Effect of the Addition of Exogenous Fibrolytic Enzymes and Malate on Ruminal Fermentation Characteristics and Methane Emission *In vitro*

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ABSTRACT

Ruminal inoculum enriched with particle-associated microorganisms was collected from six rumen cannulated Santa Inês wethers grazing tropical grass pasture and a supplement based on ground corn and soybean before feeding. It was then used to evaluate the effects of commercial exogenous fibolytic enzymes (Natuzyme; Alboraq®; Egypt) and malate (Rumalato; Norel-Misr®; Egypt; composed of disodium malate–calcium malate) supplementation on ruminal fermentation in short-term *in vitro* incubations using semi automatic system of gas production (GP). Exogenous fibolytic enzymes were supplemented to basal diet (Tifton hay (50%), ground yellow corn (34.5%), soybean meal (15%) and mineral mixture (0.5%) at 0, 0.3, 0.5 and 0.7g/kg DM, while malate was added at 0, 2, 4 and 6 g/kg DM for *in vitro* assays. The results showed no significant differences ($P > 0.05$) in cumulative GP for the inclusion of either exogenous fibrolytic enzymes or malate at different levels compared to the control diet. The second and third levels of exogenous fibrolytic enzyme supplementations depressed methane production ($P<0.05$) in comparison with control diet by 59 and 39% respectively, which was accompanied with reduction in protozoa count. The inclusion of exogenous fibrolytic enzymes improved the true degradation of dry and organic matter compared to the control diet but this enhancement was not significant. In regard to the partitioning factor (PF), which is considered as an index of microbial protein synthesis, exogenous fibrolytic enzymes or malate supplementation improved ($P>0.05$) the mean values of PF. Ammonia N concentrations and pH mean values did not differ ($P>0.05$) by the exogenous fibrolytic enzymes and malate supplementation compared to the control diet. Inclusiuon of exogenous fibrolytic enzymes increased ($P<0.05$) the specific activities of carboxy methylecellulase (CMCase) and α amylase, while malate supplementation decreased ($P<0.05$) specific activities of CMCase and α amylase compared to the control diet *in vitro*. It is concluded that exogenous fibrolytic enzymes could be used as favorable feed additives through enhancing degradation and declining methane emission *in vitro* which reduce livestock's contribution to climate change and global warming. No beneficial effects of malate supplementation on rumen microbial fermentation characteristics were observed.

Key words: gas production, methane, protozoa, degradation, ammonia

INTRODUCTION

Forages are the basis of ruminant diets. However, digestibility of plant cell walls in the total digestive tract is, at best, less than 0.65 (Van Soest, 1994). Improving degradation of fibrous and nonfibrous carbohydrates in the rumen is important for feed utilization in ruminants. Because forage dry matter (DM) typically contains 0.40–0.70 cell walls, attempts to improve ruminal fibre degradability have been an on-going research focus area. Increasing awareness of hazards associated with the use of antibiotic and chemical feed additives has accelerated investigation into natural feed additives. Legislators in Europe have moved to prohibit the use of growth - promoting antibiotics in animal feed from the end of 2005 (Chesson, 2004). This has prompted interest in seeking more natural approaches to feed antibiotics, such as exogenous polysaccharide-degrading enzymes, as a means of modulating rumen fermentation to improve feed intake, cell walls utilization, and animal performance. During the last few decades, exogenous fibrolytic enzymes have improved feed intake, which could be attributed to increased ruminal fibre digestion (Yang *et al.*, 1999; Kung *et al.*, 2000; Eun *et al.*, 2007; Jalilvand *et al.*, 2007; Eun and Beauchemin, 2008; Krueger *et al.*, 2008).

Furthermore, methane production from ruminants fed poor quality diets is higher than from animals fed better quality forages. The increased concentration of greenhouse gases (e.g. methane) in the troposphere has been implicated in climate change and global warming. Reducing methane emission from ruminant animals, therefore, has implications not only for efficient animal production but also for global environmental protection. Recent research is showing that supplementing livestock diets with fibre degrading enzymes can improve the efficiency of feed utilization, resulting in improved animal performance and a reduction of methane emissions (Colombatto *et al.*, 2003a). Therefore, the aims of these experiments were to investigate the inclusion of either exogenous polysaccharide-degrading enzymes or dicarboxylic acid (malate) on rumen fermentation characteristics and methane production *in vitro*.

MATERIALS AND METHODS

This study was conducted at the Centre for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil. It utilized the *in vitro*

gas production technique described by Theodorou *et al.* (1994) and adapted to the semi automatic system of Mauricio *et al.* (1999), using a pressure transducer and a data logger (Pressure Press Data 800, LANA, CENA–USP, Piracicaba, Brazil).

Description of substrate

The substrate used was a total mixed ration, which was composed of tifton hay (50%), ground yellow corn (34.5%), soybean meal (15.0%) and mineral premix (0.5 %) on DM basis. The proximate analysis of the total mixed ration was 92.4% dry matter (DM), 13.1% crude protein (CP), 2.0% ether extract (EE), 4.3% ash, 71.8% neutral detergent fiber (NDF), and 34.3% acid detergent fiber (ADF). The substrate was ground using a Wiley mill (Marconi, Piracicaba, SP, Brazil) to pass a 1-mm screen. The DM was determined by oven drying at 105°C for 24 h, and OM after ashing at 550°C for 4h (A.O.A.C., 1990). Ether extract was also determined according to AOAC (1990). The CP (N × 6.25) was determined by using a Leco FP528 (Leco Corporation, St. Joseph, MI, USA) combustion nitrogen analyzer (A.O.A.C., 1997). Concentrations of dietary NDF and ADF were ash-corrected and determined by the non-sequential method using beakers according to Van Soest *et al.* (1991) and Goering and Van Soest (1970), respectively. The NDF analysis was performed with the addition of heat stable α-amylase (Ankom Technology, Tecnoglobo Equipamentos, Curitiba, Brazil) and sodium sulfite.

Treatments

Two types of feed additives, exogenous fibrolytic enzymes (Natuzyme) and malate (Rumalato) were investigated at various doses on ruminal fermentation in short-term *in vitro* incubations using semi automatic system of gas production. Exogenous fibrolytic enzymes (Natuzyme) and malate (Rumalato) were received from Alboraq® and Norel-Misr® Companies, respectively in Egypt. Exogenous fibrolytic enzymes were supplemented to the total mixed ration at 0, 0.3, 0.5 and 0.7g/kg DM, while Malate was added at 0, 2, 4 and 6 g/kg DM for *in vitro* assays. Exogenous fibrolytic enzymes, contained 10.000 units of xylanase, 6.000 units of cellulose, 700 units of β gluconase, 700 units of α amylase, 200 units of phytase and 70 units of pectinase per gram. Rumalato is a commercial product composed of disodium malate–calcium malate.

Inoculum donors and preparation

Six adult rumen cannulated Santa Inês wethers (50 kg of BW) grazing tropical grass pasture and a supplement based on ground corn and soybean meal (0.7 kg/100 kg of live weight, 20% crude protein) plus a mineral mixture were used as inoculum donors. Three inoculums were used (two animals/each inoculum) at the same time. Both solid and liquid rumen contents (50:50v/v) were collected before morning feeding through the cannula using a stainless steel probe(2.5 mm screen) attached to a large capacity syringe. Liquids and solids were placed in pre-warmed (39°C) insulated flasks and transported under anaerobic conditions to the laboratory. Pooled rumen contents for each two animals were squeezed through four layers of cheese-cloth and kept in a water bath at 39°C with CO_2 saturation until inoculation took place.

In vitro gas production

The *in vitro* GP assay was carried out as described by Theodorou *et al.* (1994) and adapted to the semi automatic system of Mauricio *et al.* (1999), using a pressure transducer and a data logger (Pressure Press Data 800, LANA, CENA–USP, Piracicaba, Brazil) in 160ml serum bottles incubated at 39°C for 24h.

Ground substrate (0.5 g as-fed) was incubated in 75 ml of diluted rumen fluid (25 ml mixed rumen fluid +50 ml of Menke's buffered medium) in serum bottles. Once filled all the bottles were closed with rubber stoppers shaken and placed in the incubator at 39 °C. The bottles were shaken manually after the recording of the gas headspace pressure at 6, 12 and 24 h incubation using a pressure transducer (Theodorou *et al.*, 1994). The amount of GP at each measuring time was calculated according to the regression equation obtained in our system and conditions from unpublished data on 500 samples between gas volume versus pressure:

$GP\ (ml) = 0.0112\ psi^2 + 7.3358\ psi\ (r^2=0.98)$.

For each inoculum, four bottles contained only buffered rumen fluid without substrate were considered as the blank to correct the GP from the inoculum, four bottles contained Tifton hay as an internal standard, four bottles contained the substrate without additives were considered as a control diet and four bottles contained the substrate supplemented with the independent fibrolytic enzyme concentrations were investigated. The gas values were expressed as ml per g of incubated DM.

Methane emission and analyses

Ten ml from the gas samples were collected from each bottle by syringe (3.0, 3.0 and 4.0 ml at 6, 12 and 24 h incubation times respectively and accumulated in vacutainer tubes) for methane analyses. Methane determination was done in a Shimadzu 2014 gas chromatography equipped with a thermal conductivity detector. Separation was achieved using shincarbon ST micro packed column. Helium was the carrier gas with a flow rate of 10.0 ml/min. The detector and column temperatures were 250 and 60°C, respectively. The test of linearity and calibration were accomplished using the standard gas curve in the range of probable concentration of the samples. Methane production at the end of incubation period was

estimated from the volume of gas and the gas composition data as "CH_4 = [GP + HS] x Conc"; where CH_4 is the volume (ml) of methane, GP is the volume (ml) of gas produced at the end of the incubation, HS is the headspace volume (ml) of the serum bottle and Conc is the percentage of methane in the gas sample analyzed (Tavendale *et al.*, 2005).

Degradation partitioning factor, ammonia-N and protozoa count

After termination of the incubation at 24 h, two bottle contents were used for determination of true degredation of dry and organic matter (TDDM, TDOM) and partitioning factor (PF) as an index of microbial protein efficiency. The bottles contents were quantitatively transferred into a 600 ml spout-less beaker with a total of 70 ml of ND solution (double strength, Blummel and Becker 1997) and refluxed for 3 h at 105°C. Residual DM and ash were determined. The partitioning factor (PF) is the ratio between mg of organic matter truly degraded and gas volume (ml) at 24 h incubation (Blummel and Becker, 1997; Blummel *et al.*, 1997). Another two bottle contents were used for measuring the pH, NH_3-N concentration, protozoa counting and enzyme assays. The NH_3-N concentration was measured according to Preston (1995). Protozoa were counted microscopically following the procedure described by Kamra *et al.* (1991). Carboxy methyl celllulase (CMCase) and α amylase activities were estimated according to Somogyi method (1960) using glucose as standard. Activity (one unit of enzyme activity) were defined as the amount of enzyme that produced 1 nmol glucose equivalent of reducing sugar per minute. A further aliquot of thawed sample was analyzed for soluble protein according to Bradford method (1976).

Statistical analysis

Data were subjected to analysis of variance (ANOVA), using the General Linear Model procedure of the SAS software package (2002). The used model was: $Y = \mu + Fi + e$, where μ is overall mean, Fi the plant effect, e is the error. Experimental units were run and replicates in the same run were considered as repetitions. The significant differences between individual means were identified using Tukey test (SAS, 2002).

RESULTS AND DISCUSSION

The effects of inclusion of different levels (0, 0.3, 0.5 and 0.7 g/kg DM of exogenous fibrolytic enzymes or 0, 2, 4 and 6 g/kg DM of malate on gas and methane production *in vitro* for 24h incubation are presented in Table 1. The results showed that there were no significant differences ($P > 0.05$) in cumulative GP for the inclusion of either exogenous fibrolytic enzymes or malate at different levels compared to control diet.The second and third levels of exogenous fibrolytic enzyme supplementations depressed methane production ($P<0.05$) in comparison with control diet by 59 and 39%, respectively. On the other hand, all the levels of malate supplementation and the first level from exogenous fibrolytic enzymes did not show significant effect on methane production in comparison to control diet either expressed on methane volume per g DM or g truly digested organic matter.

The effects of inclusion of different levels from Exogenous fibrolytic enzymes or malate on true dry and organic matter degradation, partitioning factor (PF), protozoa count and NH_3-N concentration are given in Table 2. The inclusion of exogenous fibrolytic enzymes has improved the true degradation of dry and organic matter (TDDM, TDOM g/kg) compared to the control diet but this enhancement was not significant. Also, malate supplementation did not affect ($P>0.05$) the TDDM or TDOM compared to the control diet. In regard of PF which is considered as an index of microbial protein synthesis, exogenous fibrolytic enzymes or malate supplementation improved ($P>0.05$) the mean values of PF.

Table 1: Effect of different levels of exogenous fibrolytic enzymes or malate on gas and methane production for 24 h incubation *in vitro*.

	Level	GP (ml/g DM)	CH_4 (ml/g DM)	CH_4 (ml/g TDOM)
Control diet	0	149.4	18.6^{ab}	26.4^{ab}
Exogenous fibrolytic enzymes	0.3 g	141.5	15.3^{b}	24.3^{b}
	0.5 g	140.5	7.6^{d}	10.5^{c}
	0.7 g	143.3	11.4^{c}	15.8^{c}
Malate	2.0 g	145.6	20.0^{a}	29.8^{ab}
	4.0 g	140.7	20.5^{a}	29.5^{ab}
	6.0 g	139.4	21.0^{a}	30.3^{a}
SEM	-	7.9	1.11	1.65

a,b,c;d within columns, means with different superscripts differ significantly (Tukey test; $P<0.05$). GP: gas production, DM: dry matter, TDOM: True degradation of organic matter. SEM: standard error of difference between means.

Table 2: Effect of different levels of exogenous fibrolytic enzymes or malate on true degradation of dry and organic matter and rumen partitioning factor (PF) *in vitro*.

	Level	TDDM	TDOM	PF
Control diet	0	689	706	4.27^{ab}
Exogenous	0.3 g	700	714	4.33^{ab}
fibrolytic	0.5 g	711	726	4.80^{a}
enzymes	0.7 g	704	719	4.48^{ab}
	2.0 g	656	675	4.13^{b}
Malate	4.0 g	685	700	4.50^{ab}
	6.0 g	682	693	4.43^{ab}
SEM	-	12.7	11.8	0.15

a,b within columns, means with different superscripts differ significantly (Tukey test; P<0.05).
TDDM: True degradation of dry matter (g/kg DM), TDOM: True degradation of organic matter (g/kg DM), PF: partitioning factor (mg of truly digested organic matter /ml gas at 24 h), SEM: standard error of difference between means.

The protozoa count decreased significantly ($P< 0.05$) with the supplementation of the third dose of exogenous fibrolytic enzymes, while malate did not affect ($P>0.05$) the protozoa compared to the control diet. NH_3-N concentrations and mean values of pH did not differ ($P>0.05$) due to the exogenous fibrolytic enzymes and malate supplementation compared to the control diet

Data in Table 3 presented the effect of different levels of exogenous fibrolytic enzyme (exogenous fibrolytic enzymes) or malate supplementation on rumen pH, carboxy methyl cellulase (CMCase), and α amylase activities (nmole/min/mg protein) *in vitro*. No significant effects of exogenous fibrolytic enzymes or malate supplementation were observed on the mean values of pH compared to the control diet. Inclusiuon of exogenous fibrolytic enzymes increased ($P<0.05$) the specific activities of CMCase and α amylase compared to the control diet, while malate supplementation decreased ($P<0.05$) specific activities of CMCase and α amylase compared to the control diet in the supernatant of sonicated bottle contents *in vitro*.

Enzymes in feed have been used to improve utilization of a wide range of livestock diets (Beauchemin *et al.*, 2003). The use of fibrolytic enzymes as feed additives to improve degradation of fibre has been studied under *in vitro*, *in sacco* and *in vivo* conditions, but the responses have been highly variable. Their effectiveness is partly due to improved hydrolysis of fiber (Colombatto *et al.*, 2003b), which often increases digestibility (Rode *et al.*, 1999) and voluntary intake (Pinos-Rodriguez *et al.*, 2002). Several factors such as enzyme doses (Colombatto *et al.*, 2007) and type of diet (Pinos-Rodriguez *et al.*, 2008) could affect the fibrolytic activity of exogenous enzymes (Beauchemin *et al.*, 2003). Indeed, fibrolytic enzymes increased degradation of substrates, but it depends on proportion of concentrate in the diet (Giraldo *et al.*, 2008) and enzyme doses (Jalilvand *et al.*, 2008). Besides, the optimal level of the enzyme could depend on the diet, indicating the need to determine the optimum application rate of enzyme preparation for individual feeds (Yang *et al.*, 1999). Proposed modes of action include solublization of dietary fiber before entering the rumen, provision of readily fermentable substrate for ruminal microorganisms, and/or enhancement of microbial enzymatic activities in the rumen (McAllister *et al.*, 2001).

Table 3: Effect of different levels of exogenous fibrolytic enzymes or malate on pH, NH_3-N, protozoa count and enzymes activity (nmole/min/mg protein)

Levels	Level	pH	NH_3-N	Protozoa	CMCase	α amylase
Control	0	6.76	263	3.68^{ab}	49.4	54.2
	0.3 g	6.74	261	3.43^{ab}	62.1^{a}	66.7
Exogenous fibrolytic enzymes	0.5 g	6.70	268	3.95^{a}	66.1^{a}	73.9
	0.7 g	6.74	259	3.08^{b}	59.9^{ab}	64.7
	2.0 g	6.73	275	3.30^{ab}	50.2	53.2
Malate	4.0 g	6.71	264	3.78^{a}	33.1	35.3
	6.0 g	6.79	268	3.63^{ab}	36.1	41.5
SEM[1]		0.38	2.7	0.17	4.85	4.92

a,b within columns, means with different superscripts differ significantly (Tukey test; P<0.05).
NH_3-N: Ammonia nitrogen concentration (mg/l), Protozoa count ($x10^5$ ml^{-1}), SEM: standard error of difference between means.

The investigated fibrolytic enzymes by different levels at the current study did not affect total GP. This finding is in agreement with those of Colombatto *et al.* (2003b) and Jalilvand *et al.* (2008) who evaluated two levels of two enzyme products on GP and concluded that final GP values of forages were not increased by enzyme addition. The lack of effects on final GP suggests that the substrates degraded by the enzymes, would have been degraded in the medium anyway, albeit at a later time and increment in lag phase (Colombatto *et al.*, 2007). In contrast, Colombatto *et al.* (2003b) reported positive responses to the level of added enzyme on the rate of GP. Exogenous fibrolytic enzymes have been shown to increase both *in vitro* and *in situ* fibre degradation of forages (Pinos-Rodriguez *et al.*, 2002), although results are not consistent (Elwakeel *et al.*, 2007) as noted in the current experiment because responses to enzyme addition can be nonlinear (Kung *et al.*, 2000).

Colombatto *et al.* (2003b) investigated 22 commercial products of fibrolytic enzymes using two types of forages (alfalfa hay and corn silage) *in vitro*. They concluded that the most effective enzymes against alfalfa hay were not the most effective ones against corn silage, suggesting a strong enzyme-feed specificity. Enzyme-feed specificity is a well-known phenomenon and is believed to be one of the factors contributing to the observed inconsistencies in enzyme research (McAllister *et al.*, 2001). The non-significant effect of enzyme supplementation on degradation at the current study may be attributed mainly to the thick secondary cell walls in tifton hay which are slowly degraded. Therefore, it is likely that active degradation was still under way during the 30 to 48 h incubation period. In agreement with our finding, Gallardo *et al.* (2010) reported that the potentially degradable and soluble fractions and kinetics of *in vitro* degradation for DM, NDF and ADF of corn silage, corn stover, elephant grass, Guinea grass and oat straw were not affected by enzyme treatments with the exception of alfalfa hay. Therefore, chemical composition of forages could influence enzyme efficiency. Indeed, differences in chemical composition of forages may be due to differences of forage species, stage of maturity at harvest, soil type, fertilization level, season, and weather conditions (Van Straalen and Tamminga, 1990).

The results of the current study showed that exogenous fibrolytic enzymes supplementation decreased methane emission, which is in contrast with the results of Dong *et al.* (1999) who found an increase in methane production as a result of addition of exogenous enzyme. In agreement with our finding, Carro *et al.* (1999) concluded that total methane production was not reduced by the addition of malate when compared with the control diet (Table 2). In contrast, Carro and Ranilla (2003) reported that malic acid supplementation decreased methane production *in vitro* through stimulating succinate and/or propionate production by *Selenomonas ruminantium*, thereby decreasing the availability of H_2 to methanogenic bacteria (Castillo *et al.*, 2004). The higher methane production associated with greater fermentation of the diet when adding malate might have compensated the reduction of methane production due to the competition for available H_2 between malate reduction and methanogenesis. These contrasting results could be due to differences in the composition of the diet and/or to the dose of malate.

In agreement with Dean *et al.* (2008) and Avellaneda *et al.* (2009) who did not find positive effects of fibrolytic enzymes on degradation of high fibre grasses, our study showed that the fibrolytic enzyme did not impact the degradation of tifton hay evaluated in TMR. The lack of effects of the fibrolytic enzyme on high fibre forages could also resulted from enzymatic activity not the lower investigated doses because the responses to enzyme addition could be not linear (Kung *et al.*, 2000); therefore, high levels of addition can be less effective than low levels (Jalilvand *et al.*, 2008). It has been speculated that an excess of enzymes in the diet may bind to sites used by rumen bacteria and make them unavailable, creating a barrier against microbial colonization (Beauchemin *et al.*, 2003). However, some exogenous fibrolytic enzymes increase cell wall digestibility *in vitro* (Colombatto *et al.*, 2003b) or *in vivo* (Schingoethe *et al.*, 1999), but not all products are effective (Vicini *et al.*, 2003).

In consistent with our findings, Giraldo *et al.* (2008) noted no effects of fibrolytic enzyme treatments on ruminal pH, NH_3-N and total VFA concentration. Several *in vivo* (Pinos-Rodriguez *et al.*, 2002; Beauchemin *et al.*, 2003) and *in vitro* (Giraldo *et al.*, 2007) studies have shown that treating different feeds with fibrolytic enzymes produced a shift in the molar proportions of VFA, but changes in rumen fermentation pattern seem to be affected by the characteristics of the diet fed to the animals and by the type of supplemented enzyme (Beauchemin *et al.*, 2003). In agreement with these results, Yang *et al.* (1999) and Giraldo *et al.* (2008) showed no effects of fibroyltic enzymes supplementation on microbial protein synthesis.

There was no significant effect ($P>0.05$) by inclusion of malate on the average pH (Table 3). Castillo *et al.* (2004) suggested that malic acid supplementation could prevent a decrease in ruminal pH and acidosis by stimulating lactic acid uptake by *Selenomonas ruminantium*. Diet composition in the current study may explain the lack of effect of malic acid supplementation on total VFA production and pH.Positive responses to malic acid supplementation on ruminal fermentation and pH have been noted

with relatively high-grain diets. On a lactating cow diet with a 50:50 forage to concentrate ratio (Kung *et al.*, 1982), ruminal VFA concentrations and pH were not affected by malic acid supplementation. In agreement with our finding, Sniffen *et al.* (2006) reported that malic acid supplementation had no ($P>0.05$) effect on NH_3-N concentration.

Most experiments conducted on the effects of malate on rumen fermentation have been carried out *in vitro*. In most of these studies (Callaway and Martin, 1996; Carro *et al.* 1999), malate treatment resulted in changes in final pH, CH_4 and volatile fatty acid concentrations that are analogous to the effects of ionophores. However, the mode of action of malate appears to be completely different and, in contrast with antimicrobial compounds, it seems to stimulate rather than inhibit some specific rumen bacterial populations (Nisbet and Martin, 1993). Malate is a key intermediate in the production of succinate or propionate in some rumen bacteria and therefore could stimulate propionate production. In fact, propionate production was increased by adding malate to *in vitro* cultures (Callaway and Martin 1996) or semi-continuous fermenters (Carro *et al.*, 1999).

Enhanced fibrolytic activities in rumen fluid produced by the treatment of feed with exogenous fibrolytic enzymes as observed at the current study have been reported *in vitro* (Giraldo *et al* ., 2007a) and *in vivo* (Morgavi *et al.*, 2000). Giraldo *et al.*, (2008) reported that compared with control sheep, enzymatic activity of endoglucanase and xylanase in fibrolytic enzymes-supplemented animals was increased. This increment in the enzyme activity was not exclusively due to a direct effect of the exogenous enzyme and that exogenous enzyme supplementation stimulated fibrolytic activity of ruminal fluid. Morgavi *et al.* (2000) demonstrated synergism between exogenous enzymes and those produced by rumen microorganisms such that the net combined hydrolytic effect in the rumen was much greater than that estimated from the individual activities.

Malate supplementation in ruminant animal diets has been shown to improve average daily gain and feed efficiency in steers (Martin *et al.* 1999) and to increase milk persistency and feed efficiency in dairy cows (Kung *et al.*, 1982). In contrast, no effects of malate on rumen digestion and rumen microbial efficiency were found by Montano *et al.* (1999) in steers fed a diet containing 770 g steam-flaked barley, 100 g hay, 60 g cane molasses and 40 g yellow grease (fats and oils from cooking)/kg. In addition, Kung *et al.* (1982) reported no effect of malate on diet digestibility and N retention in steers fed a diet based on whole-shelled maize–maize silage (50:50, w/w) *ad libitum*. These contrasting results could be due to differences in the composition of the diet and/or to the dose of malate fed to animals.

CONCLUSION

This study suggested that the commercial fibrolytic enzyme product (Natozyme) supplementation improved dry and organic matter degradation accompanied with mitigation of methane production *in vitro*. It follows that this enzyme product has significant potential to be included as a feed additive for ruminants and reducing livestock's contribution to climate change and global warming. Further animal trials are required to test the effect of supplementation of fibrolytic enzymes on feed intake, fermentation pattern and animal performance.

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الملخص العربي

تأثير إضافة الإنزيمات المحللة للألياف والمالات علي خصائص تخمرات الكرش الميكروبية وإنتاج الميثان معملياً

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أجريت الدراسة بهدف اختبار تأثير إضافة مستويات مختلفة من الإنزيمات الخارجية المحللة للألياف أو المالات علي خصائص تخمرات الكرش الميكروبية وإنتاج الميثان معملياً باستخدام تقنية إنتاج الغاز شبه الآلي معملياً لمدة ٢٤ ساعة. تم إضافة مستويات صفر، ٠,٣، ٠,٥ ، ٠,٧ جم من الإنزيمات المحللة للألياف لكل كجم مادة جافة ومستويات صفر، ٢، ٤ ، ٦ جم من المالات لكل كجم مادة جافة إلي العليقة الأساسية المكونة من ٥٠% علف مالئ (دريس التيفتون) و ٥٠% علف مركز. أوضحت النتائج أن إنتاج الغاز لم يتأثر معنوياً عند إضافة المستويات المختلفة من الإنزيمات المحللة للألياف أو المالات مقارنة بالكنترول. إضافة الإنزيمات المحللة للألياف بمعدل ٠,٥ أو ٠,٧ جم أدي لحدوث انخفاض معنوي في إنتاج الميثان بنسب ٥٩ و٣٩% علي الترتيب مقارنة بالكنترول. انخفاض إنتاج الميثان كان متصاحباً مع انخفاض في عدد البروتوزوا. علي الرغم من تحسن الهضم الحقيقي للمادة الجافة والعضوية عند إضافة الإنزيمات المحللة للألياف فإن هذا التحسن لم يكن معنوياً. وقد تم تقدير الـ partitioning factor (PF) وهو العلاقة مابين المادة العضوية المهضومة حقيقيا وحجم إنتاج الغاز كمقياس لكفاءة البروتين الميكروبي معملياً، وأظهرت النتائج حدوث زيادة معنوية في قيم الـ PF سواء بإضافة الإنزيمات المحللة للألياف أو المالات مقارنة بالكنترول. لم تؤثر معنويا إضافة الإنزيمات المحللة للألياف أو المالات علي قيم الـ pH أو تركيز الأمونيا مقارنة بالكنترول. أحدثت إضافة الإنزيمات المحللة للألياف زيادة معنوية في نشاط إنزيمات الأميلاز والكربوكسي ميثيل سيليولاز ولكن إضافة المالات خفضت من نشاط هذه الإنزيمات مقارنة بالكنترول.

ويستخلص من هذه الدراسة أن الإضافة الخارجية للإنزيمات المحللة للألياف من الإضافات الغذائية الجيدة لتحسينها لدرجة تحلل المادة الجافة والعضوية وخفض إنتاج الميثان الذي يقلل من نسبة مساهمة الحيوانات المزرعية في مشكلة التغيرات المناخية والإحتباس الحراري، وأن إضافة المالات لم يؤثر إيجابيا علي تخمرات الكرش الميكروبية معملياً.