



# Mangosteen Peel Liquid-Protected Soybean Meal Can Shift Rumen Microbiome and Rumen Fermentation End-Products in Lactating Crossbred Holstein Friesian Cows

Kampanat Phesatcha<sup>1</sup>, Burarat Phesatcha<sup>2</sup> and Metha Wanapat<sup>3\*</sup>

<sup>1</sup> Department of Animal Science, Faculty of Agriculture and Technology, Nakhon Phanom University, Nakhon Phanom, Thailand, <sup>2</sup> Department of Agricultural Technology and Environment, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand, <sup>3</sup> Department of Animal Science, Faculty of Agriculture, Tropical Feed Resources Research and Development Center, Khon Kaen University, Khon Kaen, Thailand

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### \*Correspondence:

Metha Wanapat  
metha@kku.ac.th

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Rumen bypass protein can enhance protein availability in the lower gut. This study investigated the use of liquid-containing phytonutrients in dairy cows as a dietary additives to reduce rumen protein degradation. Four crossbred lactating Holstein Friesian cows (75% Holstein Friesian with 25% Thai native breed) with an initial body weight (BW) of  $410 \pm 20$  kg were randomly assigned to a  $2 \times 2$  factorial arrangement [two crude protein (CP) levels with soybean meal (SBM) or mangosteen peel liquid-protected soybean meal (MPLP)-SBM] in a  $4 \times 4$  Latin square design experiment. Dietary treatments were as follows: T1 = SBM in low crude protein concentrate (LPC) (SBM-LPC); T2 = MPLP-SBM in LPC (MPLP-SBM-LPC); T3 = SBM in high crude protein concentrate (HPC) (SBM-HPC); T4 = MPLP-SBM in HPC (MPLP-SBM-HPC). Apparent digestibilities of organic matter (OM) and neutral detergent fiber (aNDF) were increased ( $p < 0.05$ ) by CP level in the HPC diet (19% CP), with higher OM and aNDF digestibilities. High crude protein concentrate increased ( $p < 0.05$ ) the propionic acid in the rumen but reduced ( $p < 0.05$ ) the acetic acid-to-propionic acid ratio and methane (CH<sub>4</sub>) production. Rumen microbial populations of the total bacteria, *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens* were increased ( $p < 0.05$ ) by HPC. Real-time PCR revealed a 30.6% reduction of rumen methanogens by the MPLP-SBM in HPC. Furthermore, efficiency of microbial nitrogen synthesis (EMNS) was 15.8% increased ( $p < 0.05$ ) by the MPLP-SBM in HPC when compared to SBM-LPC. Milk yield and milk composition protein content were enhanced ( $p < 0.05$ ) by both the CP level in concentrate and by MPLP inclusion. In this experiment, a high level of CP and the MPLP-SBM enhanced the ruminal propionate, shifted rumen microbiome, and enhanced milk yield and compositions.

**Keywords:** rumen protected protein, microbiome, fermentation, lactating cows, soybean meal (SBM)

## INTRODUCTION

In the dairy industry, feed cost can contribute about 65–70% of production cost; therefore, improvement of feed utilization efficiency of milk production can have a significant impact on the profitability of dairy cow farming (1). Balancing protein requirement of both rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) could improve the supply of metabolizable protein and reduce the mobilization of the endogenous protein (2, 3). Protein utilization in lactating dairy cattle can improve rumen fermentation and reduce nitrogen loss, which would be beneficial to both animal stockholders and the people (4).

Soybean meal (SBM) is a highly degradable protein in the rumen and has a balance of highly available amino acids (5, 6). Furthermore, SBM is an excellent source of RDP and up to 65% of rumen degradability by rumen microbes (7). However, the excessive degradability of SBM may not be beneficial to high-producing dairy cows, since it increases urine nitrogen loss (6). Some of the treatment methods to reduce rumen degradability of SBM include extrusion, roasting, or expeller or the addition of lignosulfonate, xylose, or formaldehyde and the use of plant secondary compounds. These methods can increase RUP fraction of SBM up to 70% (8, 9). However, the use of phytonutrient to protect protein sources and enhance protein utilization and ruminant performance has very limited information (10, 11). Because of their natural characteristics as compared to chemical additives, phytonutrients condensed tannins (CTs) and saponins (SPs) are important ruminant feed additives, in particular for use as a CH<sub>4</sub> mitigation strategy and to improve the rumen volatile fatty acid (VFA) profiles (12, 13). Tannins are polyphenolic compounds with high affinity to proteins. Under typical ruminal conditions, these active components can form stable rumen complexes and protect dietary protein from degradation (4, 10). As a consequence of CT supplementation, decreasing protein degradation in the rumen can decrease rumen ammonia nitrogen (NH<sub>3</sub>-N) concentration. However, most of the tannin–protein complexes are required to be dissociated under the acid condition in abomasum, releasing both digestive and absorbent compounds dependent on the binding affinity (14, 15). Naumann et al. (16) stated that CT can decrease CH<sub>4</sub> synthesis in the rumen either directly or indirectly by decreasing methanogens or protozoal populations, respectively. The CTs have a direct effect on rumen methanogenic archaea by binding the proteinaceous adhesin or parts of the cell envelope, thereby impairing the formation of the methanogen protozoa complex, decreasing interspecies hydrogen transfer, and inhibiting methanogen growth. However, Ku-Vera et al. (17) reported that CTs operate as hydrogen sinks,

reducing their availability for carbon dioxide reduction to CH<sub>4</sub>. Makkar and Becker (18) found that SPs have the ability to form complexes with the lipid membranes of bacteria, increasing their permeability, causing an imbalance and, as a result, lysis of the bacterium. The majority of SPs have an effect on protozoa.

Mangosteen (*Garcinia mangostana*) peel is a tropical country agricultural by-product with 16.7% CT and 9.8% SP, which may inhibit some rumen microbes to minimize enteric CH<sub>4</sub> production. Mangosteen peel as a source of phytonutrients has the potential to be used in feeds for ruminants, with the benefit of reducing the production of CH<sub>4</sub> and biohydrogenation without adverse effects on ruminal pH and VFA but reduced methanogenic population in the rumen, thus likely reducing the production of CH<sub>4</sub> in beef cattle and swamp buffaloes (19, 20). The anti-methanogenic activities of CT are linked to a combination of direct toxicity on methanogenic archaea, decreased fiber degradation, or OM digestibility (21, 22). Wanapat et al. (23) stated that mangosteen peel supplementation at 100 g/head/day decreased numbers of methanogens, whereas it increased microbial protein synthesis in swamp buffaloes. Furthermore, Polyorach et al. (24) revealed that mangosteen peel supplementation in lactating dairy cows increased the total bacteria, while the protozoal and methanogen populations decreased. Furthermore, feeding non-protein nitrogen (NPN) such as urea in concentrate may enhance feed intake, digestibility, microbial protein production, and rumen fermentation efficiency, increasing the performance of ruminants fed low-quality roughages (25). Synchronization of ruminal ammonia and energy availability utilizing highly degradable carbohydrates in combination with easily available NPN sources such as urea has been shown to improve the productivity of ruminant though and increased the efficiency of utilization of NH<sub>3</sub>-N for microbial protein synthesis (25).

Rumen bypass protein is essentially required at the lower gut for digestion, absorption, and utilization by ruminants. A novelty of using mangosteen peel liquid (MPL) containing phytonutrients (CTs and SPs) was developed to protect SBM from rumen degradation and enhance the concentration of protein available in the lower gut. The hypothesis of the experiment was that mangosteen peel liquid-protected soybean meal (MPLP-SBM) could improve the nutrition of feed protein, thus improving milk yield and milk quality in lactating dairy cows. The objective was to determine the effect of MPLP-SBM on rumen fermentation, microbiomes, synthesis of microbial protein, and production of milk yield and composition in lactating dairy cows.

## MATERIALS AND METHODS

### Preparation of Mangosteen Peel Liquid-Protected Soybean Meal

Fresh mangosteen fruits were purchased from a local market in Muang Khon Kaen, Khon Kaen, Thailand. The fruit pulps were removed, and the peels were dried at 60°C for 48 h and ground to pass through a 1-mm sieve (Cyclotech Mill, Tecator, Hoganas, Sweden). The mangosteen peel meal was mixed with water at 1:5 ratio by adding 1 g of mangosteen peel meal into

**Abbreviations:** ADF, acid detergent fiber; BUN, blood urea nitrogen; BW, body weight; C<sub>2</sub>, acetic acid; C<sub>3</sub>, propionic acid; C<sub>4</sub>, butyric acid; CH<sub>4</sub>, methane; CP, crude protein; CT, condensed tannins; DM, dry matter; EMNS, efficiency of microbial nitrogen supply; FCM, fat-corrected milk; HPC, high crude protein concentrate; LPC, low crude protein concentrate; MCP, microbial crude protein; MPLP-SBM, mangosteen peel liquid-protected soybean meal; aNDF, apparent neutral detergent fiber; NH<sub>3</sub>-N, ammonia nitrogen; OM, organic matter; OMDR, organic matter digested in the rumen; MUN, milk urea nitrogen; PCR, polymerase chain reaction; RDP, rumen-degradable protein; RUP, rumen-undegradable protein; SBM, soybean meal; SP, saponins; TVFA, total volatile fatty acid.

5 ml of distillate water. After thorough mixing, it was drained to obtain the mangosteen peel extract solution. The solution was then sprayed onto SBM at 100 g, mixed well by a mixing rotary, and then oven-dried at 60°C for 12 h to obtain the MPLP-SBM. Representative samples of MPLP-SBM were collected and composited, and subsamples were chemically analyzed for dry matter (DM), organic matter (OM), crude protein (CP), apparent neutral detergent fiber (aNDF), acid detergent fiber (ADF), CT, and SP.

## Animals, Experimental Design, and Dietary Treatments

Four crossbred Holstein Friesian cows (75% Holstein Friesian with 25% Thai native breed) with initial body weight (BW) of  $410 \pm 20$  kg,  $125 \pm 24$  days in milk, and with initial milk yield of  $15 \pm 5$  kg/cow/day were used for the study. The experiment was evaluated in a  $4 \times 4$  Latin square design with four periods, each lasting 21 days, and dietary treatments were as follows: T1 = SBM in low crude protein concentrate (LPC) (SBM-LPC); T2 = MPLP-SBM in LPC (MPLP-SBM-LPC); T3 = SBM in high crude protein concentrate (HPC) (SBM-HPC); T4 = MPLP-SBM in HPC (MPLP-SBM-HPC). The concentrate diet was fed to each cow at the ratio of 1:2 of concentrate to milk yield, while rice straw was *ad libitum*. Feed ingredients and chemical compositions of concentrate and rice straw used in this experiment are presented in **Table 1**. Cows were kept in individual pens and were fed twice daily at milking time. There was clean fresh water and mineral blocks available all the time. Cows were weighed at the start and at the end of each period.

## Data Collection, Analysis, and Sampling Procedures

Feed, feces, and urine samples were collected during the last 7 days of each period. The feces were collected by rectal sampling, while urine sample was collected by spot sampling following manual stimulation of the vulva to stimulate urination. The urine samples were continuously kept by the addition of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to prevent volatilization of  $\text{NH}_3\text{-N}$ . One hundred milliliters of urine were sampled daily, filtered, and frozen to  $-18^\circ\text{C}$  for nitrogen analysis later.

The feeds and fecal samples were oven-dried at 60°C; each sample was ground to pass through a 1-mm sieve (Cyclotech Mill, Tecator, Hoganas, Sweden) and was chemically analyzed using the method of AOAC (26) for DM (ID 967.03) and ash (ID 942.05). The ANKOM fiber analyzer (ANKOM A200, ANKOM Technology, NY, USA) was used for determining aNDF and ADF. The ADF content was analyzed according to an AOAC (26) method (ID 967.03) and was expressed inclusive of residual ash; aNDF in samples was estimated according to Van Soest et al. (27) with addition of  $\alpha$ -amylase but without sodium sulfite, while acid-insoluble ash was measured according to Van Keulen and Young (28). Total nitrogen (N) was determined according to AOAC (26) (ID 984.13). Content of CT was analyzed by using the modified vanillin-HCl method; SPs were analyzed by using the modified vanillin-sulfuric acid method as described by Wanapat and Pongchompu (29).

Urine samples were chemically analyzed for allantoin and creatinine concentrations using high-performance liquid chromatography (HPLC; instruments by Water and Novapak model 600E; water mode 484 UV detector; column Novapak  $\text{C}_{18}$ ; column size  $3.9 \times 300$  mm; Waters Corporation, Milford, MA, USA) and were used to determine microbial purines and total protein of microbes (30). Microbial crude protein (MCP) (g/day) =  $3.99 \times 0.856 \times$  mmoles of purine derivatives excreted was determined by the method of Galo and Knapp (31). The efficiency of microbial nitrogen synthesis (EMNS) [g of N/kg of organic matter digested in the rumen (OMDR)], assuming that rumen digestion = 65% of OM digestible in total tract (32).

Milk production of all cows was collected every day. Milk samples were randomly collected twice per day in the morning at 5:00 a.m. and in the afternoon at 4:00 p.m. and mixed at 70:30 (from morning and afternoon milk collected) for analysis of fat, protein, lactose, total solids, and solids-not-fat content using Milko-Scan33 (Foss Electric, Hillerod, Denmark). Milk urea nitrogen (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO, USA).

On the last day of each sampling period, rumen fluid and jugular blood samples were collected at 0 and 4 h after feeding. Using a stomach tube connected to a vacuum pump, approximately 200 ml of rumen fluid was obtained from the rumen. Rumen fluid was immediately measured for temperature and pH using a portable pH meter (Hanna instrument HI 8424 microcomputer, Pte. Ltd., m 161 Kallang Way, Singapore) and then thoroughly filtered through four layers of cheesecloth. Samples were divided into three portions: first portion of rumen fluid was used for rumen  $\text{NH}_3\text{-N}$  analysis and total VFA using 5 ml of 1 M  $\text{H}_2\text{SO}_4$  mixed to 45 ml of rumen fluid, which was then centrifuged at  $16,000 \times g$  for 15 min, and the supernatant was kept at  $-20^\circ\text{C}$  before  $\text{NH}_3\text{-N}$  analysis (Kjeltech Auto 1030 Analyzer, Tecator, Höganäs, Sweden). The concentrations of rumen VFA profiles were analyzed using HPLC (instruments by Water and Novapak model 600E; water mode 484 UV detector; column Novapak  $\text{C}_{18}$ ; column size  $3.9 \times 300$  mm; mobile phase 10 mM  $\text{H}_2\text{PO}_4$ , pH 2.5; Waters Corporation, Milford, MA, USA) (33). According to Moss et al. (34), the calculation of ruminal  $\text{CH}_4$  production was based on using VFA proportions as follows:  $\text{CH}_4$  production =  $0.45 (\text{C}_2) - 0.275 (\text{C}_3) + 0.4 (\text{C}_4)$ .

A second portion of rumen fluid was kept with 10% formalin solution in sterilized 0.9% saline solution for the total direct counts of protozoa (35). The final portion of rumen fluid was preserved at  $-20^\circ\text{C}$  for extraction of deoxyribonucleic acid (DNA) (36).

Blood samples were collected from a jugular vein at each sampling time as for rumen fluid and kept into the tubes to which ethylene diamine tetra acetic acid was added. Samples were refrigerated for 1 h and then centrifuged at  $3,500 \times g$  for 20 min (Table Top Centrifuge PLC-02, Taipei, Taiwan). The plasma was removed and stored at  $-20^\circ\text{C}$  for the analysis of blood urea nitrogen (BUN) (37).

## Rumen Microbial Population

Community DNA was extracted using Yu and Morrison (36) method. The DNA was purified using columns from QIAGEN

DNA Mini Stool Kit (QIAGEN, Valencia, CA, USA). Standard PCR conditions for *Fibrobacter succinogenes* were as follows: 30 s at 94°C for denaturing, 30 s at 60°C for annealing, and 30 s at 72°C for extension (48 cycles). For the first cycle, 9-min denaturation time was used. In the last cycle, extension was for 10 min. Amplification of 16S rRNA for *Ruminococcus flavefaciens* and *Ruminococcus albus* was defined in a similar method, with the exception that the annealing temperature was set to 55°C according to Koike and Kobayashi (38). The isolation of genomic DNA was used in real-time quantitative PCR assays with power SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), forward and reverse primers, and template DNA. Specified primers were used to measure the microbial population of total bacteria according to Edwards et al. (39), *F. succinogenes*, *R. flavefaciens*, and *R. albus* (38), *Butyrivibrio fibrisolvens* and *Megasphaera elsdenii* (40), protozoa (41), and methanogenic archaea (42). The DNA standards Real-time PCR amplification and detection were determined using a Chromo 4™ system (Bio-Rad, CA, USA). The data of microbial population were transferred to log10 prior to statistical analysis.

## Statistical Analysis

The results were analyzed in a 4 × 4 Latin square design with 2 × 2 factorial arrangement of treatments with two CP levels (low and high) and two treatments (no treatment and MPLP treatment) using the mixed procedure of the SAS program (43). Data were analyzed using the model:

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

where  $Y_{ijk}$  is the observation from animal  $j$ , receiving diet  $i$ , in period  $k$ ;  $\mu$ , the overall mean;  $M_i$ , effect of treatment ( $i = 1-4$ );  $A_j$ , the effect of animal ( $j = 1-4$ );  $P_k$ , the effect of period ( $k = 1-4$ ); and  $e_{ijk}$ , the residual effect. Significance was declared at  $p < 0.05$ .

## RESULTS

### Feed Intake and Nutrient Digestibilities

There was no interaction effect between CP levels of concentrate diet and MPLP-SBM on total DM intakes and digestibilities of nutrients as presented in **Table 2**. The nutrient digestibilities of OM and aNDF were increased ( $p < 0.05$ ) by HPC (19% CP). Digestibility of CP was decreased ( $p < 0.05$ ) when cows were supplemented with MPLP-SBM. In contrast, no effects on digestibilities of DM and ADF by CP level and MPLP-SBM supplementation were found ( $p > 0.05$ ).

### Rumen Fermentation

There was no interaction effect between CP levels of concentrate diet and MPLP-SBM on ruminal pH, NH<sub>3</sub>-N concentration, rumen ecology, total VFA, and VFA profiles (**Table 3**). Protein levels of concentrate diet and MPLP-SBM supplementation did not reveal any effects on ruminal pH and ruminal temperature in dairy cow. Rumen NH<sub>3</sub>-N concentration and BUN were decreased with MPLP-SBM supplementation ( $p < 0.05$ ). The molar proportions of the respective VFA profiles revealed that there was no interaction between CP level of concentrate diet and MPLP-SBM on C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, and C<sub>2</sub>:C<sub>3</sub> ratio. Both CP levels of concentrate diet and MPLP-SBM increased total VFA and C<sub>3</sub>

**TABLE 1** | Chemical composition of concentrate and rice straw used in the experiment.

| Ingredients                 | Low crude protein |        | High crude protein |        | Rice straw |
|-----------------------------|-------------------|--------|--------------------|--------|------------|
|                             | - MPLP            | + MPLP | - MPLP             | + MPLP |            |
| <b>Feed ingredients</b>     |                   |        |                    |        |            |
| Cassava chip                | 500               | 500    | 490                | 490    |            |
| Rice bran                   | 180               | 180    | 180                | 180    |            |
| Untreated soybean meal      | 200               | -      | 200                | -      |            |
| MPLP soybean meal           | -                 | 200    | -                  | 200    |            |
| Palm kernel meal            | 70                | 70     | 70                 | 70     |            |
| Urea                        | 15                | 15     | 25                 | 25     |            |
| Molasses                    | 5                 | 25     | 5                  | 25     |            |
| Salt                        | 5                 | 5      | 5                  | 5      |            |
| Sulfur                      | 5                 | 5      | 5                  | 5      |            |
| Di-calcium                  | 5                 | 5      | 5                  | 5      |            |
| Premix                      | 5                 | 5      | 5                  | 5      |            |
| <b>Chemical composition</b> |                   |        |                    |        |            |
| Dry matter, g/kg            | 916               | 914    | 911                | 913    | 921        |
| Organic matter              | 946               | 958    | 949                | 948    | 893        |
| Crude protein               | 161               | 162    | 191                | 192    | 28         |
| Neutral detergent fiber     | 181               | 179    | 175                | 178    | 716        |
| Acid detergent fiber        | 156               | 154    | 150                | 153    | 514        |
| Condensed tannins           | 5                 | 18     | 6                  | 19     | -          |

MPLP, mangosteen peel liquid-protected treated.

( $p < 0.05$ ). However, CP level of concentrate diet decreased ( $p < 0.05$ ) the C<sub>2</sub>:C<sub>3</sub> ratio, while MPLP-SBM decreased the rumen CH<sub>4</sub> production ( $p < 0.01$ ).

### Ruminal Microbes

**Table 4** shows the effect of CP level and/or MPLP-SBM supplementation on the rumen microorganism population. There was no interaction effect between CP level in concentrate diet and MPLP-SBM supplementation on rumen microorganism population. Moreover, total bacteria, *F. succinogenes* and *B. fibrisolvens* populations were increased by both higher CP level in concentrate and presence of MPLP-SBM ( $p < 0.05$ ). Methanogens and protozoal population were decreased when cows were supplemented with MPLP-SBM ( $p < 0.05$ ).

### Microbial Protein Synthesis

There was no interaction effect between CP level in concentrate diet and MPLP-SBM supplementation on purine derivative, MCP, and EMNS, as shown in **Table 5**. The excretion of allantoin was not altered in any treatment ( $p > 0.05$ ). However, the absorption of allantoin and MCP were increased by MPLP-SBM supplementation ( $p < 0.05$ ). The allantoin absorption ranged from 261.4 to 315.2 g/day. In addition, the EMNS was increased in cows supplemented with MPLP-SBM ( $p < 0.05$ ).

### Milk Yield and Compositions

There was no significant interaction effect between CP level in concentrate diet and MPLP-SBM supplementation on milk yield



**TABLE 2** | Effect of crude protein level and mangosteen peel liquid-protected soybean meal on voluntary feed intake and apparent digestibility in lactating dairy cows.

| Items                            | Low crude protein |        | High crude protein |        | SEM   | p-Value |      |            |
|----------------------------------|-------------------|--------|--------------------|--------|-------|---------|------|------------|
|                                  | - MPLP            | + MPLP | - MPLP             | + MPLP |       | Pro     | MPLP | Pro × MPLP |
| <b>Dry matter intake, kg/day</b> |                   |        |                    |        |       |         |      |            |
| Rice straw                       | 3.4               | 3.7    | 3.6                | 3.7    | 0.071 | 0.16    | 0.56 | 0.84       |
| Concentrate                      | 8.9               | 9.5    | 10.5               | 11.0   | 0.584 | 0.55    | 0.54 | 0.58       |
| Total                            | 12.3              | 13.2   | 14.1               | 14.7   | 0.069 | 0.13    | 0.73 | 0.66       |
| <b>Apparent digestibility</b>    |                   |        |                    |        |       |         |      |            |
| Dry matter                       | 0.641             | 0.644  | 0.653              | 0.662  | 0.032 | 0.47    | 0.09 | 0.72       |
| Organic matter                   | 0.651             | 0.652  | 0.670              | 0.665  | 0.014 | 0.04    | 0.12 | 0.65       |
| Crude protein                    | 0.638             | 0.610  | 0.642              | 0.613  | 0.028 | 0.10    | 0.04 | 0.09       |
| aNeutral detergent fiber         | 0.581             | 0.590  | 0.616              | 0.609  | 0.025 | 0.04    | 0.09 | 0.08       |
| Acid detergent fiber             | 0.442             | 0.454  | 0.439              | 0.456  | 0.014 | 0.13    | 0.51 | 0.66       |

Pro, protein level; MPLP, mangosteen peel liquid-protected treated; Pro × MPLP, protein level × mangosteen peel liquid-protected treated; SEM, standard error of the mean. aNDF, neutral detergent fiber; Neutral detergent fiber, amylase-treated neutral detergent fiber.

**TABLE 3** | Effect of crude protein level and mangosteen peel liquid-protected soybean meal on ruminal fermentation and blood urea nitrogen in lactating dairy cows.

| Items   | Low crude protein |        | High crude protein |        | SEM   | p-Value |      |            |
|---|-------------------|--------|--------------------|--------|-------|---------|------|------------|
|   | - MPLP            | + MPLP | - MPLP             | + MPLP |       | Pro     | MPLP | Pro × MPLP |
| <b>Ruminal fermentation</b>                   |                   |        |                    |        |       |         |      |            |
| pH  | 6.7               | 6.8    | 6.8                | 6.8    | 0.564 | 0.62    | 0.41 | 0.54       |
| Temperature, °C                               | 39.7              | 39.6   | 39.5               | 39.6   | 0.311 | 0.42    | 0.75 | 0.18       |
| NH <sub>3</sub> -N, mg/dl                     | 13.9              | 12.1   | 17.1               | 15.0   | 0.791 | 0.02    | 0.04 | 0.09       |
| Blood urea nitrogen, mg/dl                    | 14.7              | 12.6   | 18.1               | 16.8   | 0.609 | 0.04    | 0.02 | 0.41       |
| <b>VFA</b>                                    |                   |        |                    |        |       |         |      |            |
| Total VFA, mmol/l                             | 106.7             | 110.6  | 111.6              | 118.7  | 0.508 | 0.04    | 0.04 | 0.71       |
| Acetic acid (C <sub>2</sub> ), mol/100 mol    | 64.8              | 64.4   | 65.4               | 64.6   | 0.971 | 0.12    | 0.11 | 0.88       |
| Propionic acid (C <sub>3</sub> ), mol/100 mol | 23.9              | 25.7   | 24.2               | 26.0   | 0.633 | 0.21    | 0.02 | 0.09       |
| Butyric acid (C <sub>4</sub> ), mol/100 mol   | 11.3              | 9.9    | 10.4               | 9.4    | 0.442 | 0.32    | 0.02 | 0.68       |
| p-Value                                       | 2.7               | 2.5    | 2.7                | 2.5    | 0.194 | 0.14    | 0.03 | 0.65       |
| Methane, mM/l <sup>a</sup>                    | 27.1              | 25.9   | 26.9               | 25.6   | 0.709 | 0.08    | 0.03 | 0.21       |

Pro, protein level; MPLP, mangosteen peel liquid-protected treated; Pro × MPLP, protein level × mangosteen peel liquid-protected treated; SEM, standard error of the mean.

<sup>a</sup>Calculated according to Moss et al. (34).

Methane production = 0.45 (acetate) - 0.275 (propionate) + 0.4 (butyrate).

and compositions (Table 6). Milk yield and 3.5% FCM yield were found the highest in cows supplemented with MPLP-SBM in concentrate containing high crude protein. Moreover, milk compositions including fat, lactose, solids-not-fat, total solids, and MUN were not affected ( $p > 0.05$ ) by CP level and MPLP-SBM supplementation, but milk protein was increased ( $p < 0.05$ ) in the high crude protein level in concentrate diet.

## DISCUSSION

### Feed Intake and Nutrient Digestibilities

Dietary CP concentration in ruminant rations is an important factor supporting growth and lactation. Colmenero and

Broderick (44) reported that increased level of CP resulted in an improvement of CP and ADF digestibilities ( $p < 0.05$ ), while Dung et al. (45) found that HPC increased CP digestibility but had no effect on DM, OM, and NDF digestibilities. In the present study, the nutrient digestibilities of OM and aNDF were increased ( $p < 0.05$ ) by the HPC (19% CP).

Mangosteen peel liquid used in this study contained CT 16.9% and SP 9.6%, which was comparable to that of Wanapat et al. (23) who reported that mangosteen peel contained CT 17.9% and SP 9.2%. Under this trial, MPLP-SBM did not change the DM intakes. Manasri et al. (46) found when supplemented CT from mangosteen peel at 0.12 g/head/day did not have an effect on DM intake in beef cattle, while Polyorach et al. (24) also found

**TABLE 4** | Effect of crude protein level and mangosteen peel liquid-protected soybean meal on rumen microbial population in lactating dairy cows.

| Items   | Low crude protein |        | High crude protein |        | SEM   | p-Value |       |            |
|---|-------------------|--------|--------------------|--------|-------|---------|-------|------------|
|   | - MPLP            | + MPLP | - MPLP             | + MPLP |       | Pro     | MPLP  | Pro × MPLP |
| <b>Direct count, cell/ml</b>                  |                   |        |                    |        |       |         |       |            |
| Protozoa, ×10 <sup>5</sup>                    | 7.2               | 4.9    | 7.8                | 5.3    | 0.981 | 0.642   | 0.019 | 0.438      |
| <b>Real-time PCR, copies/ml rumen content</b> |                   |        |                    |        |       |         |       |            |
| Total bacteria, ×10 <sup>8</sup>              | 0.7               | 4.6    | 7.7                | 9.8    | 0.210 | 0.02    | 0.02  | 0.33       |
| <i>F. succinogenes</i> , ×10 <sup>7</sup>     | 2.6               | 3.4    | 6.8                | 7.5    | 0.135 | 0.02    | 0.02  | 0.41       |
| <i>R. flavefaciens</i> , ×10 <sup>6</sup>     | 4.7               | 4.4    | 5.7                | 5.2    | 0.047 | 0.72    | 0.51  | 0.22       |
| <i>R. albus</i> , ×10 <sup>7</sup>            | 3.5               | 4.1    | 4.6                | 5.4    | 0.065 | 0.68    | 0.31  | 0.42       |
| <i>B. fibrisolvens</i> , ×10 <sup>6</sup>     | 2.1               | 3.2    | 5.5                | 6.1    | 0.081 | 0.02    | 0.02  | 0.12       |
| <i>M. eleinii</i> , ×10 <sup>7</sup>          | 3.5               | 4.1    | 4.6                | 5.4    | 0.042 | 0.36    | 0.05  | 0.71       |
| Methanogens, ×10 <sup>3</sup>                 | 5.7               | 3.2    | 4.9                | 3.4    | 0.059 | 0.41    | 0.03  | 0.16       |
| Protozoa, ×10 <sup>4</sup>                    | 4.8               | 3.2    | 5.1                | 3.6    | 0.198 | 0.71    | 0.03  | 0.53       |

Pro, protein level; MPLP, mangosteen peel liquid-protected treated; Pro × MPLP, protein level × mangosteen peel liquid-protected treated; SEM, standard error of the mean.

**TABLE 5** | Effect of crude protein level and mangosteen peel liquid-protected soybean meal on microbial protein synthesis in lactating dairy cows.

| Items                         | Low crude protein |        | High crude protein |        | SEM   | p-Value |      |            |
|-------------------------------|-------------------|--------|--------------------|--------|-------|---------|------|------------|
|                               | - MPLP            | + MPLP | - MPLP             | + MPLP |       | Pro     | MPLP | Pro × MPLP |
| Purine derivatives, mmol/day  |                   |        |                    |        |       |         |      |            |
| Allantoin excretion           | 236.9             | 254.4  | 261.9              | 272.7  | 3.198 | 0.43    | 0.71 | 0.42       |
| Allantoin absorption          | 261.4             | 283.1  | 287.7              | 315.2  | 2.654 | 0.07    | 0.04 | 0.06       |
| Urine creatinine              | 26.2              | 26.5   | 27.3               | 28.6   | 0.614 | 0.06    | 0.05 | 0.05       |
| MCP <sup>a</sup> , g/day      | 702.4             | 720.3  | 735.7              | 764.6  | 1.637 | 0.03    | 0.01 | 0.21       |
| EMNS <sup>b</sup> , g/kg OMDR | 25.9              | 27.1   | 28.4               | 32.9   | 0.879 | 0.04    | 0.01 | 0.41       |

Pro, protein level; MPLP, mangosteen peel liquid-protected treated; Pro × MPLP, protein level × mangosteen peel liquid-protected treated, SEM, standard error of the mean.

<sup>a</sup>Microbial crude protein (MCP) (g/day) = 3.99 × 0.856 × mmol of purine derivatives excreted (31).

<sup>b</sup>Efficiency of microbial nitrogen supply (EMNS), g/kg of organic matter (OM) digested in the rumen (OMDR) = {[MCP (g/day) × 1,000]/OMR (g)}, assuming that rumen digestion = 65% of digestion in total tract.

supplementation of MSP at 300 g/head/day with YEFECAP as a protein source in concentrate mixture remarkably improved the microbial fermentation efficiency. Supplementation of mangosteen peel at 300 g/head/day remarkably improved the microbial fermentation efficiency in dairy cows. However, when high CT was used, the apparent digestibility of nutrients was reduced ( $p < 0.05$ ) (4, 19). Dschaak et al. (47) showed that the supplementation of 3% quebracho CT resulted in reduced DM intake, while the production of milk and milk composition were unchanged. The variety and concentration of phytonutrients of the plant may also be due to many effects including type and growth stages. There were no significant interaction effects of the two factors found on DM intakes and nutrient digestibilities under this experiment.

## Rumen Fermentation

Ruminal temperature and pH remained unchanged among the dietary treatments, and both were in optimum range (pH 6.5–7.0)

especially for fiber digestion (48). In the present study, increasing the dietary CP level increased ( $p < 0.05$ ) the concentration of NH<sub>3</sub>-N. Similarly, Xia et al. (49) reported that the NH<sub>3</sub>-N concentration of the bulls receiving the high crude protein level was significantly higher than those receiving the low crude protein diet. In contrast, Bahrami-Yekdangi et al. (50) observed that the concentration of NH<sub>3</sub>-N diet was unchanged.

The concentration of NH<sub>3</sub>-N was reduced ( $p < 0.05$ ) by MPLP-SBM. This could be due to increased microbial protein synthesis that can consequently reduce the NH<sub>3</sub>-N concentration in the rumen. However, CTs have a high capacity for CP binding in the rumen and would reduce dietary protein loss by ammonia production, thus improving protein utilization (22). El-Waziry et al. (51) found that the concentration of ruminal NH<sub>3</sub>-N was decreased with protected SBM. Reducing NH<sub>3</sub>-N in the rumen means that treated SBM reduced peptide degradation, proteolysis, and amino acid deamination in the rumen (52). Both factors exhibited significant effects, but there were no

**TABLE 6** | Effect of crude protein level and mangosteen peel liquid-protected soybean meal on milk yield and composition in lactating dairy cows.

| Items              | Low<br>crude protein |           | High<br>crude protein |           | SEM   | p-Value |      |            |
|--------------------|----------------------|-----------|-----------------------|-----------|-------|---------|------|------------|
|                    | -<br>MPLP            | +<br>MPLP | -<br>MPLP             | +<br>MPLP |       | Pro     | MPLP | Pro × MPLP |
| Production         |                      |           |                       |           |       |         |      |            |
| Milk yield, kg/day | 17.8                 | 19.2      | 21.8                  | 23.5      | 0.886 | 0.039   | 0.04 | 0.41       |
| 3.5% FCM, kg/day   | 18.6                 | 21.1      | 24.3                  | 26.2      | 0.879 | 0.010   | 0.04 | 0.49       |
| Fat                | 3.8                  | 4.1       | 4.2                   | 4.2       | 0.418 | 0.195   | 0.29 | 0.61       |
| Protein            | 3.1                  | 3.4       | 3.7                   | 3.8       | 0.154 | 0.020   | 0.13 | 0.07       |
| Lactose            | 4.6                  | 4.7       | 4.7                   | 4.7       | 0.104 | 0.187   | 0.36 | 0.12       |
| Solids non-fat     | 9.2                  | 9.1       | 9.2                   | 9.3       | 0.207 | 0.081   | 0.45 | 0.33       |
| Total solids       | 13.8                 | 14.1      | 14.3                  | 14.0      | 0.388 | 0.652   | 0.51 | 0.21       |
| Milk urea N, mg/dl | 12.4                 | 12.6      | 12.8                  | 13.1      | 0.547 | 0.174   | 0.19 | 0.29       |

Pro, protein level; MPLP, mangosteen peel liquid-protected treated; Pro × MPLP, protein level × mangosteen peel liquid-protected treated; SEM, standard error of the mean; FCM, fat-corrected milk.

interactions. Ampapon et al. (20) stated that plant phytonutrients such as CT exhibited selective suppression of cellulolytic bacteria in the rumen. Due to the complexation of protein–tannin, MPLP-SBM supplementation in the diet has beneficial effects, reducing the availability of feed protein for possible ruminal degradation to produce ammonia–nitrogen. The concentrations of total VFA and C<sub>3</sub> were increased ( $p < 0.05$ ) by protein level or by MPLP inclusion. Wanapat et al. (23) reported that the mangosteen peel supplementation in buffaloes impacted total rumen VFA production and increased the C<sub>3</sub> concentration, while reducing C<sub>2</sub>:C<sub>3</sub> and the production of CH<sub>4</sub>. Additionally, Polyorach et al. (24) also demonstrated that the use of CT from mangosteen powder could increase total VFA concentration especially C<sub>3</sub>. Moreover, under the present study, CH<sub>4</sub> production was reduced ( $p < 0.05$ ) by MPLP-SBM supplementation. These results could be due to the suppression of methanogens that adhered to protozoa in the rumen for additional activity. Pongchompu et al. (19) revealed that plants containing CT and SP greatly improved ruminal feed degradation by mitigating the C<sub>2</sub> concentration and CH<sub>4</sub> production, hence enhancing the C<sub>3</sub> concentration. Recently, Ampapon et al. (20) revealed that supplementing mangosteen peel could reduce the production of CH<sub>4</sub> by suppressing ruminal protozoa. However, the results of mangosteen peel supplementation did not change total VFA and individual VFA as reported by Ngamsaeng et al. (53). Many previous studies have stated that CT and SP or their extracts were effective in reducing CH<sub>4</sub> production in both *in vitro* and *in vivo* studies (54, 55). Only the MPLP supplementation resulted in a reduction ( $p < 0.05$ ) of C<sub>2</sub>:C<sub>3</sub> ratio and CH<sub>4</sub> production, and no interactions were found.

## Ruminal Microbes

The populations of cellulolytic bacteria, *F. succinogenes* and *B. fibrisolvans* were increased ( $p < 0.05$ ), while those of *M. elsdenii* were not changed ( $p > 0.05$ ) by the MPLP-SBM. The populations of *R. albus* and *R. flavefaciens* were not changed while methanogen was decreased ( $p < 0.05$ ) when cows were

supplemented with MPLP-SBM. Dong et al. (56) found that *Moringa oleifera* containing CT altered the composition and diversity of methanogens, hence mitigating CH<sub>4</sub> emissions in dairy cows. However, Anantasook et al. (57) revealed that the population of *F. succinogenes* was increased when dairy cows were fed with *Samanea saman* (rain tree pod meal) containing CT and SP possibly as an effect of suppression of protozoa and methanogen numbers. Norrapoke et al. (58) described the fibrolytic bacteria species in the rumen and noted that *R. flavefaciens*, *R. albus*, and *F. succinogenes* to a higher extent degraded crystalline cellulose more effectively than the ruminococcal species (38). In the present study, methanogens were significantly reduced ( $p < 0.05$ ) when dairy cows were supplemented with MPLP-SBM. Wanapat et al. (23) stated that mangosteen peel supplementation at 100 g/head/day in swamp buffaloes increased the total number of rumen bacteria and *R. flavefaciens*, while methanogens were decreased ( $p < 0.05$ ). Both factors under this investigation showed significant effects on total bacteria, *F. succinogenes* and *B. fibrisolvans*, but no interaction effects between the CP level and MPLP-SBM were obtained.

## Microbial Protein Synthesis

In the present study, CP in concentrate diet and MPLP-SBM did not affect allantoin excretion and urine creatinine in milking dairy cows. Zhang et al. (4) stated that CT supplementation in milking dairy cows altered the N excretion route, led to less urinary N excretion, but failed to alter the N utilization efficiency for milk production. Under this work, only a high level of CP in concentrate impacted MCP. Most of the proteins supplied to the small intestine of ruminants could be supplied by microbial protein synthesis in the rumen, comprising 50–80% of overall absorbable protein (59), while MCP in the present study ranged from 702.4 to 764.6 g/day. Furthermore, high crude protein supplementation with MPLP-SBM inclusion resulted in the highest MCP among the treatments. Wanapat et al. (23) noted that when total dietary N intake was low, urea supplementation enhanced the EMNS. Higher amount of

urea recycling could enhance microbial protein synthesis, hence the EMNS, accordingly (60). Wanapat et al. (23) stated that ruminal microbial CP synthesis is primarily dependent on the sufficiency of carbohydrates as an energy source and the available NPN. Also, lactating dairy cows supplemented with CT resulted in improved EMNS (24). Both the ruminal dietary protein degradation and the synchrony of nitrogen and energy balance will affect the effectiveness of any dietary supplement, including CT supplements, on the EMNS (61, 62). Among the parameters MCP and EMNS were significantly increased by both factors, and no interactions were found.

## Milk Yield and Compositions

In the present study, increasing the dietary CP level resulted in improved milk yield. This finding is consistent with Law et al. (63) who found increases in milk yield as dietary CP was increased. However, Xia et al. (49) revealed that milk yield did not differ across CP levels in the diet.

Milk yield and milk protein were significantly increased with the supplementation of MPLP-SBM. The CT's MPLP-SBM content may interact with SBM to produce a CT-protein bound complex that would prevent the digestion of dietary protein in the rumen with higher dietary protein flow to the duodenum that could be achieved for the dairy cows (21). Anantasook et al. (57) stated that supplementation with CT from rain tree pod meals resulted in a higher milk protein and solids non-fat when compared with the control. Broderick et al. (64) suggested that silage containing 0.5% of CT improved milk yield and milk protein. Tannins have the potential to increase RUP in a diet, and when combined with high-quality protein that contains a significant amount of limiting amino acid like methionine and lysine, this could lead to an improvement in N efficiency and milk production by increasing the flow of an adequate supply of amino acid to the small intestine (11). In addition, Polyorach et al. (24) found that mangosteen peel supplementation could enhance DM intake and digestibility of nutrients and improve the fermentation process, milk yield, and composition in lactating dairy cows. However, Benchaar et al. (65) noted that when plants rich in tannins were supplemented in the diets of dairy cows, milk composition remains unchanged. Both factors resulted in significant enhancement of milk yield and 3.5% FCM, but there were no interactions found.

Blood urea nitrogen has been shown to be an indicator of the metabolism of nitrogen in ruminants, and higher BUN can imply greater degradation of the ruminal protein (66). The concentration of BUN and MUN were not changed among

treatments, and the values were in the normal range. Roseler et al. (67) and Jonker et al. (68) reported the normal ranges of 15 mg/dl for MUN and 5–6 mg/dl for BUN.

## CONCLUSIONS

High crude protein level in concentrate and MPLP-SBM supplementation enhanced rumen  $C_3$ , but reduced  $C_2:C_3$  ratio and  $CH_4$  production. Total bacterial numbers and *F. succinogenes* population were increased ( $p < 0.05$ ) with high protein level in concentrate and MPLP-SBM supplementation. Allantoin absorption was higher with MPLP-SBM supplementation, while protein content influenced MCP and EMNS with higher values ( $p < 0.05$ ) in the high CP level in concentrate. Importantly, milk yield and 3.5% FCM were remarkably enhanced ( $p < 0.05$ ) by the MPLP-SBM with HPC. Hence, it is recommended that MPLP treatment of SBM should be exploited as a feeding strategy to improve milk yield and compositions in lactating dairy cows.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The animal study was reviewed and approved by the Khon Kaen University Animal Ethics Committee.

## AUTHOR CONTRIBUTIONS

KP and MW designed the research. KP and BP conducted the research. KP analyzed the data and wrote the manuscript. All authors approved the final manuscript.

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