THE USE OF A GINSENG EXTRACT IN RABBITS

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ABSTRACT

In recent years, essential oils and other extracts of plants have evoked interest as sources of natural substances. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases, improvement in animal welfare and hygiene, food production techniques as well as food safety. The aim of this work was to study the efficacy of *Eleutherococcus senticosus* dry extract on the zootechnical, microbiological (pathogenic and harmful bacteria), immunological and biochemical blood parameters, occurence of *Eimeria* sp. oocysts in rabbits as well as the quality of rabbit meat. Forty eight rabbits (weaned at 35 days) were divided into experimental (EG) and control (CG) groups. Rabbits in EG received dry extract of *Eleutherococcus senticosus* (15 g/100 kg feed) for 21 days; the experiment lasted for 42 days. All animals were found in good health conditions throughout the trial. The application of *Eleutherococcus* extract had a beneficial effect on the mortality (EG: 8% vs. CG: 17%) and feed conversion ratio (EG: 3.55 vs. CG3.63); average daily weight gain significantly increased in EG (35.5 g vs. 29.5 g; $P<0.001$). The antimicrobial effect of *Eleutherococcus* extract was observed against coagulase-positive staphylococci (CPS), *Staphylococcus aureus*, *Clostridium*-like species and *E.coli* in faeces ($P<0.01$). Haemolytic *E. coli* were absent in faecal samples. Caecal microbial profile was slightly influenced during the extract application. Reduced counts of *Eimeria* sp. oocysts was observed through the whole experiment ($P<0.001$). The values of blood parameters were changed in the framework of physiological level; increased levels of serum total proteins, total lipids, cholesterol were observed in EG at day 21. Phagocytic activity was not influenced during the *Eleutherococcus* addition and the measurement of the activity of glutathione-peroxidase in rabbits indicated that oxidative stress was not evoked during the experiment. Concerning the quality of rabbit meat, in rabbits receiving *Eleutherococcus* extract, increased contents of fat (EG: 2.33±1.16 g/100 g vs. CG: 1.40±0.20 g/100 g) and energy value (EG: 448.6±3.5 kJ/100 g vs. CG: 415.1±6.2 kJ/100 g) were observed.

Key words: Bacteria, *Eleutherococcus senticosus*, Meat, Rabbit.

INTRODUCTION

Eleutherococcus senticosus better known as the Siberian ginseng, belongs to the Araliaceae family. Extracts – liquid or dry prepared of *E. senticosus* are used all over the world to stimulate the immune system, to strengthen resistance to infection, to increase physical and mental performance levels in humans as well as in animals. Roots and rhizome of *E. senticosus* contain characteristically eleutheroside B (0.50%), derivates of phenyl propane and eleutheroside E (0.13%), lignanes in addition to traces of coumarins as well as ceffeic acid derivates and considerable amounts of polysaccharides (2-6%; Steinmann *et al*., 2001).

Natural herbs are being explored as alternatives to antimicrobials, due to their contents of different active substances. The use of these natural antimicrobial substances – additives in rabbit breeding represents a promising way to improve health, welfare as well as meat quality of rabbits (Lauková and Mareková, 2001; Marounek *et al*., 2003). However, data concerning the effects of essential oils and

herbal extracts on the growth performance, microbiological and physiological characteristics of rabbits are scarce.

Therefore, the objective of our experiment was to study the effect of *E. senticosus* extract in rabbits ecosystem and determined its effect on zootechnical, microbiological, immunological and biochemical blood parameters, occurence of *Eimeria* sp. oocysts as well as the quality of rabbit meat.

MATERIALS AND METHODS

A total of 48 weaned rabbits (35 days old, Hy-plus breed, male sex; 945 ± 90 g initial weight) were divided into experimental (EG) and control (CG) groups of 24 rabbits in both. 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, Table 1) and had access to water *ad libitum*. In EG, the *Eleutherococcus senticosus* dry extract was applied (15 g/100 kg feed) for 21 days. Treated animals were compared with untreated (in control). All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals (1996) which was accepted by Slovak Governmental Veterinary Office and the trials were agreed by the Ethical Commission of Institute of Animal Physiology in Košice.

Table 1: Ingredients and chemical composition and nutritive value of diets

Ingredients (%):		Chemical composition (g/kg)	
Extracted clover (grass) meal	27.00	Crude protein	197.00
Extracted sugar beel	10.00	Crude fibre	166.50
Oats	13.00	Crude fat	39.00
Wheat bran	6.00	Ash	80.00
Soybean meal	7.50	Organic matter	921.00
Sunflower meal	14.00	Starch	178.00
Monocalcium phosphate	0.60	Lysin	7.50
Dicalcium carbonate	0.90	Methionine+cysteine	6.50
Salt	0.30	Cholinchloride	0.80
Carob	2.50	Metabolizable energy (MJ/kg)	10.0
DL-Methionine+Wheat meal	0.10+0.10		
Mineral and vitamin premix[1]	3.00		

[1]Premix (per kg diet): vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E acetate, 30 mg; vitamin B_2, 5 mg; vitamin B_6, 2 mg; vitamin B_{12}, 8 mg; Ca, 9.25 g; P, 6.2 g; Na, 1.6 g; Mg, 1.0 g; k, 10.8 g; Fe, 327.5 mg; Mn, 80 mg; Zn, 0.7 mg

Body weight of rabbits was checked every week of the experiment. Sampling of faeces was done at days 0 (at the start of the experiment), 7 (1 week after *E. senticosus* extract application), 21 (at the end of the *E. senticosus* extract application) as well as at day 42 (at the end of the trial, 28 days after the strain cessation) to monitor the counts of bacteria and *Eimeria* sp. oocysts. Bacteria from faecal and caecal samples were isolated by the standard microbiological method using the appropriate dilutions in Ringer solution (pH 7.0; Oxoid Ltd., Basingstoke, Hampshire, England). Dilutions were plated onto following media: Baird-Parker agar enriched with Egg Yolk Tellurite supplement (Becton & Dickinson, Cockeysville, USA) for coagulase-positive staphylococci and *S. aureus*, *Clostridium* difficile agar (Oxoid Ltd.) for *Clostridium*-like species, Mac Conkey agar for *E. coli* and incubated at 37°C for 24-48 h. The bacterial counts were expressed in log 10 of colony forming units per gram ($\log_{10}$ CFU/g ± SD). *Eimeria* sp. oocysts were examined in the faecal samples microscopically. The samples were stored at 4°C and then evaluated by the quantitative flotation technique - McMaster method (Ministry of Agriculture, Fischeries and Food,UK, 1986). The intensity of the infections was estimated in counts of oocysts per 1 g of faeces – OPG. Three animals from each group were slaughtered at days 21 and 42 and samples from caecum of each rabbits were collected to count caecal bacteria ($\log_{10}$ CFU/g ± SD).

Blood samples for biochemical and haematological analyses were obtained from the marginal ear vein (*Vena auricularis*) into dry non-heparinized glass tube at days 0, 21 and 42 and blood serum was separated by centrifugation at 3000 x g for 10 min. Serum was stored frozen in plastic vials until

analyzed for total lipids and proteins (g/l ± SD), glucose and calcium (mmol/l ± SD). Biochemical parameters were performed by an enzymatic colorimetric procedure using commercial kits of Randox (the United Kingdom). The activity of blood glutathione-peroxidase (GSH-Px) was determined by standard set RANSEL from Randox (UK). The phagocytic activity (PA) was monitored and expressed as percentage of counting the number of bacteria ingested per phagocyte during a limited period of incubation of particules suspension and phagocytes in serum (Hrubiško *et al*., 1981).

The ultimate pH was determined 48 h *post mortem* (p.m.) by a Radelkis OP-109 with a combined electrode penetrating 3 mm into the MLD. Total water, protein and fat contents were estimated using an INFRATEC 1265 spectroscop and expressed in g/100 g; from these values, the energy value was calculated [EC(kJ/100g) = 16.75 x Protein content + 37.68 x Fat content].

Statistical Analysis

Statistical evaluation of the results was performed by the Student *t*-test with the level of significance set at $P<0.05$.

RESULTS AND DISCUSSION

All animals were found in good health conditions throughout the trial; lower mortality was detected in EG compare to CG (8% vs. 17%). Average daily gain significantly increased in EG (35.5 g vs. 29.5 g; $P<0.001$). Feed conversion ratio was improved by adding *Eleutherococcus* extract (3.55 vs. 3.63). Low mortality, improved weight gain and feed conversion ratio presented also Szabóová (2007) during the experimental application of sage and oregano extracts to rabbits.

The antimicrobial effect of *Eleutherococcus* extract was observed by reduction of coagulase-positive staphylococci (CPS), *Staphylococcus aureus*, clostridia and *E.coli* in faeces ($P<0.01$; Table 2). Haemolytic *E. coli* were absent in faecal samples. Microbial counts in caecum were lower than in faeces and were slightly influenced during the extract application (Table 2). Bacteriostatic and bacteriocidal effects of plant essential oils, extracts and essences are well known and reviewed in many papers (Smith-Palmer *et al*., 1998; Burt, 2004; McGaw *et al*., 2007). The antimicrobial effect of *Eleutherococcus* extract against CPS and *S. aureus* isolates was also confirmed under *in vitro* conditions (non published data). Reduced counts of clostridia, *E. coli* and *S. aureus* was presented by Simonová *et al*. (2007a,b) during the application chamomile essential oil as well as of bacteriocin-producing and probiotic strain *Enterococcus faecium* CCM7420 and its bacteriocin in rabbits, similarly to our results. The anticoccidial effect of plant and herbal extracts and essential oils are also known (Youn and Noh, 2001). The reduction of *Eimeria* sp. oocysts was demonstrated through the whole experiment ($P<0.001$; Table 2); according to results of our previous studies (Simonová et al., 2007a; Szabóová, 2007).

Table 2: Counts of bacteria and *Eimeria* sp. oocysts in faeces and caecum of rabbits

	EG – *Eleutherococcus senticosus*				CG - control			
	Day 0	Day 7	Day 21	Day 42	Day 0	Day 7	Day 21	Day 42
Bacteria in feces (log_{10} cfu/g):								
CPS	1.6±0.5	3.7±1.0	1.7±0.5	1.9±0.5	1.6±0.5	3.7±0.5	2.6±1.0	3.0±0.5
S. aureus	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	1.6±1.0	2.1±1.4	< 1.0
E. coli	1.6±1.0	3.2±1.1	< 1.0	1.5±1.1	1.6±1.0	< 1.0	< 1.0	< 1.0
Haemolytic *E. coli*	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Clostridium-like species	1.3±0.4	2.2±0.7	1.3±0.5	2.0±0.8	1.3±0.4	2.5±0.5	2.1±0.9	2.9±0.4
Bacteria in caecum (log_{10} cfu/g):								
CPS	ND	ND	< 1.0	1.5±0.8	ND	ND	1.6±0.8	1.1±0.3
S. aureus	ND	ND	< 1.0	< 1.0	ND	ND	< 1.0	< 1.0
E. coli	ND	ND	1.3±0.3	< 1.0	ND	ND	< 1.0	< 1.0
Haemolytic *E. coli*	ND	ND	< 1.0	< 1.0	ND	ND	< 1.0	< 1.0
Clostridium-like species	ND	ND	1.2±0.3	1.2±0.3	ND	ND	< 1.0	< 1.0
Eimeria sp. oocysts (OPG)	117±18	334±20[a]	67±7[a]	217±22[a]	117±18	566±41	1307±338	1435±145

[a] - $P<0.001$

The values of blood parameters were changed in the framework of physiological level (Post Graduate Committee in Veterinary Science). Higher levels of serum total proteins, total lipids, cholesterol were observed in EG at day 21 than in CG; increased levels of the biochemical parameters in blood serum could be explained as a result of better resorption and utilization of these nutrients from the gastrointestinal tract by *Eleutherococcus* addition; similar effect of other natural substances - probiotic bacteria, bacteriocins was also described in our previous study (Simonová *et al*., 2007b). The feeding of *Eleutherococcus* by rabbits did not significantly influence biochemical parameters in blood serum and phagocytic activity (PA). In comparison with the results of PA in rabbits during the application of *Eleutherococcus*, sage, oregano extracts and commercial phytoadditive XTRACT (Haviarová *et al.*, 2006), the higher values of PA were observed in samples from rabbits receiving *Eleutherococcus* extract. The measurement of the activity of GSH-Px as well as the evidently good health of rabbits indicated that oxidative stress was not evoked during the experiment.

Table 3: Biochemical parameters in blood serum of rabbits

	EG – *Eleutherococcus senticosus*			CG - control		
	Day 0	Day 21	Day 42	Day 0	Day 21	Day 42
Total proteins (g/l)	52.8±6.6	63.5±6.9	54.9±5.9	52.8±6.6	58.2±9.8	66.1±4.6
Total lipids (g/l)	6.7±2.1	5.2±0.8	3.2±0.5	6.7±2.1	3.3±1.2	3.3±0.9
Glucose (mmol/l)	6.8±1.1	5.0±0.5	6.3±0.5	6.8±1.1	6.3±0.9	5.9±0.4
Cholesterol (mmol/l)	3.7±2.7	2.3±0.5	0.8±0.2	3.7±2.7	1.9±0.3	1.1±0.4
Calcium (mmol/l)	3.0±0.7	3.3±0.3	2.8±0.5	3.0±0.7	3.2±0.1	2.8±0.3

No significant differences were noted in the meat samples concerning the pH_{48} (EG: 5.63; CG: 5.68), the protein content (EG: 21.53 ± 0.47 g/100 g; CG: 21.63 ± 0.15 g/100 g) as well as the total water content (EG: 75.07 ± 0.76 g/100 g; CG: 75.97 ± 0.15 g/100 g). Fat content and energy value seems to be higher in rabbits meat from the animals which diet was supplemented by *Eleutherococcus* (2.33 ±1.16 g/100 g; 448.6 ± 3.5 kJ/100 g) than in rabbits from CG (1.40 ±0.20 g/100 g; 415.1 ± 6.2 kJ/100 g). The energy value of meat is closely related to protein and lipid contents. Positive correlation between lipids and energy was found in our study, similarly to results presented by Chrastinová *et al*. (2007).

CONCLUSIONS

Concluded from the results, antibacterial and anticoccidial effect of *Eleutherococcus senticosus* was observed. *Eleutherococcus* did not influence negatively biochemical parameters in blood.. That is, dry extract of *Eleutherococcus senticosus* is promising for its use in rabbit breeding by supporting physiological and zootechnological parameters and by.increasing the meat energy value. Studies are in progress to spread the recent knowledge.

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