International Journal of Veterinary Science and Agriculture Research
Volume 6 Issue 4, November-December 2024
ISSN: 2582-4112, Available at www.ijvsar.com

Changes in metabolic parameters in blood of pigs after administration of probiotics

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Abstract: *The aim of our work was to evaluate the efficacy of probiotic preparation, which contain Bacillus licheniformis and Bacillus subtilis, on value of some indices of protein and energetic profile in blood of piglets. In the experiment were used 18 weaned pigs of the age 42 days divided into two groups. The first group was the experimental group and the second served as a control group. The trial lasted eight weeks and we collected blood for determination in fourteen-day periods. Probiotic preparation had not significant effect on value of parameters of protein profile, but we found out significant differences in levels of cholesterol and total lipids between groups on day 28 of the experiment. Probiotics significantly decrease cholesterol in blood of weaned pigs.*

Keywords: probiotics, weaned pigs, metabolic indices

I. Introduction

Probiotics are characterized as microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host [1]. In addition to traditional probiotics, based on the genera *Lactobacillus* and *Bifidobacterium*, probiotics based on representatives of the genus *Bacillus* are also used. Currently, 77 species in the genus *Bacillus* are known, of which the most commonly used as probiotics are: *coagulans, subtilis, clausii, cereus, toyoi*. *Bacillus licheniformis* is also used in agriculture to improve the health and increase the growth of pigs. The effectiveness of the spores as probiotics was confirmed by La Ragione et al. [2] when they were able to suppress all signs of infection after oral application of *Bacillus subtilis* to day-old chicks, at a dose of 2.5 x 10^8 CFU, following previous inoculation with *E. coli* 078:K80. After application of 10^8 CFU of *Bacillus subtilis* to mice, was found that *Bacillus subtilis* did not cross the membranes of the gastrointestinal apparatus and thus was safe. On the other hand, a higher number of *Bacillus subtilis* bacteria excreted than the inoculum administered indicates the germination and multiplication of probiotic spores [3]. Germination of *Bacillus cereus* var. *toyoi* spores in gut samples from broiler chickens and piglets was also confirmed by Jadamus et al. [4]. Germination of spores in the gastrointestinal tract appears to be essential for the potential probiotic effect, which is manifested by improved health and increased weight gains in livestock.

In our experiment, we investigated the effect of BioPlus 2B, containing probiotic strains of *Bacillus licheniformis* and *Bacillus subtilis*, on selected metabolic and immunological parameters.

II. Material and methods

Eighteen weaned pigs aged 42 days, crossbred Landrace x Large White, were included in the experiment. The experiment lasted eight weeks to three months of age. The experimental group (n=9) received BioPlus 2B (Christian Hansen's bio systems, Hørsholm, Denmark) mixed at a ratio of 1:1000 into the feed, so that the number of probiotic spores of *Bacillus licheniformis* and *Bacillus subtilis* was 10^9/kg of feed. The control group (n=9) did not receive BioPlus 2B in the feed. The pigs were fed starter feed until the 56th day of age and subsequently the complete pre-fattening feed until the end of the experiment.

Blood for selected parameters of protein and energy profile in blood serum was collected at two-week intervals, and for immunological examinations at four-week intervals. Blood was collected from *sinus ophtalmicus* [5], and in the case of samples for immunological examination, we used 1.5% EDTA solution in a ratio of 1:10 with blood as anticoagulant.

The protein and energy profile in blood serum was determined on a multiparametric spectrophotometric analyser Alizé, company Lisabio. The assays used for the determination were from Bio Merieux. For immunological parameters, we determined the metabolic activity of phagocytes according to the method of Lokaj and Oburkova [6] and the metabolic index of lymphocytes according to Bendixen et al. [7].

Statistical data were processed by Student's *t* - test. We determined significant differences at significance levels $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

III. Results

Total protein increased gradually during the experiment in both groups. Albumin levels were balanced throughout the experimental period within the reference norm. Blood serum urea increased gradually at each collection but was within the normal range for pigs. Creatinine levels were balanced throughout the experiment in both groups. We did not observe significant differences between the groups at each collection (Table 1).

Table 1: Levels of total protein, albumin and urea in blood serum

	Collection 0	Collection 1	Collection 2	Collection 3	Collection 4
TP – E (g/l)	55.8±2.88	55.8±5.34	52.7±2.33	60.5±3.43	60.4±4.79
CB – C (g/l)	58,.±3.33	58.1±7.18	55.7±3.8	63.7±3.44	61.1±4.63
Alb - E (g/l)	38.8±2.46	37.8±3.74	35.5±2.4	39.2±2.97	40.5±2.57
Alb - C (g/l)	39.9±1.67	38.6±4.15	35.1±2.1	38.6±2.86	39.0±2.7
Urea - E(mmol/l)	3.7±0.54	4.7±0.68	5.3±0.69	5.7±0.92	6.0±0.96
Urea - C (mmol/l)	4.1±0.63	5.0±0.87	5.8±0.57	6.1±0.89	5.4±0.59
Creatinine – E (µmol/l)	111.3±17.1	96±9.2	93.9±12	96.8±12.7	94.3±12.4
Creatinine – C (µmol/l)	107±13	92.9±11.4	97.2±10.6	91.2±11.1	90.3±6.9

Note: TP – total proteins, Alb. – albumin, E – experimental group, C – control group

Glucose levels were balanced in both groups at the upper limit of the reference standard for pigs. No significant differences were observed between groups. In cholesterol level, we found a significant difference between the groups in the 2nd sampling because cholesterol increased significantly in the control group. In the next sampling, the significant difference disappeared. During the experiment, cholesterol levels in both groups were within the reference normal range. For total lipids, we also observed a significant difference in the 2nd sampling (Table 2). In immunological parameters, we found no significant differences between the groups (Tab. 3).

Table 2: **Values of energetic profile in blood serum of weaned pigs**

	Collection 0	Collection 1	Collection 2	Collection 3	Collection 4
Glu - E (mmol/l)	6.34±0.75	6.51±0.83	6.67±0.73	6.61±0.34	6.84±0.57
Glu – C (mmol/l)	6.1±0.69	6.35±0.58	6.88±0.94	6.42±0.34	6.53±0.4
Chol – E (mmol/l)	2.37±0.23	2.34±0.23	2.34±0.3*	2.9±0.31	2.79±0.45
Chol - C (mmol/l)	2.35±0.41	2.37±0.57	2.82±0.46*	3.06±0,.1	2.65±0.38
TL – E (g/l)	2.83±0.81	2.33±0.61	2.48±0.52*	3.41±1.61	2.45±0.33
TL – C (g/l)	2.84±0.66	2.66±1.05	3.39±0.56*	3.74±1.49	2.29±0.2

Note: Glu – glucose, Chol – cholesterol, TL – total lipids, E – experimental group, C – control group, * – $p < 0,05$

Table 3: The metabolic activity of phagocytes and the metabolic index of lymphocytes

	Collection 0	Collection 2	Collection 4
MAP - E	2.71±0.31	2.66±0.27	2.81±0.17
MAP - C	2.75±0.32	2.53±0.31	2.65±0.25
MI - E	0.74±0.07	0.71±0.08	0.69±0.13
MI - C	0.72±0.07	0.73±0.10	0.71±0.12

Note: MAP - the metabolic activity of phagocytes, MI - the metabolic index of lymphocytes, E – experimental group, C – control group

IV. Discussion

In our experiment, we found no significant differences between groups for immunological parameters. On the contrary, in the work of Yu et al. [8] where they investigated the response of weaned pigs after *Bacillus licheniformis* feeding to intraperitoneal administration of lipopolysaccharide (LPS) at a concentration of 100 μg/kg, they found an improvement in immune parameters. Piglets were immunized 4 hours after LPS application. The results showed that prophylactic administration of *B. licheniformis* significantly attenuated LPS-induced intestinal mucosal damage. Supplementation with *B. licheniformis* enhanced immune function and suppressed the inflammatory response by increasing serum immunoglobulin IgA and mucosal IgA and IgG concentrations in jejunum and decreasing serum IL-6 and mucosal IL-1β. In addition, pre-treatment with *B. licheniformis* prevented LPS-induced intestinal damage by up-regulating the inflammatory mediator NLRP3.

Similarly, in the work of Li et al. [9] was found an improvement in immune parameters after application of *B. licheniformis*. The study investigated the effect of *B. licheniformis* on gut microbiota, metabolites and piglet health. The main findings were that this probiotic increased growth increments and improved the health status of the piglets. Specifically, it reduced serum levels of the pro-inflammatory cytokines IL-1β and TNF-α, while increasing IL-10 levels. In the intestine, *B. licheniformis* reduced the amount of pathogenic bacteria such as *Mycoplasma*, *Vibrio*, and increased butyrate-producing bacteria, including *Oscillospira*, *Coprococcus*, and *Roseburia faecis*, leading to increased butyric acid production. In addition, *B. licheniformis* effectively improved the metabolic status of the gut, allowing the gut microbiota to provide the host with improved metabolic capabilities for nutrient utilization. Also, in the experiment by He et al. [10], the probiotic-treated group showed significantly increased expression of claudin, Zonula Occludens 1 (ZO-1), and Interleukin 10 (IL-10) in jejunum and ileum, while exhibiting a remarkable decrease in interleukin 1β (IL-1β) expression. Overall, these findings suggest that *B. subtilis* may protect gut health by modulating the gut microbiota and its metabolites.

In our work, the potential cholesterol lowering after probiotic administration was confirmed. Three mechanisms of action of probiotics in lowering blood cholesterol levels are hypothesized. One pathway is the conversion of cholesterol in the gastrointestinal tract to coprostanol, which is then not absorbed. So far, only *Eubacterium* spp. are known to have this effect, but it is thought that more species of microorganisms are capable of this conversion. In germ-free mice, cholesterol levels are higher than in conventional mice [11]. The second cholesterol-lowering pathway is the deconjugation of bile acids that are formed from cholesterol, making their reabsorption more difficult and thus forcing their further synthesis in the liver. The deconjugation of bile acids in the intestine takes place only via bacterial enzymes. *Lactobacillus* and *Bifidobacterium* bacteria can deconjugate bile acids, and Gedek [12] found an increase in lactobacilli after BioPlus 2B treatment. A third mechanism for lowering cholesterol is the direct incorporation of cholesterol into the walls of *Lactobacillus*.

Reduction in blood cholesterol after administration of probiotics was also found by Kumar et al. [13]. They suggested that the indigenous *Lactobacillus plantarum* strain Lp91 has the potential to be explored as a probiotic in the treatment of hypercholesterolemia, as they discovered the hypocholesterolemic effect of *L. plantarum* in rats. In addition, Mohania et al. [14] found that supplementation with *L. plantarum* Lp9 may have therapeutic potential to reduce plasma and hepatic lipid levels and ameliorate diet-induced hypercholesterolemia in rats fed a hypercholesterolemic diet. Administration of the probiotic strain *Lactobacillus curvatus* HY7601, combined or not with *L. plantarum* KY1032, reduced plasma cholesterol levels and hepatic lipid content (triglycerides and cholesterol) in mice fed a high-fat, high-cholesterol diet [15].

V. Conclusion

Administration of a probiotic preparation containing probiotic strains of *Bacillus licheniformis* and *Bacillus subtilis* to weaned pigs stabilized serum cholesterol and total lipid (TL) levels compared to the control group. At the 2nd collection, we found a significant increase in cholesterol and TL in the control. The level of protein profile parameters in blood serum was not affected by the administration of probiotics.

VI. Acknowledgement

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0457 and VEGA 1/0040/24: Research on the prevalence, etiological and predisposing factors of gastric ulcerative changes in pigs.

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