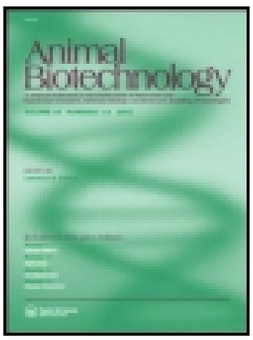

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Influence of dietary probiotic inclusion on growth performance, nutrient utilization, ruminal fermentation activities and methane production in growing lambs

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ABSTRACT

The use of two probiotic products as a means of improving *in vitro* and ruminal dry matter digestibility, fermentation characteristics and growth performance of Barki lambs were evaluated. The probiotic products in powder (PP) or liquid (PL) forms were produced from *Ruminococcus flavefaciens*, through an anaerobic fermentation process. Barki lambs ($n = 30$; 24.5 ± 0.5 kg body weight) were used in a completely randomized block design and randomly assigned among three experimental groups and fed for 75 days. Lambs were fed an equal amount of concentrate feed mixture with either no probiotic product (control), or with 20 g of PP, or 10 ml of PL per animal/d, with rice straw *ad libitum*. Both PP and PL treatments resulted in an increase ($p < 0.05$) in nearly all of the digestibility coefficients, nitrogen utilization, cell wall constituents, total volatile fatty acids, rumen volume, microbial nitrogen synthesis, gas production and average daily gain compared to the control group. Ruminal ammonia nitrogen, acetic acid and *in vitro* methane concentrations and protozoa count decreased ($p < 0.03$) in the treatment groups. Overall, the probiotic in a powder or liquid form increased in vitro dry matter digestibility, improved lambs daily gain and nutrient digestibility.

KEYWORDS

Probiotic; performance; methane; ruminal fermentation

Introduction

The use of probiotics (direct-fed microbials) as ruminant feed additives can improve dry matter intake, fiber digestibility and growth performance.[1,2] Probiotics are non-pathogenic living microorganisms that have been shown to produce no drug resistance or drug residues when fed to animals. Probiotics have the potential to be used in place of antibiotics and have been widely used in the food and feed industries during the past few decades.[3] Overall, results from using feed additives have been mixed and are thought to be partially due to enzyme characteristics, composition of the target forage, and ruminal conditions (temperature and pH); thus, feed additive selection should first be tested in a ruminal environment.[4] Numerous probiotic products are commercially available for ruminants, especially for dairy cows. These products are either of bacterial or yeast (fungi) origin and responses attributed to yeast probiotics, are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen.[3]

There is interest in enhancing the nutritive value of poor quality forages, especially when harvested at an advanced stage of maturity (>500 g NDF; >400 g ADF). Rice straw has very low crude protein concentration, less palatability and low organic matter degradation. Increasing the digestibility of low quality feeds using enzyme technologies has resulted in improvements in ruminant performance. Beauchemin et al.[5] reported that adding enzymes to animal diets increases the overall hydrolytic capacity of the rumen. Colombatto et al.[4] reported that exogenous enzymes increased microbial attachment to diets and increased total number of viable rumen bacteria. The beneficial impact of the addition of exogenous enzymes depends on several factors such as type of enzyme preparation, specific enzyme activities, enzyme stability, amount of enzyme added, diet composition and application

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method.[6] Studies have shown that probiotics can enhance feed efficiency and daily weight gain of feedlot cattle and health performance of neonates.[7,8] Probiotics are utilized in the feedlot to maintain gut health and improve feed efficiency by increasing the absorption rate of volatile fatty acids and lactate produced.[8] There is limited information on using probiotics to improve fiber digestibility of low-quality crop residues.

Probiotic products produced from anaerobic fermentation of *Ruminococcus flavefaciens*, has shown to improve live weight gain and feed conversion of wheat straw in sheep and goats.[9] It has also shown to improve nutrients digestibility and ruminal fermentation of cows fed diets containing Egyptian by-product feeds.[1] A previous report noted that yeast probiotics favor the proliferation of rumen fungi population by increasing the supply of vitamin B_1 to these microbes.[10] They also stimulate activities of fibrolytic bacteria with concomitant increase in fiber degradation by scavenging oxygen to make an anaerobic condition conducive for cellulolytic bacteria. The main actions of the probiotics are on rumen kinetics and improvements on microflora effectiveness in utilizing the feed ingredients that usually reflects on animal performance.[1] Thus, the objective of this study was to evaluate the feasibility of using the probiotics in two forms (powder and liquid) to improve growth performance, nutrients digestibility and ruminal fermentation of Barki lambs.

Materials and methods

Three trials were used to evaluate the use of probiotics (direct-fed microbial) as a potential means of improving growth performance and ruminal digestion, fermentation, and gas production characteristics.

Trial 1

Thirty weaned Barki male lambs between 6 and 8 months of age (24.5 ± 0.15 kg body weight) were used in a completely randomized block design. After 2 weeks of adaptation, where lambs consumed a ration of concentrate feed mixture plus rice straw, lambs were weighed and randomly divided into three equal groups (10 lambs/each) and then randomly distributed into individual cages according to their assigned treatment. All lambs were fed an equal amount of concentrate feed mixture with or without the probiotic products, and fed on rice straw *ad libitum* (Table 1). Animals were randomly assigned to one of three experimental treatments: control and two probiotic products supplementation for 75 d. All lambs were fed the basal diet and without the probiotics product preparation (control) and the other two treatments received 20 g/animal/d of probiotics product in powder form (i.e., PP) and 10 ml/animal/day of probiotics product preparation in liquid form (i.e., PL), respectively. The probiotics product was manually mixed with the diet before the morning feeding. Feed intake was recorded daily and lambs body weight was recorded weekly until day 75. Average daily gain was calculated as the difference between two successive weights divided by the time period (days). Lambs were vaccinated against pneumonia (Pneumococcal polysaccharide vaccine protects against 23 types of pneumococcal bacteria), injected with a broad-spectrum antibiotic of amoxicillin, and drenched a broad spectrum anthelmintic (Ivomec).

The probiotics product in powder (PP) or liquid (PL) forms were produced from *Ruminococcus flavefaciens*, obtained through an anaerobic fermentation process. The PP was 0.28 × 1014 CFU with one gram of PP/gram of corn flour and PL was 1.1 × 1013 CFU with one gram of PL/ml of water. Furthermore, the PL is a biotechnical solution product made from natural sources to elevate the level of cellulase from anaerobic bacteria and contained specific enzymes such as cellulase (7.1 IU), hemicellulase (2.3 IU/mg),

Table 1. Chemical composition (g/kg, DM basis) of the concentrate feed mixture and rice straw.[a]

	Concentrate feed mixture	Rice straw
Ingredients		
Corn grain, ground	412	
Wheat bran	128	
Barley grain, ground	113	
Soybean meal	234	
Corn gluten	39	
Molasses	44	
Calcium carbonate	10	
Dicalcium phosphate	10	
Common salt (NaCl)	5	
Mineral and Vitamin premix[a]	5	
Chemical composition of concentrates and hay (g/kg, dry matter basis)		
Organic matter	935	906
Crude protein	139	37
Ether extract	27	10
Nitrogen free extract	700	462
Neutral detergent fiber	367	732
Acid detergent fiber	257	499
Acid detergent lignin	43	105
Acid detergent insoluble crude protein	14	75
Hemicellulose	110	233
Cellulose	214	394

[a]Vitamin premix provided per kg of diet: vitamin A: 200,000 IU; vitamin D3: 300,000 IU; vitamin 10,000 IU; vitamin K: 2 mg and Anti-oxidant: 1000 mg/kg, Cu: 3300 mg/kg; Fe: 100 mg; Zn: 16,500 mg/kg; Mn: 9000 mg; I: 120 mg/kg; Co: 90 mg/kg and Se: 90 mg/kg.

amylase (61.5 IU) and protease (29.2 IU) per ml or gram. The PP is a biotechnical powder product containing similar enzymes as PL but also *Saccharomyces cerevisiae* yeast.

Trial 2

Digestibility and nitrogen balance trials were carried out using nine Barki rams (52.3 ± 1.4 kg; 3 rams/treatment). Sheep were fed twice daily according to NRC[11] at 0800 and 1500, and water was offered *ab libitum*. Rams were fed on the rations for two weeks served as the adaptation period followed by 7 days of total feces and urine collection. Animals were placed in individual metabolic cages for feces and urine collection and daily feces and urine from each ram was collected and weighed in the morning before feeding. Approximately 10 g/kg of the daily feces from each ram was sampled after thorough mixing and frozen until subsequent analysis. A 15 ml urine sample was collected and mixed with 40 ml of 100 ml/l of HCl to keep the final pH below 3. Urine samples were stored at −18 °C until taken for nitrogen analyses. Subsamples (0.20 of total collected) of feces and urine were taken once daily then stored at −18 °C until analyses. Fecal samples were dried at 60 °C for 72 h.

Samples of feed, orts and feces were ground through 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and a sample of 50 g/ration per sheep was analyzed according to AOAC.[12] Samples were analyzed for dry matter (DM, method 934.01), ash (method 942.05), nitrogen (N, method 954.01) and ether extract (EE, method 920.39). The neutral detergent fiber (NDF, Van Soest et al.[13]), acid detergent fiber (ADF) and lignin (method 973.18) analyses used was sequentially done using an ANKOM 200 fiber analyzer unit (ANKOM Technology Corporation, Fairport, NY, USA). Neutral detergent fiber was assayed without use of an alpha amylase but with sodium sulfite. Both NDF and ADF are expressed with residual ash. Hemicellulose was calculated by the difference between NDF and ADF, while the cellulose was calculated by difference between ADF, lignin concentrations.[14] Urine samples were analyzed for N according to AOAC (Table 1).[12]

Rumen liquor samples were taken at 0, 1, 3 and 6 h after feeding in the morning from three fistulated Barki ewes (48.0 ± 0.8 kg body weight) for each ration. Ewes fed on the treatment diets for 15 days as adaptation period and then followed by two consecutive days for ruminal sampling. The pH of the rumen fluid was measured immediately using an Orian 680 digital pH meter and the remaining fluid was strained through four layers of chesses cloth at each sampling time for other analyses. Rumen fluid NH_3–N was determined by using MgO as described by Al-Rabbat et al.[15]. Total volatile fatty acid concentration was estimated by using steam distillation as described by Warner.[16] Microbial protein synthesized (g of microbial protein/day) in the rumen of goats fed the treatment diets were calculated using the equation developed by Borhami et al.[17]:

$$\text{Microbial protein g/day} = \text{mole VFA produced/day} \times 2 \times 13.48 \times 10.5 \times 6.25/100,$$

where one mole of VFA yields about 2 mole ATP,[18] one mole ATP produces 13.48 Yield ATP (g of DM microbial cell), and percentage of N of dry microbial cell = 10.5.[19]

Trial 3

Gas production was measured using an adaptation of the technique described by Theodorou et al.[20] The same diet used during the *in vivo* experiment (600 g concentrate feed and 400 g of rice straw) was formulated in triplicates for the *in vitro* evaluation.

One gram of the ground samples, of each triplicate diet samples, without or with the addition of PP (20 mg) or PL (10 μl) were incubated in 120-mL serum bottles (4 bottles per diet sample) with 50 ml of diluted rumen fluid (10 mL mixed rumen fluid + 40 mL medium prepared under continuous flushing with CO_2; Theodorou et al.[20] Incubations, for each diet, were performed in three 24-h runs using rumen fluid mixed from the three fistulated sheep on different weeks of incubation, with 4 blank bottles per run. The blank bottles containing only diluted rumen fluid were used to compensate for gas production in the absence of substrate. All bottles were closed with rubber stoppers, crimped with aluminum seals, shaken and placed in an incubator at 39 °C. The volume of gas produced in each bottle was recorded after 24 h using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona). A 10-ml gas sample was collected into vacuum tubes and stored until analyzed for CH_4 concentration by gas chromatography.

Total protozoa counts were determined according to the method of Dehority.[21] Two mL of rumen fluid was pipetted into a screw-capped test tube and 10 ml of formalinized physiological saline (20 ml formaldehyde in 100 ml saline (0.85 g sodium chloride in 100 ml distilled water)) added. Two drops of Lugol's

iodine were added to the test tube that was then mixed thoroughly and stood overnight at room temperature. Total counts of protozoa were made in 30 microscopic fields at a magnification of 200 × in a Haemocytometer (Neubauer improved, Marienfeld, Germany).

Statistical analysis

Data of each experiment (Trials 1, 2 and 3) was statistically analyzed as a completely randomized design using the PROC MIXED procedure of SAS.[22] In Trials 1 and 2, the experimental unit was the animal. In Trial 3, data of each one of the 3 runs within the same diet sample, with or without the probiotic product were averaged and used as an experimental unit.[23] The following statistical model was used for the three trials:

$$Yij = \mu + Di + eij$$

where
Yij = observation on experimental unit
μ = overall mean
Di = effect of enzyme
eij = random error

Tukey's test was used for the multiple comparisons among mean values for different treatments.

Results

Nutrient digestibility study

Results showed increased total DM intake ($p = 0.02$) for lambs fed the probiotics product diets *versus* lambs fed the control diet (Table 2). Digestibility coefficients of nutrients were greater ($p < 0.05$) in lambs supplemented with probiotics than those on the control diet. Similarly, nitrogen was efficiently utilized in the probiotics treated lambs. Approximately 0.52 of ingested nitrogen was retained in the treatment group compared with the control group (0.40). There were no differences ($p < 0.05$) in pH values between treatments (Table 3). Ammonia-nitrogen was reduced ($p = 0.03$) in lambs supplemented with probiotics. Lambs fed diets supplemented with PP or PL had the highest TVFA, propionate and acetate:propionate. The PP or PL increased TVFA by 22 and 25%, and propionate by 3.9, and 4.0%, respectively. Rumen microbial N synthesis of sheep was improved by feeding PP or PL.

Performance study

The results of the initial and final body weight, average daily gain, G:F, and economic efficiency are presented in Table 4. All performance variables were

Table 2. Effects of probiotic additives on dry matter intake, nutrient digestibility, nutritive value, and nitrogen utilization of male barki sheep.

	Treatment[1]				
	Control	PP	PL	SEM[2]	*p*-Value
Dry matter intake, kg					
Concentrate	0.81	0.81	0.81		
Rice straw	0.37	0.42	0.42[a]	0.03	0.67
Total Dry matter intake	1.18[b]	1.23[a]	1.23[a]	0.02	0.02
Digestibility coefficients (g digested /g ingested)					
Dry matter	0.62[b]	0.64[a]	0.65[a]	0.001	0.01
Organic matter	0.65[b]	0.66[a]	0.67[a]	0.073	0.01
Crude protein	0.62[b]	0.65[a]	0.65[a]	0.052	0.01
Ether extract	0.69[b]	71.4[a]	0.72[a]	0.032	0.02
Neutral detergent fiber	0.55[b]	0.59[a]	0.59[a]	0.043	0.01
Neutral detergent fiber	0.52[b]	0.54[a]	0.54[a]	0.031	0.02
Acid detergent lignin	0.41[b]	0.43[a]	0.43[a]	0.033	0.02
Nutritive value, g/kg intake					
Total digestible nutrients	615[b]	632[a]	637[a]	15.1	0.02
Digestible crude protein	66	67	67	6.3	0.85
Nitrogen utilization					
N intake, g/d	20.3[b]	20.5[a]	20.5[a]	0.1	0.01
N absorbed, g/d	12.5[b]	13.2[a]	13.2[a]	0.2	0.01
N retained, g/d	4.9[b]	6.7[a]	7.0[a]	0.3	0.02
N retained, fraction of N-intake	0.24[b]	0.33[a]	0.34[a]	0.903	0.03
N retained, fraction of N-absorbed	0.40[b]	0.51[a]	0.53[a]	0.101	0.04

[a,b,c]Means in the same row with different superscripts were different ($p < 0.05$).
[1]Control (lambs were fed 600 g of a concentrate feed mixture plus 400 g of rice straw per kg of DM without any probiotic); PP (control diet supplemented with 20 g of the probiotic in powder form per animal/day); and PL (control diet supplemented with 10 ml of the probiotic in liquid form per animal/day) in a completely randomized block design.
[2]SEM: greatest standard error of the mean.

Table 3. Effects of probiotic additives on ruminal fermentation activities and microbial protein synthesis of fistulated barki ewes.

	Treatment[1]				
	Control	PP	PL	SEM[2]	*p*-Value
pH	6.44	6.51	6.49	1.11	0.63
NH_3–N, mg/100 ml	15.1[a]	14.1[b]	14.0[b]	1.23	0.03
VFA, meq/100 ml	9.3[b]	11.3[a]	11.6[a]	1.31	0.02
Molar proportion					
Acetic acid	0.58[a]	0.57[b]	0.56[b]	0.023	0.03
Propionic acid	0.25[b]	0.26[a]	0.26[a]	0.014	0.03
Butyric acid	0.10[a]	0.08[b]	0.08[b]	0.003	0.03
Acetic:Propionic	2.2[b]	2.3[ab]	2.4[a]	0.12	0.04
Rumen volume, L	3.05[b]	3.24[a]	3.26[a]	0.090	0.04
Rate of out flow, /h	0.063[a]	0.061[b]	0.061[b]	0.0007	0.04
Microbial nitrogen yield, g/d	12.3[b]	13.8[a]	13.5[a]	0.22	0.03

[a,b]Means in the same row with different superscripts were different ($p < 0.05$).
[1]Control (lambs were fed 600 g of a concentrate feed mixture plus 400 g of rice straw per kg of DM without any probiotic); PP (control diet supplemented with 20 g of the probiotic in powder form per animal/day); and PL (control diet supplemented with 10 ml of the probiotic in liquid form per animal/day) in a completely randomized block design.
[2]SEM: greatest standard error of the mean.

Table 4. Effects of probiotic additives on growth performance and economic efficiency of male barki lambs.

	Treatment[1]				
	Control	PP	PL	SEM[2]	*p*-Value
Initial body weight, kg	24.5	24.6	24.4	1.91	0.75
Final body weight, kg	33.9[b]	35.9[a]	36.4[a]	1.72	0.02
Total gain (G), kg	9.4[b]	11.8[a]	12.5[a]	0.44	0.01
Average daily gain, g/day	125.3[c]	157.2[b]	166.9[a]	1.41	<0.001
Dry matter intake (F), kg/day	1.06[c]	1.18[b]	1.19[a]	0.025	0.009
G:F	0.118[c]	0.133[b]	0.140[a]	0.01	0.002
Economic efficiency[3]					
Average daily feed cost, L.E	2.15	2.30	2.34		
Price of daily gain, L.E	4.387	5.502	5.842		
Economical return, L.E per head/d	2.237	3.202	3.502		
Economic efficiency	0.0204	0.0239	0.0259		
Relative economic efficiency	100	117	127		

[a,b,c]Means in the same row with different superscripts were different ($p < 0.05$).
[1]Control (lambs were fed 600 g of a concentrate feed mixture plus 400 g of rice straw per kg of DM without any probiotic); PP (control diet supplemented with 20 g of the probiotic in powder form per animal/day); and PL (control diet supplemented with 10 ml of the probiotic in liquid form per animal/day) in a completely randomized block design.
[2]SEM = greatest standard error of the mean.
[3]Economic efficiency = Price of average daily gain (L.E)/average daily feed cost (L.E). Calculated based on the following prices in Egyptian pounds (L.E.) per ton, 2015: Rice straw = 240 L.E/ton., Commercial feed mixture = 2250 L.E./ton. The price of one kg of body weight was 35 L.E.

enhanced ($p < 0.05$) with probiotics supplementation. Calculated feed cost was higher for probiotics treatments *versus* the control but returns, economic efficiency and relative economic efficiency were higher for the probiotics treatments.

In vitro *study*

Effects of addition of the probiotics product (i.e., PL and PP) on *in vitro* gas production parameters, methane emission and protozoa count of diets are shown in Table 5. Gas production was greater ($p < 0.05$) in probiotics diets compared with control diet. Methane emission and protozoa count were significantly less ($p < 0.05$) in the treated groups *versus* control group.

Table 5. Effects of probiotic additives on *in vitro* ruminal dry matter and organic matter degradability (g degraded/g incubated), and gas and CH_4 production, and protozoa populations in female barki sheep.

	Treatment[1]				
	Control	PP	PL	SEM[2]	*p*-Value
Dry matter degradability	0.45[b]	0.48[a]	0.48[a]	0.006	0.009
Organic matter degradability	0.53[b]	0.55[a]	0.55[a]	0.008	0.01
Gas production, ml/g of DM	44.8[b]	47.3[a]	47.3[a]	0.54	0.02
CH_4, ml/g of DM	9.3[a]	8.1[b]	8.1[b]	0.71	0.02
Protozoa, log count/mL	5.0[a]	4.1[b]	4.2[b]	0.52	0.03

[a,b]Means in the same row with different superscripts were different ($p < 0.05$).
[1]Control (lambs were fed 600 g of a concentrate feed mixture plus 400 g of rice straw per kg of DM without any probiotic); PP (control diet supplemented with 20 g of the probiotic in powder form per animal/day); and PL (control diet supplemented with 10 ml of the probiotic in liquid form per animal/day) in a completely randomized block design.
[2]SEM: greatest standard error of the mean.

Discussion

Increased DM intake noted in the present study is consistent with a previous study, which reported greater DM intake of steers supplemented with multi-enzymes sourced from the same probiotic product.[2]

Improvement of nutrient digestibility by addition of probiotics, especially the liquid form (i.e., PL) was probably due to the beneficial effects on fiber hydrolysis and rumen fermentation activity.[1] Consistent with the present study, Deng et al.[24] reported greater apparent digestibility of DM, OM, N and NDF in Dorper wethers supplemented with *Bacillus licheniformis*. In contrast, Le et al.[25] reported no differences in apparent digestibility of DM, OM and NDF in *B. amyloliquefaciens* supplemented ewes. Greater NDF digestibility with the addition of probiotics product rich in exogenous enzymes and bacterial cells may be due to greater ruminal degradability, which could reduce physical fill and allow greater DM intake.[26] However, Nsereko et al.[27] suggested that improved digestibility caused by exogenous enzyme supplement might be related to improved microbial colonization. Both enzyme forms improved colonization and increased activity of the exogenous enzyme within the rumen.[5] This view is similar to previous hypotheses that exogenous enzymes increased fibrolytic activity due to increased numbers of ruminal microbes, and increased bacterial attachment and synergistic effects with hydrolysis of ruminal microorganisms.[4]

It is noteworthy that greater nutrient digestibility as a result of enzyme supplementation will result in improvement in nutritive value. This trend was noted in the present study with improvement in the diets supplemented with the probiotics. Dean et al.[28] found that the nutritive value and fermentation of Bermudagrass silage can be improved by treating it with fibrolytic enzymes compared with control silages. This may be as a result of partial digestion of feed or weaken cell wall barriers that limit rumen microbial digestion. The direct action of exogenous enzymes before feed consumption can cause the release of reducing sugars arising from partial solubilization of cell wall components. This may, therefore, increase available carbohydrates in the rumen thereby shortening the lag time needed for microbial colonization and can also enhance rapid microbial attachment and growth.[28] These factors reflect an increase in the hydrolytic capacity of the rumen, which indirectly reduces gut fill and hence enhances DM intake.[26] Greater N intake and absorption in probiotics groups is in agreement with a previous study, which found N utilization improvements in tree fodder forages as a result of application of the exogenous fibrolytic enzyme preparation form.[29]

However, the low concentration of NH_3–N and the greater amount of total VFA obtained with probiotics inclusion in the present study suggested that they could favor the carbon flow and VFA production. Consistent with this assumption, Le et al.[25] reported lower ruminal NH_3–N concentration for *B. amyloliquefaciens* supplemented ewes.

Probiotics product use can improve stimulation of ruminal microorganism activity by reducing NH_3–N concentration in the rumen liquor by incorporation of NH_3–N into microbial protein.[1] This effect is attributed to an increase in microbial colonization of feed particles and that exogenous enzymes may act similarly to primary bacterial colonization.[30] Gado et al.[1] observed greater VFA production in lambs due to enhanced fiber digestibility of the diet. Increases in passage rate can be associated with a faster rate of particle size reduction in the rumen and a corresponding increase in feed intake.[26]

It is well known that microbial protein synthesis is a good indicator of beneficial effect of feed utilization. Microbial protein has the most significant impact on both quantity and quality of protein absorbed from the small intestine. Salem et al.[31] indicated that the same probiotics product used in the present work increased the amount of microbial protein available for animal metabolism, which might be more efficient for enhancing fiber digestibility and consequently providing more nutrients for ruminal microorganisms beneficial for microbial growth. Feeding the enzyme preparation may have stimulated or increased total viable rumen bacterial numbers, or both, because rumen microbial N synthesis was increased, which may be partially due to greater fiber digestion or an improved capacity of rumen bacteria to digest feed.

Hirstov et al.[32] showed that enzymatic activities declined when two different exogenous fibrolytic enzyme products where administered directly into the rumen due to enzyme inactivation and passage of fluid from the rumen. Similarly, maximizing the proportion of the diet to which the enzyme is added is considered to increase the chances that the enzymes will remain active in the rumen.[5]

Improvement of the average daily gain, G:F and economic efficiency by addition of probiotics product (i.e., PP or PL) was probably due to the improvement in nutrient digestibility of the diets. Supplementing diets with probiotics product rich in enzymes and bacterial live cells has been shown to improve average daily gain and G:F of feedlot cattle. Salem et al.[2] indicated that weight gain can be improved with the same probiotics product, although the response will vary as a function of the selected enzymes, doses and substrate.

Increasing level of fibrolytic enzymes increased rate and production of *in vitro* gas with some raw agricultural waste[33] or total mixed rations of different roughage concentrate ratios.[34] Colombatto et al.[4] reported short-term effect of enzymes on the degradation of feeds, with limited effects during fermentation. This continuous effect might be partly due to the pre-incubation effect that may form stable enzyme-feed complex.

Methane production reduction may be related to microflora change of methanogenium leaded by enzyme addition.[35] In an in vivo study undertaken by Deng et al.,[24] methane production was reduced by 6% in wethers supplemented with 2.5×10^8 CFU of *B. licheniformis* per head per day. Consistent with the present study, they reported higher concentration of propionate and lower concentration of acetate. It is known that propionate production is an alternative hydrogen sink, which competes with methanogens for available hydrogen.[36] Therefore, increases in propionate concentration is likely to result in a reduction in methane production with concomitant decrease in acetate concentration.[36,37] Acetate production is associated with the release of H_2, which can be used by methanogenic bacteria to form methane.[30] However, only a few studies have

investigated effects of exogenous enzymes on methane production and results are conflicting.[24,38]

Conclusion

Results showed that the probiotics product preparations used in the present enhanced nutrient intake and nutrient digestibility with concomitant increase in animal performance. Additionally, the probiotic products reduced both NH_3–N and methane production but increased VFA concentration and microbial nitrogen yield.

Disclosure statement

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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