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HYGIENA ALIMENTORUM XXXV

INTERNATIONAL SCIENTIFIC CONFERENCE

under the auspices of the Ministry of Agriculture
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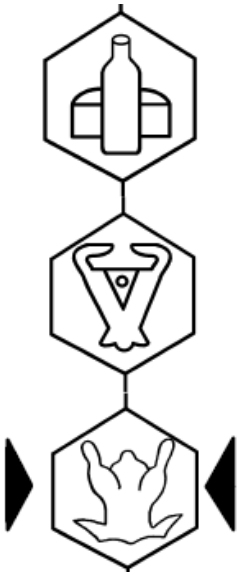
**"SAFETY OF MEAT AND MEAT PRODUCTS
IN THE SPIRIT OF TRADITIONAL QUALITY"**

May 21—23, 2014



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RADIOCAESIUM (^{137}CS) ACTIVITY CONCENTRATION REDUCTION IN WILD-BOAR (*SUS SCROFA*) MEAT AFTER PRESSURE COOKING — REPRODUCIBILITY OF RESULTS

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ABSTRACT

Reproducibility of radiocaesium isotope ^{137}Cs activity reduction in contaminated wild-boar rump-meat was analysed. The rump-meat was cut into cubes, salted (5 g NaCl.100 g^{-1} of meat), and cooked in a pressure cooker for additional 15 minutes after the pressurization conditions were achieved. Radiocaesium activity reduction by 50 % and 15 % variability in results reproducibility showed that a pressure cooker usage is an appropriate technology for contaminated meat treatment. The ^{137}Cs activity was determined using a Canberra gamma-spectrometry system.

Key words: pressure cooking technology; radiocaesium isotope ^{137}Cs; reproducibility of results; wild-boar (*Sus scrofa*) meat

INTRODUCTION

One of the most important radionuclides released into the environment after the Chernobyl nuclear accident was radiocaesium isotope ^{137}Cs, with its half-life survival in the environment of 30.2 years and similar chemical behaviour as potassium. Since Scandinavia was after the Chernobyl nuclear accident significantly affected by the radiocaesium isotope ^{137}Cs, the human food chain and food safety was closely monitored and significant attention focused on salmon [6] and caribou meat [8]. At the same time marine animals (e. g. crabs) from the north of the Ireland were monitored as well [3].

In case of radioactive contamination of wild animals it is not possible to carry out preventive steps applicable for livestock, and the only solution is a careful monitoring of each animal caught. If increased contamination by radiocaesium isotope ^{137}Cs is detected, further meat processing is required, especially if the content of radioactive caesium exceeds internationally established standards valid for the Chernobyl nuclear accident contamination by caesium (600 Bq.kg^{-1}).

Livestock decontamination is based on understanding of chemical and physical properties of specific radionuclides and must be complex. In such animals it is important to respect their food composition, digestive tract physiology and condition. Various complex or chelate additives, with the ability to bind radionuclides and form chemical compounds, have been successfully used. These additives are not absorbed by the digestive tract. Good results were achieved by adding clay minerals into the animal diet [4], [7]. There is little information published on radiocaesium isotope ^{137}Cs reduction in the meat. However, heat treatment of meat in salty water (1 % solution) can reduce the radiocaesium activity concentration by 80 % [5]. In addition, Dvorak and Kunova [1] published a significant (50 %) reduction of radiocesium isotope ^{137}Cs activity concentration in the wild boar meat using heat pressure meat treatment.

The purpose of this study was to analyze the reproducibility of methods published by Dvorak and Kunova [1], and to establish to what extent the published technology can guarantee reduction in the radiocaesium isotope ^{137}Cs activity concentration in the contaminated wild-boar meat.

MATERIALS AND METHODS

In our experiment naturally contaminated meat of a wild boar (*Sus scrofa*) was examined. We used the standard kitchen technology for meat preparation. The wild boar weight was 15 kg and the specimen was collected in Staré Ransko location (close to Havlíčkuv Brod, Czech Republic). Defrosted rump-meat was cut into cubes with a side length of approximately 1.5 cm and put into a Marinelli beaker (450 ml). Radiocaesium activity concentration of the specimen was measured before further processing of meat. Subsequently, the meat was dry-salted (5 g sodium chloride per 100 g of meat) and placed into a pressure cooker with 400 ml of water. The pressure cooker was placed on a stove, and the meat specimen was cooked for additional 15 minutes after the pressurization conditions were achieved. After cooling down, the meat samples were separated from the broth by decantation. Both, the meat samples and the broth were measured separately. Gamma spectrometry in the Marinelli beaker geometers was used, and no part of the original sample was been omitted. The experiment was repeated five times. The activity concentration of ^{137}Cs gamma radiation was determined using a Canberra gamma-spectrometry system consisting of a Desktop Inspector MCA and HPGe GC2020 germanium semiconductor detector (20 % efficiency, resolution 1.8 keV). The high-resolution gamma-spectrometry system was certified by the Czech Meteorological Institute certificate — Prague No. 911-OL-Z 2888b/2003. The assay was performed in the Marinelli beaker geometers (450 ml) and the measurement time was 18 hours (T = 64 800 s). For evaluation of results Genie 2000 (Canberra) and Gamat (PK-Servis Prague) evaluation softwares were used.

RESULTS AND DISCUSSION

As published by Dvorak and Kunova [1], the average radiocaesium activity concentration in the meat before heat pressure boiling was 106 Bq.kg^{-1}. After the heat pressure treatment, the average radiocaesium activity concentration was 53 Bq.kg^{-1} in the meat and 69 Bq.kg^{-1} in the broth, representing a 50 % reduction of radiocesium activity concentration on average.

The purpose of our study was to determine the level of reproducibility of the Dvorak and Kunova [1] experiment, which was repeated five times. Table 1 shows results in Bq per sample, and thus not the weight nor volume activity. Separate measurements were made for the radiocaesium activity concentration in both the meat and the broth. The third column shows the sum of the first two. If we consider that an average activity measured before the experiment was 49 Bq.sample^{-1}, then the final average activity of 51 Bq.sample^{-1}, (± 13.3 %) indicates an excellent analytical accuracy. Our results showed good reproducibility of Dvorak and Kunova [1] experiment, as the variability of all results represented by relative standard deviation did not exceed 15 % (Table 1).

Table 1. ^{137}Cs activity concentration reduction in the meat after heat-pressure cooking. The initial activity of ^{137}Cs in the meat before cooking was 49 Bq.sample^{-1}

Measurement No.	^{137}Cs [Bq. sample^{-1}]		
	Meat after heat-pressure cooking	Broth	Total (meat + broth)
1	15	31	46
2	19	37	56
3	20	38	58
4	19	34	53
5	16	26	42
Mean	**18**	**33**	**51**
SD	**2.2**	**4.9**	**6.8**
RSD [%]	**12.2**	**14.8**	**13.3**

RSD [%] — relative standard deviation
(SD expressed as % of an average)

One-shot application of radiocaesium under experimental conditions can yield quite different results. Lofti et al. [5] reported an 80 % radiocaesium activity reduction after cooking meat in a saline solution. However, neither the cooking nor the use of a different meat (meat from different animal species), could have such a significant effect on radiocesium activity reduction. Rather, it is likely, that some importance may have a repeated exchange of a saline solution, which corresponds with the results published by Dvorak et al. [3]. After two consequent meat samples treatment in a salt-pit (a mixture of sodium chloride and potassium nitrate) the ^{137}Cs activity concentration was reduced by 72 % compared to its default (starting) value of 103 Bq.kg^{-1}. It was also shown that a single brine replacement at different time intervals (after 24 hours, and after 7 days) did not affect the processes occurring in the meat.

CONCLUSION

Radiocaesium activity concentration reduction by 50 % with 15 % variability in results reproducibility shows, that a pressure cooker usage is an appropriate technology for contaminated meat treatment.

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This study was carried out in the framework of VEGA 1/0415/13.

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OXIDATIVE STABILITY OF MEAT PRODUCT AFTER APPLICATION OF OREGANO

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ABSTRACT

The aim of this study was to compare and evaluate the impact of different additions of oregano (*Origanum vulgare* L.) aqueous extract and oregano essential oil on the oxidative stability of meat product during cold storage. To 1 kg meat product we added 5 ml or 10 ml of aqueous extracts, or 300 µl or 500 µl of essential oils. In all experimental groups of products with water extract and essential oil we noticed higher oxidation stability compared to the control group throughout the observed storage period. On day 10 of storage we observed the highest oxidative stability in the experimental group to which we added oregano essential oil oregano at a dose of 500 µl.kg^{-1}. The malondialdehyde (MDA) value was 0.265 mg kg^{-1}.

Key words:, meat product; *Origanum vulgare;* oxidation stability

INTRODUCTION

Meat and meat products are the source of substances important for human health and nutrition [17]. Currently, production of meat products is considered the largest and the most complicated phase of fresh meat processing [12]. Lipids oxidation is one of the most common causes which deteriorate the quality of meat and meat products. Lipids oxidation is associated with production of many undesirable substances. Substances with antioxidant effect are able to slow down or eliminate the oxidative processes of lipids [13]. The use of antioxidants is the simplest method of increasing the oxidative stability of meat products and improving the most important sensory and storing parameters. One of the protection possibilities is application of natural antioxidants. An important source of natural antioxidants is plant material, which contains substances preventing oxidation as well as substances capable of improving the product taste. Several authors [1], [2], [4], [5], [6], [14] described application of plant extracts, e.]g. oregano extracts.

The aim of the study was to determine the oxidative stability of cured meat product after application of aqueous extracts and essential oils of oregano (*Origanum vulgare* L.) during 10 days of cold store.

MATERIALS AND METHODS

The tested meat product was made from pork meat and additional raw materials purchased on market and processed according to the recipe for the product type (Table 1).

We tested 5 groups of meat products. The groups were formed on the basis of various additions of oregano (*Origanum vulgare* L.) extract or essential oil, during the mixing in a bowl cutter, as follows:

- control group (C),
- experimental group 1 (EG 1) — 5 ml of oregano extract per 1 kg of meat mixture,
- experimental group 2 (EG 2) — 10 ml of oregano extract per 1 kg of meat mixture,

- experimental group 3 (EG 3) — 300 µl of oregano essential oil per 1 kg of meat mixture,
- experimental group 4 (EG 4) — 500 µl of oregano essential oil per 1 kg of meat mixture.

Oregano (*Origanum vulgare* L.) herb (Refka Company) was used to prepare a water extract. The extract was prepared by maceration of dried and milled herbs (3 g) in distilled water (100 ml) in a water bath at 70 °C for 1 hour. The macerate was filtered and made up to 100 ml with distilled water. Oregano essential oil (100 %) used in our study originated from Nobilis Tilia Company (ESP).

The oregano-treated meat product was smoked and heat-treated (temperature in a core of product reached 70 °C and persisted for 10 minutes). After the heat-treatment the product was cooled to 4 °C. Product samples were stored at 4 ± 1 °C during the testing (10 days). On days 1, 5 and 10 of storage, oxidative stability of samples was determined in a laboratory by the method of Marcinčák et al. [9]. Absorbances of samples were measured at wavelength 532 nm using a UV spectrophotometer T80 (PG Ltd. Instruments, UK). Results were calculated as malondialdehyde (MDA) quantity per 1 g of sample.

Table 1. Composition of the cured meat product [g]

Component	Amount [g]
Pork meat	1000
Water	200
Pepper, black (*Piper nigrum*)	1.5
Nutmeg (*Myristica fragrans*)	0.2
Allspice (*Pimenta dioica*)	0.2
Curing salt	18
Garlic (*Allium sativum*)	0.5
Paprika (*Capsicum annuum*)	1.0
Chilli (*Capsicum frutescens*)	1.5
Polyphosphate	7

RESULTS AND DISCUSSION

Results of oxidative stability determined in the heat-treated cured meat product are recorded in Table 2. On day 1 of storage the MDA content was 0.189 mg.kg^{-1} in control group. This level was higher compared to experimental groups with oregano water extract. In these groups the MDA values ranged from 0.165 mg.kg^{-1} (EG 1) to 0.185 mg.kg^{-1} (EG 2). In experimental groups with oregano essential oil the MDA values were 0.154 mg.kg^{-1} (EG 3) and 0.154 mg.kg^{-1} (EG 4).

Similar tendency was recorded after 5 days of storage compared with day 1 of storage when the lower malondialdehyde level and higher oxidative stability was detected in experimental groups in comparison with control group (0.267 mg.kg^{-1} MDA). The lowest oxidative damage to the fatty component of cured meat product was observed in EG 4 (0.172 mg.kg^{-1}). The same tendency of oxidative stability changes was observed at the end of the evaluated period on day 10 of storage, compared to the previous storage time. It implies, that oxidative stability of the fatty component was higher in experimental groups compared to control group (0.396 mg.kg^{-1}). The highest oxidative stability was observed in the group with 500 µl addition of oregano essential oil (EG 4; 0.265 mg.kg^{-1} MDA). The lowest oxidative stability was found in the group with 5 ml oregano extract (EG 1; 0.341 mg.kg^{-1} MDA). Macháčková [7] found lower MDA values in experimental groups on day 10 of storage compared with our results and stated that plant water extracts of oregano and thyme improve oxidative stability of cured meat product. Results of our experiment are comparable with those of Marcinčák et al. [8], who also recorded lower damage to the fatty component in meat products with addition of natural antioxidants. Results obtained in this experiment agree with findings of several authors [3], [10], [11], [15], [16] who stated that oxidative stability of meat and meat products decreases by prolongation of storage period and has negatively impact on technological and sensory quality of products.

ACKNOWLEDGEMENT

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Table 2: Values of thiobarbituric number during cold storage — measured as MDA quantity [mg kg^{-1}]

	C	EG 1	EG 2	EG 3	EG 4
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Day 1 of storage	0.189 ± 0.074	0.165 ± 0.028	0.185 ± 0.014	0.167 ± 0.007	0.154 ± 0.004
Day 5 of storage	0.267 ± 0.024	0.198 ± 0.023	0.258 ± 0.018	0.194 ± 0.027	0.172 ± 0.023
Day 10 of storage	0.396 ± 0.032	0.341± 0.035	0.300 ± 0.042	0.267 ± 0.040	0.265 ± 0.017

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RESULTS OF THE FIRST *YERSINIA ENTEROCOLITICA* MONITORING PROGRAMME IN SLOVAKIA

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ABSTRACT

The aim of the study was to monitor the production area and domestic market in the frame of occurrence a food-borne pathogen *Yersinia enterocolitica* in food. Depending on the knowledge and the monitoring programmes provided in the EU countries, only several types of food were selected. Raw pork meat, fermented heat-untreated sausages, minced meat and raw vegetables were identified as the most probable contaminated food. Isolation was made according the relevant ISO STN 10273 in the Reference Laboratory for *Yersinia* spp. of the Veterinary and Food Institute in Dolny Kubin.

Key words: food; method; monitoring; *Yersinia*

INTRODUCTION

Yersinia belongs among food-borne pathogens for which higher infectious dose is necessary in order to develop a disease [4]. Of species included in the genus *Yersinia* main human pathogens are *Yersinia pestis* (agent of plague) and *Yersinia enterocolitica* [7]. There are only few serotypes (O:3 and O:9) pathogenic for humans which were isolated from the tonsils and intestinal tract of pig carcasses. Pigs are therefore considered to be a major source of infection. The most common occurrence is in faeces, but food and water should also be a subject of greater concern [10]. Secondary infection of food is caused either by manipulation or during the storage. Alimentary yersinioses occur mainly for a short time in one district and are sporadic in most cases. The main clinical signs are gastroenteritis, mesenteric lymphadenitis and enlargement of nodes in the appendix area.

The aim of the study was to monitor the production area and domestic market in the frame of occurrence a food-borne pathogen *Yersinia enterocolitica* in food.

MATERIALS AND METHODS

Monitoring for occurrence of *Yersinia enterocolitica* in food was conducted in Slovakia for the first time. All analyzes were made in the Veterinary and Food Institute in Dolny Kubin, which is the Reference Laboratory for *Yersinia* spp. Hundred samples with different food matrices were examined.

The samples were taken by personnel of 19 different Regional Veterinary and Food Administrations (RVFA) from various supermarkets of domestic or foreign ownership. Types of samples taken were planned according to reports in scientific sources about occurrence of *Yersinia enterocolitica* and the experiences in other countries where similar monitoring programmes were implemented. As the initial running of the monitoring programme based on cultivation method having low sensitivity and high labour demands could not be very successful in detecting the pathogen, it was recommended to continue the programme with molecular methods.

As the most risky food we chose the following: chilled raw pork meat, minced meat, raw vegetables and fermented meat products

which were not subjected to heat treatment. Samples were taken at retail shops and were of domestic origin or came from various EU countries (Fig. 1).

Detection of *Yersinia enterocolitica* was performed by the cultivation method ISO STN 10273. Five typical colonies were chosen from each sample for further confirmatory testing. The suspect colonies were further sub-cultured on blood agar and Engo agar and incubated at 30 °C for 24 hours. The suspect *Yersinia* colonies were identified with oxidase (Erba-Lachema), Kligler iron (Immuna) and urea (Oxoid) tests. Oxidase-negative, glucose-positive, H2S-negative and urease-positive colonies were finally identified with API 20E (bioMérieux) strips and ENTEROtest 24 (Erba-Lachema). Serotyping was performed by slide agglutination with commercially available O-antisera for the serogroups O:1—2, O:3, O:5, O:8 and O:9 involved in most cases of human yersiniosis in Europe (*Yersinia enterocolitica* Antisera, Denka Seiken, Japan). The colonies that produced typical reaction patterns were further confirmed as *Y. enterocolitica* and biotyped according the revised scheme of W a u t e r s et al. [9] using the following tests: indole production and fermentation of xylose, salicin, trehalose and aesculin hydrolysis.

RESULTS

There was only one case from among all 100 analyzed samples where *Yersinia enterocolitica* was detected using the cultivation method. It was a sample of chilled raw pork meat

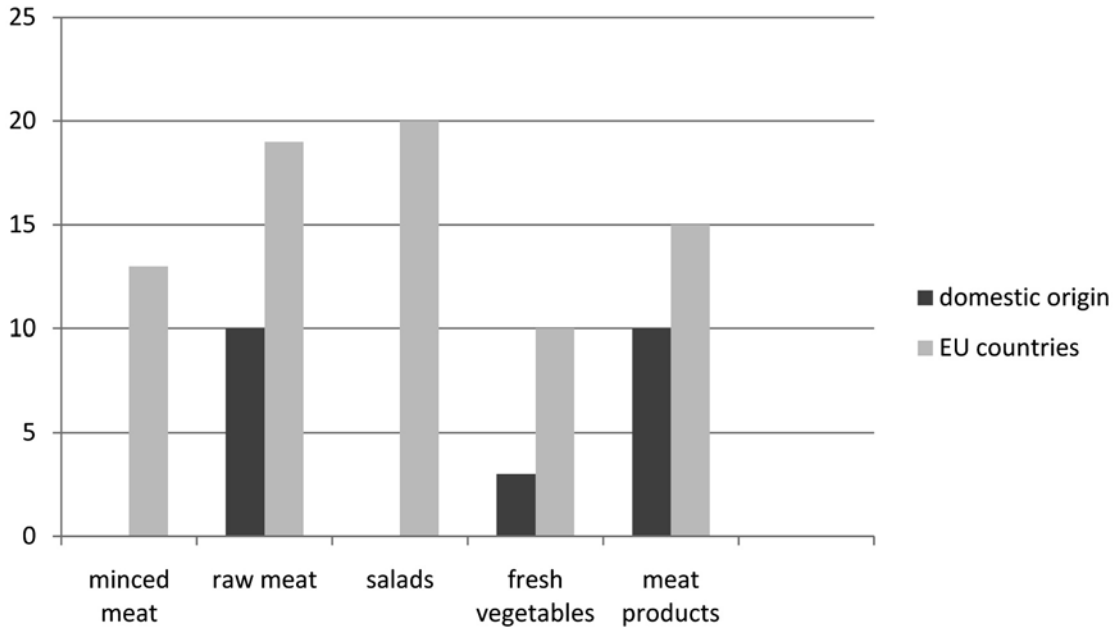

Fig. 1. Comparison of domestic and EU market samples

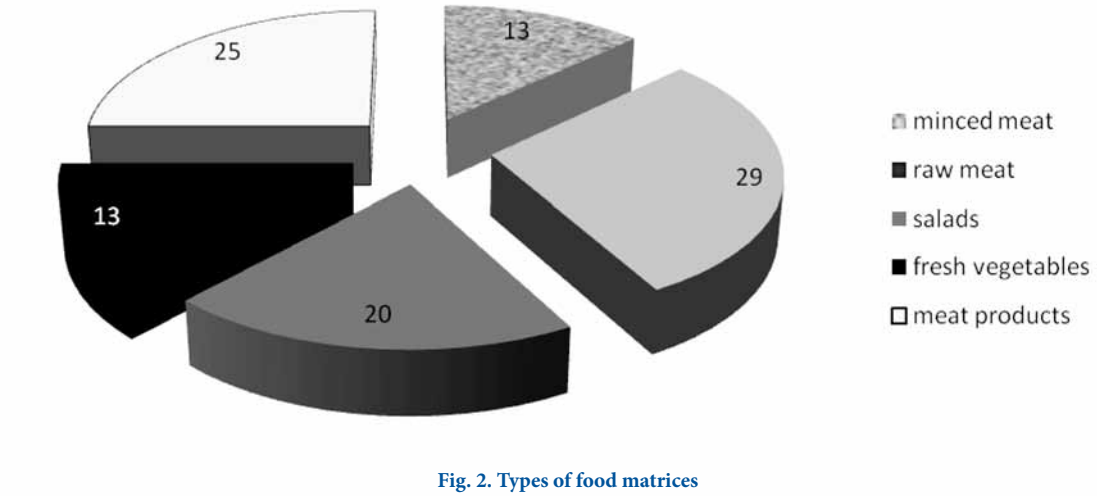

Fig. 2. Types of food matrices

(pork stew). The sample was taken by the Regional Veterinary Food Administration (RVFA) in Bardejov (northern Slovakia) at retail shop LIDL, and the country of origin was Poland.

The second positive sample was another type of chilled meat, pork neck with bone, of domestic origin, sample was taken by RVFA in Banská Bystrica (central Slovakia) at retail shop CBA. There was a positive finding of *Yersinia intermedia*. All other samples were negative for *Yersinia* sp. The types of sampled food are illustrated in Fig. 2.

DISCUSSION

Based on the monitoring programme for detection of *Yersinia enterocolitica* in food there were only 2 cases positive from all 100 analyzed samples. The positive samples were chilled raw pork meat. In other food matrices (vegetables, fermented meat products) *Yersinia* sp. were not detected by the cultivation method.

The scientific literature [5] indicates that the cultivation method is not sensitive enough. In practice it could be seen that the method has high labour demands and the cultivation media for isolation are not specific. Therefore it was necessary to identify many suspicious colonies which finally became others than *Yersinia* sp. Traditional microbiological methods were found to be insufficient for the specific identification of the *Y. enterocolitica* pathogen [8]. In another study [3], prevalence of enteropathogenic *Y. enterocolitica* in individual pigs was significantly lower by the cultivation method (9 %) compared to PCR (35 %). The authors ascribed the difference to low sensitivity of the culture method compared to PCR.

The study by Amasiani et al. [1] showed that *Y. enterocolitica* is present and active in human population and some transmission vehicles in Anambra State, Nigeria. However it may go undetected because of ineffective isolation procedures or because of its similarity to other enterobacteria with respect to biochemical reactions. Efforts should be made to include the isolation of this organism in our routine laboratory practice.

Another scientific study [5] showed that in case of using molecular methods, the prevalence of occurrence is up to 40 %. The Reference Laboratory recommended to analyse the samples also by PCR methods. It is planned, with the agreement of the State Veterinary and Food Administration of SR, that PCR methods will be used in the monitoring performed during the year 2014.

With regard to the biotypes of *Y. Enterocolitica,* the most frequent in Europe is the biotype 1A — non-pathogenic and pathogenic biotype 4. Although it was not possible to identify the isolated strain by the available sera, according the Wauter's scheme it was identified as biotype 1A — non-pathogenic.

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ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM THE ENVIRONMENT DURING PROCESSING OF POULTRY MEAT

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ABSTRACT

The aim of the study was to investigate concentration of airborne and environmental micro-organisms and the antibiotic resistant *Escherichia coli* strains in slaughterhouse environment as a possible source for poultry meat contamination. The examined air samples contained *E. coli* resistant to ampicillin, tetracycline and enrofloxacin but ESBLs were not found. However, we detected ESBLs and *E.coli* strains in swabs from every surface of slaughterhouse with multiresistance to chinolons (nalidixic acid, ciprofloxacin and enrofloxacin), streptomycin, tetracycline and cotrimoxazol. The total count of bacteria was very high in every tested section of the dirty and clean zones of the investigated slaughterhouse (more than 10^5 CFU.m^{-3}). Microbial contamination of poultry meat is affected by rearing conditions, feeding, manipulation before slaughtering, slaughter treatment hygiene, slaughterhouse hygiene and worker's hygiene.

Key words: antibiotic resistance; Escherichia coli; poultry; slaughterhouse

INTRODUCTION

Hygiene level of animal rearing points to rearing sophistication, and significantly affects the animal products. The key role of animal hygiene is to minimize contact of animals with disease agents and other adverse components of excrements.

Microbial contamination of poultry meat surfaces is affected by conditions in animal houses (microclimate parameters, density of animals, ventilation rate, cleaning and disinfection programmes, etc.), feeding, pre-slaughtering manipulation, hygiene during slaughtering and personal hygiene of workers.

Micro-organisms found on slaughtered poultry, originate from two main sources: the environment in the slaughter house (live poultry, equipment, staff) and the digestive tract of the animals [6].

To be able to control infectious diseases in the field of intensive poultry meat production, it is necessary to use some veterinary health supporting products and measures, vaccines, antibiotics and antiparasitics. Increase in bacterial resistance to antibiotics results in outbreaks of diseases with serious consequences, which are the major threat to human and animal health [5].

Although the European Union banned the use of antibiotics for growth promotion, antibiotics are still widely used in poultry production for treatment of sick animals or for prophylactic purposes. Many similar or identical antibiotics are used in both human and veterinary medicine. Tetracyclines are the group of antibiotics most frequently used in veterinary practice, followed downwardly by macrolids, lincosamids, penicillins, sulfonamides, aminoglycosides, fluorochinolons, cephalosporins and phenicols [3].

MATERIAL AND METHODS

Samples of bioaerosols and bacteriological swabs were taken from different sections of poultry slaughterhouse. The slaughter-

house was divided into a clean and dirty zone to minimize contamination of final products and ensure continuous technological processes and material flow. The dirty zone included shackling by feet and the section for poultry carcasses after electrical immobilization. The dead birds were scaled with hot water in a closed tunnel. The clean zone consisted of the evisceration section, water-chilling, cutting, de-boning and packaging sections.

Bioaerosols were collected by means of a sampler MAS-100 Eco onto standard Petri dishes with the following nutrient media: meat-peptone agar; Endo agar; McConkey agar. After incubation at 37 °C during 24 hours, the colony forming units (CFU) grown on the agars were counted and recalculated per 1 m^3 of examined air. By means of surface swabbing we obtained colonies of bacteria (*E. coli*).

For determination of minimal inhibitory concentration (MIC) from *E. coli* isolates we used colorimetric microdilution method according to CLSI guidelines M31-A3 [1]. We tested their sensitivity to ampicillin (AMP), ampicillin and sulbactam (A+IB), ceftazidime (CAZ), ceftazidime with clavulanic acid (CAC), ceftriaxon (CTR), ceftiofur (CFF), cefquinome (CFQ), gentamycin (GEN), streptomycin (STM), neomycin (NEO), nalidixic acid (NAL), enrofloxacin (ENR), chloramphenicol (CMP), florphenicol (FLO), tetracycline (TET) and cotrimoxazol (COT). Phenotype interpretation of mechanisms of β-lactamases (ESBLs) was red according the β-lactams (AMP, A+IB, CTR, CAZ, and CAC) MIC levels. ESBL genes for CTX-M and CMY-2 were determined by PCR.

RESULTS AND DISCUSION

The total count of bacteria was very high in every tested section of the dirty and clean zones of the investigated slaughterhouse (more than 10^5 CFU.m^{-3}). The highest concentration of coliform bacteria in the air was in the shackling room, killing room and during evisceration of poultry (more than 10^4CFU.m^{-3}).

E. coli strains from swabs showed high resistance to ampicillin (89 %), ceftiofur (62 %) and cefquinome (22 %), while proportion of strains resistant to ampicillin with sulbactam was only 6 %. High MIC to ceftazidime (3.6 mg.l^{-1}) and ceftriaxon (5.2 mg.l^{-1}) indicated presence of extended spectrum betalactamases (ESBLs) in 43 % of isolates. Using the PCR method, we phenotypically detected 19 positive ESBL strains. In 16 of them we detected CMY-2 genes but CTX-M genes were not detected.

Poultry may be contaminated by intestinal bacteria during processing During scalding, internal contamination of broiler carcasses can occur through feather follicles. The

Table 1. Total count of bacteria and coliforms in the air in some of the tested sections of poultry slaughterhouse

Place of sampling	Total count of bacteria [CFU.m^{-3}]	Coliform bacteria [CFU.m^{-3}]
Packaging room	7.6×10^3	0.5×10^2—2×10^2
Portioning room	6.8×10^3	0.5×10^2—0.75×10^3
Evisceration room	$>10^5$	0.4×10^3—2.6×10^4
Killing room	$>10^5$	1.0×10^2—2.5×10^4
Shackling room	$>10^5$	1.0×10^2—5.9×10^4

Table 2. Percentage of antibiotic resistant *E.coli* isolates from slaughterhouse environment

Antibiotic	% of resistance	Antibiotic	% of resistance
ampicillin	89 %	enrofloxacin	43 %
ampicillin+sulbactam	6 %	chloramphenicol	10 %
ceftiofur	62 %	florphenicol	18 %
cefquinome	22 %	tetracycline	33 %
gentamycin	14 %	cotrimoxazol	35 %
streptomycin	43 %		

water-chilling system has been often the source of bacterial contamination, because bacteria can be transferred from chicken to chicken through contact with water. The microorganisms can easily spread from one carcass to another during de-feathering and evisceration [2].

A significant risk to human population arises from resistant *E. coli* with production of ESBLs, including cefotaxims (CTX-M), AmpC beta-lactams, carbapenems (KPC) and plasmid chinolon resistance (PMQR) (4). The use of third and fourth generations of cephalosporins in farm animals may result in selection and occurrence of these dangerous resistant phenotypes of bacteria (ESBLs) [3].

Animal samples examined in Slovakia showed high resistance to tetracycline (60—80 % due to long lasting administration of chlortetracycline as a growth promotor), streptomycin (50—60 %), neomycin and spektinomycin (35 %), gentamycin (10—14 % and showing a growing trend), apramycin (2 %) and gentamycin (9 %). Resistance to chloramphenicol was detected in 22 % of samples and to fluorophenicol in about 5 %. Also 50 % resistance to fluorochinolons was observed in broiler chickens and predominance of *E.coli* strains with high enrofloxacin MIC [4].

CONCLUSION

The examined air samples contained *E. coli* resistant to ampicillin, tetracycline and enrofloxacin but ESBLs were not found. However, we detected ESBLs and *E.coli* strains in swabs from every surface of slaughterhouse with multiresistance to chinolons (nalidixic acid, ciprofloxacin and enrofloxacin), streptomycin, tetracycline and cotrimoxazol. Our results indicated prolonged circulation of similar *E.coli* strains in the investigated poultry slaughterhouse. Good health and welfare of poultry and hygiene in poultry houses and sanitation measures in slaughterhouses significantly affect the quality, safety and harmlessness of poultry meat.

ACKNOWLEDGEMENT

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EFFECTS OF DIETARY PROBIOTICS AND LINSEED SUPPLEMENTATION ON PERFORMANCE OF PIGLETS FROM PROBLEMATIC HERD

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ABSTRACT

Piglets (36) infected with *E. coli* and *Coronavirus* were divided into 2 groups. In the period from 10 days before weaning up to 14 days after weaning they were fed as follows: control group (CG) was fed commercial mixed feed OŠ-02, Norm Type, supplemented with cheese (8 g.pig^{-1}.day^{-1}). The experimental group (EG) was provided feed supplemented with probiotics (*L. plantarum* and *L. fermentum*) in cheese (4 g.pig^{-1}.day^{-1}) and 10% linseed. Lactic acid, phosphate and pH in the muscles of piglets were determined on day 1, 7 and 14 after weaning. A significant increase in lactic acid ($P \leq 0.05$) was found in CG on day 7 compared to day 1 of the experiment followed by significant decrease ($P \leq 0.05$) by day 14. In the group supplemented with probiotics and linseed lactic acid was decreased significantly ($P \leq 0.05$) on days 7 and 14. Phosphate level in CG was slightly increased on day 7 and decreased on day 14 while in EG it was decreased significantly on days 7 and 14. Ph levels reflected changes in the levels of phosphates and lactic acid during the experiment.

Key words: *Coronavirus*; *E. coli*; flaxseed; meat; pig; probiotic; quality

INTRODUCTION

Meat quality of pigs is greatly influenced by factors to which the animals are exposed before slaughter, such as a method of nutrition and exposure to disease agents [9]. Weaning often causes changes resulting in higher susceptibility of pigs to infection by enteropathogens. Probiotics have a beneficial effect on the health of the host, selectively stimulate the activity of beneficial bacteria and improve the growth of young [2]. The effectiveness of probiotics can be improved by the polyunsaturated fatty acids, which the organism receives exclusively from food (e. g. linseed). Extruded flaxseed may be used to improve meat quality without adverse effect on swine gut microbiota or animal performance [4]. The positive effect of combination of lactobacilli and polyunsaturated fatty acids was manifested by increased production of lactic acid in the pig intestine [3]. After killing the pigs, anaerobic metabolism of glycogen has a significant impact on the process of conversion of muscle to meat. With regard to meat quality, an important role plays the dynamic of LDH activity during meat ripening [7]. LDH is an enzyme involved in the production of lactic acid from pyruvic acid. Total LDH activity is composed from the activities of five isoenzymes. Isoenzyme 5 is located in skeletal muscles [10]. LDH isoenzymes in tissues are present in different quantities and their occurrence is species specific [11]. The dynamic of the concentration of lactic acid in the meat reflects the quantitative transformation of glycogen into lactic acid *via* intermediates [6]. These components affect the total pH value of meat.

The aim of this study was to determine the effects of dietary supplementation of probiotics and linseed to infected piglets and the related biochemical and physical changes in meat at different times after weaning.

MATERIALS AND METHODS

The experiment was carried out on 36 piglets (Slovak white × Landrace), 28 days old. The piglets were purchased from a commercial farm (Pozdišovce, KOAN, Slovak Republic) with confirmed infection of *E. coli* and coronavirus. The animals were transferred to the experimental unit of the Institute of Microbiology and Gnotobiology, UVMP in Košice, SR. They were randomly allocated to 2 groups: control group CG (n = 18) and experimental group EG (n = 18). The piglets were housed in stainless steel cages (1 300 × 940 × 1 300 mm) with slatted floors (6 piglets in one cage). Temperature of the environment was 20—22 °C. The cages included feeder for bulk mixture, drinker and space for excrements.

Starting from 10 days before weaning up to 14 days after weaning the animals were fed as follows: piglets from control group were fed commercial mixed feed OŠ-02, Norm Type (Spišské Vlachy, SR) with addition of Cheddar cheese at a dose of 8 g.pig^{-1}.day^{-1}. The cheese was offered to piglets once per day on the surface of feed. The experimental piglets were fed the same commercial diet supplemented with extruded linseed *Linum usitatissimum L.,* variety *Flanders* (10 % in rations, with 56.8 % of a α-linolenic acid) as a source of n–3 polyunsaturated fat acids. The Cheddar cheese was administered at dose of 4 g.pig^{-1}.day^{-}1 once per day on the surface of feed. The cheese was enriched with a probiotic strains (*Lactobacillus plantarum – Biocenol*^{TM} LP 96 (CCM 7512) isolated from jejunum and ileum of one-week old piglets and *Lactobacillus fermentum, Biocenol*^{TM} LF 96 (CCM 7514), isolated from digestive tract of chickens. Both strains were provided at concentration of 1 × 10^{9} CFU.g^{-1} of cheese. The pigs had access to feed and water *ad libitum.*

Twelve piglets from both groups were sacrificed on day 1, at the age of28 days, on day 7 and 14 after weaning by intracardial euthanasia with 1 ml.kg^{-1} BW T61^{R} (Intervet International B. V. Boxmeer, The Netherlands). The animals were handled and sacrificed in a humane manner in accordance with the guidelines established by the relevant UVMP commission. Samples of thigh muscle (*m. semimembranosus*) were taken from all carcasses and stored until analysis (24 h) in a refrigerator at 4 °C.

The concentrations of lactic acid and phosphates were determined on days 1, 7 and 14 of the experiment, in a water extract of meat using an Electrophoretic analyser, type EA 102 (Villa Labeco, SR) with a conductive detector [5]. The separation analytical system in the analyzer consisted of a leading electrolyte 10 mM HCL, β-alanine and 0.1 % mHEC, pH 3.2. The terminating electrolyte consisted of 5 mM caproic acid and 5 mM TRIS. The direct currents used in pre–separation and analytical columns were 250 μA and 50 μA. The results of analysis were evaluated by the software ITP-Pro 32. The concentrations of lactic acid and phosphates were expressed in g.100g^{-1} of meat.

The pH values were determined in a water extract of pig muscles by a pH meter (pH 720, WTW, Weilheim, Germany) with a glass electrode. The electrophoretic analysis is an appropriate method for determination of lactic acid, phosphoric acid and phosphates in meat which are important indicators of quality [1].

The results were processed by Student *t*-test (Microsoft Office Excel 7.0), setting significance levels at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001. All data were presented as mean values with the standard deviations (mean ± SD)

RESULTS AND DISCUSSION

Our results showed a positive effect of probiotic and linseed in the experimental group immediately on day 1 after weaning, when the highest mean levels of lactic acid and phosphates were recorded. Significant differences (P ≤

Table 1. Lactic acid, phosphates and pH in thigh muscles of experimental piglets

Parameters	Animal groups	Day 1	Day 7	Day 14
Lactic acid	CG	1.210 ± 0.255	1.546 ± 0.163^{+}	1.259 ± 0.173^{+}
	EG	1.830 ± 0.393*	1.280 ± 0.184*$^{+}$	1.349 ± 0.125^{+}
Phosphates	CG	0.366 ± 0.060	0.407 ± 0.073	0.335 ±0.084
	EG	1.012 ± 0.087*	0.544 ± 0.042**$^{+++}$	0.441 ± 0.026*$^{+++}$
pH	CG	5.490 ± 0.120	5.439 ± 0.075	5.598 ± 0.105^{+}
	EG	5.660 ± 0.094*	5.592 ± 0.076**$^{+}$	5.623 ± 0.024

Significant differences between CG and EG: * — P ≤ 0.05; ** — P ≤ 0.01;*** P ≤ 0.001
Significant differences between experimental days: $^{+}$ — P ≤ 0.05; $^{++}$ — P ≤ 0.01; $^{+++}$ — P ≤ 0.001

0.05) in levels of lactic acid between control and experimental group were observed on day 1 and 7 of weaning. These changes were minimal on day 14, due to decreased levels of lactic acid in both groups. Significantly higher levels of phosphates were recorded on day 1 ($P \leq 0.05$), day 7 ($P \leq 0.01$) and day 14 ($P \leq 0.05$) after weaning of piglets in EG in comparison with CG (Table 1).

Assessment of dynamic of lactic acid concentrations in control group showed a significant increase ($P \leq 0.05$) in levels of this acid in meat on day 7 of the experiment compared with day 1 and significant decrease ($P \leq 0.05$) on day 14 of the experiment. On the other hand, we observed a significant decrease in lactic acids ($P \leq 0.05$) on day 7 as well as on day 14 of the experiment in comparison with day 1 in the experimental group.

The results of lactic acid obtained in our study showed the same course of total activity of LDH in CG and EG as well as of the mutual ratio of the activity of total LDH in individual days [8]. This fact confirms the relevance and reciprocal relation of obtained results of the total activity of LDH in blood and concentrations of lactic acid in the meat of experimental pigs.

Similar course of changes such as those of lactic acid levels were recorded for phosphates. The control group showed increased levels of phosphates on day 7 compared to day 1 of the experiment. On the other hand, on day 14 there was a decrease in phosphate levels. In EG we recorded a significant decrease in phosphates on day 7 as well as on day 14 of the experiment ($P \leq 0.001$).

The pH value of meat is not influenced only by lactic acid [12]. The pH of pork meat reflected the course of changes in lactic acid and phosphates in all experimental intervals. The lowest pH values were measured on day 7 in control group.

CONCLUSIONS

Our study investigated the effect of supplementation of a symbiotic combination of probiotics (*L. plantarum* and *L. fermentum*) in cheese, as a food safe product with acceptable taste and with the stabilisation of the active substance, and of linseed as a source of polyunsaturated fatty acids for pigs. The effectiveness of these supplements in weaned piglets from an infected animal herd (*E. coli* and *Coronavirus*) was reflected in the improved nutritional quality of pork and different dynamics of biochemical parameters in meat.

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QUALITY OF SAUSAGES "SPIŠSKÉ PÁRKY" ON SLOVAK AND CZECH MARKETS

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ABSTRACT

Sausages "Spišské párky" sausages are a meat product which is considered a guaranteed traditional speciality. Their basic ingredients, composition and chemical and organoleptic characteristics are clearly defined. In our experiment we examined the quality of sausages "Spišske párky" put on the market by Slovak and Czech producers. None of the evaluated sausages met the quality referred to in the application for registration of traditional guaranteed specialities registered under EC number SK-TSC-0007-0051-18.01.2007. In four products we detected ingredients not permitted in this type of product. The legislation defines that the fat content can reach maximally 24 ± 4 %, but in two products higher values were found. Also, in two products pure muscle protein content was lower than 10 %, and organoleptic properties of one product were unsatisfactory.

Key words: counterfeit food; guaranteed traditional specialties; histological analysis; starch

INTRODUCTION

The production of sausages "Spišské párky" goes back to more than one century in Slovakia. It was first recorded when a local butcher in Spišské Podhradie, Štefan Varsányi, took advantage of the frequent visits of the Hungarian nobility to the grand fairs held by Spiš Castle. He began selling "Spišské párky" at those fairs as a special attraction. His recipe, based on a delicate mix of seasonings with sweet and hot paprika, was clearly very successful, for after a while he was selling them in Hungary and Poland as well [8].

The European Union, by the adoption of the Council Regulation (EC) 509/2006, has decided to support the diversification of agricultural production by promoting products with specific characteristics. In 2011, "Spišské párky" were registered as the traditional specialty guaranteed (TSG) in the Czech and Slovak Republics. Their material compositions as well as physical, chemical and organoleptic characteristics are clearly defined. If the manufacturer wants to produce "Spišské párky", he has to comply with the prescribed method of production and the materials used, as stated in the application for registration of the TSG [8]. The producer shall be guilty of misleading the consumer by not adhering to these regulations, and such practices may be sanctioned by the relevant authorities of the Slovak Republic.

The aim of our study was to verify whether the producers add to the products other than the permitted materials. The aim was also to verify the basic chemical and organoleptic characteristics of the product "Spišské párky", offered on the market of the Slovak and the Czech Republics.

MATERIALS AND METHODS

Samples of sausages "Spišské párky" were purchased on the market in the Czech Republic and Slovakia. Overall, products of five manufacturers of "Spišské párky" were examined; 3 producers

Table 1. Results of histological examination

Country of origin	Producer	Detected component				
		Allowed component	Not allowed components			
		Paprika	Starch	Bone fragments	Soy protein	Wheat protein
CZ	A	+	+	+	–	–
	B	+	+	+	–	–
	C	+	–	+	–	–
SR	D	+	–	–	–	–
	E	+	–	+	–	–

Table 2. The results of chemical analysis

Producer	Dry matter [%]	Fat [%]	Total protein [%]	Collagen [%])	Net muscle protein [%]	Salt [%]	Starch [+/–]
A	34.08 ± 0.11	15.69 ± 1.28	12.14 ± 0.27	2.14 ± 0.08	10.0 ± 0.19	2.18 ± 0.05	+
B	38.73 ± 0.17	21.82 ± 0.12	13.98 ± 0.54	3.78 ± 0.07	10.2 ± 0.47	2.5 ± 0.10	–
C	47.78 ± 0.09	32.48 ± 0.12	12.50 ± 0.18	4.05 ± 0.11	8.45 ± 0.14	2.26 ± 0.15	–
D	43.50 ± 0.17	26.27 ± 0.96	14.14 ± 0.29	2.66 ± 0.07	11.48 ± 0.22	2.15 ± 0.17	–
E	43.12 ± 0.59	28.82 ± 0.67	11.65 ± 0.46	3.29 ± 0.11	8.36 ± 0.35	2.09 ± 0.22	–

+/– — presence/absence

Table 3. Results of sensory evaluation

Producer	Odour	Taste	Consistency	Juiciness	Appearance in cross-section	Σ
A	4.00 ± 1.40	3.25 ± 0.96	3.25 ± 0.50	4.00 ± 1.15	3.75 ± 0.96	18.25 ± 2.10
B	3.50 ± 0.58	3.50 ± 1.00	3.25 ± 0.96	4.00 ± 0.82	4.25 ± 0.96	18.5 ± 2.51
C	3.50 ± 0.50	4.25 ± 0.96	4.00 ± 0.82	4.25 ± 0.96	3.25 ± 0.96	18.75 ± 2.21
D	2.75 ± 0.95	1.25 ± 0.50	2.50 ± 0.60	2.75 ± 0.96	3.75 ± 0.96	12.25 ± 2.63
E	4.00 ± 0.82	4.25 ± 0.96	4.25 ± 0.50	4.25 ± 0.96	3.75 ± 0.96	20.50 ± 3.10

from the Czech Republic (A, B, C) and 2 producers from Slovakia (D and E).

Determination of the proportion of dry matter, fat, total protein, collagen, net muscle protein as well as percentage of salt and presence of starch by the Lugol's solution in products was carried out according to the methods of Popelka et al. [4].

For histological examination, the samples were processed by the paraffin sections technique. One consumer packaging of each producer was examined (4 subsamples from a package; 3 slices for each staining). The samples were sliced by a rotary microtome RM 2255 (Leica, GER) to the slice thickness of 4 µm. Analysis of the histological slides was performed by an Eclipse E220 microscope (Nikon, JPN) at 100—400× magnification. Lugol-Calleja staining was used to prove starch and Alizarin Red was used for the detection of bone

fragments. Manual staining procedures were used [2]. Vegetable proteins were determined by immunohistochemical method. Soy protein was determined according to the procedure of Pospiech et al. [5], wheat protein according to ŘezáčováLukášková et al. [7].

Sensory evaluation was performed 24 hours after the purchase by an expert panel. The samples were evaluated after cooking. Smell, taste, juiciness, texture and appearance in cross-section were evaluated [6]. The samples were classified using a 5 points ranking system and the maximum possible number of allocated points was 25.

RESULTS AND DISCUSSION

"Spišské párky" are included among TSG in the Czech Republic and Slovakia (3). This product is registered under the number ES: SK-TSG-0007-0051-18.01.2007. Based on this registration, materials that can be used for production of "Spišské párky" are specified. They include fresh beef with fat content up to 10 %, fresh pork with a fat content up to 10 %, fresh pork with fat content up to 50 %, pork rind, potable water, nitrite salting mix, ground sweet and hot paprika, polyphosphates E 450 and E 451, ascorbic acid and sheep intestines as a casing [8]. The use of some other than the is against relevant legislation.

The results of histological examination confirmed the use of paprika in products by all producers. Frequently used additives which are not authorized for "Spišské párky" were also assessed (Table 1). Addition of starch was found in sausages from producers A and B. The method used for detection of starch is based on reaction of iodine anion I_5^- with amylase, which gives a blue iodine complex (1). For starch, the colour of the complex after heat treatment is brown, as demonstrated in positive samples.

The mechanically separated meat was another looked for material which is not allowed for production of "Spišské párky". This material was detected in products of producers A, B, C and E. The use of plant proteins by any of the producers was not confirmed.

Results of chemical analysis of sausages "Spišské párky" are shown in Table 2. Significant differences in fat content between the analyzed samples were observed. Legislation defines maximum fat content of 24 ± 4 %. Higher content of fat was found in 2 products (C, E) and in 1 it was at the upper limit (H). The legislation also defines the content of net muscle protein, which should not decrease below 10 %. The proportion of net muscle protein was lower in 2 analyzed samples (C, E). In one examined product the presence of starch was revealed (A), which is a not-allowed ingredient. The results of chemical analysis indicated use of lower quality meat in the products by several producers.

The results of sensory evaluation of the analyzed sausages are shown in Table 3. The odour and taste of "Spišské párky" should be pleasant after freshly-smoked product, slightly hot, appropriately salty, succulent to the bite when heated up, brittle and of cohesive consistency [8]. Our results allowed us to conclude that despite legislatively clearly defined material composition, technological process of production and organoleptic characteristics of "Spišské párky", there are significant differences in the organoleptic quality of this product offered in stores. The "Spišské párky" made by producer D did not meet the requirements on the meat product and a their taste evoked abortively processed cheese (milk protein).

CONCLUSION

The obtained results showed that none of the evaluated "Spišské párky" met legislative requirements. Histological analysis revealed presence of not allowed ingredients and analysis of chemical composition and organoleptic properties confirmed the use of low quality materials. Therefore this type of product should be subjected thorough inspection because products labelled and sold as "traditional specialty guaranteed" should be of high quality. If the inspection is inadequate, indication TSG will lose its relevance. Customers will not obtain quality products and the only parties for whom the TSG labelling will be relevant will be producers and distributors, whose products labelled as TSG will sell for a much higher price than other kinds of sausages.

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MAGNET-INFRARED-LASER RADIATION AND ITS IMPACT ON BEEF QUALITY

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ABSTRACT

This study investigated the impact of quantum therapy on meat quality of slaughtered dairy cows. For this purpose the dairy cows were treated with different doses of magnet-infrared-laser (MIL) radiation. Animals were divided into four groups according to radiation doses (4096, 512, and 64 Hz, and control without application), which were applied in the lumbar area of *m. longissimus dorsi lumbalis* (short loin) at various time intervals prior to slaughter (14 d, 24 h, and 1 h). The animals were slaughtered and the meat quality was evaluated by determining of pH value (1, 3, and 24 h post slaughter), drip loss, colour, and lactic acid and phosphoric acid amounts. The results obtained showed that exposure to MIL radiation 24 hours and 1 hour before slaughter caused a change in meat quality, as reflected by the non-standard development of pH values, changes in meat colour and levels of lactic acid and phosphoric acid.

Key words: colour; drip loss; lactic acid; meat quality; pH; quantum physics

INTRODUCTION

Quantum therapy in veterinary medicine is a new direction, which began to develop in the nineties of the twentieth century together with the production of a portable semiconductor laser apparatus. A wide range of therapeutic effect is explained by biological activating of the tissues after laser radiation exposure of animals. Unlike medication therapy, laser radiation is considered a special effect and the animal body responds by increase in non-specific immunity [5]. Recently, new physical methods in the therapy of animals were discovered. They are ecological, safe, without by-products or metabolites (acupuncture, high frequency therapy, low intensive laser therapy). According to the results of the scientific studies of many authors, the laser therapy appears to be the most economical, simple, and effective method of treatment of animals [6].

The phenomenon of laser biostimulation (LBS) has been extensively used in medical practice, although its nature and mechanisms are far from being elucidated and understood [12]. Major properties of LBS and results of studies of laser radiation interaction with biologic objects may be summed as follows: selectivity of laser treatment; changes are induced in "ill" biosystems without effect on "healthy cells"; treatment effects are different *in vivo* and *in vitro*; LBS effects are seen in non-cellular objects like plant pollen; the stimulation effect has not been found during irradiation with white light; therapeutic effects of LBS are practically identical for laser radiation with any wavelength in the range of 0.4 to 1.5 µm; protein molecules do not have *in vitro* absorption bands for radiation in the wavelength range of 0.4 to 1.5 µm; LBS effects are seen during the use of 1.5 mW.cm^2 or lower intensities and of small energy doses calculated even without account for reflected light or light that has travelled through a phase object. LBS effects reported in medical practice include a decrease in blood viscosity, stimulation of microcirculation, pain relief, enhancement of motility of cell receptors and some cells (e.g. sperm cells), stimulation of the immune and nervous systems.

Due to the fact that laser devices are used for therapeutic, prophylactic and regenerative purposes in animals which are intended for slaughter, the aim of the study was to evaluate the MIL radiation impact on the quality of beef.

MATERIAL AND METHODS

The RIKTA device (type 2004/4-MV, Milt PKP, Russia) was used in the experiments. Magnet-infrared-laser (MIL) therapeutic device RIKTA combines pulse laser radiation (0.89 mm), continuous monochromatic infrared radiation (0.85 to 0.89 mm), red LED light (0.65 mm) and static magnetic field with induction of 35 mT. The RIKTA device operates in pulse mode (5, 50 and 1000 Hz of pulse frequency) with an output power not less than 8 W.

MIL radiation (the device RIKTA 04/4-MV) was applied to retired dairy cows (n = 36) with an average weight of 420 kg. The application was performed with terminal device with 100 % power (20 W) at the lumbar region of the *m. longissimus dorsi* (short loin) on both halves, and using different doses as follows:

I. 14 days before slaughter (n = 12)
- 4096 Hz, for 10 minutes (n = 3);
- 512 Hz, for 10 minutes (n = 3);
- 64 Hz, for 10 minutes (n = 3);
- without applying MIL radiation (control) (n = 3).

II. 24 hours before slaughter (n = 12)
- 4096 Hz, for 10 minutes (n = 3);
- 512 Hz, for 10 minutes (n = 3);
- 64 Hz, for 10 minutes (n = 3);
- without applying MIL radiation (control).

III. 1 hour prior to slaughter (n = 12)
- 4096 Hz, for 10 minutes (n = 3);
- 512 Hz, for 10 minutes (n = 3);
- 64 Hz, for 10 minutes (n = 3);
- without applying MIL radiation (control) (n = 3).

The dairy cows were slaughtered in a slaughterhouse Zemplínska Teplica. The carcasses were chilled at 2 °C and air velocity 1—2 $m.s^{-1}$. pH values were measured directly in the carcass. Muscle samples were taken from the region of the *m. longissimus dorsi* one hour after slaughtering and were analysed for drip loss, colour, lactic acid and phosphoric acid. Meat quality was evaluated according to the above parameters.

The pH of the meat was measured in the carcasses (*m. longissimus dorsi*, after last rib) by a digital pH meter with glass electrode (AMA digit, model AD 140, Amarell Electronic, Germany) after slaughtering of animals at 1 hour, 3 hours and 24 hours after slaughter.

The drip loss was determined by a bag method according to Honikel [2], as a drip loss 24 hours after slaughter (stored at +4 °C).

Spectrophotometric determination of meat colour, light transmission (percentage of remission — % R), was determined by a spectrophotometer (at 520 nm) equipped with an adapter (Spekol 11, Carl Zeiss Jena, Germany).

The measurement of lactic and phosphoric acid was performed in meat samples taken 1 hour after slaughtering, which were processed immediately (homogenization in the water) and then analysed by an electrophoretic analyzer, type EA 102 with a conductive detector (Villa Labeco, Spišská Nová Ves, Slovak Republic) [7]. The results of analysis were evaluated using a software ITPP pro 32.

The mean values and standard deviations were calculated using column statistics with processing of six values for each analyzed group. Statistically significant differences between groups for each quality parameter were calculated using one-way ANOVA by Tukey comparative test using software GraphPad Prism 5 (2007). The differences were evaluated as significant at $P < 0.05$.

RESULTS

Application of MIL radiation 14 days before slaughter

After application of MIL radiation 14 days before slaughter the pH values were measured at 1, 3 and 24 hours after slaughter (Table 1). Statistically significant differences between experimental groups and control group were not found. After exposure to MIL radiation 14 days before slaughter we measured also drip loss and colour of meat (Table 2).

Statistically significant differences were found in drip losses between control group and 64 Hz group ($P < 0.05$). Comparison of levels of lactic acid and phosphoric acid in experimental groups and control group (Table 3) showed no significant differences.

Application of MIL radiation 24 hours before slaughter

After exposure to MIL radiation 24 hours before slaughter, pH in meat was again measured at 1, 3 and 24 hours after slaughter (Table 4). Significant differences were found in the value of pH_1 between 4096 Hz and a control group ($P < 0.05$), with higher mean pH values in control compared to irradiated group, and in pH_3 between groups with higher exposure (4096 Hz and 512 Hz) and control and 64 Hz groups ($P < 0.05$).

Comparison of values of drip loss and colour of meat in experimental and control groups (Table 5) showed no significant differences.

By comparing the levels of lactic acid and phosphoric acid in experimental and control groups (Table 6) we found significant differences in lactic acid levels as concentrations in 4096 Hz 512 Hz and control groups were significantly lower than in 64 Hz group ($P < 0.05$).

Application of MIL radiation 1 hour before slaughter

After MIL irradiation 1 hour prior slaughter the pH values were measured at 1, 3 and 24 hours after slaughter (Table 7). Significant differences were found between the pH in pH_1 irradiated groups (4096 Hz, 512 Hz, and 64 Hz) and higher pH in control group ($P < 0.05$).

No significant differences between drip loss and colour of meat in experimental groups and control group (Table 8) were found.

Comparison of concentrations of lactic acid and phosphoric acid in experimental l groups and control group

Table 1. Mean values and standard deviations of pH

Exposure to MIL radiation	pH_1	pH_3	pH_{24}
4096 Hz, 10 min	6.28 ± 0.157	6.35 ± 0.221	5.88 ± 0.074
512 Hz, 10 min	6.21 ± 0.139	6.28 ± 0.157	5.84 ± 0.075
64 Hz, 10 min	6.28 ± 0.133	6.24 ± 0.043	5.85 ± 0.079
Control	**6.27 ± 0.099**	**6.20 ± 0.055**	**5.81 ± 0.026**

Table 2. Mean values and standard deviations of drip loss and colour of meat

Exposure to MIL radiation	Drip loss [%]	Colour [% remission]
4096 Hz, 10 min	0.407 ± 0.194	5.295 ± 0.484
512 Hz, 10 min	0.499 ± 0.137	5.621 ± 0.458
64 Hz, 10 min	0.286[a] ± 0.162	5.323 ± 0.442
Control	**0.658[b] ± 0.272**	**5.360 ± 0.331**

Table 3. Mean values and standard deviations of lactic and phosphoric acid levels

Exposure to MIL radiation	Lactic acid [g.100 g^{-1}]	Phosphoric acid [g.100 g^{-1}]
4096 Hz, 10 min	1.424 ± 0.117	0.342 ± 0.036
512 Hz, 10 min	1.346 ± 0.212	0.379 ± 0.037
64 Hz, 10 min	1.421 ± 0.319	0.393 ± 0.071
Control	**1.478 ± 0.423**	**0.437 ± 0.095**

Table 4. Mean values and standard deviations of pH

Exposure to MIL radiation	pH_1	pH_3	pH_{24}
4096 Hz, 10 min	6.69[a] ± 0.245	6.80[a] ± 0.197	6.06 ± 0.124
512 Hz, 10 min	6.92 ± 0.174	6.79[a] ± 0.078	6.12 ± 0.109
64 Hz, 10 min	6.89 ± 0.269	7.12[b] ± 0.081	6.01 ± 0.093
Control	**7.18[b] ± 0.127**	**7.11[b] ± 0.134**	**6.06 ± 0.033**

Table 5. Mean values and standard deviations of drip loss and colour of meat

Exposure to MIL radiation	Drip loss [%]	Colour [% remission]
4096 Hz, 10 min	0.787 ± 0.379	8.193 ± 3.239
512 Hz, 10 min	1.010 ± 0.647	7.386 ± 2.769
64 Hz, 10 min	2.615 ± 1.799	7.824 ± 2.871
Control	**1.928 ± 1.354**	**9.636 ± 4.667**

Table 6. Mean values and standard deviations of lactic and phosphoric acid levels

Exposure to MIL radiation	Lactic acid [g.100 g^{-1}]	Phosphoric acid [g.100 g^{-1}]
4096 Hz, 10 min	0.563[a] ± 0.108	0.383 ± 0.018
512 Hz, 10 min	0.592[a] ± 0.088	0.352 ± 0.006
64 Hz, 10 min	0.785[b] ± 0.040	0.346 ± 0.001
Control	**0.498[a] ± 0.102**	**0.362 ± 0.043**

Table 7. The mean values and standard deviations of pH values

Exposure to MIL radiation	pH_1	pH_3	pH_{24}
4096 Hz, 10 min	6.31[a] ± 0.128	6.96 ± 0.130	5.78 ± 0.081
512 Hz, 10 min	6.58[a] ± 0.287	6.87 ± 0.224	5.78 ± 0.052
64 Hz, 10 min	6.63[a] ± 0.248	6.80 ± 0.310	5.74 ± 0.092
Control	**7.03[b] ± 0.130**	**6.78 ± 0.128**	**5.77 ± 0.073**

Table 8. Mean values and standard deviations of drip loss and colour of meat

Exposure to MIL radiation	Drip loss [%]	Colour [% remission]
4096 Hz, 10 min	0.910 ± 0.631	8.794 ± 1.906
512 Hz, 10 min	0.635 ± 0.410	6.578 ± 1.584
64 Hz, 10 min	1.255 ± 0.483	8.736 ± 4.380
Control	**1.128 ± 0.473**	**7.905 ± 1.472**

Table 9. Mean values and standard deviations of lactic and phosphoric acid levels

Exposure to MIL radiation	Lactic acid [g.100 g^{-1}]	Phosphoric acid [g.100 g^{-1}]
4096 Hz, 10 min	1.456 ± 0.183	0.605^{a} ± 0.092
512 Hz, 10 min	1.520 ± 0.170	0.486 ± 0.023
64 Hz, 10 min	1.214 ± 0.209	0.550 ± 0.097
Control	**1.226 ± 0.200**	**0.477^{b} ± 0.062**

(Table 9) showed no significant differences in values of phosphoric acid. The concentrations in 4096 and control group were significantly higher than in 512 Hz and 64 groups ($P < 0.05$). The differences between 64 Hz and control group were significant ($P < 0.05$).

DISCUSSION

Medical devices based on semiconductor lasers found wide application in modern medical practice [4]. The effect of different doses of MIL radiation on meat quality (pH, drip loss, meat colour, lactic acid and phosphoric acid content) was also evaluated.

Extreme progress in pH during the conversion of muscle to meat has long been known to influence the colour characteristics of pork, and has in fact provided the basis for two of the most well-known inferior meat quality grades, namely dark-firm-dry (DFD) and pale-soft- exudative (PSE) meat [8].

Application of MIL radiation affected mainly pH_{24} in the meat samples of the animals treated 24 hours before slaughter. The highest mean pH_{24} values were measured in the meat samples after application of radiation doses 24 hours before slaughter, mainly in the meat samples after application of radiation doses MIL 512 Hz (6.12). The normal pH_1 value is greater than 5.80 and lower than 6.20 [3]. The PSE meat is characterized by pH_1 value lower than 5.8 and DFD meat is characterized by pH_{24} value greater than 6.20 [3].

Drip loss is of high importance in pig meat production due to its financial implications. The meat processing industry is particularly affected because a low water holding capacity limits the yield in further processing. In general, meat with a high drip loss has an unattractive appearance and therefore has a low consumer acceptance, which leads to loss of sales [9]. Water is lost after the slaughter as a drip during carcase chilling or during the cold meat display [1]. The pH value is known to be negatively related to drip loss but the magnitude of correlation differs between studies [9].

Exposure to MIL radiation had a negative impact also on drip losses. Drip losses were affected by radiation dose and the time interval between application and slaughter of cattle. Higher mean values of drip losses were detected mainly in the groups exposed to higher radiation doses (4096 and 512 Hz) administered 24 hours and 1 hour prior to slaughter.

Meat with high pH value has a high water-binding capacity associated with a higher absorption of light, so the meat remains translucent (appears darker). Contrariwise, very rapid lactate production with a high temperature affects the value of protein denaturation, increases the light scattering in the muscle and causes brightness of PSE meat [3]. Individual animal species have different chemical composition of tissues of the carcass, including of the haem pigments in the meat. The meat colour affected by pre-slaughter handling and stunning is related to PSE and DFD changes [11]. Meat colour (reported as percentage of remission) was affected by application of MIL radiation mainly in dairy cows which were treated 14 days prior to slaughter. Muscle samples had lower percentage of remission in comparison with control group.

Conversion of glycogen to lactic acid takes place through changes in a phosphorus-containing compound (ATP); its quantity is reduced after slaughter. On the other hand, the level of phosphoric acid increases from 0.07 to 0.15 % together with releasing of energy macro energetic bonds and their conversion into thermal energy. The amount of glycogen in muscle tissue depends on the species and muscle type. This is a complicated biochemical process undertaken by many glycolytic enzymes [10]. The highest mean values of lactic acid were measured in all samples of cattle slaughtered 14 days after MIL application and the lowest 1 hour after application. Phosphoric acid values were affected (the highest values) mainly in the 4096 Hz group administered 1 hour prior to slaughter.

CONCLUSIONS

According to the available literature sources and practical experiences, MIL therapy can be used in various fields of veterinary medicine (surgery and orthopaedics, internal medicine, dentistry, pulmonology, gastroenterology, gynaecology, urology, nephrology and dermatology). The results obtained confirm that the method of therapy used before slaughter may cause changes in meat quality reflected by the development of non-standard pH values, increased water loss and changed meat colour.

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PHYSICO-CHEMICAL CHARACTERISTICS OF TRADITIONAL AUSTRIAN COOKED-CURED MEAT PRODUCTS AND THEIR SIGNIFICANCE FOR MULTIPLICATION OF *LISTERIA MONOCYTOGENES*

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ABSTRACT

Blutwurst and Presswurst obtained from retailers in Vienna were examined for proximate composition, pH and a_w, with a view to assess the potential to support the growth of contaminant *L. monocytogenes*. Growth potential was assessed by comparing pH and a_w to limits specified in Reg. EC 2073/2005 and by predictive modelling software. The two product groups differed significantly as regards pH, moisture and fat. Within the two products groups, there was a large variation in proximate composition. With exception of a single (low-pH) Presswurst product, all samples would be classified to support growth of contaminant *L. monocytogenes*.

Key words: Austria; Blutwurst; Growth potential; *L. monocytogenes*; Presswurst

INTRODUCTION

Presswurst (Jellied pork) and Blutwurst (Blood sausage) are traditional Austrian meat products. The idea of producing such products intended for consumption within individual households is to make use of by-products from slaughter, such as head meat, tongue, internal organs and blood. These products contain cured cooked comminuted meat with gelatin/aspic or blood as binders. Nowadays, the products are usually produced by local small-scale enterprises and popularly consumed among elderly people in Austria. Despite their long history, the recipes are not well standardized as is the case for many other meat products. The standard of identity for the products in Austria laid down in the Food Codex (4) requires only the minimum amount of meat or fat particles visible on the cut surface. Standards for the proximate composition do currently not exist. Although, the products are cooked, contamination during portioning, packaging and other handling may occur. Consumption of Presswurst and Blutwurst has been reported as cause of human listeriosis (5, 7, 9), therefore, such products may be a vehicle for transmission of *L. monocytogenes*.

Thus, this study was conducted to:

1) identify physical and chemical variation of the products available in Vienna, Austria;

2) identify whether the products are likely to favour growth of *L. monocytogenes* by using the predictive microbiology software ComBase.

MATERIALS AND METHODS

Presswurst (n = 15) and Blutwurst (n = 15) obtained from 15 different producers supplying to the Viennese market were collected and analyzed for proximate composition. Samples were homogenised in a blender to minimise the heterogeneity of the product. Analyses were done in duplicate for physico-chemical parameters including pH (penetrating electrode Schott blue line on a CG820 pH-meter, Schott, Germany), water activity (a_w; LabSwift-aw, Novasina AG,

Lachen, Switzerland), NaCl (Volhard method), moisture, fat, protein and ash [1].

The mean, standard deviation and coefficient of variance of proximate composition were calculated. Student t-test was used to test differences of proximal composition between products. Statistical significance was established at $P = 0.01$ and $P = 0.05$.

ComBase, a predictive microbiology software package for prediction of bacterial growth from controlling factors, such as a_W, pH, temperature, atmosphere composition and food additives, was used to predict *L. monocytogenes* growth. The condition profile used was as follows: *L. monocytogenes* with 0 % CO_2, static temperature, 216 hrs (9 days), initial level = 2 log CFU.g^{-1} and physiological state equal to 1 for fully adapted bacteria. The extreme and average values of pH and a_W of the examined Presswurst and Blutwurst samples were used in the prediction.

RESULTS

Proximate composition

Proximate composition of Presswurst and Blutwurst are shown in Table 1. Chemical analyses revealed that pH, moisture and fat content are significantly different between the two product groups ($P < 0.01$). Presswurst in general has higher moisture content and low fat and pH than Blutwurst. Salt contents which generally provide a protective barrier from microbial growth for meat products are considerably low for both Presswurst and Blutwurst. Notably, there is no significant correlation between a_W and NaCl for both types of products ($P > 0.05$).

Table 1. Proximate composition analyses of Presswurst and Blutwurst (n = 30)

	Presswurst (n = 15)			Blutwurst (n = 15)		
	Mean ± SD	Max-Min	CV	Mean ± SD	Max-Min	CV
pH	5.74 ± 0.45[a]	6.27—4.55	7.85	6.62 ± 0.31[a]	7.09—5.71	4.68
a_W	0.968 ± 0.004	0.974—0.962	0.380	0.965 ± 0.004	0.971—0.959	0.392
Moisture [g.100 g^{-1}]	72.90 ± 3.40[b]	77.71—67.64	4.67	56.89 ± 3.02[b]	62.92—52.15	5.31
Fat [g.100 g^{-1}]	8.64 ± 3.45[c]	15.01—3.47	39.87	20.21 ± 5.22[c]	30.57—13.02	25.85
Ash [g.100 g^{-1}]	2.54 ± 0.50	3.57—1.66	19.76	2.34 ± 0.32	2.72—1.66	13.60
Protein [g.100 g^{-1}]	15.37 ± 1.58	19.49—13.79	10.31	15.32 ± 1.25	17.38—12.51	34.11
NaCl [g.100 g^{-1}]	1.73 ± 0.36	2.46—1.22	20.93	1.51±0.23	1.87—1.11	15.36

CV — coefficient of variance; means with the same uppercase letter differ significantly ($P < 0.01$)

Table 2. Growth potential of *L. monocytogenes* calculated by ComBase over 9 days using extreme and mean value of pH and a_W obtained from Presswurst and Blutwurst samples

Product type	pH	a_W	Storage temperature		
			2 °C	4 °C	8 °C
Presswurst	4.55 (min)	0.974 (max)	0.44	0.66*	1.31*
		0.962 (min)	0.22	0.44	0.88*
	6.27 (max)	0.974 (max)	1.53*	2.41*	5.36*
		0.962 (min)	1.1*	1.75*	4.15*
	5.74 (mean)	0.968 (mean)	1.1*	1.75*	3.72*
Blutwurst	5.71 (min)	0.971 (max)	1.1*	1.75*	3.93*
		0.959 (min)	0.88*	1.31*	2.85*
	7.09 (max)	0.971 (max)	1.53*	2.41*	5.36*
		0.959 (min)	1.1*	1.75*	3.93*
	6.62 (mean)	0.965 (mean)	1.31*	2.19*	4.77*

* — For a growth potential > 0.5 log, it is assumed that the food is able to support the growth of *L. monocytogenes*

Growth potential using ComBase prediction software

The predicted growth potentials of Presswurst and Blutwurst are shown in Table 2. As expected, growth potential increased with increasing storage temperature.

DISCUSSION

From proximate composition analyses, the pH and protein content of Blutwurst are quite high, similar to the traditional Spanish blood sausages [10]. This is probably due to neutral pH and high protein content of blood [8]. Compared to Spanish products, Austrian blood sausages demonstrated lower a_W and contained smaller amount of fat and ash. In Presswurst, lower pH could be due to adding vinegar as an ingredient to enhance its unique flavour. The coefficient of variation of fat in Presswurst and protein in Blutwurst was > 30 % suggesting the different quality of raw material and technological processing used for producing such products. Also, considerable variation was found in ash and NaCl content.

High protein, pH and moisture of the products provide a rich environment for bacteria including foodborne pathogens to grow. Technological protectives such as low pH and high salt contents are limited by the characterization of the product itself, especially in Blutwurst, where blood is used as the main ingredient. For Blutwurst, low pH and high amount of NaCl could change the product appearance and affect to consumer preference. However, adding binders other than NaCl could be used to adjust a_W of the product and improve food safety.

Comparing pH and a_W value of all samples with Reg. EC 2073/2005 [6], all samples fall in the range of products which favour growth of *L. monocytogenes* therefore evaluation of growth potential of *L. monocytogenes* in the products are necessary in order to control risk of *L. monocytogenes* to consumer. Notably, there is no statistical correlation between a_W and NaCl for both type of products ($P > 0.05$), which must be considered when growth prediction software is used which allows to substitute a_W by NaCl content.

Since it is usually assumed that the contamination occurs after cooking and during packaging process, bacterial would already adjust to the temperature of the product. Thus, physiological state of the bacteria equal to 1 is preferred. From the prediction, all samples would enhance growth of the bacteria except only Presswurst sample with extremely low pH kept under 2 °C. Therefore, from the prediction base on physiochemical characteristics both products are considered to have potential for *L. monocytogenes* growth. Recently, we presented a simple and instructive graphical decision tool which would allow to assess the contribution of storage temperature, pH and a_W shifts to the multiplication of *L. monocytogenes* in cooked cured meat [3]. It was suggested that such chart could be beneficial for food safety inspectors working the field as well as small food business. In a view of possible inter-batch variation within the food business, it still would be practical to conduct batchwise pH and a_W measurement and assess listeria safety within a few minutes.

However, this approach does not considered other factors that might retard growth of bacteria such as food additives, anti-listeria substance as well as competitive background microflora, etc. It is conceivable that in inhomogeneous products component with higher pH and a_W can create niches for *L. monocytogenes* to thrive. Preliminary study on meat jelly indicates that this is not necessarily the case [2]. Thus, further studies should be conducted.

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OCCURRENCE OF DIOXINS IN FOOD AND THEIR DETERMINATION USING DR CALUX® BIOASSAY

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ABSTRACT

The reduction of human exposure to dioxins and dioxin-like polychlorinated biphenyls (PCBs) through food consumption is important and necessary to ensure consumer protection. As food contamination is directly related to feed contamination, an integrated approach must be adopted to reduce dioxin and dioxin-like PCBs incidence throughout the food chain, e.g. from feed materials through food-producing animals to humans. Every year since 2005, about 90 food samples were examined per year for dioxins and dioxin-like PCBs by DR CALUX ® method in food of animal origin. In 2011 the limit for dioxins was exceeded in 5 samples, 4 of them were of bovine fat and 1 of bovine meat. In 2012 one sample of raw cow milk exceeded the limit. Our results showed that it is important to examine food for the presence of dioxins in order to protect the public health.

Key words: bovine meat; dioxin; dioxin-like PCBs; food chain

INTRODUCTION

Dioxins are some of the most toxic chemicals known. Many natural sources can release dioxins. For instance, the presence of these compounds was confirmed in food even before the large-scale manufacturing and use of chlorinated chemicals [1], [5].

Dioxins are acquired by the human body from food, which makes up 90 % exposure through animal fat (milk, meat, fish), as well as breathing and through skin contact. Scientific Committee on Food [2], established on May 30, 2001, suggested a tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs of 14 pg toxic equivalent of the World Health Organization (WHO-TEQ)/kg body weight.

In the cell, PCDD, PCDF (polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans) and dioxin-like PCBs bind to an intracellular receptor called aryl hydrocarbon receptor (Ah receptor) [4] and form a complex that is transported to the nucleus, followed by binding to specific DNA sequences.

The toxicological effects of dioxins and related compounds begin with the observation of changes in gene expression and the induction of a number of enzymes that cause a variety of metabolic activities, some of them carcinogenic.

Dioxins have a negative impact on various systems of a living organism. They damage the immune and hormonal systems to cause infertility and sterility in men and endometritis in women. They have teratogenic and carcinogenic effects and damage the nervous and cardiovascular systems. In acute forms they cause skin diseases, developmental and neurological disorders in newborns and can damage the liver and other organs. Dioxins and dioxin-like PCBs degrade slowly and remain in the environment for a long time. Therefore, most of the current exposure comes from the release of these chemicals in the past.

The aim of this study was to determine the contamination of food by dioxins.

MATERIALS AND METHODS

Every year since 2005, about 90 food samples were examined per year by a biological screening method DR CALUX® for the detection of dioxins and dioxin-like PCBs. The method uses a genetically modified cell line H4IIE (from rat liver), which contains the luminescent luciferase gene, as a "reporter" of polychlorinated aromatic compounds present. When these cells are exposed to the extract containing dioxins, they start to produce enzyme luciferase. After reaction with the substrate luciferin, cells capable of emitting radiation are measured by a luminometer (Lumistar Galaxy, BMG Labtech, Germany). The amount of emitted light is proportional to the amount of dioxins and dioxin-like compounds in the mixture.

The method consists of the chemical and biological part. Extraction of fat from animal or plant matrix is followed by purification of the extract on a glass column packed with silica gel with H_2SO_4. The eluate is evaporated and then dissolved in a solvent – dimethyl sulfoxide (DMSO). The test was carried out on a 96-well plate with growing cells which were cultured in a CO_2 incubator at 37 °C, until a continuous 100 % monolayer was produced on the wells. The sample was prepared in three dilutions with a growth medium. The series of reference solutions consisted of 8 concentrations, starting from 0—300 pM 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in the wells. The relevant dilution ratio was strictly observed in all the wells. Each dilution was dispensed in triplicate, exactly according to the respective schedule. Each plate contained a negative sample (DMSO), positive (reference) material and a blank.

After 24 hours of incubation, the inoculum was removed, cells were washed and then lysed to release the enzyme luciferase. The plate was placed into a luminometer. After the addition of a solution containing luciferin, the luminescence was measured. To establish calibration curves we used 2, 3, 7, 8-TCDD calibration solutions.

The total amount of TCDD-TEQ in the sample was calculated by interpolation of the sample response to the calibration curve. Standard deviation for triplicate measurements was max. 15 %. Standard deviation between measurements was up to 30 %.

RESULTS AND DISCUSSION

Investigation focused on food of domestic origin and other foods inside the social exchange.

Every year since 2005, about 90 food samples were examined for dioxins and dioxin-like PCBs by the method CALUX®. Five limit-exceeding samples were confirmed in 2011, of which 4 were fat and one was meat from cattle. In 2012, one sample of raw cow milk exceeded the limit. In 2013, no limit-exceeding sample was detected.

The samples, which exceeded the maximum acceptable level of dioxins were sent for confirmatory analysis to the National Reference Centre for dioxins and related compounds at the Slovak Medical University in Bratislava, where confirmatory examination was carried out by gas chromatography combined with high resolution mass spectrometry (HRGC/HRMS).

The most efficient way of reducing the levels in the food chain is to reduce environmental contamination and avoid new release of these substances (particularly polyvinyl chloride) into the environment (3).

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SCREENING OF ANTIBIOTIC RESIDUES IN ANIMAL LIVER, KIDNEY AND MUSCLE BY TUBE MICROBIAL INHIBITION TESTS

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ABSTRACT

Microbial inhibition screening tests (MITs) play an important role in the detection of antibiotic residues in animal food products. The presented paper shows the results of the screening of antibiotic residues in animal matrices by two tube MITs, the widely used Premi®Test and the newly developed test Total Antibiotics. Both these tests are based on the inhibition of the growth of *Bacillus stearothermophilus* var. *calidolactis* test strain in the presence of antibiotics. The tests were applied to liver, kidney and muscle samples from pigs and cattle. Of a total of 117 samples, Premi®Test yielded a total of 7 positive and 4 dubious results, while Total Antibiotics gave a total of 16 positive and 11 dubious results. The higher number of positive or dubious results obtained by Total Antibiotics indicated higher sensitivity of this test to the residues of antibiotics presented in the examined samples than the Premi®Test.

Key words: antibiotics; microbial inhibition tests; screening; tissues

INTRODUCTION

Antibiotics are substances commonly used for the treatment of the infectious diseases not only in humans but also in animals. Due to the treatment of livestock, the presence of antibiotic residues could be found in food of animal origin. Presence of antibiotic residues in food may cause organ toxicity, teratogenicity, hypersensitivity and allergic reactions, or in other ways harm the human consumer. Presence of antibiotic residues renders animal products unfit for human consumption, and is in this way an important barrier to trade, and has implications on technological processes in dairy and meat industries [5], [10].

To protect consumers from exposure to high residue levels, the maximum residues limits (MRLs) for veterinary drugs and their monitoring in live animals and animal products have been established in the European Union by the Commission Regulation (EU) No. 37/2010 and the Council Directive 96/23/EC [3], [4]. The MRL value is defined as the maximum concentration of residue resulting from administration of an animal medicine which is legally permitted or recognizes as acceptable in or on a food [9].

The control of residues of veterinary drugs in the Slovak Republic is carried out according to the Act of National Council of the Slovak Republic No. 39/2007 and the Government Regulation of the Slovak Republic No. 320/2003 [1], [7]. The methods used for official control of residues must be validated according to common procedures and performance criteria established by the Commission Decision 2002/657/EC and provide the antibiotic residues at the level of MRL [2]. The validated methods should be in conformity with Council Directive 96/23/EC [4].

The detection of residues is generally divided into 2 steps, the first is a qualitative screening test which tests positive or negative, and the second one is a quantitative confirmatory analysis served for the determination of residues present in the positive sample. An effective screening method must to be low-cost and high-through-

put [10]. MITs were the earliest methods and they are still widely used for the detection of antibiotic residues. Principally, two main test formats can be distinguished: the tube test and plate test systems. The most commonly used bacterial strain for tube test is *Bacillus stearothermophilus* var. *calidolactis* [8].

The Premi®Test was developed by R-Biopharm (R-Biopharm, Darmstadt, Germany). This test is one of officially approved broad-spectrum MITs in Slovakia [6], designed for detection of the presence of antibiotic residues in meat juice (muscle, kidney and liver), fish, eggs and urine of pigs treated with antibiotics. The newly developed screening test is the Total Antibiotics. It was developed by the EuroClone S. p. A. (Euroclone S. p. A., Pero, Italy). The Total Antibiotics is designed for rapid screening of antibiotic residues in milk and meat samples [11].

The aim of our study was to use both tube MITs mentioned above for the screening of antibiotic residues in the liver, kidney and muscle from cattle and pigs.

MATERIAL AND METHODS

We analysed 117 samples including porcine liver (27), kidney (23) and muscle (12) and bovine liver (25), kidney (20) and muscle (10). The samples were collected from different retail and slaughter facilities during the period from spring 2012 to spring 2014. They were stored at –20 °C until analysis.

Premi®Test procedure: 3 g of the sample were defrosted in a microwave oven for 2 minutes to obtain the kidney, liver and muscle juices. An aliquot of 100 µl of the juice was pipetted onto agar in an ampoule with *Bacillus stearothermophilus* var. *calidolactis*. The ampoules were closed with the provided plastic foil to avoid evaporation. The ampoules containing 100 µl of kidney and liver juice were pre-incubated in a digital dry bath (Labnet Accublock Digital Dry Bath D 1200, Labnet, Edison, USA) at 80 °C for 10 min to eliminate false-positives. After this pre-treatment step, all the ampoules were further incubated for 3 to 3.5 h at 64 ± 0.5 °C. After the incubation period, an evident colour change from purple to yellow indicated that a sample was considered negative for antibiotic residues. The absence of colour change from purple to yellow indicated that a sample was considered positive for antibiotic residues. The samples with slight shades of purple colour were considered dubious.

Total Antibiotics procedure: 2.5 g of minced kidney, liver and muscle samples were transferred into individual 15 ml tubes containing 10 ml of working extraction buffer prepared by 1 : 1 dilution of the provided extraction buffer with demineralised water. The samples were shaken vigorously and incubated at 37 °C for 2 h. The clear supernatant was used directly in the test. A 200 µl sample aliquot was placed in a relative tube with *Bacillus stearothermophilus* var. *calidolactis*. The capped test tubes were placed in a digital dry bath (65 °C) for approximately 3 h. The sample was considered negative, when the colour of tube content turned from purple to yellow. The sample was considered positive, when the colour remained unchanged (purple). All the possible colour shades between yellow and purple were considered as dubious.

Table 1. Positive and dubious results of the screening for antibiotic residues by the Premi®Test and the Total Antibiotics test

Animal species	Tissue	Premi®Test	Total Antibiotics
Pigs	Liver (n = 27)	–	+
		–	±
		–	±
		+	+
	Kidney (n = 23)	±	+
		–	+
		+	+
		–	+
		+	+
		±	+
	Muscle (n = 12)	–	+
		±	±
Cattle	Liver (n = 25)	–	+
		–	±
		+	+
		–	±
		–	±
		–	±
	Kidney (n = 20)	±	±
		+	+
		–	+
		–	+
		+	+
		–	±
	Muscle (n = 10)	–	±
		–	±
		+	+

+ — positive result; ± — dubious result; – — negative result

RESULTS

The positive and dubious results of the screening of antibiotic residues in the livers, kidneys and muscles from cattle and pigs by the Premi®Test and the Total Antibiotics are presented in Table 1.

The Premi®Test yielded a total of 7 positive and 4 dubious results, while with Total Antibiotics test we obtained 16 positive and 11 dubious results. The higher number of positive or dubious results obtained by Total Antibiotics allowed us to declare that this test showed higher detection sensitivity to the antibiotic residues presented in the samples than the Premi®Test.

CONCLUSIONS

The use of antibiotics in food-producing animals may lead to the presence of residues in their tissues and other animal products. Antibiotic residues constitute a serious health and food safety problem. In order to protect human health, monitoring and checking of live animal and animal products for the presence of antibiotic residues are performed. It is essential to have effective screening tests today. The presented paper presented results of screening of antibiotic residues in animal samples by the widely used Premi®Test and the newly developed test Total Antibiotics. The Total Antibiotics yielded a much higher number of positive or dubious results than the Premi®Test and seems to be more suitable for residue screening purposes. However, to reach a final conclusion, all positive samples should be subjected to a confirmatory test.

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COMPARISON OF THE EFFECT OF AGRIMONY EXTRACT AND HUMIC ACIDS ON THE REDOX STATE OF SOME BROILER CHICKEN ORGANS

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ABSTRACT

We determined the ability of agrimony extract and humic acids to affect the antioxidant status of the broiler chicken when administered for 42 days in the diet. The application of both substances generally did not show change in superoxide dismutase activity and thus not invoked for the increased production of superoxide. Administration of agrimony extract suggests an increased decomposition of peroxides as a result of influence the metabolism of xenobiotics in the kidney as well as entitlement to GSH consumption, probably for conjugation reactions. The observed *in vivo* antioxidant effects of humic acids are close to that observed *in vitro*.

Key words: agrimony; antioxidant; broiler chicken; mitochondria; humic acid

INTRODUCTION

Essential oils are volatile plant extracts, soluble in organic solvents commonly used for a pleasant smell, flavour, antiseptic or preservative properties, and antimicrobial effects [3]. Therefore, they are recommended as a natural replacement of antibiotic growth promoters in feed. Furthermore, they have been shown to improve the digestibility of nutrients and the production performance of broilers by preventing the colonization and growth of pathogenic bacteria [13], [5]. It turned out that the improvement in the monitored parameters is in close relation with changes in immunity, intestinal microflora, improved digestion and absorption of nutrients [4]. Ban on the use of antibiotic growth promoters in animal feed is reason to investigate alternative strategies for improving the health of broiler chickens [1]. From traditional medicine, an extract from agrimony (*Agrimonia Eupatoria*) known for potent antioxidant and anti-inflammatory effects is one of the options. Chemically, it contains mainly polyphenols, terpenoids, and coumarins [14] responsible for the antioxidant properties. Humic substances, including humic and fulvic acids, are not antibiotics but if used properly, along with nutritional measures can be a useful tool for maintaining and improving the health of poultry and poultry performance [16], [19].

The aim of this paper was to study the effect of selected active compounds applied in broiler chickens feed on the activity of antioxidant enzymes in plasma and mitochondria of heart, liver and kidneys, because in addition to the liver, the most intense inactivation of xenobiotics and then elimination occurs in the kidneys.

MATERIALS AND METHODS

The experiment was carried out on 120 one-day old broilers COBB500. Chicks were randomly divided into 3 groups (n = 40). The control group (C) was fed conventional feed mixtures, the second group (AE) was administered extract from the crop tops of agrimony (*Agrimonia Eupatoria L.*) (Calendula o. j. s. c., Stará Ľubovňa, SR) in drinking water at a final concentrations of 0.2 %.

The third group (HA) was supplemented with 0.6 % of humic acic preparation, HUMAC® Natur (Hamac, Ltd., Košice, SR). Feed and water was provided *ad libitum* throughout the experiment (42 days). The broilers were killed by cervical dislocation, followed by tissue harvesting and collection of blood for obtaining of plasma. Liver, kidney and heart mitochondria were isolated by the method described by Fernández-Vizarra et al. [8], [9]. The activity of glutathione peroxidase (GPx; E.C. 1.11.1.9) was measured as described by Flohe and Gunzler [10], and of glutathione reductase (GR; E.C.1.6.4.2) according to a modified method previously described by Carlberg and Mannervik [6]. Superoxide dismutase (SOD; E.C.1.15.1.1) activity was measured by means of SOD-Assay Kit-WST (Sigma-Aldrich, Switzerland) following the user manual provided. Reduced glutathione (GSH) levels were measured by a method of Floreani et al. [11]. All the measured parameters were calculated per mg or g of mitochondrial protein determined using the bicinchinonic acid assay.

RESULTS AND DISCUSSION

As an antioxidant enzyme, SOD is responsible for the dismutation of superoxide radical ($O_2^{\cdot-}$) into H_2O_2 and O_2. In the HA group, SOD activity in the heart was significantly higher when compared to the control group ($P<0.001$), while decrease in the activity in the liver indicates the ability to scavenge $O_2^{\cdot-}$ by HA alone, thereby reducing demands on its detoxification in the body. HAs were found to significantly reduce the activity of SOD in liver mitochondria and also to scavenge $O_2^{\cdot-}$ *in vitro* [18]. SOD activities significantly increased in the kidney mitochondria in both, AE and HA groups, but the plasma and control group did not indicate any change (Table 1). This may be due to modulation of the enzymes related to the phase I metabolism of xenobiotics (CytP450 and Cytb5), those catalytic cycle lead to $O_2^{\cdot-}$ formation and subsequently influence the activity of the phase II enzymes (as SOD, catalase and glutathione-S-transferase) and thus the antioxidant parameters, but help to enhance the detoxification [17].

The activity of GPx, which reduces peroxides, was significantly reduced in heart in both groups. In the HA group, the decrease in the liver was more pronounced ($P<0.01$). Balyutite et al. [2] found a lower sensitivity of liver mitochondria against certain flavonoids compared to those in the heart. Decrease in the activity of GPx may be a sign of the ability of compounds either to reduce the amount of peroxides, or inactivate the enzyme itself. An increased production of peroxides is accompanied by increased activity of GPx. This was measured in both groups in the kidney mitochondria and even more in plasma. The amount of GPx in the kidney is almost double that of the plasma and forms a reservoir of GPx, which can be mobilized, if necessary, to other parts of the body. Moreover, plasma GPx is a selenoprotein synthesized almost exclusively in the kidney [15]. It is likely that an increase in the plasma activity of GPx reflects the enhanced conversion of peroxides in the kidney itself.

Table 1. The activity of antioxidant enzymes and the level of reduced glutathione in the plasma and the liver, heart and kidney mitochondria

Examined organ/blood	Group	SOD [μkat.mg $_{prot}^{-1}$]	GPx [U.mg $_{prot}^{-1}$]	GR [nkat.mg $_{prot}^{-1}$]	GSH [Nmol.mg $_{prot}^{-1}$]
Liver	C	2,77 ± 0,41	0,318 ± 0,02	10,40 ± 3,15	7,50 ± 0,09
	AE	3,76 ± 0,53	0,296 ± 0,02	231,6 ± 25,71***	10,86 ± 0,46***
	HA	2,07 ± 0,43	0,246 ± 0,01**	16,71 ± 3,97	5,80 ± 0,69*
Heart	C	3,92 ± 0,73	0,283 ± 0,04	18,65 ± 5,5	13,34 ± 1,48
	AE	4,09 ± 0,75	0,182 ± 0,01*	94,05 ± 9,09***	4,00 ± 0,24***
	HA	13,53 ± 0,99***	0,185 ± 0,01*	38,09 ± 16,81	7,69 ± 1,11**
Kidney	C	2.19 ± 0.28	0.522 ± 0.019	43.53 ± 4.97	11.97 ± 0.98
	AE	4.39 ± 0.87*	0.485 ± 0.005*	112.34 ± 12.47***	4.98 ± 0.31***
	HA	3.28 ± 0.47*	0.597 ± 0.010**	54.58 ± 8.07	10.11 ± 1.21
Plasma	C	2.95 ± 0.57	0.277 ± 0.013	12.64 ± 2.18	7.92 ± 0.86
	AE	2.98 ± 0.57	0.199 ± 0.009***	15.79 ± 0.55	8.59 ± 1.00
	HA	1.88 ± 0.58	0.188 ± 0.008***	12.96 ± 2.92	18.60 ± 2.76**

* — $P < 0.05$; ** — $P < 0.01$; *** — $P < 0.001$

We found a significant increase in the GR activity in mitochondria of both, heart and liver mitochondrias only in the group AE. Giao et al. [12] however, did not observe any the change in GR activity in liver mitochondria after administration of the agrimony extract. GR catalyzes the reduction of the oxidized form of glutathione to GSH, needed for a number of other reactions in the body and protection the cells from oxidative damage. Diet containing humic acids caused a reduction in the GSH level in the mitochondria of both bodies, but also in the mitochondria of the heart in the group with the addition of agrimony. Application of plant extract in the diet of broiler chickens, however, showed an increase in GSH levels in liver, which is already in agreement with the results observed by Giao et al. [12]. By compensating the oxidative stress conditions the increased activity of GR and reduced levels of GR GSH would be expected at the same time. GR activity was significantly increased only in the kidney mitochondria in the AE group together with low levels of GSH (Table. 1). In the group of HA the level of GSH in plasma increased and did not refer to the oxidative stress conditions. But one must not forget the amount of released O_2 during the decomposition of peroxides, which might be reused in the mitochondria respiratory chain for energy production.

CONCLUSIONS

The active compounds administered in the diet of broiler chickens showed an activity providing antioxidant protection to the organism. They are capable of scavenging $O_2^{\cdot-}$, which act as generators of other reactive species in the body. Administration of agrimony extract probably led to higher demands for GSH in conjugation reactions, than when using HA, and we also found an increased decomposition of peroxides, as a consequence of the effect on the metabolism of xenobiotics in the kidney. Administration of HA did not lead to conditions corresponding to the oxidative stress and finally the level of GSH in plasma increased.

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