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MICROBIAL STATUS AND QUALITY OF RABBIT MEAT AFTER RABBITS FEED SUPPLEMENTATION WITH PHYTO-ADDITIVES

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ABSTRACT

Nowdays, there is an increasing interest in public health issue due to consumption of healthy and nutritive food, e.i. rich in proteins, low in cholesterol and lipid contents. The aim of this study was to examine if oregano, sage and *Eleutherococcus senticosus* extracts, commercial Xtract as well can influence microbial status of rabbit meat after their supplementation of rabbit feed. Reduction of enterococci, coagulase-negative staphylococci and *Staphylococcus aureus* after feed supplementation by the sage and oregano was more detectable at the end of the experiment (at day 42) than after their application (at day 21). In animals with Xtract addition, antibacterial effect has been noted already at day 21. Our *in vivo* results confirmed *in vitro* antibacterial effect of the tested extracts and showed that maintaining of rabbit meat quality by plant extracts is very promising.

Keywords: sage; oregano; *Eleutherococcus senticosus*; rabbit meat; microflora

INTRODUCTION

Nowdays, there is an increasing interest of consumers in a healthy lifestyle, e.g. energetic and nutritional values of food, which are rich in protein, 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: containing of 20-21% of proteins and unsaturated fatty acids (oleic and linoleic; 60% of all fatty acids), potassium, phosphorus and magnesium; it is low in fat, cholesterol and sodium **(Bielanski et al., 2000; Dalle Zotte, 2002; Hermida et al., 2006; Dalle Zotte and Szendrő, 2011)**. That is, why the rabbit meat is better digested compare to others (beef, lamb or pork; **Enser et al., 1996**) and recommended for consumption e.g. in persons with cardiovascular illnesses **(Hu and Willett, 2002).** Moreover, the energy value (427-849 kJ/100 g of fresh meat) of rabbit meat is comparable to various commonly consumed varieties of red meat **(Dalle Zotte, 2002).**

The quality of meat is also in relation with its microbiological status, which influences nutritional and sensory traits as well. While studies of meat spoilage in red meat and poultry are reported **(Huffman, 2002)**, data concerning the microbial quality of rabbit meat are lack and limited to a few reports **(Badr, 2004; Rodríguez-Calleja et al., 2004; Rodríguez-Calleja et al., 2005).** Rabbit meat may be contaminated with organisms of various kinds, including spoilage and potentially pathogenic bacteria, like many raw foods of animal origin. These bacteria could be originated from the environment of living animals as well as there is a possible cross-contamination during preslaughter (crating, transportation, holding conditions) and processing (skinning, evisceration) operations **(Hernández, 2008).**

In recent years, natural compounds and/or substances 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; Pogány Simonová et al., 2009a).** Most of studies and reviews deal with the moderating effect of environmental, feeding, genetic and biological (age and weight) factors as well as of technological (preslaughter, transportation, processing) conditions on rabbit carcass and meat quality **(Dalle Zotte, 2002; Hernández, 2008; Dalle Zotte, Szendrő, 2011);** influence on rabbit meat microflora by natural substances (bacteria and plant extracts) have been also reported **(Pogány Simonová et al., 2009b; Pogány Simonová et al., 2009c)**. The objectives of this study were to examine if oregano and sage, *Eleutherococcus senticosus* extracts and commercial phytoadditive – Xtract can influence microbial status of rabbit meat after rabbit feed supplementation by them.

MATERIAL AND METHODOLOGY

One hundred and twenty (120) 5-weeks-old Hy-plus breed rabbits of male sex were used. All care and experimental procedures involving animals followed the guidelines stated in the *Guide for the Care and Use of Laboratory Animals*.

Rabbits were divided into 4 experimental (E1, E2, E3, E4) groups and 1 control group (C1) of 24 rabbits in each. The experiment lasted for 42 days. Rabbits were kept in standard cages, 2 animals per cage. The rabbits 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 g/kg; crude protein, 173 g. kg; crude fibre, 147 g. kg; fat, 34 g. kg, ash, 71 g. kg; organic compounds, 813 g. kg; starch, 139 g. kg; calcium, 8 g. kg; phosphorus, 5 g. kg; magnesium, 0,9 g. kg; sodium, 1,4 g. kg; potassium, 9,6 g. kg; iron, 289,6 mg. kg; zinc, 0,6 mg. kg. Every day, at the same time in the morning, the sage extract was administered to rabbits (*Salvia officinalis* L., *Labiateae*; 15 ± 1 % of cineol, 24 ± 1 % of thujon, 18 ± 1 % of borneol; 10 µL/animal/day; Calendula, Nová Ľubovňa, Slovakia) in the first experimental (E1) group and oregano extract (*Origanum vulgare* L., *Lamiaceae*; 55 ± 3 % of carvacrol; 10 µL/animal/day; Calendula, Slovakia) in the experimental group E2 for 21 days; the extracts were added into the drinking water. In the third and fourth experimental groups (E3, E4), rabbits consumed 21 days the diet supplemented with *Eleutherococcus senticosus* powder extract (E3; Dr. Poráčová, Prešov University, Slovakia; Calendula, Slovakia) and commercial phytoadditive XtTRACT of carvacrol, cinnamaldehyd and capsaicin content (E4; Cymedica SK s.r.o. Zvolen, Slovakia), each homogenized in the diet at concentration 15 mg. 100 kg. Rabbits in control group (C) did not have administered phyto-additives.

Three animals from each group were slaughtered at days 21 (8-weeks-old rabbits) and 42 (11-weeks old rabbits) of experiment; they were stunned by electronarcosis (90 V for 5 s), immediately hung by the hind legs at the processing line and quickly bled by cutting the jugular veins and the carotid arteries. After the bleeding, the *M. biceps femoris* muscles were taken from the left side of the carcasses and chilled until microbiological analysis.

Bacteria from meat samples (MBF) were selected by a standard microbiological method using the appropriate dilutions in Buffered Peptone Water (Biomark, India). Colony forming units (cfu) for bacteria were determined by plating on following media according to ISO norms 6888 and NF V 04-504: Kanamycin Esculin Azide agar (Biomark) for enterococci, Violet Red Bile Glucose agar (Biomark) for *Escherichia coli*, Mannitol Salt agar (Becton & Dickinson, Cockeysville, USA) for coagulase-negative staphylococci (CoNS), Baird-Parker agar enriched with Egg Yolk Tellurite supplement (Becton & Dickinson) for coagulase-positive staphylococci (CoPS) and *Staphylococcus aureus* and CLED agar (Imuna, Šarišské Michaľany, Slovakia) for *Proteus vulgaris* and other Gram-negative bacteria and incubated at 37°C for 24 - 48 h. The bacterial counts were expressed in log10 cfu.g.

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

At the end of the phyto-additives application (at day 21), the reduction of enterococci, CoNS and *Staphylococcus aureus* was recorded in EG4-Xtract group comparing with CG (Table 1). CoPS were reduced in group of rabbits with the sage (EG); moreover, meat samples from this group were *S. aureus* absent. *S. aureus* occured only individually in other groups (except EG4, Table 1). At the end of the experiment (at day 42), the reductive effect of oregano against all checked bacteria was detected in comparison with CG; colonies of *Proteus vulgaris* occured only individually. After the sage supplementation, the counts of enterococci, CoNS and *E. coli* were decreased. *S. aureus* and *P. vulgaris* were not determined in groups with sage and Xtract application. The counts of CoNS and *E. coli* were reduced in all experimental groups, comparing with CG (Table 3). The quality and nutritional value of meat is also depended on its microbial profile. According to our previous results **(Pogány Simonová et al. 2009b,c), t**he dominant bacteria were CoPS (3.62 ± 0.09 – 4.85 ± 0.53 cfu.g^{-1}). On the other hand**, Rodríguez-Calleja et al. (2004)** and **Hernández (2008)** presented *Pseudomonas* sp., lactic acid bacteria and yeasts as dominant contaminants on carcasses. **Badr (2004)** detected higher counts of *S. aureus* (3.98 cfu.g) and *E. faecalis* (4.26 cfu.g) in rabbit meat than it was observed by us. However, **Rodríguez-Calleja et al. (2006)** presented lower counts of *S. aureus* (1.37 ± 0.79 cfu.g^{-1}), similarly to our results (average count 1.75 cfu.g^{-1}; ranged from 1.15 ±0.15 cfu.g^{-1} to 3.11 ± 0.00 cfu. g^{-1}). The prevalence of enterobacteriae in rabbit meat was detected with different counts: to 2.00 cfu.cm^{-2} **(Berruga et al., 2005)**, 0.49 – to 4.00 cfu.g^{-1} **(Rodríguez-Calleja et al., 2005)** and/or 4.79 cfu. g^{-1} **(Badr, 2004).** Although, to low temperature tolerant *Enterobacteriaceae* are capable to multiply, they are usually a small proportion of the total flora **(García-López et al., 1998).** There are many studies dealing with the effect of natural antimicrobial substances of plant origin, e.g. plant extracts and other phyto-additives on microorganisms isolated from several sources, mainly under *in vitro* conditions **(Dorman and Deans, 2000; McGaw et al., 2007).** Up to now, only limited informations are available concerning the inhibitory activity of plant extracts on the counts of microorganisms occurred in rabbit meat **(Simonová, 2006; Szabóová, 2011)**. The use of natural substances in rabbit breeding is important and necessary, because of the residues of synthetic antimicrobial agents and additives for example coccidiostat in meat, related to consumers safety. Inhibitory effect of sage and oregano against enterococci isolated from rabbit meat was also observed under *in vitro* conditions **(Szabóová et al., 2008),** similarly to our results achieved in *in vivo* experiment. Reduction of bacteria after feed supplementation by sage and oregano was more detectable at the end of the experiment (day 42) than at the end of the application (day 21); similarly to results presented by **(Pogány Simonová et al., 2009b**) after bacteriocinogenic and probiotic *Enterococcus faecium* CCM4231 administration to rabbits. In animals with Xtract addition, antibacterial effect has been noted already at day 21. The stronger and earlier detectable effect of Xtract could be explained by heterogenity of its components, due to carvacrol, cinnamaldehyd and capsaicin. The antibacterial influence of sage and oregano was detected after their application, and by them higher reduction was noted mainly in the counts of enterococci, CoNS and *E. coli* comparing with Xtract and control groups.

The results presented in this study showed that changes in microbial profile of rabbit meat - reduction of spoilage bacteria were mainly obtained by oregano, sage and Xtract addition. We can concluded that maintaining of rabbit meat quality by plant extracts is very promising, further studies are needed to confirm and spread the recent knowledge.

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