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Comparison of Two Products of Direct-Fed Microbial Supplementation on the Nutrient Utilization and Ruminal Fermentation in Sheep

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Abstract

This experiment was undertaken to evaluate the potential impacts of supplementing two direct-fed microbial (DFM) products, namely Bactozyme and Ru-max, to the diet of 12 male Barki sheep (live body weight 46.6 ± 2.9 kg) on dry matter intake (DMI), apparent total tract digestibility of nutrients, nitrogen balance and rumen fermentation characteristics. The Bactozyme or Ru-max were supplemented at a rate of 1.0 g/head/day, mixed with the concentrate mixture. Animals were randomly allocated into 3 equal groups (n=4) and were subjected to the digestibility trails.

The results showed that the inclusion of either products of DFM had no positive impact on DMI, but non-significantly improved the apparent total tract digestibility of dry matter (DM), organic matter (OM) and crude protein (CP). However, the Bactozyme addition increased ($P<0.05$) the apparent total tract digestibility of neutral detergent fiber (NDF) and acid detergent fiber (ADF). The enhancement of the apparent total tract digestibility of cell wall was not significant for the two of DFM products and a non-significant improvement in cell wall digestion due to the Ru-max supplementation over the control group was found. The DFM products had positive impacts on the average of total digestible nutrients (TDN) and digestible crude protein (DCP) but non significant in comparison with the untreated animals. In addition, the nitrogen balance was improved ($P>0.05$) by 8 and 13% due to Ru-max and Bactozyme supplementation, respectively in comparison with the control group. The results also revealed that inclusion of DFM products had no impacts on rumen pH 3.0 and 6.0 h after feeding but Bactozyme reduced ($P<0.05$) the rumen pH 1.0 h after feeding compared to the control group. The inclusion of Bactozyme and Ru-max increased ($P>0.05$) the NH_3-N concentration in the rumen at 1.0 and 3.0 h after feeding but the Bactozyme decreased ($P<0.05$) the NH_3-N concentration and increased the volatile fatty acids (VFA) at 6.0 h after feeding compared to the control group. Overall, results indicated that the two DFM products had positive impacts on cell wall digestibility, which in turn improves metabolic energy supply and nutrients utilization in ruminants as well.

Keywords: feed additives, bactozyme, Ru-max, fermentation, nutrients digestibility

1. Introduction

Numerous studies have been conducted in an attempt to increase ruminant productivity by manipulating the rumen environment and to increase feed digestibility and nutrient utilization by the animals in order to supply sufficient nutrients to support a high level of milk production. One approach that has recently been widely investigated is the application of direct-fed microbial (DFM) preparations, in order to promote digestion and intestinal hygiene (Gourinier-Chateau et al., 1994), enhance animal performance and reduce usage of antibiotics (Jouany & Morgavi, 2007; Guedes et al., 2008; Wallace et al., 2008).

The definition of DFM is very broad and may include specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Knowlton et al., 2002). The supplementation of DFM agents in dairy rations has become a generally accepted practice with the following stated benefits: increased ruminal digestion, dry matter intake (DMI), and milk production and reduced body temperature (Piva et al., 1993; Higginbotham et al., 1994; McGilliard & Stallings, 1998). *Enterococcus faecium* produces moderate amounts of lactic acid in the rumen. This could stimulate growth of lactic acid utilizer's microrganisms and stabilize ruminal pH (Nocek et al., 2002;

2003). Yeast and yeast products have been widely used in ruminant nutrition to manipulate rumen fermentation and improve animal performance (Bruno et al., 2009; Yalçin et al., 2011). In addition, other studies have shown substantial improvement of feed digestibility, rumen fermentation and animal performance due to fibrolytic enzymes supplementation (Bala et al., 2009; Gado et al., 2009; Holtshausen et al., 2011).

However, testing DFM supplementation produced variable and inconsistent results so far (cf. also Z. Mir & P. S. Mir, 1994). One main point to explain this is the diversity of DFM origin. Several biotic factors such as the strain of yeast, bacteria, fungi, enzymes and its viability, nature of the diet, animal type and its physiological status and level of performance may play considerable role in this regard. Still, direct comparisons among direct-fed microbial products have rarely been carried out, and a comprehensive analysis of variables indicative of the complex of influence of such products is mostly lacking. Therefore, the present study was conducted to compare the responses of Barki sheep to supplementation of two commercial DFM and determine their effect on feed intake, nutrients digestibility, nitrogen (N) utilization and rumen fermentation characteristics.

2. Materials and Methods

This experiment was conducted at the Milk Production Project, Animal Production Department, Faculty of Agriculture, Alexandria University, Egypt. All analyses were carried out at the Animal Nutrition Laboratory, Department of Animal Production, Faculty of Agriculture, Alexandria University, Egypt.

2.1 Animals and Management

Twelve adult male Barki sheep (live body weight, 46.6 ± 2.9 kg), a small Egyptian fat-tailed sheep breed, were randomly allocated to equal three groups (n=4). The control group received a basal diet, which composed concentrate mixture and Egyptian clover (*Alexandrium trifolum*) hay without supplement, the other two groups received the basal diet plus either 1.0 g of Ru-max/head/day or 1.0 g of Bactozyme /head/day according to the producer recommendations. The DFM product Ru-max® (Agri-King, Inc., Fulton, USA) is composed from cellulases, β-glucanases, amlyases, *Aspergillus oryzae, Enterococcus faecium* and *Saccharomyces Cerevisiae*. Bactozyme is a microbial feed additive (Dyno Vet. Company, the Egyptian - French Factory, Alexandria, Egypt) consisting of *Saccharomyces cerevisiae* ($20x10^{10}$ CFU), total live bacteria ($2x10^{10}$ CFU), *Lactobacillus acidophilus* ($2x10^{9}$ CFU), *Lactobacillus casei* ($0.4x10^{8}$ CFU), *Lactobacillus plantarum* ($1.6x10^{9}$ CFU), *Entrococcus faeciun* ($4.0x10^{9}$ CFU), *Bacillus subtilis* ($6 x10^{9}$ CFU), *Bacillus licheniformis* ($6 x10^{9}$ CFU), phytase (2400 U), lipase (2400 U), xylanase (1200 U), cellulase (2400 U), pectinase 400 U, amylase (20000 U), protease (40000 U), β-gluconase (1000 U), fructo oligosaccharides (10 g), mannan oligosaccharides (10 g), calcium propionate (24 g), copper penta sulphate (10 g) and carrier up to 1 kg.

The animals were housed individually in metabolic crates under a protective roof and had free access to fresh water throughout the study. The basal diet consisted of clover hay and a concentrate mixture the composition of which is given in Table 2. The diet components were offered twice daily at 08:00 and 16:00 h in amounts of 750 and 750 g as fed/day foreach of clover hay and concentrate mixture, respectively. The experimental period lasted for 30 days, with the first 21 days being an adaptation period to the diet, followed by 7 days of sample collection (feces, urine, refusal feed). Individual intakes of clover hay and concentrate were recorded daily by weighing the feed offered and refused. During the collection period, also the complete output of feces was recorded by collection in buckets. Feces samples of 100 g/kg of total weight were collected and stored under 5˚C during the collection period. Directly afterwards, the samples collected during the 7 days were mixed. One kilogram of this mixture was dried at 60˚C for 72 h in a forced air oven, ground through a 1-mm screen and stored at room temperature until analysis. The remainder was kept in a freezer (–20˚C) for analysis of dry matter (DM) and total N. The urine was completely collected in plastic buckets containing 100 ml of H_2SO_4 (10%), and the amounts were recorded and samples (10%) were collected daily. These samples were stored in a freezer (–20˚C) during the collection period. Just after the collection period, the urine samples were pooled per animal and representative samples were frozen at –20˚C until further analysis.

The ruminal fluid was collected via the stomach tube at 1.0, 3.0 and 6.0 h after feeding consecutive for consecutive 2 days. The rumen pH was measured immediately after collection using pH meter. The rumen fluid was separated from the feed particles through four layers of gauze and stored at –20˚C for later analysis.

2.2 Sample Analyses

Chemical analyses were performed according to AOAC (2006). DM contents of feeds and refusals were determined by drying at 135˚C for 2 h, but of feces were dried at 105˚C overnight. OM was determined as the weight loss after ashing at 550˚C for 2 h. N content of feeds, feces and acidified urine was determined using the Kjeldahl method, and CP was calculated as 6.25 × N. Ether extract (EE) was analyzed according to AOAC (2006).

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the procedures of Van Soest et al. (1991). No sodium sulfite and α-amylase were used in the procedure for NDF determination. Both NDF and ADF are expressed without residual ash. Concentrations of NH_3-N and total volatile fatty acids (VFA) in rumen fluid were determined by distillation using Markham apparatus according to the Preston (1995) and Warner (1964), respectively.

2.3 Statistical Analysis

Data were analyzed using the generalized linear model procedure (SAS, 2002). The following model was assumed: $Y_{ij}= \mu+ T_i + e_{ij}$ where: μ is the overall mean, T_i is the treatment type, e_{ij} is the random error term. Differences among means were tested using Duncan multiple range test (Steel & Torrie, 1980).

3. Results

The mean values of the proximate analysis on DM basis of the concentrate mixture and clover hay are presented in Table 1. The results of the proximate analysis showed that OM, CP and EE of concentrate mixture and clover hay were 895 vs. 890, 142 vs. 135 and 38.6 vs. 14 g/kg, respectively, while, NDF, ADF and hemicellulose content of the concentrate mixture were 398, 176 and 222, respectively and were 448, 282 and 166 g/kg for Egyptian clover, respectively.

Table 1. Ingredients and chemical composition of concentrate mixture and clover hay fed to Barki sheep

Ingredients,	g/kg DM	
Ground yellow corn	250	
Wheat bran	300	
Cotton seed meal	170	
Sunflower meal	245	
Limestone	20.0	
Sodium chloride	10.0	
Trace minerals*	5.0	
Items (g/kg DM)	Concentrate mixture	Clover hay
OM	895	890
CP	142	135
EE	38.6	014
NDF	398	448
ADF	176	282
Hemicellulose	222	166

*Mineral mixture contained (g/kg): Manganese Sulphate12.58, Zinc Sulphate 9.3, Copper Sulphate 3.2, Ferrous sulphate 16.67 Calcium iodate 0.081, Sodium selenite 0.4, Magnesium oxide 9.4, Cobalt sulphate 0.2, Sodium chloride Add to kg (M/s, Dyno vet company, Alexandria, Egypt).

Data of feed intake, nutrients apparent total tract digestibility and feeding values of rations without (control) or with Bactozyme and Ru-max are summarized in Table 2. The results showed that the inclusion of two products of DFM had no positive impact on DMI compared to the control group. The ratios of consumed clover hay and concentrate were 44.3:55.7; 44.0:56.0 and 43.3:56.7% for the control, Ru-maz and baztozyme groups, respectively. However, the supplementation of Bactozyme or Ru-max apparently improved the apparent total tract digestibility of DM, OM and CP but the improvement was not significant compared to the control. While the Bactozyme addition increased ($P<0.05$) the apparent total tract digestibility of NDF and ADF significantly, Ru-max only showed numerical increases compared to the control group. Although, DFM had positive responses on the mean values of TDN and DCP, no significant changes in comparison to the untreated animals were observed.

Table 2. Effects of Bactozyme and Ru-max supplementation on dry matter intake (DMI), nutrients apparent total tract digestibility and nutritive value in Braki sheep (Means ± SE)

	Control	Ru-Max	Bactozyme
DMI, g/head/d	1158.9±19.5	1154.4±19.5	1165.8±19.5
Apparent total tract digestibility, %			
Dry matter	66.9±1.3	68.1±1.3	69.9±1.3
Organic matter	67.3±1.3	68.4±1.3	70.6± 1.3
Crude protein	71.5±1.2	72.5±1.2	74.3±1.2
Ether extract	75.2±1.3^{b}	81.4±1.3^{a}	78.7±1.3^{ab}
Neutral detergent fiber	58.8±1.3^{b}	61.1±1.3^{ab}	63.2±1.3^{a}
Acid detergent fiber	40.6±2.3^{b}	45.4±2.0^{ab}	49.3±2.2^{a}
TDN	63.3±1.5	64.7±1.5	68.0±1.5
DCP	10.0±0.16	10.2±0.16	10.6±0.16

Different letters (a, b) in the same row indicate significant differences (P<0.05).

TDN: Total digestible nutrients; DCP: Digestible crude protein.

The effects of Bactozyme and Ru-max supplementation on N utilization are given in Table 3. Fecal and urinary N decreased (P>0.05) when Bactozyme and Ru-Max were supplemented compared to the control group. In addition, the N balance was apparently improved (P>0.05) by 8 and 13% due to Ru-max and Bactozyme supplementation, respectively in comparison to the control group.

Table 3. Effect of Bactozyme and Ru-max supplementation on nitrogen fractions of Barki sheep (Means ± SE)

	Control	Ru-Max	Bactozyme
N intake, g/d	25.8±0.53	25.7±0.53	25.9±0.53
Fecal N, g/d	7.4±0.67	6.9±0.67	6.1±0.67
Urinary N, g/d	9.2±1.47	7.9±1.47	8.7±1.47
Nitrogen balance, g/d	9.2±1.34	10.9±1.34	11.1±1.34

Mean values of rumen pH revealed that Bactozyme inclusion reduced (P< 0.05) rumen pH after 1.0 h of feeding but, pH at subsequent intervals such as 3.0 and 6.0 h after feeding were statistically comparable with control.

Table 4. Effect of Bactozyme and Ru-max supplementation on rumen pH after different times at morning feeding of Barki sheep

Groups	Rumen pH		
	1.0 h	3.0 h	6.0 h
Control	6.43±0.07^{a}	6.49±0.07	6.62±0.09
Ru-max	6.40±0.07^{ab}	6.47±0.07	6.67±0.09
Bactozyme	6.21±0.07^{b}	6.28±0.07	6.52±0.09

Different letters (a, b) in the same column indicate significant differences (P<0.05).

Bactozyme and Ru-max supplementation showed apparent increase (P> 0.05) in the NH_3-N at 1.0 and 3.0 h of post feeding. However, it was higher on Ru-max at 6.0 h of post feeding (P< 0.05) than Bactozyme and comparable to control. Bactozyme and Ru-max supplementation had no effects on VFA concentration either 1.0 or 3.0 h after feeding but VFA concentration was increased (P< 0.05) on both supplements compared to control.

Table 5. Effect of Bactozyme and Ru-max supplementation on NH_3-N concentration after different times at morning feeding of Barki sheep

Groups	NH_3-N (mg/dL) concentration		
	1.0 h	3.0 h	6.0 h
Control	15.5±2.0	19.4±1.88	19.9±1.7[ab]
Ru-max	18.7±2.1	21.4±2.4	20.8±1.5[a]
Bactozyme	19.4±2.1	20.1±2.1	16.3±1.8[b]

Different letters (a, b) in the same column indicate significant differences (P<0.05).

Table 6. Effect of two products of direct-fed microbial supplementation on VFA concentration after different times at morning feeding in Barki sheep

	VFA concentration (meq/dL)		
	1h	3h	6h
Control	10.51±0.69	8.73±0.80	7.25±0.41[b]
Ru-max	10.85±0.69	8.61±0.81	10.09±0.61[a]
Bactozyme	9.59±0.76	9.09±0.80	9.18±0.55[a]

Different letters (a, b) in the same row indicate significant differences (P<0.05).

4. Discussion

Cellulose and hemicellulose represent about 250-300 g/kg of most ruminant diets. These plant cell wall polymers are insoluble, structurally complex and not totally physically accessible, which explains why their degradation is sometimes limited. Moreover, the host enzymes are unable to hydrolyze these kinds of molecules. Improving the bioavailability of nutrients in a feedstuff by increasing the cell wall hydrolysis through the microbial supplement is a promising solution and, DFM preparations are reported to promote digestion and intestinal hygiene (Gourinier-Chateau et al., 1994), enhance animal performance and reduce usage of antibiotics (Jouany & Morgavi, 2007; Guedes et al., 2008; Wallace et al., 2008).

Ru-max and Bactozyme supplementation did not improve DMI and digestibility of DM, OM and CP but, digestibility of EE, NDF and ADF were improved significantly. Dawson (1992) also reported that the addition of DFM resulted in increased concentration of total anaerobic bacteria and the increase was associated with fibre digesting and lactic acid utilizing bacteria (Dawson 1992). The components that are used in DFM may be classified as lactic acid utilizing bacteria *Enterococcus*, (LUB), yeast products containing *Saccharomyces cerevisiae* and fungi *Aspergillus* oryzae, fibrolytic enzymes and cobalt carbonate. The LUB potentially moderatesrumen conditions and improve feed efficiency. Yeast DFM may reduce harmful oxygen, prevent excess lactate production, increase feed digestibility, and improve fermentation in the rumen. The DFM may also compete with and consequently inhibit the growth of pathogens, stimulate immune function, and modulate microbial balance in the gastrointestinal tract.

Many workers reported that the supplementation of DFM in ruminant rations has become a generally accepted practice due to increased ruminal digestion, DMI, performance and reduced body temperature (Piva et al., 1993; Higginbotham et al., 1994; McGilliard & Stallings, 1998). Nikkhah et al. (2004) and Raeth-Knight et al. (2007) have observed non-significant improvement of DMI by animals fed with yeast culture. Similalry, Bernard et al. (2010) and Arriola et al. (2011) reported that adding fibrolytic enzymes supplementation to dairy cow diet did not enhance DMI and no difference was found between cows supplemented with or without fibrolytic enzymes. DMI is often considered as a function of the initial rate of fiber digestion. An early stimulation of ruminal activity can be expected to have a major impact on the feed consumption and can provide a driving force for improved animal performance. Although significant improvement in the NDF digestibility was observed with Ru-max and Bactozyme supplementation, it could be presumed that they might be lagging in improving the initial rate of fiber digestion that could be translated into enhanced DMI. Supplementing lactating dairy cows with DFM products containing *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* did not affect rumen fermentation in cows. On the other hand, Szasz, (2002), Ware and Zinn (2005) reported that Fibrozyme inclusion to the steers or heifer's diets increased total tract digestibility of NDF and ADF and increased DMI and average daily gain. Few

workers concluded improved feed intake, feed conversion rate, daily weight gain and total body weight in sheep, goat and cattle on administration of DFM (Torres-Rodriguez et al., 2007; Samli et al., 2007; Casey et al., 2007). Improvement from DFM supplementation could be anticipated due to positive effects on various digestive processes, especially cellulolysis, synthesis of microbial protein, stabilizers of ruminal pH and lactate, increased absorption of some nutrients and displayed a growth-promoting effect. The positive effect of the Bactozyme additive on NDF digestibility in this study might be related to stimulation of growth of cellulolytic bacteria. However, the effects of DFM on DMI appeared varying in this study probably due to variation in products, methods of applying, roughage: concentrate ratio (Yang et al., 2000; Bowman et al., 2002).

No increase in CP digestibility was observed in confirmation to Bassiouni et al. (2010) who supplemented fibrozyme to corn silage or rice straw. Mean values of DM and OM apparent total tract digestibility were not affected, while apparent total tract digestibility of NDF and ADF were improved ($P<0.05$) in treated groups compared to the control group. These results were in accordance with Beauchemin et al. (2003) and Arriola et al. (2011) who reported that exogenous enzymes improve apparent total tract digestibility of plant cell wall, and there is evidence for numerous potential modes of action suggesting their interdependence. Adding Bactozyme and Ru-max to a diet may increase the hydrolytic capacity of the rumen mainly due to increased bacterial attachment, stimulation of rumen microbial populations and synergistic effects with hydrolases of ruminal microorganisms. The net effect is increased enzymatic activity within the rumen, which enhances digestibility of the feed. Moreover, DFM supplementation to ruminant diets could also partly reduce digesta viscosity (Hristov et al., 2000) and alter ruminal fermentation (Gado et al., 2009; Arriola et al., 2011) and/or enhance attachment and colonization to the plant cell wall by ruminal microorganisms (Wang et al., 2001, Holtshausen et al., 2011) by synergism with enzymes or stimulate the rumen microbial numbers.

The TDN and DCP were not significantly changed between treatments as observed by Ismaiel et al. (2010). An apparent trend in improved N balance on study due to DFM supplementation was observed that was due to less of excretion of urinary nitrogen and fecal nitrogen in sheep fed bactozyme and Ru-max compared with control group, which in consistent with observations reported by El-Ashry et al. (2003); Ahmed and Salah (2006) and Ismaiel et al. (2010).

DFM feeding showed variable and inconsistent affect on altering rumen fermentation patterns. Some reports have demonstrated no effects of yeast culture supplementation on ruminal pH, rumen ammonia concentration, and VFA patterns *in vivo* (Wiedmeier et al., 1987) and *in vitro* (Newbold et al., 1996). Microbial conversion of peptides and amino acids to ammonia in the rumen is unfavourable to the host animal, because energy is required for microbial protein synthesis, and not all ammonia is incorporated into protein (Wallace et al., 1997). Consequently, if high levels of ammonia occur in the rumen, a large amount of N is excreted in urine and feces. For example, in animal production systems feeding high amounts of N, more than half of it is excreted in urine, mostly in the form of urea which is rapidly mineralised in NH_3/NH_4 + and then converted to nitrous oxide (N_2O), which has a global warming potential that is 296 fold that of carbon dioxide (CO_2) and more than 12 fold that of methane (Steinfeld et al., 2006). Because of the increasing concern of the role of livestock on climate change, nutritional strategies that aim at decreasing N loss in the rumen are of interest.

A decrease in NH_3 concentration is attributed to ruminal microbial proliferation, due to the increase of microbial use of available NH_3 for microbial N synthesis (Crocker et al., 1998). Some of the DFM e.g. galacto-oligosaccharides, are known to suppress ammonia producing bacteria, and stimulate the production of *Bifidobactrium,* which has the ability to assimilate ammonia as a N source (Deguchi et al., 1993). Beauchemin et al. (2000) found that enzyme supplementation decreased NH_3N value before and after feeding. This decreasing in NH_3N was likely caused by an increase in ruminal availability of slowly digestible carbohydrates due to enzyme supplementation.

Nutrients of forage cell walls are degraded to several metabolites, such as VFA, by ruminal bacteria, protozoa, and fungi. Results clearly indicated that no significant effect on VFA's concentration by Bactozyme or Ru-max supplementation at 1 or 3h after feeding but the VFA increased significantly at 6h after feeding compared to the control group. This result was associated with Kung et al. (2002); Sutton et al. (2003); Eun and Beauchemin (2005) who concluded that total VFA were not affected by enzyme supplementation, while Arriola et al. (2011) reported that fibrolytic enzymes supplementation increased ($P<0.03$) VFA concentration. However, the animal responses to DFM addition have been highly variable, apparently influenced by the composition of the diet and much remains to be elucidated about the dose- and diet-dependence of DFM effects (Chaucheyras-Durand et al., 2008). The variations among these studies may be due to differences in roughage: concentrate ratio, which may have effects on lactic acid concentration and rumen pH.

5. Conclusions

In conclusion, under the conditions of this study, both types of the investigated DFM (Ru-max and baztozyme) had no positive impacts on DMI, N utilization but improved cell wall digestibility as one of the key targets of the DFM products supplementation, which then would improve metabolic energy supply and nutrients utilization in ruminants as well.

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