfare conditions are assessed (*Ritter et al.*, 2006.; *Ritter et al*., 2007), because as a consequence of over loaded vehicle, injuries, bruises, and in worst case scenario deaths, can occur. Deaths during transportation represent objective indicator of the severity and level of suffering of animals. Based on this statement, from the aspect of animal welfare and consumer demands, the mortality percentage of over 0% cannot be accepted, but in the practice, deaths during transport happen in all animal species. Percentage of animals that died varies in different producers and transporters. Significantly lower mortality percentage occurs in pigs which come from farms whit good manufacturing practice compared to farms with poorer farming and rearing conditions (*Fitzgerald et al.,* 2009.)

Animal welfare and quality of meat

Path of the animal from the farm to the slaughter house has numerous obstacles with which the animal is faced: handling and contact with the human/operator, transportation, different conditions and environment to which the animal is not used, deprivation of food and water, changes in the social structure, separation and/or mixing of animals (*Ferguson and Warner,* 2008; Ristić, 2009.). As consequence of above mentioned, animals can experience fear, dehydration, hunger, intensive physical stress and activity, fatigue and injuries. Inability of the animals to overcome the stress factors of the environment can additionally complicate and emphasize the consequences.

As a consequence of transportation, in all animal species, certain loss in body weight occurs, mainly due to loss of water, process of sweating, respiration and urine and faeces excretion. Factors influencing the loss of body weight are the condition of the animal, if the transportation vehicle is overloaded, season and climatic factors (heats or very humid and cold weather) and duration of the transportation. Loss of body weight occurs as consequence of food and water deprivation before slaughtering and it is most obvious in the first 12 h. From the aspect of animal welfare, bigger problem is the potential rehydration of animals upon their arrival to the slaughterhouse. In the livestock depot there should be water available for animals and different systems of water distribution. In some researches it was registered that animals, although they have access to water, refuse to drink it. Reason for this can be limited access or unfamiliar system of water supply to animals in the new environment. This is especially problem in calves and young cattle (*Gregory*, 2003; *Jacob et al.,* 2006).

Bruises are defined as traumatic injuries without skin penetration with damages to blood vessels of the injured region, with extravasation of the surrounding tissue. Fattened animals are more susceptible to these injuries, also animals reared in chronically stressful conditions. Injuries of this type are most often occurring in cattle in the following regions: (a)

legs, thighs and loins (approximately 31 %); (b) in region of scapula and shoulder (approximately 36 %); (c) rib region (approximately 13 %); (d) and 20 % of injuries of other body regions. In sheep, bruises occur due to rough treatment of animals, when they are pulled by the wool or legs, in triage, sorting, weighing, loading, unloading, etc. when producer establishes that this problem occurs, it is necessary to determine the origin of the injury, if it was inflicted in the slaughterhouse or earlier in the production chain. If injuries occur in the slaughterhouse, the changes will be obvious and visible on animals from various producers. If the problem is not associated with the slaughterhouse, then by tracing the origin of the injured animals the farm/producer or transporter in question can be determined. Trauma injuries most often occur as consequence of overloaded vehicle, and the other reason is rough treatment of animals (*Whytes et al.*, 1985; *Grandin*, 2010). However, trauma injuries in cattle can occur on the body/carcass any moment before bleeding

Stress is response of the organism to harmful factors of the environment. Stress outcome can be adapting or exhausted adapting, leading to collapse. Stress as the consequence of transportation represents the condition of the anxiety of the entire organism under the influence of different stress factors (extreme temperatures – low and high, noise, rough treatment of animals, excessive food deprivation, sudden and frequent changes of the air pressure).

Upon first signs of stress in pigs, stress factors should be eliminated and animal should be left to rest. Due to stress, organism cannot neutralize microorganisms entering the digestive tract, resistance to infectious diseases is reduced and there is also negative impact on sensory meat properties, consistence, taste, smell and colour.

Negative impact on sensory meat properties can also be induced by duration of the transportation (*Warriss,* 2000). It is considered that the transportation (shorter than four hours) has slight effect on pH values 24 hours after slaughtering, provided that the transportation conditions were satisfactory (*Grandin*, 2000). *Villarroel et al.* (2003) from Spain, investigated the effect of conditions and duration of transportation (30 minutes, three and six hours) on sensory meat properties (smell, tenderness, succulence, aroma intensity, aroma quality and general impression). The authors concluded that the duration of transportation had influenced the sensory meat properties, primarily tenderness and general impression.

Every activity of the animal (aggressive behaviour, long duration of transport, excessive use of the electric prods, etc.) requires energy, i.e. use of glycogen from the muscles. Glycogen reserves in musculature before slaughtering have very important impact on meat quality attributes: pH value of meat after slaughtering, succulence, sustainability, colour and water binding capacity. Glycogen concentration in cattle and sheep ranges from 75 and 120 mmol/kg (*Immonen et al.,* 2000). So, certain losses in the quantity of glycogen can be tolerated in the meat ripening process, until the critical limit is reached (45–57 mmol/kg) bellow which the expected pH value after slaughtering cannot be achieved (5,5–5,6), (*Ferguson and Warner*, 2008; *Tarrant,* 1989). Dark, firm and dry meat occurs as consequence of stress due to low glycogen content at the moment of slaughtering, and in the process of glycolysis small amount of lactic acid is generated which slightly decreases the pH value of the muscles, which has negative effect on process of meat ripening and quality. Pale, soft and exudative meat, as consequence of stress, occurs due to denaturation of muscle proteins, by combined action of increased temperature of the musculature and higher quantity of lactic acid as consequence of intensive glycolysis.

Instead of conclusion

Numerous factors which have negative effect on meat quality, and are related to animal welfare, can be reduced in the meat production chain, only by respecting adequate legislative regulations, implementation of good manufacturing practice, permanent staff training and management support.

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Dobrobit životinja za klanje i uticaj na kvalitet mesa

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R e z i m e: Dobrobit životinja ima značajnu ulogu u lancu proizvodnje mesa. Duž celog lanca proizvodnje od farme do trenutka klanja, zadatak stručne javnosti i ostalih osoba uključenih u proizvodnju mesa, jeste obaveza da se sa životinjama postupa na human način, kako bi se poštedele straha, patnje i bola. Pošto klanice predstavljaju nepoznato okruženje za životinje i samim tim stresnu sredinu, negativni efekti mogu se umanjiti adekvatnom manipulacijom sa životinjama. Garancija odgovarajućeg odnosa prema životinjama i posledičnog kvaliteta mesa je poštovanje odgovarajuće zakonske regulative i kontinuirane edukacije osoblja uključenog u lanac hrane animalnog porekla.

Ključne reči: *dobrobit, kvalitet, meso.*

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Production and consumption of meat in Republic of Macedonia*

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A b s t r a c t: Meat and meat products participate as one of the most important items in human nutrition. According to its biochemical and physiological traits, meat has a significant place in the diet. Its presence in diet is an essential need.

That is why permanent need of each society is to increase the meat production enabling the supply to the market with this product and improving the economic position of producers. Providing high productivity, economy and profitability of producers should be taken into consideration. In this way, cheaper products, available for every category of consumers, including even those categories with lower income, should be obtained.

Key words: *production, meat consumption, Republic of Macedonia.*

1. Introduction

Demand for meat and meat products, as consequence of growth, development and rapid changes of all major aspects influencing the meat production (number and structure of population, income and its distribution, prices, export orientation and trends) constantly increase in Republic of Macedonia. However, this increase is different manifested in different periods, depending on the changes in factors influencing it (*Ažderski*, 1992).

In order to provide sufficient quantities of meat and meat products to meet the demand of the market is not an easy task. This is especially expressed in moments when the demand for these products is growing. It would be illusory to expect fast turnaround in terms of abundant supply of meat on the market in short period, because the reproduction in livestock production is very slow as we know. If we want to increase the production of meat, it is necessary to take numerous measures in the society. First of all, measures which will motivate the producers for this type of production (*Ažderski i Pejkovski,* 1997).

In livestock production, sector should move in the direction of increase of yield, to change the breed structure in order to improve the productivity and, consequently, to increase the supply of meat as major products for domestic consumtion as well as for export.

To increase the production, no major investments are necessary. Less investments and better organization give significant results in relatively short period of time.

2. Present in meat production in Republic of Macedonia

2.1 Meat production

Meat production has an important role in Macedonian agriculture. Livestock production for agricultural households is major source of income, even 60% of total income. Through production of meat the following should be realized:

- satisfaction of the domestic demand which is constantly growing,
- to ensure necessary quantities of meat as necessary reserves for the society,
- to provide sufficient meat quantities for export, based on economically rational basis (in sheep and lamb meat) and
- to provide higher profitability of production and better standard of living.

Meat production directly depends on livestock production, breed structure and gain realized per unit (head of livestock).

Feed production has direct effect on the meat production. Especially increase of production of wheat and forage leads to more stable livestock production and improvement of the product range.

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In the last years, modern technical-technological solutions cause the increase of the average gain per animal, and also contribute to increase of work productivity and economic efficiency in animal rearing (*Jovanović et. al,* 2009)**.**

Due to relatively positive natural and economic conditions, as well as to methods used in livestock production, meat production is constantly increasing. Increased production of meat comes not only from increase of gain, but also from improved breed structure, which leads to increase of gain per animal.

Meat supply depends on production, but other factors are also important. The price is the main factor which regulate the trende in meat production. Main characteristic of meat production is that it can not adapt quickly to the market demands. Only the production of poultry meat is quickly adaptable to the market demands, because of the short reproduction cycle.

In other livestock species, reproduction cycle is longer. It ranges from 2 to 4 years, giving the meat production cyclic character with tendency of constant growth.

Meat production directly depends on livestock production and it's breed structure. RM disposes with good natural and economic conditions for production of meat. This is expressed if we take into consideration increasing changes in agriculture and growing investments in it. Investment in hydro-melioration systems and changes in the sowing structure on plough fields in favour of forage plants has direct influence on livestock production and changes in its structure. Also, country disposes with high quality mountain and high-mountain pastures which, with minimum of agro-technical measures, can be excellent source of cheap food. That is excellent basis for development of sheep production.

2.2 Meat consumption per capita in Republic of Macedonia

One of the main conditions for good functioning of social reproduction is balance between two links: production and consumption.

If commodity funds are ensured on time, this results in normal consumption to certain social level. On the other hand, this creates conditions for development of a stable process of social reproduction on an extended basis. This can all be realized within the laws of economics, i.e. market mechanisms through all production and trade stages until final retail stage is reached.

Table 1. Meat production in Republic of Macedonia for the period 2000 to 2009.
Tabela 1. Proizvodnja mesa u Republici Makedoniji u periodu 2000-2009. godine

Godina/ Year	Ukupno/ Total	Govedina/ Beef	Svinjetina/ Pork	Ovčetina/ Mutton	Živinsko meso/ Poultry	Ostalo/ Other
2000	27.470	7.287	9.323	4.919	**4.840 (+)**	1.101
2001	26.041	**5.835 (-)**	8.413	5.789	4.702	1.302
2002	27.471	6.738	**10.626 (+)**	**4.637 (-)**	3.992	1.478
2003	29.835	8.691	9.609	5.895	4.116	**1.524 (+)**
2004	**29.839 (+)**	**8.824 (+)**	9.373	7.030	3.189	1.423
2005	28.264	7.604	8.897	6.857	3.809	**1.097 (-)**
2006	28.041	7.132	8.633	**7.198 (+)**	3.715	1.363
2007	27.229	7.121	8.856	6.495	3.524	1.232
2008	**25.065 (-)**	7.018	8.703	5.204	**3.012 (-)**	1.128
2009	25.362	7.307	**8.291 (-)**	5.225	3.319	1.220
Average/ Prosek 2000-2009	**27.462**	**7.356**	**9.072**	**5.925**	**3.822**	**1.287**

(+) maximum
(-) minimum
Source: State Statistics Bureau

Fast growth of population in Republic of Macedonia and also changes in the structure of the income, impose the question of increased meat production. It is a fact that Republic of Macedonia, even though it doesn't have great surplus (except in sheep and lamb meat), it wants to export meat in order to improve the financial-balance position of RM towards other countries.

Table 2. Meat consumption per household and per capita in Republic of Macedonia for the period 2000 to 2009.
Tabela 2. Potrošnja mesa po domaćinstvu i članu domaćinstva u Republici Makedoniji u periodu od 2000 do 2009 godine.

Year/ Godina	Meat type/Vrste mesa	Average per household/ Prosek po domaćinstvu, kg	Average household per capita/Prosek po članu domaćinstva, kg
2000	Fresh and processed meat/Meso, sveže i prerađeno	**113,0**	**28.8**
	Beef and veal/Govedina, teletina i junetina	25,1	6,4
	Pork/Svinjetina	29,0	7,4
	Mutton and lamb meat/Ovčetina i jagnjetina	5,5	1,4
	Poultry meat/Živinsko meso	30,4	7,7
	Other meat type/Ostale vrste mesa	5,7	1,5
	Meat products/Proizvodi od mesa	17,3	4,4
	Fish/Riba	**17,6**	**4,5**
	Fresh and frozen fish/Sveža i zamrznuta riba	16,9	4,3
	Fish products/Proizvodi od ribe	0,7	0,2
2001	Fresh and processed, meat/Meso, sveže i prerađeno	**144,3**	**29,1**
	Beef and veal/Govedina, teletina i junetina	28,7	5,8
	Pork/Svinjetina	38,7	7,8
	Mutton and lamb meat/Ovčetina i jagnjetina	7,5	1,5
	Poultry meat/Živinsko meso	38,2	7,7
	Other meat type/Ostale vrste mesa	7,9	1,6
	Meat products/Proizvodi od mesa	23,3	4,7
	Fish/Riba	**20,5**	**4,2**
	Fresh and frozen fish/Sveža i zamrznuta riba	9,6	4,0
	Fish products/Proizvodi od ribe	0,9	0,2
2002	Fresh and processed meat/Meso, sveže i prerađeno	**159,4**	**40,2**
	Beef and veal/Govedina, teletina i junetina	36,1	9,1
	Pork/Svinjetina	28,7	7,3
	Mutton and lamb meat/Ovčetina i jagnjetina	5,3	1,3
	Poultry meat/Živinsko meso	42,1	10,6
	Other meat type/Ostale vrste mesa	4,1	1,1
	Meat products/Proizvodi od mesa	43,1	10,8
	Fish/Riba	**24,2**	**6,0**
	Fresh and frozen fish/Sveža i zamrznuta riba	21,3	5,3
	Fish products/Proizvodi od ribe	2,9	0,7
2003	Fresh and treated meat/Meso, sveže i prerađeno	**157,2**	**39,4**
	Beef and veal/Govedina, teletina i junetina	37,0	9,2
	Pork/Svinjetina	24,9	6,3
	Mutton and lamb meat/Ovčetina i jagnjetina	4,9	1,2
	Poultry meat/Živinsko meso	44,9	11,3
	Other meat type/Ostale vrste mesa	3,7	0,9
	Meat products/Proizvodi od mesa	41,8	10,5
	Fish/Riba	**21,8**	**5,5**
	Fresh and frozen fish/Sveža i zamrznuta riba	19,2	4,8
	Fish products/Proizvodi od ribe	2,6	0,7
2004	Fresh and processed meat/Meso, sveže i prerađeno	**163,0**	**41,4**
	Beef and veal/Govedina, teletina i junetina	39,0	9,9
	Pork/Svinjetina	29,9	7,6
	Mutton and lamb meat/Ovčetina i jagnjetina	4,2	1,1
	Poultry meat/Živinsko meso	43,4	11,0
	Other meat type/Ostale vrste mesa	3,8	1,0
	Meat products/Proizvodi od mesa	42,7	10,8
	Fish/Riba	**22,3**	**5,7**
	Fresh and frozen fish/Sveža i zamrznuta riba	19,5	5,0
	Fish products/Proizvodi od ribe	2,8	0,7

2005	Fresh and treated meat/Meso, sveže i prerađeno	**175,0**	**39,5**
	Beef and veal/Govedina, teletina i junetina	40,7	9,2
	Pork/Svinjetina	28,7	6,5
	Mutton and lamb meat/Ovčetina i jagnjetina	4,1	0,9
	Poultry meat/Živinsko meso	47,8	10,8
	Other meat type/Ostale vrste mesa	4,5	1,0
	Meat products/Proizvodi od mesa	49,2	11,1
	Fish/Riba	**25,3**	**5,7**
	Fresh and frozen fish/Sveža i zamrznuta riba	22,0	5,0
	Fish products/Proizvodi od ribe	3,3	0,7
2006	Fresh and processed meat/Meso, sveže i prerađeno	**177,6**	**44,4**
	Beef and veal/Govedina, teletina i junetina	44,7	11,2
	Pork/Svinjetina	27,1	6,8
	Mutton and lamb meat/Ovčetina i jagnjetina	4,1	1,0
	Poultry meat/Živinsko meso	44,0	11,0
	Other meat type/Ostale vrste mesa	4,2	1,0
	Meat products/Proizvodi od mesa	53,5	13,4
	Fish/Riba	**26,4**	**6,6**
	Fresh and frozen fish/Sveža i zamrznuta riba	22,9	5,7
	Fish products/Proizvodi od ribe	3,5	0,9
2007	Fresh and processed meat/Meso, sveže i prerađeno	**168,0**	**42,6**
	Beef and veal/Govedina, teletina i junetina	41,0	10,4
	Pork/Svinjetina	24,1	6,1
	Mutton and lamb meat/Ovčetina i jagnjetina	3,3	0,8
	Poultry meat/Živinsko meso	44,1	11,2
	Other meat type/Ostale vrste mesa	4,5	1,2
	Meat products/Proizvodi od mesa	51,0	12,9
	Fish/Riba	**24,6**	**6,2**
	Fresh and frozen fish/Sveža i zamrznuta riba	21,0	5,3
	Fish products/Proizvodi od ribe	3,6	0,9
2008	Fresh and processed meat/Meso, sveže i prerađeno	**173,5**	**45,2**
	Beef and veal/Govedina, teletina i junetina	39,0	10,2
	Pork/Svinjetina	23,2	6,0
	Mutton and lamb meat/Ovčetina i jagnjetina	3,4	0,9
	Poultry meat/Živinsko meso	43,2	11,1
	Other meat type/Ostale vrste mesa	4,7	1,2
	Meat products/Proizvodi od mesa	60,0	15,8
	Fish/Riba	**25,0**	**6,4**
	Fresh and frozen fish/Sveža i zamrznuta riba	21,3	5,5
	Fish products/Proizvodi od ribe	3,7	0,9
2009	Fresh and processed meat/Meso, sveže i prerađeno	**171,5**	**45,3**
	Beef and veal/Govedina, teletina i junetina	36,4	9,7
	Pork/Svinjetina	22,4	5,9
	Mutton and lamb meat/Ovčetina i jagnjetina	3,8	1,0
	Poultry meat/Živinsko meso	45,6	12,0
	Other meat type/Ostale vrste mesa	4,6	1,2
	Meat products/Proizvodi od mesa	58,7	15,5
	Fish/Riba	**24,0**	**6,3**
	Fresh and frozen fish/Sveža i zamrznuta riba	20,5	5,4
	Fish products/Proizvodi od ribe	3,5	0,9

Average/ Prosek 2000-2009	Fresh and processed meat/Meso, sveže i prerađeno	**160,3**	**39,6**
	Beef and veal/Govedina, teletina i junetina	36,8	9,1
	Pork/Svinjetina	27,8	6,8
	Mutton and lamb meat/Ovčetina i jagnjetina	4,6	1,1
	Poultry meat/Živinsko meso	42,4	11,2
	Other meat type/Ostale vrste mesa	4,8	1,3
	Meat products/Proizvodi od mesa	44,1	11,6
	Fish/Riba	**23,2**	**6,1**
	Fresh and frozen fish/Sveža i zamrznuta riba	20,4	5,4
	Fish products/Proizvodi od ribe	2,8	0,7

Source: State Statistics Bureau

Consumption of food is satisfactory in terms of volume, but not in the structure. Average citizen of Macedonia daily consume 3000 kcal, but the energy derives mainly from cereals (*Ažderski et al.,* 2002).

Presence of animal products in consumed food is not satisfactory (daily about 30 g of animal proteins are consumed, whereas in developed countries daily intake is 80-90 grams). Therefore, , in addition to production of approx. 300.000 tons of wheat, which is only a half of the need, we import each year 250.000 to 300.000 tons of wheat.

On the other hand, high level of increase in demand for animal products causes instability on domestic market. This applies, especially, on meat as major and basic product of animal origin. Often occurs that domestic production doesn't increase proportionally to the increase of demand. This discrepancy occurring between the demand and production of meat in different time periods as a consequence has constant price increase. This influences drop in the demand to some extent.

Consumption of meat in Republic of Macedonia is not satisfactory. If we take into consideration the importance of meat in human diet, the issue of inadequate presence of meat in nutrition of Macedonian population is not underestimated.

3. Material and methods

Data from public, annuals, bulletins and journals published by the State Statistics Bureau was used in preparation of this study. For quantitative analysis of data different statistical methods were used: tables, index method and linear trend.

In calculation of average growth rate, geometrical mean was used and, subsequently, comparative method, etc.

4. Conclusion

Livestock is one of the agriculture branches very important for Macedonian economy. Through livestock production, everything that is unusable by humans or cannot be made into products, is transferred into usable products for animals and therefore has irreplaceable party. Livestock production is and will be treated in future as branch of exceptional social importance.

However, livestock production is rather extensive that results in small supply of livestock products on the market. Recently, society has been making great efforts to improve this progressive branch (annually, over 100 million euros investments, of which 50 million euros is for livestock production). But, in spite of this, this social activity is insufficient. Frequent losses of livestock breeders because of high cost of food resulted in oscillations on the livestock production, especially meat. This has as a consequence reduced supply of meat, which leads to increase of price and reduced demand of meat from the population.

In order to avoid such incidence, a great social intervention is necessary. Intervention would consist of social-economic measures, of current policy such as: price policy, state reserves, funds for improvement of livestock production, compensations, subsidies, premiums, credits with subsidized interest rates, longer repayment period, etc.

Consumption of meat in Republic of Macedonia exceeds the supply, which is expressed through big deficit and import of large quantities of meat, except mutton and lamb meat.

Therefore it is necessary to engage more social means in development of livestock production, which will bring benefit to the population, in terms of consumption as well as increase of income for producers, and ultimately whole society.

It can be concluded that the demand for meat is unsatisfactory.

Average consumer in RM uses annually 39.6 kg of meat (*Statistički godišnici na DZS,* 2000– 2009;). If this is compared to meat consumption per capita in developed countries, it is obvious that we lag behind significantly. For instance, in USA, in average, meat

consumption per capita is 208 kg, in Australia 200 kg, France 195 kg, Canada 193 kg. This means that we are behind in meat consumption by 5 times. Meat consumption in RM is also behind Greece (101 kg), Spain (97 kg) and Serbia (87 kg) over 2 times (*Matekalo-Sverak et. al.*, 2009; *Prevolnik et al.,* 2010; *Stamenković et. al.,* 2008; *Usaleski*, 1981).

The question is raised: What is the reason for such low meat consumption? The answer is low national income per capita realized in RM and high retail prices of meat. Also, habits in nutrition of population should be considered, as well as consumption of cheaper products in diet, relatively low supply of meat on the market and high retail prices. All of the formely mentioned contributed to present situation in regard to meat consumption.

Also, if we look at the structure of the consumed meat it can be observed that situation changes in favour to beef, as high quality meat, as well as to poultry meat, which is cheap. Maybe the consumption of pork could be higher if the supply of this meat on the market would increase. In case of mutton, a slight drop in structure of consumed meat is recorded. This indicates that consumer orientation has shifted towards meat of better quality, indicating changes in the nutritional habits and choosiness.

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Proizvodnja i potrošnja mesa u Republici Makedoniji

Ažderski Jovan, Pejkovski Zlatko, Stojanova Marina, Jakovlev Zlatko, Angelkova Tanja, Bunevski Goko

R e z i m e: Meso i proizvodi od mesa učestvuju u ishrani kao jedan od najvažnijih njenih činilaca. Prema svojim biohemijskim i fiziološkim osobinama, meso ima važno mesto u ljudskoj ishrani. Njegovo prisustvo u ishrani je od ključne važnosti.

Zbog toga je stalna potreba svakog društva povećanje proizvodnje mesa, čime se omogućava snabdevanje tržišta proizvodima i poboljšanje ekonomske situacije proizvođača. Obezbeđivanje visoke produktivnosti, ekonomičnosti i rentabilnosti proizvođača, takođe, mora biti uzeto u obzir. Na ovaj način, jefitinij proizvodi će biti dostupni svim kategorijama potrošača, čak i onih sa manjim prihodima.

Ključne reči: *proizvodnja, potrošnja mesa, Makedonija.*

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Risk assessment in traditional production of Herzegovina ham

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A b s t r a c t: Production of traditional food products can pose safety problems in conditions of individual family farms, which is very difficult to monitor. Traditional production of Herzegovina ham and other foods of animal origin intended for public consumption does not follow the existing regulations on food, which poses a potential threat to human health.

Bearing in mind what we mentioned before, the aim and research tasks in this paper are aimed at the risk assessment of different contaminants in the traditional production of Herzegovina ham. Risk assessment is done on the basis of testing the Herzegovina ham in the municipality of Nevesinje. The results showed that the production of ham is not standardized, which resulted in considerable variability in the quality of the tested samples on the sensory properties. Assessment process of traditional production of Herzegovina ham found that there is a risk in production unless you don't follow the prescribed norms of production and trade of this productare not followed. By analysing of production conditions, the difference in the way of work and given results, some measures have been proposed for the goal of standardizing production, which will be helpful during making manufacturing specifications, registration of Herzegovina ham and obtaining indications of geographical origin.

Key words: *risk assessment, Herzegovina ham.*

Introduction

Food used by human population is excellent foundation for various dangers and hazards, whether of biological, chemical or physical origin, with potentially harmful effect on human health. Food can be contaminated primary and secondary. Primary contamination is during all production stages, preparation, processing, treatment and distribution and secondary contamination occurs due to inadequate and improper storage. Intake of food that has been exposed to various dangers results in incidence of diseases in human organism.

Considering the above mentioned, subject of our research in this study was risk assessment from various contaminants in traditional production of Herzegovina ham, evaluation of properties and characteristic as well as of safety of products manufactured according to traditional production methods, i.e. products which potentially can be subject to the protection of the geographical indication of origin.

Food safety refers to ultimate reduction of risks. The European Union takes very seriously its responsibility in managing and controlling of risks occurring in the global food market that is constantly changing. It makes decisions based on scientific studies which are transparent and available to all: the scientists, agricultural producers, food producers and consumers. At the same time, EU believes that the food safety standards would also have impact on product range and quality and not limit them. Objective is not to stifle and suppress the innovation or to homogenize the wide range of food products available on the European market, but to establish the general principles of safety to be used as basis upon which the quality and diversity can develop and prosper.

Risk can never be completely eliminated. However, by setting high standards, permanent risk assessment and use of best available independent scientific advice, European Union can by boast of good food safety policy (*Markus*, 2008).

Today, in global market, there is huge competition in marketing of food products. In this struggle to stay competitive, traditional products are becoming more recognized, products bearing the designation of origin or geographical indications, i.e. products whose special properties derive from

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the value of their ingredients, modes of production and processing, and the region from which they come. Higher education on nutritive and protective food properties and increased consumer purchasing power in developed countries contributed to the increase of demand for these food products which are indispensable part of culture and tradition of a region. Traditional products help promote national gastronomic and tourist-restaurant services.

According to the definition (*Regulation Council (EC)* No 509/2006) term traditional means mode of production and/or processing which is passed on from generation to generation and traditional image of food product is recognition on the market as the product that is characterized by special property or properties. *Special property* means one or several properties which clearly distinguish this product from similar products of the same category. Also, persistence of a food product, especially its form or package, how and where it is arranged and exhibited, are not considered as special property.

Even though the production of ham has relatively long history in Herzegovina, there is insufficient data on this production. In former Yugoslavia, although it was produced all around the country, there were four geographical regions where hams named after these regions were produced:

1. Dalmatian,
2. Užice,
3. Kras, and
4. Njeguš ham.

In Herzegovina, production of ham is also traditional and it takes place on agricultural holdings, mainly for own use. There is very little data in literature on traditional technology of ham production on the territory of East Herzegovina, so main source of information are producers. In regard to production method, there aren't any significant differences between the Njeguš ham, Dalmatian ham, Užice ham and Kras ham (*Joksimovic et al*., 1983) and Herzegovina ham. Main difference is in regard to sensory properties which are caused by differences in climate and duration of certain stages in production process. This refers to the salting, smoking, drying, etc.

According to *Antonic et al.,* (2006c), during production of pork ham, due to dehydration, changes in mass as well as in size, primarily length and diameter of the treated/processed musculature (piece of meat) occur. Dynamics of these changes is not the same in all production stages (salting, smoking, drying) and it primarily depends on conditions in which certain stages of production process take place. For instance, salting takes place at the cooling temperature 0–4° C, and prepared musculature is stacked in special dishes (containers). It is certain that in such temperature conditions, all physical-chemical and biochemical process have slower rate. Also, the process of dehydration is closely associated with other processes and changes which occur within the muscles (piece of meat). In other words, intensity of dehydration and distribution of moisture in certain muscle layers influence the course of physical-chemical, biochemical, microbiological and other changes (*Antonic et al.*, 2006c).

In production of Herzegovina ham, pig legs are used from animals of different breeds and ages. Body mass of pigs ranges from 100 to over 200 kg, age between 8 to 15 months. Fattening is mainly extensive and the economic effect of fattening is often neglected. Meat deriving from older animals contains less moisture and process of drying and ripening is associated with less risk.

Cutting of carcass sides is done according to the still current Rulebook on quality of slaughter pigs (Official Journal of SFRY, No. 2/85). In case of ham production, in general, legs are separated from the carcass by a cut going through pelvic joint, so that the *fovea capitis* – head of femur remains in the leg. Sacral and pelvic bones, as well as tail bones are removed. Fatty tissue on the inside of the leg is removed as much as possible, to facilitate better penetration of salt. Skin is left on the leg and feet are cut.

Discovery that the shelf life of meat can be extended by salt treatment dates from ancient times, so salting, in addition to smoking and drying, is considered as one of the oldest methods of conservation (*Antonic,* 2006a).

In Herzegovina, immediately after shaping, legs are salted. Salting is done by strong rubbing of salt over all leg surfaces. Content of salt ranges from 6–10% of the leg mass. Mainly sea salt is used, and in certain cases coarse kitchen salt.

In conditions of dry salting, i.e. procedure which is mainly applied in production of dry meat products, including the ham, first the dissolving of salt crystals in separated meat fluid occurs on the superficial area of the leg. Formed concentrated solution on the muscle surface directly effects creation of salt concentration gradient which is the driving force for the diffusion movement. Simultaneously with the diffusion of salt, also water molecules are diffusing in attempt to equalize the concentrations (*Antonic et al.*, 2006b).

After salting, hams are stacked into wooden or plastic barrels, where they remain for 15 to 20 days. Stacking is done in a way to keep the *fovea capitis* – head of femur turned upwards. During salting it is

necessary to, at least once, remove the meat fluid and add slightly more salt.

Subsequent to the salting period in barrels, hams are taken out, additionally salted, slightly, and stacked on special areas (cleaned stone area, or wooden plank or concrete) for pressing. In practice this operation is most often done in the following way: above stacked hams cloth or tarpaulin is placed to protect them from dust, or even pests (rodents and insects); over the covers a plank is placed with adequate load (usually rocks) of mass from 100 to 150 kg. For this operation also a screw press can be made.

Hams are stacked placing the *fovea capitis* – head of femur facing downwards. This stage lasts until all drained fluid/juice is released and, in practice, this is usually 10 to 14 days. During the pressing, hams need to be turned at least once. Pressing stage is considered final when hams obtain flat shape, and duration of this stage greatly depends on the load (pressure). However, the main task is releasing of juices, and not forming/shaping of hams.

After pressing, excess salt is removed from the legs and certain amount of garlic, red pepper and pepper (garlic, in addition to specific flavour also has known action in prevention of bacterial growth). Legs rubbed in previously illustrated way are left to drain for couple of hours (usually overnight) and subsequently hanged in dryer where they remain for some time to drain. In the dryer equal slight air ventilation must be provided. The following is process of smoking which is at the same time drying of products.

The smoke is obtained exclusively by burning of hardwood (beech, oak or elm). Combustion should be slow (without flame) in order not to overstep the temperature of cold smoking. If the weather is more humid, smoking is done continuously, but during dry days several hours of smooking daily is enough.

Duration of smoking ranges from 30 to 40 days and after completion of this period smoking as technological stage is over, but the process of penetration of smoke particles from superficial to deeper layers continues.

In traditional approach to smoking and drying, which applies also to drying of ham, a conventional type of dryer is present, with burning stoke within the dryer and poorly solved system of slight air ventilation and influx of fresh air.

In such products it is very difficult to control the concentrations of carcinogen elements from smoke in the final product. Today, we know (*Knezevic,* 2007; *Toroman et al.,* 2009) that smoke contains polycyclic aromatic hydrocarbons which give the pleasant flavour to dry meat but also are carcinogenic, which is why smoked products are suspected as risk factor in incidence of carcinoma (*Djinovic et al*., 2008).

According to EU standards, maximum residue limit of 3,4 benzopyrene present in food in the form in which it is consumed resulting from the use of aromas is 0,03 μg/kg (**Council Directive 88/388/EEC).** Maximum residue limit of benzo[a]pyrene in Bosnia and Herzegovina for smoked meat and smoked meat products is 5,0 μg/kg of wet mass (*Rulebook on maximum reside limits of certain contaminants in food, official Journal of B and H, No.* 37/09).

Traditional approach to smoking and drying includes conventional dryer with stoke within the dryer and habit to produce smoke during entire drying and fermentation stage/period. In such conditions, control of the microclimate conditions of the process of fermentation, drying and ripening of products and, especially, control of the content of carcinogenic substances in the final product, is very difficult (*Djinovic*, 2008).

Studies of the content of benzo[a]pyrene done by controlling the process of smoking of hams where the stoke temperature was kept at the level of 300-350^{o} C *(Toroman et al.,* 2009), showed that the content of benzo[a]pyrene decreased in smoked meat. Mean value of the benzo[a]pyrene content in the first group was 4.18 μg/kg, when temperature of stoke was not under control, whereas at controlled stoke/furnace temperature mean value was significantly reduced to 2,818 μg/kg.

After stages of smoking and drying, fermentative and other processes are still taking place in the leg meat, and moisture is still not removed to the extent necessary for durable products. Therefore, it is necessary to continue with the gradual process of ripening and additional drying of ham. In order for these processes to continue in desired directions, it is necessary to provide room with adequate conditions for smoked products (dark room, without temperature fluctuations, uniform humidity, good air ventilation without draft, etc.).

According to *Antonic* (2006a), during ripening process different changes in tissues occur, resulting in drying, forming of stable colour, pleasant aroma and firm texture of the product. Basic changes of structural components, associated with process of ripening of dry meat products, include degradation, primarily of proteins but also lipids, under the action of endogenous enzymes and microbial enzymes (*Antonic,* 2006a).

In this regard, numerous proteolytic and lipolytic reactions are included in the process of creation of flavour and/or flavour precursors.

Ripening period lasts at least 5 to 6 months. From the beginning of the production of obtaining of the final products, the leg mass decreases by approx. 30% (total salting, smoking and ripening loss). Legs with thinner layer of adipose/fatty tissue can loose mass of up to 35%. Desirable quality properties are achieved after 10 months, and one year is considered to be optimum (*Joksimovic et al.,* 1983). There are no precise data for Herzegovina ham and these parameters are changeable depending on the holding.

Material and methods

Objective of our study was the evaluation of Herzegovina ham produced on the territory of the municipality of Nevesinje in the years of 2008 and 2009. During the research, activities of pig breeders were monitored on pig holdings producing the Herzegovina ham. Authors of this paper have never intervened in activities of holding or gave any suggestions to the farmers.

Production took place on six individual agricultural holdings, which produce each in average annually eight hams from own production. Holdings are located on different locations of the municipality of Nevesinje.

In general, in production of Herzegovina ham legs deriving from different breeds, age and masses are used. Mass of pigs ranged from 120 to 160 kg/pig, age from 8 to 10 months. Pigs were fattened extensively, and economical results/performance in pig fattening were not in foreground.

In traditional production of Herzegovina ham legs are separated from the carcass sides by a cut going through pelvic joint, so that the *fovea capitis* – head of femur remains in the leg. Sacral and pelvic bones, as well as tail bones are removed. Fatty tissue on the inside of the leg is removed as much as possible to facilitate better penetration of salt. In most cases, skin is left on the leg and feet are cut.

Some holdings in Herzegovina, which traditionally manufacture the ham, remove the skin and adipose/fatty tissue also on the outside of the leg. In both cases it is necessary to obtain the oval shape of the leg and remove all parts which are protruding and eliminate all uneven areas on cut surfaces.

In four cases, skin and adipose/fatty tissue were left on the leg and in two holdings, skin and adipose tissue were removed from the leg. All legs had adequate oval shape without protruding parts.

Sea salt was used for salting in the amount of averagely 8% of the leg mass. After salting, legs were placed into plastic barrels for two and three weeks. Legs were stacked on wooden beech planks, covered with tarpaulin, beech planks were placed over it carrying the load (rocks) of around 100kg of weight. Legs remained this way for about 10 days and during this time they were turned once so that the fluids/juices could drain.

After pressing, legs were washed with tap water and excess of salt was removed, and certain amount of garlic, red pepper and pepper was rubbed into legs. After this operation, legs were hanged and left overnight to drain.

In all agricultural holdings, dryers were conventional with furnace/stoke within the dryer. The air ventilation system was different and could be designated as very poor, uneven ventilation to very strong drafts recorded in two holdings.

For smoking healthy beech and elm wood was used. Smoking and duration of smoking varied in ranges from 30 to 45 days, with various intervals.

After smoking, none of the farmers had separate room for ripening of ham, but it took place in dryer, only the legs were not smoked and there was more free space and better air circulation. All hams were smoked together with other dry meat products prepared by this holding for winter.

The first sample was tested 6 month after smoking and other samples 8 to 10 months after smoking. Small number of samples was evaluated after 14 months (production from year 2008).

The ham during ripening stage, summer months, was protected by farmers in different ways.

Production of ham took place in six different agricultural holdings, in conditions which are identical to practices implemented in these holdings for generations, without any changes to this day. There were some differences in how the ham was manufactures in these six holdings, which were reflected in the obtained results.

Production conditions, differences in operation and results obtained in these six holdings were used by authors to give suggestions and recommendations of certain measures for the purpose of realizing better organization and standardized production, which could be of help in establishing of manufacturing specification, i.e. registration of the Herzegovina ham and obtaining designation of the protected geographical origin/indication.

Two samples from each holding were subject to sensory analysis. Evaluation was done by the panel consisting of seven representatives of producers and consumers of Herzegovina ham, connoisseurs of properties and qualities of this product.

In determining sensory properties, descriptive method was used (*Radovanović, Popov-Raljić* 2001). By this method the following was determined: exterior appearance, cross section appearance, external

colour, cross section colour, texture, smell and flavour. Maximum score was 20 points.

Results and discussion

In production of ham the musculature of the highest quality of pig carcass was used, i.e. *m. longissimus lumborum et thoracis*.. Data which refer to mean values were established for pre-slaughter pig mass, mass of warm and cooled carcasses/carcass sides are presented in Table 1.

mass during cooling 1.71%, and yield of cold carcass 79.56%.

Relatively high variation coefficients (Cv) occur because investigated carcasses were not grouped according to weight/mass groups. This can easily be observed from data on standard deviation (Sd), i.e. standard deviation from the mean value of pre-slaughter pig mass which was 138 kg (minimum value of pre-slaughter pig mass was 120 kg, and maximum 160 kg).

Also, in holdings which were included in the

Table 1. Data on pre-slaughter mass of pigs and yield of warm and cooled carcases
Tabela 1. Podaci o masi svinja prije klanja i prinosa toplih i ohlađenih svinjskih trupova/polutki

Name/Naziv		Calculated indicators (n = 12)/ Izračunati pokazatelji (n = 12)		
		Xsr	Sd	Cv (%)
Pre-slaughter pig mass/Masa svinja prije klanja (kg)		138	12.35	8.95
Mass of primarily processed carcass/Masa primarno obrađenog trupa (kg)	Warm/Topli	111.8	10.2	9,13
	Cooled/Ohlađeni	109.8	10.06	9,17
Loss of mass during cooling/Gubitak mase tokom hlađenja (%)		1.71	-	-
Yield of cold carcass/Prinos ohlađenog trupa (%)		79.56	-	-

Table 2. Results of sensory evaluation of quality of pork ham
Tabela 2. Rezultati senzorne ocjene kvaliteta svinjskog pršuta

Number/ Redni Broj	Sample code/ Šifra uzorka	Points/ Broj bodova	% of achieved in relationship to maximum of points/ % ostvarenih u odnosu na maks. broj bodova	Class/Klasa
1	2.1.	14.35	71.50	Second/Druga
2	2.2.	15.60	78.00	Second/Druga
3	2.3.	17.35	86.75	First/Prva
4	2.4.	12.75	63.75	Third/Treća
5	2.5.	15.50	77.50	Second/Druga
6	2.6.	18.75	93.75	Extra/Ekstra
7	2.7.	17.85	89.25	First/Prva
8	2.8.	17.10	85.50	First/Prva

In Herzegovina, in production of meat products in traditional way, the predominant product is pork ham. Its regular control is very expensive and is rarely carried out in the field. Also, there are no possibilities to control of the content of carcinogenic compounds in the final product. The need to find the procedure within the process which will enable the safety of the final product still remains.

In trial conditions (Table 1) it was established that the average pre-slaughter mass of pigs was 138 kg, of primarily processed warm carcasses 111.8 kg, cooled carcasses 109.8 kg, i.e. the average loss of

trial, production was occurring in uncontrolled conditions and risk from the moment of slaughtering and suitability of such meat further in processing is present:

- Time of last feeding of the animal is not considered,
- Operating in unhygienic conditions, which among other things is associated with arrangement of the slaughter premises and premises for preparation of products, as well as the fact that this production is not in compliance with veterinary-sanitary regulations,

- ~ Cooling of meat (temperature of meat depends on outside temperature),
- ~ There is possibility/danger of spreading of zoonoses and other disease, etc.

The following contribute to reduction of risk:

- ~ Slaughtering of pigs is done in late autumn, when outside temperatures are low (below 10˚C),
- ~ Hygiene of knives used to cut blood vessels is satisfactory,
- ~ Meat is tested/analyzed on the presence of *Trichinella spiralis,* which is mandatory obligation for over 10 years, and this is done in registered veterinary stations and clinics.

The next stage is salting and smoking of product. By the process of osmosis salt takes water from meat and in this way prevents spoiling of meat, and its antiseptic properties provide longer duration of meat. However, in addition to indispensable and positive effects, these procedures can have some adverse action.

Contrary to traditional production, in industrial meat production, chemical conservants, nitrites and nitrates are used to prevent spoilage of meat products.

Rulebook on conditions for use of food additives in food intended for human consumption (*Official journal of Bosnia and Herzegovina,* No. 83/08), stipulates a special procedure which can prohibit the use of certain additives in production of food which is considered as traditional. In Bosnia and Herzegovina, the list of traditional products has not yet been compiled, but this is expected very soon. Traditionally produced Herzegovina ham should be included in this list. Also, according to traditional production technology of Herzegovina ham, the use of food additives is not allowed, especially nitrites and nitrates.

All six agricultural holdings which are engaged in production of smoked ham and were subject of analysis, do not have any established habits of use of food additives which could be regarded as not allowed in traditional products.

Risk of incidence of bacteria *Clostridium botulinum* (botulism), in our case, is very low, due to following reasons:

- ~ Animals are slaughtered when the outside temperatures are low and pig meat is quickly cooled,
- ~ Salt is used in sufficient amounts (about 8% of the mass of pig leg),
- ~ Legs are left in the salt long enough for the salt to penetrate the inside of the leg,
- ~ Temperature at the beginning of drying is below 15 ˚C, which prevents growth of *Clostridium botulinum* in insufficiently ripe legs.

In the process of manufacturing of dry meat products, the content of certain substances in regulated limits must be ensured. In smoke, in addition to great number of useful compounds, there are also some undesirable substances, such as tar, soot and carcinogenic compounds from the group of polycyclic aromatic hydrocarbons. As indicator of the presence of carcinogenic smoke compounds in smoked products, benzo[a]pyrene is used, because of its greatest carcinogenic potential. In Bosnia and Herzegovina, like in European Union, content of benzo[a]pyrene in smoked meat products must be below 5,0 μg/kg. It is well known that application of high temperature of pyrolysis will lead to forming of carcinogenic benzo[a]pyrenes and, because of that, first of all, in the drying stage and smoking phase it is necessary to provide controlled temperature of furnace/stoke and sufficient air in order to create small amounts of benzo[a]pyrene.

The creation of polycyclic aromatic hydrocarbons starts at temperatures of 400° C and at temperatures of pyrolysis of 600° C they are created in significant extent. At the same time, content of polycyclic aromatic hydro carbons in smoke is higher when some organic substances (fats, proteins) are burning at the same time as wood.

All holdings, subjected to the trial, used healthy hardwood as source of smoke and made pauses during smoking.

It is recommended, where possible (building, reconstruction, etc.) to use stokes/furnaces outside the dryer. Smoke is brought to dryer through system of pipes which lower temperature of smoke, and it is possible to install wet barriers, which can collect undesirable combustion by-products of wood.

The next stage is ripening of ham. Ripening took place in inadequate premises or it continued in premises where it smoked (dryers).

From the obtained results for sensory analysis of eight hams, one sample was scored as extra class, one sample as third class, three as first and three samples as second class. The best score was given to ham which underwent ripening process of 14 months, whereas the lowest score for the ham that ripened only for 6 months. All hams which ripened for 10 months were scored as first or extra class.

Non-standardized production is one of the main observed deficits in production of ham. If producers of traditional Herzegovina ham want to improve the quality of their product and be present on the market,

either domestic or international, they need to address the issue of standardization.

One of the initial steps would be to establishe the association of all interested traditional producers of Herzegovina ham as members. Associated in this way, they could more easily agree on technology and method of production (product specification), which would than be compulsory for all producers. When creating the product specification, it is necessary to do the risk assessment. This task is best done by those individuals who are familiar with specific characteristics and properties of the product being assessed, necessary procedures and available space. This would contribute to reduction of risk of contamination and obtaining a standardized product of improved quality.

Presently, there are several important differences in production technology, which need to be agreed upon in a consensus:

- ~ Skin and adipose/fatty tissue are left on the leg or removed,
- ~ Smoking method and duration,
- ~ Ripening period.

Based on two year monitoring of the situation in the field and obtained sensory results, the opinion is prevailing that traditional Herzegovina ham has skin and adipose/fatty tissue, duration of smoking of 30 to 45 days, but with obligatory improvement of the furnace/stoke system in dryers and ripening period of at least 10 months.

Conclusion

Based on research results established in the conditions of this study and available literature data, and in accordance with determined tasks of the study, the following can be concluded:

- Production of ham in traditional way in Herzegovina is not standardized and it takes place in inadequate hygienic conditions, this primarily refers to slaughter premises and space for reception of legs.
- Average pre-slaughter body mass of pigs was 138 kg, of primarily processed warm carcasses 111.8 kg, cooled carcasses 109.8 kg, i.e. average loss of mass during cooling was 1.71%, and yield of cooled carcass was 79.56%.
- Legs were smoked in dryers with open furnaces/stokes within the dryer and poor air ventilation system and influx of fresh air.
- Ripening of meat also takes place in inadequate premises without temperature regulation and air ventilation.
- In regard to sensory properties and the quality of final product, all analyzed samples were within the limits for this type of product, even though the quality varied considerably.
- The best properties were established in ham which was subject to the longest ripening process of 14 months, whereas the worst scored product was the one which was ripened for the shortest time (6 months).
- Hams which ripened for the period of 10 months were scored as the first and extra class.
- In evaluation of the process of traditional production of Herzegovina ham, it was established that great risk in production exists if regulated and issued norms of production and trade of this type of products are not respected.

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Procena rizika u tradicionalnoj proizvodnji hercegovačkog pršuta

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R e z i m e: Proizvodnja tradicionalnih prehrambenih proizvoda može da predstavlja problem bezbednosti u uslovima proizvodnje u individualnim porodičnim gazdinstvima, koju je veoma teško nadzirati. Tradicionalna proizvodnja hercegovačkog pršuta, kao i ostalih namirnica životinjskog porekla namenjenih potrošnji ne prati postojeće propise o hrani, što predstavlja potencijalnu opasnost za ljudsko zdravlje.

Imajući u vidu napred navedeno, cilj i zadaci ispitivanja, u okviru ovog rada, usmereni su na procenu rizika od različitih kontaminenata kod tradicionalne proizvodnje hercegovačkog pršuta.

Procena rizika rađena je na osnovu ispitivanja hercegovačkog pršuta na području opštine Nevesinje. Rezultati ispitivanja su pokazali da proizvodnja pršuta nije standardizovana, što se odrazilo na znatno variranje kvaliteta ispitivanih uzoraka na senzorna svojstva. Procenom procesa tradicionalne proizvodnje hercegovačkog pršuta ustanovljeno je da postoji rizik u proizvodnji, ukoliko se ne poštuju propisane norme proizvodnje i prometa ovog proizvoda.

Na osnovu sagledavanja uslova proizvodnje, razlika u načinu rada i dobijenih rezultata, predložene su određene mere standardizovanja proizvodnje, koje će u budućnosti pomoći prilikom izrade proizvođačke specifikacije, odnosno registracije hercegovačkog pršuta i dobijanja oznake geografskog porekla.

Ključne riječi: *procena rizika, hercegovački pršut.*

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JECFA, 2005. Joint FAO/WHO Expert Committee on Food Additives. Summary and Conclusion. Sixty-Fourth Meeting, Rome, 8-17 February, JECFA/64/SC. http://www.who.int/ipcs/food/jecfa/summaries/en/.

Morgan S. K., Daly C. C., Simmons N. J., Johnson N. V., Cummings T. L., 2008. The effect of pre-slaughter events on the expression of small heat shock proteins in the muscle. 54th International Congress of Meat Science & Technology, Proceedings, General Speakers Session, Electronic Copy, Cape Town, South Africa, 10th–15th August.

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