# Meat quality of female rabbits following administration of epicatechin

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#### Abstract

The aim of this study was to determinate the effect of epicatechin on selected parameters of meat quality in rabbits. Adult female rabbits (n = 20), 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 the experiment. The animals were divided into four groups: a control group (C) and experimental groups E1, E2 and E3. The experimental groups received epicatechin in injectable form intramuscularly (Musculus biceps femoris) at 10 µg kg<sup>-1</sup> in E1, 100 µg kg<sup>-1</sup> in E2 and 1 000 µg kg<sup>-1</sup> in E3 three times a week for 30 days. The animals were slaughtered after 30 days. Meat quality was analysed from a sample of Musculus biceps femoris (50 g) for parameters characterising the content of nutrients (content of water, proteins, fat, amino acids and fatty acids) and processing technology parameters (electrical conductivity, pH, colour). Following application of epicatechin, the content of fat and total water increased insignificantly (P > 0.05) in the E3 group in comparison with the control group. Statistically insignificant changes (P > 0.05) were observed in the amino acid and fatty acid content investigated in this study. The intramuscular administration of epicatechin led to some significant changes (P < 0.05) in the electrical conductivity between the E2 (higher) and C group, and in pH between E2 vs. E1 and E2 vs. C. However, further investigation is needed to obtain a final answer concerning the health-promoting effects of epicatechin.

Epicatechin, meat quality, rabbits

## Introduction

Meat has a great potential for delivering important nutrients such as fatty acids, minerals, dietary fibre, antioxidants and bioactive peptides to the diet (Decker and Yeonhwa 2010). Meat and meat products are associated with nutrients often considered as negative, including high fat and calorie content, and high levels of saturated fatty acids (SAFA), cholesterol and sodium which are linked to cardiovascular diseases, hypertension, obesity and diabetes. Some of these negative nutrients can be minimised by careful selection of the meat portion consumed, by productive factor manipulation, in particular feeding, and by post-mortem manipulation of the carcass (i.e. fat removal by mechanical processes). Rabbit meat offers excellent nutritive and dietetic properties (Dalle Zotte 2002; Dalle Zotte 2004; Combes and Dalle Zotte 2005; Hernández and Gondret 2006), rich in protein composition (about 22% in the case of the loin - *M. longissimus dorsi* or LD - and hind-leg meat). In addition to its high protein content, rabbit meat also contains high levels of essential amino acids (EAA). The lean meat portion (water and protein content) is relatively constant (73.0 ± 2.3 g water and 21.5 ± 1.4 g protein in 100 g of meat) with a decreasing trend

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from the mid part (loin) to the hind part and then to the fore part of the carcass. Although synthetic antioxidants were once widely used in the meat industry, consumer concerns about their safety and toxicity drove the industry to find natural sources (Coronado et al. 2002). Certain herbs and spices can be used to add functional properties to meat and meat products (Zhang and Zhou 2010) without any negative affect to the meat's physical and sensory characteristics. Various natural ways of improving the oxidative stability of rabbit meat have also been studied. Earlier studies increased dietary oat levels and the oxidative stability of rabbit lipids improved significantly as a result (López-Bote et al. 1998). The aim of the present study was to determinate the effect of epicatechin on selected parameters of meat quality (content of water, proteins, fat, amino acids and fatty acids, electrical conductivity, pH, colour) in rabbit meat.

### **Materials and Methods**

Adult female rabbits (n = 20), 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 the experiment. The rabbits were obtained from an experimental farm of the Animal Production Research Centre in 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 m²) under a constant photoperiod of 14 hours of daylight. 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. The rabbits were healthy and their condition was judged as good at the commencement of the experiment. Water was available at all times from automatic drinking troughs. Groups of adult animals were balanced for age (150 days) and body weight ( $4 \pm 0.5$  kg) at the beginning of the experiment. The adult rabbits were fed 12.35 MJ·kg¹ of metabolisable diet composed of a pelleted concentrate (Table 1).

Table 1. Chemical composition of the experimental diet

Component	$[g \cdot kg^{-1}]$
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·kg <sup>-1</sup> ]

### Experimental design and diets

Animals were divided into four groups: a control group (C) and three experimental groups (E1, E2, E3). The experimental groups received epicatechin (Sigma-Aldrich Chemie, Germany) in injectable form intramuscularly at  $10~\mu g \cdot k g^{-1}$  in E1,  $100~\mu g \cdot k g^{-1}$  in E2 and  $1~000~\mu g \cdot k g^{-1}$  in E3 three times a week for 30 days (Table 2). In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by an ethical committee.

### Procedures

After 30 days, the animals were slaughtered and samples of *Musculus biceps femoris* were collected. 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 from a sample of *Musculus biceps femoris* (50 g) for parameters characterising the content of nutrients (content of water, proteins, fat, amino acids and fatty acids) and processing technology

parameters (electrical conductivity, pH, colour). The value of pH (24 hours post mortem) was recorded by an OP-109 portable battery acidometer. 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 CM-2600d spectrophotometer (Osaka, Japan). As the surface of the sample was wet, the colour with shine (SCI) was evaluated. The content of water, proteins, fat and fatty acids was analysed by the FT IR method (Fourier Transform Infrared Spectroscopy) using a Nicolet 6700 (Pragolab s.r.o.). The content of amino acids was recorded using a gas chromatography capillary (CGC), electron capture detector (ECD) and nitrogen-phosphorus detectors (NPD). The electrical conductivity was evaluated using PMV 51 (mS·cm<sup>-1</sup>).

Table 2. Intramuscular application of epicatechin in injectable form (μg·kg<sup>-1</sup>)

Group $(n = 5)$	С	E1	E2	E3
Dose	-	10	100	1 000

C - control group; E1, E2, E3 - experimental groups

#### **Results and Discussion**

In animal production, the manipulation of diets to increase the PUFA content of products can reduce the oxidative stability by increasing the degree of unsaturation in the muscle. For this reason, increased interest is being seen in antioxidants which are widely used to improve meat quality and sensory characteristics and extend shelf life. Synthetic antioxidants were formerly widely used in the meat industry, but consumer concerns about

Table 3. The effect of epicatechin on selected parameters of nutrient content in samples of *Musculus biceps femoris* of female rabbits  $(g \cdot 100 g^{-1})$ 

Item	С	E1	E2	Е3
Protein	$23.40 \pm 0.19$	$23.24 \pm 0.28$	$23.67 \pm 0.14$	$23.18 \pm 0.12$
Fat	$2.03 \pm 0.43$	$1.47 \pm 0.22$	$1.49 \pm 0.35$	$2.48 \pm 0.33$
Total water	$73.04 \pm 0.38$	$74.03 \pm 1.02$	$73.53 \pm 0.24$	$73.67 \pm 0.42$

C - control group; E1, E2, E3 - experimental groups; mean  $\pm$  SD - standard deviation

Table 4. Effect of epicatechin on amino acid content in samples of *Musculus biceps femoris* from female rabbits  $(g \cdot 100 g^{-1})$ 

Item	С	E1	E2	E3
Arg	$1.41 \pm 0.08$	$1.57 \pm 0.14$	$1.43 \pm 0.02$	$1.44 \pm 0.10$
Cys	$0.32\pm0.01$	$0.35 \pm 0.04$	$0.33 \pm 0.01$	$0.35 \pm 0.02$
Phe	$0.93 \pm 0.05$	$1.03 \pm 0.09$	$0.95 \pm 0.01$	$0.95 \pm 0.06$
His	$1.03 \pm 0.05$	$1.14 \pm 0.09$	$1.04 \pm 0.04$	$1.06 \pm 0.09$
Ile	$0.83 \pm 0.05$	$0.93 \pm 0.09$	$0.84 \pm 0.03$	$0.86 \pm 0.07$
Leu	$1.80 \pm 0.10$	$2.00 \pm 0.18$	$1.84 \pm 0.02$	$1.84 \pm 0.12$
Lys	$1.88 \pm 0.10$	$2.10 \pm 0.19$	$1.92 \pm 0.03$	$1.93 \pm 0.13$
Met	$0.69 \pm 0.03$	$0.74 \pm 0.05$	$0.68 \pm 0.03$	$0.69 \pm 0.05$
Thr	$0.98 \pm 0.06$	$1.09 \pm 0.13$	$1.02 \pm 0.02$	$1.07 \pm 0.08$
Val	$0.94 \pm 0.04$	$1.04 \pm 0.09$	$0.97 \pm 0.02$	$0.99 \pm 0.05$

Arg - Arginine, Cys - Cysteine, Phe - Phenylalanine , His - Histidine, Ile - Isoleucine, Leu - Leucine, Lys - Lysine, Met - Methionine, Thr - Threonine, Val - Valine; C - control group; E1, E2, E3 - experimental groups; mean ± SD - standard deviation

Table 5. Effect of epicatechin on the content of fatty acids (g·100 g <sup>-1</sup> FAME) and cholesterol (g·100 g <sup>-1</sup> ) in	samples
of Musculus biceps femoris from female rabbits	

Item	С	E1	E2	E3
n-3 fatty acid	$0.45 \pm 0.03$	$0.52 \pm 0.12$	$0.48 \pm 0.09$	$0.54 \pm 0.05$
n-6 fatty acid	$6.80 \pm 0.83$	$6.35 \pm 1.16$	$5.74 \pm 0.77$	$6.05 \pm 1.76$
PUFA	$7.79 \pm 0.87$	$7.34 \pm 0.90$	$6.82 \pm 0.76$	$6.94 \pm 1.33$
MUFA	$54.52 \pm 0.95$	$54.03 \pm 2.67$	$53.76 \pm 2.30$	$54.56 \pm 0.94$
SAFA	$38.39 \pm 1.73$	$39.69 \pm 1.67$	$38.72 \pm 1.40$	$38.56 \pm 0.60$
Cholesterol	$0.35 \pm 0.04$	$0.29 \pm 0.05$	$0.30 \pm 0.10$	$0.32 \pm 0.07$

Polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SAFA), fatty acid methyl ester (FAME); C - control group; E1, E2, E3 - experimental groups; mean ± SD (standard deviation)

Table 6. Effect of epicatechin on selected processing technology parameters in samples of *Musculus biceps femoris* from female rabbits.

Item	С	E1	E2	E3
pH 24	$5.86 \pm 0.06^{b}$	$5.87 \pm 0.10^{b}$	$5.66 \pm 0.04^{a}$	$5.74 \pm 0.06$
Electrical conductivity [mS·cm <sup>-1</sup> ]	$2.23 \pm 0.39^{a}$	$2.58 \pm 0.30$	$4.80 \pm 1.40^{b}$	$2.50 \pm 0.99$
Colour L*	$60.19 \pm 2.25$	$56.89 \pm 3.86$	$57.06 \pm 2.82$	$58.75 \pm 7.57$
a*	$-0.72 \pm 1.67$	$-1.43 \pm 1.23$	$-0.86 \pm 1.19$	$-1.07 \pm 0.90$
b*	$8.59 \pm 1.56$	$5.59 \pm 2.50$	$8.83 \pm 2.05$	$8.20 \pm 1.61$

C - control group; E1, E2, E3 - experimental groups; Colour L\* - lightness, a\* - redness / greenness, b\* - yellowness / blueness; mean  $\pm$  SD (standard deviation), a-b the dissimilar letters mean significant differences (P < 0.05)

safety and toxicity pressed the food industry to find natural sources (Coronado et al. 2002). The results for selected meat quality parameters (content of water, proteins, fat, amino acids and fatty acids, electrical conductivity, pH, colour) are presented in Tables 3 – 6. After application of epicatechin in injectable form at a specified quantity, the content of fat and total water increased insignificantly (P > 0.05) in experimental group E3 in comparison with the control group. Statistically insignificant changes (P > 0.05) were observed in the amino acid and fatty acid content investigated in this study. The intramuscular administration of epicatechin led to some significant changes (P < 0.05) in the electrical conductivity between the E2 (higher) and C group, and in pH between E2 and E1 and the control group. The lowered pH value was observed only in rabbits that received epicatechin in injectable form (100 µg·kg<sup>-1</sup>) which could be the result of less intense oxidation of myoglobin with consequent lower levels of metmyoglobin. These results are quite surprising in view of the demonstrated in vitro antioxidant activity of epicatechin, for which reason the lack of the same positive effect in muscle tissue is unclear (Wang et al. 2007). The addition of green tea had no significant effect on the pH, colour or overall sensory quality of sausages (Bozkurt 2006). Many studies over the last decade have emphasised the function of tea catechins in inhibiting lipid oxidation in chicken meat and raw red meat. Many studies have reported the curvilinear relationship between the tenderness and ultimate pH value of meat, with optimum tenderness occurring at a pH value of approximately 6 in beef cattle. Our study has provided valid evidence that epicatechin affected the ultimate pH value in post-mortem muscle of rabbits. 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).

### Conclusions

Epicatechin in injectable form administrated intramuscularly in various concentrations affected the values of pH (lower in comparison with the control group and E1 group) and electrical conductivity between the E2 group and the control group. Other parameters of meat quality were relatively stable. Antioxidant supplementation could enhance the health of rabbits at sensitive stages such as the transition period, but may also have an additional value in giving the final product (meat) added value that benefits consumer health. Research in the field of epicatechin in connection with meat quality is worthy of further investigation. The detailed physiological mechanism should also be further investigated.

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#### References

- Azam S, Hadi N, Khan N, Hadi S 2004: Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties. J Toxicol in Vitro 18: 555-561
- Bozkurt H 2006: Utilization of natural antioxidants: green tea extract and Thymbra spicata oil in Turkish dryfermented sausage. J Meat Sci 73: 442-450
- Combes S, Dalle Zotte A 2005: Le viande de lapin: valeur nutritionnelle et particularités technologiques. Journées de la Recherche Cunicole. 167-180 (In French)
- Coronado SA, Trout G.R, Dushea FR, Shah NP 2002: Antioxidants effects of rosemary extract and whey powder on the oxidative stability of wiener sausages during 10 months frozen storage. Meat Sci 62: 217-224
- Chen L, Yang X, Jiao H, Zhao B 2002: Tea catechins protect against leadinduced cytotoxicity, lipid peroxidation, and membrane fluidity in HepG2 cells. J Toxicol Sci 69: 149-156
- Decker E.A, Yeonhwa P 2010: Healthier meat products as functional foods. Meat Sci 86: 49-55
- Dalle Zotte A 2002: Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livestock Prod Sci 75: 11-32
- Dalle Zotte A 2004: Avantage diététiques. Le lapin doit apprivoiser le consommateur. Viandes produits carnés 23: 1-7 (In French)
- Hernández P, Gondret F 2006: Rabbit meat quality. Maertens and Coudert (Eds.) Recent Advances in Rabbit Sciences. COST and ILVO Publication, Melle, Belgium: 269-290
- Kinjo J, Hitoshi M, Tsuchihashi R, Korematsu Y, Miyakoshi M, Murakami T 2006: Hepatoprotective constituents in plants 15: protective effects of natural-occurring flavonoids and miscellaneous phenolic compounds as determined in an HepG2 cell cytotoxicity assay. J Nature Med 60: 36-41
- López-Bote CJ, Sanz M, Rey A, Castaño A, Thos J 1998: Lower oxidation in the muscle of rabbits fed diets containing oats. Anim Feed Sci Tech 70: 1-9
- Wang L, Pan B, Sheng J, Xu J, Hu Q 2007: Antioxidant activity of Spirulina platensis extracts by supercritical carbon dioxide extraction. J Food Chem 105: 36-41
- Zhang L, Zhou JLK 2010: Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation. Biores Technol 101: 2084-2089