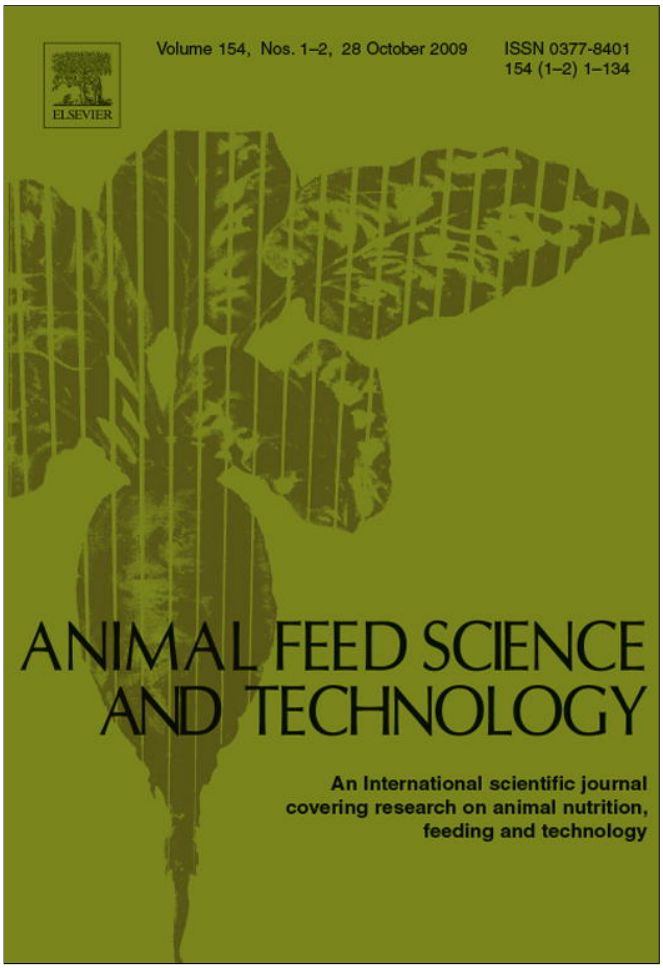

Animal Feed Science and Technology 154 (2009) 36–46

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows

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ARTICLE INFO

Article history:
Received 22 October 2008
Received in revised form 22 May 2009
Accepted 23 July 2009

Keywords:
ZADO®
Enzymes
Digestibility
SCFA
Rumen microbial protein
Milk production

ABSTRACT

This experiment studied effects of a mixture of exogenous enzymes (ZADO®) from anaerobic bacteria on ruminal fermentation, feed intake, digestibility, as well as milk production and composition in cows fed total mixed rations (TMRs; 0.7 corn silage and 0.3 of a concentrate mixture). Twenty lactating multiparous Brown Swiss cows (500 ± 12.4 kg live weight) were randomly assigned into two experimental groups of 10 immediately after calving and fed a TMR with or without (CTRL) addition of 40 g/cow/d of enzymes for 12 weeks. Addition of enzymes increased ($P<0.05$) rumen microbial N synthesis. Intake of dry matter (DM) and organic matter (OM) was positively influenced ($P<0.05$) by supplementation, and digestibility of all nutrients was higher ($P<0.05$) in the total tract of supplemented cows, although the magnitude of the improvement varied among nutrients, with the highest improvement in aNDFom and ADFom (418–584 and 401–532 g/kg respectively;

Abbreviations: ADFom, acid detergent fiber; CTRL, control diet; CP, crude protein; DM, dry matter; aNDFom, neutral detergent fiber; OM, organic matter; TMR, total mixed ration.

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0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2009.07.006

$P<0.05$) than the other nutrients. Supplementation of enzymes also increased ($P<0.05$) rumen ammonia N and total short chain fatty acid (SCFA) concentrations, and individual SCFA proportions were also altered with an increase in acetate (61.0–64.8 mol/100 mol; $P=0.05$) before feeding, and acetate and propionate increased 3 h post-feeding (60.0–64.0 and 18.3–20.8 mol/100 mol respectively; $P<0.05$). Milk and milk protein production was higher (12.8–15.7 and 0.45–0.57 kg/d respectively; $P<0.05$) for cows fed the ZADO® supplemented diet. This exogenous enzyme product, supplemented daily to the TMR of cows in early lactation, increased milk production due to positive effects on nutrient intake and digestibility, extent of ruminal fermentation and microbial protein synthesis.

1. Introduction

Recent research has demonstrated that supplementing diets of dairy cows and feedlot cattle with fiber degrading enzymes can improve feed utilization and animal performance by enhancing fiber degradation *in vitro* (Hristov et al., 1996; Gado et al., 2007; El-Adawy et al., 2008; Rodrigues et al., 2008), *in situ* (Feng et al., 1996; Lewis et al., 1996; Tricarico et al., 2005; Krueger et al., 2008), and *in vivo* (Yang et al., 1999; Gado et al., 2007; Salem et al., 2007; Gado and Salem, 2008). Feeding enzymes is often accompanied by increased feed intake, which may partly be due to increased palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis. However post-ingestive enzyme effects, such as increased digestion rate and/or extent of digestion (Beauchemin and Rode, 1996; Feng et al., 1996; Gado and Salem, 2008; Krueger et al., 2008) may increase hydrolytic activity in the rumen to reduce gut fill and enhance feed intake (Adesogan, 2005).

Proposed modes of action of direct-fed enzymes include solubilization of dietary fiber before ingestion, provision of readily fermentable substrate for ruminal microorganisms and/or enhancement of microbial enzyme activity in the rumen (McAllister et al., 2001). A variety of factors, such as the specific activity of the enzymes, their mode and level of application, as well as the type of animal and its diet, may affect enzyme efficacy. Direct-fed enzymes can also enhance microbial colonization of feed by increasing numbers of ruminal fibrolytic microbes (Morgavi et al., 2000; Nsereko et al., 2000) to increase rate of degradation of fiber in the rumen (Yang et al., 1999; Tricarico et al., 2005; Giraldo et al., 2008), rumen microbial protein synthesis (Yang et al., 1999; Nsereko et al., 2002) and forestomach digestibility.

Positive effects of adding exogenous enzymes to ruminant diets have been reported for lactating dairy cows and growing cattle. Dairy cows fed forage treated with a fibrolytic enzyme additive ate more feed and produced 5–25% more milk (Lewis et al., 1995; Tricarico et al., 2005; Stella et al., 2007), improved the energy balance of transition dairy cows (DeFrain et al., 2005) and increased milk production in small ruminants (Titi and Lubbadeh, 2004; Stella et al., 2007). In feedlot cattle, fibrolytic enzymes have improved live weight (LW) gain by as much as 35% and feed conversion ratio by up to 10% (Beauchemin et al., 1995).

A commercial exogenous enzyme mixture (ZADO®), prepared from anaerobic bacterium, has been shown to improve ruminal fermentation, N balance and nutrient digestibility, as well as milk yield of cows fed diets containing Egyptian by-product feeds (Gado et al., 2007; Soliman, 2006), as well as LW gain and feed conversion of wheat straw in sheep and goats (Gado and Salem, 2008; Salem et al., 2007). Our objective was to evaluate impacts of ZADO® supplemented to a total mixed ration (TMR) of dairy cows on feed intake and digestibility, ruminal fermentation and microbial protein synthesis, as well as on milk production and composition.

Table 1
Ingredient and chemical composition (g/kg of DM) of the concentrate mixture as a part of the TMR[a] fed to the dairy cows.

	g/kg
Ingredient composition	
Yellow corn grain, ground	330
Cotton seed cake	315
Soya bean meal	60
Wheat bran	112
Rice bran	95
Molasses, liquid	45
Yeast	5
Magnesium sulfate	2
Salt	5
Calcium bicarbonate	25
Mineral and vitamin mixture[b]	6
Chemical composition	
Organic matter	943
Crude protein	156
Ether extract	43
aNDFom	441
ADFom	251

[a] TMR: total mixed ration consisted of 0.7 corn silage (DM, 320 g/kg; CP, 50 g/kg DM; aNDFom, 460 g/kg DM; ADFom, 310 g/kg DM) and 0.3 of the concentrate mixture on a DM basis.

[b] Mineral and vitamin mixture: Ca, 190 g/d; P, 115 g/d; Mg, 63 g/d; Cl, 167 g/d; K, 380 g/d; Na, 70 g/d; S, 53 g/d; Co, 3.3 mg/d; Cu, 197 mg/d; Fe, 360 mg/d; Mn, 900 mg/d; Se, 2 mg/d; Zn, 810 mg/d; Vit.A, 940 (1000 IU/d); Vit.D, 165 (1000 IU/d); Vit.E, 374 (1000 IU/d).

2. Materials and methods

The study was conducted at a private dairy farm at Sharkia, Egypt and the chemical and statistical analysis was completed at the molecular laboratory of the Department of Animal Production of Ain Shams University in Cairo as well as the laboratory of Animal Nutrition of the Faculty of Agriculture of Alexandria University.

2.1. Animals and feeding

Twenty lactating multiparous Brown Swiss cows (500 ± 12.34 kg LW) with similar milk production were randomly assigned into two groups of 10 and fed a TMR without (CTRL) or with addition of 40 g/d of ZADO® enzyme powder. Cows were randomly assigned to the experimental groups after being sorted by parity. The enzyme product was made from natural sources of anaerobic ruminal bacteria including 7.1 unit/g of cellulase, 2.3 unit/g of xylanase, 61.5 unit/g of α-amylase and 29.2 unit/g of proteases (patent no. 22155 of Egypt, Molecular Biology Laboratory of the Ain Shams University (Cairo) according to Gado, 1997).

Cows were fed a TMR of 0.7 corn silage and 0.3 concentrate on a DM basis (Table 1) with fed refusals of 0.10–0.15. Cows were individually fed in tie-stalls at 0600, 1500 and 1800 h for the first 12 weeks of lactation. The TMR was balanced for minerals and vitamins and formulated to meet the nutrient requirements of cows according to NRC (2001) recommendations (Table 1). The daily amount of enzymes was mixed individually for each cow with the TMR fed at 0600 h.

2.2. Measurements of digestibility, rumen metabolism and milk production

Feed and orts samples were collected twice weekly to calculate dry matter (DM) intake. Apparent digestibility was determined by adding chromic oxide (55.1 mg of Cr/kg of DM) to the diets and sam-

pling feces from the rectum of each cow at five equally spaced times per day on week 12 of the study. Fecal samples, for determination of digestibility, were composited by cow, dried at 55 °C, ground to pass a 1 mm screen and retained for chemical analyses.

On the 12th week of the study, samples of rumen fluid were withdrawn from each cow by stomach tube before the morning feeding (*i.e.*, 0 h) and 3 h after feeding on one day. Samples (50 ml/cow) were immediately filtered and stored for short chain fatty acids (SCFAs; *i.e.*, acetic, propionic, butyric acids) and ammonia N analysis. To determine microbial N synthesis, urine from each cow was collected for a period of 24 h and diluted to a fixed volume with water, and one sub-sample was stored at −20° for later analysis of purine derivatives.

Cows were milked in their tie-stalls at 0700 and 1700 h, and milk yield was recorded daily and sampled on two days in weeks 11 and 12 of the study. Milk samples were preserved with potassium dichromate, stored at 4 °C, and sent to the laboratory for compositional analysis.

2.3. Sample analysis

Amounts of TMR offered and refused were recorded daily by cow. Feed refusals from individual cows were collected, mixed within treatment and a representative sample frozen daily. Twice weekly composite samples of feed refusals were analyzed for DM, and the DM content of feed ingredients was determined weekly to adjust dietary formulations (if necessary) to account for small changes in ingredient DM contents. To determine DM, feed samples were dried at 60 °C for 48 h in a forced air oven.

Dried samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 1 mm screen. Analytical DM content of the samples (feed, orts, feces) was determined by drying at 135 °C for 3 h, and organic matter (OM) was determined as the weight loss on ashing at 550 °C. Neutral detergent fiber (aNDFom) and acid detergent fiber (ADFom) were determined using the procedures of Van Soest et al. (1991). Sodium sulfite was not used in the procedure for aNDFom determination, but pre-treatment with heat stable amylase (type XI-A from *Bacillus subtilis*; Sigma, Sharkia, Egypt) was included. Both aNDFom and ADFom are expressed without residual ash.

Chromium, as a marker to calculate nutrient digestibility, was determined in fecal samples by atomic absorption spectrophotometry according to AOAC (2000; ID 952.02). Collected feed, refusals and fecal samples were analyzed for N, aNDFom and ADFom. A Kjeldahl method (AOAC, 1990; ID 954.01) was used to determine N.

Ruminal pH was determined before rumen liquor was stored with a digital pH meter. Acetic, propionic and butyric acids in rumen fluid were quantified using crotonic acid as the internal standard using gas chromatography (model 5890, Hewlett Packard, Little Falls, DE, USA) with a capillary column (30 m × 0.25 mm i.d., 1 m phase thickness, Supelco Nukol; Sigma–Aldrich, Mississauga, ON, Canada), and flame ionization detection. Oven temperature was 100 °C for 1 min, which was then ramped by 20 °C/min to 140 °C, and then by 8–200 °C/min, and held at this temperature for 5 min. The injector temperature was 200 °C, the detector temperature was 250 °C, and the carrier gas was He. Concentrations of NH_3-N were determined using the colorimetric method described by Rhine et al. (1998). A standard curve was made to determine whether a linear relationship existed between varying concentrations of ammonium sulfate standard solution and intensity of color produced by Nesslerization. Ten test tubes containing 0.10–1.0 ml of standard solution were prepared. To each test tube, 0.04 ml of Nessler's reagent was added and volume was made up to 5 ml with distilled water. The intensity of color thus developed was measured at a wavelength of 420 nm on Spectronic 21 within 5–10 min after setting it at 0 absorbance with the blank.

Sub-samples of urine were analyzed for allantoin by high-performance liquid chromatography with pre-column derivation according to Chen et al. (1993) and for uric and hypoxanthine plus xanthine according to Chen et al. (1990). In the latter method, hypoxanthine and xanthine were determined collectively as uric acid after treatment with xanthine oxidase. Urine samples were diluted with distilled water before the assays, by 40 times for allantoin and 10 times for uric and hypoxanthine plus xanthine. The N content of urine was determined by the method of Davidson et al. (1970). All daily urine samples were analyzed individually. Rumen microbial N was calculated depending on the total purine derivatives (*i.e.*, allantoin and uric acid measured) according to Chen et al. (1990).

Daily milk weights were recorded during weeks 11 and 12 of the experiment. Milk samples were analyzed for fat, crude protein (CP) and lactose with Milk-O-Scan 605 (Foss Electric, Hillerod, Denmark) based on infrared technology. Final milk composition for each week was expressed as the weighted yield of the two daily milkings. Average fat and CP yields were calculated by multiplying milk yield by fat and CP content of milk on an individual cow basis. Milk energy (MJ/kg) was calculated on an individual cow basis using the milk fat, CP and lactose content of the milk (Tyrrell and Reid, 1965).

2.4. Statistical analysis

Statistical analysis was completed on data from weeks 11 and 12 of the experiment, considering the period from weeks 1 to 10 to be an adaptation period. Collected data on nutrient intake and digestibility, ruminal fermentation parameters and milk yield and composition in the two cows groups (*i.e.*, CTRL and ZADO® enzymes) were analyzed as a factorial design using the general linear models procedure of SAS (2001), with methods of Steel and Torrie (1980) to determine differences due to enzymes addition. The significance level of the test was $P<0.05$.

3. Results

Intake of DM and OM was positively influenced by enzyme supplementation (Table 2), but aNDFom intake was not. Digestibility of DM, OM, aNDFom and ADFom was higher ($P<0.05$) in the total tract of ZADO® enzyme supplemented cows by 8–16%.

Enzyme supplemented cows had higher ($P<0.05$) SCFA and ammonia N concentrations (Table 3) pre-feeding (122 *versus* 111 mmol/100 ml; 126 *versus* 110 mg/l respectively) and at 3 h post-feeding (128 *versus* 119 mmol/100 ml; 67 *versus* 55 mg/l respectively). Acetate was increased before feeding, and acetate and propionate 3 h post-feeding, with enzyme supplementation. There was an increase in N in the ZADO group. There were also an increase in excretion of N in urine and feces in the ZADO group, but this was not statistically significant. The N balance in the ZADO group was higher than in the control group. Microbial N synthesis was higher in enzyme supplemented cows (220 *versus* 190 g/d; $P<0.05$).

Output of milk and milk energy was higher ($P<0.05$) for cows fed the enzyme supplemented diet (Table 4). However, increased milk yield for cows fed enzymes was not accompanied by increases in yield of milk components, except CP, which was increased ($P<0.05$) by addition of enzymes product.

Table 2
Intake, whole tract nutrients digestibility and nitrogen balance of the TMR[a] fed to dairy cows supplemented with (ZADO®) or without (CTRL) the exogenous enzymes mixture.

	CTRL	ZADO®	SEM	Significance (P)
Intake (kg/d)				
DM	16.1	18.2	0.21	0.049
OM	14.1	16.4	0.14	0.048
aNDFom	7.1	7.4	0.23	0.192
ADFom	4.04	4.57	0.11	0.087
Digestibility (g/kg)				
DM	663	743	2.1	0.045
OM	667	741	2.9	0.047
aNDFom	418	584	2.8	0.049
ADFom	401	532	2.3	0.046
Nitrogen balance				
N intake (g/d)	1654	1870	54	0.07
Urinary N (g/d)	509	542	12.6	0.11
Fecal N (g/d)	659	711	14.3	0.17
N balance (g/d)	486	617	11.9	0.06

DM, dry matter; OM, organic matter; ADFom, acid detergent fiber; aNDFom, neutral detergent fiber.

[a] TMR: total mixed ration without (CTRL) or with (ZADO®) the commercial exogenous enzyme mixture.

Table 3
Ruminal pH, short chain fatty acids (SCFAs, total and individual), ammonia N concentrations (after 0 and 3 h of feeding); microbial nitrogen synthesis of the TMR[a] fed to dairy cows supplemented with (ZADO®) or without (CTRL) the exogenous enzymes mixture.

	CTRL	ZADO®	SEM	Significance (*P*)
pH	6.1	5.9	0.24	0.41
Before feeding (0 h)				
Total SCFA (mmol/l)	111	122	2.1	0.34
Individual SCFA (mol/100 mol)				
Acetate (A)	61.0	64.8	1.30	0.05
Propionate (P)	17.8	18.1	0.83	0.13
Butyrate	11.3	11.9	0.81	0.24
A:P ratio	3.43	3.85	1.162	0.14
Ammonia N (mg/l)	55	67	0.37	0.04
Post-feeding (3 h)				
Total SCFA (mmol/l)	119.2	128	3.6	0.04
Individual SCFA (mol/100 mol)				
Acetate (A)	60.0	64.0	1.2	0.04
Propionate (P)	18.3	20.8	0.87	0.01
Butyrate	10.9	11.0	0.96	0.31
A:P ratio	3.28	3.08	0.070	0.01
Ammonia N (mg/l)	110	126	2.3	0.05
Microbial N (g/d)	190	220	9.6	0.04
Uric acid (mmol/d)	22.4	24.6	0.67	0.16
Allantoin (mmol/d)	308	304	10.4	0.26

[a] TMR: total mixed ration without (CTRL) or with (ZADO®) the commercial exogenous enzyme mixture.

4. Discussion

4.1. Nutrient intake and digestibility

Increased DM intake by addition of enzymes may be partly due to increased nutrient digestibility, which is consistent with previous results with the same enzyme mixture (Soliman, 2006; Gado et al., 2007; Salem et al., 2007; El-Adawy et al., 2008; Gado and Salem, 2008). However the magnitude of DM intake increase was much higher in the present study, although the increase is consistent with sheep and goats (Salem et al., 2007).

In the current study, DM intake and digestibility were improved by about 13 and 9% respectively with enzyme addition. Other reports have also shown increases in DM, particularly fiber, digestibility

Table 4
Milk production and composition of the TMR[a] fed to dairy cows supplemented with (ZADO®) or without (CTRL) the commercial exogenous enzymes mixture.

	CTRL	ZADO®	SEM	Significance (*P*)
Milk				
Production (kg/d)	12.8	15.7	0.85	0.046
Energy (MJ/d)	58.2	70.7	8.15	0.041
Milk composition (g/kg)				
Fat	39	38	2.4	0.163
Protein	35	36	1.7	0.242
Lactose	45	45	2.2	0.361
Milk component yield (kg/d)				
Fat	0.50	0.60	0.041	0.126
Protein	0.45	0.57	0.022	0.047
Lactose	0.58	0.71	0.054	0.141

[a] TMR: total mixed ration without (CTRL) or with (ZADO®) the commercial exogenous enzyme mixture.

with fibrolytic enzyme addition (Gado and Salem, 2008; Hristov et al., 2008). Bowman et al. (2002), for example, reported a 25% increase in total tract aNDFom digestibility with a fibrolytic enzyme product, but it appeared that most of the impact was post-ruminal. Our results are consistent with Beauchemin et al. (2001), who reported that the average increase in DM intake due to enzyme supplementation was 1.6 kg/d in dairy cows. In contrast, Schingoethe et al. (1999) treated forage with 0.7–1.5 l/t forage DM with concentrated cellulase and xylanase enzymes and reported that cows fed treated and untreated forage had similar DM intake.

Generally, digestion of aNDFom varies due to the chemical composition of the diet (Varga et al., 1998), the size of the indigestible aNDFom fraction, the digestion rate of potentially digestible aNDFom and rumen outflow rate (Firkins et al., 1998), as well as use of feed additives. Exogenous fibrolytic enzymes would be expected to increase fiber digestion by increasing the rate of ruminal digestion of the potentially digestible aNDFom fraction (Yang et al., 1999), but increases in fiber digestion may also be, in part, due to reduced digesta viscosity (Hristov et al., 2000), alterations in ruminal fermentation (Nsereko et al., 2002) and/or enhanced attachment and colonization to the plant cell wall by ruminal microorganisms (Nsereko et al., 2000; Wang et al., 2001) and/or by synergism with enzymes in rumen fluid (Morgavi et al., 2000). However, increased fiber digestion is unlikely the result of supplemental enzyme activity alone because the contribution of added exogenous enzymes to total ruminal activity is relatively small (Beauchemin et al., 2001). Morgavi et al. (2000) demonstrated synergism between exogenous enzymes and ruminal enzymes such that the net combined hydrolytic effect in the rumen was much greater than that estimated from individual enzyme activities. Wang et al. (2001) reported that enzyme supplementation increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system with rumen fluid. Stimulation of rumen microbial numbers by the use of enzymes could result in higher microbial biomass, which would provide more total polysaccharidase activity to digest feedstuffs. Consistent with this hypothesis, Yang et al. (1999) reported that enzyme supplementation of dairy cow diets increased feed digestion in the rumen and flow of microbial protein from the rumen. The beneficial effects on animal performance are likely to be highest for ruminants in negative energy balance, such as cows in early lactation (Rode et al., 1999), which is consistent with results of our study.

Previous studies in our laboratory (*i.e.*, Gado et al., 2007) showed that this ZADO enzyme product improved *in vitro* DM and aNDFom digestibility of wheat straw in the first 24 h of incubation, which is consistent with Feng et al. (1996) and Wang et al. (2004), who suggested that exogenous fibrolytic enzymes increase rate of DM digestion. Our exogenous enzyme, rich in xylanolytic, cellulase, α-amylase and protease activity, had positive effects on digestion of aNDFom in TMR, consistent with Krause et al. (1998), who suggested that enzymes can improve nutrient degradation in high-concentrate diets. Perhaps the net effects of fibrolytic enzyme mixtures are not limited to the dietary component to which the enzymes are applied, which may explain why fibrolytic enzymes can be effective in improving digestibility of the non-fiber carbohydrates in addition to increasing digestibility of fiber when enzymes are added to the concentrate portion of a diet, or to high-concentrate diets (Beauchemin et al., 2003).

4.2. Ruminal fermentation and microbial protein synthesis

Total and individual SCFA, in particular acetate and propionate, in the cows fed the enzyme diet suggest that enzymes improved ruminal fiber fermentation. Several studies (Lewis et al., 1999; Wang et al., 2001) have demonstrated that treatment of feed with fibrolytic enzymes before feeding increased ruminal enzyme activities. Our enzyme product contained some materials, such as sugars, that are fermented by rumen microorganisms, which could have increased rumen SCFA concentrations (Wallace et al., 2001; Colombatto et al., 2003; Gado and Salem, 2008; Giraldo et al., 2008; Ranilla et al., 2008). However these levels were low, and unlikely to have had a substantive impact.

Previous studies have shown that treatment of diets with enzymes before feeding, or incubation with ruminal fluid, enhanced beneficial effects of enzymes on ruminal fermentation (Wang et al., 2001; Giraldo et al., 2004; Gado and Salem, 2008). As pointed out by Colombatto et al. (2003), some have suggested that this could be due to creation of a stable enzyme–feed complex (Kung et al., 2000), but others have indicated the possibility of alteration in the fiber structure, which

would stimulate microbial colonization (Nsereko et al., 2000; Giraldo et al., 2008; Ranilla et al., 2008). Wang et al. (2001) suggested that changes in rumen fermentation patterns may reflect a shift in the species profile of colonizing bacteria in response to enzyme treatment of feed with exogenous enzymes. That feeding enzymes increased the proportion of acetate in the rumen fluid may suggest that methane production was also increased, since acetate production is associated with release of H^+ which can be used by methanogens to form methane (Stewart et al., 1997), which represents a loss of energy to the host. Increased propionate proportion with enzyme supplementation may suggest synergism between exogenous enzymes in feeds and lactic acid bacteria in the rumen.

Our results may be consistent with Beauchemin et al. (2003), who reported that supplementing the diet of feedlot cattle with lactic acid bacteria increased the proportion of propionate and, consequently, decreased the proportion of butyrate in rumen fluid compared with the control. Results suggest that our enzyme supplemented diet stimulated lactic acid-utilizing bacteria which also produce propionate. Similarly, Kim et al. (2000) reported increased rumen propionate concentrations in steers fed lactic acid-producing bacterium.

The lower acetic/propionic acid ratio is consistent with increases in fiber degradation for cows supplemented with enzymes, indicating that the added enzymes made the fermentation more gluconeogenic, and thereby improved the energetic efficiency of fermentation. This shift in SCFA proportions could increase precursor availability, and improve nutrient utilization, particularly for dairy cows in early lactation when nutrient intake lags nutrient demand (Eun et al., 2007), possibly due to fermentation of sugars released by cell wall hydrolysis by enzymes. This agrees with Dawson and Tricarico (1999) and Krueger and Adesogan (2008), who noted that enzyme mixtures containing cellulase were more inclined to alter the relative proportions of SCFA thereby resulting in more acetate and less propionate and less butyrate.

Feeding the enzyme preparation may have stimulated and/or increased total viable rumen bacterial numbers because rumen microbial N synthesis was increased which may be due, at least in part, to increased fiber digestion and an improved capacity of rumen bacteria to digest feed. Although this possibility may not be supported by Nsereko et al. (2002) and Krueger et al. (2007) who showed that while cellobiose and glucose utilizing bacteria were stimulated, effects on the fibrolytic population were negligible. Our results indicate that enzyme supplementation increased the quantity of microbial protein available to animal metabolism, and that increased fiber digestibility increased the net energy density of the ZADO enzyme diet.

Increased ammonia N concentration in cows fed the enzyme supplemented diet supports its capability to enhance rumen protein degradation, probably because it contained protease enzymes. However increased protein degradation may also reflect the more neutral rumen pH with enzyme addition, thereby increasing ruminal bacterial colonization of feed particles (Yang et al., 1999; Morgavi et al., 2000; Nsereko et al., 2000). However, Colombatto et al. (2007), working with an enzyme product rich in xylanolytic activity, concluded that exogenous enzymes had higher activity close to pH neutrality and that the hypothesis that exogenous enzymes have an effect on digestion when pH values were not optimal for fiber degradation is not supported.

4.3. Milk production and composition

A major finding of our study was that milk production was higher in enzyme supplemented cows (12.75 *versus* 15.70 kg/d). The enzyme diet was provided immediately after calving and a higher milk yield and DM intake was observed immediately. The large increase in DM intake, digestibility and ruminal fermentation activities suggest that increased milk production was due to feeding enzymes. Soliman (2006) reported 23% higher milk production of dairy cows fed peanut hay ensiled with enzymes for 45 d, and explained the improvement as being due to increased nutrient digestibility and microbial protein synthesis.

Studies on enzyme supplementation to dairy cow diets have shown increased milk yields of 7–15% (Beauchemin et al., 1999; Lewis et al., 1999; Rode et al., 1999; Schingoethe et al., 1999; Yang et al., 2000), probably due to increased digestibility (Rode et al., 1999; Yang et al., 1999; Tricarico et al., 2005), as well as alteration of acetic/propionic acid ratio in the rumen (Rode et al., 1999; Giraldo

et al., 2008), which increased energy available for milk production (Lewis et al., 1999; Yang et al., 1999).

5. Conclusions

The exogenous enzyme product (ZADO®), sourced from anaerobic bacterium and added to the TMR of cows in early lactation, increased milk production due to enhanced nutrient intake, and nutrient digestibility, as well as increased rumen microbial protein synthesis.

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