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THE INFLUENCE OF PROBIOTIC *BACILLUS LICHENIFORMIS* ON SELECTED METABOLIC PARAMETERS OF CALVES

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ABSTRACT

The aim of the study was to evaluate the effect of *Bacillus licheniformis* administration on selected metabolic indices in blood of calves. We also compared phagocytic activity of neutrophils between groups. Eighteen calves, 7–8 days old, were included in the trial and they were divided into three groups. Experimental groups were given 200 ml of *Bacillus licheniformis* culture twice a day for ten days. Calves in the first group (n = 6) got culture at the concentration of $3x10^8$. ml^{-1} and the second experimental group (n = 6) got culture at the concentration of $3x10^7$. ml^{-1}. Control group (n = 6) got only milk. Blood samples were colected on the 0th, 2th, 5th and 10th day of the experiment. The *Bacillus licheniformis* significantly increased level of haemoglobin, values of packed cell volume (PCV), number of erytrocytes on day 10 of the trial. Level of total cholesterol in blood of the experimental groups was steady while cholesterol in the control group increased on day 10, which expressed significantly compared with experimental groups. Application of *Bacillus licheniformis* do not influenced index of phagocytic activity of neutrophils and level of minerals in blood.

KEYWORDS: *Bacillus licheniformis*, calves, metabolic indices, phagocytic activity.

INTRODUCTION

Probiotics are viable microorganisms in sufficient numbers capable of altering the microflora of the digestive tract of the host.[1] Several microorganisms, mainly lactic acid bacteria

(*Lactobacillus* spp., *Bifidobacterium* spp.) and few non-lactic acid bacteria (*Bacillus licheniformis*, *Bacillus subtilis*) are considered as probiotics.[2]

In order to reduce morbidity of calves, the main effort should be focused to diarrhoeic syndrome. Diarrhoeic syndrome results not only in direct losses due to deaths of animals, but also indirect losses – loss of weight, expensive therapy, and weight gains lower by up to 20 % during reconvalescence. In the past, in the prevention of diarrhoea, antibiotic premixes were frequently used, however, because of required reduction of antibiotic residua in the foods, pre- and probiotics are used nowadays.[3]

Although several experiments with *Bacillus* probiotics were done, their mode of action is not clear until now. To understand the mode of the action of *Bacillus* feed additives, the first step is to determine if the ingested spores germinate in the gut. Enzymes, antimicrobials and other substances can only be synthesized by growing vegetative cells, whereas immunomodulation and competition for adhesion could be accomplished by spores.[4]

The experiments showed that 70-90% of dietary-supplemented *Bacillus* spores germinate in the proximal part of the gastrointestinal tract and only limited outgrowth of the vegetative cell population occurs. Less spores and more vegetative cells were detected after 24 hours, but total counts increased only 2.14 fold compared to time zero. The two *Bacillus* strains can temporarily remain in the GI system, but will be unable to permanently colonize the GI tract.[5]

Although there are a lot of results about probiotics and their effect in prevention gastrointestinal disorders much less is known about influence of probiotics on values of metabolic indices in blood of animals. Our work was aimed at the study of effects of probiotic culture of *Bacillus licheniformis* in potentiation of calves' non-specific immunity, and examination of selected indices of haematological and mineral profiles.

MATERIAL AND METHOD

In the experiments, 18 calves (Holstein x Slovak spotted crossbreds, 7–8 days old) were used, divided into three groups. The first experimental group (n = 6) was given *Bacillus licheniformis* culture at the concentration of $3x10^8$. ml^{-1} and the second experimental group (n = 6) was given culture of *Bacillus licheiformis* at the concentration of $3x10^7.ml^{-1}$ in the milk; the control group (n = 6) was fed only the milk.

The lyophilised powder preparation contained $18x10^6$ *Bacillus licheniformis* per gram of lyophilisate. Prior to administration, either 0.1 g or 0.01 g of the lyophilisate was mixed with 1 L of milk and cultured at 37 °C for 10 hours. The final culture, which contained 3.10^{11}, respectively 3.10^{10} bacteria per l of milk, was given to calves in the first experimental group and the second group at the dose of 200 ml twice a day.

Blood samples were collected from jugular vein on 0th, 2th, 5th, 10th days. Phagocytic activity (PhA) was estimated with the use of L-hydroxymethyl-metacrylate particles according to Toman et al.[6] Haematological indices were analysed by automatic cell analyser SERONO 150 Plus (*Switzerland*). Blood concentrations of mineral elements were analysed by flame absorption spectrophotometry (A Analyst 100, Perkin Elmer, U.S.A.). Level of total cholesterol was analysed by automatic analyser ALIZE (Lisabio, France) with the use of Bio Merieux kits (France). The health state of the animals was checked every day. The results were analysed by Student's *t*–test.

RESULTS

In the first experimental group, haemoglobin (Hb) levels increased insignificantly from 8.8 $g.dl^{-1}$ to 9.3 $g.dl^{-1}$ during the experiment and in the 2^{nd} experimental group increased from 7.9 to 8.1 $g.dl^{-1}$. In the control group Hb level decreased significantly from 9.15 $g.dl^{-1}$ to 7.5 $g.dl^{-1}$. We recorded significant differences between the groups on the 10^{th} day.

During the experiment, values of packed cell volume (PCV) were quite steady. While in the 1st experimental group PCV ranged from 0.29 to 0.31 $l.l^{-1}$, in the 2_{nd} experimental group and in control group PCV slightly decreases at the end of experiment. On the 10^{th} day we found significant difference among the groups.

At some sampling intervals, numbers of erythrocytes (Er) even exceeded the reference values. Higher Er numbers we recorded in the first group, in which they increased from 8.7 $T.l^{-1}$ to 9.1 $T.l^{-1}$ and persisted till the end of experiment. In the 2^{nd} group number of Er was steady in the whole period of the trial. On the other hand, in the control group Er decreased from 9.5 $T.l^{-1}$ to 7.6 $T.l^{-1}$. In the control group, significant decrease was observed on 10^{th} day. Between the groups, significant difference was observed on 10^{th} day.

Numbers of leukocytes (Leu) increased in all groups during the experiment. In the first group, Leu number gradually increased from initial 10.6 $G.l^{-1}$ up to 15.1 $G.l^{-1}$ on 10^{th} day of the

experiment, in the 2nd group from 8.6 G.l^{-1} to 15.5 G.l^{-1}. In the control group Leu numbers were constant up to the 5th day of experiment and then increased suddenly on day 10. We found no significant difference (Tab. 1).

Phagocytic activity of neutrophils (PhAneu) was steady during the experiment without any significant differences between the groups. In all groups PhAneu decreased to 40-41 % on 2th day and later increased to 44–45 %. During the whole experiment we did not record any significant differences. In the experimental groups, steady cholesterol (Chol) concentrations were recorded. In the control group, cholesterol levels gradually increased till the end of experiment. We revealed significant differences on 10th day between experimental groups and control group (Tab. 2).

Table 1: Haematological profile in calves.

Parameter	0th day	2th day	5th day	10th day
Hb – 1st exp. group (g.dl^{-1})	8.8 ± 1.07	9.46 ± 0.84	9.05 ± 0.92	9.3 ± 1.03^{a}
Hb – 2nd exp. group (g.dl^{-1})	7.88 ± 1.07	7.4 ± 2.16	8.6 ± 0.83	8.12 ± 1.38
Hb – control group (g.dl^{-1})	9.15 ± 1.69	8.06 ± 2.08	8.5 ± 0.96	7.5 ± 1.32^{a}
PCV – 1st exp. group (l.l^{-1})	0.29 ± 0.02	0.31 ± 0.02	0.31 ± 0.04	0.31 ± 0.03 ^{a,b}
PCV – 2nd exp. group (l.l^{-1})	0.28 ± 0.13	0.25 ± 0.06	0.28 ± 0.03	0.24 ± 0.03^{a}
PCV – control group (l.l^{-1})	0.31 ± 0.07	0.25 ± 0.05	0.29 ± 0.04	0.24 ± 0.03^{b}
Er – 1st exp. group (T.l^{-1})	8.7 ± 0.92	9.2 ± 1.06	9.1 ± 0.94	9.6 ± 1.18 ^{a,b}
Er – 2nd exp. group (T.l^{-1})	7.3 ± 1.55	7.2 ± 1.5	7.3 ± 1.3	7.5 ± 0.87 ^{a}
Er – control group (T.l^{-1})	9.6 ± 1.68	7.44 ± 1.35	7.7 ± 1.23	7.6 ± 0.65 ^{b}
MCV – 1st exp. group	33.8 ± 2.12	33.5 ± 1.66	33.6 ± 1.8	32.5 ± 1.49
MCV – 2nd exp. group	33.8 ± 1.08	33.7 ± 1.12	33.75 ± 1.42	32.2 ± 2.15
MCV – control group	32.7 ± 2.84	33.6 ± 2.36	33.68 ± 2.77	30.1 ± 2.6
Leu – 1st exp. group (G.l^{-1})	10.9 ± 4.5	13.65 ± 3.37	12.3 ± 3.6	15.1 ± 6.2
Leu – 2nd exp. group (G.l^{-1})	8.58 ± 0.89	10.4 ± 1.5	10.7 ± 0.39	15.5 ± 6.9
Leu – control (G.l^{-1})	11.2 ± 6.65	11.8 ± 5.4	11.3 ± 5.5	15.2 ± 8.8

Note: ^{a,b} – significant differences between groups

Table 2: Values of phagocytic activity and cholesterol.

Parameter	0th day	2th day	5th day	0th day
PhAneu – 1st exp. group (%)	44.1 ± 9.6	41.0 ± 9.3	43.4 ± 9.5	44.7 ± 5.9
PhAneu – 2nd exp. group (%)	48.9 ± 10.0	41.8 ± 6.9	45.2 ± 9.1	45.3 ± 4.0
Phaneu – control group (%)	44.4 ± 7.6	40.3 ± 14.4	45.8 ± 6.7	44.4 ± 4.9
Chol – 1st exp. group (mmol.l^{-1})	1.58 ± 0.31	1.57 ± 0.21	1.58 ± 0.09^{a}	1.87 ± 0.14^{a}
Chol – 2nd exp. group (mmol.l^{-1})	1.87 ± 0.4	1.87 ± 0.4	1.94 ± 0.32^{a}	1.7 ± 0.32^{b}
Chol – control group (mmol.l^{-1})	1.59 ± 0.23	1.73 ± 0.31	1.87 ± 0.45	2.37 ± 0.4^{a,b}

Note: PhA neu – phagocytic activity of neutrophils; Chol – cholesterol

^{a,b}– significant differences between groups

In all groups, calcium (Ca) concentrations varied, but was in physiological range. In the beginning of the experiment, the lowest Ca concentrations were recorded in the first group. On 5th day, decrease in Ca concentrations was observed in all groups. Significant differences between the 1st and the 2nd experimental groups were observed only on 0th day of the experiment. In the first group, Ca concentration was 2.24 mmol.l^{-1} and in the 2nd group 2.47 mmol.l^{-1}; next sampling revealed no significant differences.

During the experiment, magnesium (Mg) concentrations ranged within the reference limits in all groups without any significant differences between the groups. Phosphorus (P) concentrations slightly decreased in all groups during the experiment. In the first group, the highest P concentration was recorded on 0th day – 2.9 mmol.l^{-1} and then P levels gradually decreased to 2.64 mmol.l^{-1} on 10th day, however the decrease was insignificant. In the 2nd experimental group P slightly decreased from 3.0 (day 0) to 2.73 (day 10). In the control group, P levels ranged from 2.9 to 2.56 mmol.l^{-1} (Tab. 3).

Table 3: Concentrations of minerals in the blood of calves.

Parameter	0th day	2th day	5th day	10th day
Ca – 1st exp. group (mmol.l^{-1})	2.24 ± 0.1^{a}	2.55 ± 0.12	2.13 ± 0.06	2.18 ± 0.06
Ca – 2nd exp. group (mmol.l^{-1})	2.47 ± 0.1^{a}	2.55 ± 0.1	2.21 ± 0.07	2.18 ± 0.05
Ca – control group (mmol.l^{-1})	2.35 ± 0.15	2.53 ± 0.06	2.24 ± 0.07	2.2 ± 0.05
P – 1st exp. group (mmol.l^{-1})	2.9 ± 0.22	2.7 ± 0.4	2.8 ± 0.29	2.64 ± 0.26
P – 2nd exp. group (mmol.l^{-1})	3.04 ± 0.55	2.86 ± 0.25	2.83 ± 0.12	2.73 ± 0.18
P – control group (mmol.l^{-1})	2.96 ± 0.24	2.92 ± 0.23	2.9 ± 0.29	2.56 ± 0.3
Mg – 1st exp. group (mmol.l^{-1})	0.88 ± 0.16	0.97 ± 0.14	0.84 ± 0.1	0.78 ± 0.1
Mg – 2nd exp. group (mmol.l^{-1})	1.05 ± 0.1	1.0 ± 0.11	0.89 ± 0.12	0.91 ± 0.21
Mg – control group (mmol.l^{-1})	0.92 ± 0.14	0.98 ± 0.05	0.86 ± 0.08	0.82 ± 0.05

DISCUSSION

Haematological indices (Hb, PCV, Ec) were significantly more favourable in the first experimental group compared with the control one. This is related to use of probiotics, which improve haematopoesis. Our results are similar to these reported by Herich et al.[7] who found significant increase in PCV and Hb values (compared with the controls) after 10 days of *Lactobacillus casei* administration to newborn piglets. Author Huska[3] reported significantly higher mean corpuscular volume (MCV) of erythrocytes in pigs after administration of *Lactobacillus reuteri.* In our trial we did not observed significant differences in MCV between groups. Koudela et al.[8] observed favourable effects of *Enterococcus faecium* administration on increasing of Hb concentrations in piglets and poultry.

In young, growing animals, concentrations of minerals strongly depend on their content in the milk. In our experiment Ca, P and Mg levels were in physiological range and significant differences between groups were not observed.

Cholesterol concentrations slightly increased on 10th day in the first experimental group and in the control group. We revealed significant differences control group in comparison with experimental groups. Some authors[9] found out that *Bifidobacterium longum* strain BL1 significant decreased serum concentration of total cholesterol, low-density lipoprotein cholesterol and triglycerids in rats. On the other hand administration *Streptococcus thermophilus* did not decrease these indices. It is supposes that in hyperlipidemic subjects, any effects that do occur result primarily in reductions in cholesterol, whereas in normal lipidemic subjects, effects on serum triglycerides are the dominant feature.[10] Decreasing cholesterol levels after administration of *Lactobacillus acidophilus* related to changes of micelles of cholesterol and its subsequent precipitation by bile acids was reported.[11]

Bacillus licheniformis and *Bacillus subtilis* produce proteases, amylase, and catalases. It has been proved that proteases produced by the genus *Bacillus* stimulate the growth of lactobacilli.[12] Similar was reported by Gedek[13] who observed increased numbers of lactobacilli after administration of *Bacillus* probiotics. Lactobacilli support resistance to pathogens through production of volatile fatty acids and thus decrease pH in the intestine.

Mojžišová et al.[19] reported significant increase in leukocyte phagocytic activity in fattening bulls after one-month administration of *Lactobacillus plantarum.* However, our experiment lasted shorter time and therefore immunostimulating effects of the probiotic could not be manifested.

CONCLUSION

We can conclude that *Bacillus licheniformis* significantly increased level of haemoglobin, values of PCV, number of erytrocytes on 10th day of the trial. Level of total cholesterol in blood of the experimental groups was steady while cholesterol in the control group increased on 10th day, which expressed significantly compared with experimental groups.

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