Macrominerals and trace minerals in rabbit meat after application of epicatechin and patulin

Anna Kalafová[1], Jozef Bulla[1], Ondrej Bučko[2], Mária Poláčiková[3], Katarína Zbyňovská[1], Jaroslav Kováčik[1], Monika Schneidgenová[1], Ľubica Chrastinová[3], Ľubomír Ondruška[3], Rastislav Jurčík[3], Marcela Capcarová[1]

[1]Department of Animal Physiology, Faculty of Biotechnology and Food Sciences
[2]Department of Animal Husbandry, Faculty of Agrobiology and Food Resources
Slovak University of Agriculture
[3]National Agricultural and Food Centre, Research Institute of Animal Production
Nitra, Slovak Republic

Abstract

The goal of this study was to analyse the effect of the natural antioxidant epicatechin (E) and the mycotoxin patulin (P) on the content of minerals in the meat of rabbits. Adult female rabbits (n = 25) were randomly divided into 5 groups: control group C and experimental groups E1, E2, E3 and E4. Patulin was applied intramuscularly in groups E1, E2, E3 and E4 (10 $\mu g \cdot kg^{-1}$) twice a week, while animals in groups E2, E3 and E4 received E three times a week for 30 days. Samples of *M. longissimus dorsi* (50 g) were analysed after slaughter. The concentration of macroelements (phosphorus, potassium, sodium, magnesium and calcium) and microelements (zinc, copper and manganese) were measured using the atomic absorption spectrophotometry method. A significant decrease ($P < 0.05$) in the content of manganese was recorded in groups E3 and E4 vs. the control. The values in experimental groups E1 and E2 were lower when compared to the control group, although with no significant difference ($P > 0.05$). The significantly lowest ($P < 0.05$) content of copper in this study was measured in groups E3 and E4 with a higher dose of E (100 and 1 000 $\mu g \cdot kg^{-1)}$ in comparison with groups E1 and E2. No significant differences ($P > 0.05$) in the content of phosphorus, potassium, sodium, magnesium and calcium were found between the groups. We assumed that the intramuscular application of higher doses of E may contribute to the occurrence of oxidative stress and support the pro-oxidative effects of mycotoxin. However, further investigation is needed to explain the mechanism of action concerning the effects of epicatechin and patulin.

Epicatechin, macrominerals, patulin, rabbit meat, trace minerals

Introduction

In recent years, substances derived from plants have been successfully used to reduce lipid oxidation in meat products; their antioxidant properties are apparently related to their phenolic content (Karami et al. 2010). The potential antioxidant role of natural plant extracts has been considered in various studies, suggesting an interesting field of exploration in line with the consumer's criteria relating to any additive: safety (Cho et al. 2006; Huang et al. 2010; Castillo et al. 2012; Gálik et al. 2013). The results of Ahmed et al. (2013) showed that another natural antioxidant – resveratrol – has significant potential as an antibiotic alternative for reversing the adverse effects of weaning stress on growth performance, immunity and the microbial environment in *E. coli* and Salmonella-challenged piglets. The positive effects of natural antioxidants on meat characteristics are retarding lipid oxidation, colour loss and microbial growth (Descalzo et al. 2005; Velasco and Williams 2011). Meat quality can be improved by incorporating these natural antioxidants into animal diets, adding these compounds to the meat surface or using active packaging. Some authors have reported that natural antioxidants have no effect on the sensory characteristics of meat. There are studies which demonstrate that the addition of essential oil compounds to the diet of growing lambs (carvacrol and cinnamaldehyde) does not affect the sensory characteristics of sirloin (Chaves et al. 2008). The only evidence

Address for correspondence:

Ing. Anna Kalafová, Ph.D.
Department of Animal Physiology
Faculty of Biotechnology and Food Sciences
University of Agriculture in Nitra
Trieda A. Hlinku 2, 949 76 Nitra, Slovak Republic

Phone: +421 376 414 258
E-mail: anna.kalafova@uniag.sk

of the effect of natural antioxidants in inhibiting off-odour formation and discoloration of meat is active packaging (Coma 2008). Mycotoxins can cause serious health problems in animals and humans known as mycotoxicosis. The major problem associated with animal feed contaminated with mycotoxins is not acute disease episodes, but rather the ingestion of low levels of toxins which may cause a range of metabolic, physiologic and immunologic disturbances (Akande et al. 2006). The short-lived epoxide Aflatoxin B1 (AFB1) has also been associated with coagulopathy due to reduced synthesis of vitamin K and other clotting factors as a result of the sub-lethal intoxication of animals (Bababunmi et al. 1978). With regard to cytotoxic effects, AFB1 has been shown to induce lipid peroxidation in rat livers leading to oxidative damage to hepatocytes (Shen et al. 1995). In a study with white-tailed deer fawn fed 800 mg·kg^{-1} AF over an 8-week period (Quist et al. 1997), acute injuries in the liver were indicated by increased serum bile acid concentrations and hepatic lesions. Patulin has a strong affinity for sulfhydryl groups which explains why it inhibits the activity of many enzymes (Askar 1999). In addition, it has also been reported that patulin at a concentration of 295 mg·L^{-1} in drinking water for 4 weeks caused effects on the gastro-intestinal tract such as gastric ulcers in rats (Speijers et al. 1988).

The aim of this work was to evaluate the content of certain macrominerals, such as phosphorus, potassium, sodium, magnesium and calcium, and some trace elements, such as zinc, copper and manganese, in the meat of rabbits following intramuscular application of patulin and epicatechin.

Materials and Methods

Animals and diets

Adult female rabbits (n = 25), maternal albinotic line (crossbreed New Zealand white, Buskat rabbit, French silver) and paternal acromelanistic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver), were used in the experiment. The rabbits were obtained from an experimental farm of the National Agricultural and Food Centre, Research Institute of Animal Production, Nitra, Slovak Republic. The rabbits (age 4 months, weighing 4.0 – 4.5 kg) were housed in an individual flat-deck wire cages (area 0.34 m^2) under a constant photoperiod of 14 h of daylight. The temperature (18 – 20°C) and humidity (65%) in the building were recorded continually by means of a thermograph positioned at the same level as the cages. The 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. The adult rabbits were fed a metabolisable diet of 12.35 MJ·kg^{-1} (Table 1) composed of a pelleted concentrate.

Experimental design

The animals were divided into 5 groups: control group C and experimental groups E1, E2, E3 and E4. The animals in experimental groups E1, E2, E3 and E4 received patulin (Sigma-Aldrich Chemie, Germany) by intramuscular injection (10 µg·kg^{-1}) twice a week, while the animals in groups E2, E3 and E4 received epicatechin (Sigma-Aldrich Chemie, Germany) three times a week (Table 2) by intramuscular injection *(Musculus longissimus dorsi)*. The experiment lasted 30 days. Institutional and national guidelines for the care and use of animals were observed in this animal study, and all experimental procedures involving animals were approved by an ethical committee.

Procedures

The samples were analysed for the concentration of macroelements (phosphorus, potassium, sodium, magnesium and calcium) and microelements (zinc, copper and manganese) using the atomic absorption spectrophotometry (AAS) method (wavelength for P 430 nm; K 766.5 nm; Na 589 nm; Ca 422.7 nm; Mg 285.2 nm; Zn 213.9 nm; Cu 324.7 nm; Mn 279.5 nm). A reaction with a solution of molybdenum and vanadium (Microchem, Pezinok, Slovakia) was performed before the spectrophotometric method for determination of phosphorus. Biological material (*Musculus longissimus dorsi* samples) was taken from the animal organisms with chromo-nickel surgical instruments. Preparation samples were dried until dry mass was obtained. To obtain the dry mass, small pieces of tissue of a weight of 0.050 to around 1.000 g were placed on a Petri dish and placed in a dryer regulated by a thermostat at 60 °C for 24 h; following this, the dryer temperature was set to 105 °C. The samples were regularly weighed until their loss of mass was unnoticeable. The dried samples were mineralised by wet mineralisation.

During the process of wet mineralisation, all dry material from each sample was placed in separate mineralisation tubes, dissolved with the addition of 2 mL of a concentrated $HNO_3 – HClO_4$ mixture at a proportion of 4:1 and heated in a thermostat digestion block at 120 °C for 90 min. The resulting solution was diluted to 10 mL with demineralised water. All element concentrations are expressed on a wet-weight basis in $\mu g \cdot kg^{-1}$. The recovery of the method was 96 – 98% and the reproducibility was better than 1%.

Statistical analysis

To compare the results of the analysis of variance, one-way ANOVA tests were applied to calculate basic statistic characteristics and to determine significant differences between the groups. The statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA) was used. Duncan's test for the analysis of variance was used to calculate basic statistic characteristics and to determine significant differences between experimental and control groups. Differences were compared for statistical significance at a level of $P < 0.05$.

Table 1. Chemical composition of the basal diet

Nutrient	$[g \cdot kg^{-1}]$
Dry matter	926.26
Crude protein	192.06
Fat	36.08
Fibre	135.79
Non-nitrogen compounds	483.56
Ash	78.78
Organic matter	847.49
Calcium	9.73
Phosphorus	6.84
Magnesium	2.77
Sodium	1.81
Potassium	10.94
Metabolisable energy	12.35 $MJ \cdot kg^{-1}$

Table 2. Intramuscular application of epicatechin and patulin in injectable form

Group (n = 5)	Epicatechin $[\mu g \cdot kg^{-1}]$	Patulin $[\mu g \cdot kg^{-1}]$
Control C	0	0
Experimental E1	0	10
Experimental E2	10	10
Experimental E3	100	10
Experimental E4	1000	10

C – control group; E1,E2,E3,E4 – experimental groups

Results and Discussion

Mycotoxins are secondary metabolites of some fungi belonging to *Aspergillus, Penicillium* and *Fusarium* species and are common contaminants of human foodstuffs, such as wine, coffee beans, nuts and animal feed. Mycotoxins can enter the food chain through contaminated cereals and foodstuffs (e.g. milk, meat, eggs) obtained from animals fed mycotoxin-contaminated feedstuffs. This experiment assesses the status of mineral nutrients in rabbit meat and the influence of intramuscular administration of epicatechin and patulin.

Macrominerals in rabbit meat

The results obtained for macrominerals are presented in Table 3. Following the intramuscular application of epicatechin and patulin in female rabbits, a slight effect or no effect on macroelements (phosphorus, potassium, sodium, magnesium and calcium) in rabbit meat was measured and the differences between the groups were insignificant ($P > 0.05$). There is a great variability in the mineral element content of rabbit meat in the various studies (López-Alonso et al. 2000; Combes 2004; Hermida et al. 2006). This fact may be related to the mineral distribution in the carcass, for which reason further studies are needed to evaluate the distribution of mineral elements in rabbit meat.

Table 3. Effect of epicatechin and patulin on macroelements in samples of *Musculus longissimus dorsi* of female rabbits [$g \cdot kg^{-1}$]

Parameter	C	E1	E2	E3	E4
Na	1.17 ± 0.15	1.12 ± 0.04	1.01 ± 0.13	0.74 ± 0.31	0.75 ± 0.02
K	11.24 ± 0.29	11.43 ± 0.26	11.37 ± 0.26	10.61 ± 0.68	10.53 ± 0.30
Ca	0.14 ± 0.06	0.13 ± 0.08	0.10 ± 0.01	0.09 ± 0.01	0.14 ± 0.01
Mg	0.28 ± 0.01	0.30 ± 0.04	0.27 ± 0.01	0.27 ± 0.01	0.30 ± 0.01
P	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.24 ± 0.01

C – control group; E1,E2,E3,E4 – experimental groups; mean ± SD (standard deviation)

Trace minerals in rabbit meat

The results are presented in Table 4. A significant decrease in the concentration of manganese in groups E3 and E4 vs. the control group was recorded ($P < 0.05$). The values in the other experimental groups (E1 and E2) were also lower in comparison with the control group, though without any significant difference ($P > 0.05$). We believe that the application of higher doses of pure epicatechin could lead to a pro-oxidant imbalance causing oxidative stress which may contribute to the effects of mycotoxin-patulin. Scott et al. (1986) reported that manganese had been shown to be an essential requirement for patulin biosynthesis, though its site of action was unknown. Inhibitor studies did show, however, that manganese exercised its effect on patulin biosynthesis by influencing the coordinate appearance of pathway enzymes by means of an effect at the level of transcription. The significantly lowest ($P < 0.05$) content of copper in this study was measured in groups E3 and E4 with a higher dose of epicatechin (100 and 1 000 $\mu g \cdot kg^{-1}$) in comparison with groups E1 and E2. Free flavonoid aglycones were also detected in the study by Moon et al. (2000) in which pharmacological doses were administered, indicating possible saturation of the conjugation pathways. The formation of anionic derivatives by conjugation with glucuronides and sulphate groups facilitates their urinary and biliary excretion and explains their rapid elimination. Lambert et al. (2010) and Yang et al. (2011) reported that although catechins are chemical antioxidants which can quench free radical species and chelate transition metals, there is evidence that some of the effects of these compounds may be related to induction of oxidative stress. These pro-oxidant effects may also induce endogenous antioxidant systems in normal tissues that offer protection against carcinogenic insult. The presence of transition metals has also been shown to play an important role in EGCG-mediated (epigallocatechin-3-gallate) H_2O_2. Incubation of HL-60 human leukaemia cells with tea catechins in the presence of Cu (II) resulted in the formation of 8-oxo-guanosine (Oikawa et al. 2003). Green tea polyphenols are unstable and undergo auto-oxidative reactions resulting in the production of ROS (Sang et al. 2005). Co-incubation with the catalase or the copper chelator bathocuproine reduced catechin-mediated oxidative damage. Although polyphenols have generally been regarded as antioxidants, the emerging evidence for the pro-oxidant effects of these compounds is interesting and raises many potential questions. Although a limited amount of data has shown that these pro-oxidant effects can occur *in vivo*, there have been no careful dose–response studies and there is no strong data relating to these pro-oxidant effects and cancer prevention *in vivo* (Hou et al. 2006). Lambert et al. (2010) reported some antioxidant and pro-oxidant effects of polyphenols in cancer prevention. For example, increased endogenous antioxidant capacity may be more important prior to carcinogen exposure, whereas pro-oxidant cell-killing effects may be more important in clearing transformed cells from the body and limiting tumour growth. Careful mechanistic studies in animal models representing different stages of carcinogenesis and an integrated approach to analysing the data from these studies will be essential to understanding when and to what extent the antioxidant or pro-oxidant effects of tea polyphenols are important to cancer prevention. A delicate balance between antioxidants and pro-oxidants in the body in general, and specifically in the cell, is responsible for regulation of various metabolic pathways leading to maintenance of immunocompetence, growth and development, and protection against stress conditions (Surai and Dvorska 2001).

Table 4. Effect of epicatechin and patulin on microelements in samples of *Musculus longissimus dorsi* of female rabbits [$mg \cdot 100g^{-1}$]

Parameter	C	E1	E2	E3	E4
Zn	20.17 ± 8.05	15.06 ± 2.46	13.29 ± 0.97	13.48 ± 3.30	12.80 ± 1.32
Cu	0.91 ± 0.11	1.08 ± 0.19^{a}	1.00 ± 0.08^{a}	0.46 ± 0.09^{b}	0.38 ± 0.19^{b}
Mn	0.55 ± 0.28^{a}	0.32 ± 0.05	0.30 ± 0.09	0.13 ± 0.07^{b}	0.14 ± 0.02^{b}

C – control group; E1, E2, E3, E4 – experimental groups; mean ± SD (standard deviation); a, b - the dissimilar letters mean significant differences in the line

Conclusions

In modern rabbit farming systems, in which the main objective is to obtain products of high quality (meat), the concept of quality does not only take in a safe product for the consumer, but also the use of farming practices that respect animal health, whether in intensive or extensive systems. Epicatechin in injectable form administrated intramuscularly in different concentrations affected the values of manganese (a significant decrease in groups E3 and E4 vs. the control group) and copper (significantly lower in E3 and E4 in comparison with E1 and E2). The intramuscular application of higher doses of epicatechin may contribute to the occurrence of oxidative stress and support the pro-oxidative effects of mycotoxin. Other parameters of mineral analysis were relatively stable. The most recent research has indicated that some antioxidants with prolonged use of pure forms have a pro-oxidant effect (there is a reversal of antioxidant). In addition, limited information regarding the interaction between mycotoxins and flavonoids is available. This knowledge may help feed formulators to better utilise flavonoids when formulating diets for rabbits. However, it is impossible to prevent the detrimental effects of mycotoxins on various metabolic processes in the body using antioxidants alone. Mycotoxin binders show promise in decreasing mycotoxin toxicity, as well as in preventing damage to antioxidant systems. Some species of moulds have the ability of producing mycotoxins. Mycotoxin contamination of diets can result in losses in production and financial losses. Mould inhibitors and mycotoxin binders can be effective tools in controlling mould and mycotoxin problems. Antioxidants, on the other hand, can help preserve the palatability of feed ingredients or complete diets.

Acknowledgements

This work was financially supported by VEGA scientific grant No. 1/0760/15. This work was co-funded by the European Community under project No. 26220220180: "AgroBioTech" Building Research Centre.

References

Ahmed ST, Hossain ME, Kim GM, Hwang JA, Ji H, Yang CJ 2013: Effects of resveratrol and essential oils on growth performance, immunity, digestibility and fecal microbial shedding in challenged piglets. Asian Austr J Anim Sci **26(5)**: 683-690

Akande KE, Abubakar MM, Adegbola TA, Bogoro SE 2006: Nutritional and health implications of mycotoxins in animal feeds: A review. Pakistan J Nutr **5(5)**: 398-403

Askar A 1999: Patulin in apple juice and children's apple food. Fruit Process **3**: 74-77

Bababunmi EA, Uwaifo AO, Bassir O 1978: Hepatocarcinogen in Nigerian foodstuffs. World Rev Nutr Diet **28**: 188-209

Castillo C, Hernandez J, Pereira V, Benedito JL 2012: "Update about nutritional strategies for preventing ruminal acidosis." Advances in Zoology Research, O. P. Jenkins. 4th Ed. 84 p

Coma V 2008: "Bioactive packaging technologies for extended shelf life of meat-based products." Meat Sci **78(1-2)**: 90-103

Descalzo AM, Insani M, Biolatto A, Sancho AM, Garcia PT, Pensel N 2005: "Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef." Meat Sci **70(1)**: 35-44

Gálik B, Arpášová H, Bíro V, Rolinec M, Šimko M, Juráček M, Novotná I 2013: The effect of dietary *Rhus coriaria L*. supplementation on fatty acids composition in the table eggs. Acta fytotech zootech **16(2)**: 49-52

Hou Z, Xiao H, Lambert J, You H, Yang CS 2006: Green tea polyphenol, (−)-epigallocatechin-3-gallate, induces oxidative stress and DNA damage in cancer cell lines, xenograft tumors, and mouse liver. The 97th AACR (American Association for Cancer Research) annual meeting. Washington, DC

Huang Y, Yoo JS, Kim HJ, Wang Y, Chen YJ, Cho JH, Kim IH 2010: Effect of dietary supplementation with blended essential oils on growth performance, nutrient digestibility, blood profiles and fecal characteristics in weanling pigs. Asian Aust J Anim Sci **23(5)**: 607-613

Chaves AV, Stanford K, Gibson LL, Mcallister TA, Benchaar C 2008: "Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and carcass characteristics of growing lambs." Anim Feed Sci Technol **145(1)**: 396-408

Cho JH, Chen YJ, Min BJ, Kim HJ, Kwon OS, Shon KS, Kim IH, Kim SJ, Asamer A 2006: Effects of essential oils supplementation on growth performance, IgG concentration and fecal noxious gas concentration of weaned pigs. Asian Austr J Anim Sci **19**: 80-85.

Karami M, Alimon AR, Sazili AQ, Goh YM 2010: "Meat quality and lipid oxidation of infraspinatus muscle and blood plasma of goats under dietary supplementation of herbal antioxidants." J Anim Vet Advances **9**: 3039-3047

Lambert JD, Kennett MJ, Sang S, Reuhl KR, Ju J, Yang CS 2010: Hepatotoxicity of high oral dose (−)-epigallocatechin-3-gallate in mice. Food Chem Toxicol **48(1)**: 409-16

López-Alonso M, Benedito JL, Miranda M, Castillo C, Hernández J, Shore RF 2000: Toxic and trace elements in liver, kidney and meat from cattle slaughtered in Galicia (NW Spain). Food Addit Contam **17(6)**: 447-57

Moon JH, Nakata R, Oshima S, Inakuma T, Terao J 2000: Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women. Am J Physiol Regul Integr Comp Physiol **279**: 461-467

Oikawa S, Furukawa A, Asada H, Hirakawa K, Kawanishi S 2003: Catechins induce oxidative damage to cellular and isolated DNA through the generation of reactive oxygen species. Free Radic Res **37(8)**: 881-890

Quist CF, Howerth EW, Fischer JR, Wyatt RD, Miller DM, Nettles VF 1997: Evaluation of low-level aflatoxin in the diet of white-tailed deer. J Wild Dis **33(1)**: 112-121

Sang S, Lee MJ, Hou Z, Ho CT, Yang CS 2005: Stability of tea polyphenol (−)-epigallocatechin-3-gallate and formation of dimers and epimers under common Experimental conditions. J Agric Food Chem **53(24)**: 9478-9484

Scott R E, Jones A, Gaucher GM 1986: Manganese and antibiotic biosynthesis. III. The site of manganese control of patulin production in *Penicillium urticae.* Can J Microbiol **32(3)**: 273-279

Shen HM, Ong CN, Shi CY 1995: Involvement of reactive oxygen species in aflatoxin B1-induced cell injury in cultured rat hepatocytes. Toxicology **99(1-2)**: 115-123

Speijers GJ, Franken MA, Van Leeuw FX 1988: Subacute toxicity study of patulin in the rat: effects on the kidney and gastro-intestinal tract. Food Chem Toxicol **26(1)**: 23-30

Surai PF, Dvorska JE 2001: Effects of T-2 Toxin, Zeolite and mycosorb on antioxidant systems of growing quail. Asian Austr J Anim Sci **14(12)**: 1752-1757

Velasco V, Williams P 2011: "Improving meat quality through natural antioxidants." Chilean J Agricult Res **71(2)**: 313-322

Yang CS, Wang H 2011: Mechanistic issues concerning cancer prevention by tea catechins. Mol Nutr Food Res **55(6)**: 819-831