

Effect of growth promoters (probiotics) supplementation on performance, rumen activity and some blood constituents in growing lambs

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Abstract

The current study examined the effects of probiotics (Pronifer and More-yeast) supplementation to growing lambs. 25 crossbred (Osimi×Rahmani) growing lambs of about 6-8 months age and an average initial live body weight of 25 kg were used. Lambs divided into five groups (5 lambs for each group) and the experimental period lasted for 169 days. The control group T_1 received concentrate feed mixture (CFM), group T_2 and T_3 received 1.5 and 3 kg Pronifer/Mg (megagram) CFM and group T_4 and T_5 received 2.5 and 5 kg More-yeast/Mg CFM, respectively. Rumen liquor and blood samples were taken and digestibility trial was conducted at the end of the experiment. Results showed higher digestibility values for crude protein ($P<0.05$) in T_3 and T_4 when compared to control. Nutritive ratio was lower ($P<0.05$) in groups T_3 and T_4 than in group T_2 . More-yeast supplementation improved dry matter intake ($P<0.001$), while a reverse trend was observed with Pronifer supplementation. All treatments had little effect on ruminal liquor except ruminal ammonia concentration in T_5 was lower ($P<0.05$) than other treatments. The differences in most blood plasma parameters due to treatment effect were not significant, except plasma urea and globulin concentrations increased ($P<0.05$) with T_5 treatment. The obtained results appear to indicate that More-yeast supplementation improved lambs performance and digestibility, whereas, Pronifer supplementation enhanced digestibility.

Keywords: probiotics, performance, rumen activity, blood constituents and growing lambs

Zusammenfassung

Auswirkung der Wachstumsförderer (Probiotika) Supplementierung auf die Leistung, Pansenaktivität und einige Blutbestandteile in wachsende Lämmer

Die aktuelle Studie untersuchte die Auswirkungen von Probiotika (Pronifer und More-yeast) Supplementierung auf wachsende Lämmer. Es wurden 25 Kreuzungstiere (Osimi×Rahmani), ca. 6-8 Monaten alt, mit einem durchschnittlichen Körpergewicht von 25 kg zu Beginn der Studie verwendet. Die Lämmer wurden in 5 Gruppen mit je 5 Lämmern aufgeteilt. Das Experiment dauerte 169 Tage. Die Kontrollgruppe (T_1) erhielt eine Kraftfutter-Mischung (CFM), die Gruppen T_2 und T_3 erhielten 1,5 bzw. 3 kg Pronifer/Tonne CFM und die Gruppen T_4 und T_5 erhielten 2,5 und 5 kg More-yeast/Tonne CFM. Es wurden Pansensaft und Blutproben entnommen und am Ende des Experiments wurde eine Verdaulichkeitsstudie

durchgeführt. Die Ergebnisse zeigten eine höhere Verdaulichkeit für Rohprotein ($P < 0,05$) in T_3 und T_4 im Vergleich zur Kontrollgruppe. Das nutritive Verhältnis war niedriger ($P < 0,05$) in den Gruppen T_3 und T_4 als in der Gruppe T_2 . More-yeast Supplementierung verbesserte die Trockensubstanzaufnahme ($P < 0,001$) der Lämmer, während ein umgekehrter Trend mit Pronifer Supplementierung erkennbar war. Alle Behandlungen hatten wenig Einfluss auf die gemessenen Parameter im Pansen außer, dass die ruminale Ammoniakkonzentration in Gruppe T_5 am geringsten war ($P < 0,05$). Die meisten Blutplasmaparameter wurden durch die Behandlung nicht beeinflusst mit der Ausnahme, dass Harnstoff und Globulin erhöht waren ($P < 0,05$) in der T_5 Behandlung. Die erhaltenen Ergebnisse deuten darauf hin, dass die Lämmer, die mit More-yeast gefüttert wurden, eine bessere Leistung und Verdaulichkeit zeigten, während Pronifer Supplementierung die Verdaulichkeit verbessert.

Schlüsselwörter: Probiotika, Leistung, Pansenaktivität, Blutbestandteile und Lämmer

Introduction

Probiotics are being explored as substitutes of antibiotic feed additives that improve gut health and promote animal performance. Microbial products are known to promote rumen metabolic development by modulating rumen function and fermentation activity of its microflora, which improves ruminant production performance (Tripathi & Karim 2011). Probiotic growth promoters have been used as supplements in animal feeds for more than seven decades to enhance growth and decrease feed:gain ratio (Dawson 1993). Probiotics are viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance (Cremonini *et al.* 2002).

Most evidences for such effects come from results of intensive studies of microbial activities in the rumen of cattle and sheep (Windschitl 1992 and Dawson 1993). Pronifer is a fermentation product consisting of a bacterial cocktail of specific lactic acid producing bacteria. As a probiotic agent, it may act through improving the balance of the intestinal microflora (Games 1987). In addition, Dawson *et al.* (1990) reported a decrease in ruminal pH and an increase in cellulolytic ruminal bacterial numbers in steers fed hay supplemented with lactic acid bacteria (*L. acidophilus*). Also, Pronifer improves health, performance, minimize diarrhoea and increase growth rates (Bohm & Srou 1995).

Yeast product supplementation has several benefits in ruminant nutrition which have been demonstrated an increase in nutrient digestibility, alteration of the proportion of volatile fatty acids produced in the rumen, reduction in ruminal ammonia, and increase of ruminal microorganism population (Chaucheyras-Durand *et al.* 2008). Furthermore, yeast culture supplementation in growing lamb has the potential to improve intake and growth and can substitute antibiotics as growth promoting feed additive (Tripathi & Karim 2011). Dawson (1992) mentioned that yeast stimulates rumen bacteria and enhance lactate and ammonia utilization resulting in moderate ruminal pH and increases in microbial population which lead to increased in fibre digestion and protein synthesis in the rumen. Yeast culture improved feed intake, weight gain and feed conversion in growing ruminants (Salem *et al.* 2000 and El-Waziry *et al.* 2000). Yeast also provides vitamins to support the growth of rumen fungi (Chaucheyras-Durand *et al.* 1995).

The objective of this study was to investigate the effect of adding Pronifer and More-yeast supplements as growth promoters on performance, digestibility, rumen activity and blood parameters in growing crossbred lamb.

Material and methods

Lambs, treatments and samples

Twenty five crossbred (Osimix×Rahmani) growing lambs, about 6-8 months old with an average initial live body weight (LBW) of 25.04 ± 0.36 kg were used in this experiment. The experiment was carried out at Moshtohor Animal Farm, Ministry of Agriculture, Egypt. The animals were divided into five groups on the basis of initial LBW and age. Each group contained five animals. The feeding trial lasted for 168 days and animals were fed individually. Lambs were assigned at random to one of the five experimental diets as shown in Table 1.

Table 1
Feeding regimes

Treatments	Experimental diets
T ₁	Control diet (CD): CFM* + wheat straw <i>ad libitum</i>
T ₂	CD +1.5 kg Pronifer/Mg CFM
T ₃	CD +3 kg Pronifer/Mg CFM
T ₄	CD +2.5kg More-yeast/Mg CFM
T ₅	CD +5kg More-yeast /Mg CFM

*CFM: concentrate feed mixture consisted of yellow corn 65 %, wheat bran 15 %, decorticated soybean 10 %, decorticated lin seed 5 %, molasses 3 %, calcium carbonate 1.4 %, sodium chloride 0.5 % and Min and Vit. Premi 0.1 %

The diets used in this study consisted of wheat straw, concentrate feed mixture (CFM) and Pronifer or More-yeast.

The chemical analysis of the experimental feeds used is presented in Table 2. Pronifer is a probiotic feed additive which contains:

- viable lactic acid bacteria (approx. 10^6 CFU/g) (*Lactobacillus planetarium*, *L. brevis*, *L. fermentum*, *L. casei*, and *Pediococcus acidilacticii*)
- lactic acid fermentation metabolites and enzymes (organic acids, glucosidase, and peptidase enzymes)
- free (soluble) amino acids and short-chain peptides (Srouf *et al.* 2000).

Table 2
Chemical analysis of the experimental diets

Items	DM, %	Composition on DM basis, %					Ash, %
		OM, %	CP, %	CF, %	EE, %	NFE, %	
Wheat straw	95.95	88.60	2.87	43.88	1.80	40.05	11.40
CFM	91.31	95.62	12.00	4.24	12.91	66.47	4.38

DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fibre, EE: ether extract, NFE: nitrogen-free extract

This probiotic was purchased from Schwester P.G.E. Company (Austria) by Egyptian Ven Thred Company.

More-yeast is a probiotic including live yeast (*saccharomyces cerevisiae*) and other direct-fed microbials including strains of *lactobacillus*, *streptococcus*, *aspergillus* and several others. The live yeast contained pure strains of *saccharomyces cerevisiae* grown in purified cane and beet molasses solutions under the most stringently controlled chemical, bacteriological and sanitary conditions. More-yeast was imported from Norchem, Inc. (Hauppauge, NY, USA) Company by International Marketing Center (Cairo, Egypt).

Lambs were adapted to the experimental diets for two weeks before the start of the feeding trial. Wheat straw was offered *ad libitum*, while CFM was allowed according to the NRC (1985) recommendations based on LBW. Diets were offered twice daily in two equal portions at 8.00 a.m. and 4.00 p.m. Feed intake and refusals (if any) were recorded daily. Lambs were weighed every second week in the morning before feeding. At the end of the experiment, a digestibility trial was conducted using three animals from each experimental group assigned at random and maintained in individual digestibility cages. Lambs were weighed at the start and end of each trial.

The digestibility trial consisted of 15 days as a preliminary period, followed by 10 days as the collection period. The experimental rations were weighed and fed according to NRC (1985). Wheat straw was offered *ad libitum*. Feed intake was recorded daily once at 8.00 a.m. Faeces were collected from each individual animal once daily before the morning meal, the refusal of feeds (if any) were collected and recorded daily before offering the new feed. Daily faeces excreted from each lamb was weighed and 10% of the fresh faeces was taken and dried at 60°C for 24-72 h to determine dry matter of the faeces. Composite samples from the daily dried faeces, for each lamb, were mixed, ground and stored in refrigerator for subsequent chemical analysis. Representative samples of experimental diets, refusals and faeces were analysed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash contents according to AOAC (1999) methods, while nitrogen-free extract (NFE) was calculated by differences.

Rumen liquor samples were taken individually from three lambs of each experimental group at four times during the feeding trial period (0, 8, 16 and 24 weeks). Rumen samples were taken using a rubber stomach tube connected to a vacuum pump (Nagah 2002), before feeding and at 3 and 6 h post-feeding. Rumen samples were strained through four layers of cheese cloth. Rumen liquor analysis included ruminal pH value (pH meter), Ammonia nitrogen (N-NH_3) concentration determined immediately according to Conway (1962). Total volatile fatty acid (TVFA) concentrations were determined by the steam distillation method (Abou-Akkada & El-Shazly 1964).

Blood samples were taken at 0, 8, 16 and 24 weeks throughout the feeding period. The blood samples were taken from jugular vein of three lambs from each experimental group before morning feeding by vein puncture and were put into 10 ml heparinized test tubes. Blood samples were immediately centrifuged at 3500 rpm for 15 min to separate plasma. Blood plasma samples were immediately frozen at -20°C and stored until analysis. Commercial kits (Diamond Diagnostics Co., Egypt) were used to determine total protein (TP), albumin (Al), urea (U), cholesterol (Ch), creatinine and plasma transaminases; glutamic-oxaloacetic-transaminase (GOT) and glutamic-pyruvic-transaminase (GPT), according to Armstrong & Carr (1964), Doumas *et al.* (1971), Patton & Crouch (1977), Allain *et al.* (1974), Henry (1965) and Reitman & Frankel (1957), respectively. While, globulin (G) was calculated by

subtracting the AI concentration from the TP value and AI:G ratio was measured by dividing albumin value by its corresponding globulin value.

Statistical analysis

The data were statistically analysed according to SAS (1999). The statistical models used in this study were as follows:

$$Y_{ij} = U + A_i + E_{ij} \quad (1)$$

where Y_{ij} is the observed response, U is the overall mean, A_i is the effect of treatment (growth promoters), E_{ij} is the experimental error. The differences among groups were tested by Duncan's multiple rang test (Duncan 1955).

Results

Nutrients digestibility and feeding values

The average digestion coefficients and feeding values of experimental diets are shown in Table 3. The present results showed that lambs fed T_1 (control) recorded numerically lowest digestibility values for all feed nutrients (DM, OM, CP, CF, EE and NFE). The differences in nutrients digestibility due to treatment effect were not significant except for CP digestibility. Lambs of T_3 (3 kg Pronifer/Mg CFM) and T_4 (2.5 kg More-yeast/Mg CFM) showed the highest CP digestibility values. The lowest digestible crude protein (DCP) values were shown by T_2 and T_1 ($P < 0.05$) while the highest ones were recorded by T_3 and T_4 ($P < 0.05$). In general, T_3 (3 kg Pronifer/Mg CFM) and T_4 (2.5 kg More-yeast/Mg CFM) showed the best feeding values (DCP) and narrowest nutritive ratio (NR) values, compared with either the control or other treatments.

Table 3
Averages of nutrients digestibility and feeding values of the experimental diets

Variable	Treatments					SEM	P-values
	T_1	T_2	T_3	T_4	T_5		
Apparent digestibility, %							
Dry matter, DM	82.9	84.1	85.8	85.6	83.1	1.50	0.53
Organic matter, OM	85.1	86.9	88.4	88.0	86.6	1.25	0.42
Crude protein, CP	79.8 ^b	80.3 ^b	85.9 ^a	85.5 ^a	83.2 ^{ab}	1.53	0.05
Crude fibres, CF	55.1	66.7	66.1	65.9	64.0	5.11	0.50
Ether extract, EE	86.0	88.0	89.4	89.8	88.5	1.27	0.31
NFE	89.2	90.4	90.9	90.3	89.6	0.96	0.73
Feeding values, % DM							
SV	89.5	90.7	93.5	93.4	90.7	1.35	0.30
TDN	93.8	95.4	97.7	97.5	95.4	1.37	0.23
DCP	9.0 ^b	8.9 ^b	9.8 ^a	9.8 ^a	9.3 ^{ab}	0.17	0.01
Nutritive ratio	9.4 ^{ab}	9.7 ^a	9.0 ^b	9.0 ^b	9.3 ^{ab}	0.15	0.02

^{a,b}averages with different superscripts in the same column are significantly ($P < 0.05$) different, Apparent digestibility (%): [(nutrient intake - nutrient excretion in feces)/nutrient intake] × 100, DCP% (digestible crude protein): (digested protein/DMI) × 100, TDN%: digested CP + digested CF + digested NFE + (digested EE × 2.25), SV% (starch value): (digested CP × 0.94) + digested CF + digested NFE + (digested EE × variable factor1) - (CF% × variable factor2), Nutritive ratio: TDN - DCP/DCP; Nagah (2002)

Lamb performance

Average LBW, daily weight gain (DWG), dry matter intake (DMI) and feed conversion (FC) of lambs fed experimental diets are illustrated in Table 4. Lambs of More-yeast supplementations T₄ and T₅ recorded the highest values of LBW, DWG without significant, also, lambs of T₄ and T₅ consumed the highest amount of daily DMI ($P < 0.001$), whereas, those of T₂ and T₃ (Pronifer supplemented groups) recorded the lowest amount compared with the control. Data for feed conversion did not show any diet effects.

Table 4
Averages of lamb performance and feed conversion values of lambs fed the experimental diets

Variable	Treatments					SEM	P-values
	T ₁	T ₂	T ₃	T ₄	T ₅		
Lamb performance							
Body weight, kg	55.20	54.25	54.6	60.25	58.80	3.09	0.49
Daily gain, kg	0.180	0.180	0.181	0.188	0.194	0.01	0.89
Daily dry matter intake, kg	1.175 ^b	1.135 ^c	1.161 ^{bc}	1.243 ^a	1.225 ^a	0.01	0.001
Feed conversion							
kg DM intake/Kg gain	6.528	6.306	6.414	6.612	6.314	0.42	0.93
kg SV intake/Kg gain	5.839	5.717	5.994	6.176	5.727	0.39	0.74
kg TDN intake/Kg gain	6.122	6.017	6.265	6.447	6.021	0.41	0.78
kg DCP intake/Kg gain	0.589	0.561	0.630	0.649	0.588	0.04	0.33

^{a,b,c}Averages with different superscripts in the same column are significantly ($P < 0.05$) different.

Ruminal liquor and blood parameters

Values of ruminal pH, NH₃-N and TVFA concentrations of lambs in the different experimental treatments are shown in Table 5.

Table 5
Averages of ruminal pH and blood parameters values of lambs fed the experimental diets

Variable	Treatments					SEM	P-values
	T ₁	T ₂	T ₃	T ₄	T ₅		
Rumen activity							
Ruminal pH	6.35	6.26	6.69	6.45	6.51	0.12	0.98
NH ₃ -N, mg/100ml	25.98 ^a	24.11 ^a	24.93 ^a	23.49 ^{ab}	21.00 ^b	0.98	0.05
TVFA, mg/100ml	12.34	12.42	12.49	12.34	13.07	0.39	0.99
Blood parameters							
Total protein, g/dl	6.76	7.22	7.33	7.36	7.62	0.24	0.29
Albumin, g/dl	4.80	4.30	4.84	4.81	4.66	0.14	0.07
Globulin, g/dl	1.96 ^b	2.92 ^a	2.48 ^{ab}	2.55 ^{ab}	2.96 ^a	0.24	0.05
Al/G ratio	2.67	1.7	2.06	2.49	1.75	0.28	0.07
Urea, mg/dl	23.04 ^b	21.52 ^b	24.2 ^{ab}	24.5 ^{ab}	27.98 ^a	1.32	0.03
Creatinine, g/dl	1.64	1.56	1.62	1.46	1.40	0.08	0.42
Cholesterol, mg/dl	58.46	58.01	56.48	54.36	47.74	3.95	0.68
GOT, u/l	28.74	29.18	31.84	32.41	38.73	3.54	0.53
GPT, u/l	20.75	20.05	22.17	20.76	21.67	0.71	0.06

^{a,b}Averages with different superscripts in the same column are significantly ($P < 0.05$) different.

Results showed that the differences in pH and TVFA values at the different experimental treatment were very limited and without any significant effect. However, differences in $\text{NH}_3\text{-N}$ concentration due to treatments effect were significant ($P<0.05$). Differences in blood plasma concentrations due to treatment effects were seen for globulin (G) and urea (U) concentrations ($P<0.05$) (Table 5).

Discussion

Nutrients digestibility and feeding values

The improvement in nutrients digestibility is possibly explained on the basis that yeast culture enhanced microbial activity and also due to that some beneficial activities of lactic acid bacteria in the gastrointestinal tract, i.e., its anti diarrhoea activities, anti tumour activities and its ability to alter enzyme activities (Dawson 1988).

The main effect of yeast culture supplementation on ruminants include improvement of gut health and ecology through rumen maturity by favouring microbial establishment, stabilisation of rumen pH and interaction with lactate utilizing bacteria (Yang *et al.* 2004). The establishment of complex rumen microbial ecosystem subsequently improve rumen function that promotes absorption ability and feed digestion (Hopper *et al.* 2001), thus live yeast additives improve gut health.

These results are in agreement with those reported by Bohm & Srour (1995) who reported that supplementing the diet of crossbred calves with 5 g Pronifer/(kg concentrate \times d) improved the digestibility of most feed nutrients. El-Ashry *et al.* (2003) with sheep and goats, Nagah (2002) and Khattab *et al.* (2003) with crossbred lambs and Ragheb *et al.* (2003) with Friesian calves indicated that the digestibility of most feed nutrients improved by yeast culture (YC), yea-sac (YS) or lacto-sac (LS) supplementation. Feeding values have improved with supplementations due to positive effect on digestible tract. Nagah (2002) and Khattab *et al.* (2003) reported that the use of either 3 g YS or 3 g LS/h/d improved SV and TDN values of crossbred lambs. Also, Gado *et al.* (1998), Salem *et al.* (2000) and El-Ashry *et al.* (2003) found that yeast culture supplementations had positive effect on TDN and DCP values of growing lambs.

Lamb performance

Results showed significant increase in DM intake due to More-yeast supplementation (T_4 and T_5). In addition, LBW and DWG are enhanced slightly with growth promoter supplementations probably due to enhance in rumen activity and digestibility.

The active yeast supplementation has positive effects in young ruminant's performance through increased DM intake and daily gain, Thus, The performance promoting effects of live yeast additives could be correlated to an improvement in rumen development parameters such as papillae length and width, and rumen thickness (Lesmeister *et al.* 2004), early establishment and stabilization of rumen microbial communities (Chaucheyras-Durand & Fonty 2001, 2002) and reduced number of days of diarrhoea (Galvano *et al.* 2005). Moreover, YC, YS and LS supplements improved the growth performance of growing lambs (Salem *et al.* 2000, Fayed 2001, Nagah 2002 and El-Ashry *et al.* 2003).

On the other hand, supplements had no significant effects on FC agreement with Nagah (2002) and El-Ashry *et al.* (2003), they indicated that YC, YS and LS supplements had no effect on feed conversion values (as DM, TDN and DCP/kg gain) of growing lambs. Similarly, Mir & Mir (1994) with steers and Ragheb *et al.* (2003) with Friesian calves found that live yeast and LS had no positive effect on feed utilization values (kg gain/kg DM, TDN and DCP).

Ruminal liquor parameters

The significant differences in the values of ruminal $\text{NH}_3\text{-N}$ showed the lowest value T_5 . In accordance with the previous results, Nagah (2002) and Mussa (2001) reported that the ruminal $\text{NH}_3\text{-N}$ concentrations for YS and LS supplementations were lower than the control but the differences were not significant.

The reduction in ruminal $\text{NH}_3\text{-N}$ of More-yeast treated animals in the present study may be probably attributed to the inhibitory effect of this growth promoter on proteolysis amino acid deamination and ruminal urease activity (Fuller & Johnson 1981, Starnes *et al.* 1984 and Gomez *et al.* 1991).

The mechanism of yeast action may be due to competition between yeast cells and the bacteria for energy supply and by a direct inhibitory effect of yeast on small peptides and bacterial peptidases (Chaucheyras-Durand *et al.* 2008). Yeast culture supplementation is reported to enhance microbial growth and decrease N loss by incorporating more digestible carbohydrates into microbial mass (Sniffen *et al.* 2004).

Blood parameters

The differences in plasma globulin concentrations between T_1 and T_2 or T_5 were significant, whereas no significant differences were observed among other treatments. Sadik (1989) found that lactobacillus concentrate supplement significantly increased plasma globulin concentrations for buffalo heifers. Also, Mohanna (2000) with lactating buffaloes, Salem *et al.* (2000) with growing crossbred sheep, and El-Ashry *et al.* (2003) with Barki lambs, reported that YC supplementation significantly increased plasma G values.

Result obtained revealed that plasma urea concentration in blood plasma of lambs fed the experimental diets ranged from 21.52 mg/dl for T_2 (1.5 kg Pronifer/Mg CFM supplemented group) and 27.98 mg/dl for T_5 (5 kg More-yeast/Mg CFM supplemented group). The differences in plasma urea concentrations were only significant between T_5 and those of T_1 and T_2 , whereas no significant differences were detected between other treatments. Ragheb *et al.* (2003) who reported that dietary YS and LS supplementation increased blood plasma urea concentrations compared with the control group.

In general results of blood plasma parameters for lambs fed the experimental diets indicated that all treatments had little effect on blood plasma parameters estimated in the present study as the differences in all parameters due to treatment effect were not significant except for globulin and urea values.

However, values of most blood plasma parameters estimated in the present study are within the normal ranges for ruminants published by several workers in the literatures (Owen *et al.* 1954, Mahmoud 1993, Nagah 2002, EL-Ashry *et al.* 2003 and Ragheb *et al.* 2003), and

suggested that the experimental growth promoters are safe for physiological and healthy status of all experimental lambs.

In conclusion, supplementation of probiotics as growth promoters (Pronifer and More-yeast) in diets of growing lambs enhanced protein digestibility and dry matter intake, whereas these supplements had little effect on feed conversion and rumen activity. Both, Pronifer and More-yeast supplementation in diet improved plasma globulin which may be related to immunity in these animals.

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