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Effect of feeding lambs on some feed additives

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Abstract

This study was conducted to determine the effect of adding different levels of a commercial microbial and enzyme mixture (Bactozym®) to the rations of Osimi lambs on growth performance, nutrients digestion, nutritional values, rumen fermentations, feed conversion ratio, and blood parameters. Fifteen Osimi lambs aged 5–6 months with an average body weight of 24.02 kg ± 1.5 were divided into 3 treatment groups of 5 males each. All experimental lambs were fed a basal diet consisting of a concentrated feed mixture and wheat straw at levels 2 and 1% of live body weight, respectively. Lambs in the first group were fed the basal diet without additives and considered a control group. While those in the second and third groups were fed the basal diet supplemented with Bactozym® at levels of 1 and 2 g/head per day, respectively. Blood samples were collected once at the end of the experiment from three lambs from each treatment to estimate some blood components. A digestion experiment was conducted at the end of the experiment to estimate the digestibility parameters and nutritional value of all experimental treatments. Rumen fluid samples were also collected once, three hours after eating, to measure the characteristics of the rumen fluid. The results indicated significant differences in total dry matter, feed conversion ratio, average daily body weight gain, pH values of rumen fluid, volatile fatty acid concentration, ammonia concentration, globulin, and glucose concentrations, albumin level, and liver enzyme levels between the treated and control groups. The second and third treatments showed improved feed conversion ratio, higher daily weight gain, and higher concentrations of volatile fatty acids in rumen fluid. The treatments also showed increased blood concentrations of globulin, and glucose, while ammonia concentration and albumin levels decreased. The levels of liver enzymes also decreased in the second and third treatments compared to the control group. The results obtained indicate that adding the microbial and enzymes mixture (Bactozyme®), available in Egyptian markets, to the lambs' diets at a rate of 1 g/h/day produced notable nutritional outcomes (*i.e.*, the best nutritional and economic efficiency).

Keywords: Bactozym, Ossimi lambs, performance, rumen fermentations, digestibility.

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1. Introduction

The world's population density is expected to reach more than 9 billion by 2050, presenting majestic food security challenges, particularly for developing countries. Moreover, economic growth has increased the demand for livestock products, putting pressure on the livestock sector to produce more with restricted resources. However, the livestock sector is one of the fastest-growing agricultural sectors, contributing about 40 percent of the global value of agricultural production and supporting the livelihoods and food security of almost 1.3 billion people (Bruinsma, 2003). This growth poses issues related to the most efficient use of resources to produce nutrition for humans, the effects of land transformation and more intensified use on the maintenance of ecological services and biodiversity, the effects of ruminant methane production on weather variation, and the effects of climate transformation-induced temperature rise on animal production. The most important objectives for using probiotics in animal feed are to maintain and improve the performance (productivity and growth) of the animal and prevent and control enteric pathogens. In the context of the growing concern with the sub-therapeutic use of AGP in animal feed and a greater appreciation of the role of the microbial ecology of the gastrointestinal tract in determining animal productivity, increasing numbers of probiotic products are being developed and used in animal nutrition (FAO, 2016). Adding probiotics to sheep improves the benefit of poor-quality feeds (Khattab *et al*., 2020). Anaerobic rumen organisms help in the process of digesting fiber, which leads to the decomposition of the cell walls of plant cells (Galindo *et al*., 2017). As metabolic processes increase, there is a need for operational support of the digestive system by complex biologically active feed additives (Devyatkin *et al*., 2021), particularly probiotics and prebiotics, which mostly correspond to the features of the digestive system of ruminants and increase the biological value of the diet and the efficiency of feed assimilation. Live competitive strains of microorganisms or their metabolites in probiotic supplements are well colonized and take root in the host organism's gastrointestinal tract, optimizing the normal flora (McNeill *et al*., 2016). Improving feed digestibility, biological status, and natural resistance of the body. It is known that probiotic drugs have pronounced enzymatic and proteolytic properties (Magomedaliev *et al*., 2019). Many studies have been devoted to the use of probiotics in agriculture. Extensive scientific and practical material has been accumulated. European Commission (2017) describe their role in improving the microbiocenosis of the gastrointestinal tract, increasing the body's natural resistance, and changing the host's biochemical reactions by optimizing its microbiological status, immunomodulatory, and anti-infective effects (Vladimir *et al*., 2021).

2. Materials and methods

This study was carried out at the experiment Station Faculty of Agriculture, Al-Azhar Univresity, Assiut branch, Assiut, Egypt, during the period from January 2021 to March 2021 and

was lasted for 3 months. This study aimed to investigate the effects of adding a commercial microbial and enzymes mixture (Bactozym®) to the diets of growing lambs on growth performance, nutrients digestibility and blood parameters.

2.1 Animals, diets and experimental design

Fifteen male Osimi lambs aged 5 to 6 months were randomly assigned into three dietary treatment groups of 5 animals each. All experimental lambs were fed a basal ration consisting of concentrate feed mixture (CFM) and wheat straw at levels of 2 and 1% of their live body weight, respectively. The diets were formulated to satisfy the nutrient requirements of rams according to the recommendations of NRC (1985). The first group was fed the basal diet without additives and served as a control group, while the second and third groups were fed the basal diet supplemented with a commercial microbial and enzymes mixture (Bactozym®) at levels 1 and 2 g/head/day, respectively. The probiotics in Bactozym include *Enterococcus faecalis* (4 billion CFU/kg), *Lactobacillus casei* (0.4 billion CFU/kg), *Lactobacillus plantarum* (1.6 billion CFU/kg), *Saccharomyces cerevisiae* (200 billion CFU/kg), *Bacillus subtilis* (6 billion CFU/kg), and *Bacillus licheniformis* (6 billion CFU/kg). Combined with a variety of enzymes, such as beta-gluconase (1,000 U/kg), amylase (20,000 U/kg), protease (40,000 U/kg), xylanase (1,200 U/kg), cellulase (2,400 U/kg), pectinase (400 units/kg), fructose oligosaccharides (10 g/kg), mannan oligosaccharides (10 g/kg), calcium propionate (24 g/kg), and copper penta sulfate (10 g/kg).The chemical composition analysis of CFM and wheat straw are presented in Table (1).

Table (1): Chemical composition of experimental rations (on DM basis).

Feed stuffs	Chemical composition of the ingredients (on DM basis, %)						
	DM	OM	CP	CF	EE	Ash	NFE
CFM	93.28	94.47	16.30	10.31	2.66	5.53	65.20
WS	86.62	90.20	1.74	37.85	1.01	9.90	49.60

WS = wheat straw, CFM = Concentrate feed mixture.

2.2 Growth performance

The initial and final live body weights of lambs were weighed before morning feeding, and the weight gain was calculated as the difference between the initial and final live body weights. Feed conversion was calculated and expressed as kg dry matter (DM), total digestible nutrients (TDN), and digestible crude protein (DCP) per kg/kg body weight gain.

2.3 Rumen liquor parameters

At the end of the digestibility trial, rumen fluid samples were collected three hours after morning feeding from each lamb using a stomach tube and then filtered through three layers of cheesecloth. The filtrated portion was then immediately

used to measure pH using a digital pH meter (Hanna Instruments Hi 3424 micro-computer-pH meter) and ammonia N concentration according to the method of Conway (1957). Three to four drops of formalin solution were added to the filtrated portion to stop the microbial activity before its storage for analysis, and then the samples were kept frozen at -20 ℃ for the determination of total volatile fatty acids (TVFAs). The TVFAs were measured according to the procedures of Warner (1964).

2.4 Digestion trial

At the end of the feeding trial, three animals from each treatment group were selected to carry out the digestibility trial. Each animal was kept individually in a metabolic cage that allowed for the separation of feces and urine. The digestibility trial lasted 17 days. The daily feed consumption was accurately estimated during the whole experimental period. The daily feces and urine excreted were collected before morning feeding during the last 7 days of the experimental period (collection period) and kept in the refrigerator. The individual urine samples were pooled together, and subsamples were picked for N-determination. A proximate chemical analysis of feeds, ingredients, feces, and urine was performed according to AOAC (2005). Urinary N was determined using the microKjeldahl technique. The apparent digestion coefficients of nutrients and feeding values were calculated.

2.5 Blood sampling and biochemical analysis

At the end of the experimental period, blood samples were withdrawn from the jugular vein without an anticoagulant before morning feeding (fasting). Blood samples were centrifuged at 4000 rpm for 15 minutes. Subsequently, the separated serum was kept in a clean, dried glass vial and stored at -20 °C until analysis. Serum total protein and glucose were analyzed according to Armstrong and Carr (1964), and serum albumin was determined according to Doumas *et al*. (1971). While serum globulin was obtained as the difference between the total protein and albumin concentrations, Serum GOT (AST) and GPT (ALT) were determined according to the method described by Reitman and Frankel (1975).

2.6 Statistical analysis

The data were statistically analyzed using general linear model (GLM) procedure of SAS (2004) program, version 8.2. Differences among groups for feed intake, daily gain, feed conversion, blood parameters and nutrient digestibility were evaluated by one-way ANOVA. The significant differences between treatments means were tested by Duncan Multiple Range Test (Snedecor and Cochran, 1980).

3. Results and Discussion

3.1 Growth performance and economic efficiency

The data presented in Table (2) showed

that the dietary treatments had no effect on final live body weight or total weight gain; however, they significantly increased the average daily weight gain compared with the control group. Additionally, lambs in the T2 group had the highest values of TDMI, TDNI, and TDCPI, followed by those in the T1 group compared with the control group. In terms of feed intake values expressed as DMI, TDN, and DCP relative to kg of body weight, the treated groups had the lowest values compared with the control group. Generally, increasing the levels of Bactozym® improved feeding values based on TDN and DCP. The improvement in weight gain may be attributed to the level of probiotics in the diet (Khalid *et al*., 2011). In this respect, Gebregiorgis *et al*. (2012) reported that an increase in protein intake increases feed intake, digestibility, and, consequently, growth rate. These results agree with the findings of Soliman *et al*. (2016), who found that feeding high-energy diets resulted in greater daily body weight gain. Also, Vladimir *et al*. (2021) found that dietary supplementation of probiotics (Enzimsporin™) improved the lambs' body weight gain by 18.8%.

Table (2): The effect of adding different levels of commercial microbial and enzyme mixtures to the diets of growing Osimi lambs on growth performance and feed conversion ratio.

Item	Treatment		
	Control	T1	T2
No. of. Lambs	5	5	5
Duration day	90	90	90
Initial weight, kg	24.00 ±1.64	24.26 ± 1.01	23.80 ± 1.36
Final live wt., Kg	38.88 ± 2.30	40.60 ± 0.51	40.20 ± 0.73
Total gain, kg	14.8	16.6	17.2
Av. Daily gain, g	164.40±1.50 ^{b}	184.40± 3.88 ^{a}	191.00±2.92 ^{a}
CFM	470	470	490
Roughage	230	250	260
T DM intake	700 ± 0.86^{c}	720± 1.28^{b}	750 ± 1.59^{a}
TDN intake	554.40	618.04	640.42
T DCP intake	44.80	47.09	49.95
DM/Kg	4.3	3.9	3.9
TDN/Kg	3.37	3.34	3.35
DCP/Kg	0.039	0.035	0. 035
Cost/ kg gain	18.49	17.17	17.67
Economic Efficiency	3.25	3.49	3.38

$^{a, b, c}$ means with different superscripts on the same row are different at ($P<0.05$) and ($P<0.01$). T2= lambs group fed ration supplemented with 2 g Bactozym® / head / day.

Regarding the economic efficiency (EE), the lambs fed rations supplemented with 1 g of probiotics had the highest economic efficiency, followed by those fed rations supplemented with 2 g/head/day of Bactozym®, while lambs in the control group recorded the lowest value. These results agree with the results obtained by Soliman *et al*. (2016), who stated that the probiotic-supplemented group showed the

highest return value compared with the control group.

3.2 Rumen fluid variables

Table (3) shows that rumen pH was affected by Bactozym® supplementation, with significant differences between the T2 and control groups (6.14 to 6.19) and no significant differences between the T1 and control groups (6.14 to 6.18). The values were within normal ranges 3 hours after feeding. Cellulolytic bacteria need a rumen pH between 6.2 and 7.0 to multiply rapidly and colonize the epidermal surfaces of plant fragments within 5 minutes. This result may be related to the fermentation process of both nonstructural and structural carbohydrates and the production of volatile fatty acids, which increased with the passage of time and caused a reduction in ruminal pH until they were proportionally more absorbed from the rumen wall, resulting in an increase in pH again. These results agreed with the findings of El-Shinnawy (2010) and El-Shinnawy *et al*. (2011). In the present study, lambs in T1 and T2 treatment groups had the highest (P<0.05) value of TVFAs in rumen fluid (26.27 m.eq/100 ml, 27.43 m.eq/100 ml) compared with the control group (24.37 m.eq/100 ml). These results agree with Sanchez *et al*. (2010), who provided evidence that probiotics increase total TVFAs and microbial protein concentrations and stabilize the rumen pH. Similar results were obtained by Komonna (2007) and Soliman *et al*. (2016). In terms of ammonia-N concentration, data in Table 4 showed that ruminal ammonia nitrogen values were significantly lower (P<0.05) in T2 group (25.67 mg/100 ml) than those in T1 group (27.34 mg/100 ml), and control group (28.37 mg/100 ml).

Table (3): Effects of adding different levels of a microbial and enzyme mixture to the diet of growing Osimi lambs on rumen fluid variables.

Items	Treatment			Sig.
	Control	T1	T2	
Ruminal p^H (3h after feeding)	6.19 ±0.003[a]	6.18±0.09[a]	6.14 ±0.44[b]	*
TVFA's (meq/dl) (3h after feeding)	24.37 ± 0.32 [b]	26.27 ± 0.15 [a]	27.43 ± 0.35[a]	**
NH_3-N (mg / dl) (3h after feeding)	28.37 ± 1.56 [a]	27.34 ± 1.72 [a]	25.67± 3.10 [b]	**

[a, b] means with different superscripts on the same row are different at (P<0.05) and (P<0.01), T1 = lambs group fed ration supplemented with 1g Bactozym® / head / day, T2 = lambs group fed ration supplemented with 2 g Bactozym® / head / day, sig. = significant, * = p-value < 0.05, ** = p-value < 0.01.

The decrease in NH3-N concentration with animal-fed rations supplemented with probiotics (DFM) could be a result of increased incorporation of ammonia into microbial protein. The ranges of NH3-N concentration were 12.16–14.03 at zero time and 16.05–16.82 mg/100 ml at 6 hours post-feeding. These ranges could cover the required amounts for microbial protein synthesis, since the minimum value in this concern is 3.3 to 8.5 mg/100 mL ruminal fluid (Kang-Meznarich and

Broderick, 1981). The obtained results are consistent with those obtained by Soliman *et al*. (2016). On the contrary, Sanchez *et al*. (2010) found that the concentrations of NH_3-N were not modified by supplemented prebiotics.

3.3 Apparent digestibility coefficients and nutritive values

Results shown in Table (4) indicated that the apparent digestion coefficients of various nutrients and feeding values improved with higher levels of Bactozym® supplementation in animal diets. The improvement was significant for OM digestibility, CP digestibility, CF digestibility, EE digestibility, and NFE digestibility compared to animals fed the control ration, which had the lowest digestion coefficients. The positive effect of the probiotic's additive on CF digestibility in this study might be related to stimulation of the growth of cellulolytic bacteria (Michael *et al*., 2011). Several studies have demonstrated that the addition of probiotics improves nutrient digestibility and the degradation of fiber (El-Waziry and Ibrahim, 2007). Also, Ismaiel *et al*. (2010) showed that CP and CF digestibility significantly improved in lambs fed rations supplemented with probiotics (Tonilisat or Roemin). Our results agree with sallam *et al*. (2014), who reported that adding DFM Ru-max to a diet may increase enzymatic activity within the rumen, which enhances the digestibility of the feed. Whitley *et al*. (2009) also reported improved apparent DM, OM, and CP digestibility in goats fed a diet supplemented with commercial probiotics compared with the control group. Soliman *et al*. (2016) reported that animals given rations supplemented with probiotics recorded the highest ($P<0.05$) values. Regarding nutritive values, lambs fed a diet supplemented with Bactozym® at a 2 g/head/day level had higher TDN and DCP values compared to the control group, while those fed a 1 g/head/day level had an intermediate value. Live competitive strains of microorganisms or their metabolites in probiotic supplements are well colonized and take root in the host organism's gastrointestinal tract, optimizing the normal flora, improving feed digestibility, biological status, and natural resistance of the body. These results agree with Soliman *et al*. (2016), who found that probiotics (Ru-Max) had positive responses to the mean values of TDN and DCP. The nutritive values, expressed as TDN and DCP, showed the lowest values recorded by control (57.95% and 7.77%, respectively), while the highest values were recorded by lambs receiving 0.5 and 1 g PRO/d (62.32%, 64.28%, and 8.432%, 9.21%, respectively). Saleem *et al*. (2017) found during the post-weaning phase, the final BW, ADG, total gain, and feed conversion ratio of the lambs receiving probiotic treatments tended to be greater ($p \leqslant 0.10$) than the control group. Except for ether extract digestibility, all nutrient digestibility was improved with the inclusion of probiotics in the post-weaning diets.

Table (4): Effects of adding different levels of microbial and enzyme mixture (Bactozym®) to the diets of growing Osimi lambs on apparent digestibility coefficients and nutritive values.

Item	Treatments			sig.
	Control	T1	T2	
Digestion coefficients (%)				
Dry matter	50.33 ±0.24	53.68 ± 1.56	54.84 ± 1.67	Ns
Organic matter	54.88 ± 0.90[b]	58.57 ± 1.41[ab]	60.03 ± 1.50[a]	*
Crude protein	56.26 ±1.87	58.52 ± 1.99	59.98 ± 1.28	Ns
Crude fiber	61.54 ± 0.88[b]	69.77 ± 1.19[a]	71.09 ± 1.38[a]	*
Ether Extract	64.04 ± 4.17[b]	74.62 ± 1.22[a]	73.69 ± 1.45[a]	*
NFE	51.87 ± 0.59[b]	54.00 ± 1.58[ab]	57.45 ± 1.17[a]	*
Nutritive values				
TDN %	73.72± 0.42[b]	77.58 ± 1.95[ba]	80.73 ± 1.84[a]	*
DCP (gm)	7.06 ± 0.23	7.49 ± 0.16	7.52 ± 0.15	Ns

[a, b] means with different superscripts on the same row are different at (P<0.05) and (P<0.01). T2 = lambs group fed ration supplemented with 2 g Bactozym® / head / day, sig. = significant, * = p-value < 0.05, NS = insignificant.

3.4 Blood biochemical variables

The effects of dietary Bactozym® supplementation on the blood parameters of lambs are presented in Table 5. The results revealed that adding Bactozym® to lamb's diet had no effect on serum total protein, but increased serum globulin and lowered albumin concentrations significantly compared with the control group. Increasing globulin concentrations in the treated groups may be related to the beneficial effect of probiotic supplementation on increasing protein digestibility through the enzymatic effect of protease and altering the amino acid profile of the digested due to increasing microbial protein synthesis. Our results are partly in agreement with the findings of Abdel-Khalek *et al*. (2000), who reported that supplementation of 10 g/kg diet probiotics showed significantly (P<0.05) higher values of serum albumin, which are about 4.58 and 19.65% higher than supplementation of 5 g/kg diet probiotics and control, respectively. Abdel-Salam *et al*. (2014) found that Najdi male lambs fed a high level of synbiotic had the highest concentration of blood serum albumin and globulin, followed by those fed a low level of synbiotic, while the lowest value was in the control group. Also, Hussein (2014) reported increased values of plasma total protein, albumin, and globulin in lambs supplemented with probiotics (5 g and 10 g of probiotics per kg of diet; Biovet-YC + a concentrate feed mixture). In terms of serum glucose, lambs in T1 and T2 had the highest glucose concentration compared with the control group. This increase might be related to a temperate improvement in gluconeogenesis and increased lactose absorption (De Valdez *et al*., 1997). In contrast, Antunovic *et al*. (2006) reported that there was no change in blood glucose levels in lamb fed diets containing probiotics. Also, Ding *et al*. (2008) reported that no differences were noticed in blood glucose concentration in lambs fed diets with or without probiotics. In terms of liver enzymes

(AST and ALT), the dietary Bactozym® supplementation significantly decreased the liver enzyme levels compared with the control group.

Table (5): Effects of dry feed microbial and enzyme supplement on blood parameters of growing Osimi lambs.

Items	Treatments			Sig.
	Control	T1	T2	
Total protein (g/dl)	5.90 ± 0.58	6 .00 ± 0.061	6 .08 ± 0.060	NS
Albumin (g/dl)	3.55 ± 0.070[a]	3.28 ± 0.070 [b]	3.23 ± 0.088 [b]	*
Globulin (g/dl)	2.35± 0.031[b]	2.72 ± 0.128 [a]	2.84 ± 0.127[a]	*
Glucose (mg/dl)	51.00 ± 0.50 [b]	53.07 ± 0.12 [b]	60 .17± 0.44 [a]	**
AST (IU/L)	108.07 ± 0.28 [a]	103.00 ± 0.29 [b]	102.27 ± 0.22 [b]	**
ALT (IU/L)	21.68 ± 0.11[a]	19.84 ± 0.15[b]	19.09 ± 0.20[b]	**

[a, b] mean with different superscripts on the same row are different at (P<0.05) and (P<0.01). T2= lambs group fed ration supplemented with 2g Bactozym® / head / day, sig.= significant, * = p-value < 0.05, ** = p-value < 0.01, NS = insignificant.

These results were in accordance with those found by Hill *et al*. (2014), who reported that differences in AST and ALT concentrations and blood plasma parameters of crossbred (Osimi × Rahmani) growing lambs receiving different levels of probiotic (Pronifer) were not significant. However, the values of most previous blood serum parameters estimated in the present study are within the normal ranges for ruminants published by several workers in the literature (Ragheb *et al*., 2003). Positive changes in the direction of protein metabolism are confirmed by the indicators of transamination enzyme activity, particularly the revealed tendency to increase the level of Alanine transaminase (ALT) in sheep of the experimental groups. Simultaneously, the lower indicators of levels (AST) were found in experimental groups 1 and 2, with a general tendency to lower the AST/ALT ratio.

4. Conclusion

The results obtained indicate that adding the microbial and enzymes mixture (Bactozyme®), available in Egyptian markets, to the lambs' diets at a rate of 1 g/h/day produced notable nutritional outcomes (*i.e*., the best nutritional and economic efficiency).

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