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**REGULAR ARTICLE**

# THE EFFECT OF DIETARY SUPPLEMENTATION OF HERBAL EXTRACTS ON GROWTH PERFORMANCE AND HEALTH STATUS OF RABBITS

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## ABSTRACT

Seventy two rabbits (Hycole, (♂+♀) 5 weeks old) were divided into 2 experimental (EG) and one control (CG) group. The feed mixture A in control (CG) did not contain any coccidiostat. In the first experimental group (EG1) was tested a complete granulated mixture B enriched with 30 g dry extract *Eleutherococcus senticosus* per 100 kg mixture. The animals were fed mixture B in combination with probiotic strain *Enterococcus faecium* EF AL 41 applied orally at a dose ($10^9$ CFU. $ml^{-1}$) 500 µl per animal in drinking water during 21 days. In the second group (EG2) was tested complete granulated mixture B. The experiment lasted 42 days. Body weight and feed consumption were registered weekly. Samples of faeces were taken at days 1, 21 and 42 to monitor the counts and effect of natural substances. Blood samples were also taken to check biochemical parameters using commercial kits. The samples for microbiological analyses were treated by the standard microbiological method according to ISO with appropriate media. Results presented in this study introduce different mechanisms of action for different classes of feed additives and how these may influence the observed live performance benefits. Application of *Eleutherococcus* extract in combination with EF AL 41

strain in rabbits was beneficial for performance. Additives reduced counts of *S. aureus* and *Clostridium-like sp* in faeces. Bioactive substances can be beneficial for animal health as was repeatedly confirmed by our results (mortality in EG1- 12%, EG2 - 8% compared to CG- 17%).

**Keywords:** rabbit; probiotic; enterococci; *Eleutherococcus senticosus*; health

## INTRODUCTION

Natural herbs are being explored as alternatives to antimicrobials. *Eleutherococcus senticosus* better known as Siberian ginseng, belongs to the Araliaceae family. *Eleutherococcus senticosus* contains different bio active substances. The root contains varieties of glucosides (0.6~0.9%): daucosterol, acanthosides A, B, B1, C, D, E, isofraxidinglucoside, ethyl-α-D-galactoside, syringaresinol glucoside, syringin, hyperin, eleutheroside B (0.5%), eleutheroside E (0.13%) and others. The use of herbal extract like feed supplement is widespread and increases both in human and veterinary medicine in the Slovak Republic. The combined effect of these results is to confirm a rightful place for plant extracts as consistent, science - based, naturally - occurring performance enhancers for widespread use. The significance of this in one dimension is global and, in another, spans the entire food chain from producer to consumer. Enterococci present Gram - positive, facultative anaerobic lactic bacteria from the genus Enterococcus, division Firmicutes. They are ubiquitous microbiota constituting a large proportion of autochthonous microflora in animals **(Franz *et al.*, 2007; Mareková *et al.*, 2007; Simonová *et al.*, 2008; Chrastinová *et al.*, 2010; Lauková *et al.*, 2012).** Some produce bacteriocins mostly enterocins; some of bacteriocin - producing strains also possess probiotic properties. *Enterococcus faecium* AL 41 strain (represents environmental isolate, produces *Ent* M) was tested for beneficial effect in rabbits.

The aim of this study was to compare combined effect of strain EFAL41 and *Eleutherococcus senticosus* dry extract on selected parameters in rabbits.

## MATERIAL AND METHODS

The experiment was performed on the experimental farm at the Animal Production Research Centre, Nitra: Institute of Nutrition. A total of 72 rabbits (Hycole, (♂+♀) 5 weeks old) were divided into 2 experimental (EG) and 1 control (CG) groups. The feed mixture A without coccidiostat was fed in the control group (CG). In the first group (EG1) was tested complete granulated mixture B enriched with 30 g dry extract *Eleutherococcus senticosus* per 100 kg mixture. The animals were fed mixture B in combination with probiotic strain *Enterococcus faecium* EF AL 41 that was applied orally at a dose ($10^9$ CFU. $ml^{-1}$) 500 µl per animal in drinking water during 21 days. In the second group (EG2) was tested complete granulated mixture B. The experiment lasted 42 days. Body weight and feed consumption were registered weekly. Samples of faeces were taken at days 1, 21 and 42 to monitor the counts and effect of natural substances. The samples for microbiological analyses were treated by the standard microbiological method according to ISO with appropriate media, expressed in colony forming units (log 10) per g. Rabbits were kept in standard cages (0.61 m x 0.34 m x 0.33 m), 2 animals per cage.

The samples of individual feeds were analyzed for content of nutrients (Tab 1) according to procedures of the AOAC (1995), and starch according to the alpha-amyloglucosidase method. Rabbits were fed *ad libitum* (Tab 1) and they had free access to drinking water from nipple drinkers during the experiment. Blood samples were obtained from the marginal ear vein (*vena auricularis*). Blood samples were also taken to check biochemical parameters using commercial kits. In blood serum the total proteins and lipids ($g.l^{-1}$), cholesterol, glucose, calcium ($mmol.l^{-1}$) were analysed (Randox, the United Kingdom). The results were quoted as mean ± standard deviation (SD); statistical evaluation of the results was performed by the one-way ANOVA and post-hoc Tukey test.

**Table 1** Ingredients and chemical composition of the experimental diets for rabbits

| Ingredients (%) | Diet A | Diet B | Nutrients ($g.kg^{-1}$) | Diet A | Diet B |
|---|---|---|---|---|---|
| Lucerne meal | 25 | 25 | Dry matter | 904.8 | 900 |
| Dried beet pulp | 17 | 17 | Crude protein | 171.4 | 172.1 |
| Sunflower extr. meal | 17.4 | 17.4 | Crude fibre | 173.6 | 165.2 |
| Wheat bran | 8.7 | 8.7 | Fat | 41.4 | 43.2 |
| Apple pomace | 7 | 7 | N free extract | 448.1 | 454.1 |
| Oats | 5 | 5 | Organic matter | 834.4 | 834.7 |
| Barley | 4.7 | 4.5 | Ash | 70.4 | 65.4 |
| Olive press cakes | 6 | 5 | Starch | 157.4 | 174.0 |
| Carob meal | 0 | 0.2 | ADF | 210.3 | 196.2 |
| Mineral &Vitamins* | 2.5 | 2.5 | NDF | 365.1 | 313.51 |
| Wheat meal+30g *Eleutherococcus* dry extract | 0 | 1 | Calcium | 6.7 | 8.44 |
| DDGS from maize | 5 | 5 | Phosphorus | 4.1 | 3.56 |
| Rape oil | 1.7 | 1.7 | ME ($MJ.\ kg^{-1}$) | 10.8 | 11.0 |

Legend: ADF – acid detergent fibre; NDF – neutral detergent fibre; * Composition of mineral and vitamins premix (per 1 kg): 45% heat treated soya, 22% dicalcium phosphate, 13% dicalcium carbonate, 10% sodium chloride, 5% sodium bicarbonate, 2% methionine and 3% vitamin premix vit. A 12000 IU; vit.$D_2$ 2500 IU; vit. E 20 mg; vit.$B_1$ 1.5 mg; vit. $B_2$ 7.5 mg; vit. $B_6$ 4.5 mg; vit.$B_{12}$ 30 µg; vit.K 3 mg; nicotic acid 45 mg; folic acid 0.8 mg; biotin 0.08 mg; choline chloride 450 mg; premix minerals (per kg diet) Ca 6.85 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

**Table 2** Performances of rabbits in response to dietary supplementation of plant extracts ($\overline{X} \pm SD$)

| Performances (n=24) | 1-EG Ginseng + EFAL41 | 2-EG Ginseng | 3-CG Control |
|---|---|---|---|
| Initial weight (g) | 1050±123 | 950±100 | 964±109 |
| Live weight on 56th day of age (g) | 1933±216 | 1734±280 | 1810±208 |
| Final weight (g) | 2652±281* | 2521±263 | 2465±180 |
| Dairy weight gain ($g.day^{-1}$) | 38.14 | 37.40 | 37.11 |
| Feed conversion ratio between day 35 and 56 ($g.g^{-1}$) | 2.47±0.03* | 3.01±0.03 | 2.81±0.07 |
| Feed conversion ratio during the experiment ($g.g^{-1}$) | 3.03±0.02 | 3.49±0.02 | 3.63±0.11 |
| Daily feed intake ($g.day^{-1}$) | 131.5±10 | 130.5±10 | 129.4±8 |
| Mortality (n) | 3 | 2 | 4 |

*P<0.05 Significant differences from control

## RESULTS AND DISCUSSION

No significant differences were found among experimental groups in feed intake and body weight in the fattening experiment (Tab 2). The values of blood parameters were in the framework of the physiological levels. Feeding of complete granulated mixture B enriched with 30 g dry extract *Eleutherococcus senticosus* per 100 kg mixture to rabbits did not influence biochemical and mineral parameters in blood, as well as it had no negative effect on growth performance of rabbits. At day 42, concentration of total lipids and cholesterol was decreased (Tab 4) after the application of bioactive substances in experimental groups (**Simonová *et al.*, 2008; Lauková *et al.*, 2012**). Globally were in all groups bacterial counts balanced in the faeces of rabbits after the application of the experimental diets (Tab 3).

**Table 3** Counts of bacteria in faeces of rabbits (log 10 CFU.g$^{-1}$)

| Bacterial strains | Test sampling | 1-EG Ginseng + EF AL41 | 2-EG Ginseng | 3-CG Control |
|---|---|---|---|---|
| *Enterococcus faecium EFAl41* | 1st day | nd | nd | nd |
| | 21st day | <1,0 | nd | nd |
| | 42nd day | <1,0 | nd | nd |
| *Coagulase-positive staphylococci (CoPS)* | 1st day | 2.57±0.64 | 1.6±0.5 | 1.6±0.5 |
| | 21st day | 2.18±0.46 | 1.7±0.5 | 2.6±1.0 |
| | 42nd day | 4.36±0.41 | 1.9±0.5 | 3.0±0.5 |
| *Staphylococcus aureus* | 1st day | 2.81±0.67 | <1.0 | <1.0 |
| | 21st day | 1.14±0.69 | <1.0 | 2.1±1.4 |
| | 42nd day | 1.49±0.74 | <1.0 | <1.0 |
| *Escherichia coli* | 1st day | 2.43±1.18 | 1.6 ±1.0 | 1.6 ±1.0 |
| | 21st day | 3.91±1.42 | <1.0 | <1.0 |
| | 42nd day | 3.47±1.01 | 1.5±1.1 | <1.0 |
| *Clostridium-like sp.* | 1st day | 2.2±0.4 | 1.3±0.4 | 1.3±0.4 |
| | 21st day | 1.31±0.2 | 1.3±0.5 | 2.1±0.9 |
| | 42nd day | 2.8 ±0.3 | 2.0±0.8 | 2.9±0.4 |
| *Pseudomonas-like sp.* | 1st day | 3.07±1.1 | 3.07±1.1 | 3.07±1.1 |
| | 21st day | 4.4±0.4 | 2.9±0.4 | 4.6±0.2 |
| | 42nd day | 4.9±0.3 | 4.6±0.3 | 4.9±0.2 |

**Table 4** Biochemical parameters in the blood of rabbits

| Bacterial strains | Test sampling | 1-EG Ginseng + EF AL41 | 2-EG Ginseng | 3-CG Control |
|---|---|---|---|---|
| Total proteins ($g.l^{-1}$) | 1st day | 51,1±1,95 | 52.8±6.6 | 52.8±6.6 |
| | 21st day | 53,6±3,1 | 63.5±0.8 | 58.2±9.8 |
| | 42nd day | 59,0±1,71 | 54.9±5.9 | 66.1±4.6 |
| Total lipids ($g.l^{-1}$) | 1st day | 6.7±2.1 | 6.7±2.1 | 6.7±2.1 |
| | 21st day | 5.1±0.4 | 5.2±0.8 | 3.3±1.2 |
| | 42nd day | 3.3±0.4 | 3.2±0.5 | 3.3±0.9 |
| Glucose ($mmol.l^{-1}$) | 1st day | 7.7±0.7 | 6.8±1.1 | 6.8±1.1 |
| | 21st day | 9.7±1,5 | 7.5±1.3 | 8.66±1.9 |
| | 42nd day | 6.2±0.5 | 5.7±1,0 | 6.1±0.5 |
| Cholesterol ($mmol.l^{-1}$) | 1st day | 2.97±0.3 | 3.7±2.7 | 3.7±2.7 |
| | 21st day | 2.62±0.4 | 2.3±0.5 | 1.9±0.3 |
| | 42nd day | 1.87±0.35 | 0.8±0.2 | 1.1±0.4 |
| Calcium ($mmol.l^{-1}$) | 1st day | 2.2±0.4 | 3.3±0.7 | 3.0±0.7 |
| | 21st day | 2.6±0.1 | 3.3±0.3 | 2.6±0.1 |
| | 42nd day | 2.4±0.1 | 2.8±0.5 | 2.7±0.1 |

After the application of *Eleutherococcus* extract in combination with EF AL 41 strain was detected beneficial effect on performance of rabbits. However, *Enterococcus faecium* AL 41 strain colonized in rabbits' digestive tract (**Lauková *et al.*, 2009; Lauková *et al.*, 2012).** Additives reduced counts of *Staphylococcus aureus* and *Clostridium*-like sp. in faeces. Bioactive substances can be beneficial for animals health as repeatedly confirmed by our results (mortality in EG1- 12%, EG2 - 8% compared to CG- 17%).

## CONCLUSION

Results presented in this study showed the benefits on monitored rabbit physiological parameters. The health status of animals was good.

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