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EFFECT OF THE PROBIOTIC "LACTINA" ON SOME BIOLOGICAL PARAMETERS AND NONSPECIFIC RESISTANCE IN NEONATAL PIGS**

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Abstract: Twenty four pregnant Danube white sows were allocated to 4 groups. All animals were fed basal diet formulated to meet their nutrient requirements. In addition to the basal diet, the sows from the II group were given the probiotic "Lactina" (500 g/ton) from the 5 day of pregnancy until the weaning of their offspring (35 days of age) and their piglets were offered 800 g/ton Lactina during the pre-starter period and 500 g/ton throughout the starter period. The probiotic was only supplemented to the sows in the III group and to the piglets of the IV group in the same quantities as those in the second group.

Both sows and piglets diets in the I (control) group were not supplemented with Lactina. Blood samples were taken from 20 randomly chosen piglets (5 in each group) at 5 and 35 days of age.

The probiotic Lactina, supplemented both to the sows and piglets diets (II group) , increased complement activity in the piglets ($P<0.05$) at 5 days of age compared to control group, while the addition of Lactina to sows only (III group) or to piglets only (IV group) did not produce significant effect.

Supplemental Lactina decreased plasma cholesterol level ($P<0.05$) at 35 days of age in II group of piglets. Plasma indol level declined in the piglets of the II group at the age of 35 days, while urea level were not changed in any of the Lactina supplemented groups, with the exception of the IV group where the piglets had higher ($P<0.001$) urea level at 5 days of age.

Taken together our results indicate that the beneficial effect of Lactina on the nonspecific response and on the studied biological parameters is most emphasized when the probiotic is supplemented both to the sows and piglets diets.

Besides, Lactina stimulated complement activity at the beginning of the neonatal period (5 day) and had no effect at the end of the neonatal period (35 day).

Introduction

Despite the immense amount of literature on health benefits of probiotics, there is not yet a clear picture of their effect, because of the diversity of microorganisms contained in each commercial probiotic and also because of the animal species, used in the experimental models. Probiotics have been found to stimulate nitrogen utilization (*Mohan et al., 1996*) and to lower serum (*Jin et al., 1998*) and yolk (*Haddodin et al., 1996*) cholesterol concentration in broilers and hens. Plenty of studies demonstrate the ability of probiotics to stimulate immunity (*Perdigon et al., 1995; Park et al., 2002*) and support cellular health by inhibiting the same enzymes that convert non–harmful pro-carcinogens into mutagens (*Gorbash, 1979; Wolowski et al., 2001*).

The objective of the present study was to investigate the effect of probiotic "Lactina" on nonspecific resistance and plasma levels of cholesterol, urea and indol in neonatal pigs.

Material and methods

Twenty four pregnant Danube white sows were allocated to 4 groups. The sows were kept in stalls equipped with a feeding trough and nipple waterer. The stalls had solid concrete floors.

All sows were fed concentrate mix, formulated to meet established nutrient allowances.

The piglets were fed pre-starter and starter diets. In addition to their basal diet some of the sows were given the probiotic "Lactina" (500 g/ton) from the day (d) of pregnancy until the weaning of their offspring (35 d of age). The piglets were offered Lactina during the pre-starter (800 g/ton) and starter (500g/ton) periods.The probiotic was given orally (10 g/100 ml distilled water from 1 to 14 d of age. Besides the piglets were offered Lactina supplemented pre-starter from 5 d of age onwards.

The probiotic Lactina contained lyophilized species of *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus lactis, Streptococcus thermophilus, Enterococcus faectum.*

Lactina was supplemented to the basal diets of sows and their offspring according to the corresponding group as shown in the following experimental scheme:

Groups/Grupe	I	II	III	IV
Sows/Krmače	-	+	+	-
Piglets/Prasad	-	+	-	+

***Legends**: (-) sows and piglets in the respective groups did not receive supplemental Lactina; (+) sows and piglets in the respective groups were given supplemental Lactina*
***Legenda**: (-) krmače i prasad u respektivnim grupama bez dodatnog probiotika Lactina; (+)krmače i prasad u respektivnim grupama sa dodatnim probiotikom Lactina*

Five randomly chosen piglets from a single litter in the corresponding groups were sampled by puncture of *v. jugularis* at 5 and 35 days of age (immediately before weaning).

All samples were taken at 9h, within 5 min from the onset of the immobilization of each piglets in order to reduce stress eliciting effect of the handling.

The nonspecific resistance was evaluated by the complement hemolytic activity, determined by the methods of *Grislova et al*.(1978). Plasma cholesterol levels were measured by the method of Watson (1960). Plasma urea and indol levels were assayed as described by *Rerat et al.* (1976) and *Balahovskii* cited by *Chilov,* (1959), respectively.

Results and discussion

Serum complement activity was significantly higher ($P<0.05$) in the II group of piglets at 5 d of age and tended to be higher ($P>0.05$) in the other experimental groups (III and IV) at the same age , compared to control piglets (Fig. 1). Serum complement activity at 35 d of age, unlike that at 5 d of age, was within the range of control piglets in the II and III groups and tended to be lower in IV group.

These results indicate that the effect of Lactina on complement activity is most pronounced during the first days of neonatal life, when piglets are more vulnerable to detrimental environmental factors and are more likely to develop gastrointestinal infections. The observed effect of Lactina on complement activity at 5 d was most probably due to the fact that the palliative effect of the probiotics is evident only if the resident flora is insufficient and if the microorganisms, contained in the probiotic have immunomodulatory properties that are equivalent to those of resident intestinal flora (*Moreau and Gaboriau – Routhtan, 1991; Cebra,1999*). This assumption is consistent with the established lack of stimulatory effect of the probiotic on complement activity at 35 d of age. It is expected that intestinal flora at that time is dominated by

friendly bacteria, thus procuring a beneficial effect on both innate and acquired immunity (*Bourliox et al., 2003*).

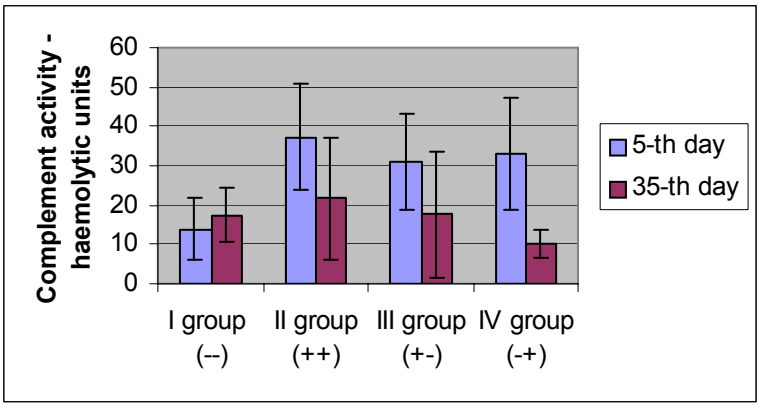

Figure 1. Effect of Lactina supplemented to the diet of sows and piglets alone or to both of them on complement haemolytic activity
Slika 1. Efekat probiotika Lactina dodatog u obroke za krmače i prasad, pojedinačno, ili u obroke za obe grupe na njihovu hemolitičku aktivnost

The very fact that the most spectacular stimulatory effect of Lactina on complement activity is established in II group of piglets suggests that the beneficial effect of Lactina at 5 d of age is more pronounced when Lactina is supplemented both to sows (during pregnancy and lactation) and piglets diets.

The obvious beneficial effect of Lactina on complement activity at 5 d of age supports the view that piglets intestinal flora at that age is not stable and it is known that the quality of resident flora has a crucial effect on the intestinal immune system (*Bourlioux et al., 2003*).

Plasma cholesterol level tended to be lower at 5 d of age and was significantly lower at 35 days of age ($P<0.05$) in the group of piglets as compared with the control piglets (Fig.2). Similar trend, although less emphasized, was observed in the III group of piglets. Plasma cholesterol level in IV group of piglets, unlike that of the other 3 group, tended to be higher.

The observed cholesterol lowering effect of Lactina is consistent with the results of *De Smet et al.* (1998) who found significant lowering of LDL-cholesterol concentration and no change in HDL – cholesterol in pigs fed Lactobacillus supplemented diet.

Presently it is assumed that the cholesterol lowering effect of the probiotics is due to the ability of many intestinal species to resist the detergent – like anti-microbial properties of bile salts by hydrolysis of conjugated bile salts. Bile salts are continuously present in gastrointestinal tract via enterohepatic

circulation (*Bahar and Andrew, 1999*). They are synthesized mainly from cholesterol. Therefore their hydrolysis by the probiotic bacteria could lead to decline of plasma cholesterol level, because of the enhanced cholesterol requirements for bile salt synthesis (*Geun-Bae Kim et al., 2004*).

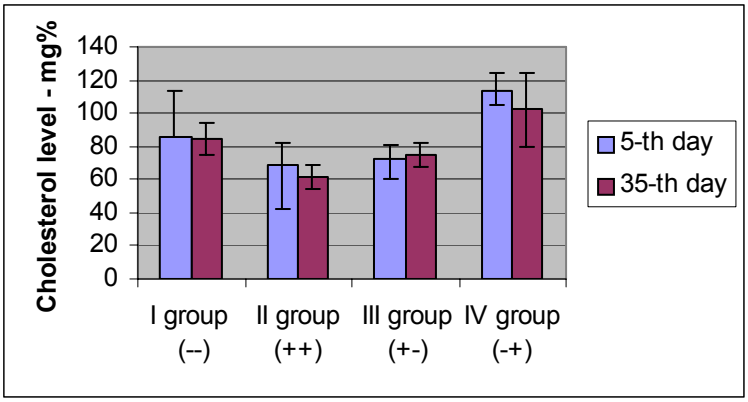

Figure 2. Effect of Lactina supplemented to the diet of sows and piglets alone or to both of them on plasma cholesterol levels
Slika 2. Efekat probiotika Lactina dodatog u obroke za krmače i prasad, pojedinačno, ili u obroke za obe grupe na nivoe holesterola u plazmi

Our results have shown that cholesterol – lowering effect of Lactina is most pronounced when it is supplemented both to sows and piglet diets. This effect was less pronounced when Lactina was only supplemented to sows and was absent when Lactina was only supplemented to piglets diets both at 5 and 35 days of age.

Plasma urea level in the piglets of II group tended to be lower at 5 days of age in comparison with that of control piglets and (Fig. 3) was not changed in the piglets of III group. Plasma urea level in the piglets of IV group, unlike the other groups was significantly higher at 5 days of age ($P<0.001$).

There were no significant differences in plasma urea level between I, II and III group at 35 days of age.

These findings come to show that the urea – lowering effect of Lactina, although not significant is only present at the beginning of the neonatal period, when floral colonization of the gastrointestinal tract progresses and intestinal flora are fragile and not well diversified. The fact that Lactina had no effect on urea level at 35 days of age was probably due to improved quality and stability of the intestinal microflora at that time. Our interpretation of the observed trend of lower urea level in Lactina supplemented piglets is consistent with the

established urea hydrolysis by microbial urease in pigs (*Metges, 2000*). Furthermore the probiotics have been found to regulate the beneficial effect of intestinal bacteria (MacFarlane and Cummings, 1999). In addition it has been established that the microbial lysine and threonine contribution to the host homeostasis ranged from 8 to 17 % and from 5 to 21 % , respectively (*Metges, 2000*).

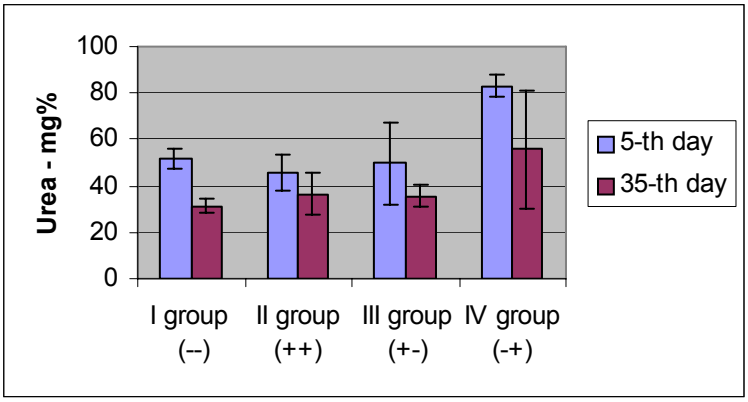

Figure 3. Effect of Lactina supplemented to the diet of sows and piglets alone or to both of them on plasma urea levels
Slika 3. Efekat probiotika Lactina dodatog u obroke za krmače i prasad, pojedinačno, ili u obroke za obe grupe na nivoe uree u plazmi

The increased urea level both at 5 and 35 d ($P>0.05$) of age in the piglets of the IV group could be due to gastrointestinal problems.

The plasma indol level was significantly lower ($P<0.05$) in the second group of piglets at 35 d of age while at 5 d of age the lowering effect of Lactina was not significant (Fig. 4). Plasma indol level in III group tended to be lower both at 5 and 35 d of age as compared to the control group.

Our data suggest that the used probiotic improves the quality of the intestinal microflora which in turn reduces the “unfriendly” bacteria, including those responsible for indol production.

Plasma indol level in the IV group of piglets was significantly higher ($P<0.05$) than that of control animals. The increased indol level in this group of piglets coincides with the increased cholesterol (Fig.2) and urea levels and confirms once again our assumption that gastrointestinal function in these pigs was compromised.

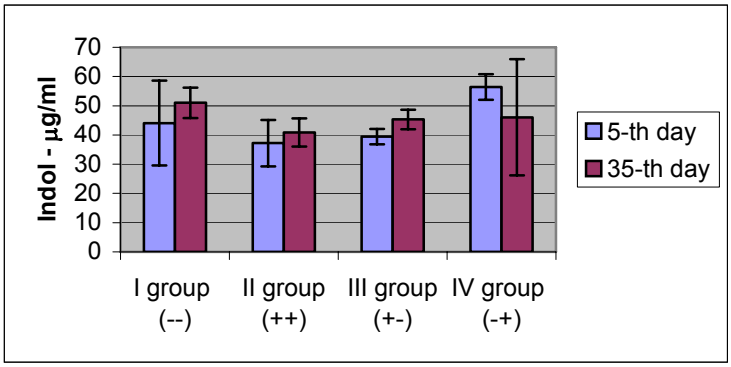

Figure 4. Effect of Lactina supplemented to the diet of sows and piglets alone or to both of them on plasma indol levels
Slika 4. Efekat probiotika Lactina dodatog u obroke za krmače i prasad, pojedinačno, ili u obroke za obe grupe na nivoe indola u plazmi

Conclusion

We have provided evidence showing that the effect of Lactina on complement activity is most pronounced at 5 d of age, when it is supplemented both to sows and piglets diets. Lactina has no effect on complement activity at 35 d of age. Supplementation of Lactina to both sows and piglets produces significant ($P<0.05$) reduction of plasma cholesterol level at 35 days of age. The cholesterol – lowering effect of Lactina is observed as a trend when Lactina is supplemented to sows diet only. Lactina caused significant ($P<0.05$) reduction of plasma indol level at 35 days of age when supplemented both to sows and piglets, but has no effect on plasma urea levels irrespective of the supplementation scheme.

Lactina supplementation is less effective, when given to piglets diet only.

Efekat probiotika "Lactina" na neke biološke parameter i nespecifičnu otpornost kod neonatalnih svinja

D. Gudev, S.Popova-Ralcheva, P. Moneva, M. Ignatova

Rezime

Dvadesetčetiri suprasne krmače dunavske bele rase su podeljene u 4 grupe. Sve životinje su hranjene osnovnim obrokom koji je formulisan da zadovolji sve njihove potrebe za hranljivim materijama. Svinje iz grupe II su u obroku dobijale i probiotik "Lactina" (500 g/t) od 5 dana gestacije do odbijanja njihovog potomstva (u uzrastu od 35 dana) a njihova prasad su dobijala 800 g/t Lactina probiotika tokom pred-starter perioda i 500 g/t tokom starter perioda. Probiotik je takođe dodavan u obroke samo za krmače III grupe i samo za prasad IV grupe u istim količinama kao u drugoj grupi.

Obroci za krmače i prasad u grupi I (kontrola) nisu dopunjavani probiotikom. Uzorci krvi su uzimani od 20 slučajno odabrane prasadi (5 u svakoj grupi) u uzrastu od 5 i 35 dana.

Probiotik Lactina, kojim su dopunjavani obroci za krmače i prasad (grupa II) uticao je na povećanje aktivnosti komplementa kod prasadi ($P<0.05$) u uzrastu od 5 dana u poređenju sa kontrolom, dok je dodavanje probiotika Lactina samo obrocima za krmače (grupa III) ili samo obrocima za prasad (grupa IV) nije imalo signifikantan uticaj.

Dodatak probiotika lactina je uticao na smanjenje nivoa holesterola u plazmi ($P<0.05$) kod prasadi u uzrastu od 35 dana u grupi II. Nivo indola u plazmi se smanjio kod prasadi II grupe u uzrastu od 35 dana, dok je nivo uree ostao nepromenjen u svim grupama hranjenim obrocima sa dodatkom lactina probiotika, sa izuzetkom grupe IV gde su prasad imala viši nivo uree ($P<0.001$) u uzrastu od 5 dana.

Rezultati ukazuju da je povoljan uticaj probiotika lactina na nespecifičnu reakciju i ispitivane biološke parameter najizraženiji kada je probiotik dodavan u obroke za svinje i prasad.

Takođe, probiotik Lactina je stimulisao aktivnost kompetenta na početku neonatalnog perioda (5 dana) i nije imao uticaj na kraju neonatalnog perioda (35 dana).

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