

INFLUENCE OF FEEDING THE PROBIOTIC PIONEER PDFM® TO GROWING LAMBS ON PERFORMANCES AND BLOOD COMPOSITION

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The research has been conducted on 48 weaned lambs divided into two groups (C-control and E-experimental) and lasted for 35 days. To lambs in group E the probiotic PIONEER PDFM® was added to the fodder. Lambs in group E had larger average body weights, greater daily weight gain, increased average daily intake and a better feed efficiency, compared to group C. However, differences between groups have not been statistically significant. The concentration of calcium in the blood serum of group E (2.16 mmol/l) on the 35th day has been statistically lower ($P < 0.01$) compared to group C (2.38 mmol/l). The concentration of chloride was statistically higher in group E ($P < 0.05$) on the 19th day (104.75:103.38 nmol/L) and statistically lower ($P < 0.05$) on the 35th day (106.00: 107.33 mmol/L). The concentration of iron in the blood serum was statistically higher ($P < 0.05$) on the 19th and the 35th day in group E (26.29 and 33.00 mmol/l) than in group C (21.97 and 29.15 mmol/l); concentrations of phosphorus and potassium in the blood serum were statistically higher on the 35th day in group E (2.74 and 4.95 mmol/l) than in group C (2.41 and 4.65 mmol/l). Lambs in group E had in the blood sera statistically lower ($P < 0.01$) concentrations of glucose and urea. The activities of the enzymes ALT, AST and CK measured on the 35th day of fattening were lower ($P < 0.01$) compared to group C. The concentrations of total bilirubin and triglycerides were statistically higher in group E than in group C.

Key words: Lambs, probiotic, conversion, blood, metabolic acidosis

INTRODUCTION

Various biologically active substances added to the fodder have been used for a very long time with the aim to increase production and reduce expenses in animal breeding. In the last decade, because many countries have introduced a partial or a complete ban on nutritive antibiotics various probiotic preparations are

used as an alternative. The most interesting probiotic preparations are those with certain types of microorganisms such as *Lactobacillus* (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus casei*) and *Streptococcus* (*Streptococcus faecium*, being *Enterococcus faecium*, etc) which help to maintain the balance of the desirable microorganism population within the digestive tract. Probiotics are mainly live microorganisms (bacteria, fungi) that can be normally found in nature and in the digestive tract (Fox 1988). They restore and maintain the balance of desirable microorganisms during the times of stress or disease and improve the growth of young animals. Wolter and Henry (1988) quote that probiotics select microorganisms within the digestive tract and increase the number of desirable lactic acid bacteria. The probiotics control microorganisms in the digestive tract, stimulate immunity and improve food absorption (Teeler and Vanabelle 1991). Several authors have established the positive effect of probiotics in fodder (Haryanto *et al.* 1994; Feist *et al.* 1997; Strzetelski *et al.* 1998; Erhard *et al.* 2000; Daniecke *et al.* 2001; Ghorbani *et al.* 2002; Görgülü *et al.* 2003 and Krehbiel *et al.* 2003) in polygastric animals - sheep and calves or cattle.

Data concerning by which *Enterococcus faecium* improves animal performance, particularly in weaned lambs are very limited. Since weaning is a great stress to lambs the aim of this research was to establish the influence of feeding the bacteria directly on metabolic indicators in lambs.

MATERIAL AND METHODS

The research has been conducted on 48 lambs of the Merinolandschaf breed immediately after weaning. The lambs were, on average, 60 days old and divided into two groups (50% males and 50% females). The growing period lasted for 35 days. During the experiment, the lambs were kept under equal conditions in two separate groups. Both groups were fed with a feed mixture and meadow hay (*ad libitum*). Probiotic preparation PIONEER PDFM® has been added to the feed mixture in the experimental group of lambs in a concentration of 0.1%. The chemical composition of the feed mixture and meadow hay has been analyzed to the AOAC (1984) and data are presented in Table 1.

Individual weighing has been performed at the beginning (1st day), in the middle (19th day) and the end of the experiment (35st day). On the last day (35th day) the daily gain has been calculated, as well as the average daily intake and the feed efficiency. The foremixture with the probiotic and a vitamin mineral additive has been previously prepared with the aim to balance and mix the active ingredients of the probiotic preparation. The probiotic preparation is a granulate that has to be added to the feed and contains microspherically protected bacteria of lactic fermentation (two types of the *Enterococcus faecium*). The bacteria *Enterococcus faecium* are facultative anaerobic bacteria which turn carbohydrates into lactic acid without the generation of gases. They are round or oval shaped, in pairs, Gram-positive, immobile, and do not create spores. They belong to the Lancefield D group and, they are part of the intestinal flora and are

not genetically modified. The probiotic preparation PIONEER PDFM® contains 2×10^{11} CFU.kg⁻¹ (colony forming unit per kilogram) of *Enterococcus faecium*.

Table 1. Material and chemical composition of the feed mixture and meadow hay

Forages (%)	Feed mixture		Meadow hay
	Control (C)	Experiment (E)	
Oat	50	50	
Corn	26	26	
Soybean grits	14.5	14.5	
Wheat bran	3.2	3.2	
Lactic substitute	5.0	5.0	
Salt	0.3	0.2	
VAM (vitamin and mineral mixture)*	1.0	1.0	
Probiotic PIONEER PDFM®	–	0.1	
Water		13.57	9.35
Crude proteins		15.95	9.58
Crude fats		3.83	3.06
Crude ash		6.50	6.70
Crude fibres		4.76	31.29
Calcium		1.76	0.97
Phosphorus		0.61	0.33
NET (non-nitrogen extractive matters)		55.65	35.02
Oat feeding unit (kg)		1.12	0.30

*Contents in 1 kg= Vitamin A 1000000 IU/g; Vitamin D₃ 150000 IU/g; vitamin E 1500 mg; vitamin K₃ 50 mg; vitamin B₁ 100 mg; vitamin B₂ 200 mg; nicotin acid 1000 mg; pantotenic acid 500 mg; vitamin B₆ 200 mg; vitamin B₁₂ 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, antioxidanse 10000 mg.

After weighing the lambs on the 19th and 35th day and registering the food consumption, we have collected blood samples (10 ml) from the jugular vein into sterile vacuum tubes Venoject® (Terumo Europe. Leuven, Belgium). Mineral indicators (Ca, P-inorganic, Na, K, Cl and Fe), biochemical indicators (glucose, urea, creatinin, total bilirubin, total proteins, albumin, cholesterol and triglycerides) and enzyme activities (ALT-alanine aminotransferase, AST-aspartate aminotransferase, AP - alkaline phosphatase, GGT-γ-glutamyltransferase, cholinesterase, alpha amylase, CK - creatinine kinase, LDH- lactate dehydrogenase) have been measured on Olympus 640 analyser.

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

RESULTS

Body weight and daily gain of the lambs are shown in Table 2.

Table 2. Body weight and daily gain of experimental lambs

Indicator	Statistic size	Group	
		Control (C)	Experiment (E)
<i>Body weight (kg):</i>			
Initial body weight (1 st day)	Mean ± SD	19.75 ± 2.25	20.08 ± 2.24
19 st day	Mean ± SD	24.69 ± 2.74	25.13 ± 2.52
	%	100.00	101.75
35 th day	Mean ± SD	28.56 ± 2.49	29.23 ± 2.91
	%	100.00	102.29
<i>Daily gain (g):</i>			
From 1 st to 19 th day	Mean ± SD	259.87 ± 61.26	265.35 ± 43.15
	%	100.00	102.07
From 20 st to 35 th day	Mean ± SD	236.98 ± 74.25	256.51 ± 62.49
	%	100.00	107.61
From 1 st to 35 th day	Mean ± SD	251.79 ± 47.81	261.34 ± 39.40
	%	100.00	103.65

SD- standard deviation

The lambs from group E had a larger body weight on the 19th and 35th day of the growing period. Daily weight gains of lambs from group E have been measured from the 1st to the 19th day and from the 1st to the 35th day of growth. They were 2.07, 7.16 respectively and in average 3.65% higher in group E than group C. However, the differences between body weight and the daily gain of both groups have not been statistically significant (Table 2).

The lambs in group E had a higher average daily intake (feed mixtures and meadow hay) in all growing phases by 3.02% and 6.25% (Table 3). The feed efficiency (feed mixtures and meadow hay) was improved in group E compared to group C.

Table 3. Consumption of food and feed efficiency in the fattening lambs

Indicator	Statistic size	Group			
		Control (C)		Experiment (E)	
		Feed mixture	Meadow hay	Feed mixture	Meadow hay
<i>Food consumption (kg/day):</i>					
From 1 st to 19 th day	Mean	0.80	0.28	0.82	0.30
	%	100.00	100.00	102.50	107.14
From 20 st to 35 th day	Mean	0.85	0.35	0.88	0.38
	%	100.00	100.00	103.53	108.57
From 1 st to 35 th day	Mean	0.83	0.32	0.85	0.34
	%	100.00	100.00	103.02	106.25
<i>Feed efficiency (kg/kg):</i>					
From 1 st to 19 th day	Mean	3.08	1.08	3.09	1.13
	%	100.00	100.00	100.32	104.63
From 20 st to 35 th day	Mean	3.54	1.46	3.44	1.48
	%	100.00	100.00	97.18	101.37
From 1 st to 35 th day	Mean	3.31	1.28	3.27	1.31
	%	100.00	100.00	98.79	102.34

The concentration of minerals in blood serum of lambs is shown in Table 4.

Concentration of Ca in the blood serum of group E measured on the 35th day was statistically lower ($P < 0.01$) than in group C. The concentration of Cl was lower ($P < 0.05$) on the 35th and higher ($P < 0.05$) on the 19th day of the growing period in group E compared to group C. In both measurements the concentrations of Fe were significantly higher ($P < 0.05$) in group E. On the 35th day the concentration of inorganic P and K were higher in group E.

The concentration of biochemical indicators within the blood serum of lambs is shown in Table 5.

Lambs in group E had a very low concentration of glucose and urea, measured on the 35th day of the growing period, while the concentration of total bilirubin and triglycerides was significantly higher in the same group.

The activity of enzymes in the blood sera of lambs is shown in Table 6.

Table 4. Mineral indicators in the blood serum (mmol/l)

Indicator	Reference range (Kaneko, 1997)	Time of taking blood samples			
		19 st day		35 st day	
		Control (C) n=24	Experiment (E) n=24	Control (C) n=24	Experiment (E) n=24
Ca, mmol/l	2.88-3.20	2.57 ± 0.24	2.53 ± 0.22	2.38 ± 0.17	2.16 ± 0.22**
P – inorg., mmol/l	1.62-2.36	2.92 ± 0.64	3.12 ± 0.54	2.41 ± 0.36	2.74 ± 0.54*
Na, mmol/l	139.00-152.00	150.46 ± 3.16	149.29 ± 2.53	151.63 ± 3.20	150.42 ± 2.52
K, mmol/l	3.90-5.40	4.97 ± 0.40	4.81 ± 0.34	4.65 ± 0.36	4.95 ± 0.34**
Cl, mmol/l	95.00-103.00	103.38 ± 2.12	104.75 ± 2.40*	107.33 ± 2.96	106.00 ± 2.60*
Fe, µmol/l	29.70-39.70	21.97 ± 7.04	26.29 ± 7.06*	29.15 ± 9.64	33.00 ± 9.58*

Mean ± SD; *P<0.05; **P<0.01

Table 5. Biochemical indicators in blood serum of lambs (mmol/l)

Indicator	Reference range (Kaneko, 1997)	Time of taking blood samples			
		19 st day		35 st day	
		Control (C) n=24	Experiment (E) n=24	Control (C) n=24	Experiment (E) n=24
Glucose, mmol/l	2.78-4.44	5.31 ± 1.13	5.12 ± 0.92	6.96 ± 1.87	5.71 ± 1.04**
Urea, mmol/l	2.86-7.14	8.91 ± 1.95	8.20 ± 1.65	8.16 ± 1.85	6.68 ± 1.51**
Creatinine, µmol/l	88.40-168.00	59.46 ± 8.67	58.08 ± 10.66	52.58 ± 8.92	52.42 ± 8.19
Bilirubin – total, µmol/l	1.71-8.55	3.96 ± 0.46	4.25 ± 0.65*	3.63 ± 0.50	4.17 ± 0.70**
Total proteins, g/l	60.00-79.00	61.87 ± 4.99	62.32 ± 4.95	60.63 ± 4.15	61.12 ± 4.26
Albumin, g/l	24.00-30.00	30.06 ± 2.30	29.95 ± 2.04	30.18 ± 2.30	29.88 ± 1.65
Cholesterol, mmol/l	1.10-2.30	1.12 ± 0.30	1.15 ± 0.23	1.07 ± 0.25	1.07 ± 0.26
Triglycerides, mmol/l	0.1-0.5	0.34 ± 0.10	0.39 ± 0.09	0.23 ± 0.06	0.29 ± 0.11*

Mean ± SD; *P<0.05; **P<0.01

Table 6. Activity of enzymes in blood sera of lambs (U/l)

Indicator	Reference range (Kaneko, 1997)	Time of taking blood samples					
		19 st day		35 st day			
		Control (C) n= 24	Experiment (E) n= 24	Control (C) n= 24	Experiment (E) n= 24	Control (C) n= 24	Experiment (E) n= 24
ALT	30.00	27.79 ± 10.75	22.50 ± 9.68	36.54 ± 9.23	20.88 ± 5.40**		
AST	60.00-280.00	166.46 ± 6.54	155.42 ± 5.48	188.88 ± 34.77	120.63 ± 22.88**		
AP	83.40-666.80	361.08 ± 132.75	390.25 ± 108.43	385.46 ± 114.99	420.29 ± 161.08		
GGT	20.00-52.00	61.21 ± 10.13	58.63 ± 10.67	61.04 ± 10.89	58.38 ± 12.15		
Choline sterase		167.38 ± 18.45	181.63 ± 22.97*	173.13 ± 14.08	179.38 ± 18.79		
Alpha amylase		31.42 ± 25.85	26.38 ± 18.22	26.71 ± 16.88	23.92 ± 19.92		
CK	8.10-12.90	365.08 ± 110.29	299.75 ± 122.26*	407.50 ± 218.36	207.88 ± 120.43**		
LDH	238.00-440.00	1393.67 ± 230.38	1568.50 ± 447.29	1288.75 ± 251.87	1351.58 ± 442.51		

Mean ± SD; *P<0.05; **P<0.01

The activities of ALT, AST and CK enzymes on the 35th day of the growing period were statistically lower ($P < 0.01$) in group E compared to group C and activity of CK in group E was statistically lower ($P < 0.05$) on the 19th day of growth. In opposition to them, the activities of cholinesterase were statistically higher ($P < 0.05$) in the blood of group E measured on the 19th day, but on the 35th day of the fattening period this difference was not statistically significant. Among the activities of AP, GGT, LDH and alpha amylase there has not been a significant difference between the groups.

DISCUSSION

The addition of probiotics did not have a greater influence on the body weights of lambs or the daily gain of group E in the 19th and 35th day of the experiment. 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 hay, as well as a better feed efficiency (Table 5). Pond and Goode (1985) found improved daily gain for 24.7% and 6.4% as well as better feed efficiency for 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.

Harayanto *et al.* (1994) and Birch *et al.* (1994) have also accomplished higher daily weight increases of lambs fed with feeds enriched with probiotics, but they were not statistically significant. Various authors have achieved significantly higher daily weight gain with the usage of probiotics in feeds. Strzetelski *et al.* (1998) have used the probiotic Probios® and *Streptococcus faecium*® in the feed for calves and have accomplished higher daily increases (10.0% and 6%), plus a better conversion of food (1.00% and 6.00%). Daniecke *et al.* (2001) have accomplished a higher daily weight gain (3.8% and 8.0%), as well as a better conversion ration (up to 2.2%) in calves when fed milk replacement up to 4.4%. Similarly, Gill *et al.* (1987) fed a bacterial DFM (direct fed microbials) during a 28-d receiving period and reported a 9.3% increase in daily weight gain, 9.5% improvement in feed efficiency and a 10.9% reduction in morbidity in calves. Supplementing cattle diets on daily weight basis with lactate-utilizing bacteria and (or) lactate-producing bacteria has been shown to improve the feed efficiency and average daily gain of feedlot cattle (Swinney-Floyd *et al.*, 1999; Rust *et al.*, 2000).

Results of electrolytes determined in the serum of lambs (Table 4) show that the addition of the probiotic in the feed mixture of group E influenced its composition.

Concentration of Ca at the end of the experiment in group E was significantly lower ($P < 0.01$), but concentration of K was significantly higher, compared to group C. Higher values have been also noticed in the Cl level in the first part of the experiment in group E and at the end of the experiment in group C (Table 4).

Added probiotic contained lactic fermentation bacteria (two types of *Enterococcus faecium*) which produce the lactic acid by substrate fermentation. Lactic acid exists in two isomers: D-lactic acid and L-lactic acid. L-lactic acid is often called the natural form because it is the main isomer formed in animals and

humans, whereas D-lactic acid, mainly produced by some microorganisms, is considered as the unnatural isomer (Counotte 1981). The metabolism of D-lactate in the body is slower than the metabolism of L-lactate which results in D-lactate accumulation in the blood (Giescke *et al.* 1976). The differences described in electrolyte concentrations in the serum could be explained by the state of the organism during development of metabolic acidosis. K^+ and hydrogen (H^+) concentrations in the extracellular fluid are parallel to each other (Ganong 2001) from which results that the increase of K in group E has been caused by the increase of H^+ , which means that the lactate was accumulated in the organism. Significantly lower Ca in group E could be the consequence of the metabolic acidosis, too. The parathyroid hormone (PTH) has a decisive role in the notable reduction of Ca level in blood (Brown 1991). PTH influences indirectly by operating the synthesis of 1.25-dihydroxycholecalciferol ($1.25-(OH)_2D_3$) in the kidneys and in other organs, as well. $1.25-(OH)_2D_3$ directly influences the absorption of Ca from the intestine and the kidneys by operating the synthesis of calbindin-D proteins (Hurwitz 1996). It also increases the number of osteoclasts in the bone tissue. $1.25-(OH)_2D_3$ production is depressed by metabolic acidosis (Ganong 2001). Acidifying diets induce the release of cations (including Ca) into the blood in order to correct its pH (Riond 2001). Metabolic acidosis first stimulates the physicochemical mineral dissolution then cell mediated bone resorption by increasing the activity of osteoclasts and osteoblasts (Bushinsky *et al.* 1999; Bushinsky and Frick 2000). Ca reabsorption in renal tubules is directly inhibited in metabolic acidosis and leads to increased urinary Ca excretion (Beck and Webster 1976; Scott *et al.* 1993).

The increased level of alkaline phosphatase (AP) in the serum of group E is the indicator of reinforced activities of bone tissue cells. A notably higher level of phosphate ($P < 0.05$) at the end of the experiment in group E is a consequence of the drop of Ca level. There is usually a tendency for plasma P to rise in chronic calcium deficiency (Underwood and Suttle, 2001). The lambs fed with Ca deficient feed suffered from hyperphosphataemia. Ca concentration in the sera of both groups was within the normal physiological range from 2.00 mmol/l to 2.5 mmol/l (Field *et al.* 1975) which is above the medium values from 1.8 to 2.0 mmol/l (Underwood and Suttle 2001). The level of Cl in the sera of group E was on the 19th day of the experiment significantly higher ($P < 0.05$) and, at the 35th day lower, compared to group C. Cl is the main anion in the plasma. The bicarbonate ion (HCO_3^-) represents the measureable anion. Higher concentration of Cl on the 19th day in group E could be the consequence of a drop in HCO_3^- because of the respiratory compensation lactic acidosis. At the end of the experiment the lower level of Cl could be caused by renal compensation of acidosis by production of acidic urin. Renal compensation, to some measure, restores the HCO_3^- supplies, but because of the anion gap (with the aim of keeping the ionic balance), the Cl plasma level is reduced.

Among the biochemical values, the glucose and urea concentrations (Table 5) in group E are notably lower ($P < 0.01$), and the concentration of total bilirubin is higher ($P < 0.05$) in comparison to group C. Glycolysis is inhibited by acidosis (Macklear and Guest 1953). Therefore, acidemia produced the increase of blood

glucose in rats (Alberti and Cutbert 1982). Gluconeogenesis is also inhibited by increased H^+ concentration (Iles *et al.* 1977). Gluconeogenesis, in ruminants, is also the main source of glucose and it has a decisive influence on its level in the blood. Glucose precursors are propionate (40-55%), lactate (17%), amino acids (16-25%) and glycerol (1%) (Huntington and Eisemann 1988). Lower levels of glucose in lambs in group E could be explained by lowered gluconeogenesis, whether it is directly a result of the increased H^+ concentration or indirectly, because of the influence of insulin. It is known that insulin inhibits phosphorylase and gluconeogenic enzymes. Potassium stimulates the secretion of insulin (Genuth 1988), while K depletion inhibits its secretion (Ganong 2001). Acidaemia caused by ammonium chloride in rats influenced a marked increase of insulin concentration. Significantly higher levels of triglycerides in group E ($P < 0.05$) could be assigned to the influence of insulin on increased lipid synthesis in the liver.

Lowered activity of ALT ($P < 0.01$) and AST ($P < 0.01$) gives us information on gluconeogenesis (Table 6). These two transaminases are highly active in the liver, and their activity could be considered as a measure of gluconeogenesis. Increased gluconeogenesis in the experiment *in vitro* was followed by the increased activity of ALT and AST (Lenna *et al.* 1999).

Metabolic acidosis influences the level of urea in blood. In rodents, chronic metabolic acidosis, induced by either HCl or NH_4Cl , results in suppression of syntheses of urea, but with concomitant stimulation of amino acid oxidation, net glutamine synthesis, and urinary NH_4^+ release (May *et al.* 1992; Karim *et al.* 2002). Under acidotic condition, there is a large change in glutamine utilisation and synthesis in liver, i.e. transfer of ammonia through the glutamine amino group is stimulated (Lobley *et al.* 2000). In creating significantly lower levels of urea in group E ($P < 0.01$), besides the complete uptake of NH_3 for synthesis of glutamine, there could be a slight absorption of NH_3 from the rumen. Higher insulin level and its anabolic effect did not influence urea production in cattle (Eisemann and Huntington 1994). The increased separation of aldosterone due to K increase with consequently increased glomerular filtration rate could be excluded since creatinine levels between the groups are completely equal. A marked lowered level of the CK activity in group E ($P < 0.01$) could be considered as a part of the protective mechanism in the compensation for acidosis. Acidosis induces, to a great extent, the increase of the adenine nucleotide level in skeletal muscle cells, therefore, the infusion of NH_4Cl led to a significant increase of ATP, ADP and AMP within the muscles as well as in the liver of experimental rats (Alberti and Cutbert 1982). The increase of ATP level should mean a higher creatine phosphate level, as well. A decrease of the CK serum activity in our experimental lambs, presuming high ATP and creatine phosphate levels in the muscle cell (which is almost exclusively the CK source), indicates a reduction in the dynamics of the creatine phosphate consumption and restitution in the cell. A decreased level of CK was found in rabbits fed with a feed which presumably induced hypovitaminosis B_1 (Liker *et al.*, 1998). Cholinesterases activity, which was higher in group E and markedly higher only in the middle of the duration of the experiment ($P < 0.05$), could be a consequence of an increased separation of catecholamines that,

among others, is frequently found in metabolic excitatory acetylcholine in sympathetic ganglia and adrenal medulla. A significant increase of bilirubin has been already marked after the 19th day of the experiment in group E ($P < 0.05$). At the end of the experiment the difference was much higher ($P < 0.01$) (Table 5). Almost the same changes have been found in commotion of concentration of Fe. They were also significantly higher in group E ($P < 0.05$) (Table 4). This can be explained by an increased sensibility of red blood cells followed by their more intensive substitution, that is by shorter life-span. We have observed that a higher sensibility of red blood cells was among the first signs of a disturbed acid-base balance. The increase of bilirubin in serum could be a consequence of a damaged liver, but the values of the enzymes measured do not confirm this hypothesis.

CONCLUSION

The group of lambs fed with probiotic had larger body weight, greater daily gain, increased average daily intake and a better feed conversion ratio, compared to group C, but differences between groups have not been statistically significant. Probiotic usage in lambs shows that it has a mild influence to the acido-basic balance by which it did not influence the fattening results. The differences in the indicators of production (higher average daily intake and a better feed efficiency) could be also a consequence of lowered catabolic processes and/or higher level of insulin due to the influence on the acid-base balance.

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REFERENCE

1. Alberti KGMM, Cutbert C, 1982, The hydrogen ion in normal metabolism: a review. In: Porter R, Lawrenson G, editors, *Metabolic acidosis*. Ciba Foundation Symposium, Pitman Books, London, 1-19.
2. A.O.A.C., 1984, Official Methods of Analysis. (14th Ed.). Association of Official Analytical Chemists, Arlington, VA, USA.
3. Beck N, Webster SK, 1976, Effects of acute metabolic acidosis on parathyroid hormone action and calcium mobilization, *Am J Physiol*, 230, 127-31.
4. Birch KS, Thomas JD, Ross TT, 1994, Growth and carcass characteristics of newly received feeder lambs treated with probiotics and vitamin E, *Sheep Goat Res*, J. 10, 3, 201-6.
5. Brown EM, 1991, Extracellular Ca^{2+} sensing, regulation of parathyroid cell function end role of Ca^{2+} and other ions as extracellular (first) messengers, *Physiol Rev* 71, 371-411.
6. Bushinsky DA, Frick KK, 2000, The effects of acid on bone, *Curr Opin Nephrol Hypert*, 9, 369-79.
7. Bushinsky DA, Chabala JM, Gavrilov KL, Levi-Setti R, 1999, Effects of in vivo metabolic acidosis on midcortical bone ion composition, *Am J Physiol*, 277, 813-19.

8. *Counotte GHM*, 1981, Regulation of lactate metabolism in the rumen, PhD. Thesis, Veterinary faculty, University of Utrecht.
9. *Daenicke R, Böhme H, Flachowsky G*, 2001, Efficacy of various probiotics on the performance of raising calves. In: Book of Abstract of the 52th Annual Meeting of EAAP, Budapest, Hungary, August, 26-9, 2001, 107.
10. *Eisemann JH, Huntington GB*, 1994, Metabolite flux across portal-drained viscera, liver, and hindquarters of hyperinsulinemic, euglycemic beef steers, *J Anim Sci*, 72, 2919-29.
11. *Erhard MH, Leuzinger K, Stangassinger M*, 2000, Studies on the prophylactic effect of feeding probiotics, pathogen-specific colostrum antibodies or egg yolk antibodies in ne newborn calves, *J Anim Physiol Anim Nutr*, 84, 3-4, 85-91.
12. *Feist K, Nagel S, Voigt J*, 1997, Microbial cultures versus germs causing diarrhea. Probiotic against calf diarrhea, *Neu Landwirtschaft*, 2, 66-73.
13. *Field AC, Suttle AF, Nisbet DI*, 1975, Effect of diets low in calcium and phosphorus on development of growing lambs, *J Agri Sci Camb*, 85, 435-42.
14. *Fox SM*, 1988, Probiotics: Intestinal inoculants for production animals, *Vet Med*, 83, 806-30.
15. *Ganong WF*, 2001, Review of Medical Physiology. 20th ed. Lange/McGraw-Hill Companies, New York, 795.
16. *Genuth SM*, 1998, Endocrine system. In: Berne RM, Levy MN, editors, Physiology, 4th Edition, The C.V. Mosby Company, 919-1083.
17. *Ghorbani GR, Morgavi DP, Beauchemin KA, Leedle JAZ*, 2002, Effect of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle, *J Anim Sci*, 80, 1977-86.
18. *Gill DR, Smith RA, Ball RL*, 1987, The effect of probiotic feeding on health and performance of newly-arrived stocker calves, *Okl Agr Exp Stn*, MP-119, 202-4.
19. *Giescke D, Bartelmus C, Stangassinger M*, 1976, Untersuchungen zur Genese und Biochemie der Pansenacidose. 3. Stoffwecheldynamik von D(-) und L(+) Milchsäure nach Verabreichung von Zückern and nach Überfütterung mit Stärke, *Zbl Vet Med*, 23, 353-63.
20. *Görgülü M, Siuta A, Yurtseven S, Öngel E, Kutlu HR*, 2003, Effect of probiotics on growing performance and health of calves, *Cub J Agric Sci*, 37, 2, 125-9.
21. *Haryanto B, Diviyanto K, Isbandi S*, 1994, Effect of probiotic supplement on the growth and carcass yield of sheep. In: Proceedings of the 7th AAAP Animal Science Congress on Sustainable animal production and the environment, Bali, Indonesia, July 11-16, 1994, 2, 549-50.
22. *Huntington GB, Eisemann JH*, 1988, Regulation of nutrient supply by gut und liver tissues, *J Anim Sci*, 66, (suppl. 3), 35-48.
23. *Hurwitz S*, 1996, Homeostatic control of plasma calcium concentration. *Crit. Rev. Biochem, Mol Biol*, 31, 41-100.
24. *Iles RA, Cohen RD, Rist AH, Baron PG*, 1977, The mechanism of inhibition by acidosis of gluconeogenesis from lactate in rat liver, *Biochem J*, 164, 185-91.
25. *Kaneko J J*, 1997, Clinical Biochemistry of Domestic Animals. 5th Ed. Academic Press. San Diego, California, USA, 923.
26. *Karim Z, Attmane-Elakeb A, Bichera M*, 2002, Renal handling of NH₄⁺ in relation to the control of acid-base balance by the kidney, *J Nefrol*, 15, (suppl 5), 128-34.
27. *Krehbiel CR, Rust SR, Zhang G, Gilliland SE*, 2003, Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action, *J Anim Sci*, 81, Suppl. 2, 120-32.
28. *Leena S, Shameena B, Oommen O V*, 1999, Studies on the effect of growth hormone in vivo and in vitro on lipogenic enzymes and transaminases in a teleost *Anabus testudineus* (Bloch), *Endocr Res*, 25, 341-55.
29. *Liker B, Božac R, Husdžinović I., Mužic S, Biočina B, Tumpić A, Rupić V*, 1998, Activity of some enzymes in the blood plasma of Hyla rabbits fed various proportions of substrate from production of the *Pleurotus pulmonarius* mushroom, *Acta Vet Brno*, 67, 27-36.
30. *Lobley GE, Milano GD, Van der Walt JG*, 2000, The Liver: Integrator of Nitrogen Metabolism. In: Cronje P., editor, Ruminant physiology: digestion, metabolism, growth, and reproduction, CABI Publishing, Oxon and New York, 149-68.

31. Macklear B, Guest GM, 1953, Blood glycolysis: mechanisms inhibited by glycolysis, *Am J Physiol*, 174, 269-72.
32. May RC, Masud T, Logue B, Bailey J, England B, 1992, Chronic metabolic acidosis accelerates whole body proteolysis and oxidation in awake rats, *Kidney Int*, 41, 1535-42.
33. Pond KR, Goode L, 1985, The Evaluation of Probios® for wean-stressed Lambs. Microbial Genetics Division, Pioneer Hi-Bred International, Des Moines, Iowa.
34. Riond JL, 2001, Animal nutrition and acid-base balance, *Eur J Nutr*, 40, 245-54.
35. Rust SR, Metz K, Ware DR, 2000, Effect of Bovamine rumen culture on the performance and carcass characteristics of feedlot steers. Mich. Agric. Exp. Sta. Beef Cattle, Sheep and Forage Sys. Res. Dem. Rep. No. 569, East Lansing, 22-6.
36. Scott D, Loveridge N, Abu Damir H, Buchan W, Milne J, 1993, Effects of acute acid loading on parathyroid hormone secretion and on urinary calcium and cAMP excretion in the growing lamb, *Exp Physiol*, 78, 157-63.
37. Strzetelski JA, Kowalczyk J, Krawczyk K, 1998, Effect of various probiotics on calf performance, *J Anim Feed Sci*, 7, (Suppl. 1), 241-4.
38. Statistica, Stat Soft, Inc., 2001, Statistica (data analysis software system), version 6, www.statsoft.com.
39. Swinney-Floyd D, Gardiner BA, Owens FN, Rehberger T, Parrott T, 1999, Effect of inoculation with either strain P-63 alone or in combination with *Lactobacillus acidophilus* LA53545 on performance of feedlot cattle, *J Anim Sci*, 77 (suppl.1), 77.
40. Teeler E, Vanabelle M, 1991: Probiotics: fact and fiction, *Mededelingen van de faculteit Landbouwwetenschappen, Rijksuniversitet Gent*, 56, 1591-9.
41. Underwood EJ, Suttle N, 2001, The mineral nutrition of Livestock. 3rd Ed., CABI Publishing, Oxon, New York, 624.
42. Wolter R, Henry N, 1988, Bacteries lactiques et alimentation animale, *GTV*, 6, 19-29.

UTICAJ ISHRANE PROBIOTIKOM PIONEER PDFM® NA PROIZVODNE PERFORMANSE I SASTAV KRVI KOD JAGNJADI U TOVU

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DOMAĆINOVIĆ M i ŠPERANDA T

SADRŽAJ

Ova ispitivanja su izvršena na 48 jagnjadi podeljenih posle zalučivanja u dve grupe i trajala su 35 dana. Jagnjad eksperimentalne (E) grupe bila je hranjena koncentratom sa dodatkom probiotika PIONEER PDFM®. Na kraju oglada, ona su imala veću telesnu masu, veći dnevni prirast, povećanu dnevnu konzumaciju hrane i bolju konverziju u poređenju sa kontrolnom grupom, ali ove razlike nisu bile statistički značajne. Jagnjad ove grupe imala su nižu koncentraciju kalcijuma 35. dana oglada (2.16 mmol/l) u poređenju sa kontrolnom grupom (2.38 mmol/l) i ova razlika je bila statistički značajna ($P < 0.01$). Koncentracija hlora u krvi jagnjadi ove grupe je bila veća 19. dana (104.75:103.38nmol/L) a manja ($P < 0.05$) 35. dana (106.00:107.33 mmol/L). Koncentracija gvožđa u serumu je bila veća u grupi E ($P < 0.05$) 19. i 35. dana (26.29 i 33.00 mmol/l) nego u kontrolnoj grupi (21.97 i 29.15 mmol/l). Koncentracije fosfora i kalijuma u serumu su bile veće 35. dana u

grupi E (2.74 i 4.95 mmol/l) nego u kontrolnoj grupi (2.41 i 4.65 mmol/l). Jagnjad grupe E imala je u serumu nižu koncentraciju glukoze, uree i manju aktivnost enzima ALT, AST i CK 35. dana oglada i ove razlike su bile statistički značajne ($P < 0.01$) dok je koncentracija ukupnog bilirubina i triglicerida bila veća.