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Effects of Mangosteen Peel (*Garcinia mangostana*) Supplementation on Rumen Ecology, Microbial Protein Synthesis, Digestibility and Voluntary Feed Intake in Cattle

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Abstract: Four, rumen fistulated cattle were randomly assigned according to a 4 x 4 Latin square design. The experiment was to study effects of crude saponins and condensed tannins in mangosteen peel on rumen microorganisms and fermentation, microbial protein synthesis and nutrient digestibility in cattle. The dietary treatments were as follows: T1 = Control (without Mangosteen peel supplementation, MSP); T2 = 50 g DM of MSP/hd/d; T3 = 100 g DM of MSP/hd/d; T4 = 150 g DM of MSP/hd/d with urea-treated rice straw (UTS) fed *ad libitum*. Roughage dry matter intakes in terms of kg/d and %BW were slightly higher in 100 gDM/hd/d supplemented cattle. Apparent digestibilities (%) of DM, OM, CP, NDF and ADF were similar among treatments. The values of ruminal temperature, pH, NH₃-N and BUN were not significantly affected by MSP supplementation. However, MSP supplementation increased bacterial population, and was highest at 150 gDM/hd/d supplementation. The protozoal population was significantly decreased while fungal zoospore populations were not changed, and were highest at the 100 gDM/hd/d supplementation group. However, lower values of TVFAs and C2/C3, and higher proportions of C3 were found at 100 gDM/hd/d of MSP supplemented than in the control group. In addition, microbial nitrogen supply, efficiency of rumen microbial protein synthesis and P/E ratio tended to be higher in MSP supplemented groups and were highest at 100 gDM/hd/d MSP supplementation. These results suggest that MSP supplementation at 100-150 gDM/hd/d could be used as a dietary source to manipulate rumen ecology thus improving rumen fermentation and potential productivity in cattle.

Key words: Mangosteen peel, saponins, condensed tannins, rumen ecology, urea-treated rice straw, cattle

Introduction

In the tropics, most ruminants are fed low-quality roughages, agricultural crop-residues and industrial by-products, which basically contain high levels of ligno-cellulosic materials, low levels of fermentable carbohydrate and low levels of good-quality protein (Wanapat, 1999). It is also essential to understand what happens when a feed is utilized by the ruminants. The feed conveyed into the rumen is digested by enzymes secreted by the microorganisms living in the rumen. The microbes mainly are bacteria (~ 10¹⁰-10¹² cells/ml of rumen fluid), protozoa (~ 10⁴-10⁶ cells/ml of rumen fluid) and fungi (~ 8% of total rumen microbes). However, bacteria are the major group to digest feeds before the host animals do. It has been reported that the elimination of the rumen protozoal population (defaunation) leads to improved animal performance (Leng, 1990). Moreover, it has been shown that protozoa can engulf bacteria in the rumen (200 cells/minute or 1% bacteria/minute). As a result of protozoal activity, a significant reduction in the flow of microbial biomass to the small intestine has been found (Hsu *et al.*, 1991).

Several antiprotozoal agents have been used, such as copper sulfate, zinc, molybdenum, dioctyl sodium sulphosuccinate (Jouany *et al.*, 1988). Moreover, considering the difficulties of on-farm defaunation (Moss *et al.*, 2000) a reduction, rather than the total elimination, of rumen protozoal population, which was shown to have similar effects (Veira *et al.*, 1983) has been suggested as a way to improve productivity for tropical diets (Dominguez Bello and Escobar, 1997). However, widespread use of the method as an on-farm has not been seen, due to its complexity and high cost. Tropical plants normally contain high or medium contents of secondary compounds. Among these compounds are the crude saponins (CS) and condensed tannins (CT), which have been shown to exert a specific effect against rumen protozoa while the rest of the rumen biomass remains unaltered (Wang *et al.*, 2000). Numerous studies have been conducted to determine the effects of feeding ruminants with saponin-rich plants, such as alfalfa (Klita *et al.*, 1996), *Enterolobium cyclocarpum*, *Spinadus saponaria*, *Sapindus rarak*, *Sesbania sesban*, *Quillaja saponaria*

Table 1: Composition of concentrate feed

Ingredients	% DM
Cassava chip	80
Rice bran	6
Brewer's meal	5
Urea	3.5
Molasses	3.5
Sulfur	0.5
Salt	0.5
Mineral mix	1
Chemical composition by calculation (%)	
DM	90
CP	12
TDN	76

and *Acacia auriculiformis* and *Yucca schidigera* (Wang *et al.*, 2000). Results have indicated that the saponins have strong antiprotozoal activity and may serve as an effective defaunating agent for ruminants. The detergent action of saponins is believed to be responsible for killing the rumen protozoa (Makkar *et al.*, 1998). However, investigation of condensed tannins and saponins in mangosteen (*Garcinia mangosteen*) peel has not been investigated, for such effect.

Therefore, the aim of the present experiment was to study effects of crude saponins and condensed tannins in mangosteen peel on rumen microorganisms and fermentation, microbial protein synthesis and nutrients digestibility in cattle.

Materials and Methods

Animals and management: Four, ruminal fistulated cattle were used (200-250 kg of live weight). The animals were kept individually penned. Before the start of the experiment, each of animals was dewormed with IVOMEC, and injected with AD₃E vitamins. The animals were adjusted to the feed for 2 weeks before starting the experiment. In each period the animals were raised in the pen for 14 days and then moved to metabolism crates for 7 days (2 days for adjustment and 5 days for collection of samples). Clean fresh water and a mineral block were available at all times.

Experimental design and treatments: A 4 x 4 Latin square design was used, and there were 21 days in each period. The dietary treatments were as follows: T1 = Control (without Mangosteen peel supplementation, MSP); T2 = 50 g DM of MSP/hd/d; T3 = 100 g DM of MSP/hd/d; T4 = 150 g DM of MSP/hd/d, respectively.

Experimental feeds and feeding: Urea-treated rice straw (UTS) (Wanapat, 1985; Wanapat, 1990) was used as a roughage and fed *ad libitum* during feeding in the pen, and 90% of *ad libitum* when the animals moved to the metabolism crates. Concentrate (12 %CP, 65 %TDN; Table 1) was fed at 0.5% of body weight in two equal portions, at 0800 h and at 1600 h. The MSP were collected from fruits and were sun-dried and ground into

powder form. Ground MSP was mixed with the concentrate before feeding according to treatments. All animals were gradually adapted to experimented feeds. Data collection, chemical analysis and samplings:

During the first 14 days of each period, feed offered and feed refused were weighed daily for voluntary feed intake measurement and feed samples were randomly collected twice a week for DM analysis using hot air oven (AOAC, 1990). During the last 5 days of each period, feed samples were collected daily and divided into two parts. The first part analyzed for DM daily while the second part was kept and pooled at the end of each period for analyses of ash, CP (AOAC, 1990), NDF and ADF (Goering and Van Soest, 1970). Condensed tannins content in MSP was analyzed by using the vanillin-HCl method as modified by Wanapat and Pongchompu (2001) and crude saponins were measured by using methanol extraction following the method of Kwon *et al.* (2003).

Total faeces were collected and weighed during the last 5 days of each period. The faecal samples were collected at about 5% of the weight and divided into two parts. The first part was analyzed for DM daily; the second part kept stored in refrigerator and pooled at the end of each period for chemical analyses, similarly with the feed samples.

Total urine was collected on the same days as feces by using plastic containers with concentrated sulfuric acid added to prevent nitrogen loss. The urine samples were collected (about 10% of the volume) and kept in a refrigerator and pooled at the end of each period to be analyzed for NH₃-N by the hypochlorite-phenol procedure (Beecher and Whitton, 1978) and nitrogen balance and purine derivatives determined for estimating microbial protein synthesis according to the procedure of Zinn and Owen (1986).

Heparinized blood samples were collected from the jugular vein at 0, 2, 4 and 6 h post-feeding from each animal at the end of each period. Samples were refrigerated for 1 h and then centrifuged at 3500 x g for 20 min. The plasma was removed and analyzed for BUN composition according to the method of Roseler *et al.* (1993).

Rumen fluid samples were collected from fistulated rumen at the same time as blood sampling and analyzed immediately for rumen pH using a glass electrode pH meter. Fifty ml of rumen fluid samples were collected and 5 ml of 2N H₂SO₄ added to stop fermentation by microbes and then centrifuged at 3,000 x g for 10 min. About 20-30 ml of supernatant was collected and frozen at -20°C until analyzed in the laboratory for VFAs using High Performance Liquid Chromatography (HPLC; Model Water 600; UV detector, Millipore Corp.) according to the method of Samuel *et al.* (1997) for NH₃-N by the hypochlorite-phenol procedure (Beecher and Whitton, 1978). The subsequent rumen

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Table 2: Chemical compositions of feed and feed stuff

Item	DM	Ash	OM	CP	NDF	ADF	CT ¹	CS ²
	-----%DM-----							
Urea-treated rice straw	57.7	17.1	82.8	8.0	69.8	49.0	-	-
Mangosteen peel	93.0	2.8	97.2	18.3	56.8	51.3	16.8	10.0
Concentrate feed	87.5	6.5	93.5	10.2	10.8	6.7	-	-

¹ CT = condensed tannins; ² CS = crude saponins.

Table 3: Effect of mangosteen peel supplementation on voluntary feed intake and digestibility in cattle

Item	Mangosteen peel supplementation (g/hd/day)				SEM
	0	50	100	150	
Roughage DM, Kg/d	4.7	4.80	5.1	4.7	0.42
%BW	1.6	1.6	1.8	1.6	0.11
g/kg BW ^{0.75}	67.8	68.0	74.1	66.8	4.48
Total DM, Kg/d	6.2	6.2	6.6	6.3	0.50
%BW	2.1	2.1	2.3	2.1	2.14
g/kg BW ^{0.75}	88.3	89.6	96.0	89.6	4.54
Weight change (kg)	0.02	0.36	0.07	0.32	0.23
Digestibility (%)					
DM	73.5	69.7	71.8	74.4	2.14
OM	78.1	74.7	76.4	78.4	1.90
CP	61.4	61.9	60.8	63.0	4.14
NDF	76.4	73.4	75.3	76.2	1.82
ADF	69.5	65.3	68.4	70.3	2.12

Values on the same row with different superscripts differed (p<0.05)

fluid was immediately fixed with 10% formalin solution (1:9 v/v, rumen fluid : 10% formalin) (Galyean, 1989) for measuring the microbial population. The total direct count of bacteria, protozoa (Holotrich and Entodiniomorhp) and fungal zoospores were made using the procedure of Galyean (1989) by a haemocytometer (Boeco). Differentiation of rumen fungal zoospores from small protozoa was based on characteristics of having flagellae, while large protozoa had ciliates around the cells. Rumen fluid was diluted using autoclave distilled water (121°C for 15 minutes) as a medium by 100, 10 and 10 times, and counting using a 10 x 40, 10 x 10 and 10 x 40 ocular x objective microscope for bacteria, protozoa and fungal zoospores, respectively.

Statistical analysis: The means of each parameter measured in the digestibility studies, nutrient intake and rumen microorganisms were analyzed by the analysis of variance (ANOVA) techniques using the General Linear Model (GLM) procedures of the Statistical Analysis System Institute (1998). Mean separations with a significant F (P<0.05) for treatment were statistically compared using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

Results and Discussion

Characteristics of feed-stuffs: The composition of the concentrate and chemical composition of feeds and

feedstuffs are shown in Tables 1 and 2, respectively. The CP, NDF and ADF were 8.0, 18.3 and 10.3 % of DM, 69.8, 56.8 and 10.8 % of DM and 49.0, 51.3 and 6.7 % of DM in urea-treated rice straw, MSP and concentrate feed, respectively. Ground MSP contained 16.8 % and 10.0 % (w/w) of condensed tannins and crude saponins, respectively. The CP value in the concentrate was 10.3%. Furthermore, CP, NDF and ADF contents in urea-treated rice straw were similar to the values reported earlier by Wanapat (1985). Moreover, the CT value in MSP (16.8%) was slightly higher than other values reported, which ranged from 7-15% (wt/wt). Crude saponins in MSP (10.0%) were higher than in other plants, such as *Yucca schidigera* (4.4%) (Eryavuz and Dehority, 2004), seed of *Moringa oleifera* (2.2%) (Anhwange *et al.*, 2004), *Enterolobium cyclocarpum* (1.9%) and *Pithecellobium saman* (1.7%), but were lower than in *Sapindus saponaria* (12%) (Hess *et al.*, 2003). It should be observed that MSP contained relatively high level of CP and minerals especially, K and Ca. These minerals could be beneficial for ruminants.

Feed intake and digestibility of nutrients: Roughage and total dry matter intakes in term of kg/d and %BW in steers with supplementation of MSP at 100 g/hd/d were slightly higher than those supplemented with 0, 50 and 150 g/hd/d (Table 3). However, response of MSP supplementation was not consistent. Apparent digestibilities (%) of DM, OM, CP, NDF and ADF were not significantly different among treatments (P>0.05). In addition, body weight changes were not affected by MSP supplementation. These data are in agreement with previous studies by Eryavuz and Dehority (2004). On the other hand, Diaz *et al.* (1993) demonstrated that high levels of saponins and/or tannins in diets resulted in decreased apparent digestibility, especially of N. Tannins are known to decrease protein degradability by complexing with feed protein, which may lead to inhibition of protein degradation in the rumen by the high concentrations of condensed tannins.

Ruminal temperature, pH, ammonia-nitrogen (NH₃-N) and blood-urea nitrogen (BUN): Rumen ecology parameters, including temperature, pH, NH₃-N and BUN are presented in Table 4. Mean values of ruminal temperature, pH, NH₃-N and BUN were similar among treatments. Values were stable at 38°C, 6.5 and 13 mg/dl for temperature, pH and BUN, respectively. No effects of MSP supplementation on NH₃-N concentrations were found (11.5 to 14.2mg/dl) and were

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Table 4: Effect of mangosteen peel supplementation on ruminal temperature, ruminal pH, ammonia nitrogen (NH₃-N) and blood urea nitrogen (BUN) in cattle

Item	Mangosteen peel supplementation (g/hd/day)				SEM
	0	50	100	150	
Temperature (°C)					
0 h- post feeding	38.0	38.1	38.4	37.8	0.14
2	38.5	38.4	38.0	38.2	0.28
4	38.5	38.4	38.9	38.0	0.39
6	38.5	38.6	38.5	39.0	0.24
mean	38.4	38.4	38.4	38.3	0.16
Ruminal pH					
0 h- post feeding	6.5	6.4	6.6	6.5	0.08
2	6.5	6.6	6.7	6.7	0.05
4	6.4	6.6	6.5	6.5	0.09
6	6.5	6.4	6.4	6.3	0.08
mean	6.5	6.5	6.5	6.5	0.06
NH ₃ -N (mg/dl)					
0 h- post feeding	7.6	6.7	6.0	6.0	1.32
2	20.4	21.5	25.3	20.9	2.08
4	13.1	11.7	14.9	10.2	0.98
6	9.4	9.9	10.5	8.8	1.14
mean	12.6	12.4	14.2	11.5	0.79
0 h- post feeding	11.1	10.9	11.8	11.9	1.00
2	13.4	13.0	13.1	14.9	1.14
4	14.8	14.0	13.8	14.6	1.18
6	13.7	14.3	14.3	12.9	1.30
mean	13.2	13.1	13.3	13.6	1.04

Values on the same row with different superscripts differed (p<0.05)

highest at 100 g/hd/d supplementation. Ruminal temperature, pH, NH₃-N and BUN values, as influenced by MSP, were in normal ranges, as reported for optimal microbial digestion of fiber and digestion of protein (Wanapat, 1990). The adhesion to cellulose of the three cellulolytic species was completely inhibited at temperatures below 4°C, and in *R. albus* and *F. succinogenes* adhesion also decreased at temperatures above 50°C, and achieved maximal values at 30 to 38°C (Pell and Chofield, 1993). Moreover, the rumen processes that ensure maximum feed intake and digestibility, efficiency, normal buffer levels and healthy cows all operate within a narrow pH range of 6.4 to 6.8. According to numerous reports, the optimal level of ruminal ammonia concentration for efficient digestion is from 5.0 to 25.0 mg/dl (Preston and Leng, 1990) and 15 to 30 mg/dl (Wanapat and Pimpa, 1999). Preston (1996) suggested that the quantity of ammonia absorbed from the rumen was reflected in circulating BUN. In addition, BUN was also depending on P/E ratio balance. BUN levels lower than 12.7 mg/dl could be due to an insufficiency in CP per unit of digestible energy (Hwang *et al.*, 2001).

Ruminal microorganism population: Effects of MSP supplementation on ruminal microorganisms, total counts of bacteria, protozoa and fungal zoospore, as

Table 5: Effect of mangosteen peel supplementation on ruminal microorganism population in cattle

Item	Mangosteen peel supplementation (g/hd/day)				SEM
	0	50	100	150	
Bacteria (x 10 ⁹ cells/ml)					
0 h- post feeding	2.8	4.5	4.1	5.0	6.59
2	3.4	4.4	3.8	5.9	5.43
4	2.7 ^a	4.6 ^b	4.3 ^b	4.8 ^b	3.51
6	2.5	3.6	3.9	3.9	7.68
mean	2.8 ^a	4.3 ^b	4.0 ^b	5.3 ^c	1.78
Protozoa					
Entodiniomorph (x 10 ⁵ cells/ml)					
0 h- post feeding	12.0	11.5	9.5	10.5	2.89
2	15.0	13.0	10.3	9.0	1.85
4	27.0 ^a	19.3 ^b	10.2 ^c	19.5 ^b	1.82
6	29.0 ^a	24.5 ^{ab}	20.5 ^b	21.5 ^b	1.31
mean	20.8 ^a	13.8 ^b	12.6 ^b	15.1 ^{ab}	0.09
Holotrich (x 10 ³ cells/ml)					
0 h- post feeding	12.0	11.5	9.5	10.5	2.90
2	15.5	13.5	10.3	9.0	1.66
4	27.5 ^a	20.1 ^b	10.2 ^c	19.5 ^b	1.16
6	29.0 ^a	24.5 ^{ab}	20.5 ^b	21.5 ^b	1.31
mean	21.0 ^a	17.4 ^b	12.6 ^c	15.4 ^b	0.47 ^{ax}
Zoospore (x 10 ⁵ cells/ml)					
0 h- post feeding	2.9	2.0	4.0	2.1	6.87
2	3.2	2.3	3.5	2.5	6.10
4	2.7	2.2	3.5	2.4	7.00
6	2.9	2.4	3.0	1.4	4.42
mean	2.9	2.2	3.5	2.1	4.03

Values on the same row with different superscripts differed (p<0.05)

measured at 0, 2, 4 and 6 h post feeding, are shown in Table 5. MSP supplementation increased (P<0.05) the bacterial population, and the total count of bacteria was highest at 150 g/h/d of MSP supplementation. Protozoal populations decreased in all treatments with supplementation of MSP when compared with the control group, while, the fungal zoospore populations were not affected. However, increasing the level of MSP supplementation tended to change the rumen microbial population. It appeared that MSP might play an important role in changing rumen microorganism populations as a result of CT and/or CS. Finlay *et al.* (1994) described a symbiosis of protozoa with methanogenic archaea. Other studies have shown that 9-25% of total methanogens are associated with the protozoa (Newbold *et al.*, 1995). Furthermore, in a pure culture study Wang *et al.* (2000) who found that steroidal saponins from *Y. schidigera* inhibited cellulolytic ruminal bacteria and fungi, but their effects on amylolytic bacteria were species dependent and similar to the effects of ionophores. Eryavuz and Dehority (2004) showed no effect of *Y. schidigera* extract on ruminal microbial concentration in sheep. Moreover, results on saponin contents in plants showed that the inclusion of *Enterolobium ciclocarpum* as a supplement, or as the basal diet in crossbred sheep, did not eliminate

Table 6: Effect of mangosteen peel supplementation on ruminal total volatile fatty acids in cattle

Item	Mangosteen peel supplementation (g/hd/day)				SEM
	0	50	100	150	
TVFAs (mmol/L)					
0 h- post feeding	99.0	99.2	87.7	101.9	4.76
2	91.9	84.0	88.8	88.2	4.25
4	88.7	96.1	88.6	90.9	4.00
6	89.4	93.4	96.1	100.5	3.79
mean	92.2	93.2	90.3	95.4	1.72
Acetate (mmol/100mol)					
0 h- post feeding	63.3	59.6	59.2	58.8	3.16
2	63.1	61.9	60.0	65.6	3.39
4	61.7	65.1	63.1	62.2	2.81
6	61.3	61.4	61.4	63.7	1.78
mean	62.4	62.0	60.9	62.6	1.36
Propionate (mmol/100mol)					
0 h- post feeding	22.1	26.6	25.9	26.6	2.85
2	22.6	22.3	24.7	19.7	2.82
4	23.1	20.5	22.1	23.4	1.66
6	24.1	24.1	25.4	23.3	1.29
mean	23.0	23.4	24.5	23.5	1.36
Butyrate (mmol/100mol)					
0 h- post feeding	14.5	13.7	14.8	14.5	2.02
2	14.1	15.7	15.2	14.5	1.43
4	15.1	14.3	14.7	14.3	1.87
6	14.4	14.4	13.0	12.8	1.72
mean	14.5	14.5	14.4	14.0	0.63
Acetate : Propionate ratio					
0 h- post feeding	3.1	2.2	2.4	2.4	0.46
2	2.9	2.8	2.7	3.4	0.45
4	2.7	3.1	2.9	2.7	0.29
6	2.5	2.5	2.4	2.7	0.17
mean	2.8	2.7	2.6	2.8	0.22

Values on the same row with different superscripts differed (p<0.05)

Table 7: Effect of mangosteen peel supplementation on nitrogen balance in cattle

Item	Mangosteen peel supplementation (g/hd/day)				SEM
	0	50	100	150	
Nitrogen intake (g/d)	82.2	85.5	85.0	88.7	7.42
Faeces nitrogen (g/d)	32.0	32.8	33.7	33.4	5.28
Urine nitrogen (g/d)	12.8	13.1	11.9	14.9	1.96
Nitrogen absorption (g/d)	50.2	52.7	51.3	55.3	4.49
Nitrogen absorption (%)	61.4	61.9	60.8	63.0	3.08
Nitrogen retention (g/d)	37.4	39.6	39.3	40.3	5.67

Values on the same row with different superscripts differed (p<0.05)

protozoa from the rumen, but caused a significant reduction of total protozoal number and a variation in the species of protozoa present in the rumen. Holotrichs were the most susceptible species to *E. ciclocarpum* (Navas *et al.*, 1993). On the other hand, Diaz *et al.* (1993), in a study on the use *Sapindus saponaria* as a defaunating agent in mature tropical crossbred sheep, reported that the protozoal population was significantly

reduced (84%), and total viable bacteria, cellulolytic bacteria and fungi were significantly increased in a treatment which included 50 g of *S. saponaria* compared with a control (0 g of *S. saponaria*). Recently, research evaluated the effect of saponin containing plant materials such as *Morinda citrifolia* (fruit), *Nothopanax scutellarium* (leaves), *Sesbania sesban* (leaves) and *Sapindus rarak* (fruit) on *in vitro* fermentation, that protozoal populations were lowest in the treatment with *Sapindus rarak*, and concluded that saponin rich plants have a potential as a natural defaunating agent. In addition, *in vitro* fermentation and supplementation with *S. saponaria* can decrease protozoal count (by 54%) and daily methane release (by 20%) relative to a control (without saponins) (Hess *et al.*, 2003). Although most studies indicated a reduction in ruminal protozoal numbers, however, effects of saponins and/or condensed tanins on overall ruminal fermentation were not consistent among studies. Due to the effects of saponins/condensed tannins-rich plants, protozoal concentrations in the rumen have been variable markedly both with diets and with feeding (Odenyo *et al.*, 1997), which could result in variable findings.

Effect on ruminal volatile fatty acids production: The influence of level of MSP supplementation on TVFAs, acetate acid, propionic acid and butyric acid proportions, and acetic to propionic (C2/C3) ratio are shown in Table 6. There were no significant differences (P>0.05) for TVFAs production and proportions of VFAs among treatments. However, lower values in terms of TVFAs and C2/C3 ratio were obtained, and higher proportion of C3 were found at 100 g/hd/d of mangosteen supplementation. However, the response to level of MSP supplementation was not consistent. The results were similar to an *in vitro* trial with *Yucca Schidigera*, by Hristov *et al.* (2004), a feeding trial with *Sapindus saponaria*, by Diaz *et al.* (1993) and with alfalfa root, by Klita *et al.* (1996). Hess *et al.* (2003) found a significant increase in propionate production studies with both animals and *in vitro* trials, respectively. Moreover, effects of saponins on ruminal propionate and reduced acetate to propionate ratio have been found to vary with diets and applications. Grobner *et al.* (1982) reported a significant increase in propionate production *in vitro* with 60 ppm of saponins, while Valdez *et al.* (1986) found no significant change in propionate production *in vivo* with 77 ppm of sarasaponins. Hussain and Cheeke (1995) indicated numerically decreased ruminal propionate concentrations on high-forage and high-concentrate diets when *Y. schidigera* was included at 75 ppm (as-fed-basis). Increased molar proportions of propionate in the rumen are often found in studies with defaunated sheep (Williams and Coleman, 1991). As the single most important rumen bacterium involved in decarboxylation of succinate, *Selemonas ruminantium*

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Table 8: Effect of mangosteen peel supplementation on microbial protein synthesis estimation from urine purine derivative (PD) excretion and microbial protein and energy (P/E) ratio in cattle

Item	Mangosteen peel supplementation (g/hd/day)				SEM
	0	50	100	150	
Allantoin, mmol/L	7.6	8.1	8.6	8.0	0.50
mmol/d	20.7	22.8	26.6	22.8	3.26
Total PD, mmol/d	24.4	26.8	31.8	29.7	3.83
mmol/kg BW ^{0.75}	0.36	0.39	0.44	0.41	0.05
PD absorption (mmol/d)	16.4	19.2	24.6	22.5	4.55
Calculated microbial N synthesis, gN/d	11.9	13.9	17.9	16.3	3.31
gN/kgOMDR	5.0	5.1	7.3	5.6	1.04
Microbial protein synthesis (g/d)	74.5	87.3	112.0	102.3	20.69
VFA production (MJ/d)	19.9	20.4	18.8	20.9	1.42
P/E ratio (g/MJ)	4.1	4.3	6.1	4.6	0.87
CH ₄ production (mol/100mol)	32.7	32.4	31.6	32.5	0.99

Values on the same row with different superscripts differed ($p < 0.05$)

is apparently responsible for most of propionate production in the rumen arising from the randomizing pathway (Wolkin and Miller, 1988). Wallace *et al.* (1994) determined that growth of *S. ruminantium* was not affected by yucca saponins, whereas growth of some other rumen bacterial species (*Streptococcus bovis* and *Butyrivibrio fibrisolvens*) was strongly inhibited. In our study, MSP did not show any adverse effects on rumen fermentation with regard to VFAs production. However, higher levels of MSP supplementation should be investigated in order to explore more potent effects.

Nitrogen balance, urinary purine derivative excretion, microbial protein synthesis and P/E ratio: As shown in Table 7, N-balance in terms of N absorption and retention, was not significantly different ($P > 0.05$) among treatments. Nevertheless, N retention and absorption were slightly higher in group supplemented with MSP than in the control group, but the response was not consistent. Excretion of allantoin in the urine, microbial nitrogen supply, efficiency of rumen microbial protein synthesis and P/E ratio were not significantly ($P > 0.05$) different among treatments, but were highest at 100 g/hd/d MSP supplementation. Microbial nitrogen supply in the rumen is summarized in Table 8. The microbial nitrogen supply ranged from 11.9 to 17.9 gN/day. Furthermore, the efficiency of rumen microbial protein synthesis and P/E ratio was slightly higher in steers fed MSP, where the values ranged from 5.0 to 7.3 gN/kg of OMDR and 4.1 to 6.1 g/MJ, respectively. However, the response to MSP supplementation was not consistent. In this experiment, MSP supplementation did not affect nitrogen utilization, microbial protein synthesis and P/E ratio although. Lu and Jorgensen (1987) found high levels of alfalfa saponins to be strongly inhibitory of N digestion in the forestomach when a forage-basal diet was fed. These researchers also reported a trend towards reduced N retention with increasing saponins levels. Preston and Leng (1987) suggested that when

the objective of a feeding strategy is production of milk, meat, hair or wool then microbial protein output from the rumen should be at a maximum relative to the energy in VFAs. The more microbial protein that is produced from a low-cost carbohydrate source, the less will be the requirement for supplementary by pass protein, which is usually the most expensive portion of the diet.

Conclusion and Recommendations: Mangosteen peel (MP) could be used as a supplement at 100-150 g/hd/d. The results revealed improvement of rumen ecology in terms of increasing microbial population and decreasing protozoal population. In addition, microbial protein synthesis were also enhanced by MP supplementation as MP is a by-product, it could be easily collected and be used by farmers in Mangosteen plantation area. However, research on MP supplementation in lactating or fattening cattle should be investigated further.

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References

- AOAC., 1990. Official Methods of Analysis (15thEd), Association of Official Analytical Chemistry Washington, D.C., U.S.A.
- Anhwange, B.A., V.O. Ajibola and S.J. Oniye, 2004. Chemical study of the seeds of *Moringa oleifera* (Lam) and *Detarium microcarpum* (Guill and Sperr). J. Biol. Sci., 4: 711-715.

- Beecher, G.R. and B.K. Whitton, 1978. Ammonia determination: Reagents modification and interfering compounds. *Anal. Biochem.*, 36: 243.
- Diaz, A., M. Avendano and A. Escobar, 1993. Evaluation of *Sapindus saponaria* as a defaunating agent and its effects on different ruminal digestion parameters. *Livest. Res. Rural Dev.*, 2: 1-6.
- Dominguez Bello, M.G. and A. Escobar, 1997. Diurnal variations in milk and blood urea-nitrogen and whole blood ammonia nitrogen in dairy cows. *Anim. Feed Sci. Tec.*, 69: 91-102.
- Eryavuz, A. and B. A. Dehority, 2004. Effect of *Yucca schidigera* extract on the concentration of rumen microorganisms in sheep. *Anim. Feed Sci. Tec.*, 117: 215-222.
- Finlay, B.J., G. Esteban, K.J. Clarke, A.G. Williams, T.M. Embley and R.P. Hirt, 1994. Some rumen ciliates have endosymbiotic methanogens. *FEMS. Microbiol. Lett.*, 117: 157-162.
- Galyean, M., 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Rang Sciences. New Mexico State University, U.S.A.
- Goering, H. and K. Van Soest, 1970. Forage Fiber Analysis. Agriculture hand book No.379. United State Department of Agriculture, Washington D. C., USA.
- Grobner, M.A., D.E. Johnson, S.R. Goodall and D.A. Benz, 1982. Glycofractions of *Yucca* plant and their role in ammonia control. In: *Proc. West. Sect. Am. Soc. Anim. Sci.*, 33: 64-65.
- Hess, H.D., M. Kreuzer, T.E. Diaz, C.E. Lascano, J.E. Carulla, C.R. Soliva and A. Machmuller, 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. *J. Biol. Sci.*, 109: 79-94.
- Hristov, A.N., K.L. Grandeen, J.K. Ropp and D. Greer, 2004. Effect of *Yucca schidigera* based surfactant on ammonia utilization *in vitro*, and *in situ* degradability of corn grain. *Anim. Feed Sci. Tec.*, 115: 341-355.
- Hsu, J.T., G.C. Fahey, N.R. Merchen and R.I. Mackie, 1991. Effect of defaunation and various nitrogen supplementation regimens on microbial numbers and activity in the rumen of sheep. *J. Anim. Sci.*, 69: 1279-1289.
- Hussain, I. and P.R. Cheeke, 1995. Effect of dietary *Yucca Schidigera* extract on rumen and blood profiles of steers fed concentrate- or roughage-based diets. *Anim. Feed Sci. Tec.*, 51: 231-242.
- Hwang, S.H., M.J. Lee and H.C. Peh, 2001. Diurnal variations in milk and blood urea-nitrogen and whole blood ammonia nitrogen in dairy cows. *Asian-Aust. J. Anim. Sci.*, 14: 1683-1689.
- Jouany, J.P., D.I. Demeyer and J. Grain, 1988. Effect of defaunating the rumen. *Anim. Feed Sci. Tec.*, 21: 229-265.
- Klita, P.T., G.W. Mathison, T.W. Fenton and R.T. Hardin, 1996. Effects of alfalfa root on digestive function in sheep. *J. Anim. Sci.*, 74: 1144-1156.
- Kwon, J.H., J.M.R. Belanger and V.A. Yaylayan, 2003. Application of the microwave-assisted process (MAP™) to the fast excretion of ginseng saponins. *Food. Res. Int.*, 36: 491-498.
- Leng, R.A., 1990. Factors affecting the utilization of poor-quality forage by ruminants particularly under tropical conditions. *Nutr. Res. Rev.*, 3: 27-91.
- Lu, C.D. and N.A. Jorgensen, 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. *J. Nutr.*, 117: 919-927.
- Makkar, H.P.S., S. Sen, M. Blummel and K. Becker, 1998. Effect of fractions containing saponins on rumen fermentation. *J. Agri. Food Chem.*, 46: 4324-4328.
- Moss, A.R., J.P. Jouany and J. Newbold, 2000. Methane production by ruminants: its contribution to global warming. *Ann. Zootech.*, 49: 231-253.
- Navas-Camacho, A., M.A. Laredo, A. Cuesta, H. Anzola and J.C. Leon, 1993. Effect of supplementation with a tree legume forage on rumen function. *Livest. Res. Rum. Dev.*, 5: 58-71.
- Newbold, C.J., B. Lassalas and J.P. Jouany, 1995. The importance of methanogenesis associated with ciliate protozoa in ruminal methane production *in vitro*. *Lett. Appl. Microbiol.*, 21: 230-234.
- Odenyo, A.A., P.O. Osuji and O. Karanfil, 1997. Effects of multipurpose tree (MPT) supplements on ruminal ciliate protozoa. *Anim. Feed Sci. Tec.*, 67: 169-180.
- Pell, A.N. and P. Chofield, 1993. Microbial adhesion and degradation of plant cell walls. In: *Forage cell wall Structure and Digestibility*. (Eds. R.D. Hatfield, H.G. Jung, J. Ralph and P.J. Weimer), ASA-CSSA-SSSA, Madison, WI., pp: 397-423.
- Preston, R.L. and R.A. Leng, 1987. Matching Ruminant Production Systems with Available Resources in the Tropic and Sub-Tropics. Penambull Book Armidale, Australia.
- Preston, R.L., 1996. Protein requirements for growing-finishing cattle and lambs. *J. Nutr.*, 90: 157-160.
- Preston, R.L. and R.A. Leng, 1990. Effect of supplementation with protein meal on the growth of cattle given a basal diet of untreated or ammoniated rice straw. *Asian-Aust. J. Anim. Sci.*, 3: 269-279.
- Roseler, D.K., J.D. Ferguson, C.J. Sniffen and J. Herrema, 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk non-protein in Holstein cows. *J. Dairy Sci.*, 76: 525.
- Samuel, M., S. Sagathewan, J. Thomas and G. Mathen, 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. *Indian. J. Anim. Sci.*, 67: 805.
- SAS., 1998. SAS/STAT User's Guide. Version 6.12. SAS Inst., Inc., Cary, NC., USA.

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- Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedure of Statistics. A Biometrical Approach. 2ndedn. McGraw- Hill Book Company, New York.
- Valdez, F.R., L.J. Bush, A.L. Goetsch and F.N. Owens, 1986. Effect of steroidal saponins on ruminal fermentation and on production of lactating dairy cows. *J. Dairy Sci.*, 69: 1568-1575.
- Veira, D.M., M. Ivan and P.Y. Jui, 1983. Rumen ciliate protozoa: Effects on digestion in the stomach of sheep. *J. Dairy Sci.*, 66: 1015-1022.
- Wallace, R.J., L. Arthaud and J.C. Newbold, 1994. Influence of *Yucca schidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. *Appl. Environ. Microbiol.*, 60: 1762-1767.
- Wanapat, M., 1985. Improving rice straw quality as ruminant feed by urea-treatment in Thailand. In: *Relevance of Crop Residues as Animal Feed in Developing Countries*. (Ed. M. Wanapat and C. Devendra), C. (Eds.), Funny Press, Bangkok, Thailand.
- Wanapat, M., 1990. Nutrition Aspect of Ruminant Production in Southeast Asia with Special Reference to Thailand. Funny Press, Ltd., Bangkok, Thailand. 217 pp.
- Wanapat, M., 1999. Feeding of Ruminants in The Tropics Based on Local Fed Resources. Department of Animal Science, Khon Kaen University, Khon Kaen 40002, Thailand.
- Wanapat, M. and O. Pimpa, 1999. Effect of ruminal NH₃-N levels on ruminal fermentation, purine derivatives, digest digestibility and rice straw intake in swamp buffaloes. *Asian-Aus. J. Anim. Sci.*, 12: 904-907.
- Wanapat, M. and O. Pongchompu, 2001. Method for Estimation of Tanin by Vanillin-HCL Method (A modified method of Burns, 1971). Department of Animal Science, Khon Kaen University, Khon Kaen 4002, Thailand.
- Wang, Y., T.A. McAllister, L.J. Yanke and P.R. Cheek, 2000. Effect of steroidal saponin from *Yucca schidigera* extract on ruminant microbes. *J. Appl. Microbiol.*, 88: 887-896.
- Williams, A.G. and G.S. Coleman, 1991. The Rumen Protozoa. 60, Springer-Verlag New York INC, New York, pp: 1762-1767.
- Wolkin, M.J. and T.L. Miller, 1988. Microbe-microbe interactions. In: *Rumen Microbial Ecosystem* (Ed. P.N. Honson), Elsevier Scientific Publishers, London, U.K., pp: 1762-1767.
- Zinn, R.A. and F.N. Owen, 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *J. Anim. Sci.*, 66: 157-166.