Livestock Science 141 (2011) 148–154

Contents lists available at ScienceDirect

Livestock Science

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

Effect of coconut oil and mangosteen peel supplementation on ruminal fermentation, microbial population, and microbial protein synthesis in swamp buffaloes

R. Pilajun, M. Wanapat *

Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

ARTICLE INFO

Article history:
Received 20 March 2011
Received in revised form 18 May 2011
Accepted 23 May 2011

Keywords:
Coconut oil
Mangosteen peel
Rumen fermentation
Microbial population
Swamp buffalo
Rice straw

ABSTRACT

Four rumen fistulated swamp buffalo bulls were randomly assigned to receive dietary treatments according to a 4×4 Latin square design. The four dietary treatments were unsupplementation (control), supplementation with coconut oil (CO) at 50 g/kg, supplementation with mangosteen peel (MP) at 30 g/kg, and supplementation with CO at 50 g/kg and MP at 30 g/kg (COM), of total dry matter intake. Animals received concentrate at 10 g/kg BW, together with a basal diet of *ad libitum* rice straw. Feed intake, digestibility of dry matter, organic matter and crude protein were not significantly affected; however, MP supplementation resulted in significantly lower overall NDF and ADF digestibility when compared with COM supplementation. Ruminal pH and BUN concentrations were not significantly different between treatments, while supplementation with CO increased concentration of ruminal NH_3–N. Total volatile fatty acid concentrations were increased with MP supplementation. The proportion of ruminal acetic acid was decreased while propionic acid was increased by either CO or COM supplementation. The ruminal protozoal population was dramatically decreased with either CO or MP supplementation; whereas, COM treatment had a lower fungal zoospores population when compared with the control group. Supplementation with MP resulted in a lower population of cellulolytic bacteria than supplementation with COM. In addition, CO supplementation resulted in a higher population of proteolytic bacteria than the control or supplementation with COM. Nitrogen balance and microbial protein synthesis were not affected by CO or MP supplementation. In conclusion, CO or MP supplementation could improve rumen fermentation by positively affecting the ruminal on microbial population in swamp buffalo on rice straw diets.

1. Introduction

Manipulation of the rumen microbial ecosystem to enhance fibrous feed digestibility, reduce methane emission and reduce nitrogen excretion by ruminants such as to improve their performance are some of the most important goals for animal nutritionists (Patra et al., 2006). Dietary lipids are potent modifiers of ruminal fermentation and may offer a nutritional strategy to reduce protozoal predation and intraruminal recycling of bacterial protein, thus improving the efficiency of dietary protein utilization and mitigating N losses in the ruminant animals (Hristov and Jouany, 2005). Moreover, medium chain fatty acid such as lauric and myristic acids, which are present in high concentrations in coconut oil, are known to modify ruminal fermentation (Henderson, 1973) and to mitigate greenhouse gas emissions (Machmüller, 2006). However, feeding an animal with vegetable fat blend will not only inhibit methanogenesis but could also inhibit fiber-degrading Gram-positive bacteria in the rumen (Palmquist, 1994); and accordingly depress fiber degradation (Beauchemin and McGinn, 2005). Plant secondary compounds such as

* Corresponding author. Tel./fax: +66 43 202368.
E-mail address: metha@kku.ac.th (M. Wanapat).

1871-1413/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.livsci.2011.05.013

condensed tannins and saponins have been shown to inhibit protozoa and methanogens presumably by lowering methanogenic activity of protozoal-associated methanogens (Guo et al., 2008). However manipulation of ruminal fermentation of swamp buffalo by the use of lipids and/or tropical plants is still limited.

Our previous study (Pilajun and Wanapat, unpublished) illustrated that supplementation with coconut oil (CO) or mangosteen peel (MP) (*Garcinia mangostana*), a tropical evergreen tree, could improve *in vitro* fermentation using rumen liquor of swamp buffalo. They were shown to reduce methane production and alter microbial populations, although the methanogen population was not affected. The aim of the present study was to investigate microbial population and diversity in the rumen of swamp buffalo fed on rice straw as influenced by supplementation with CO and MP.

2. Materials and methods

2.1. Animals and feeds

Four, 4-year old rumen fistulated swamp buffalo bulls with a body weight of 420 ± 15 kg were randomly assigned to receive dietary treatments according to a 4×4 Latin square design with 4 dietary treatments. The four treatments were unsupplementation (control), supplementation with CO at 50 g/kg, supplementation with MP at 30 g/kg, and supplementation with CO at 50 g/kg and MP at 30 g/kg (COM), of total dry matter intake. Coconut oil contained 403 ± 3 g/kg DM of lauric acid and 161 ± 2 g/kg DM of myristic acid, while mangosteen peel powder contained 146 ± 2 g/kg DM of condensed tannins and 95 ± 2 g/kg DM of crude saponins. Feed ingredients and chemical composition are presented in Table 1. The experiment was conducted for 4 periods, and each period lasted 21 days. All animals were fed with control diet for adaptation during the first 7 days, which should be sufficient for microbial adjustment due to rumen retention time not to exceed 48 h (Church, 1969). Then, the animals were fed with respective dietary treatments during the last 14 days. All buffaloes were supplemented with their respective treatment concentrates at 10 g/kg BW divided between two daily feeds (07.00 and 16.00) with rice straw given *ad libitum*. Mineral blocks and water were available *ad libitum* for all animals which were housed in individual pens.

Table 1
Feed ingredients and chemical composition.

	Concentrate	Rice straw	Mangosteen peel
Feed ingredient, g/kg DM			
Cassava chip	650		
Rice bran	100		
Palm kernel meal	202		
Urea	15		
Molasses	15		
Salt	5		
Sulfur	3		
Mineral	10		
Chemical composition, g/kg DM			
Organic matter	941	883	976
Crude protein	142	24	207
Ether extract	34	6	15
Neutral detergent fiber	160	840	562
Acid detergent fiber	84	604	509
Condensed tannin	–	–	146
Saponins	–	–	95

2.2. Samples and analysis

Measurements of feed intake and samples of feeds, refusals, feces, rumen fluid, and blood were made during the 21 days of each period. Rumen fluid and blood samples were collected at 0, 2, 4 and 6 h-post feeding while urine and feces from total collection of each individual buffalo were made during the last 7 days of each period. The samples were stored at −20 °C before analysis, while feces, collected daily in each period, were bulked, mixed and a 50 g/kg sub-sample taken for later chemical analysis.

Feed, orts, and feces were collected daily, and a 10% sample was composited per buffalo and period and stored (−20 °C) until analysis. Samples were dried in a forced-air oven at 60 °C for 96 h, ground through a 1-mm stainless steel screen (Cyclotec 1093 Sample mill, Tecator, Hoganas, Sweden), and analyzed for DM (967.03) and OM (942.05) according to the AOAC (1995). The CP (984.13) content was determined by using a Kjeltec Auto 1030 Analyzer (Tecator). The method of Van Soest et al. (1991) was used to determine NDF (amylase added) and ADF on an ash-free basis using an Ankom Fiber Analyzer incubator (Ankom Technology, Fairport, NY). Rumen fluid was immediately measured for pH using a portable pH temperature meter (HANNA, instruments HI 8424 microcomputer, Singapore) and NH_3–N by Kjeltech Auto 1030 Analyzer (Bremmer and Keeney, 1965). Volatile fatty acids were analyzed using High Pressure Liquid Chromatography (instruments by controller water model 600E; water model 484 UV detector; column novapak C18; column size 3.9 mm×300 mm; mobile phase 10 mM H_2PO_4 [pH 2.5]) according to Samuel et al. (1997). Rumen fluid was collected for direct count of protozoa and fungal zoospores using the methods of Galyean (1989) by a hemacytometer (Boeco, Singapore) and bacteria groups (total viable, cellulolytic, proteolytic and amylolytic) were measured using the roll-tube technique of Hungate (1969).

Total urine excretion was conducted and acidified using 10 mL of H_2SO_4 solution (2 M). Urine samples were analyzed for allantoin concentration by high-performance liquid chromatography as described by Chen et al. (1993). Microbial purine derivative absorption was calculated by the equation of Liang et al. (1994).

$$Y = 0.12X + \left(0.20BW^{0.75}\right)$$

The supply of microbial N (MN) was estimated by urinary excretion of purine derivatives (PD) according to the predictive equation of Chen and Gomes (1995):

$$MN(g/d) = 70X/(0.116 \times 0.83 \times 1000) = 0.727X$$

where X and Y are, respectively, absorption and excretion of PD in mmol/d. Efficiency of microbial N supply (EMNS) was calculated using the following formula:

$$EMNS = \text{microbial N}(g/d)/\text{DOMR}$$

where DOMR = apparently digested OM in the rumen.

2.3. Statistical analysis

All data were statistically analyzed according to a 4×4 Latin square design using the ANOVA procedure of SAS (1996). Data were analyzed using the model $Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$ where Y_{ijk} is the observation from animal j, receiving diet i, in period k; μ, the overall mean; M_i, effect of treatment (i = 1 to 4); A_j, the effect of animal (j = 1 to 4); P_k, the effect of period (k = 1 to 4); and ε_{ijk}, the residual effect. Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Differences between means with $P<0.05$ were accepted as representing statistically significant differences.

3. Results

Feed intake and nutrient digestibility as influenced by CO and/or MP supplementation are presented in Table 2. Total feed intake, intake of rice straw, digestibility of dry matter, organic matter, and crude protein were not affected by feed supplementation ($P>0.05$); however, fiber digestibility (NDF, ADF) was changed following supplementation ($P<0.05$). Mangosteen peel supplementation resulted in lower NDF and ADF digestibility when compared with the COM treatment ($P<0.05$), though not different from the control group ($P>0.05$).

Table 3 shows the effects of CO and/or MP supplementation on blood urea nitrogen (BUN) and ruminal fermentation in swamp buffalo in which they were not significantly different among treatments at any times of sampling ($P>0.05$), except for NH_3–N concentration ($P<0.05$). Supplementation with CO increased concentration of ruminal NH_3–N ($P<0.05$), while MP and COM supplementation had no effects ($P>0.05$). Supplementation with MP resulted in a higher total volatile fatty acid concentration when compared with CO or COM supplementation ($P<0.05$) while not significantly different from the control group ($P>0.05$). There were variations in the proportions of acetic acid and propionic acid ($P<0.05$) in which acetic acid was decreased while propionic acid was increased by CO supplementation ($P<0.05$). In addition, COM supplementation also changed the proportions of acetic acid and propionic acid in the same direction as with CO supplementation ($P<0.05$). Butyric acid was not influenced by any of the dietary supplements ($P>0.05$).

The protozoal population was dramatically decreased with either CO or MP supplementation ($P<0.05$); however, supplementation with COM did not have a synergist inhibitory effect. Supplementation with CO or COM resulted in a lower zoospores population when compared with the control treatment ($P<0.05$) but was not significantly different when compared with MP supplementation ($P>0.05$). Populations of total viable bacteria and amylolytic bacteria were not affected by either CO or MP supplementation ($P>0.05$). Supplementation with MP resulted in a lower population of cellulolytic bacteria compared to supplementation with COM ($P<0.05$), but it was not different to the control and CO supplementation treatments ($P>0.05$). In contrast, CO supplementation resulted in a higher population of proteolytic bacteria compared to both the control and COM treatments ($P<0.05$) and was similar to the MP supplementation treatment ($P>0.05$, Table 4).

Nitrogen excretion via urine was lower in buffalo supplemented with MP when compared with CO ($P<0.05$), but it was not significantly different from the control group ($P>0.05$). Nitrogen absorption and retention were not significantly different between treatments ($P>0.05$). Microbial protein synthesis in terms of quantity and efficiency were not affected by either CO or MP supplementation ($P>0.05$, Table 5).

Table 2
Effect of coconut oil and mangosteen peel supplementation on voluntary feed intake and nutrient digestibility.

Items	Control	CO[1]	MP	COM	SEM	P-value
Total DM intake						
kg/d	5.11	5.22	5.33	5.19	0.06	0.186
g/kg BW	1.57	1.57	1.59	1.55	0.01	0.358
g/kg $BW^{0.75}$	66.5	67.1	68.1	66.3	0.67	0.307
Rice straw intake						
kg/d	1.85	1.89	1.98	1.85	0.05	0.302
g/kg BW	0.57	0.57	0.59	0.55	0.01	0.358
g/kg $BW^{0.75}$	24.0	24.3	25.4	23.51	0.66	0.329
Apparent digestibility, %						
Dry matter	62.8	60.8	59.7	61.8	3.71	0.536
Organic matter	62.4	61.1	58.9	60.8	4.19	0.549
Crude protein	61.7	69.3	61.7	66.4	3.58	0.424
Neutral detergent fiber	44.6[ab]	44.9[ab]	42.6[b]	52.7[a]	3.95	0.041
Acid detergent fiber	44.0[ab]	42.0[ab]	41.0[b]	50.7[a]	3.45	0.047

[ab] Values on the same row with different superscripts differed ($P<0.05$).
[1] CO = coconut oil 50 g/kg, MP = mangosteen peel 30 g/kg, COM = coconut oil 50 g/kg + mangosteen peel 30 g/kg.
SEM = standard error of the means.

Table 3
Effect of coconut oil and mangosteen peel supplementation on blood urea nitrogen and rumen fermentation.

Items	Control	CO[1]	MP	COM	SEM	P-value
Blood urea nitrogen, mg/dL	8.6	8.9	8.5	8.9	0.23	0.318
Ruminal pH,	6.5	6.5	6.5	6.4	0.03	0.286
Ruminal temperature, °C	38.3	38.4	38.4	38.3	0.16	0.966
NH_3–N, mg/dL	6.0[b]	11.5[a]	7.0[b]	5.7[b]	1.06	0.027
Total VFA, mmol/L	88.5[ab]	83.3[b]	91.9[a]	82.0[b]	2.34	0.039
mol/100 mol total VFA						
Acetic acid	68.4[a]	63.5[b]	68.5[a]	63.6[a]	1.69	0.045
Propionic acid	18.6[b]	24.0[a]	19.2[b]	25.8[a]	2.28	0.028
Butyric acid	13.0	12.4	12.2	10.6	0.76	0.225

[ab] Values on the same row with different superscripts differed ($P<0.05$).
[1] CO = coconut oil 50 g/kg, MP = mangosteen peel 30 g/kg, COM = coconut oil 50 g/kg + mangosteen peel 30 g/kg.

4. Discussion

Although feed intake was not affected by feed supplementation, fiber digestion was changed by either MP or COM supplementation. Palmquist (1994) proposed that fiber digestion will be restricted when ruminants receive diets with a fat content higher than 70 g/kg DM intake, a level which is higher than in the present study (~60 g/kg DM intake). However in a previous study (Pilajun and Wanapat, unpublished) it was found that DM and OM disappearances were reduced with CO addition. The depression following oil supplementation can most probably be attributed to the negative effect of CO on the extent of NDF digestion (Dohme et al., 1999; Machmüller et al., 2003). It has been expected as coconut oil is toxic to certain ruminal bacteria. The medium-chain fatty acids are small

Table 4
Effect of coconut oil and mangosteen peel supplementation on volatile fatty microbial population (log quantity).

	Control	CO	MP	COM	SEM	P-value
Direct count technique						
Protozoa, $\times 10^5$ cell/mL						
0 h-post feeding	4.4[a]	3.2[b]	3.5[ab]	3.0[b]	0.37	0.035
4 h-post feeding	4.7[a]	2.9[b]	3.3[b]	2.7[b]	0.41	0.021
Mean	4.7[a]	3.0[b]	3.3[b]	2.8[b]	0.35	0.027
Fungal zoospore, $\times 10^6$ cell/mL						
0 h-post feeding	6.3	6.0	6.2	6.0	0.40	0.098
4 h-post feeding	7.0[a]	5.7[b]	6.0[ab]	5.5[b]	0.38	0.042
Mean	6.7	5.9	6.0	5.8	0.34	0129
Roll-tube technique						
Total viable bacteria, $\times 10^{10}$ CFU/mL						
0 h-post feeding	9.0	8.7	8.5	9.2	0.37	0.293
4 h-post feeding	9.4	8.5	8.5	9.4	0.43	0.376
Mean	9.2	8.6	8.5	9.3	0.39	0.391
Cellulolytic bacteria, $\times 10^{10}$ CFU/mL						
0 h-post feeding	5.4	5.2	4.7	6.0	0.44	0.087
4 h-post feeding	5.5[ab]	4.9[ab]	4.5[b]	6.3[a]	0.54	0.038
Mean	5.5[ab]	5.1[ab]	4.6[b]	6.2[a]	0.47	0.046
Proteolytic bacteria, $\times 10^8$ CFU/mL						
0 h-post feeding	7.5	8.5	8.0	7.2	0.44	0.107
4 h-post feeding	7.0[b]	8.8[a]	7.8[ab]	6.7[b]	0.35	0.041
Mean	7.3[b]	8.7[a]	7.9[ab]	7.0[b]	0.38	0.044
Amylolytic bacteria, $\times 10^8$ CFU/mL						
0 h-post feeding	8.1	8.5	8.0	8.7	0.46	0.318
4 h-post feeding	8.4	8.9	8.2	9.0	0.42	0.273
Mean	8.3	8.7	8.1	8.9	0.40	0.300

[ab] Values on the same row with different superscripts differed ($P<0.05$).
[1]CO = coconut oil 50 g/kg, MP = mangosteen peel 30 g/kg, COM = coconut oil 50 g/kg + mangosteen peel 30 g/kg.
SEM = standard error of the means.

enough to be readily dissolved in the lipid phase, to penetrate and physically disrupt cell membranes, and to inhibit enzymes involved in energy production and nutrient transfer, leading to reversible and irreversible changes that may lead to the death of the cell (Machmüller, 2006). Moreover, dietary fat influences digestion of fiber in the rumen by coating both the plant fibrous material and the surface of the microbes, hence preventing the rumen microbes from degradation of feeds (Oldick and Firkins, 2000). Beauchemin et al. (2007) also found that adding 20 g/kg quebracho tannin extract to the diet had no effect on either DM or NDF digestibility in cattle. However, other authors have found that feeding high levels of dietary saponins and/or tannins decreased apparent digestibility (Ngamsaeng et al. 2006; Poungchompu et al., 2009). The present study may suggest that selective suppression of cellulolytic bacteria by saponins and tannins did occur as was reported by McSweeney et al. (2001). Moreover, this variable effect could be due to the type and concentration of saponins and tannins contained in the plants. The mangosteen peel used in this study contains 146 ± 2, and 95 ± 2 g/kg DM of condensed tannins and crude saponins, respectively, which were relatively similar to the levels used by Kanpakdee and Wanapat (2007).

Earlier studies (Hristov et al., 2009; Kongmun et al., 2011) found that NH_3–N concentration in the rumen of swamp buffalo

Table 5
Effect of coconut oil and mangosteen peel supplementation on nitrogen balance, and microbial protein synthesis.

Items	Control	CO[1]	MP	COM	SEM	P-value
Nitrogen balance, g/d						
Nitrogen intake	86.6	86.5	88.0	87.2	1.36	0.452
Fecal nitrogen	32.8	26.6	33.6	29.2	2.96	0.102
Urinal nitrogen	49.1[ab]	55.7[a]	49.0[b]	54.7[ab]	1.37	0.044
Nitrogen absorbed	53.8	59.9	54.3	58.0	3.44	0.391
Nitrogen retained	4.6	4.2	5.2	3.3	1.23	0.593
Microbial protein synthesis						
PD excreted, mmol/d	26.8	28.3	27.4	28.3	4.24	0. 672
PD absorbed, mmol/d	79.2	88.9	80.4	93.7	10.34	0.740
Microbial nitrogen supply, gN/d	56.3	64.1	58.0	68.0	8.24	0.740
EMPS, gN/kg OMDR	23.9	30.8	26.6	33.5	6.47	0.691

[ab] Values on the same row with different superscripts differed ($P<0.05$).
[1]CO = coconut oil 50 g/kg, MP = mangosteen peel 30 g/kg, COM = coconut oil 50 g/kg + mangosteen peel 30 g/kg.
PD = purine derivative, EMPS = efficiency of microbial protein synthesis, OMDR = apparently digested organic matter in the rumen (65% of apparently digested organic matter in total tract) according to ARC (1984).
SEM = standard error of the means.

was not affected by CO supplementation. Moreover, Wanapat et al. (2010) stated that supplementation of a 60 g/kg mixture of CO and sunflower oil decreased NH_3–N concentration by depressing protein degradation. Concentration of ammonia–nitrogen is directly affected by protein degradation however; it is able to fluctuate by assimilation of rumen microbes also (Atasoglu et al., 1998). The ruminal NH_3–N concentration was not affected by any of the following: dietary condensed tannin intake (Perez-Maldonado and Norton, 1996), supplementation of MP (Kanpukdee and Wanapat, 2008), and soapberry fruit–mangosteen peel pellet (Poungchompu et al., 2009); however, addition of ethanol extract of soapnut (*Sapindus mukorossi*) onto *in vitro* rumen fermentation of feed with buffalo rumen liquor decreased NH_3–N concentration (Kamra et al., 2006). Moreover, Grobner et al. (1982) found a reduction in ammonia concentration when saponins were included at 60 mg/kg in the incubation medium. Condensed tannin in the diet has beneficial effects which are mediated by protein–tannin complexation, decreasing availability of feed protein for ruminal degradation and ammonia nitrogen release (Makkar, 2003).

Increases in ruminal propionate concentration due to lauric acid or CO supplementation have been reported previously (Kongmun et al., 2011; Yabuuchi et al., 2006). Some part of the lipids can be degraded in the rumen by microbes, with propionic acid being produced from glycerol while acetic acid was synthesized from fatty acid oxidation (Doreau and Chilliard, 1997). However, Hristov et al. (2009) reported that CO supplementation did not affect the proportion of propionic acid, while butyric acid was increased in the rumen of the lactating cow. In addition, Wanapat et al. (2010) supplemented vegetable oils (CO and sunflower oil) in feedlot swamp buffalo and found decreased proportions of propionic acid in rumen fluid. These variations could be caused by proportion of fat in the total diets. Earlier work reported that condensed tannins and saponin had a variable effect on ruminal VFA concentration. The concentrations of total VFA and propionic acid were significantly increased, while proportion of acetic acid was decreased by supplementation with saponin rich tropical fruit (Poungchompu et al., 2009). However, Ngamsaeng et al. (2006) found no significant effects of feeding level of MP on either total VFA or individual VFA concentration. Moreover, cattle fed a forage-based diet supplemented with quebracho tannin extract did not exhibit a changed proportion of propionic acid, although acetic acid percentage was linearly decreased (Beauchemin et al., 2007). The expected shift in the VFA profile from acetate to propionate was associated with an increased fiber digestion.

Ruminal protozoa count was reduced through the addition of either CO or MP in agreement with the our previous work (Pilajun and Wanapat, unpublished), earlier *in vitro* work (Dohme et al., 1999; Kongmun et al., 2010) and *in vivo* work (Kongmun et al., 2011; Wanapat et al., 2010). The likely mode of action is of the medium-chain saturated fatty acids, specifically lauric acid, being particularly toxic to ruminal protozoa (Machmüller and Kreuzer, 1999). On the other hand, Poungchompu et al. (2009) found that populations of protozoa and fungi were dramatically decreased when dairy heifers were fed with plants containing condensed tannins and saponins. Moreover, the ethanol extract of soapnut (*S. mukorossi*) seed pulp completely inhibited *in vitro* methane production along with a significant reduction in protozoa count and acetate/ propionate ratio (Agarwal et al., 2006). The sensitivity of protozoa towards plant secondary compounds may be explained by the presence of sterols in cell membranes (Newbold et al., 1997). However, as suggested by Williams and Coleman (1992), the effect of defaunation on propionate concentration is likely to not be a direct effect of decreased protozoal counts, but an indirect effect of the defaunating agent on ruminal bacteria. It is likely that defaunation resulted in increased propionate concentration as the defaunating agent inhibited bacteria and protozoa that are not involved in propionate production, thus, indirectly promoting the growth of species such as *S. ruminantium* that are central to propionic acid production in the rumen.

The population of fungal zoospores was affected by CO supplementation as mentioned by Kongmun et al. (2011) and Pilajun et al. (2010). However, the number of fungal zoospores was decreased by soapberry fruit–mangosteen peel pellet supplementation in dairy heifers (Poungchompu et al., 2009). Methanogens serve as scavengers removing the hydrogen produced by protozoa and ruminal fungi have been related to fiber digestion (Bauchop, 1979) which leads to hydrogen production for methanogenesis. Thus, reduction of the protozoal population could be a cause of the decreasing fungal population due to the lack of an electron sink.

The population of proteolytic bacteria was increased by CO supplementation which may be related to increasing NH_3–N concentration in the rumen. In contrast, Kongmun et al. (2011) reported that proteolytic bacteria were not affected by CO supplementation, although they were increased when supplementation was a combination of CO and garlic powder. Although either CO or MP supplementation by themselves did not affect cellulolytic bacteria, a combination of CO and MP could improve digestibility of swamp buffalo. However, McSweeney et al. (2001) found that cellulolytic bacteria in the rumen of sheep were depressed by the condensed tannins present in calliandra (*Calliandra calothyrsus*). A reduction in the cellulolytic population could be explained by direct inhibition of the micro-organisms through tannin interactions with the cell wall and secreted catabolic enzymes (Jones et al., 1994), and/or by reduced substrate availability due to complexing of tannins with nutrients. The absence of any effect in this study may be due to adaptation of the microbes by alteration in the feeds (Makkar, 2003). Similarly *in vitro* studies have also demonstrated that some rumen bacteria have developed mechanisms to prevent these specific interactions (tannin tolerance) (Jones et al., 1994).

Although NH_3–N concentration in the rumen and microbial populations was changed by feed supplementation, nitrogen balance and microbial protein synthesis were not different among treatments. McSweeney et al. (2001) also reported that supplementation with the tropical forage calliandra which is rich in condensed tannins, did not change microbial protein synthesis in the rumen of sheep. Similar results were reported by McNeill et al. (1998) who found that microbial flow from the rumen was not inhibited by tannins present in *Leucaena leucocephala* (73 g/kg condensed tannin). However, Makkar et al. (1995) have shown in *in vitro* experiment that tannins can either reduce or increase the efficiency of microbial protein synthesis. It may be that the *in vivo* situation is not mimicked or the effects are too small to be detected in animal studies. In addition, the absence of an effect of feed addition on microbial protein synthesis may have resulted from higher recycling of

plasma purine derivatives in swamp buffalo than in cattle (Pimpa et al., 2003) which is one of the nitrogen conservation systems of ruminant.

5. Conclusion

Supplementation with either CO or MP did not affect feed intake and nutrient digestibility; however, ruminal fermentation was improved particularly increasing propionic acid concentration. The protozoal population was dramatically decreased with dietary supplementation, and fungal zoospore numbers were also decreased when a combination between CO and MP was supplemented. Groups of bacteria were not influenced by dietary treatment except proteolytic bacteria which was increased with CO supplementation. CO and MP peel have specific effect on rumen fermentation or microbial protein synthesis however; combination of the sources could improve rumen fermentation by enhancing on microbial population in the rumen of swamp buffalo when fed with rice straw.

References

Agarwal, N., Kamra, D.N., Chaudhary, L.C., Patra, A.K., 2006. Effect of *Sapindus mukorossi* extracts on in vitro methanogenesis and fermentation characteristics in buffalo rumen liquor. J. Appl. Anim. Res. 30, 1–4.

Agricultural Research Council, 1984. The Nutrient Requirements of Ruminant Livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp. 38–39.

AOAC, 1995. Official Method of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, Virginia, USA, pp. 1–18.

Atasoglu, C., Valdes, C., Walker, N.D., Newbold, C.J., Wallace, R.J., 1998. De novo synthesis of amino acids by the ruminal bacteria *Prevotella bryantii* B14, *Selenomonas ruminantium* HD4, and *Streptococcus bovis* ES1. Appl. Environ. Microbiol. 64, 2836–2843.

Bauchop, T., 1979. Rumen anaerobic fungi of cattle and sheep. Appl. Environ. Microbiol. 38, 148–158.

Beauchemin, K.A., McGinn, S.M., 2005. Methane emissions from feedlot cattle fed barley or corn diets. J. Anim. Sci. 83, 653–661.

Beauchemin, K.A., McGinn, S.M., Martinez, T.F., McAllister, T.A., 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. J. Anim. Sci. 85, 1990–1996.

Bremmer, J.M., Keeney, D.R., 1965. Steam distillation methods to determination of ammonium, nitrate and nitrite. Anal. Chem. Acta. 32, 485–495.

Chen, X.B., Gomes, M.J., 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives —an overview of the technical details. Occasional Publication 1992. International Feed Resources Unit. Rowel Research Institute, Aberdeen, UK.

Chen, X.B., Kyle, D.J., Orskov, E.R., 1993. Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization. J. Chromatogr.. 617, 241–247.

Church, D.C., 1969. Digestive Physiology and Nutrition of Ruminants. Digestive Physiology., vol. I. Metropolitan Printing, Oregon, Portland.

Dohme, F., Machmüller, A., Wasserfallen, A., Kreuzer, M., 1999. The role of the rumen ciliate protozoa for methane suppression caused by coconut oil. Lett. Appl. Microbiol. 29, 187–192.

Doreau, M., Chilliard, Y., 1997. Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows. Reprod. Nutr. Dev. 37, 113–124.

Galyean, M., 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Range Sciences, New Mexico State University, New Mexico.

Grobner, M.A., Johnson, D.E., Goodall, S.R., Benz, D.A., 1982. Sarsaponin effects on in vitro continuous flow fermentation of a high grain diet. J. Anim. Sci. 55, 491–497.

Guo, Y.Q., Liu, J.-X., Lu, Y., Zhu, W.Y., Denman, S.E., McSweeney, C.S., 2008. Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro-organisms. Lett. Appl. Microbiol. 47, 421–426.

Henderson, C., 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. 81, 107–112.

Hristov, A.N., Jouany, J.-P., 2005. Factors affecting the efficiency of nitrogen utilization in the rumen. In: Hristov, A.N., Pfeffer, E. (Eds.), Nitrogen and Phosphorus Nutrition of Cattle and Environment. CAB International, Wallingford, UK, pp. 117–166.

Hristov, A.N., Vander Pol, M., Agle, M., Zaman, S., Schneider, C., Ndegwa, P., Vaddella, V.K., Johnson, K., Shingfield, K.J., Karnati, S.K.R., 2009. Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. J. Dairy Sci. 92, 5561–5582.

Hungate, R.E., 1969. A roll tube method for cultivation of strict anaerobes. In: Norris, J.R., Ribbons, D.W. (Eds.), Methods in Microbiology, Edited by Norris. New York. Academic. NY, USA, pp. 117–313.

Jones, G.A., McAllister, T.A., Cheng, K.-J., Muir, A.D., 1994. Effect of sainfoin (*Onobrychis viciifolia* Scop) on growth and proteolysis by 4 strains of rumen bacteria: resistance of *Prevotella* (Bacteroides) ruminicola B14. Appl. Environ. Microbiol. 60, 1374–1378.

Kamra, D.N., Agarwal, N., Chaudhary, L.C., 2006. Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. Int. Cong. Ser. 1293, 156–163.

Kanpakdee, S., Wanapat, M., 2007. Effects of mangosteen (Garcinia mangostana) peel and sunflower and coconut oil supplementation on rumen fermentation, milk yield and milk composition in lactating dairy cows. MSc Thesis MEKARN-SLU http://www.mekarn.org/MSC2005-07/thesis07/contsuch.htm.

Kanpukdee, S., Wanapat, M., 2008. Effects of mangosteen (*Garcinia mangostana*) peel and sunflower and coconut oil supplementation on rumen fermentation, milk yield and milk composition in lactating dairy cows supplement. From Livest. Res. Rural Devel. V. 20 http://www.cipav.org.co/lrrd/lrrd20/supplement/such2.htm.

Kongmun, P., Wanapat, M., Pakdee, P., Navanukraw, C., 2010. Effect of coconut oil and garlic powder on in vitro fermentation using gas production technique. Livest. Sci. 127, 38–44.

Kongmun, P., Wanapat, M., Pakdee, P., Navanukraw, C., Yu, Z., 2011. Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. Livest. Sci. 135, 84–92.

Liang, J.B., Matsumoto, M., Young, B.A., 1994. Purine derivative excretion and rumen microbial yield in Malaysian cattle and swamp buffalo. Anim. Feed. Sci. Technol. 47, 189–199.

Machmüller, A., 2006. Medium-chain fatty acids and their potential to reduce methanogenesis in domestic ruminants. Agric. Ecosyst. Environ. 112, 107–114.

Machmüller, A., Kreuzer, M., 1999. Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. Can. J. Anim. Sci. 7, 65–72.

Machmüller, A., Soliva, C.R., Kreuzer, M., 2003. Effect of coconut oil and defaunation treatment on methanogenesis in sheep. Repord. Nutr. Dev. 43, 41–55.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin rich feeds. Small Rum. Res. 49, 241–256.

Makkar, H.P.S., Borowy, N., Becker, K., Degen, R., 1995. Some problems in fiber determination in tannin-rich forages. Anim. Feed. Sci. Technol. 55, 67–76.

McNeill, D.M., Osborne, N., Komolong, M., Nankervis, D., 1998. Condensed tannins in the *Leucaena* genus and their nutritional significance for ruminants. In: Shelton, H.M., Gutteridge, R.C., Mullin, B.F., Bray, R.A. (Eds.), Leucaena — Adaptation, Quality and Farming Systems, ACIAR Proceedings No. 86. ACIAR, Canberra, pp. 205–214.

McSweeney, C.S., Palmer, B., Bunch, R., Krause, D.O., 2001. Effect of the tropical forage calliandra on microbial protein synthesis and ecology in the rumen. J. Appl. Microbiol. 90, 78–88.

Newbold, C.J., Hassan, S.M., Wang, J., Ortega, M.E., Wallace, R.J., 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. Brit J Nutr. 78, 237–249.

Ngamsaeng, A., Wanapat, M., Khampa, S., 2006. Effects of mangosteen peel (*Garcinia mangostana*) supplementation on rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake in cattle. Pakistan J. Nutr. 5, 445–452.

Oldick, B.S., Firkins, J.L., 2000. Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily. J. Anim. Sci. 78, 2412–2420.

Palmquist, D.L., 1994. The role of dietary fats in efficiency of ruminants. J. Nutr. 124, 1377S–1382S.

Patra, A.K., Kamra, D.N., Agarwal, N., 2006. Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim. Feed. Sci. Technol. 128, 276–291.

Perez-Maldonado, R.A., Norton, B.W., 1996. The effects of condensed tannins from *Desmodium intortum* and *Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. Br. J. Nutr. 76, 515–533.

Pilajun, R., Wanapat, M., Wachirapakorn, C., Navanukroaw, C., 2010. Effect of coconut oil and sunflower oil ratio on ruminal fermentation, rumen microorganisms, N-balance and digestibility in cattle. J. Anim. Vet. Adv. 9, 1868–1874.

Pimpa, O., Liang, J.B., Balcells, J., Jelan, Z.A., Abdullah, N., 2003. Urinary purine derivative excretion in swamp buffaloes after duodenal purine base infusion. Anim. Feed Sci. Tech. 104, 191–199.

Poungchompu, O., Wanapat, M., Wachirapakorn, C., Wanapat, S., Cherdthong, A., 2009. Manipulation of ruminal fermentation and methane production by dietary saponins and tannins from mangosteen peel and soapberry fruit. Arch. Anim. Nutr. 5, 389–400.

Samuel, M., Sagathewan, S., Thomus, J., Mathen, G., 1997. An HPLC method for estimation of volatile fatty acids of rumen fluid. Indian J. Anim. Sci. 67, 805–807.

SAS, 1996. User's Guide: Statistic, Version 5. Edition. SAS. Inst Cary, NC, U.S.A.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd Ed. McGraw-Hill Inc, New York.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods of dietary fiber, neutral detergent fiber and non-starch carbohydrates in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Wanapat, M., Mapato, C., Pilajun, R., Toburan, W., 2010. Effects of vegetable oil supplementation on feed intake, rumen fermentation, growth performance, and carcass characteristic of growing swamp buffaloes. Livest. Sci.. doi:10.1016/j.livsci.2010.06.006

Williams, A.G., Coleman, G.S., 1992. The Rumen Protozoa. Springer-Verlag New York, Inc, New York, NY. USA.

Yabuuchi, Y., Matsushita, Y., Otsuka, H., Fukamachi, K., Kobayashi, Y., 2006. Effects of supplemental lauric acid-rich oils in high-grain diet on in vitro rumen fermentation. Anim. Sci. J. 77, 300–307.