Praca oryginalna *Original paper*

Effect of a high dose of probiotic preparation on some blood indices of suckling piglets

ROBERT LINK, GABRIEL KOVÁČ

University of Veterinary Medicine, Košice 041 81, Komenského 73, Slovak Republic

Link R., Kováč G.

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Summary

The aim of the project was to observe the influence of a high dose of BioPlus 2B on selected parameters and mean weight gain of suckling piglets. The body weight of piglets was controlled and blood samples were taken on days 0, 7, 14, 21, 28 and 35 of the experiment. The red blood cells (RBC) increased gradually in both groups and at the end of the experiment, the number of erythrocytes in the experimental group was significantly higher ($P < 0.05$) compared with the control group. The level of urea in the control piglets began increasing from the fourth week of life. At the fifth, sampling the urea level in the control group was considerably higher and the difference between groups was significant ($P < 0.05$). By the week five the mean weight gains differed significantly between the groups ($P < 0.05$). The final mean weight of experimental piglets was 10.6 kg and the control piglets 9.9 kg.

Keywords: piglets, probiotics, blood indices

One of the principal priorities of increasing profitability of pig rearing is prevention of direct and indirect losses of suckling and weaned piglets. The most frequent causes of diarrhoea in piglets are *E. coli*, *Isospora suis* and rotavirus. Other important cause are *Clostridium perfringens* and agent of viral gastroenteritidis (6). At the present, the in-feed antibiotics are used to treat and prevent post-weaning diarrhoea and maintain uniformity of piglets in a pen (3). However, unprofessional administration of antibiotics can result in residues in meat and organs of slaughtered animals and may lead to resistance of micro-organisms. For example, in the 1970's enterococci were not considered important human pathogens and prior to 1986 they were in general considered susceptible to glycopeptides. However, shortly after the first observations of glycopeptide resistant enterococci this resistance spread rapidly world-wide (1). In 1993 they were isolated from food animals in England (4) and soon after that in Germany (12). The use of antibiotic growth stimulators had been banned in Sweden and Finland before admittance of these countries to EU and a little later Denmark also joined in this effort (22). The states mentioned initiated gradual elimination of antibiotic stimulators throughout EU.

Administration of probiotics is one of the alternative ways of potentiating the growth of suckling and weaned piglets and preventing the diarrhoeic syndrome. Probiotics are defined as biopreparations containing live cells or metabolites of stabilised autochtonous micro-organisms which affect the optimum colonisation and composition of gut microflora, stimulate digestive processes and immune responses of macroorganisms (8).

Probiotics should be administered as soon as possible after birth. Early exposure of the intestine to live micro-organisms and bacterial colonisation together with dietary antigens is very important for the development of the gut barrier (21). Administration of probiotics can influence the microflora composition by increasing the number of lactobacilli and other anaerobes (18). Dietary supply of probiotic bacteria stimulates the transport of target antigens through Peyer's patches (11).

Recently, in addition to conventional probiotics based on genera *Lactobacillus* and *Bifidobacterium*, preparations based on representatives of the genus *Bacillus* have come into the foreground. At present, we recognise 77 species belonging to the genus *Bacillus*, of which the following are used most frequently: *coagulans*, *subtilis*, *clausii*, *cereus*, *toyoi* (17). In the agricultural sector, *Bacillus licheniformis* has also been used to improve the health status of pigs and increase their weight gains.

The aim of the study was to determine the influence of a dose 3.2×10^{10} *Bacillus licheniformis* and *Bacil-*

lus subtilis in probiotic preparation BioPlus 2B on some indices of haematological, protein and energy profile and weight gains of suckling piglets.

Material and methods

Animals. The experiment was carried out on 16 piglets, Landrace × Slovak White cross-breeds, from two litters. The piglets were divided into two groups, experimental (n = 8) and control (n = 8). Each group consisted of three piglets from first litter and 5 from the second one. In each group there were five females and three males.

Experimental design. The investigations started after farrowing and lasted till weaning of piglets at the age of 35 days. During the experiment, the experimental piglets (n = 8) were given a probiotic preparation BioPlus 2B (Christian Hansen's bio systems, Hørsholm, Denmark) which consisted of equal proportions of *Bacillus licheniformis* and *Bacillus subtilis* at a concentration of $3.2 \times 10^9.g^{-1}$ of the preparation. Each experimental piglet received 10 g of the probiotic powder per day, i.e. 32×10^9 *Bacillus licheniformis* and *Bacillus subtilis*. The probiotic preparation was administered individually in the form of a solution prepared in distilled water.

No probiotic preparation was administered to the control piglets (n = 8).

During the trial, average daily weight gains, haematological, protein and energy indices in blood plasma of piglets were determined.

Blood sampling. Blood samples were collected on the first day of piglets lives (at birth), and subsequent samples were collected on days 7, 14, 21, 28 and 35 of the experiment. Blood was sampled from the eye sinus. Except for blood used for haematological examination, all blood samples for laboratory analyses were treated with heparin in order to obtain plasma. Blood samples for haematological examination were treated with 1.5% K_2-EDTA solution at a ratio of 1 : 10.

Laboratory test. Haematological profile was determined with a blood cell analyser Serono 150 and Dilutor 106 Plus (Switzerland). Protein and energy profiles were determined using a multi-parametric spectrophotometric analyser Alizé of firm Lisabio (France). Kits for individual determinations were obtained from Bio Merieux firm. Concentration of bilirubin was determined spectrophotometrically. Total serum immunoglobulins were determined by precipitation with $ZnSO_4$ splotion and subsequent measurement of produced turbidity.

Statistical evaluation. The obtained data were analysed on average value and standard deviation (SDE). They were also processed by unpaired Student t-test and the respective increase or decrease of particular parameters compared to the starting values was evaluated by paired Student t-test using $p < 0.05$ as the level of significance.

Results and discussion

All haematological parameters with the exception of mean corpuscular volume (MCV) were below the lower reference limit (7) at birth. In the course of the trial, the number of erythrocytes (RBC) increased gradually in both groups. At the last sampling (d 35) the number of RBC in the experimental group was significantly higher ($p < 0.05$) compared to the control. Haematocrit values were within the physiological range in both groups. The MCV of erythrocytes was slightly above the reference level (7), probably as a result of slightly lower number of RBC. A decrease in MCV was observed at the two final samplings when the number of RBC was increased. The differences in haemoglobin concentration between the groups were insignificant (tab. 1).

The influence of probiotics on haematological parameters differs depending on length of application and type of probiotics. Herich et al. (9) reported a significant increase in PCV and haemoglobin concentration in germ-free newborn piglets which were given *Lactobacillus casei* for ten days. In the experiment carried out by Morill et al. (15) on calves, the numbers of white and red blood cells were not influenced by supplement of probiotics. However, MCV of erythrocytes and mean corpuscular haemoglobin concentrations were different ($p < 0.01$) at d 10 (16).

The level of total proteins (TP) did not show any marked fluctuation. Albumin and the level of total immunoglobulins (TIg) increased at the first sampling

Tab. 1. Haematological profile of suckling piglets in particular days of life

Parameter	Treatment	Day of sampling					
		At birth	7th	14th	21st	28th	35th
RBC, $10^{12}.l^{-1}$	E	3.97 ± 1.08	3.95 ± 0.82	4.36 ± 1.45	4.76 ± 1.83	5.34 ± 0.76	6.28 ± 0.39*
RBC, $10^{12}.l^{-1}$	C	3.89 ± 0.84	4.02 ± 0.71	4.30 ± 1.32	4.63 ± 1.86	5.14 ± 0.74	5.77 ± 0.45*
HCT, $l.l^{-1}$	E	0.26 ± 0.05	0.28 ± 0.02	0.29 ± 0.05	0.30 ± 0.07	0.32 ± 0.01	0.37 ± 0.03
HCT, $l.l^{-1}$	C	0.24 ± 0.03	0.29 ± 0.04	0.29 ± 0.05	0.32 ± 0.09	0.32 ± 0.01	0.37 ± 0.03
MCV, $f.l^{-1}$	E	64.7 ± 6.22	72.6 ± 12.35	70.6 ± 15.02	65.8 ± 12.47	59.9 ± 8.58	59.4 ± 4.59
MCV, $f.l^{-1}$	C	61.7 ± 4.40	71.7 ± 8.12	70.1 ± 12.28	72.3 ± 13.42	63.5 ± 8.26	63.9 ± 6.43
WBC, $10^9.l^{-1}$	E	8.31 ± 1.16	21.55 ± 7.25	10.32 ± 2.33	10.12 ± 2.52	12.06 ± 3.05	13.71 ± 6.29
WBC, $10^9.l^{-1}$	C	7.90 ± 2.46	20.48 ± 6.38	11.34 ± 3.50	10.80 ± 3.71	10.18 ± 1.43	11.28 ± 1.50
Hb, $g.dl^{-1}$	E	7.72 ± 1.33	8.03 ± 1.29	9.80 ± 1.34	9.49 ± 0.74	10.60 ± 1.26	9.80 ± 0.74
Hb, $g.dl^{-1}$	C	7.68 ± 1.31	8.34 ± 1.27	9.81 ± 0.90	10.05 ± 0.71	10.39 ± 0.64	10.01 ± 0.52

Explanations: RBC – red blood cells; HCT – haematocrit; MCV – mean corpuscular volume; WBC – white blood cells; Hb – haemoglobin; E – experimental group; C – control group; * $p < 0.05$

Tab. 2. Protein profile in blood of suckling piglets in particular days of life

Parameter	Treatment	Day of sampling					
		At birth	7th	14th	21st	28th	35th
TP, g.l^{-1}	E	63.6 ± 6.60	62.9 ± 9.12	64.4 ± 9.55	59.0 ± 9.48	58.4 ± 10.69	64.4 ± 12.84
TP, g.l^{-1}	C	51.6 ± 12.19	59.4 ± 11.98	60.4 ± 10.82	54.3 ± 8.18	60.5 ± 13.58	63.0 ± 14.25
TIg, UZST	E	17.64 ± 5.20	19.93 ± 2.75	20.10 ± 2.24	18.75 ± 2.52	18.09 ± 1.56	18.10 ± 1.39
TIg, UZST	C	14.38 ± 6.21	16.78 ± 3.52	17.96 ± 3.52	17.06 ± 2.50	17.70 ± 2.33	17.44 ± 1.63
Alb, g.l^{-1}	E	14.46 ± 1.70	28.01 ± 2.67	35.34 ± 3.18	37.22 ± 3.57	34.62 ± 3.15	37.30 ± 2.75
Alb, g.l^{-1}	C	13.41 ± 1.46	28.14 ± 2.53	34.54 ± 2.33	34.68 ± 2.56	35.86 ± 3.27	37.32 ± 2.86
Urea, mmol.l^{-1}	E	4.51 ± 0.61	2.59 ± 0.89	2.45 ± 1.21	2.41 ± 1.31	2.82 ± 0.71	2.64 ± 0.69*
Urea, mmol.l^{-1}	C	4.57 ± 0.74	2.75 ± 1.20	2.52 ± 1.16	2.46 ± 0.97	3.44 ± 1.76	3.82 ± 1.36*

Explanations: TP – total proteins; TIg – total immunoglobulins; Alb – albumin; * $p < 0.05$; E – experimental group; C – control group

and then remained relatively stable until the end of the experiment (tab. 2).

Other authors presented frequently controversial results concerning the influence of probiotics on immunological parameters. It was found that probiotics increased phagocytic activity after ten-day administration to gnotobiotic piglets (9). On the other hand, concentration of immunoglobulins did not differ significantly after 10 day application of 2 g of *Lactobacillus paracasei* to newborn piglets (10).

The plasma urea level was similar in both groups within 3 weeks of piglets' lives (tab. 2). In experimental group it remained almost constant till the end of the experiment, whereas in control group piglets from 4th week this parameter began to increase and in 5th week the difference in comparison with experimental group was significant ($p < 0.05$).

Samanya and Yamauchi (19) revealed, that also chickens fed dried *Bacillus subtilis* var. *natto* for 28 days, had decreased blood ammonia concentration in the experimental group ($p < 0.05$). These results suggest that *B. subtilis natto* was responsible for decreased ammonia concentration.

The results reported by Osadchaia (16) point to the influence of composition of rations on potentiation of production of amino acids or saccharides. The high content on nitrogen substances in mixed feed for piglets supports production of amino acids in the digestive tract and production of urea is decreased under the action of probiotic bacteria.

With the exception of the 1st day of live, plasma glucose was slightly above the reference value (5). Total cholesterol (TCh, mmol.l^{-1}) increased from 1.49 at birth in both treatments to 4.6 and 5.05 (experimental and control group, respectively) at day 14 of life. Since the 3rd week of life, TCh decreased to about 3.9 in both groups and remained relatively constant (tab. 3).

The initial mean weight of piglets from the experimental and control group was similar in both groups (tab. 4). At the end of the experiment the mean weight

Tab. 3. Level of glucose and total cholesterol in blood of suckling piglets in particular days of life

Parameter	Treatment	Day of sampling					
		At birth	7th	14th	21st	28th	35th
Glu, mmol.l^{-1}	E	6.01 ± 1.45	7.33 ± 0.84	8.06 ± 0.66	6.66 ± 0.39	7.16 ± 1.56	7.04 ± 0.63
Glu, mmol.l^{-1}	C	5.44 ± 1.72	7.06 ± 1.62	8.51 ± 0.84	6.64 ± 1.44	6.90 ± 0.62	7.32 ± 1.01
TCh, mmol.l^{-1}	E	1.49 ± 0.68	3.93 ± 0.68	4.60 ± 0.85	3.88 ± 1.20	3.94 ± 1.92	3.89 ± 2.55
TCh, mmol.l^{-1}	C	1.49 ± 0.73	3.78 ± 0.61	5.05 ± 1.28	3.93 ± 0.89	3.57 ± 0.84	3.34 ± 0.67

Explanations: Glu – glucose; TCh – total cholesterol; E – experimental group; C – control group

Tab. 4. Body weight (BW) and average daily gains (ADG) of suckling piglets in particular weeks (kg)

Parameter	Treatment	Day of sampling					
		At birth	7th	14th	21st	28th	35th
BW	E	1.56 ± 0.39	2.74 ± 0.62	4.50 ± 0.77	6.14 ± 1.11	8.27 ± 1.46	10.60 ± 1.73
BW	C	1.55 ± 0.32	2.71 ± 0.74	4.30 ± 1.13	6.06 ± 1.37	8.23 ± 1.87	9.92 ± 1.59
ADG	E		0.168 ± 0.05	0.252 ± 0.04	0.234 ± 0.06	0.304 ± 0.05	0.332 ± 0.05*
ADG	C		0.171 ± 0.05	0.227 ± 0.06	0.252 ± 0.04	0.309 ± 0.08	0.242 ± 0.08*

Explanations: E – experimental group; C – control group; * $p < 0.05$

gains of experimental piglets were equal to 0.332 kg.day^{-1} and those of control group reached only 0.242 kg.day^{-1} (tab. 4). The difference was significant ($p < 0.05$).

In other studies, chickens fed dietary *B. subtilis natto* for 28 days had also a tendency to display greater growth performance (19). In the experiment carried out by Alexopoulos et al. (2), the probiotic preparation BioPlus 2B increased weight gain and improved health status of piglets and sows which were administered this preparation before and after farrowing.

Increased mean daily weight gains in experimental group of piglets may have resulted from better utilisation of nutrients as *Bacillus licheniformis* and *Bacillus subtilis* produce amylases, proteases and lipases. Additional cause of better weight gains may have been the preventive effect of probiotics against diarrhoea. In the trial with probiotics, contained equal proportion of *Bacillus licheniformis* and *Bacillus subtilis*, was revealed that all groups supplemented with probiotics exhibited a reduced incidence and severity of diarrhoea (14).

The weight of experimental piglets in the experiment was within the standard range (13) while the weight of control piglets after two and three weeks of the experiment was close to the lower standard limit. By the end of the experiment the body weight of piglets corresponded to the medium level of growth intensity (20), with mean body weight reaching 10.6 kg in experimental piglets and 9.9 in the control ones.

The experiment proved that administration of probiotics increases weight gains of piglets, improves utilisation of nutrients, particularly of feed proteins, which is reflected in lower concentration of urea in blood plasma of experimental piglets. Erythropoiesis was also better in the experimental group which could contribute to better condition and health of experimental animals.

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Author's address: MVDr. Robert Link, University of Veterinary Medicine, Komenskeho 73, Košice, 041 81, Slovakia; e-mail: robolin65@hotmail.com