Effect of Mangosteen Peel, Garlic and Urea Pellet Supplementation on Rumen Fermentation and Microbial Protein Synthesis of Beef Cattle

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Abstract: The objective of this study was to determine the effect of mangosteen peel, garlic and urea pellet supplementation on rumen fermentation and microbial protein synthesis of beef cattle. Four crossbred (Brahman x Holstein) beef cattle were randomly assigned according to a 4×4 Latin square design to receive four dietary treatments of different mangosteen peel pellets in concentrate. The treatments were as follows: T1, none supplementation; T2, supplementation with mangosteen peel pellet at 200 g/head/day (Mago-pel); T3, supplementation with mangosteen peel and garlic pellet at 200 g/head/day (Mago-pic) and T4, supplementation with mangosteen peel, garlic and urea pellet at 200 g/head/day (Mago-ulic). Rice straw was offered at ad libitum and concentrate was fed at 0.5% of BW. The results were found that total DMI and digestibility of DM and CP were not significantly affected by pellet supplementation whereas digestibility of NDF and ADF were higher in the pellet supplementation than in the control (p<0.05), ruminal temperature, pH, NH, total VFA and butyrate were similar among treatments although NH3-N tended to be higher in supplemental treatments and the highest was in Mago-ulic supplemental treatment. There was significantly different in propionate production (p<0.05) between treatments in which the highest was in Mago-ulic supplementation. In addition, the acetate, acetate to propionate ratio and methane production were reduced, bacterial population was increased and the highest was in Mago-ulic treatment. In contrast, protozoal population was reduced while fungal zoospores were not affected by feed supplementation. Microbial protein synthesis was increased by pellet supplementation although there was not significantly different between Mago-pel and control. In this study, supplementation of Mago-ulic at 200 g/head/day has shown the greatest for improving rumen fermentation, microbial protein synthesis and lower protozoa population in beef cattle.

Key words: Mangosteen peel pellets, garlic, urea, rumen fermentation, beef cattle, rice straw

INTRODUCTION

Ruminants establish a symbiotic relationship with rumen microorganisms. However, this symbiotic relationship has energy (losses of methane) and protein (losses of ammonia N) inefficiencies (Van Nevel and Demeyer, 1988). Utilization of feed additive has proved to be a useful strategy to improve the efficiency of energy and protein utilization in the ruminant. One of possible alternatives is using the secondary compound in natural plants such as saponins, tannins and essential oils. Mangosteen peel contains high amount of secondary compounds, especially condensed tannin (15.8%) and saponin (9.8%) (Ngamsaeng *et al.*, 2006). Which are also

assumed to be responsible for anti-protozoa effects and decreased methane concentration in rumen atmosphere (Poungchumpu et al., 2009) or in in vitro (Hess et al., 2003). Garlic is another kind of herb that has been used by humans as a source of antimicrobial agents for the gastrointestinal. Therefore, it could manipulate rumen fermentation. Busquet et al. (2005) reported that garlic supplementation decreased in the proportion of acetate and increased proportion of propionate and butyrate, inhibition of methanogenesis and decreased in the CH₄:VFA ratio. Many researches have shown that urea treatment could be used to add ammonia nitrogen for ruminal microbes, increased nutritive value particularly the crude protein content, digestibility and voluntary feed

intake by ruminant (Wanapat, 1984). However, manipulating ruminal fermentation of beef cattle by using combination of mangosteen peel, garlic and urea still limit of data, thus the objectives of this study were to determine effect of mangosteen peel, garlic and urea pellet supplementation on rumen fermentation and microbial protein synthesis of beef cattle.

MATERIALS AND METHODS

Animals, feeds and experimental design: Four, ruminally fistulated crossbred steer (Brahman x Holstein Friesian) cattle with initial BW of 220±15 kg were randomly assigned to receive four dietary treatments according to a 4×4 Latin square design. The dietary treatments were as follow: T1 none supplementation (control); T2 (Mago-pel) = Supplementation with mangosteen peel pellet at 200 g/head/day; T3 (Mago-lic) = Supplementation with mangosteen peel and garlic pellet at 200 g/head/day and T4 (Mago-ulic) = Supplementation with mangosteen peel, garlic and urea pellet at 200 g/head/day. Pellets were prepared from mangosteen peel, garlic and urea. Mangosteel peel and garlic are dried under the sun for 2-3 days after that bake temperature 60°C for 2-3 days and then crush thoroughly through a screen of 0.1 mm by using Cyclotech Mill (Tecator, Sweden) and were ground and then dried under the sun for 2-3 days after that baked temperature 60°C for 2-3 days. The mixture was pelleted to a size of 1.0 cm in diameter. Feed ingredients and chemical composition of concentrate, pellets and rice straw are shown in Table 1. Experimental animals were housed in individual pens and were supplemented with their respective treatment concentrates at 0.5% BW divided between two daily feeds (7.00 and 16.00) with rice straw given *ad libitum*. Mineral blocks and clean fresh water were available for all animals at all time.

Sample collection and analysis: The experiment was conducted for 4 periods, each lasted for 21 days. Feed intake, refusals and feed samples were collected daily during 21 days of each period and pooled prior to analyses. During the last 7 days of each period, fecal and urine samples were collected every day for chemical analysis. Fecal samples were divided into two parts, first part was analyzed for DM and Ash, the second part was kept at -20°C and pooled at the end of each period for analyzing CP according to AOAC (1990), NDF and ADF according to Van Soest et al. (1991). Rumen fluid was collected from fistulated rumen at 0, 2, 4 and 6 h post feeding in the end of each period. Rumen fluid was immediately measured for pH using a portable pH temperature meter (HANNA, instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through 4 layers of cheesecloth. Samples were divided 3 portions.

The first portion was used for ammonia-nitrogen with 5 mL 1 M. H₂SO₄ added to 45 mL of rumen fluid. The mixture was centrifuged at 16,000×g for 15 min and the supernatant was stored -20°C before NH₃-N analysis using the Micro-Kjeldahl Methods (AOAC, 1990) and volatile fatty acids by using HPLC (Instruments by controller water model 600E; water model 484 UV detector; column Novapak C18; column size 3.9×300 mm; mobile phase 10 mM H₂PO₄ [pH 2.5]) according to procedure of Samuel *et al.* (1997). The second portion was fixed with 10% formalin solution to determine total direct count of

Table 1: Ingredients and chemical compositions of the diets

Ingredients (%)	Concentrate	Mago-pel	Mago-lic	Mago-ulic	Rice straw
Cassava chip	65.0	-	-	-	-
Cassava starch	-	0.5	0.5	0.5	-
Rice bran	8.0	-	-	-	-
Coconut meal	8.0	-	-	-	-
Palm meal	3.0	-	-	-	-
Soybean meal	14.0	-	-	-	-
Molasses	0.5	1.0	1.0	1.0	-
Sulfur	0.5	-	-	-	-
Mineral premix	0.5	-	-	-	-
Salt	0.5	-	-	-	-
Urea	-	0.0	0.0	2.0	-
Mangosteen peel powder	-	98.5	93.5	91.5	-
Garlic powder	-	0.0	5.0	5.0	-
Chemical composition (%)					
Dry Mater (DM)	89.0	93.3	93.1	92.7	94.0
DM (%)					
Organic Mater (OM)	93.3	96.5	96.4	96.5	87.2
Crude Protein (CP)	14.3	21.2	21.5	22.1	2.4
Neutral Detergent Fiber (NDF)	24.6	57.3	57.2	57.0	72.3
Acid Detergent Fiber (ADF)	11.1	48.6	48.2	48.3	59.6
Condensed tannins	-	17.4	17.3	17.0	-
Saponins	-	11.3	11.3	11.1	-

bacteria, protozoa and fungal zoospores by using methods of Galyean (1989). The third portion was cultured for group of bacteria using a roll tube technique (Hungate, 1969) for identifying bacteria groups (cellulolytic, amylolytic, proteolytic and total viable count bacteria). Total urine excretion was conducted and acidified using 10 mL of $\rm H_2SO_4$ solution (2 M). Urine samples were analyzed for allantoin concentration by HPLC as described by Chen and Gomez (1995). Microbial purine derivative absorption was calculated by the equation of Chen and Gomez (1995).

$$Y = 0.85 X + 0.385 B W^{0.75}$$

The supply of microbial N was estimated as follows:

Microbial N (g day⁻¹) =
$$X \times 70/(0.116 \times 0.83 \times 1000)$$

= $0.727 \times X$

where, X and Y are absorption and excretion of PD in mmol day⁻¹, respectively. The Efficiency of Microbial Nitrogen Synthesis (EMNS) was calculated by equation:

$$EMNS = MN (g day^{-1})/DOMR$$

where, DOMR is apparently digested OM in the rumen (65% digestible OM intake).

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:

$$Y_{ijk} = \mu + M_i + A_j + P_k + \epsilon_{ijk}$$

Where:

 Y_{ijk} = The observation from animal j receiving diet, i in period, k

 μ = The overall mean

 M_i = Effect of treatment (i = 1-4)

 A_i = The effect of animal (i = 1-4)

 P_k = The effect of period (k = 1-4)

 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.

RESULTS AND DISCUSSION

Dry matter intake and apparent digestibility: The effects of pellets supplementation on total Dry Mater Intake (DMI) and nutrients digestibility are shown in Table 2. It was found that rice straw DMI, total DMI in term of kg per day and BW% were not significantly affected (p>0.05) by pellet supplementation. This result was similar with the result of Poungchompu et al. (2009) who found that DMI was not affected by mangosteen peel supplementation. Apparent digestibility (%) of DM, OM and CP were not significantly different among treatments. However, apparent digestibility of NDF and ADF was higher in pellet supplementation than those in control treatment (p<0.05) but not among the supplemental groups. Higher digestibility in experimental diets could be due to better activity of fiber fermentation as reported in the study of Patra et al. (2006). Saponin and condensed tannin in mangosteen peel affected on fiber fermentation as reported by Patra and Saxena (2009) that saponin supplementation increased in NDF digestibility in sheep probably due to increased microbial populations with the depressed protozoal activity.

Rumen fermentation: Effect of pellet supplementation on rumen fermentation characteristics in beef cattle are shown in Table 3. Ruminal pH, temperature and NH₃-N were not altered by pellet supplementation and the values were stable at pH 6.8 and temperature 38.3-38.6°C. Rumen NH₃-N was ranged between 14.77 and 15.53 mg dL⁻¹ which were in optimal range (15-30 mg dL⁻¹, Wanapat and Pimpa, 1999) for increasing microbial protein synthesis in ruminant fed low-quality roughages, although rumen NH₃-N tended to be higher in Mago-ulic supplemental treatment. This effect was due to higher digestibility and

Table 2: Effect of different pellet supplementation on feed intakes and digestibility

Items	Control	Mago-pel	Mago-lic	Mago-ulic	SEM	p-values
Rice straw DM intake						
kg day ⁻¹	2.6	2.4	2.6	2.4	0.19	0.723
BW day ⁻¹	1.3	1.1	1.4	1.2	0.13	0.562
Total DM intake						
kg day ⁻¹	3.6	3.3	3.6	3.4	0.20	0.724
BW% day ⁻¹	1.8	1.6	1.9	1.7	0.13	0.566
Apparent digestibility (%)						
DM	58.2	60.8	58.4	58.4	4.06	0.974
OM	59.8	61.2	60.1	61.2	3.28	0.892
CP	66.5	65.4	67.3	65.7	0.95	0.522
NDF	52.6ª	64.7°	61.9 ^b	62.5 ^b	2.04	0.021
ADF	55.2ª	61.5 ^b	60.4 ^b	60.9 ^b	1.02	0.043

a,bMeans in the same row with different superscripts differ (p<0.05), SEM: Standard Error of the Mean

Table 3: Effect of different pellet supplementation on rumen fermentation

Items	Control	Mago-pel	Mago-lic	Mago-ulic	SEM	p-values
Ruminal pH	6.8	6.8	6.8	6.9	0.04	0.920
Ruminal temperature (°C)	38.6	38.5	38.3	38.4	0.17	0.702
NH_3 - $N (mg dL^{-1})$	14.8	14.8	15.0	15.5	0.34	0.921
Total VFA (mmol L ⁻¹)	112.3	111.6	113.8	112.8	2.21	0.903
Acetate (C2) (%)	63.4ª	61.2^{b}	61.3 ^b	60.6 ^b	0.54	0.043
Propionate (C ₃) (%)	23.1 ^b	25.3ª	24.9 ^a	25.6ª	0.51	0.051
Butyrate (C ₄) (%)	13.5	13.5	13.8	13.8	0.36	0.882
$C_2:C_3$	2.8^{a}	2.4 ^b	2.5 ^b	2.4^{b}	0.07	0.051
CH ₄ (mmol L ⁻¹)	27.6^{a}	26.0°	26.3 ^b	25.8 ^b	0.62	0.040

a-bMeans in the same row with different superscripts differ (p<0.05); SEM: Standard Error of the Mean; CH₄ = 0.45C₂-0.275C₃+0.4C₄ (Moss et al., 2000)

Table 4: Effect of different pellets supplementation on rumen microorganism populations

Items	Control	Mago-pel	Mago-lic	Mago-ulic	SEM	p-values
Total direct count (cell mL ⁻¹)						
Bacteria × 10 ¹⁰	9.1 ^b	9.8ab	9.8 ^{ab}	11.7^{a}	0.62	0.050
Protozoa × 10 ⁵	6.4^{a}	3.96	4.3 ^b	4.0°	0.25	0.001
Fungal zoospore × 105	7.9	8.0	7.9	8.3	0.95	0.980
Viable bacteria population (CFU	mL ⁻¹)					
Total bacteria × 108	8.2	11.8	10.0	13.3	0.50	0.630
Amylolytic bacteria × 107	16.7	20.7	19.9	22.4	0.52	0.810
Proteolytic bacteria × 107	9.8⁴	4.6⁰	4.5 ^b	4.9 ⁶	0.51	0.050
Cellulolytic bacteria × 10 ⁸	1.5	2.0	2.7	3.0	0.55	0.510

a, b Means in the same row with different superscripts differ (p<0.05), SEM: Standard Error of the Mean

ammonia was added for rumen microbial synthesis. Although, there was generally agreement that tannin decreased ruminal protein degradation through formation of tannin-protein complexes. Kongmun reported that NH₃-N was reduced when increasing garlic powder supplementation at 16/200 mg diet *in vitro*. Hussain and Cheeke (1995) reported saponins in the Yucca extract can bind NH₃ when ruminal NH₃ concentrations were high and released it again when ruminal NH₃ was low providing a continuous and adequate supply of NH₃ for microbial protein synthesis. This increases the availability of nutrients to rumen bacteria and reduces environmental damage by decreasing losses of NH₃ to the environment. Poungchompu *et al.* (2009) reported NH₃-N was not affected by mangosteen peel supplementation.

Total VFA concentrations in all treatments ranged from 111.59-113.82 mol dL⁻¹ and were similar to those reported by Wanapat *et al.* (2008). Total VFA and butyrate (C₄) were not significantly different among treatments (p>0.05). However, propionate (C₃) increased with supplemental treatments (p<0.05) and the highest was in Mago-ulic treatment.

As reported by Ngamsaeng *et al.* (2006) that supplementation of 100 g/h/day mangosteen peel powder *in vivo* study in beef cattle did not affect on total VFAs while Pilajun and Wanapat (2011) reported that supplementation of 30 g kg⁻¹ mangosteen peel powder in *in vivo* study in swamp buffaloes increased in total VFA. The increasing of propionate proportion by mangosteen peel and garlic powder supplementation had been reported by Poungchompu *et al.* (2009), respectively

as a result of rechannelling of hydrogen from methane to propionate and decrease acetate:propionate ratio which is beneficial nutritionally for ruminants. The acetate (C_2) , C_2 - C_3 ratio and methane production were reduced by pellet supplementation.

These results were in agreement with Hu *et al.* (2005) and Poungchompu *et al.* (2009) who showed that methane emission was reduced by condensed tannin, saponin and garlic supplementation.

Rumen microorganism populations: Microorganism populations are affected by pellet supplementation (Table 4) on microorganism populations. Bacteria populations were found significantly increased with the supplementation while protozoal populations were reduced. However, there were not significantly different among supplemental groups.

The highest bacteria populations were found in Mago-ulic treatment while fungal zoospores and viable bacteria population were not effected except for proteolytic bacteria which were reduced by pellet supplementation as reported by Aerts *et al.* (1999) that proteolysis in *in vitro* studies was inhibited probably by supplementation about 400 µg CT mL⁻¹ or greater. Reduction of protozoal population demonstrated that compositions of mangosteen peel and garlic could impact on growth of protozoa via unique membrane lipid of protozoa.

However that was not in bacteria (De Rosa *et al.*, 1986). Saponin and condensed tannin in mangosteen peel increased bacteria as reported by Newbold *et al.* (1997)

Table 5: Effect of pellet supplementation on purine derivative

Items	Control	Mago-pel	Mago-lic	Mago-ulic	SEM	p-values
PD excretion (mM day ⁻¹)	55.4°	59.0 ^{bc}	64.7°	78.5ª	1.75	0.002
PD absorption (mM day ⁻¹)	41.2°	45.2^{bc}	52.5 ^b	68.4ª	2.78	0.002
MN supply (g day ⁻¹)	29.9°	32.9^{bc}	38.1 ^b	49.7ª	2.02	0.002
EMPS (gN kg ⁻¹ DOMD)	16.6°	$18.5^{\rm b}$	20.7⁰	29.2ª	2.04	0.019

*Means in the same row with different superscripts differ (p<0.01), SEM: Standard Error of the Mean, 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)

who conducted on *Yucca schidigena* and reduced protozoal populations as reported by Hu *et al.* (2005) who conducted on tea saponin at 0.2 g L⁻¹ and garlic (Busquet *et al.*, 2005). Elimination of protozoal in the rumen consequently increased numbers of bacteria in the rumen fluid (Preston and Leng, 1987).

Microbial protein synthesis: Purine derivative excretion, PD absorption, microbial N and Efficiency Microbial N Synthesis (EMNS) were significantly different among treatments (p<0.01) ranged from 55.41-78.47; 41.20-68.38 mM day⁻¹; 29.95-49.71 and 16.57-29.24 g kg⁻¹, respectively. The highest was in Mago-ulic supplementation following by Mago-lic, Mago-pel and then control.

This results were agreement with the normal range for cattle rumen (EMNS 9.1-27.9 g kg⁻¹ DOMD for forage concentrate mix diet) as reported by Karsli and Russell (2001). Hoover and Stokes (1991) proposed that the rate of digestion of carbohydrate would have great impact on EMNS. In addition, matching the release of ammonia N from dietary with the release of usable energy may improve N utilization, improve EMNS as well.

Therefore, Mago-ulic treatment was greater than others. Makkar *et al.* (1995) who indicated that the EMNS was greater in forage containing saponin and tannins which reduced ruminal N degradable. It was evident that tannins at low levels had the potential to modulate rumen fermentation towards maximising microbial protein synthesis (Wanapat, 2000). The decrease in the rate of digestion of feeds by tannins could help synchronising the release of various nutrients which in turn might be responsible for increased efficiency of microbial synthesis (Karsli and Russell, 2001).

However in this experiment, PD, MN and EMNS in Mago-pel treatment were not significantly different with those in the control while there were significant among Mago-lic, Mago-ulic and control treatment (p<0.01) (Table 5).

CONCLUSION

Based on these results, it could be said that supplementation of mangosteen peel, garlic and urea pellet could improve efficiency of feed utilization through increased NDF and ADF digestibility, improved rumen ecology by increasing propionate concentration, decreasing protozoal numbers and improved microbial protein synthesis. Mago-ulic pellet is therefore recommended for use as supplement.

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