Bulgarian Journal of Agricultural Science, 18 (No 1) 2012, 112-116
Agricultural Academy

PERIPHERAL BLOOD LEUCOCYTES DISTRIBUTION AND ADRENAL FUNCTION IN GROWING PIGS, FED PROBIOTIC SUPPLEMENTED DIET*

D. GUDEV[1], S. POPOVA-RALCHEVA[2], P. MONEVA[1] and M. IGNATOVA[1]
[1]*Institute of Animal Science, BG – 2232 Kostinbrod, Bulgaria*
[2] *Agricultural Academy, Institute for Information Serving of the System, BG – 1113 Sofia, Bulgaria*

Absrtract

GUDEV, D., S. POPOVA-RALCHEVA, P. MONEVA AND M. IGNATOVA**,** 2012. Peripheral blood leucocytes distribution and adrenal function in growing pigs, fed Lactina supplemented diet. *Bulg. J. Agric. Sci,* 18: 112-116

The objective of this study was to evaluate adrenal status, peripheral blood leucocytes distribution and activity of the enzymes (AST) and (ALT) in piglets during the neonatal period.

Pregnant Danube white sows (n=24) were allotted to 4 groups as follow: the sows and their piglets did not receive supplemental probiotic („Lactina"); both the sows and piglets diets were supplemented with probiotic; the supplemental probiotics was only given to the sows (their piglets were deprived of „Lactina"); the supplemental probiotic was only given to the piglets. The diet for sows was supplemented with „Lactina" (500 g/ton) from the 85 day of pregnancy until weaning of their piglets (35 days of age). Piglets were given 800 g same probiotic per ton diet during the pre-starter period and 500 g/ton during the starter period.

Probiotic „Lactina" had no significant effect on plasma cortisol level, neutrophil/ lymphocyte ratio and aspartate aminotransferase activity at both 5 and 35 d age. Alanine aminotransferase activity was higher in II group at 5 ($P>0.05$) and 35 d of age ($P<0.01$). It is concluded that the increased ALT activity in the second group of piglets was due to psychological stress induced by the screaming of the previously handled pigs during the sampling.

Key words: cortisol, neutrophil/lymphocyte ratio, aspartate aminotransferase activity, neonatal piglets

Introduction

The investigations during the recent years have demonstrated that the nutritive antibiotics can be successfully replaced by probiotics as growth promoters in animal husbandry (Grigorova et al., 2004; Stoyanov, 2005). The probiotics, based on lactic acid bacteria have been found to boost immune function. (Bourliox et al., 2002) and protect against unfriendly bacteria (Majamaa et al., 1995). Furthermore, some studies have shown that ingestion of viable probiotics is associated with anticancerogenic effects (Wollowski, 2001).

It is well known that the two protective systems of all mammals (immune and hypothalamic-pituitary-adrenal axis) are in a permanent relationship - each system exerting control on the other.

However, to our knowledge there is no evidence about the effect of probiotics on adrenal function. In the current study, we investigated peripheral

**This study was financially supported by Science Research Fund in Bulgarian Ministry of Education and Science*
E-mail: simona_raltcheva@abv.bg

blood leucocyte distribution and adrenal function in pigs during the neonatal period, when pigs are most sensitive to detrimental environment factors.

Material and Methods

Pregnant Danube white sows (n=24) were allocated to 4 groups. The sows were kept in stalls equipped with feeding troughs and nipple watered. The stalls had solid concrete floors. All sows were fed on 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 85 day of pregnancy until the weaning of their offspring (35 d). Some of the piglets were offered „Lactina" (during the pre-starter period (500 g/ton diet). The probiotic „Lactina" (contains lyophilized species of *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus faectum).*

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

Groups	I	II	III	IV
Sows	-Lactina	+ Lactina	+ Lactina	-Lactina
Piglets	-Lactina	+ Lactina	-Lactina	+ Lactina

Piglets were given probiotic orally (10 g/100 ml distilled water) from one to 14 days of age. Besides, piglets were offered „Lactina" as supplemented pre-starter from 5 d of age onwards. Five randomly chosen piglets from a single litter of the corresponding groups were sampled by puncture of *v. jugularis* at 5 and 35 d of age immediately before weaning.

All samples were taken at 9 h within 5 min. from the onset of the immobilization of each piglet in order to reduce stress-eliciting effect of the handling. Adrenal function was assessed by radioimmunoassay (Kanchev et al., 1976). Leucocytes were counted microscopically in smears made after staining with Giemsa-Romanovski. Aspartate aminotransferase and alanine aminotransferase were determined by the methods of Berymeyer and Bernt (1963).

The results of the statistical analysis are expressed as means ± S.E.M and were analyzed by ANOVA. When the results were statistically processed by the use of two factors analysis the difference was less than 3%.

Results and Discussion

The diet supplemented with probiotic „Lactina" had no significant effect on adrenal function, as judged by plasma cortisol level, at the beginning (5th d) and in the end (35 d) of the neonatal life (Figure 1). However, plasma cortisol levels tended to be higher at both 5th and 35th d in II group of piglets. The observed trend of higher cortisol level in these pigs could be due either to increased stress susceptibility in some individuals or to „Lactina"-induced enhancement of adrenal response. The second assumption is less probable, since not all piglets had increased cortisol level compared to their control counterparts (II group) at both 5 and 35 d of age. Therefore, the most likely reason for the increased cortisol level (P>0.05) in II group of piglets is the increased stress-sensitivity of some individuals to the alarm-inducing screams of the

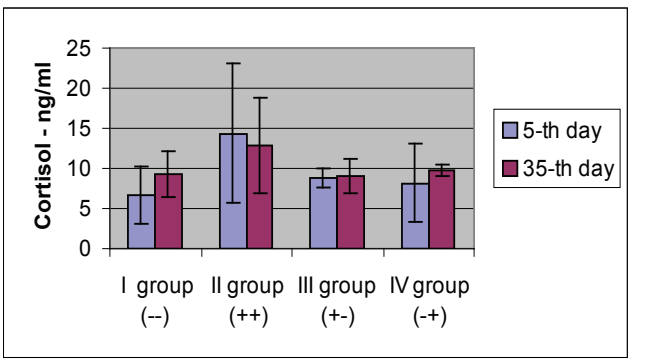

Fig. 1. Plasma cortisol level in neonatal piglets, fed "Lactina" supplemented diet

previously handled piglets during the blood sampling. This assumption is further supported by the percentages of leucocytes subpopulations in the peripheral blood (Figure 2). There is no doubt that the observed increase of cortisol level in the piglets of second group was caused by the alarm – inducing screaming of the previously sampled piglets. Our view is based on the fact, that cortisol enhancement induces significant decrease in numbers and percentages of lymphocytes and an increase in numbers and percentages of neutrophils (Dhabhar et al., 1995), which take place at least 1 h after the start of the stress-eliciting stimuli. However, the ratio neutrophils/lymphocytes (Figure 3) did not indicate any stress in the II group. Therefore, the observed trend of cortisol elevation in the II group was most probably due to stress – inducing stimuli initiated in less than an hour before the sampling of the respective piglets. In addition, the highest rate of cortisol enhancement in some individuals of the II group did not exceed normal cortisol range more than twice, which is indicative for low level of stress load. Our interpretation is consistent with the neutrophil/ lymphocyte ratio (Figure 3), which was lowest in the II-nd group of pigs, both at 5 and 35 d of age, compared to that of the rest groups. The relative neutrophil/lymphocyte ratio is considered as a sensitive and reproducible method for stress evaluation (Jong et al., 2002; Post and Rebel, 2003). Therefore the observed reduction in neutrophil/lymphocyte ratio, confirms our assumption that the increased cortisol level in II group was due to a short – term psychological stress and that cortisol, when used as a single stress indicator might mislead the researcher.

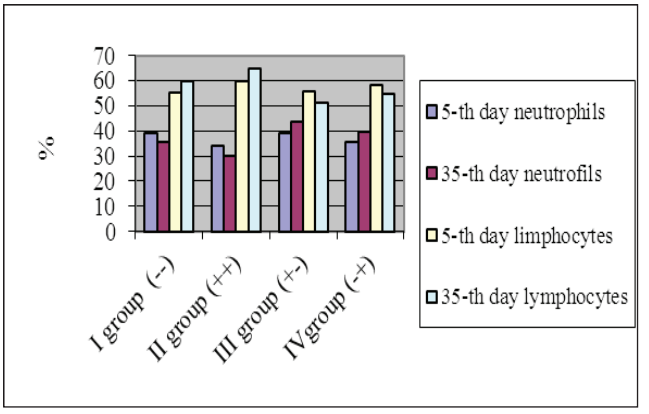

Fig. 2. Neutrophils and lymphocytes in the blood of neonatal piglets, fed "Lactina" supplemented diet

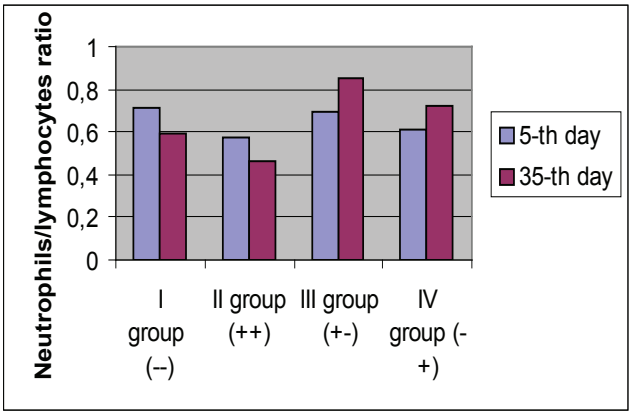

Fig. 3. Neutrophils and lymphocytes ratio in the blood of neonatal piglets, fed "Lactina" supplemented diet

Our view, that the observed trend of cortisol enhancement in the II group of piglets was attributable to higher stress sensitivity of some individuals is further supported by the activity of the enzymes aspartate aminotransferase (Figure 4) and alanine aminotransferase (Figure 5).

Aspartate aminotransferase activity tended to be higher in II group of piglets at 5 days of age

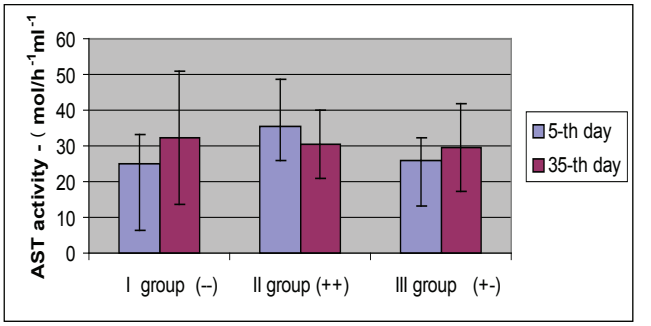

Fig. 4. Serum AST activity in the blood of neonatal piglets, fed "Lactina" supplemented diet

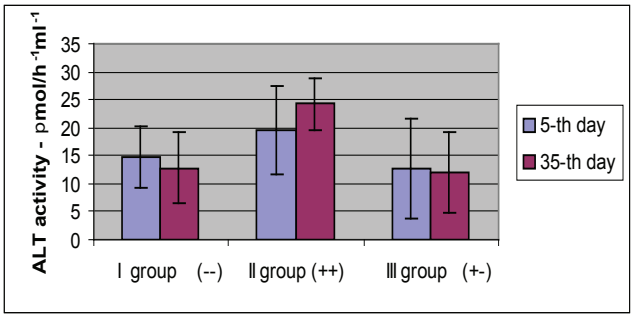

Fig. 5. Serum ALT activity in the blood of neonatal piglets, fed "Lactina" supplemented diet

compared to that in control animals. Alanine aminotransferase activity was significantly higher ($P<0.001$) in II group of piglets at 35 days of age. The increased ALT activity coincided with the observed trend of higher cortisol levels and indicate that the activity of this enzyme is more sensitive indicator of psychological stress than the level of cortisol. The illustrated increase in the activities of both enzymes ($P>0.05$) is consistent with the reported stimulatory effect of cortisol on these amonotransferases (Smirnov, 1974).

The increased ALT activity in the II group of pigs at 35 d of age (Figure 5) was not accompanied with an increase of AST (Figure 4). This finding could be because AST is localized mostly in mitochondria and it is less susceptible to stress than ALAT, which is localized mainly in cytoplasma. These results support our view that the intensity of the stress was not strong enough to induce a sizable change in the activity of the less stress-sensitive enzyme at the age of 35 days.

The calculated AST/ALT ratio (Figure 6), known as coefficient of Ritis, was not significantly changed in both experimental groups at the age of 5 d but declined sharply in the II group of piglets at the age of 35 d, mainly because of the enhancement of ALT activity. This data confirm once more that some individuals in the II group of piglets were responsive to the possible psychological stress, and that the extent of adrenal response was not strong enough to produce changes in the AST activity.

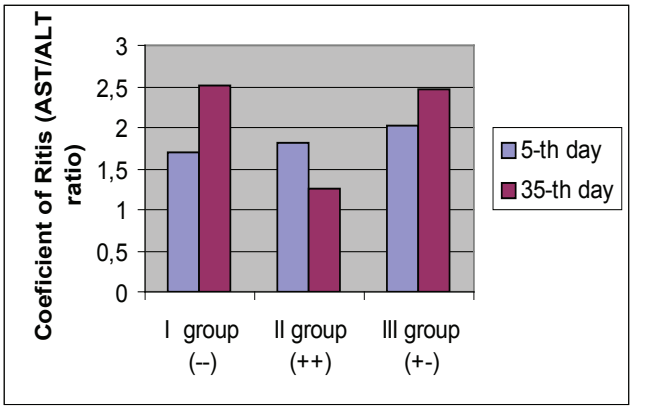

Fig. 6. Coeficient of Ritis (AST/ALT ratio) in the blood of neonatal piglets, fed "Lactina" supplemented diet

Conclusions

Probiotic „Lactina" supplemented both to pregnant sows (from 85 d of pregnancy up to 35 d after delivery) and their piglets (from 1 day up to 35 day of age) had no significant effect on plasma cortisol level, peripheral blood leucocyte distribution and plasma AST activity, but caused significant ($P<0.001$) increase in plasma ALT activity at 35 days of age. Our results come to show that cortisol level may mislead the investigator unless it is used with other indicators of stress.

References

Bergmeyer, H. and E. Bernt, 1963. Methods of Enzymatic Analysis.

Bourliox, P., B. Koletzko, F. Guanrner and V. Braesco, 2002. The intestins and its microflora are partners for the protection of the host: report on the danone Symposium "The inteligent intestine". *Journal of Clinical Nutrition*, **78**: 675-683.

Dhabhar, F. S., A. H. Miller, B. S. McEwen and R. L. Spencer, 1995. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *The Journal of Immunology*, **154:** 5511-5527.

De Jong, I. C., S. van Voorst, D. A. Ehlhard and H. J. Brokhuis, 2002. Effect of restricted feeding on physiological stress in growing broilers breeeders.

Br. Poult. Sci., **43**: 156-168.

Grigorova, S., S. Surdjiska, D. Stoyanov and M. Dimitrova, 2004. Influence of probiotic "Lactina" on the productivity of layers. *Bulgarian Journal of Agricultural Science*, **10**: 634-642.

Gross, W. B. and H. S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis., **27:** 972-979.

Kanchev, L. N., H. Dobson, W. K. Ward and R. J. Fitzpatric, 1976. Concentration of steroids in bovine peripheral plasma during oestrus cycle and effect of betamethasone treatment. *J. Repr. Fert.,* 48: 341-345.

Majamaa, H., E. Isolauri, M. Saxelin and T. Vesikari, 1995. Lactate acid bacteria in treatment of acute rotavirus gastroenteritis. *J. Pediatr. Gastroenterol. Nutr*., 20: 333-339.

Post, J., J. M. Rebel and A. Alder Huurne, 2003. Automated blood cells count: a sensitive stress in broilers. *Poult. Sci.,* 82: 591-595.

Smirnov, O. K., 1974. Early determination of animal productivity. Moskva. *Kolos (*Ru).

Stoyanov, D., 2005. Study on the possibilities for replacement of nutritive antibiotics in concentrate mixture with the probiotic "Lactina". Disertation, Sofia (Bg).

Wollowski, I., G. Rechkemmer and B. L. Pool-Zobel, 2001. Protective role of probiotics and prebiotics in colon cancer. *American Journal of Clinical Nutrition,* 73: 4519-455.

Received July, 1, 2011; accepted for printing December, 2, 2011.