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Effect of probiotics supplementation on productive performance of growing lambs

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Abstract

The aim of this study was to investigate the effect of Grow K Probio (commercial product) supplementation in the rations of growing lambs at different levels on performance, digestion coefficients, nutritive values, nitrogen utilization, daily gain, feed conversion and economic efficiency. Twenty-four Frafra lambs (23.70 $\pm$ 2.56 Kg body weight) were randomly distributed into four groups, each with six animals. The first group was a control group and animals fed on1 kg concentrate feed mixture (CFM) and 1% Alfalfa hay of live body weight (LBW), while animal in groups T1, T2 and T3 fed the same control diet supplemented with 2.5, 5 and 7.5 g/h/d commercial probiotic (Grow K Probio/h/day), respectively. The feeding trial lasted for 120 days for average daily gain, feed intake and fed conversion ratio measurements. Four digestibility trials were also carried out using twelve local rams randomly assigned into four groups (4 rams/trial) for nutrients digestibility, nutritive values and nitrogen utilization measurements. Results indicated that the digestibility of all nutrients and feeding value of experimental rations containing Grow K Probio increased ($P<0.05$) with increasing the level of Grow K Probio supplementation as compared to the control ration. Daily dry matter intake expressed as DM and TDN was significantly ($P<0.05$) higher in lambs fed rations containing different levels of Grow K Probio than those fed control ration. Lambs received 5 g/h/d Grow K Probio recorded highest ($P<0.05$) average daily weight gain (ADG) than those received other Grow K Probio levels and control ration. The lambs fed different levels of probiotic (Grow K Probio) had ($P<0.05$) significant effect, on rumen NH3-N and total volatile fatty acids. However, rumen pH not affected. Feed conversion and economic efficiency of lambs fed T2 and T1 were improved when compared with T3 and control groups. It could be concluded that, supplementation of probiotics particularly with the level 5 g /h/d probiotic (Grow K Probio) to growing lambs improve nutrient digestibility, daily gain, feed conversion and economic efficiency as compared with other groups.

Keywords: probiotics, digestive parameters, sheep performance, nutritional value, rumen ferments.

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1. Introduction

Recently Havenaar *et al.* (1992) defined the probiotics as "Mono or mixed cultures of live microorganisms which, applied to animal or man, beneficially effect on the host by improving the properties of the indigenous microflora. Probiotics are non-pathogenic microbes occur in nature and the gastrointestinal tract of ruminants that has a positive influence on the host animals (Dunne *et al.*, 1999). Probiotics improve microbial ecosystem (Musa *et al.*, 2009), nutrient synthesis and their bioavailability resulting in better growth performance in farm animals (Oyetayo and Oyetayo, 2005). In addition to the probiotics also improve nutrient absorption (Teeler and Vanabelle, 1991), reduce the incidence of intestinal infection (Casas and Dobrogosz, 2000) and restore the gut microflora in case of diarrhoea (Musa *et al.*, 2009). They are also known to increase ruminal pH (Umberger *et al.*, 1989), total volatile fatty acids (VFAs) and ruminal biomass (NewBold *et al.*, 1996) and thus influence the cellulolytic activity and microbial protein synthesis and fiber degradation (Martin and Nisbet, 1990). It is also considered that they compete with other pathogenic micro-organisms for the provision of nutrients and other growth factors (Rolfe, 2000). They enhance immunity by promoting the antibodies, IgA and cytokines production (Trebichavsky and Splichal, 2006). A positive impact of probiotics supplementation on nutrient intake, weight gain and feed conversion ratio (FCR) in ruminants has been reported by many workers (Chiofalo *et al.*, 2004; Whitley *et al.*, 2009). However, the actual mechanism of probiotics for improvising animal performance is not known (Koop-Hoolihan, 2001). However, different environmental factors may affect the gut microbial ecology; they include diet, medication, stress, age and general living conditions (Vlková *et al.*, 2009). Positive effects of probiotics on the rumen environment and performance of ruminants have been intensively studied due to beneficially effect for altering microbial activities, fermentative and digestive functions in the rumen. The probiotics can also stimulate specific groups of beneficial bacteria in the rumen, and has provided mechanistic models that can explain their effects on animal performance (Dutta *et al.*, 2009). The aim of the present study was to investigate the effect of different levels of Probiotic (Grow K Probio) supplementation in the rations of growing lambs on nutrient digestibility, nitrogen retained and performance of growing lambs.

2. Materials and methods

This experiment was carried out at Animal Production Research Station of Malawi, Animal Production Research Institute, Agriculture Research Center, Cairo, Egypt. The present study was carried from January 2021 to April 2021 and was lasted for 4 months.

2.1 Animals, diets and management

Twenty-four healthy lambs (6 months old with an average body weight 23.70 ±

2.56 kg) were divided into four groups (6 lambs each), according to their average live body weight. The control group animals fed on1 kg concentrate feed mixture (CFM) with 1% Alfalfa hay of LBW. The groups T1, T2 and T3 animals received the same control ration supplemented with 2.5, 5 and 7.5 g/h/d of Grow K Probio (commercial probiotic). The composition of Grow K Probio was *Lactobacillus acidophilus* 5×10^9 cfu, *Bacillus subtilis* 2×10^9 cfu, *Bacillus licheniformis* 2×10^9 cfu and *Enterococcus faecium* 1×10^9 cfu). The composition of CFM was 39% wheat bran, 38% yellow corn grain, 17% sunflower meal solvent extract, 3% molasses, 2% limestone powder and 1% salt. The chemical composition of the concentrate mixture, Alfalfa hay and experimental ration are shown in Table (1). The requirements of growing lambs provided based on NRC (1985) guidelines. The rations were offered twice daily at 8.00 a.m. and 4.00 p.m. The feed remainders were collected and weighed daily to determine the daily feed intake. The quantity of concentrate mixture was adjusted every two weeks according to the change in body weight (NRC, 1985). Drinking water was freely available all times. Feed efficiency was expressed as DM, TDN, DCP which was calculated by dividing the daily total dry matter intake (in gram) by the daily weight gain (in gram).

Table (1): chemical composition of concentrate feed mixture, Alfalfa hay and experimental ration

Item	DM%	Nutrients% (DM basis)						GE, MJ /kg DM
		OM	CP	CF	EE	NFE	Ash	
Concentrate feed mixture (CFM)	88.49	92.35	14.01	15.10	4.65	58.59	7.65	1.83
Alfalfa hay	81.28	83.90	14.90	30.90	1.02	37.08	16.10	1. 63
Experimental ration	80.79	90.40	14.23	18.83	3.83	53.53	9.60	1.79

**GE, MJ/kg DM = 0.0226 CP + 0.0407 EE + 0.0192 CF + 0.0177 NFE (MAFF, 1975).

2.2 Digestibility trail

The digestibility trials were carried out using twelve local rams. Each trial lasted for 3 weeks; the first 2 weeks were considered as a preliminary period followed by one week collection period. Animals were randomly distributed into four experimental groups, each with three rams.

2.2.1 Chemical analysis and digestion coefficients measurements

The diet samples were taken daily during the collection period. At the end of the collection period, samples were mixed and grounded through 1 mm. screen for chemical analysis. Feces were collected daily and 10% of its weight were taken and dried at 60-70 ºC for 24 hours. The fecal samples from each animal were composited and grounded through a 1mm mill screen for subsequent chemical analysis. The chemical analysis of feeds, residuals and feces were carried out using the procedures of Association of the Official Analytical Chemists (AOAC, 2005). The apparent digestion coefficients of nutrients were calculated by expressing the difference between the content of nutrients in both consumed feed and feces as a percentage of its intake. The digestible energy (DE) and metabolizable energy (ME) MJ/kg DM of the tested ration were calculated according to Aldermann *et al.* (1975).

2.2.2 Rumen liquor parameters

Rumen content samples were collected once from each ram, using a stomach tube, at the end of the digestibility trial. Samples were taken at 0, 3 and 6 hours after the morning feeding. Rumen liquor samples were filtered through four layers of cheesecloth. The filtrated portion was used immediately for the measurement of pH using a digital pH meter (Hanna instruments Hi 3424 micro-computer–pH meter), and ammonia N concentration according to Conway (1957). Few drops of saturated solution of mercuric chloride were added to the filtrate to stop the microbial activity before its storage for analysis, and then the samples were kept frozen at -20ºC for determination of total volatile fatty acids (VFAs). The total VFAs acids were measured using the procedures of Warner (1964).

2.3 Statistical Analysis

Data for all traits were statistically analyzed according to Snedecor and Cochran, 1980 in one way analysis of variance using general linear model (GLM) procedure by computer program of SAS (1995) using the model:

$$X_{ij} = \mu + A_i + e_{ij}$$

The Rumen liquid parameters data was analyzed according to the following statistical model:

$$X_{ijk} = \mu + A_i + B_j + e_{ij}$$

Where: X_{ij} = represents observation, μ = overall mean, A_i = effect of treatments (rations) where i=control, T1, T2 and T3, B_j= Time of rumen liquor where j= zero, three hours and six hours after feeding and e_{ij} = experimental error (common error). Duncan's Multiple Range Test (Duncan, 1955) was used to compare among means of each trait.

3. Results and Discussion

3.1. Nutrient digestibility

The effect of dietary supplementation of probiotics Grow K Probio on nutrient digestibility and the nutritive values of different treatments are presented in Table (2). Results showed that the apparent digestibility coefficients of all nutrients were significantly (P<0.01) increased with increasing the level of probiotic (Grow K Probio) to animal as compared with control group. The positive effect of supplement direct fed microbial (DFM) additive on CF digestibility in this study might be related to stimulation of growth of cellulolytic bacteria (Michael *et al*., 2011). This result is agreement with reported by Sallam *et al*. (2014) who reported that adding DFM Ru-max to diet may increase enzymatic activity within the rumen and enhances digestibility of the feed. Also, Galip (2006) reported that addition of probiotic (DFM) at 5 or 10 g/ day has significantly modified the proportions of the different protozoa types and improved ruminal cellulolytic activity. Similar results were reported by Abd El-Ghani, (2004). Krehbiel *et al*. (2003) reported that addition of probiotic (DFM) to diet of animal improved the CP and CF digestibility. Also, Ismaiel *et al*. (2010) showed that CP and CF digestibility significantly improved in lambs fed rations supplemented with probiotic (Tonilisat or Roemin). Also, Whitley *et al*. (2009) found improve in apparent DM, OM, CP, NDF and ADF digestibility in goats fed diet supplemented with commercial probiotics when compared with the control group. In contrast, Titi *et al*. (2008) reported that the addition of probiotics had no effect on DM, CP and NDF digestibilities.

Table (2): Digestion coefficients and nutritive values of the experimental rations, by sheep.

Item	Treatments				SEM	Sig
	Control	T1	T2	T3		
Digestion coefficients (%)						
DM	62.68[c]	68.11[b]	71.75[a]	70.31[a]	1.13	*
OM	73.69	72.80	74.84	74.48	0.86	NS
CP	66.95 [c]	72.76[b]	79.77 [a]	80.23 [a]	0.76	*
CF	61.02 [b]	62.10 [b]	65.21 [a]	66.16[a]	2.24	*
EE	71.44[c]	73.96 [b]	78.11[a]	78.92[a]	0.89	*
NFE	72.85[c]	77.31[ab]	78.18[a]	78.21[a]	1.82	*
Nutritive values						
TDN	62.02[c]	66.25[b]	68.31[a]	68.71[a]	0.83	*
DE (MJ/kg DM)*	1400	1383	1421	1415	---	
ME (MJ/kg DM)**	1148	1113	1165	1160	---	
DCP(gm)	8.81[c]	10.35[b]	11.55[a]	11.42[a]	0.11	**

[a, b and c] Means within the same row with different superscripts differ (P<0.05). T1: lambs received 2.5 g GKP, T2: received 5 g GKP, T3: received 7.5 g GKP. *DE and **ME calculated according to Aldermann *et al*. (1975) using equations being DE (MJ/kg DM) = Digestible organic matter (DOM X 19) and ME (MJ/kg DM) = DE X 0.82.

3.2 Nutritive values

The nutritive values of the experimental rations expressed as total digestible nutrient (TDN) and digestible crude protein (DCP) are presented in Table (2). The results showed that TDN and DCP% values were higher (P<0.05) for animals received 7.5 g/h/d probiotic (Grow K Probio) as compared with other treatment group and control group. The improve feeding value of experimental ration with addition different level of probiotic may be attributed to improve nutrients digestibility (Table 2). The high energy value of rations containing high levels of probiotic was primarily due to its high digestibility of EE and NFE content. These results are in agreement with reported by Sallam *et al.* (2014) and Soliman *et al.* (2016) found a positive response with supplement probiotics (Ru-Max) on feeding value of TDN and DCP. Also, Ismaiel *et al*. (2010) reported that the highest value of DCP% was recorded with DFM (Tonilisat) group when compared with the other groups.

3.3 Nitrogen balance

Table (3) showed that the nitrogen intake was similar in all groups. However, the nitrogen retained, Retain–N ⁄Absorbed–N, Retain–N /Intake-N were significantly (P<0.05) higher in all probiotic groups than the control one. The improve in nitrogen retained in treatment groups may be attributed to improve in N digestion and lower urinary N excretion (Mc-Allister *et al.*, 1998). These results agree with those obtained by Nocek and Kautz (2006) stated that the supplement probiotic increase the microbial crude protein and protein escapes from rumen degradation to be available for enzymatic digestion in the small intestine. Also, the other positive effect of probiotics (Grow K Probio), it could improve nitrogen utilization from the reduction of nitrogen excretion from fecal and urine (Ahmed and Salah 2006; El-Ashry *et al.*, 2000; Soliman *et al.*, 2016).

Table (3): Nitrogen balance of growing lambs fed different levels of probiotic.

Item	Treatment				SEM	Sig
	Control	T1	T2	T3		
Total N-Intake (TNI)	30.78	31.21	31,94	31,12	0.012	NS
Fecal -N	5.36^{a}	4.18^{b}	4.22^{b}	4.01^{b}	0.43	*
Urinary - N	16.12^{a}	15.28^{a}	14.21^{b}	13.25^{b}	1.74	*
Total N-Execration	21.48^{a}	19.46^{b}	18.43^{b}	17.26^{c}	1.74	*
N Balance (NB)	9.30^{c}	11.75^{b}	13.51^{a}	13.88^{a}	1.43	*
N – Absorption (NA)	25.42^{b}	27.03^{a}	27.72^{a}	27.11^{a}	1.21	*
NB/NA%	36.59^{c}	43.47^{b}	48.74^{a}	51.20^{a}	1.88	*
NB/TNI%	30.21^{c}	37.65^{b}	42.30^{a}	44.60^{a}	1.65	*

$^{a, b, c}$ and ^{d} means with different superscripts on the same row are different at (P<0.05). T1: lambs received 2.5 g GKP, T2: received 5g GKP, T3: received 7.5 g GKP.

3.4 Rumen parameters

The results shown in Table (4) indicated that the rumen pH was not significantly affected with supplement probiotic Grow K Probio when compared with control group. The obtained values were within the normal ranges (6.15-6.38) as reported by Hungate (1966). The same author indicated that the suitable rang for cellulytic bacteria was bout 6.2 and 7.0 for multiply rapidly and colonize the epidermal surfaces of plant fragments within 5 minutes. Concerning the effect of sampling time, the results indicated the lowest pH value was recorded at zero time then reached to the peak after 3hrs form feeding then decrease gradually to reach 6.38 after 6hrs from feeding. These results may be due to the changes in the quantities of metabolites especially VFAs and ammonia produced in the ruminal digested. This result agreed with the finding of El-Shinnawy (2010). The average ruminal NH_3-N concentration was decreased ($P<0.05$) in the groups of lambs received 5 and 7.5 gm from probiotic Grow K Probio as compared with control group (Table 4). However no significantly differences were found between the groups received 2.5 gm from probiotic and control one. Effect of sampling times on NH_3-N concentration showed the mean values of NH_3-N concentration were lower before feeding and increase gradually to reach its maximum value at 6 hrs after feeding. The lower NH_3-N concentration with animal received probiotics (DFM) may be due to increase incorporation of ammonia into microbial protein (Shibata, 1985). The results obtained in this study are consistent with the results obtained by Biggs and Hancock (1998) and Soliman *et al.* (2016). However, Sánchez *et al*. (2010) reported that the concentrations of NH_3-N were not affected by supplement prebiotic.

Table (4): Effects of supplementation of different levels of probiotic on ruminal parameters of growing lambs.

Items	Treatment				Average	SEM	Sig
	Control	T1	T2	T3			
Ruminal pH							
Zero time	6.33^{b}	6.38^{b}	6.15^{c}	6.18^{c}	6.26^{c}	0.05	NS
3 h after feeding	6.65^{a}	6.53^{a}	6.60^{a}	6.58^{a}	6.89^{a}	0.04	NS
6 h after feeding	6.38^{ab}	6.33^{c}	6.45^{b}	6.35^{b}	6.38^{b}	0.05	NS
Average	6.45	6.41	6.40	6.38	----	0.04	NS
SEM	0.03	0.03	0.03	0.03	0.04	----	----
Sig	*	*	*	*	**	----	---
TVFA’s (meq/dl)							
Zero time	9.75^{c}	10.38^{c}	10.38^{c}	10.13^{c}	10.16^{c}	0.14	NS
3 h after feeding	10.50b^{b}	11.50^{ab}	12.00^{ab}	12.00^{ab}	11.50^{b}	0.21	* *
6 h after feeding	10.75^{ba}	11.63^{aba}	12.63^{aa}	12.75^{aa}	11.94^{a}	0.28	*
Average	10.33^{c}	11.17^{b}	11.67^{a}	11.63^{a}	----	0.20	**
SEM	0.17	0.17	0.17	0.17	0.20	----	----
Sig	***	***	***	***	***	----	----
NH_3-N (mg/dl)							
Zero time	14.03^{c}	12.62^{c}	12.62^{c}	12.16^{c}	12.86^{c}	0.34	NS
3 h after feeding	16.36^{b}	15.90^{b}	15.43^{b}	16.36^{b}	16.01^{b}	0.24	NS
6 h after feeding	20.08^{aa}	19.62^{aba}	19.16ab^{a}	18.23^{ba}	19.27^{a}	0.28	*
Average	16.82^{a}	16.05^{ab}	15.74^{b}	15.58^{b}	----	0.32	*
SEM	0.42	0.42	0.42	0.42	0.32	----	----
Sig	***	***	***	***	***	----	----

$^{A, B, C}$ Means in the same Colum a, b, c in the same row with different superscripts are significantly different ($P<0.05$), NS = not significant. TVFA’S = total volatile fatty acids, NH3-N = ammonia. T1: lambs received 2.5 g GKP, T2: received 5g GKP, T3: received 7.5 g GKP.

As shown in Table (4) the total volatile fatty acids (VFAs) concentration increased ($P<0.05$) in all treated groups as compared with control group. Also, the VFAs concentration was higher ($P<0.05$) in lambs received 5gm in T3 and 7.5 gm in T4 than those received 2.5 gm in T2. Concerning the effect of sampling time on VFAs concentration, it is clear that the concentration VFAs of was lower before feeding then increases gradually until reached the peak at 6 hours post feeding. These results are agreement with Sánchez *et al*. (2010) stated that the probiotics increase volatile fatty acid (VFA), microbial protein concentrations, and stabilize the rumen pH. El-Ashry *et al*. (2000) found that TVFA's concentration in the rumen was low before feeding and increased with time after feeding.

3.5 Growth performance:

The results in Table (5) revealed that the body weight gain and average daily gain was increased (P<0.05) in lambs fed on all treatment groups in compassion to control one. Also, the average daily gain was higher in T2 than other treatment groups. The improve performance in probiotics groups may be due to improve nutrient digestibly and rumen fermentation in these groups (Tables 2 and 4). These results are in agreement with those reported by Sarwar *et al.* (2010) reported that the supplementation probiotic increase weight gain of growing lambs. In addition, Khaled *et al.* (2011) sated that the improvement of weight gain may be attributed to the level of probiotic in diet.

Table (5): Performance of growing Frafra lambs fed different levels of probiotic (Grow K Probio).

Item	Treatment				SEM Sig
	Control	T1	T2	T3	
Av. Initial weight, kg	23.60	23.60	23.80	23.80	2.12 NS
Av. Final live wt., Kg	46.70^{c}	50.50^{b}	57.22^{a}	50.06^{b}	2.02 *
Total gain, kg	23.10^{c}	26.90^{b}	33.42^{a}	26.26^{b}	2.47 **
Av. Daily gain, g	193^{c}	224^{b}	279^{a}	219^{b}	3.02 **
Feed consumption					
Av. CFM, g	1000	1000	1000	1000	---
Av. Alfalfa hay intake, g	352^{c}	371^{b}	400^{a}	364^{b}	1.43 *
Av. daily DM intake, g	1352^{c}	1371^{b}	1400^{a}	1364^{b}	2.18 NS
Av. daily TDN, kg	0.879^{c}	0.934^{b}	0.977^{a}	0.949^{b}	2.17 *
Av. daily DCP, g	148^{c}	156^{b}	159^{a}	156^{b}	2.94 *
Feed Efficiency					
Kg DM/Kg gain	7.01^{a}	6.12^{b}	5.02^{c}	6.23^{b}	0.43 *
Kg TDN/Kg gain	4.55^{a}	4.17^{b}	3.50^{c}	4.33^{a}	0.03 *
Kg DCP/Kg gain	0.766^{a}	0.696^{b}	0.570^{c}	0.712^{b}	0.14 *
Revenue, L.E.	612.36	831.36	1207.68	796.32	---
Economic efficiency	0.792	1.062	1.517	1.022	---

$^{a, b, c \text{ and } d}$ means with different superscripts on the same row are different at (P<0.05). R1: control, R2: lambs received 2.5 g GKP, R3: received 5g GKP, R4: received 7.5 g GKP. *Based on free market prices of feed ingredients 2021, the cost of experimental rations was estimated as the total prices of ingredients used in the concentrate feed mixture, Alfalfa hay, and Grow K Probio (Commercial probiotic), being, 5060, 3940 L.E./ ton and 100 L.E./kg, respectively and the price of one kg body weight on selling, 60 L.E. **Economic efficiency Y = [(A-B/B)], where A= selling cost of obtain gain, and B=feeding cost of this gain.

The average daily feed intake in terms of dray matter (DM), Total digestible nutrient (TDN) was increased with supplements different levels of probiotic Grow K Probio to growing lambs. Among the treatment groups the lambs revised 5 gm in T2 of probiotic were the highest one of feed intake. The feed conversion ratio expressed as DM, TDN and DCP was significantly (P<0.05) improved on all probiotic groups when compared with control group (Table 5). The beast value of feed conversion ratio among treatment groups was recoded in T2 followed T1 then T3. Increase feed intake without significant improvement in feed conversion ratio (Afsharmanesh and Sadaghi, 2014).

3.6 Economic efficiency

The results in Table (5) clear that the economic efficiency was improved in T2 and T1 as compared with T3 and control group (1.35 and 0.98 vs. 0.81 and 0.88, respectively). These results are in agreement with reported by Soliman *et al.* (2016) who stated that probiotics supplemented improve net revenue compared with control group. It could be concluded that, supplements different levels of probiotic (Grow K Probio) as a commercial probiotic particularly 5g/head/day improved nutrient digestibility, rumen fermentation and growth performance of growing lambs. Accordingly, it could be concluded that, feeding growing lambs 1 kg concentrate feed mixture plus Alfalfa hay 1% of LBW and 5 g/head/day probiotic (Grow K Probio) (commercial probiotic), resulted in superior nutrition, better daily gain and feed efficiency and better economic efficiency.

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