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Influence of Prosimbiont E product on growth and haematological indices in young goats

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Abstract

The aim of the current study was to assess the effect of a nutritive supplement (Prosimbiont E), consisting of minerals, vitamins and live cultures (Lactobacillus plantarum, Streptococcus faecium, Saccharomyces cerevisiae) incorporated in the concentrate mixture (1.5%), on growth and haematological indices in young goats. For the trial, four experimental groups were set up (n = 11), consisting of Carpathian breed young goats (120-160 days of age), which were ad libitum fed with two rations (R1 and R2) consisting of Lolium hay (60% in R1 and 40% in R2), and a concentrate mixture (40% in R1 and 60% in R2) with 14% crude protein. Average daily gain was significantly higher in supplemented groups compared to their controls, by 18 g (p<0.02) and 37 g (p<0.01) in R1 and R2, respectively. The nutritive supplements administration increased the number of protozoa / ml of ruminal fluid, by 153.2% in R1 and 165.3% in R2. Also, the nutritive supplement doubled the share of Diplodinium and Dasytricha protozoa genera to the detriment of Entodinium genus. Haematological indices were within the reference range of the species, with values of Hgb, RBC, MCV and MCHC significantly higher (p<0.05) in the experimental groups compared with the controls.

Keywords: goat, nutritive supplement, growth, protozoa, haematological

1. Introduction

Feed additives are substances added in small amounts to help complete the rations and balancing it to a better use of its components, the stimulation of growth, which is focused on increasing the efficiency of feed conversion and growth rate improvement. Different nutrients and feed additives fed to fattening goat kids can positively influence haematological indicators and bio-productive indicators (D. DRINCEANU [1]).

In this study three probiotics were used: *Sacharomyces cerevisiae* with a role in higher concentrations cellulolytic bacteria, those that use lactic acid and proteolytic bacteria in the rumen (K.A. DAWSON & al. [2]); *Lactobacillus sp.* are the first organisms that inhabit the rumen and milk used as a substrate (the most common species are: *Lactobacillus acidophilus, Lactobacillus lactis, Lactobacillus casei, Lactobacillus plantarum*), *Streptococcus sp.* which converts sugars and starches into lactate (D. DRINCEANU [1]).

Throughout supplementation with Sel-Plex and Yea-Sacc[1026] of the concentrates mixture fed to the young goat significant differences (p<0.02) were registered for the final body weight, total weight gain and average daily gain when compared to the control group.

Haematological indices are ranging within physiological limits and no significant difference ($p>0.05$) was found between the control group and experimental groups (O.S. VOIA & al. [3]). Other studies pointed out an increase of the performance of unweaned lambs by using *Saccharomyces cerevisiae* yeast supplements (S. MILEWSKI & al. [4,5]).

Protozoa number decrease could be influenced by the feeding supplements. Variation of protozoa count in the rumen fluid has an important function, being an indicator for acute and subacute acidosis determined by the lactic acid relative to the volatile fatty acids in rumen. Thus, protozoa count in rumen could be used to diagnose the ruminal acidosis (T.G. NAGARAJA & al. [6]).

Supplementation of feed with *Saccharomyces cerevisiae* induces a significant structural change in rumen bacterial populations by increasing the percentage of protozoa and decreases the percentage of other groups of bacteria (L.J. ERASMUS & al., [7]).

Absence of ciliate protozoa in lambs' rumen could have negative effects on ruminal microbial ecology, feed sources biodegradation processes and muscle fatty acids composition (D.R. YANEZ-RUIZ & al., [8]).

Yeast culture supplementation does not affect some serological blood parameters in goats (B. ÖZSOY & al., [9]). However, F.K. ÖGUZ [10], reported that blood parameters were not statistically affected by supplementation with the yeast culture in male goat kids.

The purpose of this study was to evaluate the effect that it has Prosimbiont E (nutritive supplement with an original structure) on the bio indicators, ruminal microflora and haematological blood parameters.

2. Materials and Methods

Animals, Diets

Researches were carried out in a commercial farm from Timis county (western Romania) on weaned, single-born, Carpatina breed, male young goats, during their 120-160 days of age. At the beginning of the experiments the young goats were randomly distributed in 4 experimental groups, each group consisting of 11 heads according to the experimental design presented in Table 1. All young goats were kept under identical rearing conditions.

Table 1. Experiment organization design

Specification	G_1 (control)	G_2	G_3 (control)	G_4
Male young goat (n), 120-160 days	11	11	11	11
Nutritive factors	*Lolium* hay (60 %) Concentrate mixture A (40 %) R_1	*Lolium* hay (60 %) Concentrate mixture A (40 %) R_1+Prosimbiont E	*Lolium* hay (40 %) Concentrate mixture B (60 %) R_2	*Lolium* hay (40 %) Concentrate mixture B (60 %) R_2+ProsimbiontE

Young goats from groups 1 (controls) and 2 (experimental group) were fed *ad libitum* with *Lolium* hay 60% and the mixture of concentrate A representing 40% of the ration R_1 structure. In groups 3 (controls) and 4 (experimental group) was administered, *ad libitum,* *Lolium* hay 40% and the concentrate B, representing 60% of the ration R_2 structure. In concentrated mixtures administered to the experimental groups 2 and 4, Prosimbiont E feed additive was included, in a proportion of 1.5%.

Prosimbiont E product contains: chelated micronutrients, vitamins, glucose, methionine, lysine and live cultures (Table 2), in quantities which to ensure the organism completing specific requirements, and population of the rumen with microorganisms' species, to create a favorable environment for the development of the microflora.

Table 2 Level of supplementing nutrients by adding Prosimbiont E at 1 kg mixture of concentrates

Composition Prosimbiont E Nutrients	Unit	Additional nutrient level
Zn	[mg]	7
Fe	[mg]	18
Cu	[mg]	2
Mn	[mg]	2,6
Ca	[mg]	2,4
P	[mg]	510
Na	[mg]	500
Vitamin A	[UI]	15000
Vitamin D_3	[UI]	3000
Vitamin E	[mg]	25
Vitamin K_3	[mg]	1,25
Vitamin B_1	[mg]	2,5
Vitamin B_2	[mg]	7,5
Vitamin B_6	[mg]	5
Vitamin B_{12}	[mg]	0,025
Folic acid	[mg]	1
Nicotinic acid	[mg]	37,5
Ca pantothenate	[mg]	11
Glucose	[g]	10
Methionine	[g]	0,2
Lysine	[g]	0,2
Lactobacillus plantarum, Streptococcus faecium	[g]	0,14
Sacharomyces cerevisiae strain *Yea-Sacc*^{1026}	[g]	3,4

Experimental groups were made with 14 days before the actual start of the experiments, in order for the goat kids to become accustomed to the experimental feeding and housing conditions.

Body weight evolution was calculated by individual weighing of the young goats at the age of 120 and 160 days of age.

Ruminal fluid protozoa count and genera determination

Infusoria number determination consisted in assessment of the number per volume unit (mm^3) after immobilization with a 1% formaldehyde solution, method described by DEHORITY [11]. Materials and reagents used were: 1% formaldehyde solution; Fuchs-Rosenthal haemocytometer, cover glasses, pipettes, and Optika B100 microscope. One milliliter ruminal fluid was treated with 4 ml 1% formaldehyde solution. The lamina was applied by pressure to haemocytometer until the Newton circles appeared. With the Pasteur pipette 1-2 ruminal fluid drops were applied at the edge of the lamina, which by capillarity penetrated under the lamina. It was kept undisturbed for 2-3 minutes in order for the infusoria to distribute and stabilize within the haemocytometer grid. After that the infusoria were counted in 100 microscopic fields at 100x magnitude.

Calculation was carried out using the following formula: $N = \frac{n \times 5 \times 1000}{3.2}$

where: N – infusoria number per mm^3; n – number of counted infusoria; 5 - dilution; 3.2 counting camera volume; 1000 – correction coefficient.

The main ruminal infusoria genera were determined taking into account that they belong to subclass Ciliata, are about 30 genera, and are grouped in large, medium, and small categories according to their size (20-200 μm).

Blood haematological indices analysis

Blood was sampled at the beginning and at the end of the research trials. The number of erythrocytes (RBC) and leukocytes (WBC) was determined by using the Burker-Turk haemocytometer. For the measurement of haematocrit (PCV) capillary tubes were used, with centrifugation at 12,000 rpm / min for two minutes. The concentration of haemoglobin (Hgb) was determined colorimetrically with potassium ferrocyanide method, with the semi-automatic biochemistry analyzer Vet Screen (L.C. ANDERSON & al., [12], I. PADEANU [13]).

Red cells indices are mean corpuscular volume (MCV) and mean cell haemoglobin concentration (MCHC) were calculated using the following formula: MCV = PCV x 10 / no.E and MCHC = Hgbx100/PCV.

Statistical analysis

Data was analyzed using variance analysis. The software employed was MIMITAB 14. Variance analysis was based on the following equations: $X = \frac{\Sigma x}{n}$; $SD = \sqrt{S^2}$

where: X = average around which the variable values are grouping; n = individuals number; Σx = individuals sum; S^2 = variance; SD = standard deviation.

Significant difference in variables was tested using Mann-Whitney u test at 0.05 level of probability.

3. Results and discussion

The results regarding the body weights and growth rates are being presented in Table 3. In the R_1 feeding trial, through supplementation with Prosimbiont E in G_2, no significant differences in terms of final body weight was registered, however the total gain difference of 0.71 kg/head/interval and the average daily gain of 18 g/head becomes significant for $p < 0.05$.

In the trial where R_2 ration was given, by the supplementation with Prosimbiont E in G_4, the differences regarding total growth gain of 1.43 kg/head/interval and the average daily gain of 37 g/head were at a significance level of $p < 0.01$.

Goat kids which received probiotic supplementation were found to have increased body weight and growth parameters as compared to the nonsupplemented kids, possibly due to increased dry matter intake and crude fiber digestibility, and decrease in the incidence of diarrhoea due to alteration of the rumen microflora by way of decrease in the number of pathogenic microorganisms and increase in the number of beneficial microorganisms (S.A. KOCHEWAD & al., [14]).

BUGDAYCI & al., [15], opined that dietary live yeast culture significantly ($p<0.05$) increased live weight gain compared with other groups at the hot (middle of summer) final weeks of the study however this result was not reflected to average live weight gain at the end of the experiment.

Table 3. The influence of the feed additive on the growth indices of goat kids (n=10)

Indicators	$G_1 - R_1$ Control	$G_2 - R_1$ Prosimbiont E	G_3 - R_2 Control	$G_4 - R_2$ Prosimbiont E
	$X \pm SD$			
Initial weight [kg]	19.07 ± 1.68	19.20 ± 1.58	19.16 ± 1.61	19.02 ± 1.54
Final weight [kg]	23.36 ± 1.78	24.20 ± 1.75	26.94 ± 2.13	28.26 ± 1.76
Weight gain [kg]	4.29 ± 0.61	5.00^{*} ± 0.66	7.77 ± 0.93	9.20^{**} ± 0.88
Average daily gain [g]	107 ± 15.18	125^{*} ± 16.43	194 ± 23.03	231^{**} ± 22.03

Note: * $p<0.05$; $^{**}p<0.01$

Out of the ruminal fluid collected has been determined the number of protozoa and have been identified the main types, the results are presented in Table 4.

Table 4. Number and genus of protozoa identified in the ruminal fluid sampled from the goat kids at the end of the experiment

Indicators		$G_1 - R_1$ Control	$G_2 - R_1$ Prosimbiont E	G_3 - R_2 Control	$G_4 - R_2$ Prosimbiont E
Number/ml ruminal fluid		7.96×10^5	1.22×10^6	8.84×10^5	1.46×10^6
Genus %	*Entodinium sp.*	97.73	95.56	96.30	93.68
	Diplodinium sp.	0.45	1.24	1.12	2.12
	Dasytricha sp.	1.82	3.20	2.58	4.20

According to data presented, it can be concluded that under the influence of feed additive the number of protozoa/ml ruminal fluid collected from young goats in G_1-R_1 increased from 7.96×10^5 to 1.22×10^6 in G_2-R_1, hence a growth of 153.2%. Among the identified genus, it was registered that the predominant one was *Entodinium*, at a rate of 97.23 - 95.56%, and the proportion increased from 0.45% to 1.24% in *Diplodinium* and from 1.82% to 3.2% *Dasytricha* genus.

In the intensive feeding system (proportion of concentrates of 60%) there was an increase in the number of protozoa/ml fluid under the influence of the additive Prosimbiont E, from 8.84×10^5 in G_3-R_2, to 1.46×10^6 in G_4-R_2, resulting an increase of 165.3%. Regarding the identified genus, *Entodinium* share is lower (96.3% - 93.68%), again the trends are for increase of *Diplodinium* genus from 1.12% to 2.12% and from 2.58% to 4.2% in *Dasytricha* genus.

The frequencies of appearances of *Entodinium* species in goats are significantly higher than of the other ciliates. This probably stems from the better adaptation of those species to the goat rumen with respect to the other entodiniomorphid (Order: Entodiniomorphida) ciliates (B. GÖÇMEN & al., [16]).

DENEV & al. [17], studied the effects of yeast culture (YC) on the gastrointestinal ecosystem of the rumen population species. Researchers have shown that activity preparation (YC) can stimulate beneficial bacteria in the rumen, thus improving animal performance. Effects (YC) of the animal productivity depend on the culture strain. Research has clearly established strategies for modifying and optimizing microbial activity in the gastrointestinal ecosystem and techniques to boost performance and health of ruminants.

Feed additives influence on the haematological indices is being presented in Table 5.

Haemoglobin (Hgb) had significantly higher values ($p<0.05$) in the experimental groups with 6.93% (G_2-R_1) and 8.23% (G_4-R_2) compared to controls groups (G_1-R_1 and G_3-R_2, respectively). BUGDAYCI & al., [15], reported that **s**ome haematologycal parameters were not altered -except HGB and HCT- by dietary live yeast culture supplementation in goat kids.

Haematocrit (PCV) expressed as a percentage is at the upper limit of the reference 27% in G_4-R_2, the lowest value recorded in controls groups 25.67% and 26% respectively.

Red blood cells (RBC) have a significantly higher value ($p<0.05$), with 0.91 mil/mm3 in the experimental group G_2-R_1, compared to group G_1-R_1, and significantly lower ($p<0.05$) with 1.38 mil/mm3 in the experimental group G_4-R_2 compared with group G_3-R_2.

Table 5. The influence of the feed additive on the haematological indices of goat kids at the end of the experiment

Indicators	Normal limits [12]	$G_1 - R_1$ Control	$G_2 - R_1$ Prosimbiont E	G_3 - R_2 Control	$G_4 - R_2$ Prosimbiont E
		$X \pm SD$			
Hgb [g /dl]	8-12	8.80 ± 0.66	9.41* ± 0.22	8.87 ± 0.31	9.60* ± 0.50
PCV [%]	22-38	25.67 ± 1.53	26.67 ± 0.58	26.00 ± 1.00	27.00 ± 1.01
RBC [$x10^6/mm^3$]	8-18	12.18 ± 1.52	13.09* ± 2.19	12.26 ± 0.71	10.88* ± 0.62
WBC [thousands/mm^3]	4000-13000	6700 ± 916	7366 ± 763	6500 ± 953	7566 ± 1929
MCV [μ^3]	16-25	21.19 ± 1.56	20.83* ± 4.10	21.23 ± 0.80	24.86* ± 1.13
MCHC [g/dl]	30-36	34.27 ± 0.87	35.30* ± 0.45	34.11 ± 0.28	35.54* ± 0.54

Note: * $p<0.05$; Hgb- concentration of haemoglobin; PCV- haematocrit; RBC-erythrocytes; WBC-leukocytes; MCV- mean corpuscular volume; MCHC- mean cell haemoglobin concentration

Leukocytes (WBC) are higher in the experimental groups, between 7366 thousands /mm^3 (G_2-R_1) and 7566 thousands /mm^3 (G_4-R_2) compared to the control group G_1-R_1 and G_3-R_2 were leucocytes took values between 6700-6500 thousands /mm^3. Lambs supplemented with probiotics shows significant improvement the values of Hgb, PCV, RBC's, and WBC's compared with control group, assert HUSSEIN [18] and SARWAR & al [19]. Similar types of results are obtained by MILEWSKI & SOBIECH [20] those who reported that yeast feeding lambs had a significant effect on blood WBC's count in and contributed to higher lymphocyte percentages in the leukogram.

MCV and MCHC has the significant differences ($p<0.05$) in G_2-R_1 (20.83 μ^3 respectively 35.30 g/dl) and in the G_4-R_2 (24.86 μ^3 respectively 35.54 g/dl) compared with control groups G_1-R_1 (21.19 μ^3 and 34.27 g/dl, respectively) and G_3-R_2 (20.83 μ^3 and 35.30 g/dl, respectively). By adding in feed the products Prosimbiont E, all values of the haematological indices are ranging in normal limits. MCHC is very significant in the diagnosis of anaemia and also serve as a useful index of the capacity of bone marrow to produce red blood cells. Increased MCV may also be observed in regenerative anaemia due to hemolysis and haemorrhages (A.A. NJIDDA & al., [21]).

According to the MILEWSKI & SOBIECH [20], the changes in the blood haematological parameters of ewes fed a diet supplemented with dried yeast were indicative of blood supply improvement and immunity enhancement.

4. Conclusion

Supplementation of concentrate with fed additive Prosimbiont E in fattening young goats had an effect a significant increase ($p<0.05$) of the average daily gain of 16.82% in R_1 trial (fiber 60%) and of 19.07% in R_2 trial (concentrate mixture 60 %). Prosimbiont E added in the concentrate feed increased both the protozoa count and genus diversity. *Entodinium* genus was the predominant genus in all the ruminal fluid samples collected. Haematological indices are ranging within physiological limits and significant difference ($p<0.05$) was found between experimental and control groups for Hgb, RBC, MCV and MCHC.

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References

1. D. DRINCEANU. Biotehnologii in alimentatia animalelor.. eds., Eurobit, Timisoara, 2000, pp. 42, 72-73.
2. K.A. DAWSON, D.M. HOPKINS. Differential effects of live yeast on the cellulolytic activities of anaerobic ruminal bacteria. *Journal of Animal Science*, 69:1, 531 (1991).
3. O.S. VOIA, D. FOALE, C. FALCA, I. PADEANU, S. DARABAN. Influence of some feed additives on growth and haematological indices in goat kids after weaning (note 1). In: *Proceedings of the 23rd International Symposium, New technologies in contemporary animal production*, Novi Sad, Serbia, 2013, pp. 134-136.
4. S. MILEWSKI, P. SOBIECH, D. BEDNAREK, R. WÓJCIK, J. MAŁACZEWSKA, B. ZALESKA, A. SIWICKI. Effect of oligosaccharides on the meat performance traits and selected indicators of humoral immunity in lamb. *Bull Vet Inst Pulawy*, 54, 175-179 (2010).
5. S. MILEWSKI, R. WÓJCIK, J. MAŁACZEWSKA, S. TRAPKOWSKA, A.K. SIWICKI. Effect of β-1,3/1,6-D-glucan on meat performance and non-specific humoral defense mechanisms in lambs. *Medycyna Wet*, 63, 360-363 (2007).
6. T.G. NAGARAJA, E.C. TITGEMEYER. Rumen acidosis in beef cattle: the current microbiological and nutritional outlook., *J. Dairy Sci*., 90, 17-38 (2007).
7. L.J. ERASMUS, P.M. BOTHA, A. KISTNER. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. *J. Dairy Sci*.,75, 3056-3065 (1992).
8. D.R. YANEZ-RUIZ, N.D. SCOLLAN, R.J. MERRY, C.J. NEWBOLD. Contribution of rumen protozoa to duodenal flow of nitrogen, conjugated linoleic acid and trans-vaccenic acid in steers fed silages differing in their water-soluble carbohydrate content. *British Journal of Nutrition*, 96, 861-869 (2006).
9. B. ÖZSOY, S. YALÇIN, Z. ERDOĞAN, Z. CANTEKIN, T. AKSU. Effects of dietary live yeast culture on fattening performance on some blood and rumen fluid parameters in goats. *Revue Méd. Vét*, 164:5, 263-271 (2013).
10. F.K. OGUZ, K.E. BUGDAYCI, M.N. OGUZ, K.M. ALBAY. The effect of yeast culture products (Rumisacc and Intetotal) on fattening performance, some blood and rumen fluid parameters in male kids. *Review on Agriculture and Rural Development*, 3 :1, 79-86 (2014).
11. B.A. DEHORITY. Rumen microbiology. Nottingham University Press, Nottingham, UK, 2003, pp. 372.
12. L.C. ANDERSON, G. OTTO, K.R. PRITCHETT-CORNING, M.T. WHARY. Laboratory Animal Medicine. 3rd eds., Academic Press, USA, 2015, pp.629.

13. I. PADEANU. Productiile ovinelor si caprinelor. eds. Mirton, Timisoara, 2002, pp. 4-7.
14. S.A. KOCHEWAD, J.M. CHAHANDE, A.B. KANDURI, D.S. DESHMUKH, S.A. ALI, V.M. PATIL. Effect of probiotic supplementation on growth parameters of Osmanabadi kids. *Veterinary World*, 2 :1, 29-30 (2009).
15. K.E. BUGDAYCI, M.N. OGUZ, F.K. OGUZ, S. SAHINDURAN. Effect of addition of live yeast culture on fattening performance on some blood and rumen fluid parameters in male kids fed with sucrose supplemented concentrate. *Review on Agriculture and Rural Development*, 3 :1, 256-264 (2014).
16. B. GÖÇMEN, S. RASTGELDI, A. KARAO˘LU, H. AÔKAN. Rumen ciliated protozoa of the Turkish domestic goats (*Capra hircus* L.). *Zootaxa*, 1091, 53–64, (2005).
17. S.A. DENEV, T.Z. PEEVA, P. RADULOVA, N. STANCHEVA, G. STAYKOVA, G. BEEV, P. TODOROVA, S. TCHOBANOVA. Yeast cultures in ruminant nutrition. *Bulgarian Journal of Agricultural Science*, 13, 357-374 (2007).
18. A.F. HUSSEIN. Effect of biological additives on growth indices and physiological responses of weaned najdi ram lambs. *Journal of Experimental Biology and Agricultural Sciences*, 2 :6, 598-607 (2014).
19. M. SARWAR, N. MUKHTAR, M.A. SHEHZAD, M. NISA. Traditional versus high input feeding system: Impact on nutrients intake, blood dynamics, hormonal profile, weight gain and economics in growing lambs. *Egyptian Journal of Sheep & Goat Sciences*, 5, 127-145 (2010).
20. S. MILEWSKI, P. SOBIECH. Effect of dietary supplementation with *Saccharomyces cerevisiae* dried yeast on milk yield, blood biochemical and haematological indices in ewes. *Bull Vet Inst Pulawy*, 53, 753-758 (2009).
21. A.A. NJIDDA , I.T. HASSAN, E.A. OLATUNJI. Haematological and biochemical parameters of goats of semi arid environment fed on natural grazing rangeland of Northern Nigeria. *Journal of Agriculture and Veterinary Science*, 3 :2, 01-08 (2013).