

QUALITY OF MEAT OF RABBITS AFTER APPLICATION OF EPICATECHIN AND PATULIN

Anna Kalafová, Jozef Bulla, Ondrej Bučko, Jana Emrichová, Katarína Zbyňovská, Peter Petruška, Monika Schneidgenová, Ľubica Chrastinová, Ľubomir Ondruška, Rastislav Jurčík, Martin Mellen, Marcela Capcarová,

ABSTRACT

The aim of the present study was to determinate the effect of epicatechin and patulin on selected parameters of meat quality of rabbits. Adult female rabbits (n=25), maternal albinotic line (crossbreed Newzealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in experiment. Animals were divided into five groups: control group (C) and experimental groups E1, E2, E3, and E4. Animals from experimental groups E1, E2, E3, E4 received patulin through intramuscular injection ($10 \mu\text{g}\cdot\text{kg}^{-1}$) twice a week and animals from groups E2, E3, E4 received epicatechin three times a week through intramuscular injection. After 30 days animals were slaughtered. For analysing of meat quality the samples of *Musculus longissimus dorsi* (50 g) were used. Application of epicatechin and patulin to rabbits had slight or no effect on the pH levels in stomach, small intestine, large intestine and urinary bladder contents, however differences among the groups were insignificant ($p > 0.05$). Application of epicatechin and patulin to rabbits had slight or no effect on total water, protein, fat and differences among the groups were insignificant ($p > 0.05$). The values of amino acids concentrations were not influenced after application of epicatechin and patulin. The fatty acid profiles in animals after application of different doses of epicatechin and $10 \mu\text{g}\cdot\text{kg}^{-1}$ patulin were similar ($p > 0.05$). Concentration of cholesterol increased in experimental groups in comparison with the control group, but differences were insignificant ($p > 0.05$). pH levels of meat of rabbits in experimental group E3 was lower when compared with the control group, but differences was not significant ($p > 0.05$). Electric conductivity parameter was increased in each experimental group (in E3 the highest) against the control but without significant differences ($p > 0.05$). Colour L parameter was slightly decreased in experimental groups with comparison to the control group (in E3 the lowest). Generally we can conclude that intramuscular application of epicatechin or patulin did not affect parameters of meat quality as well as pH values of internal organs content. Further investigations are needed to prove the final answer concerning the health promoting effects of epicatechin and patulin.

Keywords: Rabbits; epicatechin; patulin; pH level; meat quality

INTRODUCTION

Flavonoids are ubiquitous in plant foods. Important dietary sources can include tea, red wine, apples, and cocoa (Renaud and de Lorgeril 1992; Hammerstone et al., 2000). Many flavonoids are potent antioxidants in *in vitro* systems (Williams et al., 2004). Epidemiologic studies have reported a reduced risk of cardiovascular disease in subjects with a high flavonoid intake (Huxley et al., 2003). Protective lipid oxidation during storage of meat is indispensable in order to preserve the quality standards and the shelf life of this product (Nieto et al., 2010). That is the reason for increasing numbers of studies examined dietary additions of natural (no synthetic) antioxidants (Nieto et al., 2010; Bodas et al., 2012; Morán et al., 2012a; Morán et al., 2012b; Capcarova et al., 2012). This strategy is especially interesting because if antioxidants are deposited in the meat during the life of the

animal no addition of exogenous products would be required after slaughter. This alternative, perceived by the consumer as a high quality standard (Sebranek and Bacus, 2007), might be especially useful to prevent meat lipid peroxidation when diets rich in polyunsaturated fatty acids (PUFAs) are administered to the animals. In this sense, attention has been paid to phenolic compounds, a group of substances present in fruits, vegetables, nuts and seeds which have shown potent antioxidant effects as metal chelators or free-radical scavenging activities (McBride et al., 2007). Most of these compounds also have shown antimicrobial properties when added directly to the meat as additives (McBride et al., 2007). However, results have been different when included in the diet of the animals. Patulin, 4-hydroxy-4H-furo(3,2c)pyran-2(6H)-one, is a mycotoxin produced by molds including *Penicillium expansum*, *Aspergillus*, and *Byssachlamys*,

also occurring world-wide in apple, apple products and sometimes in a number of foods including peaches, pears, and grain, or their products (Sommer et al., 1974; Frank et al., 1977; Scott et al., 1977; Chan et al., 2006; Morales et al., 2008; Kwon et al., 2005). Patulin level of 50 µg.kg⁻¹ in apple juice was suggested to be sufficient for protection of human health (Kwon et al., 2010). Patulin exerts its toxic effect by covalently binding to reactive sulfhydryl groups in cellular proteins, as well as by glutathione depletion, resulting in oxidative damage and generation of reactive oxygen stress (ROS) (Renaud and de Lorgeril 1992; Hammerstone et al., 2000; Wu et al., 2008). The aim of present study was to determinate the effect of epicatechin and patulin on selected parameters of meat quality (content of total water, proteins, fat, content of amino acids and fatty acids, electric conductivity, pH, colour) in muscle of rabbits and pH levels of stomach, small intestine, large intestine and urinary bladder content.

MATERIAL AND METHODOLOGY

2.1. Animals

Adult female rabbits (n = 25), maternal albinotic line (crossbreed New Zealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in

experiment. Rabbits were obtained from an experimental farm of the Animal Production Research Centre Nitra, Slovak Republic. Rabbits (age 4 months, body weight 4.0 – 4.5 kg) were housed in individual flat-deck wire cages (area 0.34 m²) under a constant photoperiod of 14 h of day-light. The temperature (18 – 20 °C) and humidity (65%) of the building were recorded continually by means of a thermograph positioned at the same level as the cages. Rabbits were healthy and their condition was judged as good at the commencement of the experiment. Water was available at any time from automatic drinking troughs. Adult rabbits were fed diet of a 12.35 MJ.kg⁻¹ of metabolizable diet (Table 1) composed of a pelleted concentrate.

2.2. Experimental design and diets

Animals were divided into five groups: control group (C) and experimental groups E1, E2, E3, and E4. Animals from experimental groups E1, E2, E3, E4 received patulin through intramuscular injection (10 µg.kg⁻¹) twice a week and animals from groups E2, E3, E4 received epicatechin three times a week (Tab 2) through intramuscular injection. Experiment lasted 30 days. In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures

Table 1 Chemical composition (g.kg⁻¹) of the experimental diet.

Component	
Dry matter	926.26
Crude protein	192.06
Crude Fat	36.08
Crude Fibre	135.79
nitrogen free extract	483.56
Ash	78.78
Organic matter	847.49
Calcium	9.73
Phosphorus	6.84
Magnesium	2.77
Sodium	1.81
Potassium	10.94
Metabolizable energy	12.35 MJ.kg ⁻¹

Table 2 Application of epicatechin and patulin in injectable form (*intramuscular*).

group	n	epicatechin (µg.kg ⁻¹)	patulin (µg.kg ⁻¹)
Control C	5	0	0
Experimental E1	5	0	10
Experimental E2	5	10	10
Experimental E3	5	100	10
Experimental E4	5	1000	10

involving animals were approved by ethical committee.

2.3. Meat quality analysis

At the end of the experimental period, which lasted 30 days, 25 adult female rabbits were slaughtered in an experimental slaughterhouse. The carcasses were prepared by removing the stomach, small intestine, large intestine and urinary bladder with contents. In these organs pH levels using pH 213 Microprocessor pH meter (Hanna instruments, USA) were determined. Samples of *Musculus longissimus dorsi* (50 g) were collected from each animal. The meat samples were collected one hour after slaughter, wrapped in aluminium foil and stored at 4 °C for 24 hours. Meat quality was analysed for parameters characterizing the content of nutrients (content of total water, content of proteins, fat, content of amino acids and fatty acids) and processing technology parameters (electric conductivity, pH, colour).

The content of water, proteins, crude fat and fatty acids were analysed by method FT IR (Fourier Transform infrared Spectroscopy) using Nicolet 6700. Content of amino acids was detected using gas chromatography GC-ECD/NPD. In 1997, Oh-shin et al. [4, 5] reported a simultaneous GC-ECD/NPD method that included quantitation, for insecticides including carbaryl in drinking water. Low LOD of 0.1 ng/mL, and excellent linearity ($R^2 = 0.998-1.000$) were demonstrated.

The value of pH (24 hour post mortem) was detected by portable battery acidometer OP-109. The colour was measured on the surface of the *M. longissimus dorsi* (10 mm thickness), at 24 h post mortem. Colour data were expressed in terms of Lightness (L^*), redness (a^*), yellowness (b^*) in accordance with CIELAB colour space (CIE, 1976).

Instrumental colour measurements were recorded for L^* (lightness; 0: black and 100: white), a^* (redness/greenness; positive values: red and negative values: green), and b^* (yellowness/blueness; positive values: yellow and negative values: blue) using a spectrophotometer CM-2600d (Konica, Minolta, Japan). Due to wet surface of the sample, we evaluated the colour with shine (SCI).

2.4. Statistical analyses

The data used for statistical analyses represent means of values obtained in three blood collections performed on separate days. To compare the results, one-way ANOVA test was applied to calculate basic statistic characteristics and to determine significant differences among the experimental and control groups. Statistical software SIGMA PLOT 11.0 (Jandel, Corte Madera, CA, USA) was used. Differences were compared for statistical significance at the level $p < 0.05$.

RESULTS AND DISCUSSION

3.1. pH levels in stomach, small intestine, large intestine and urinary bladder with contents

The results of pH levels in stomach, small intestine, large intestine and urinary bladder with contents are presented in Figure 1. Application of epicatechin and patulin of rabbits had slight or no effect on the levels of pH in selected organs and differences among the groups were insignificant ($p > 0.05$). Chao- Zhi ZHu et al., (2014)

published that the antioxidant activity of peptides from Jinhua hams exhibited the strongest scavenging activity at the neutral pH, and there was no significant decrease under acidic conditions. Even when the pH was reduced to 3, it still maintained 90% of its DPPH radical scavenging activity. When the pH was increased to 9, the DPPH radical scavenging activity sharply declined and at pH 11, the activity was reduced by 40% compared with that under neutral pH condition. Deamination is promoted at higher pH values resulting in changes with structure and conformation and loss of antioxidant capacity. Different pH values will affect the actual degradation pathway used (Patel and Borchardt 1990; Bell and Labuza 1991). It is important to study the theoretical and practical aspects of the stability of the antioxidants during processing, storage, and in the gastrointestinal tract (Sannaveerappa et al., 2007).

3.2. Nutritional composition of rabbits meat

Results of total water, crude protein, crude fat are presented in Table 3. Application of epicatechin and patulin of rabbits had slight or no effect on measured parameters and differences among the groups were insignificant ($p > 0.05$). The values of amino acids (Table 4) were not influenced after application of epicatechin and patulin. The fatty acid profiles of different doses of epicatechin and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of patulin were similar and did not differ among the groups ($p > 0.05$) (Table 5). Concentration of cholesterol (Table 5) increased in experimental groups in comparison with the control group, but differences were insignificant ($p > 0.05$). Although oxidation is recognized as the main cause of deterioration of meat quality during storage and processing, it is a crucial reaction to develop typical flavor of meat products, especially for many kinds of dry-cured meat products with long-term ripening process (Chizzolini et al., 1998). It is clear that the main oxidation occurring during meat processing is auto-oxidation (Gandemer, 1999), which involves with initiation, propagation and termination steps (Frankel, 1984). It is known that polyunsaturated fatty acids undergo auto-oxidation much more readily than mono or saturated fatty acids (Chizzolini et al., 1998). Therefore, during meat products processing, the PLs, which contain greater proportion of polyunsaturated fatty acids, are more important source for volatiles compared to triacylglycerols (TGs) (Toldrá, 1998). A large number of volatiles such as alkanes, aldehydes, alcohols, esters and carboxylic acids are produced from this process, of which the volatiles with low odour threshold play important roles for meat flavour perception development. Tang et al., (2000) reported that tea catechin supplementation at levels of 200 and 300 $\text{mg}\cdot\text{kg}^{-1}$ in feed were found to be more effective in retarding lipid oxidation in all tissues. Several studies in animals have demonstrated that epicatechin has beneficial effects in chronic degenerative diseases (Al-Gayar et al., 2011; Si et al., 2011; Gómez-Guzmán et al., 2011; Mohamed et al., 2011). Animal studies have shown that patulin is carcinogenic, mutagenic, teratogenic and highly toxic (Lee and Roschenthaler 1986; Yanagisawa et al., 1987; Alves et al., 2000; Schumacher et al., 2005). There is no documented evidence of any adverse effects of patulin to man. Nevertheless, it is considered to be a contaminant in most countries. The

wide numbers of health studies done on patulin have demonstrated that it inhibits several enzymes (Ashoor, 1973). This may be attributed to its reactivity with sulfhydryl groups (Arafat, 1985). Dickens and Jones (1961) found that localized tumours developed in rats when they were repeatedly injected with sub-lethal doses of patulin. Patulin had no effect on the parameters. High doses of patulin, administered via the drinking-water, caused effects on the kidney and gastro-intestinal tract of Wistar rats. No changes in the relative weight or histological appearance of the adrenal glands were observed (Speijers et al., 1988). There are a variety of supplemental antioxidants employed in practical rabbit nutrition of which tocoferol (TOH) and vitamin C are the most widely used. TOH protects cellular membranes against oxidative damage. It reacts or functions as a chain-breaking antioxidant, thereby neutralizing free radicals and preventing oxidation of lipids within membranes (Morrissey et al., 1994; McDowell, 2000). Vitamin C can reduce the generation of ROS and might regenerate α -tocopherol from its oxidized form (Reed, 1992). Some authors suggested that phenolic compounds

could influence secondary metabolites biosynthesis (Sanzani et al., 2009), but there is no information in literature regarding the effect of these flavanones on polyacetyltrehalose (PAT) biosynthesis. Mallozzi et al., (1996) worked with quercetin and found that this flavonol reduced 55% the accumulation of aflatoxin B1 at 25 ppm, and that naringenin, the aglycone of the flavanone naringin, also reduced aflatoxin B1 in almost 41% at the same concentration.

3.3. The effect of epicatechin and patulin on processing technology parameters

The effect of epicatechin and patulin on processing technology parameters was monitored in this study (Table 6). In the experimental groups we found relatively equal levels of pH 24h, electric conductivity, colour L and a*, b* in comparison with the control group. In this study pH 24h levels in muscle samples in experimental group E3 was lower when compared with the control group, but differences were not significant ($p > 0.05$).

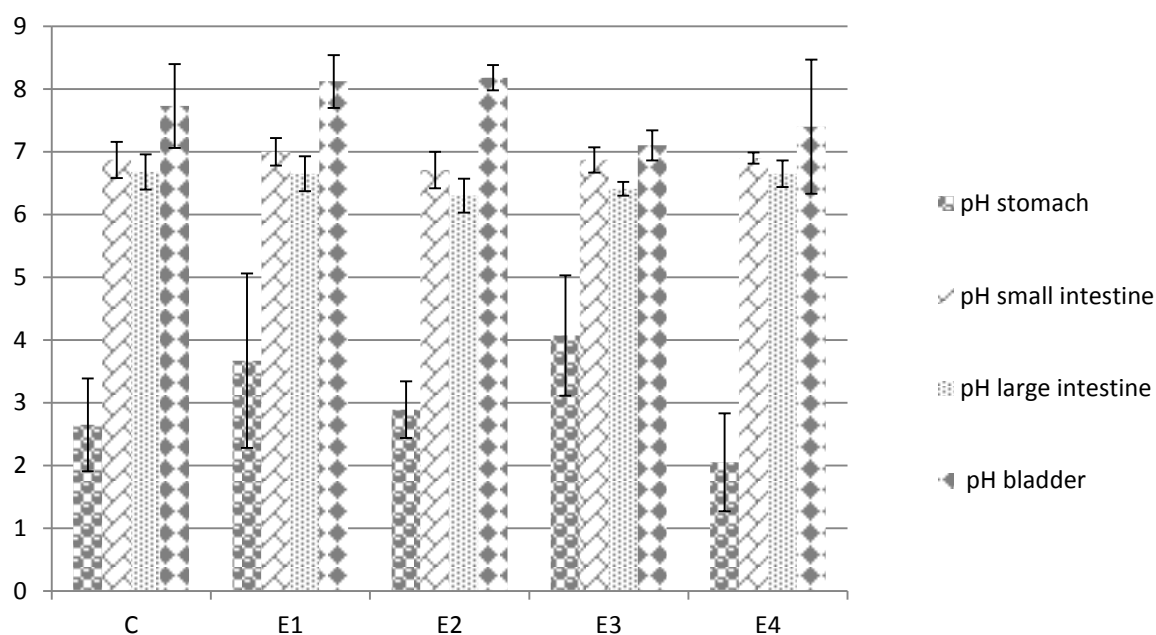


Figure 1 pH levels of stomach, small intestine, large intestine and urinary bladder with contents.

Table 3 Effect of epicatechin and patulin on selected nutrients content in samples of *Musculus longissimus dorsi* of rabbits (g.100g⁻¹).

Parameter	C	E1	E2	E3	E4
Crude protein	24.29 ±0.46	24.67 ±0.70	24.26 ±0.26	23.91 ±0.41	23.84 ±0.07
Crude fat	1.14 ±0.25	1.19 ±0.17	1.06 ±0.24	1.21 ±0.22	1.26 ±0.43
Total water	73.61 ±0.38	73.58 ±0.41	73.83 ±0.28	73.95 ±0.34	73.88 ±0.41

C - control group; E1 10 $\mu\text{g.kg}^{-1}$ patulin; E2 10 $\mu\text{g.kg}^{-1}$ epicatechin and 10 $\mu\text{g.kg}^{-1}$ patulin; E2 100 $\mu\text{g.kg}^{-1}$ epicatechin and 10 $\mu\text{g.kg}^{-1}$ patulin; E3 1000 $\mu\text{g.kg}^{-1}$ epicatechin and 10 $\mu\text{g.kg}^{-1}$ patulin – experimental groups ; mean \pm SD (standard deviation).

Table 4 Effect of epicatechin and patulin on content of amino acids in samples of *Musculus longissimus dorsi* of rabbits (g.100g⁻¹).

Parameter	C	E1	E2	E3	E4
Arginine	1.47 ±0.01	1.46 ±0.13	1.52 ±0.08	1.62 ±0.02	1.54 ±0.06
Cysteine	0.34 ±0.02	0.34 ±0.03	0.35 ±0.02	0.36 ±0.01	0.35 ±0.01
Phenylalanine	0.97 ±0.07	0.96 ±0.08	1.00 ±0.05	1.06 ±0.01	1.01 ±0.04
Histidine	1.04 ±0.09	1.02 ±0.10	1.14 ±0.07	1.24 ±0.04	1.16 ±0.09
Isoleucine	0.86 ±0.08	0.86 ±0.09	0.90 ±0.05	0.98 ±0.03	0.92 ±0.05
Leucine	1.87 ±0.14	1.85 ±0.15	1.94 ±0.10	2.05 ±0.02	1.96 ±0.08
Lysine	1.96 ±0.16	1.95 ±0.17	2.04 ±0.11	2.18 ±0.03	2.06 ±0.09
Methionine	0.70 ±0.05	0.70 ±0.06	0.74 ±0.04	0.81 ±0.03	0.74 ±0.03
Threonine	1.02 ±0.08	1.02 ±0.09	1.08 ±0.05	1.13 ±0.02	1.09 ±0.06
Valine	0.96 ±0.07	0.96 ±0.07	1.03 ±0.05	1.08 ±0.02	1.04 ±0.05

C - control group; E1 10 µg.kg⁻¹patulin; E2 10 µg.kg⁻¹ epicatechin and 10 µg.kg⁻¹patulin; E3 100 µg.kg⁻¹epicatechin and 10 µg.kg⁻¹patulin; E4 1000 µg.kg⁻¹epicatechin and 10 µg.kg⁻¹patulin – experimental groups ; mean ± SD (standard deviation)

Table 5 Effect of epicatechin and patulin on content of fatty acids (g.100g⁻¹ FAME) and cholesterol (g.100g⁻¹) in samples of *Musculus longissimus dorsi* of rabbits.

Parameter	C	E1	E2	E3	E4
n-3 fatty acid	0.46 ±0.07	0.45 ±0.12	0.45 ±0.07	0.58 ±0.06	0.47 ±0.04
n-6 fatty acid	7.42 ±0.91	7.09 ±1.09	7.99 ±1.72	10.13 ±2.83	8.07 ±2.07
PUFA	8.06 ±0.74	8.52 ±0.79	8.48 ±1.22	10.59 ±2.23	8.57 ±1.51
MUFA	54.91 ±0.59	55.32 ±1.59	56.28 ±1.78	54.97 ±2.07	55.37 ±2.98
SAFA	40.12 ±0.70	40.44 ±0.69	40.47 ±2.04	38.75 ±1.72	40.47 ±2.40
Cholesterol	0.18 ±0.02	0.26 ±0.04	0.21 ±0.07	0.26 ±0.01	0.24 ±0.07

Polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SAFA), Fatty acids methyl ester (FAME); C - control group; E1 10 µg.kg⁻¹patulin; E2 10 µg.kg⁻¹ epicatechin and 10 µg.kg⁻¹patulin; E3 100 µg.kg⁻¹epicatechin and 10 µg.kg⁻¹patulin; E4 1000 µg.kg⁻¹epicatechin and 10 µg.kg⁻¹patulin – experimental groups; mean ± SD (standard deviation).

Table 6 Effect of epicatechin and patulin on selected processing technology parameters in samples of *Musculus longissimus dorsi* of rabbits.

item	C	E1	E2	E3	E4
pH 24 h	5.70 ±0.06	5.70 ±0.06	5.69 ±0.06	5.61 ±0.05	5.74 ±0.14
electric conductivity	0.98 ±0.21	1.35 ±0.47	1.53 ±0.32	2.17 ±0.99	1.15 ±0.10
colour L	56.83 ±1.07	56.43 ±3.14	57.40 ±0.99	56.35 ±1.02	54.50 ±1.92
a*	-2.17 ±0.87	-1.23 ±0.73	-1.35 ±0.98	-0.79 ±0.89	-1.37 ±1.17
b*	6.48 ±1.91	6.99 ±1.25	5.61 ±0.36	6.39 ±0.79	5.25 ±1.20

C - control group; E1 10 µg.kg⁻¹patulin; E2 10 µg.kg⁻¹ epicatechin and 10 µg.kg⁻¹patulin; E3 100 µg.kg⁻¹epicatechin and 10 µg.kg⁻¹patulin; E4 1000 µg.kg⁻¹epicatechin and 10 µg.kg⁻¹patulin – experimental groups; mean ± SD (standard deviation).

Meat colour is related to the energy metabolism of muscles, the processing, storage stability of meat and can be affected by many factors. **Hernández et al., (1997); Pla et al., (1998); Fushi et al., (2006)** reported a pH 24h in muscle *M. longissimus dorsi* (MLD) 5.6 and 5.7 in the thigh muscle. Similar values published **Ludewig et al., (2003)**. **Blasco and Piles (1990)** reported pH of MLD ranging from 5.66 to 5.71. In this study electric conductivity parameter was increased in each experimental groups (in E3 the highest). Colour L parameter was slightly decreased in experimental groups when compared to the control group (in E3 the lowest). The meat samples of animals from experimental groups were reddish and greenish as the control group. This was possibly due to partial cell breakdown and blood migration caused by the slow freezing process or to the oxidation of the meat pigment, which stability depends on animal species, muscle biochemical characteristics, and some external parameters (**Lyon and Lyon 2002**). The lowered pH values were observed only in rabbits received epicatechin in injectable form (100 µg.kg⁻¹), what could be a result of less intense of oxidation of myoglobin with consequent lower levels of metmyoglobin. These results are quite surprising because of the demonstrated *in vitro* antioxidant activity of epicatechin, thus the reason of the lack of the same positive effect in muscle tissue is unclear (**Wang et al., 2007**). Epicatechin protects cells from oxidative insults by modulating the cellular antioxidant defences and reducing reactive oxygen species (ROS) production in the presence of stressors (**Chen et al., 2002; Azam et al., 2004; Kinjo et al., 2006**).

CONCLUSION

In conclusion, intramuscular administration of epicatechin and patulin had no effect on the selected parameters of meat quality (*M. longissimus dorsi*) of broiler rabbits. Research on the field of interactions among epicatechin and patulin in animal bodies and the questions of safety levels can also have a positive impact on the safety of food and will be worthy of further investigation.

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Contact address:

Anna Kalafová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: anna.kalafova@uniag.sk.

Jozef Bulla, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: jozef.bulla@uniag.sk.

Ondrej Bučko, Slovak University of Agriculture in Nitra, Faculty of Agrobiological and Food Resources, Department of Animal Husbandry, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: ondrej.bucko@uniag.sk.

Jana Emrichová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: emrichova@gmail.com.

Katarína Zbyňovská, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: zbynovska.katarina@gmail.com.

Peter Petruška, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: petruska.peter85@gmail.com.

Monika Schneidgenová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: monika.schneidgenova@uniag.sk.

Lubica Chrástínová, National Agricultural and Food Centre, Research Institute of Animal Production, Hlohovecka 2, 949 01 Nitra, Slovakia, E-mail: chrastinova@cvzv.sk.

Ľubomír Ondruška, National Agricultural and Food Centre, Research Institute of Animal Production, Hlohovecka 2, 949 01 Nitra, Slovakia, E-mail: ondruska@cvzv.sk.

Rastislav Jurčík, National Agricultural and Food Centre, Research Institute of Animal Production, Hlohovecka 2, 949 01 Nitra, Slovakia, E-mail: jurcik@cvzv.sk.

Martin Mellen, Hydina Slovensko (Poultry Slovakia), 065 11 Nova Lubovna, E-mail: martin.mellen@gmail.com

Marcela Capcarová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: marcela.capcarova@uniag.sk.