KRMIVA

PROBIOTIC APPLICATION IN LAMBS NUTRITION

PRIMJENA PROBIOTIKA U HRANIDBI JANJADI

Z. Antunović, Marcela Šperanda, D. Amidžić, V. Šerić, Z. Steiner, M. Domaćinović, F. Boli

Original scientific paper - Izvorni znanstveni članak
UDC: 636.3.033; 636.087.8
Received - Primljeno: 27. June - lipanj 2006.

SUMMARY

The research was conducted on 28 lambs divided into two groups (C-control and E-experiment). The investigation started on day 15 of age and lasted until day 77 of age. The probiotic BioPlus 2B was added into the feed of the lamb in group B. The lambs in group E had a higher body weight (by 1.90%), greater daily gain (by 2.50%), increased average daily intake and a better feed conversion, compared to C group but differences between the groups were not statistically significant. The lambs in group E had, in the blood serum, statistically lower concentration of urea (5.51:7.97 mmol/l) and triglycerids (0.22:0.35 mmol/l) compared to group C. No significant differences between lambs in the groups C and E were observed in the blood levels of glucose (2.67:2.45 mmol/l), cholesterol (1.25:1.08 mmol/l), total proteins (66.08:63.70g/l), creatinine (52.01:48.0 µmol/l) and albumin (27.68:28.35 g/l). The results testify to the moderate increase of metabolic activity in the E group of lambs.

Key words: lambs, probiotic BioPlus 2B, nutrition, body weight, feed conversion, metabolic activity

INTRODUCTION

There are various biologically active substances added in the feed that have been used for a very long time with the aim of increasing the production and reducing the expenses in animal breeding. In the last decade, many countries have introduced partial or a complete ban on nutritive antibiotics usage, as an alternative, there are various probiotic preparations (the bacterial direct-feed microbials). The most intresting probiotic preparations are those with certain types of microorganisms *Lactobacillus (Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus casei),* S*treptococcus* (*Streptococcus faecium*, being *Enterococcus faecium*, etc) and *Bacillus* (*Bacillus subtilis, Bacillus licheniformis, Bacillus cereus)* which help maintain the balance of the desirable microorganism population within the digestive tract (Lopez, 2000). According to Fuller (1992) probiotics are biopreparations containig living cells or metabolites of stabilized autochthonous micro-organisms that optimize the colonization and composition of gut microflora in both animals and humans and have a stimulative effect on digestive

DSc. Zvonko Antunović, Associate Professor; DSc. Marcela Šperanda, Assistant Professor; Bsc. Danijel Amidžić; MSc. Zvonimir Steiner, DSc. Matija Domaćinović, Full Professor - Department of Animal Science, Faculty of Agriculture, Trg sv. Trojstva 3, 31000 Osijek, Croatia; BSc. Franjo Boli -"Belje" d.d., Farma "Prosine"; MSc. Vatroslav Šerić - Clinical Hospital Osijek, J. Hutlera 4, 31000 Osijek. The paper is a part of diploma thesis.

processes and the immunity of the host. Probiotics have been defined as non-pathogenic micro-organisms that, when ingested, exert a positive influence on the host health or physiology (Dunne et. al., 1999). They restore and maintain the balance of the desirable microorganisms in times of stress or disease and improve the young animals growth (Dawson 1993; Simon et al., 2001; Antunović, et. al. 2005). The probiotics control of the microorganisms in the digestive tract stimulates the immunity and improves the food absorption (Teeler and Vanabelle 1991). Several authors have established the positive effect of the probiotics (*Baccilus spp.*) in the fodders (Jenny et al., 1991; Roth et al., 1992; Seifert and Gessler, 1996; Daenicke et al., 1998; Gritzer and Leitgeb, 1998; Garza-Cezares et al., 2001; Bomba et al., 2002) in poligastric animals.

There is very limited published information on the mechanisms by which bacterials *Bacillus licheniformis* and *Bacillus subtilus* improve animal performance, particularly of unweaned and weaned lambs. Since weaning is a great stress to the lambs, the aim of this research was to establish the influence of feeding the bacterial direct- fed microbials on metabolic indicators in lambs.

MATERIAL AND METHODS

The research has been conducted on 28 lambs of the Merinolandschaf breed before and after weaning. The lambs were, on average, 15 days old, divided into two groups in proportion of 50% males and 50% females. After weaning lambs the lambs

Table 1. Material and chemical composition of the feed mixture and the meadow hay
Tablica 1. Sirovinski i kemijski sastav krmne smjese i livadnog sijena

Forages - Krmiva (%)	Feed mixture - Krmna smjesa		Meadow hay Liv. sijeno
	Control - Kontrola (C)	Experiment - Pokus (E)	
Maize - Kukuruz	50.0	50.0	
Oat - Zob	16.0	16.0	
Soybean extruded - Ekstrudirana soja	15.0	15.0	
Wheat bran - Pšenične posije	7.5	7.5	
Lactic substitute - Mliječni nadomjestak	10.0	10.0	
Salt - Sol	0.5	0.5	
VAM (vitamin and mineral mixture)*	1.0	1.0	
Probiotic BioPlus 2B®	-	0.1	
Water - Voda	13.37		9.35
Crude proteins - Sirove bjelančevine	15.36		9.58
Crude fat - Sirova mast	3.82		3.06
Ash - Pepeo	6.49		6.70
Crude fibres - Sirova vlakna	4.79		31.29
Calcium - Kalcij	1.76		0.97
Phosphorus - Fosfor	0.61		0.33
NET (non-nitrogen extractive matters)	55.04		35.02
Oat feeding unit (kg) - Zobena jedinica	1.12		0.30

*Contents in 1 kg (Sadržaj u 1 kg) = Vitamin A 1000000 IUIg; Vitamin D_3 150000 IU/g; vitamin E 1500 mg; vitamin K_3 50 mg; vitamin B_1 100 mg; vitamin B_2 200 mg; nicotin acid 1000 mg; pantotenic acid 500 mg; vitamin B_6 200 mg; vitamin B_{12} 1.2 mg; cholin chloride 20000 mg; Fe 4000 mg; Cu 800 mg; Mn 3500 mg; Zn 5000 mg; I 80 mg; Co 20 mg; Se 15 mg; Mg 10000 mg; S 10000 mg, antioxidant 10000 mg.

were continued with fattening and monitoring of the first week till 77 days of age. During the experiment the lambs were kept with ewes in the same conditions in two separated groups. Both groups of lambs were suckling and fed with a feed mixture and meadow hay (*ad libitum*). Probiotic preparation BioPlus 2B was added to the feed mixture in the experimental group of the lambs in concentration of 0.1%.

Material and chemical composition of the feed mixture and the meadow hay was analyzed to the AOAC (1984) and is presented in the Table 1.

Individual weighing was been performed at the beginning (1st day) and the end of the experiment (62st day). On the last day (62th day) the daily gain was calculated as well as the average daily intake and the feed efficiency. There has been prepared previously the foremixture with the probiotic preparation and a vitamin-mineral additive in the aim of equally mixing and apportioning the active ingredients of the probiotic preparation. BioPlus 2B (Chr. Hansen A/S, Hörsholm, Denmark) is a probiotic containing *B. licheniformis* (DSM 5749) and *B. subtilis* (DSM 5750) spores in a 1:1 ratio (1.6 x 10^9 spores/g : 1.6 x 10^9 spores/g). *Bacillus licheniformis* of Bioplus 2B has been isolated from soil, while *B. subtilis* has been isolated from soya bean fermentation. Both component micro-organisms of BioPlus 2B are registred in Annex II of 70%524 Directive as safe for use as feed additives when used according to the two main substances is genetically modified nor produces enterotoxins. The carrier substances are sodium silicate (1%) and whey permeate (98%), free of heavy metals, micro-organisms and mycotoxins.

After the weighing on the 62th day and registering the food consumption, we have collected the blood samples (10 ml) from the jugularis vein into the sterile vacuum tubes Venoject® (sterile Terumo Europe. Leuven, Belgium). Biochemical indicators (glucose, urea, creatinine, total proteins, albumin, cholesterol and triglycerides) in blood serum were measured on the Olympus AU640 analyser.

The values of the researched parameters after the experiment completion have been statistically processed with a computer program for analysis of the variance (Statistica Stat Soft Inc., 2001).

RESULTS AND DISCUSSION

Fattening performances of the lambs are shown in Table 2.

The lambs from the experimental group (E) had a larger body weight on the 62th day of the growing by 1.90%. Daily gain of the lambs in group E have been measured from 1st to the 62th day of the growing. It was by 2.50% higher in the group E than the group C. However, the differences between the body weight and the daily gain of both groups have not been statistically significant (Table 2). The lambs of the group E had a higher average daily intake feed mixtures by 2.90% and meadow hay by 7.69%. The feed efficiency (feed mixtures) was better in the experimental group (E) than in the control group (C). The values mentioned were higher in group E compared to the control group (Table 2) and could be explained by a higher consumption of the concentrate and the hay as well as a better feed efficiency. Pond and Goode (1985) found better daily gain by 24.7% and 6.4% as well as better feed efficiency by 17% and 0.30% with the usage of Probios® in feeding the lambs during their first two weeks and from the second to the fourth week. In the fattening trial with veal calves fed fed mixture with added probiotic preparation (*Bacillus cereus*) obtained significant higher daily weight gain by 7.6 and 8.1% and the feed conversion rate by 5.3% (Roth et al., 1992). Daenicke and Leibzien (1994), Wagner and Landfried (1999) observed a higher intake (by 4%) and live weight gain (by 6%), whereas Garza-Cezares et al (2001) for 3.4% and Gritzer and Leitgeb (1998) by 2.1% in bulls fed with probiotic (*Bacillus spp.*). Bacterial probiotics like *Bacillus spp*. are used within ruminants principally during the early stages of life in order to prevent digestive dysfunction and to enhance the rate of development of the normal intestinal flora Garza-Cezares et al (2001). Microorganisms which are not true inhabitants of the intestine (*Bacillus spp.)* different modes of action can be proposed than for intestinal bacteria. For instance, specific effects could include the release of dipicolinic acid by spore-forming *Bacillus spp*. upon germination, receptors for E. Coli fimbriae on yeast cell walls, or the release of large amounts of lactic acid by intestianl bacteria-*Enterococcus spp.* (Simon et al., 2001). Calves fed replacer containing the *Bacillus subtilis* concentrate showed higher daily gain and feed efficiency particularly in the first period of the experiment, but differences did not significantly (Jenny et al., 1991).

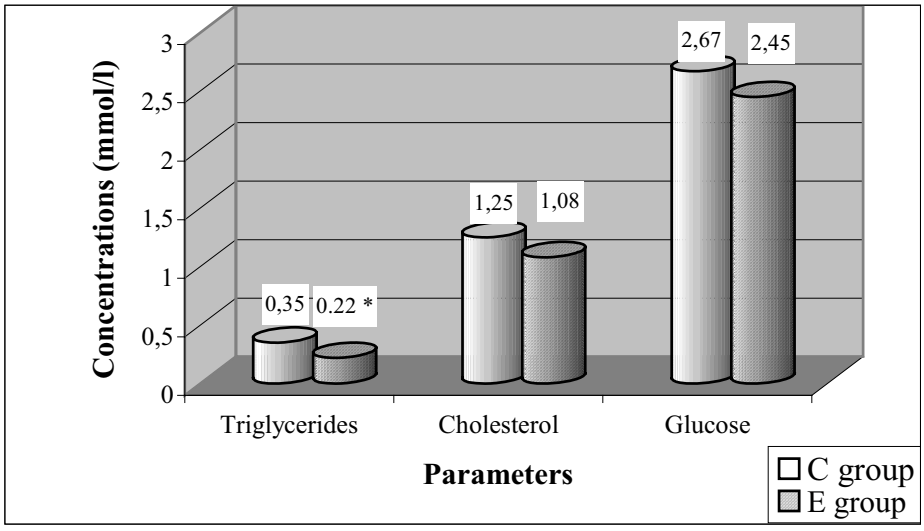
Table 2. Fattening performances of the lambs
Tablica 2. Tovna svojstva janjadi

Indicator - Pokazatelj	Statistical size Stat. veličina	Group - Grupa		
		Control - Kontrola (C)	Experiment - Pokus (E)	%
Body weight (kg) - Tjelesna masa (kg)				
Initial body weight (1st day) Početna tjelesna masa (1. dan)	Mean ± SD Prosjek ± SD	8.25 ± 1.86	8.32 ± 1.22	+ 0.85
Final body weight (62th day) Završna tjelesna masa (62. dan)	Mean ± SD Prosjek ± SD	22.08 ± 4.59	22.50 ± 3.14	+ 1.90
Daily gain (g) - Dnevni prirast (g)				
From 1st to 62th day Od 1. do 62. dana	Mean ± SD Prosjek ± SD	223.11 ± 49.62	228.69 ± 37.91	+ 2.50
Feed consumption (kg/day) - Konzumiranje hrane (kg/dan)				
Feed mixture (from 1st to 62th day) Krmna smjesa (od 1. do 62. dana)	Mean ± SD Prosjek ± SD	0.69	0.71	+ 2.90
Meadow hay (from 1st to 62th day) Livadno sijeno (od 1. do 62. dana)	Mean ± SD Prosjek ± SD	0.26	0.28	+ 7.69
Feed efficiency (kg/kg) - Konverzija hrane (kg/kg)				
Feed mixture (from 1st to 62thday) Krmna smjesa (od 1. do 62. dana)	Mean ± SD Prosjek ± SD	3.10	3.09	- 0.15
Meadow hay (from 1st to 62th day) Livadno sijeno (od 1. do 62. dana)	Mean ± SD Prosjek ± SD	1.17	1.22	+ 4.27

SD - standard deviation (standardna devijacija)

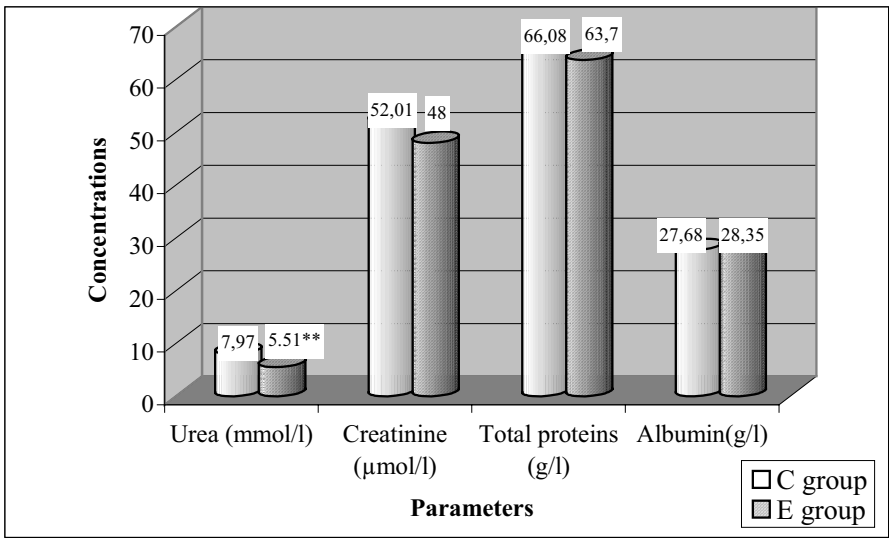
The concentration of biochemical indicators in blood serum of the lambs studied within energetic and protein metabolism parameters (Figure 1. and Figure 2.).

Graph 1. Energetic metabolism parameters in the blood serum of lambs (*P<0.05)
Grafikon 1. Pokazatelji energetskog metabolizma u krvnom serumu janjadi (*P<0.05)



Graph 2. Protein metabolism parameters in the blood serum of lambs (P<0.01)**

Grafikon 2. Pokazatelji proteinskog metabolizma u krvnom serumu janjadi (P<0.01)**



Among energetic metabolism parameters, the triglycerides concentration (Figure 1.) in group E are significantly lower (P<0.05) in comparison with group C. No significant differences between groups were observed for the blood levels of cholesterol and glucose. Significant lower content of triglycerides in gruop E showed a positive energetic balance in the lambs. Similar observations with the application of a probiotic in feed mixture for kids were obtained by Chiofalo et al. (2004). Probiotics influence blood cholesterol level by the inhibition of cholesterol synthesis, or decrease its level directly by assimilation (Zacconi et al., 1992).

Among protein metabolism parameters, the urea concentration (Figure 2.) in group E was significantly lower (P<0.01) in comparison with group C while concentrations of the total proteins and creatinine were lower in group E but differences between groups were not statistically significant. Similar results for blood urea concentration in lambs have been obtained by Antunović et al. (2005) and for goat kids by Chiofalo et al. (2004).

CONCLUSION

The group of lambs fed a probiotic had a larger body weight, greater daily gain, increased average daily intake and a better feed efficiency, compared to group C but differences between the groups were not statistically significant.

REFERENCES

1. Antunović, Z., M. Šperanda, B. Liker, V. Šerić, Đ. Senčić, M. Domaćinović, T. Šperanda (2005): Influence of feeding the probiotic PIONEER PDFM® to growing lambs on the performance and the blood structure. Acta Veterinaria Beograd 5(4): 287-300.
2. A.O.A.C. (1984): Official Methods of Analysis. (14th Ed.). Association of Official Analytical Chemists, Arlington, VA, USA.
3. Bomba, A. R. Nemcova, D. Mudronova, P. Guba (2002): The possibilities of potentiating the efficacy of probiotics. Trends in Food Science & Technology 13: 121-126.
4. Chiofalo, V., L. Liotta, B. Chiofalo (2004): Effects of the administration of *Lactobacilli* on body growth and on the metabolic profile in growing Maltese goats kids. Reprod. Nutr. Dev. 44: 449-457.
5. Daenicke, R., G. Flachowsky, P. Patschewitz (1998): Zur Wirkung eines Probiotikums (Bacillus cereus) auf die Leistung von Aufzuchtkälbern. VDLUFA- Schriftenreihe 49: 473-476.
6. Daenicke, R., P. Leibzien (1994): Zum Einfluss von Toyocerin und Monesin-Na auf die Mast- und

Schlachtleistung von Bullen. Proc. 106. VDLUFA – Kongreβband, 19.-24.09.1994. Jena, p.789-792.

7. Dawson, K. A. (1993): Probiotics and enzymes in ruminant nutrition. Proceedings of the 1st Symposium Kartause Ittlingen, Switzerland October 13-16., p.89-96.
8. Dunne, C., L. O'Mahony, L. Murphy, S. O'Halloran, M. Feeney, S. Flynn, G. Fitzgerald, C. Daly, B. Kiely, G. O'Sullivan, F. Shanahan, J. K. Collins (1999): Probiotics; from myth to reality - Demonstration of functionality in animal models of disease and in human clinical trials. Antonie van Leeuwenhoek 76: 279-292.
9. Fuller, R. (1992): The effect of probiotic on the gut microbiology of farm animals. In B. J. B. Wood (Ed.) The lactic acid bacteria. Pp. 171-192. London, UK, Elsevier Applied Science.
10. Garza-Cazares, F., R. Daenicke, G. Flachowsky (2001): Research note: Effect of Bacillus cereus on performance of growing bulls. Archi Animal Nutrition, 55: 161-165.
11. Gritzer, K., Leitgeb, R. (1998): Űberprüfung der wirksamkeit antibiotischer und mikrobieller Leistungsfőrderer in der Rindermast. Die Bodenkultur 49(1): 51-59.
12. Jenny, B. F., H. J. Vandijk, J. A. Collins (1991): Performance and Fecal Flora of Calves Fed a Bacillus subtilis Concentrate. J. Dairy Sci., 74: 1968-1973.
13. Kaneko, J. J. (1997): Clinical Biochemistry of Domestic Animals. 5th Ed. Academic Press. San Diego, California, USA, 923.
14. Lopez, J. (2000): Probiotics in animal nutrition. Asian-australian Journal of animal sciences, 13: 12-26.
15. Pond, K. R., L. Goode (1985): The Evaluation of Probios® for wean-stressed Lambs. Microbial Genetics Division, Pioneer Hi-Bred International, Des Moines, Iowa.
16. Roth, F. X., M. Kirchgessner, U. Eidelsburger, B. Gedek (1992): Zur nutritiven Wirksamkeit von Bacillus cereus als Probiotikum in der Kälbermast. 1. Einfluβ auf Wachstumsparameter, Schlactleistung und mikrobielle metaboliten im Dünndarm. Agribiol. Res. 45(4): 294-302.
17. Seifer, H. S. H., F. Gessler (1996): Continuous oral application of probiotic B cereus- An alternative to the prevention of enterotoxemia? Deutsche tierartzliche wochenschrift, 103(10): 386-389.
18. Simon, O., A. Jadamus, W. Vahjen (2001): Probiotic feed additives- effectiveness and expected modes of action. Journal of Animal and feed Sciences 10(1): 51-67.
19. Statistica, Stat Soft, Inc. (2001): Statistica (data analysis software system), version 6, www.statsoft.com.
20. Teeler, E., M. Vanabelle (1991): Probiotics: fact and fiction, Mededelingen van de faculteit Landbouwwetenschappen, Rijksuniversitet Gent, 56: 1591-1599.
21. Wagner, B., K. Landfried (1999): Bullenmast mit Mokroorganismen. Veredlungsproduktion 4: 80-81.
22. Zacconi, C., V. Bottazzi, A. Rebecchi, E. Bosi, P. G. Sarra, L. Tagliaferi (1992): Serum cholesterol levels in axenic mice colonized with Enterococcus faecium and Lactobacillus acidophilus. Microbiologica 15: 413-418.

SAŽETAK

Istraživanje je provedeno na 28 janjadi podijeljene u dvije skupine (C - kontrolna i E - pokusna). Istraživanje je započelo 15. dan starosti janjadi i trajalo do 77. dana starosti. Janjadi u skupini E dodan je u hranu probiotic BioPlus 2B. Janjad u skupini E imala je veću tjelesnu masu (za 1.90%), veći dnevni prirast (za 2.50/%), povećani dnevni unos i bolju konverziju hrane u usporedbi sa skupinom C ali razlike između skupina nisu bile statistički značajne. Janjad skupine E imala je u serumu krvi statistički manju koncentraciju ureje (5.51 : 7.97 mmol/1) i triglicerida (O.22 : 0.35 mmol/l) u usporedbi sa skupinom C. Nisu primijećene značajne razlike između janjadi skupina E i C u razini glukoze u krvi (2.67 : 2.45 mmol/l), kolesterola (1.25 : 1.08 mmol/1), ukupnih bjelančevina (66.08 : 63.70 g/l), kreatinina (52.01 : 48.0 umol/l) i albumina (27.68 : 28.35 g/l) Rezultati pokazuju umjeren porast metaboličke aktivnosti kod janjadi u skupini E.

Ključne riječi: janjad, probiotic BioPlus 2B, hranidba, tjelesna masa, konverzija hrane, metabolička aktivnost