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# Effects of mangosteen peel (*Garcinia mangostana*) supplementation on rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake in beef steers

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## Abstract

Four rumen fistulated beef cattle steers were randomly assigned according to a 4 x 4 Latin square design. The treatments were ground mangosteen peel (MSP) supplementation at 0, 50, 100 and 150 gDM/hd/d with urea-treated rice straw (UTRS) fed *ad libitum*. Roughage and total dry matter intakes in terms of kg/d and %BW were slightly higher in 100 gDM/hd/d supplemented cattle. Apparent digestibility (%) of DM, OM, CP, NDF and ADF were similar among treatments. The values of ruminal temperature, pH, $NH_3$-N and BUN were not significantly affected (P>0.05) by MSP supplementation. MSP supplementation increased (P<0.05) the bacterial population , that was highest at 150 gDM/hd/d of MSP supplementation. The protozoal population was significantly decreased while fungal zoospore populations were not affected, and were highest for 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 were not significantly different (P>0.05), but were found to be highest at 100 gDM/hd/d MSP supplementation. These results suggest that MSP supplementation at 100-150 gDM/hd/d could be used as an alternative defaunating source in ruminants to improve feed efficiency and production. Based on these results, further research on levels and type of feed resources of supplementation should be investigated, particularly in feeding trials.

*Key words*: Saponins; Condensed tannins; Mangosteen; Rumen ecology; Protozoa; Bacteria; Fungi; Microbial protein synthesis; Local feed resources; Ruminants; Rice straw.

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## 1. 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, which leads to low digestibility and voluntary feed intake and an imbalance in the absorbed nutrients (protein to energy ratio, P/E). A consequence, growth, reproductive rate, and milk production are reduced ( Leng, 1990; Wanapat, 1999). It is essential to understand what happens when a feed is utilized by the ruminant. The feed conveyed into the rumen is digested by enzymes secreted by the

microorganisms living in the rumen. The microbes mainly are bacteria (~ $10^{10}$-$10^{12}$ cells/ml of rumen fluid), protozoa (~ $10^{4}$-$10^{6}$ cells/ml of rumen fluid) and fungi (~ 8% of total rumen microbes). However, bacteria are the major group to digest the feed before the host animal does. It has been reported that the elimination of the rumen protozoal population (defaunation) leads to improved animal performance (Leng, 1990). Morover, it has been shown that protozoa engulf bacteria (200 cells/minute or 1% bacteria/minute). As a result of protozoa activity, a significant reduction in the flow of microbial biomass to the small intestine has been found (Bird and Leng, 1978; Bird *et al.*, 1979; Hsu *et al*., 1991)

Several antiprotozoal agents have been used, such as copper sulfate, zinc, molybdenum, dioctyl sodium sulphosuccinate (Jouany *et al*., 1988), alcohol ethoxylate, alkanates (Bird *et al*., 1979) and calcium peroxide (Demeyer, 1982), in attempts to improve protein nutrition for ruminants, but widespread adoption of their use has been hampered by problems with toxicity, either to other ruminal microorganisms, or to the host animal. 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 on tropical diets (Dominguez Bello and Escobar, 1997).

Tropical plants normally contain high or medium contents of secondary compounds. Among these compounds are the saponins and condensed tannins, which have been shown to exert a specific effect against rumen protozoa while the rest of the rumen biomass remains unaltered (Lu and Jorgensen, 1987; Getachew *et al.*, 2000; Wang *et al.*, 2000). Numerous studies have been conducted to determine the effects of feeding ruminants with saponin-rich plants, such as alfalfa (Lu and Jorgensen, 1987; Klita *et al*., 1996), *Enterelobium cyclocarpum* (Navas-Camacho *et al*., 1993), *Spinadus saponaria* (Diaz *et al*., 1993), *Sapindus rarak* (Thalib *et al*., 1995), *Sesbania sesban* (Newbold *et al.*, 1997; Odenyo *et al*., 1997; Teferedegne *et al*., 1999), *Quillaja saponaria* and *Acacia auriculoformis* (Makkar *et al*., 1998) and *Yucca schidigera* (Wallace *et al*., 1994; Hristov *et al*., 1999;Wang *et al*., 1998, 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 mangostan*) peel has not been investigated.

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 feed digestibility using beef cattle steers.

## 2. Materials and methods

### *2.1 Location and duration*

This experiment was carried out on-station at the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand during July to November, 2004.

### *2.2. Animals and management*

Four ruminal fistulated steers were used (about 2-3 years of age; 200-250 kg of live weight).The animals were kept individually penned. Before start of the experiment, each of animal was dewormed with IVOMEC, and injected with $AD_3E$ 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.

*2.3. Experimental design and treatments*

A 4x4 Latin square design (LSD) was used, and there were 21 days in each period. The following treatments were applied:

T1 = Control (no mangsteen peel supplemented, MSP)
T2 = 50 gDM of MSP/hd/d
T3 = 100 gDM of MSP/hd/d
T4 = 150 gDM of MSP/hd/d

*2.4 Experimental feeds and feeding*

Urea-treated rice straw (UTRS) 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. MSPs were collected from fruits and were sun-dried and ground into powder form. Ground MSP was mixed with the concentrate before feeding according to treatment.

*2.5 Data collection, chemical analysis and samplings*

*2.5.1 Weight monitoring*

Live weight of each animal was weighed at the start and at the end of each period.

*2.5.2 Feed sampling*

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 while the second part 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 (Burns, 1971) as modified by Wanapat and Poungchompu (2001) and crude saponins were measured by using methanol extraction following the method of Kwon *et al*. (2002) as modified by Wanapat and Ngamsaeng (2004).

*2.5.3 Faecal sampling*

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 every day; the second part kept in a refrigerator and pooled at the end of each period for chemical analyses, similarly to the feed samples.

*2.5.4 Urine sampling*

Total urine was collected on the same days as faeces 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 $NH_3$-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).

*2.5.5 Blood sampling*

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).

*2.5.6 Rumen fluid sampling*

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. 50 ml of rumen fluid samples were collected and 5 ml of 2N $H_2SO_4$ 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 freezed at -20 ºC until analyzed in the laboratory for VFAs using High Performance Liquid Chromatography (HPLC; Model Water 600; UV detector, Millipore Crop.) according to the method of Samuel *et al*.(1997) for $NH_3$-N by the hypochlorite-phenol procedure (Beecher and Whitton, 1978).The subsequent rumen 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 was made using the procedure of Galyean (1989) by a haemacytometer (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 (121ºC for 15 minutes) as a medium by 100, 10 and 10 times, and counting using a 10x40, 10x10 and 10x40 occular x objective microscope for bacteria, protozoa and fungal zoospores, respectively.

*2.6 Statistical analysis*

The data were analyzed by Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure of the Minitab software version 13.31 (2000). Significance between means was tested using the least significant difference (LSD) according to the following model;$Yijk = \mu + T_i + C_j + R_k + e_{ijk}$

Where $Y_{ijk}$= The criteria under study, in treatment i; column j; row k, $\mu$= Overall sample mean, $T_i$ = Effect of treatment i , $C_j$ = Effect of treatment i at column j,

$R_k$ =Effect of treatment i at row k, $e_{ijk}$ = Error.

## 3. Results

*3.1 Characteristis of feedstuffs*

The composition of the concentrate and chemical composition of feeds and feedstuffs are shown in Tables1 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.

**Table 1.** Composition of concentrate feed

| **Ingredients** | **% in fresh basis** |
|---|---|
| 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 |
| Price; Baht/kg | 5.00 |

**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

*3.2 Voluntary feed intake and digestibility*

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 in than those supplemented with 0, 50 and 150 g/hd/d (Table3). However, response of MSP supplementation was not consistent. Apparent digestibility (%) of DM, OM, CP, NDF and ADF was not significantly different in all group ($P>0.05$). Body weight changes were not affected by MSP supplementation.

*3.3 Ruminal temperature, pH, ammonia-nitrogen ($NH_3$-N) and blood-urea nitrogen (BUN)*

Rumen ecology parameters, including temperature, pH, $NH_3$-N and BUN are presented in Table 4. Mean values of ruminal temperature, pH, $NH_3$-N and BUN were similar among treatments. Values were stable at 38 º C, 6.5 mg%, 13 mg% for temperature, pH and BUN, respectively. No effects of MSP supplemtation on $NH_3$-N concentrations were found, at 11.5 to 14.2 mg%, and was highest at 100 g/hd/d supplementation.

**Table 3.** Effect of mangosteen peel supplementation on voluntary feed intake and digestibility

| 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 |

**Table 4.** Effect of mangosteen peel supplementation on ruminal temperature, ruminal pH, ammonia nitrogen ($NH_3$-N) and blood urea nitrogen (BUN)

| 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/%** | | | | | |
| 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 |
| **BUN, mg/%** | | | | | |
| 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 |

*3.4 Ruminal microorganisms population*

Effects of MSP supplementation on ruminal microorganisms, total counts of bacteria, protozoa and fungal zoospore, as measured at 0, 2, 4 and 6 h post feeding, are shown in Table 5. Total bacteria and protozoal counts were significantly different at 2 and 4 h-post feeding. As shown, 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, but were highest at the MSP supplementation level of 100 g/hd/d. However, increasing the level of MSP supplementation tended to change the rumen microbial population.

**Table 5.** Effect of mangosteen peel supplementation on ruminal microorganism population

| **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, x $10^5$ cells/ml | | | | | |
| *Entodiniomorph* | | | | | |
| 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* | | | | | |
| 0 h- post feeding | 0.0 | 0.0 | 0.0 | 0.0 | 0.00 |
| 2 | 0.5 | 0.5 | 0.0 | 0.0 | 0.40 |
| 4 | 0.5 | 0.7 | 0.0 | 0.0 | 0.51 |
| 6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.00 |
| mean | 0.3 | 0.3 | 0.0 | 0.0 | 0.02 |
| *Total* | | | | | |
| 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 |
| Zoospore, x $10^6$ 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 |

*3.5 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 observed, 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.

**Table 6.** Effect of mangosteen peel supplementation on ruminal total volatile fatty acids (TVFAs), acetic acid (C2), propionic acid (C3), butyric acid (C4) and C2/C3 ratio

| Item | Mangosteen peel supplementation, g/hd/day | | | | SEM |
|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | |
| TVFAs, m mol/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 |
| C2, m mol/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 |
| C3, m mol/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 |
| C4, m mol/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 |
| C2/C3 | | | | | |
| 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 |

*3.6 Nitrogen balance*

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.

**Table 7.** Effect of mangosteen peel supplementation on nitrogen balance

| 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 |

*3.7 Urinary purine derivative excretion, microbial protein synthesis and P/E ratio*

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 is 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 waas 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 consistant.

**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

| Item | Mangosteen peel supplementation, g/hd/day | | | | SEM |
|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | |
| Allantoin, | | | | | |
| m mol/l | 7.6 | 8.1 | 8.6 | 8.0 | 0.50 |
| m mol/d | 20.7 | 22.8 | 26.6 | 22.8 | 3.26 |
| Total PD,[1/] | | | | | |
| m mol/d | 24.4 | 26.8 | 31.8 | 29.7 | 3.83 |
| m mol/kg $BW^{0.75}$ | 0.36 | 0.39 | 0.44 | 0.41 | 0.05 |
| PD absorption, m mol/d [2/] | 16.4 | 19.2 | 24.6 | 22.5 | 4.55 |
| Calculated microbial N synthesis,[3/] | | | | | |
| gN/d | 11.9 | 13.9 | 17.9 | 16.3 | 3.31 |
| gN/kgOMDR[4/] | 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[5/] | 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 |

[1/]Allantoin in urine cattle was 80-85% of total purine (IAEA, 1997); [2/]calculated of PD absorption (Verbic *et al*., 1990); [3/]calculated of microbial N synthesis (Chen *et al*., 1992); [4/]OMDR = organic matter digestible in the rumen was 65% of organic matter digestible in total tract. [5/]VFA production = 7.5 mol VFA/1 kg dry matter digested (Czerkawski,1986); [6/] $CH_4$ production = 0.5(acetate) – 0.25(propionate) + 0.5(butyrate) (Orskov *et al*., 1968).

## 4. Discussion

All materials mixed in the concentrate feed in this study were brought from the Khon Kaen Dairy Co-operative. The CP value in the concentrate as analyzed (10.3%) was lower than the calculated value (12%). This could be due to variations in the nutritive values of the ingredients. Furthermore, CP, NDF and ADF contents in urea-treated rice straw was similar to the value reported by Wanapat (1985). Moreover, the CT value in MSP (16.8%) was slightly higher than values reported, which ranged from 7-15% (wt/wt) (http://library.rits.ac.th/journal/542841.html). 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 *Pitheecellobium 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.

*4.1 Effecst MSP supplementation on voluntary feed intake and digestibility*

As shown, dry matter intake, and apparent digestibilities of DM, OM, CP, NDF and ADF were not affected by treatments. These data are in agreement with previous studies by Hristove *et al*.(1999), Eryavuz and Dehority (2004) and Mader and Brumm (1987). On the other hand, Diaz *et al*.(1993) and Klita *et al*.(1996) 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.

*4.2 Effect of MSP supplementation on rumen ecology parameters*

Ruminal temperature, pH, $NH_3$-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 (Gong and Forsberg, 1989; Minato *et al*., 1993; Pell and Schofield, 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 rumial ammonia concentration for efficient digestion is from 5.0 to 25.0 mg% (Preston and Leng, 1987) and 15 to 30 mg% (Perdok and Leng, 1990; 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. Diets, which are balanced in P/E have BUN concentrations of 12.7 mg%. BUN levels lower than this reference could be due to an insufficiency in CP per unit of digestible energy (Hwang *et al*., 2001).

*4.3 Effect MSP supplementation on ruminal microorganism population*

It appeared that MSP might play an important role in changing rumen microorganism populations as a result of CT and/or CS. As the results showed, the bacterial population increased, while protozoal population decreased when MSP was supplemented, although the fungal zoospore populations were only slightly changed. 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) found that steroidal saponins from *Y. schidigera* inhibit cellulolytic ruminal bacteria and fungi, but their effects on amylolytic bacteria are 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 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. Holotrichas are the most susceptible species to *E. ciclocarpum* (Navas *et al.*, 1992). 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 protozoa population was significantly reduced (84%), and total viable bacteria, cellulolytic bacteria and fungi significantly increased in a treatment which included 50 g of *S.saponaria* compared with a control (0 g of *S.saponaria*). Recently, research by Wina *et al.* (2003) 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 and found 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 protozoa 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 varied markedly both with diet and with feeding (Lu and Jorgensen, 1987; Navas-Camacho *et al*., 1993; Odenyo *et al*; 1997), which could result in variable findings. It appeared that MSP could be used as defaunating source.

*4.4 Effect MSP supplementation on volatile fatty acids production*

As shown, there were no significant effects (P>0.05) of feeding level of MSP on TVFAs and individual VFA concentration, and 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). Hristov *et al*. (1999) and 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* is apparently responsible for most of propionate production in the rumen arising from the randomizing pathway (Wolin and Miller, 1988). Wallace *et al*. (1994) and Wang *et al*. (2000) determined that growth of *S. ruminantium* was not affected by yucca saponins, whereas growth of some other rumen bacterial species (*Streptococus 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.

*4.5 Effect of MSP supplementation on nitrogen balance, urinary purine derivative excretion, microbial protein synthesis and P/E ratio*

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). The amino acid supply affects a large number of biological functions within the animal. The amount of protein absorbed relative to energy will be closely related to the level of production achieved.

## 5. Conclusions and recommendations

Based on this experiment, the following conclusions and recommendations can be made:

- Supplementation of MSP did not affect ruminal parameters, microbial protein synthesis, voluntary feed intake and apparent digestibility.
- Supplementation of MSP increased rumen bacterial and decreased protozoal population, but maintained fungal zoospore population.
- MSP supplementation at 100 g/hd/d could be an alternative defaunating strategy in ruminants, particularly for small-holder farmers, to improve feed efficiency and production.
- However, further studies should be conducted in production trials, especially with lactating cows.
- Moreover, the effects of condened tannins and/or saponins on specific microbial species in the rumen should be studies to explore its effect on rumen ecology.
- As MSP is fruit by-product and can be simply collected it is therefore recommended to use it as a dietary supplement to improve rumen ecology and ruminant productivity.

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