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ANIMAL RESEARCH PAPER

Influence of cellulase addition to dairy goat diets on digestion and fermentation, milk production and fatty acid content

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SUMMARY

Twenty four French Alpine goats (39 ± 2·0 kg) were individually housed in a completely randomized design and fed a basal diet containing 146 g crude protein and 356 g neutral detergent fibre (NDF)/kg in the absence (control – CTRL) or presence (CELL) of 2 ml of cellulase/kg dry matter intake (DMI) for 70 days, which included a 10-day adaptation period. The feed was offered three times daily at 07·00, 13·00 and 19·00 h, but the single daily dose of cellulase was only fed at 07·00 h. Goats were hand-milked daily; milk production recorded and samples taken for compositional analysis. During the last 5 days of the experimental period, goats from each group were individually housed in stainless steel metabolic cages to enable separate and total collection of faeces and urine for nutrient digestibility and ruminal fermentation determinations. Goats fed CELL had greater DMI and greater digestibility of dry matter (DM), organic matter and NDF than CTRL goats. CELL goats had greater ruminal pH, concentration of acetic acid and concentration of propionic acid than CTRL goats. However, the concentration of ruminal butyric was lower in CELL goats compared with CTRL goats. CELL goats had greater milk yield, energy corrected milk, milk energy content, milk energy output and milk density than CTRL goats and the milk content for total solids, fat, protein and lactose were also greater for CELL goats than for the CTRL goats. The milk of CELL goats had greater palmitoleic acid, *cis*-10-heptadecanoic acid content and mono-saturated acids than the milk of CTRL goats and lower linoleic acid, linolenic acid contents and saturated fatty acids than the milk of CTRL goats. These results suggest that addition of 2 ml cellulase/kg DM of feed in the diet of lactating French Alpine goats elevated their milk production and improved its composition probably due to improved feed utilization.

INTRODUCTION

Many attempts have been made to improve the utilization of feeds by dairy cattle. In recent years, use of feed additives and enzymes in ruminant nutrition have gained elevated interest, e.g. yeast (Elghandour *et al*. 2014), phytogenic extracts (Cedillo *et al*. 2014; Salem *et al*. 2014) and fibrolytic enzymes (Alsersy *et al*. 2015; Salem *et al*. 2015; Valdes *et al*. 2015). Furthermore, feeding dairy animals on diets supplemented with fibre-degrading enzymes has been shown to improve feed utilization and animal performance (Khattab *et al*. 2011; Salem *et al*. 2013). However, the mode of action of these enzymes has not been fully elucidated.

Hydrolysis of dietary fibre before ingestion (Khattab *et al*. 2011), provision of readily fermentable substrates for ruminal micro-organisms (McAllister *et al*.

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2001) and synergistic enhancement of microbial enzyme activity in the rumen (Morgavi *et al.* 2000) have been suggested as possible modes of action. In addition, alteration of ruminal fermentation (Salem *et al.* 2015) and enhanced ruminal micro-organism attachment to and colonization of the plant cell wall (Wang *et al.* 2001) are other possibilities.

Although the results of applying fibrolytic enzymes in dairy cattle rations have been encouraging, they have also been inconsistent. Some studies showed elevated milk production and improved composition (Gado *et al.* 2009; Khattab *et al.* 2011) while others did not (Elwakeel *et al.* 2007; Dean *et al.* 2013). Although the reasons for this are unknown, some factors such as differences in enzyme activity, application rate and composition, stage of lactation, time of enzyme delivery, ruminal activity and enzyme stability, enzyme-feed specificity and the portion of the diet in which enzymes are applied have been implicated (Dean *et al.* 2013).

The objective of the present study, therefore, was to determine the effects of adding a fibrolytic enzyme cellulase in goat rations on feed intake, ruminal fermentation, nutrient digestibility, milk production and milk composition of French Alpine dairy goats fed a high fibre basal diet.

MATERIALS AND METHODS

All procedures involved in handling animals during the experimental period were conducted according to the official Mexican standards of animals care (NORMA Oficial Mexicana 1995).

Study location

The experiment was performed at the metabolic area of the Animal Science farm, and laboratory of animal nutrition of the Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México, México (19°02′04″N, 100°02′14″W, 1720 m a.s.l.). The climate is moderately humid with an average temperature of 15–18 °C and annual rainfall of 950–1000 mm.

Animals, housing and feeding

Twenty-four French Alpine dairy goats (39 ± 2·0 kg initial live weight, 9 months old, first calving) were housed in individual pens provided with automatic feeders and waterers. Immediately after kidding, the goats were adapted to the pens and the basal diet (Table 1) for 10 days before starting the experiment, which lasted for 60 days. Goats were injected with 3 ml/animal of fat-soluble vitamins (A, D, E; Vigantol, Bayer, Mexico City, Mexico) and treated with 0·5 ml Ivermectin (Laboratory Sanfer, Mexico City, Mexico) per animal for internal and external parasites and the health of the animals monitored throughout the experiment.

A week before the start of the experiment, goats were weighed individually and assigned randomly to two treatments (12 goats per treatment) in a completely randomized design.

The treatments were: CTRL: basal diet without addition of cellulase; CELL: basal diet + Celluase® plus (Dyadic® PLUS, Dyadic international, Inc., Jupiter, FL, USA) at 2 ml/kg dry matter (DM) of feed. The activity of the exogenous fibrolytic enzyme Celluase® plus was determined and contained 30 000–36 000 units of cellulase/g and 7500–10 000 units of beta-glucanase/g.

Goats were offered a total mixed ration (TMR; Table 1) three times daily at 07.00, 13.00 and 19.00 h. The TMR was balanced for minerals and vitamins and formulated to cover the nutrient requirements of goats according to NRC (1981) recommendations plus a margin of 0·15. The daily allocation of enzyme for each goat was mixed individually with the TMR and fed at 07.00 h. Throughout the experimental period, the amount of feed offered was recorded and orts were collected daily and weighed for determination of daily feed intake. Additionally, feeds were sampled daily, composited weekly and dried at 60 °C to constant weight and stored for later chemical analysis.

Nutrient digestibility, rumen fermentation and milk production

All goats from each group were housed individually in stainless steel metabolic cages at room temperature with free access to water during the last 5 days of the experimental period for nutrient digestibility and ruminal fermentation determinations. Beneath each cage were 4 mm stainless steel screens to retain faeces but let the urine through to enable separate and total collection of faeces and urine. Total faeces was collected once daily from each goat before the morning feeding, according to the methodology proposed by Stock *et al.* (1987), and stored at −10 °C for later analysis. A sample of about 150 g of faeces from each animal was taken daily and composited weekly.

Table 1. *Ingredients and chemical composition of the basal diet, offered to the French Alpine dairy goats during the first third of lactation*

	g/kg
Ingredients (g/kg DM)	
Sorghum grains	171
Maize grain	171
Wheat bran	90
Soybean meal	90
Urea	12
Molasses	48
Minerals and vitamins*	18
Maize stover	80
Alfalfa hay	320
Chemical composition (g/kg DM)	
Dry matter	865
Organic matter	926
Crude protein	146
Ether extract	97
Neutral detergent fibre	356
Acid detergent fibre	307

* Mineral/vitamin premix contained: vitamin A (12 000 000 IU), vitamin D_3 (2 500 000 IU), vitamin E (15 000 IU), vitamin K (2·0 g), vitamin B_1 (2·25 g), vitamin B_2 (7·5 g), vitamin B_6 (3·5 g), vitamin B_{12} (20 mg), pantothenic acid (12·5 g), folic acid (1·5 g), biotin (125 mg), niacin (45 g), iron (50 g), zinc (50 g), manganese (110 g), copper (12 g), iodine (0·30 g), selenium (200 mg), cobalt (0·20 g).

The composited samples were dried in a forced air oven at 65 °C for 72 h then ground through a 1 mm screen using a Wiley mill grinder (Wiley mill, Model 4, Thomas Scientific, Swedesboro, NJ, USA) and kept for later determination of compositional analysis. Additionally, rumen fluid was taken from the ventral sac of the rumen of each goat using a stomach tube 3 h after the morning feeding. The rumen samples (50 ml/goat) were filtered immediately using four layers of cheesecloth, strained and stored in 45 ml glass bottles. The ruminal pH was then determined using a digital pH meter (GLP 22, Crison Instruments, Barcelona, Spain) and a few drops of toluene and paraffin oil added to each bottle to cover the surface and the bottles stored at −18 °C for later individual volatile fatty acids (VFA) analyses.

Goats were hand-milked daily: milk production was measured in kg (electronic scale; OHAUS Mod. 5603, Mexico City, Mexico) and recorded. Milk was sampled twice weekly and preserved with potassium dichromate. The samples were stored at 4 °C before being sent to the laboratory for compositional analysis.

Chemical analysis and calculations

Dried samples were ground (sieve of 1 mm diameter, Wiley mill, Model 4, Thomas Scientific, Swedesboro, NJ, USA) and analysed for DM (ID 930.15), ash (ID 942.05), ether extract (ID 945.16) and crude protein (CP; ID 984.13) according to AOAC (1997). Collected feed, orts and faecal samples were analysed for neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents (Van Soest *et al.* 1991) using an ANKOM200 Fibre Analyser unit (ANKOM Technology Corporation, Macedon, NY, USA).

The concentrations of acetic, propionic and butyric acids in rumen fluid were quantified as described in Hernandez *et al.* (2014) 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 internal diameter, 1 m phase thickness, Supelco Nukol; Sigma–Aldrich, Mississauga, ON, Canada), and flame ionization detection. Oven temperature was adjusted to 100 °C for 1 min, which was then increased by 20 °C/min to 140 °C, and then by 8 °C/min to 200 °C, and held at this temperature for 5 min. The injector temperature was at 200 °C and the detector temperature at 250 °C. The carrier gas was Helium.

Ruminal methane (CH_4) production was calculated according to the equation of Moss *et al.* (2000) as:

$$\begin{aligned} CH_4(\text{g/day}) = {} & 0{\cdot}45\,\text{acetate(mmol/l)} \\ & - 0{\cdot}275\,\text{propionate (mmol/l)} \\ & + 0{\cdot}40\,\text{butyrate(mmol/l)} \end{aligned}$$

Milk samples were analysed for fat, crude protein and lactose using Milk-O-Scan 605 (Foss Electric, Hillerod, Denmark), which is based on infrared technology. For milk fatty acid profile determination, 150 ml of milk from each goat (experimental unit) were taken and stored at −20 °C and then are lyophilized to make a composited sample for fatty acid profile determination by gas chromatography (model 5890, Hewlett Packard, Little Falls, DE, USA) according to Erwin *et al.* (1961).

Average fat, crude protein and lactose yields (g/day) were calculated by multiplying milk yield by fat, crude protein and lactose content (g/kg) of milk of each individual goat. The gross energy concentration in milk

was calculated according to Tyrell & Reid (1965) as described in Kholif *et al.* (2015) as:

$$\begin{aligned}\text{Milk energy (MJ/kg)} = 4{\cdot}184 \times 2{\cdot}204 \times [(41{\cdot}63 \\ \times \text{fat (g/kg)} + 24{\cdot}13 \\ \times \text{protein (g/kg)} + 21{\cdot}60 \\ \times \text{lactose (g/kg)} - 117{\cdot}2) \\ /10000]\end{aligned}$$

The milk energy output was then calculated as milk energy output (MJ/day) = milk energy (MJ/kg) × milk yield (kg/day).

Energy corrected milk (ECM) was calculated according to Sjaunja *et al.* (1991) as:

$$\begin{aligned}\text{ECM (kg/day)} = \text{milk (kg/day)} \times [38{\cdot}3 \times \text{fat (g/kg)} \\ + 24{\cdot}2 \times \text{protein (g/kg)} + 16{\cdot}54 \\ \times \text{lactose (g/kg)} + 20{\cdot}7]/3140\end{aligned}$$

Statistical analyses

The nutrient intake and digestibility, ruminal fermentation parameters and milk yield and composition data were analysed with the PROC MIXED procedure of SAS (SAS Institute 2006) in which a completely randomized design was used with the statistical model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

where Y_{ij} is every observation of the jth goat assigned to ith treatment, T_i is the treatment effect and E_{ij} is the residual error.

Comparisons of results were performed using Tukey's test at $P < 0{\cdot}05$ (Steel & Torrie 1980).

RESULTS

Feed intake, digestion and ruminal fermentation

Feed intake (kg/day), digestion (g digested/g ingested) and ruminal fermentation data are summarized in Table 2. Goats fed the CELL diet had greater DM intake (DMI; $P = 0{\cdot}049$), organic matter (OM) intake ($P = 0{\cdot}049$) and NDF intake ($P = 0{\cdot}049$) than CTRL goats (Table 2). The digestibility of DM, OM and NDF for CELL goats was also greater ($P < 0{\cdot}001$) than for CTRL goats. Additionally, the ruminal pH for CELL goats was greater ($P = 0{\cdot}013$) than for CTRL goats. CELL goats had greater concentration (mmol/l) of acetic acid ($P < 0{\cdot}001$) and concentration (mmol/l) of propionic acid ($P = 0{\cdot}007$) than the CTRL goats. However, the concentration of ruminal butyric (mmol/l) was lower ($P < 0{\cdot}001$) in CELL goats compared with CTRL goats (Table 2).

Milk production and composition

Milk yields (kg/day) and composition (g/kg) are presented in Table 3. CELL goats had greater milk yield ($P = 0{\cdot}042$), ECM ($P = 0{\cdot}001$), milk energy content (MJ/kg, $P = 0{\cdot}008$), milk energy output (MJ/day, $P = 0{\cdot}001$) and milk density (g/ml, $P = 0{\cdot}003$) than CTRL goats. Additionally, the milk content for total solids ($P = 0{\cdot}008$), fat ($P = 0{\cdot}016$), protein ($P = 0{\cdot}003$) and lactose ($P = 0{\cdot}05$) were greater for CELL goats than for the CTRL goats. The same trends were noted for daily total solids ($P = 0{\cdot}001$), fat ($P = 0{\cdot}001$), protein ($P = 0{\cdot}001$) and lactose ($P = 0{\cdot}002$) yields. Feeding efficiency expressed as ECM/DMI tended to be greater ($P = 0{\cdot}066$) for CELL goats than for CTRL goats; however, when expressed as milk yield/DMI, there were no differences (Table 3).

Milk fatty acid profiles

The milk fatty acid profiles (g/100 g of total esterified fatty acids) are summarized in Table 4. The milk of CELL goats had greater palmitoleic acid ($P < 0{\cdot}001$), *cis*-10-heptadecanoic acid contents ($P = 0{\cdot}021$) and mono-saturated fatty acid ($P = 0{\cdot}043$) concentrations than the milk of CTRL goats, and lower linoleic acid ($P = 0{\cdot}035$), linolenic acid ($P = 0{\cdot}023$) and saturated fatty acid content ($P = 0{\cdot}047$) than the milk of CTRL goats. CELL goat milk tended to have lower oleic acid ($P = 0{\cdot}073$), greater ($P = 0{\cdot}072$) capric acid and lower short-chain fatty acids ($P = 0{\cdot}097$) than CTRL goat milk (Table 4).

DISCUSSION

Feed intake

Goats supplemented with CELL consumed about 11% more DM, OM and NDF compared with CTRL goats. Greater nutrient intake was mirrored by greater nutrient (i.e., DM, OM and NDF) digestibility. These results are consistent with the previous results of Salem *et al.* (2013), which can be explained by the greater nutrient digestibility, particularly fibre, and Gado *et al.* (2009) obtained 13% more DMI due to enzyme addition in dairy cow diets. This is in contrast to Dean *et al.* (2013) who observed no difference in

Table 2. *Feed intake, nutrient digestibility and ruminal fermentation of French Alpine dairy goats fed on the basal diet in the absence (CTRL) or presence of cellulase (CELL) enzyme (2 ml/kg DMI) during the first third of lactation (60 days) (n = 12 per treatment)*

	Diets			
	CTRL	CELL	S.E.M.	*P* value
Intake (kg/day)				
Dry matter	3·1	3·5	0·12	0·049
Organic matter	2·9	3·2	0·11	0·049
Neutral detergent fibre	1·11	1·23	0·041	0·049
Digestibility (g digested/g ingested)				
Dry matter	0·74	0·77	0·011	0·001
Organic matter	0·76	0·80	0·013	0·001
Neutral detergent fibre	0·62	0·68	0·011	<0·001
Ruminal fermentation				
pH	6·44	6·62	0·046	0·013
Acetate (A; mmol/l)	45	47	1·2	<0·001
Propionate (P; mmol/l)	14·2	15·8	0·39	0·007
Butyrate (mmol/l)	9·5	7·3	0·28	<0·001
A/P ratio	3·17	3·03	0·075	0·174
CH_4 (g/day)	20·1	19·9	0·19	0·483

Table 3. *Milk yield and composition of French Alpine dairy goats fed on the basal diet in the absence (CTRL) or presence of cellulase (CELL) enzyme (2 ml/kg DMI) during the first third of lactation (60 days) (n = 12 per treatment)*

	Diets			
	CTRL	CELL	S.E.M.	*P* value
Milk				
Yield (kg/day)	3·1	3·4	0·13	0·042
Energy corrected milk (ECM, kg/day)	2·6	3·3	0·12	0·001
Milk energy content (MJ/kg)	2·8	3·1	0·18	0·008
Milk energy output (MJ/day)	8·3	10·5	0·37	0·001
Density (g/ml)	1·026	1·028	0·0012	0·003
Milk composition (g/kg)				
Total solids	104	114	2·6	0·008
Fat	34	40	1·5	0·016
Protein	29	31	1·4	0·003
Lactose	41	44	2·0	0·050
Milk component yield (kg/day)				
Total solids	315	392	13·3	0·001
Fat	103	136	5·9	0·001
Protein	87	105	3·4	0·001
Lactose	125	151	5·1	0·002
Feed efficiency				
Milk (milk/DMI)	0·99	1·01	0·053	0·772
Energy corrected milk (ECM/DMI)	0·84	0·96	0·044	0·066

Table 4. *Milk fatty acid profile (g/100 g of total fatty acids esterified) of French Alpine dairy goats fed on the basal diet in the absence (CTRL) or presence of cellulase (CELL) enzyme (2 ml/kg DMI) during the first third of lactation (60 days) (n = 12 per treatment)*

	Diets			
	CTRL	CELL	S.E.M.	*P* value
Butyric acid (C4 : 0)	1·76	1·66	0·073	0·355
Caproic acid (C6 : 0)	2·34	2·26	0·061	0·340
Caprylic acid (C8 : 0)	2·95	2·87	0·080	0·479
Capric (C10 : 0)	11·2	10·5	0·26	0·072
Undecanoic (C11 : 0)	0·40	0·39	0·016	0·877
Lauric (C12 : 0)	5·4	5·0	0·16	0·113
Tridecanoic (C13 : 0)	0·24	0·23	0·010	0·473
Myristic (C14 : 0)	11·4	11·3	0·16	0·498
Myristoleic (C14 : 1)	0·189	0·169	0·0092	0·134
Pentadecanoic (C15 : 0)	1·11	1·10	0·037	0·781
Palmitic (C16 : 0)	28·5	27·6	0·68	0·376
Palmitoleic (C16 : 1)	1·06	1·22	0·023	<0·001
Heptadecanoic (C17 : 0)	0·97	1·01	0·031	0·432
Cis-10-heptadecanoic (C17 : 1)	0·29	0·37	0·023	0·021
Stearic (C18 : 0)	8·7	9·4	0·40	0·247
Oleic (C18 : 1)	18·9	20·5	0·57	0·073
Linoleic (C18 : 2)	2·9	2·6	0·12	0·035
Linolenic (C18 : 3)	0·53	0·44	0·028	0·023
Conjugated linoleic acids	0·68	0·76	0·050	0·231
Arachidonic acid (C20 : 4)	0·39	0·43	0·047	0·600
Short-chain fatty acids	18·3	17·3	0·39	0·097
Medium-chain fatty acids	49·6	48·5	0·64	0·234
Long-chain fatty acids	32·2	34·0	0·79	0·112
Saturated fatty acids (SFA)	75·0	73·4	0·55	0·047
Mono-saturated acids	20·5	22·2	0·58	0·043
Poly-saturated fatty acids (PSFA)	4·5	4·2	0·18	0·200
PSFA/SFA	0·060	0·057	0·0025	0·346

feed intake of dairy cows supplemented with fibrolytic enzyme.

Nutrient digestibility

One of the main aims of adding cellulolytic enzymes in ruminant diets is to improve fibre digestion (Alsersy *et al*. 2015; Salem *et al*. 2015). In the present study, greater DM (~4%), OM (~5%) and NDF (~9%) digestibility were obtained with CELL addition compared with CTRL diets. This is consistent with previous studies, which reported greater nutrient digestibility due to enzyme addition (Khattab *et al*. 2011; Salem *et al*. 2013). Although the exact mode of action of the enzyme has not been elucidated, it has been suggested that higher rate of ruminal digestion of the potentially digestible NDF fraction (Yang *et al*. 1999), changes in the site of nutrient digestion (Kung *et al*. 2000), changes in gut viscosity (Hristov *et al*. 2000), altered ruminal fermentation (Khattab *et al*. 2011), enhanced attachment and colonization to the plant cell wall by ruminal micro-organisms (Wang *et al*. 2001) and complementary actions with ruminal enzyme (Morgavi *et al*. 2000) are possible causes of greater nutrient digestibility.

However, greater fibre digestion is unlikely to be the result of supplemental enzyme activity alone because the contribution of added exogenous enzymes to total ruminal activity is relatively small (Beauchemin *et al*. 2004). Moreover, elevated numbers of non-fibrolytic and fibrolytic bacteria in rumen fluid, which elevates microbial biomass, and provides more total polysaccharidase activity to digest feedstuffs (Wang *et al*. 2001; Giraldo *et al*. 2008) has not been

explained. The Celluase® plus product used in the present study contained cellulase and beta-glucanase, which has been shown to have positive effects on digestion of NDF in TMR, although the results are inconsistent. Some studies (Gado *et al.* 2009; Khattab *et al.* 2011; Salem *et al.* 2013) reported greater nutrient digestibility, particularly fibre, with enzyme addition to the diets of dairy goats, dairy cows and feedlot cattle, but others did not (Giraldo *et al.* 2008; Dean *et al.* 2013). Dean *et al.* (2013) showed that total tract apparent digestibility of DM, NDF and CP were unaffected when the diet of dairy cows was supplemented with fibrolytic enzyme.

Ruminal fermentation

Goats fed the CELL diet showed higher ruminal pH compared with the CTRL goats. Ruminal pH is one of the most important determinants of nutrient digestion particularly fibre digestion, which is modulated via the effect it has on bacterial attachment to fibre substrates because fibrolytic bacteria are very sensitive to ruminal pH changes (Sung *et al.* 2007). Higher ruminal pH is more suitable for fibrolytic microbial activity than low ruminal pH, which changes the rumen microbial population from fibrolytic to amylolytic (Tajima *et al.* 2001). In contrast, Khattab *et al.* (2011) reported no change in ruminal pH due to enzyme addition to the diet of dairy goats.

In the present study, ruminal concentrations of propionic and acetic acids were ~12 and ~6% higher, respectively, whereas butyric acid was ~31% lower for the CELL goats compared with CTRL goats, suggesting improved ruminal fibre fermentation. Gado *et al.* (2009) and Salem *et al.* (2013) obtained higher total and individual VFA concentrations when animals were supplemented with enzymes in their diets, suggesting that enzyme addition may alter ruminal fermentation (Khattab *et al.* 2011; Salem *et al.* 2013). The shift in ruminal fermentation may be a result of altered fibre structure, which could stimulate microbial colonization (Giraldo *et al.* 2008) or a shift in the species profile of fibre-colonizing bacteria as a response to enzyme addition (Wang *et al.* 2001).

The higher concentration of propionic acid in the rumen of CELL goats may also suggest synergism between exogenous enzymes in the feed and lactic acid bacteria in the rumen (Gado *et al.* 2009). Higher propionic acid concentration could elevate precursor availability and improve nutrient utilization, particularly for dairy goats in early lactation when nutrient intake lags behind nutrient demand (Eun *et al.* 2007), possibly due to fermentation of sugars released via cell wall hydrolysis by enzymes. The results of the present study suggest that addition of cellulase enzyme in the diet made the fermentation more gluconeogenic, and thereby improved the energetic efficiency of ruminal fermentation. Moreover, the greater concentration of acetic acid in the rumen fluid of CELL goats suggests that CH_4 production was also greater, since acetic acid production is associated with the release of hydrogen, which can be used by methanogens to form CH_4 (Stewart *et al.* 1997) and results in a loss of energy to the host animal (Gado *et al.* 2009). However, calculated CH_4 production data do not support this hypothesis. Although only a few studies have investigated the effects of exogenous enzymes on CH_4 production, the results are conflicting (Togtokhbayar *et al.* 2015). Although the concentrations of individual VFA, and by extension total VFA, were greater for the CELL goats than the CTRL goats, this result did not affect ruminal pH which was also greater. The reasons for this are unclear.

Milk production and composition

Goats fed CELL diet had 13% (3·44 *v.* 3·05 kg/day) greater milk production compared with CTRL goats. The same trend was observed for ECM, which was 26% greater (3·29 *v.* 2·61 kg/day) for CELL goats compared with CTRL goats. Additionally, milk energy content and milk energy output were greater for CELL goats than CTRL goats. The greater milk production is a reflection of greater feed intake, nutrient digestibility and ruminal fermentation activities due to the feeding of enzymes (Gado *et al.* 2009; Khattab *et al.* 2011). Gado *et al.* (2009) reported 23% greater milk production in dairy cows fed a TMR supplemented with enzymes at 40 g/cow/day for 12 weeks.

In the present study, ruminal concentrations of propionic acid were greater for CELL goats than for CTRL goats. Since propionic acid is the precursor of glucose and lactose, studies suggest that increasing glucogenic precursors resulted in curvilinear elevated milk yield, linear elevated milk protein content and curvilinear decrease in milk fat content (Rigout *et al.* 2003). This is because propionic acid has greater energy available for milk production (Yang *et al.* 1999). Moreover, greater concentration of acetic acid in the ruminal fluid of CELL goats is the main reason for greater

milk fat content (Gado *et al.* 2009). The greater milk fat content for CELL goats could also be related to the greater digestibility of NDF of this diet (Kholif *et al.* 2014). One of the most surprising results was the greater milk lactose content with enzyme addition. The effect on milk lactose percentage is unusual because the content of milk lactose is typically very constant and changes only slightly (Elwakeel *et al.* 2007). Rumen acetate is the main source for milk short-chain fatty acid synthesis, thereby sparing protein and increasing milk fat and protein content (Fuller 2004). Kholif *et al.* (2014) showed that elevated milk protein content was related to greater DM, OM and fibre digestibility. In contrast, Dean *et al.* (2013) did not report any differences in milk production and component yields by enzyme supplementation of the dairy cow diet.

In the present study, addition of enzyme did not alter the concentration of many fatty acids. The milk of CELL goats had greater mono-saturated fatty acids than the milk of CTRL goats and lower saturated fatty acids than the milk of CTRL goats. Milk fatty acids originated mainly from plasma (~60%) or by the *de novo* synthesis in the mammary gland from acetate and 2-hydroxybutyrate originating as a result of rumen fermentation involving acetyl CoA carboxylase enzymes and fatty acid synthetase (Kholif *et al.* 2014). Ruminants do not synthesize polyunsaturated acids; consequently their concentration in milk depends on the amount absorbed from the intestines. These results in the present study may be due to the altered contents of acetic and propionic acid production in rumen as a result of greater fibre digestion. The direct result of shifted VFA proportions could elevate precursor availability for fatty acid synthesis, particularly during early lactation when nutrient intake lags behind nutrient demand (Eun *et al.* 2007). Gado *et al.* (2009) reported that enzyme supplemented diet showed greater ruminal acetate, propionate and butyrate proportions.

It can be concluded that under the study conditions in the tropical regions of Mexico, addition of exogenous fibrolytic cellulase at 2 ml/kg DM in the diet of lactating French Alpine goats resulted in greater feed intake and nutrient digestibility and improved milk production and composition.

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