Quality of Rabbit Meat and Phyto-Additives

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Abstract

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The consumption of healthy and nutritive food (rich in proteins and low in cholesterol and lipid contents) is a preferred factor with the contemporary consumers. In addition, natural alternatives are requested to replace the additives used up to now but recently banned. To reach the above given condition, phyto-additives represent a good alternative. The aim of this study was to examine the physicochemical properties and amino acid composition of rabbit meat after the enrichment of rabbit diet with oregano, sage, and *Eleutherococcus senticosus* extracts, and to make a comparison with the commercial product XTRACT and control samples (without plant extracts). The addition of oregano and sage extracts as well as *El. senticosus* in the rabbit diet positively influenced the physicochemical properties of rabbit meat by increasing its energy value ($P < 0.05$ – sage). Supplementing rabbits feed with oregano and sage extracts led to an improvement on the amino acid composition ($P < 0.01$; $P < 0.001$ – serine). These findings are also supported by the good health state of rabbits. Outgoing from these results, the diet enriched with the plant extracts is beneficial for the health state of rabbits involving the nutritional quality of rabbit meat in connection with consumers.

Keywords: amino acids; *Eleutherococcus senticosus*; oregano; rabbit meat; sage

Nowadays, consumers are increasingly interested in a healthy lifestyle, e.g. energy and nutritional values of foods, which are rich in protein and low in cholesterol and lipid contents. From the nutritional point of view, rabbit meat is flavourful and easily digested, with high nutritional and dietetic properties: this meat contains 20–21% of proteins, unsaturated fatty acids (oleic and linoleic; 60% of all fatty acids), potassium, phosphorus, and magnesium, it has low concentrations of fat, cholesterol, and sodium (Bielanski *et al*. 2000; Dalle Zotte 2002; Hermida *et al*. 2006). That is why the rabbit meat is better digested as compared to other kinds of meat (beef, lamb, or pork; Enser *et al*. 1996) and is recommended for consumption, e.g. for persons with cardiovascular illnesses (Hu & Willett 2002). Moreover, the energy value of rabbit meat (427–849 kJ/100 g of fresh meat) is comparable to various commonly consumed sorts of red meat (Dalle Zotte 2002).

The studies concerned with the quality of rabbit meat have focused mainly on biochemical or biophysical traits such as pH, water holding capacity, or colour (Pla *et al*. 1998; Hernández *et al*. 2000). The results on the sensory properties, e.g. appearance, texture, flavour, and others, are also

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available (Hernández *et al*. 2000; Dalle Zotte 2002; Polak *et al*. 2006), since some authors mentioned the scarce rabbit meat sensory analysis (Ariño *et al*. 2007). Most of the works deal with the fatty acid composition of rabbit meat (Cambero *et al*. 1991; Ramírez *et al*. 2006) as well as mineral analysis (Hermida *et al.* 2006), however, the studies concerning the protein and amino acids contents are rare.

In recent years, natural compounds produced by microorganisms (probiotics, bacteriocins), aromatic plants and their extracts have received increased attention as potential alternatives to growth promoters in several animals, due to their antimicrobial activity (Lewis *et al*. 2003; Lauková *et al*. 2006; Marcin *et al*. 2006; Simonová *et al*. 2008). While most of the studies deal with the moderating effects of the environmental, feeding, genetic, and biological (age and weight) factors as well as those of technological (pre-slaughter, transportation, processing) conditions on rabbit carcass and meat quality (Dalle Zotte 2002), the results concerning the influence of natural substances (probiotics) or herbal extracts on rabbit meat composition have not yet been reported, except the oxidative stability of muscle tissues in rabbits (Botsoglou *et al.* 2004). The antimicrobial effects of oregano and sage extracts are known (Marcin *et al*. 2006; Simonová *et al*. 2008); e.g. in Slovakia, oregano is a component of a commercial feed mixture for rabbits – XTRACT. To have in paramount interest the basic research as well as to follow the conclusions of our previous *in vitro* studies, oregano and sage extracts as well as *Eleutherococcus senticosus* were added into the feed and their influence on the quality of rabbit meat (biophysical and physicochemical traits and amino acid composition) was evaluated.

MATERIALS AND METHODS

Experiment schedule and diet. One-hundred and twenty-five-week-old Hy-plus breed rabbits of male sex were used. All the care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals. The rabbits were divided into 4 experimental (EG1, EG2, EG3, EG4) groups and 1 control group (CG) of 24 rabbits in each. The experiment lasted for 42 days. The rabbits were kept in standard cages, 2 animals per cage. The rabbits were fed the commercial granulated diet for growing rabbits (ANPRO.FEED, VKZ Bučany, Slovakia) and had access to water *ad libitum*. The chemical composition of the diet was as follows: dry matter (884.0 g/kg of diet); crude protein (173.0 g/kg); crude fibre (147.0 g/kg); fat (34.0 g/kg); ash (71.0 g/kg); organic compounds (813.0 g/kg); starch (139.0 g/kg); calcium (8.0 g/kg); phosphorus (5.0 g/kg); magnesium (0.9 g/kg); sodium (1.4 g/kg); potassium (9.6 g/kg); iron (0.3 g/kg); zinc (6.0 mg/kg). Every day, at the same time in the morning, the rabbits were administered the sage extract (*Salvia officinalis* L., *Lamiaceae*; 15 ± 1% of cineol, 24 ± 1% of thujon, 18 ± 1% of borneol; 10 µl/animal/day; Calendula, Nová Ľubovňa, Slovakia) in the first experimental group (EG1), and the oregano extract (*Origanum vulgare* L., *Lamiaceae*; 55 ± 3% of carvacrol; 10 µl/animal/day; Calendula, Slovakia) in the experimental group EG2 for 21 days; the extracts were added into the drinking water. In the third and fourth experimental groups (EG3, EG4), the rabbits consumed the diet supplemented with *Eleutherococcus senticosus* powder extract (EG3; kindly supplied by Dr. Poráčová, Prešov University, Slovakia), and commercial phytoadditive XTRACT (carvacrol, cinnamaldehyd, and capsaicin content; Cymedica SK s.r.o. Zvolen, Slovakia; EG4), each admixed in the diet at the concentration of 15 mg/100 kg. The rabbits in CG groos were not administered phyto-additives.

Slaughtering and sampling. Three animals from each group were slaughtered on days 21 and 42 of the experiment; they were stunned by electronarcosis (90 V for 5 s), immediately hung by the hind legs in the processing line and quickly bled out by cutting the jugular veins and the carotid arteries. After the bleeding, out the *Musculus longissimus dorsi* (MLD) muscles were separated by removing the skin, fat, and connective tissue, chilled and stored for 24 h at 4°C until physicochemical analysis.

Physicochemical analyses and amino acids detection. The ultimate pH was determined 48 h *post mortem* (p.m.) using a Radelkis OP-109 with a combined electrode penetrating 3 mm into the MLD.

The colour measurements were taken on the carcass surface of the MLD. Colour was recorded using a MINISCAN XE plus which gives the average of three measurements of lightness (L^*), redness (a^*), and yellowness (b^*) coordinates.

Total water, protein, and fat contents were estimated using an INFRATEC 1265 spectroscope and expressed in g/100 g; from these values, the energy value was calculated [EC(kJ/100 g) = 16.75 × Protein content + 37.68 × Fat content].

The ash content was determined by mineralisation of the samples at 550°C according to STN 570185. The water holding capacity was determined by the compress method at constant pressure (Hašek & Palanská 1976).

Amino acids were determined in fat-free samples by ion-exchange chromatography (free amino acids) and by liquid chromatography (total amino acids) after acid hydrolysis in 6M HCl. Sulphur amino acids were determined after hydrolysis with hydrogen peroxide and formic acid. An Amino Acid Analyzator AAA 400 (Ingos a.s., Prague, Czech Republic) was used for amino acids separation.

Statistical analysis. Statistical evaluation of the results was performed by one-way analysis of variance (ANOVA) with the *post hoc* Tukey post-test. The results are quoted as means ± SEM.

RESULTS AND DISCUSSION

No significant differences were noted in the meat samples concerning the pH_{48} as well as the lightness (L^*) and yellowness (b^*; Table 1); on the other hand, the colour measurements differed in the parameter of redness (a^*), mainly in the meat from the rabbits of the groups EG1 (oregano; 4.20) and EG4 (XTRACT; 1.49). The pH value depends on the balance of muscle energy metabolism and represents a key role in the maintenance of the meat quality during storage. It determines the environmental microbial balance, because of the bacteriostatic effect of low pH on meats (Dalle Zotte 2002). Most reports present values of pH_{24} higher than 5.70 (Dalle Zotte *et al.* 2005; Polak *et al.* 2006). In our study, lower pH_{48} values (in the range 5.61–5.71) were obtained, which could be explained by depletion of glycogen reserve in muscles during refrigeration and by a longer storage time. Meat pH affects many meat properties**,** including the water holding capacity, muscle fat kontent, and carcass colour. Losses of water in meat increases pH decrease, because of the muscle proteins are closer to the isolelectric point which results in a lower hydration level (Dalle Zotte *et al.* 1995). The properties of meat, the colour parameters in particular, are strictly related to pH, which influences the muscle texture and the oxidation of haem pigments. Also, at high pH levels oxymyoglobin is rapidly turned into reduced myoglobin with dark red colour**;** it is also related with oxidative energy metabolism (Ouhayoun & Dalle Zotte 1993). It is known, that meat lightness increases with muscle myofibrillar protein shrinkage, which is itself negatively correlated to pH value, e.g. the lower pH, the higher lightness (Dalle Zotte & Ouhayoun 1998). While the results concerning

Table 1. Biochemical and biophysical composition of *M. longissiumus dorsi* of rabbits (mean ± SEM), $n = 3$

	EG1 oregano[a]	EG2 sage[b]	EG3 *Eleutherococcus*[c]	EG4 XTRACT[d]	CG[e]
PH_{48}	5.67 ± 0.10	5.71 ± 0.08	5.63 ± 0.04	5.61 ± 0.05	5.68 ± 0.13
L^* (lightness)	48.17 ± 6.70	50.91 ± 3.93	51.07 ± 5.93	49.74 ± 1.46	49.15 ± 0.84
a^* (redness)	4.20 ± 3.10	3.01 ± 2.13	2.56 ± 1.60	1.49 ± 1.47	3.93 ± 1.84
b^* (yellowness)	9.07 ± 0.68	8.45 ± 2.30	8.94 ± 3.67	8.84 ± 1.17	8.57 ± 0.16
Water content (g/100g)	74.93 ± 0.70	74.07 ± 0.83[*d/**e]	75.07 ± 0.75	75.80 ± 0.01	75.97 ± 0.15
Protein content (g/100g)	21.77 ± 0.47	21.37 ± 0.72	21.53 ± 0.47	21.80 ± 0.10	21.63 ± 0.15
Fat content (g/100g)	2.33 ± 1.05	3.53 ± 1.53	2.33 ± 1.17	1.37 ± 0.06	1.40 ± 0.20
Energy value (KJ/100g)	451.39 ± 33.80	491.03 ± 45.69[*d,e]	448.60 ± 37.54	416.65 ± 1.11	415.11 ± 6.17
WHC (g/100g)	33.33 ± 1.08	32.80 ± 3.58	32.20 ± 0.24	32.37 ± 1.89	33.30 ± 2.94
Ash (g/100g)	1.033 ± 0.058	1.033 ± 0.058	1.070 ±0.060	1.033 ± 0.060	1.000 ± 0.001

EG – experimental group; CG – control group; WHC – water holding capacity; $^*P < 0.05$; $^{**}P < 0.01$

the lightness described by these authors are in accordance with the negative correlation with pH, in our study the rabbits meat possessed the lowest muscle lightness, even though it did not have the highest pH. The positive relationship between the pH and red colour of meat was also confirmed (Dalle Zotte & Ouhayoun 1998; Polak *et al*. 2006), contradictory to our findings. Moreover, under our conditions higher values of yellowness were measured; this could be connected with free radicals, produced by lipid oxidation during storage and/or manipulation, which can oxidise haem pigments, causing discolouration of meat and meat products (Münch 2004).

In the MLD from the EG2 rabbits (consuming sage extract), a significant decrease of water content was noted as compared to CG ($P < 0.01$) and EG4 (XTRACT; $P < 0.05$). Comparing all meat samples, the lowest protein content and the highest fat content were found in EG2 (Table 1). Fat content seems to be higher in the meat from rabbits fed the diet supplemented with oregano and *Eleutherococcus* (EG1, EG3) than in rabbits from CG and EG4. The chemical composition of meat is closely related to age; e.g. water content decreases with increasing age. This result is in agreement with that presented by the authors studying the age and genotype effects on the muscle composition (Gondret *et al*. 1998; Chrastinová *et al*. 2002). On the other hand, in our case lower and/or similar protein contents were measured than those presented by the authors cited above. The data concerning the protein content of rabbits meat as related to age are contradictory; there are reports demonstrating an increase (Gondret *et al*. 1998) or a decrease of the protein content with the increasing age (Dalle Zotte *et al*. 1996). Dalle Zotte (2002) presented the lipid content of rabbits meat in the range from 0.6 to 14.4 (average 6.8). In our study, the contents of lipids determined were below the average, although higher than those described by Chrastinová *et al*. (2002). Polak *et al*. (2006) measured a higher intramuscular fat content influenced by the genotype, age, and sex of rabbits. The increase of the meat energy value in the rabbits from EG2 group was detected ($P < 0.05$), and it was also much higher in the groups EG1 and EG3 in comparison to CG and EG4 (XTRACT). The energy value of meat is closely related to protein and lipid contents. A positive correlation was found in our study: between lipids and energy the higher lipid content, the higher energy value. No significant differences were found in ash contents and water holding capacity (WHC; Table 1). In our study, a low ash content was measured in comparison with the statements by other authors (Dalle Zotte *et al*. 1996; Gondret *et al*. 1998; Chrastinová *et al*. 2002). There is also a positive correlation between WHC and intramuscular fat content (Hernández *et al*. 2000) as well as the ultimate pH (Lambertini *et al*. 2006). Regarding oregano and sage extracts, there are no other comparable studies concerning rabbits and/or their carcass composition, but several other investigations have been carried out on other animals. Botsoglou *et al*.(2004) presented the antioxidant effect of oregano essential oil on lipid oxidation in rabbits MLD.

The concentration of essential amino acids (AAs) was lower in all experimental groups as compared to CG (Table 2) while non-essential amino acids levels were increased. Minimal changes in AA composition were noted in EG4 as compared to CG, except the lower valine concentration. A lower value of valine was also found in EG1 and EG2. A decrease of methionine + cysteine concentration was detected in EG1 as compared to other groups (EG3, EG4, CG); similarly, in EG2 methionine + cysteine and isoleucine concentrations were decreased. On the other hand, higher values of threonine were measured in EG1 ($P < 0.05$, EG1 *vs*. EG3; $P < 0.01$, EG1 *vs*. EG4, CG) and EG2 ($P < 0.05$, EG2 *vs*. EG4, CG). Among non-essential AAs, a significant increase of serine concentration was recorded in EG1 ($P < 0.01$, EG1 *vs*. EG3; $P < 0.001$, EG1 *vs*. EG4, CG) and EG2 ($P < 0.001$, EG2 *vs*. EG3, EG4, CG). A high nutritional value is also confirmed by the high content of AAs, mainly of essential AAs. There are few reports on AA content of several meats and the majority of them described the AA detection in pork, beef, poultry, or ostrich meats (Moya *et al*. 2001; Straková *et al*. 2006). The comparison of our results with the literature data is rather difficult, because of the lack of more detailed studies on rabbit meat concerning the AA composition; there are only preliminary studies on this subject. In our study, the proportion of essential AAs in rabbit meat was lower than that described by Chrastinová *et al*. (2002; 54.42% and 54.52%) after maize mixture feeding by rabbits; the authors did not describe the composition of the individual AAs. However, e.g. ostrich meat

Table 2. Amino acid composition (g/100 g) of *M. longissimus dorsi* in rabbits (n = 3)

	EG1 oregano[a]	EG2 sage[b]	EG3 *Eleutherococcus*[c]	EG4 XTRACT[d]	CG [e]
Total protein (g/100g)	21.77 ± 0.47	21.37 ± 0.72	21.53 ± 0.47	21.80 ± 0.10	21.63 ± 0.15
Threonine	4.84 ± 0.04	4.80 ± 0.03	4.70 ± 0.02	4.66 ± 0.04	4.64 ± 0.03
Valine	5.60 ± 0.15	5.71 ± 0.06	5.88 ± 0.14	5.65 ± 0.04	5.99 ± 0.03
Methionine + cysteine	2.98 ± 0.04	3.05 ± 0.08	3.47 ± 0.06	3.53 ± 0.03	3.52 ± 0.04
Isoleucine	5.61 ± 0.04	5.56 ± 0.07	5.66 ± 0.04	5.76 ± 0.04	5.75 ± 0.07
Leucine	9.37 ± 0.04	9.93 ± 0.05	9.21 ± 0.08	9.35 ± 0.07	9.34 ± 0.11
Phenylalanine	3.09 ± 0.04	3.01 ± 0.16	3.08 ± 0.09	2.99 ± 0.01	2.92 ± 0.02
Histidine	4.55 ± 0.07	4.58 ± 0.26	4.39 ± 0.05	4.51 ± 0.06	4.54 ± 0.12
Lysine	10.32 ± 0.16	10.21 ± 0.09	10.24 ± 0.12	10.31 ± 0.13	10.32 ± 0.12
Arginine	5.65 ± 0.12	5.58 ± 0.04	5.62 ± 0.04	5.60 ± 0.05	5.66 ± 0.09
Essential amino acids	52.02 ± 0.68	51.83 ± 0.83	52.25 ± 0.64	52.36 ± 0.46	52.67 ± 0.62
Aspartic acid	9.15 ± 0.03	9.19 ± 0.04	9.20 ± 0.07	9.19 ± 0.03	9.19 ± 0.05
Serine	4.07 ± 0.11[**d/***e]	4.10 ± 0.09[***d,e]	3.80 ± 0.04	3.76 ± 0.03	3.74 ± 0.02
Glutamic acid	16.94 ± 0.32	16.73 ± 0.10	16.75 ± 0.15	16.75 ± 0.09	16.73 ± 0.25
Proline	0.91 ± 0.04	0.88 ± 0.04	1.01 ± 0.26	0.84 ± 0.04	0.87 ± 0.08
Glycine	5.08 ± 0.22	5.34 ± 0.48	5.24 ± 0.33	5.07 ± 0.29	5.13 ± 0.32
Alanine	6.51 ± 0.06	6.63 ± 0.14	6.42 ± 0.01	6.46 ± 0.08	6.49 ± 0.04
Tyrosine	5.34 ± 0.12	5.32 ± 0.24	5.33 ± 0.11	5.26 ± 0.07	5.22 ± 0.11
Non-essential amino acids	47.99 ± 0.89	48.20 ± 1.13	47.75 ± 0.97	47.33 ± 0.63	47.37 ± 0.87

**$P < 0.01$; ***$P < 0.001$

contains a lower level of essential AA, except phenylalanine, than that observed by us in rabbit meat (Sales & Hayes 1996); moreover, a higher content of non-essential AAs was found in rabbit meat than in ostrich meat (except aspartic acid and arginine, which are essential AAs in rabbits, but not in apart from ostrich). Above proline and arginine, higher levels of AAs were also detected in rabbits than those described in breast and thigh muscles of broiler chicken and pheasant (Straková *et al*. 2006). Rabbit meat contains higher amounts of threonine, histidine, lysine, serine, glutamic acid, and glycine than ostrich meat and also higher amounts of threonine, lysine, glutamic acid, and glycine as compared to chicken meat (Sales & Hayes 1996; Straková *et al*. 2006). Our findings provided primary knowledge concerning the quality of rabbit meat due to its AA composition; and it indicates that rabbit meat has high nutritional and biological values.

In conclusion, it seems that oregano and sage extracts as well as *El. senticosus* can be involved in rabbits diet without any adverse effects on the carcass characteristics. The dietary supplementation is effective in improving the energy value and amino acid composition of rabbit meat. These findings are also supported by a good health state of rabbits.

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