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Original Research Article

Phytonutrient pellet supplementation enhanced rumen fermentation efficiency and milk production of lactating Holstein-Friesian crossbred cows

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ABSTRACT

The objective of this experiment was to investigate the effects of inclusion of dragon fruit peel pellet (DFPP) and dietary non-protein nitrogen (NPN) on nutrients digestibility, rumen fermentation efficiency, plasma antioxidant activity, microbial protein synthesis, milk yield and composition in lactating Holstein-Friesian crossbred cows. Four animals were randomly allotted to 4 dietary treatments according to a 2 × 2 factorial arrangement in 4 × 4 Latin square design. The treatments were as follows: 300 g DM of DFPP +100 g of urea (T1), 300 g DM of DFPP + 200 g of urea (T2), 400 g DM of DFPP + 100 g of urea (T3), and 400 g DM of DFPP + 200 g of urea (T4), respectively. The results showed that intake of rice straw was increased ($P < 0.01$) by the DFPP addition. Including DFPP and urea did not affect ($P > 0.05$) the NDF and ADF digestibilities, but increased the apparent digestibilities of dry matter, organic matter, and crude protein ($P < 0.01$). Rumen fermentation process, especially the propionate concentration, was significantly increased by the DFPP levels. The plasma antioxidant activity was increased ($P > 0.05$) with the addition of DFPP. The DFPP improved ($P < 0.01$) microbial protein synthesis. The supplementation of DFPP and urea increased ($P < 0.05$) milk fat, whereas milk yield and 3.5% fat corrected milk were only increased ($P < 0.05$) by the DFPP supplementation. Based on these results, addition of DFPP at 400 g/animal per day with urea at 100 g/animal per day improved rumen fermentation, plasma antioxidant activity, milk yield and milk fat percentage.

1. Introduction

Industrial fruit processing has been increasingly important to avail fruit-products for human consumption. The use of fruit-wastes has been receiving more attention as they contain phytonutrients (PTN) sources (Ibrahim et al., 2017). Phytonutrients are a major group of secondary metabolites commonly found in fruit peels and wastes especially the phenolic compounds (PC), condensed tannins (CT), and saponins (SP), which play a vital role in animal health and nutrition (Abbas et al., 2017). Furthermore, these compounds exert impacts on antioxidant activity which can prevent the availability of free radicals (Borges et al., 2010). Free radicals and reactive oxygen species are continuously formed in the body of animal (Aruoma, 1998). Generally, animals are naturally protected against reactive oxygen species or free radicals via various natural antioxidant enzymes. However, animals from tropical areas are inclined to oxidative stress due to prolonged exposure to high temperatures (Salles et al., 2010). In ruminant feeding, there has been a growth of interest in the study of oxidative stress determined as an imbalance between oxidants and antioxidants to understand the function of oxidant and antioxidant molecules in physiological conditions (Lykkesfeldt and Svendsen, 2007).

Dragon fruit (*Hylocereus undatus*) peel is one of the alternative sources of plant containing antioxidants, especially PC that is non-

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toxic and biologically safe. A current study has shown that antioxidant addition in the ruminant diet could alleviate oxidative stress status (Rizzo et al., 2013) and increase plasma antioxidant capacity (Tian et al., 2019). Moreover, these plants contain phytonutrients such as CT and SP, with the specific effect of improving milk profile, nitrogen efficiency in dairy cows (Frutos et al., 2004), enhancing rumen fermentation (Kang et al., 2014; Cherdthong et al., 2019), as well as a protective effect on ruminal protein in order to promote duodenal utilization and modify the volatile fatty acid pattern in the rumen (Salem et al., 2014). However, dietary non-protein nitrogen (NPN) supplementation has been shown to enhance the crude protein level of low quality agro-industrial by-products (Foiklang et al., 2016). Nevertheless, not much research has been carried out on the use of dragon fruit peel and NPN in in vivo trials especially in lactating dairy cows.

Hence, this present experiment investigates the effect of dragon fruit peel pellet (DFPP) as a source of PTN in combination with NPN on nutrient digestibility, rumen fermentation efficiency, plasma antioxidant capacity, oxidative stress, microbial protein synthesis, milk yield and composition in lactating Holstein-Friesian crossbred cows.

2. Materials and methods

2.1. Animal care and management

All Holstein-Friesian crossbred cows were approved by the Institute of Animals for Scientific Purpose Development (IAD) of Thailand (protocol no. U1-06878-2560). Dairy cows were injected with vitamin AD3E and drenched with ivermectin (1 mL per 50 kg live weight) before imposing the respective treatments. Animals were housed in a ventilated barn and in individual pens (3 m × 4 m). Mineral blocks and clean drinking water were available freely.

2.2. Dragon fruit peel pellet preparation

The fresh peels were obtained from a fruit canning factory in Thailand. The fruit peels were dried in an oven at 60 °C for approximately 3 d and were ground (Cyclotech Mill, Tecator, Höganäs, Sweden) then the dragon fruit powder was mixed with ingredients (Table 1) to pellet form by using a Ryuzo-kun pelleter (Kakiuchi Co., Ltd., Nankoku, Kochi, Japan).

2.3. Experimental procedure

Four Holstein-Friesian crossbred cows were used in a 2 × 2 factorial in 4 × 4 Latin square design. Pre-experimental live weights were 525 ± 25 kg, 123 ± 21 d-in-milk (DIM), and an average milk yield of 13.8 ± 1.8 kg/animal per day. The study lasted for 84 d, with four 21-d experimental periods; the first 14-d for adaptation to dietary treatments and last 7 d for sample collection. Animals were fed concentrate to milk yield ratio at 1:1.5 and rice straw was fed at free choice. Cows were hand milked at 06:00 and 16:00.

2.4. Dietary treatments

The dietary treatments were two factors of DFPP, fed at 300 and 400 g DM/animal per day, and urea at 100 and 200 g/animal per day. Thus, the factorial arrangement of treatments were as follows: 300 g DM of DFPP +100 g of urea (T1), 300 g DM of DFPP + 200 g of urea (T2), 400 g DM of DFPP + 100 g of urea (T3), and 400 g DM of DFPP + 200 g of urea (T4), respectively. The DFPP and urea supplementation were top-dressed on the concentrate, manually mixed and fed to animals.

2.5. Chemical composition of feeds

The DFPP and concentrate mixture were prepared following the respective ingredients shown in Table 1. The concentrate mixture (14% CP and 78% TDN) containing 55.0% cassava chip, 19.0% soybean meal, 10.5% dried brewer's grains, 10.0% rice bran meal, 4.0% molasses, 1.0% mineral premix, 0.5% sulfur was then mixed well. The dry matter (DM; No. 967.03), ash (No. 942.05), crude protein (CP; No. 984.13) in the samples were chemically analyzed by using the method of AOAC (2012; No. 973.18), and fiber contents were determined using Ankom A200i Fibre Analyser (Ankom Technology Co., New York, USA) (Van Soest et al., 1991). Furthermore, the DFPP was analyzed for SP content by using reflux distillation method (Wang and Fang, 2004), and for CT following the modified method

Table 1
Ingredients and chemical composition of the feeds.

Item	Concentrate	Rice straw	Dragon fruit peel powder	Dragon fruit peel pellet
Ingredients, % as fed				
Cassava chip	55.0			–
Soybean meal	19.0			–
Dried brewers' grains	10.5			–
Rice bran meal	10.0			–
Molasses	4.0			1.0
Sulfur	0.5			1.0
Mineral premix[1]	0.5			1.0
Urea	–			1.0
Salt	–			1.0
Dragon fruit peel powder	–			90.0
Cassava starch	–			5.0
Chemical composition, % dry matter				
Dry matter, % as fed	87.7	90.6	90.4	86.5
Ash	4.9	12.3	1.5	1.4
Crude protein	13.9	3.0	5.4	9.7
Neutral-detergent fiber	17.5	71.3	33.0	17.8
Acid-detergent fiber	10.1	55.5	29.6	14.5
Condensed tannins	–	–	6.9	6.2
Saponins	–	–	8.9	8.1
Total phenolic content, mg/100 g	–	–	35.4	34.2

[1] One kilogram of mineral premix contains the following content: vitamin A 10,000,000 IU; vitamin D 1,600,000 IU; vitamin E 70,000 IU; Fe 50 g; Mn 40 g; Zn 40 g; Cu 10 g; I 0.5 g; Se 0.1 g; Co 0.1 g.

of vanillin-HCl as described by Wanapat and Poungchompu (2001). Total phenolic content (TPC) was determined by the modification of the Folin-Ciocalteu spectrometric method (Lim et al., 2006).

2.6. Sample collection and chemical analyses

Feed refusals were recorded daily before the morning feeding and dry matter consumption of each animal was measured. Feces, urine, and milk samples were collected during the last 7 d of each experimental period. Rumen fluid (0 and 4 h after feeding) was sampled from cows, on the last day of each period and taken through a tube, connected with a vacuum pump inserted via the mouth to the middle part of the rumen and into a plastic beaker. On the last day, blood samples 10 mL (0 and 4 h-after-feeding) were collected (from the jugular vein) and 12-mg ethylenediamine tetraacetic acid (EDTA) was then added.

Approximately 5% of total fresh fecal samples were collected daily and separated into two portions; the first portion of each day was analyzed for DM (No. 967.03) content (AOAC, 2012; No. 973.18) and the last portion was pooled at the end of each period and for each animal. The pooled fecal samples (500 g) were stored at − 20 °C until later analysis for other compositions. The urine samples were corrected at 10% of total output from each animal. Individual urine (45 mL) was homogenized with 1 mol/L sulfuric acid (5 mL) to prevent volatilization of ammonia and frozen to − 20 °C until later analysis. The urinary allantoin was analyzed by using the method of Chen et al. (1993). The concentration of microbial purine derivatives (PD) absorption (X; mmol/d) corresponding to the PD excretion (Y; mmol/d) was calculated based on their relationship following to Chen and Gomes (1992): $Y = 0.85X + (0.385BW^{0.75})$. The microbial N supply (MNS) was estimated by using the method of Chen and Gomes (1992): MNS (g/d) = $(X \times 70)/(0.116 \times 0.83 \times 1{,}000) = 0.727 \times X$, where X is PD absorption (mmol/d). The ratio of purine-N to total N in mixed rumen microbes is 11.6:100. The microbial purine digestibility is 0.83 and the N concentration of purine is 70 mg N/mmol. The efficiency of microbial nitrogen synthesis (EMNS) was based on the equation defined by Chen and Gomes (1992): EMNS = microbial nitrogen (g/d)/DOMR, where DOMR is digestible organic matter fermented in the rumen (the ruminal digestion was 650 g/kg OM of digestion in total tract). DOMR = DOMI × 0.65, where DOMI is digestible organic matter intake, which followed the assumptions calculated by ARC (1984).

One of the milk samples was combined following the proportion of milk production at milking time (using a ratio of the morning to afternoon at 60:40) and preserved with potassium dichromate ($K_2Cr_2O_7$) then stored at 4 °C until later analysis for compositions. Milk samples were analyzed for content of protein, fat, solids-not-fat (SNF), total solids (TS), lactose, galactose, glucose, casein, and milk urea nitrogen (MUN) using MilkoScan FT1 (FOSS analytical A/S, Hillerod, Denmark).

After discarding the first 200 mL of rumen fluid to minimize saliva contamination, approximately 50 mL of a sample of rumen fluid was collected. Rumen fluid was immediately detected for pH using a pH meter (HANNA Instruments HI 8424 microcomputer, Singapore). Samples (45 mL) were mixed with 1 mol/L sulphuric acid (5 mL) to stop the microbe activity and then centrifuged (3,000 × g at 4 °C for 10 min) and stored at − 20 °C until analysis concentration of volatile fatty acids (VFA) (Samuel et al., 1997); using high-performance liquid chromatography (HPLC) (Model Water 600; UV detector, Millipore Crop) and ammonia nitrogen (NH_3–N) (AOAC, 2012; No. 973.18); using Kjeldahl process (Kjeltech Auto 1030 analyzer, Tecator, Hoganiis, Sweden). Rumen samples (1 mL) and 10% formalin (9-mL) were mixed well for the total direct counts of bacteria and protozoa (Galyean, 1989), which were calculated using a haemocytometer (Boeco, Hamburg, Germany). Details of the experimental protocols were stated in Wanapat et al. (2014).

The blood samples were centrifuged at 500 × g for 10 min at 4 °C to separate plasma and stored at − 20 °C for blood urea nitrogen (BUN) analysis (Croker, 1967). Furthermore, the lipid oxidation of plasma was determined with thiobarbituric acid reactive substances (TBARS) (Toaldo et al., 2015). Briefly, supernatants were measured with absorbance at 532 nm via UV absorption spectrophotometry (T80 + UV/Vis, PG Instruments Ltd., Leicestershire, United Kingdom). The results were calculated as TBARS concentration expressed in μmol/L of malondialdehyde (MDA) using the molar extinction coefficient of the pink TBA chromagen as 1.56×10^{-5}. The antioxidant capacity was determined spectrophotometrically according to the method of Martinez et al. (2006), using a stable free radical a-diphenyl-b-picrylhydrazyl (DPPH). The absorbance was determined at 517 nm using a UV–Vis spectrophotometer (SpectraMax M3 Multi-Mode Microplate Reader, Molecular Devices, San Jose, CA, USA).

2.7. Statistical analyses

The data variables were analyzed according to a 4 × 4 Latin square design with a 2 × 2 factorial arrangement (two levels of DFPP and NPN). All parameters were analyzed by ANOVA using the general linear model (GLM) procedure (SAS, 2013). Treatment effects were tested using Duncan's new multiple range test. Significant effects were identified at $P < 0.01$ and $P < 0.05$.

3. Results

3.1. Nutrient composition of diets

Ingredients and chemical composition of the diets are presented in Table 1. The concentrate used a cassava chip as an energy source and contained CP, NDF, and ADF at 13.9%, 17.5%, and 10.1% DM. The rice straw consisted of 3.0% CP, 71.3% NDF, and 55.5% ADF, and DFPP contained 9.7% CP, 17.8% NDF, and 14.5% ADF, respectively. Importantly, DFPP contained phytonutrient compounds especially total PC at 34.2 mg/100 g, 8.1% SP, and 6.2% CT, respectively.

3.2. Dry matter consumption and nutrient digestibility

As shown in Table 2, the concentrate consumption was greater ($P = 0.03$) in animals fed with the levels of DFPP at 400 g/animal per day than those at 300 g/animal per day, and there was no difference in percent body weight by the DFPP and urea supplementation. The rice straw and total DM consumption were significantly enhanced ($P < 0.001$) with the DFPP supplementation. Digestibilities of DM, OM, and CP were significantly different among treatments ($P < 0.001$). The supplementation of DFPP and urea significantly increased ($P < 0.001$) CP digestibility, especially the addition of DFPP at 400 g/animal per day combined with urea at 200 g/animal per day. Moreover, no differences were found in fiber digestibilities (NDF and ADF) of the cows.

3.3. Rumen fermentation and blood metabolite

Rumen fermentation and blood metabolite parameters are shown in Table 3. The rumen pH was not influenced among treatments. The NH_3–N concentration was higher ($P < 0.001$) in DFPP and urea supplemented with 400 and 200 g/animal per day. Rumen microbes, and total bacteria group were significantly increased ($P < 0.001$) with DFPP and urea supplementation, whilst the protozoa population was reduced ($P < 0.001$) when DFPP levels were

Table 2
Dry matter consumption and nutrients digestibility in lactating dairy cows fed the dragon fruit peel pellet and non-protein nitrogen supplementation.

Item	300 g/d DFPP		400 g/d DFPP		SEM	*P*-value		
	100 g/d Urea	200 g/d Urea	100 g/d Urea	200 g/d Urea		DFPP	Urea	DFPP × Urea
DM consumption								
Concentrate								
kg DM/d	6.3[a]	6.7[a]	6.9[b]	6.9[b]	0.17	0.03	0.37	0.42
% BW	1.2	1.3	1.3	1.3	0.04	0.06	0.36	0.38
g/kg $BW^{0.75}$	57.6[a]	60.4[a]	62.7[b]	62.8[b]	1.53	0.03	0.37	0.40
Rice straw								
kg DM/d	7.7[a]	7.7[a]	7.9[b]	8.0[b]	0.07	<0.01	0.85	0.57
% BW	1.4	1.5	1.5	1.5	0.02	0.07	0.52	0.48
g/kg $BW^{0.75}$	70.0[a]	70.0[a]	72.0[b]	72.5[b]	0.59	<0.01	0.87	0.53
Total DM intake								
kg DM/d	14.0[a]	14.4[a]	14.8[b]	14.9[b]	0.17	<0.01	0.33	0.48
% BW	2.7[a]	2.7[a]	2.8[b]	2.9[b]	0.03	<0.01	0.16	0.47
g/kg $BW^{0.75}$	127.5[a]	130.2[a]	134.7[b]	135.2[b]	1.54	<0.01	0.35	0.49
Nutrients digestibility, %								
Dry matter	57.5[a]	63.8[b]	61.3[a]	65.7[b]	1.82	<0.01	<0.01	0.48
Organic matter	57.2[a]	64.2[b]	60.1[a]	65.7[b]	2.12	<0.01	<0.01	0.18
Crude protein	56.4[a]	65.1[b]	58.8[a]	66.7[b]	2.54	<0.01	<0.01	0.26
Neutral detergent fiber	54.7	54.3	53.6	56.5	1.03	0.64	0.24	0.13
Acid detergent fiber	48.4	48.2	47.9	49.5	1.04	0.69	0.50	0.39

DFPP = dragon fruit peel pellet; SEM = standard error of the mean.
[a,b] Mean within rows with different letters differ significantly ($P < 0.05$).

Table 3
Rumen fermentation and blood metabolite in lactating dairy cows fed the dragon fruit peel pellet and non-protein nitrogen supplementation.

Item	300 g/d DFPP		400 g/d DFPP		SEM	*P*-value		
	100 g/d Urea	200 g/d Urea	100 g/d Urea	200 g/d Urea		DFPP	Urea	DFPP × Urea
Rumen fermentation								
Rumen pH	6.7	6.6	6.7	6.7	0.10	0.53	0.36	0.50
Rumen NH_3–N, mg/dL	16.8[a]	17.5[ab]	19.3[b]	19.8[b]	0.75	<0.01	<0.01	0.65
Rumen VFA, mol/100 mol								
Acetate (C2)	70.0[a]	70.2[a]	68.5[b]	68.0[b]	0.50	<0.01	0.73	0.52
Propionate (C3)	22.7[a]	22.8[a]	24.6[b]	24.8[b]	0.47	<0.01	0.71	0.91
Butyrate (C4)	7.3	7.0	6.9	7.2	0.27	0.65	1.00	0.33
Acetate:Propionate ratio	3.0[a]	3.1[a]	2.8[b]	2.7[b]	0.08	<0.01	1.00	0.53
Total VFA, mmol/L	122.2[a]	123.7[b]	123.7[b]	128.0[c]	0.58	<0.01	<0.01	0.25
Rumen microbes								
Total bacteria, × 10^9 cell/mL	31.8[a]	32.7[b]	32.2[c]	34.1[d]	0.16	<0.01	<0.01	0.60
Protozoa, × 10^6 cell/mL	16.2[a]	15.7[a]	12.9[b]	12.3[b]	0.97	<0.01	0.05	0.43
Blood metabolite								
BUN, mg/dL	14.5[a]	16.5[b]	17.0[b]	18.5[c]	0.90	<0.01	<0.01	0.19

DFPP = dragon fruit peel pellet; SEM = standard error of the mean; NH_3–N = ammonia nitrogen; VFA = volatile fatty acids; BUN = blood urea nitrogen.
[a,b,c,d] Mean within rows with different letters differ significantly ($P < 0.05$).

incremental. Furthermore, the addition of DFPP and urea were associated with differences ($P < 0.001$) in BUN concentration.

3.4. Rumen volatile fatty acids

The concentrations of acetate, propionate, acetate:propionate ratio, and total VFA in the rumen fluid of cows supplemented with DFPP were significantly enhanced ($P < 0.001$), whilst butyrate production was not impacted by the DFPP and urea supplementation. The proportion of propionate was increased ($P < 0.001$) with the level of DFPP at 400 g/animal per day (Table 3).

3.5. Plasma antioxidant activity and oxidative stress

As shown in Table 4, the supplementation of DFPP resulted in enhancements in DPPH scavenging activity measured at 0 and 4 h after feeding ($P = 0.04$ and $P = 0.03$). DPPH scavenging activity was increased ($P = 0.03$) in the group fed DFPP at 400 g/animal per day when compared with the group fed DFPP at 300 g/animal per day. MDA was not influenced between cows supplemented with the different treatments.

3.6. Urinary purine derivatives and microbial protein synthesis

Allantoin (absorption and excretion) concentrations showed higher significance ($P < 0.001$) with the DFPP and urea supplementation. Microbial nitrogen supply (MNS) and efficiency of microbial nitrogen supply (EMNS) were significantly increased when cows were supplemented with DFPP ($P < 0.001$) and urea ($P = 0.04$; Table 5).

3.7. Milk yield and composition

The data for milk yield and composition in cows supplemented with the DFPP and urea are taken in Table 6. Both milk production and 3.5% fat collected milk (kg/d) were greater ($P = 0.03$ and $P = 0.01$) when DFPP at 400 g/animal per day was added to the diet. Enhancement in milk fat was significantly higher ($P = 0.02$) when cows were fed DFPP and urea in the diet. In contrast, milk protein,

Table 4
Plasma antioxidant activity and oxidative stress in lactating dairy cows fed the dragon fruit peel pellet and non-protein nitrogen supplementation.

Item	300 g/d DFPP		400 g/d DFPP		SEM	*P*-value		
	100 g/d Urea	200 g/d Urea	100 g/d Urea	200 g/d Urea		DFPP	Urea	DFPP × Urea
Plasma antioxidant activity								
DPPH scavenging activity, %								
0 h post feeding	15.8[a]	17.2[a]	20.6[b]	22.9[b]	2.90	0.04	0.31	0.78
4 h post feeding	16.6[a]	18.1[a]	22.3[b]	23.7[b]	2.85	0.03	0.65	0.52
Mean	16.7[a]	18.6[a]	22.8[b]	24.4[b]	2.53	0.02	0.23	0.67
Oxidative stress								
MDA, μmol/L								
0 h post feeding	1.1	1.0	1.0	1.0	0.24	0.68	0.77	0.83
4 h post feeding	1.5	1.4	1.2	1.2	0.35	0.74	0.83	0.98
Mean	1.3	1.2	1.1	1.1	0.31	0.65	0.71	0.80

DFPP = dragon fruit peel pellet; SEM = standard error of the mean; DPPH = a-diphenyl-b-picrylhydrazyl; MDA = malondialdehyde.
[a,b] Mean within rows with different letters differ significantly ($P < 0.05$).

Table 5
Urinary purine derivatives and microbial protein synthesis in lactating dairy cows fed the dragon fruit peel pellet and non-protein nitrogen supplementation.

Item	300 g/d DFPP		400 g/d DFPP		SEM	*P*-value		
	100 g/d Urea	200 g/d Urea	100 g/d Urea	200 g/d Urea		DFPP	Urea	DFPP × Urea
Urinary purine derivatives								
Allantoin, mmol/d								
Absorption	196.7[a]	225.1[b]	207.7[a]	254.3[b]	1.81	<0.01	<0.01	0.08
Excretion	195.5[a]	219.7[b]	204.7[a]	244.5[b]	1.67	<0.01	<0.01	0.13
Microbial protein synthesis								
MNS, g N/d	75.2[a]	79.8[ab]	77.9[ab]	81.2[b]	0.52	<0.01	0.03	0.29
EMNS, g N/kg OMDR	18.6[a]	23.4[b]	19.4[a]	25.2[b]	1.30	<0.01	0.04	0.38

DFPP = dragon fruit peel pellet; SEM = standard error of the mean; MNS = Microbial nitrogen supply; EMNS = efficiency of microbial nitrogen supply; OMDR = 65% of organic matter digestible in total tract.
[a,b] Mean within rows with different letters differ significantly ($P < 0.05$).

Table 6
Milk production and composition in lactating dairy cows fed the dragon fruit peel pellet and non-protein nitrogen supplementation.

Item	300 g/d DFPP		400 g/d DFPP		SEM	*P*-value		
	100 g/d Urea	200 g/d Urea	100 g/d Urea	200 g/d Urea		DFPP	Urea	DFPP × Urea
Milk production, kg/d								
Milk yield	12.6[a]	13.3[a]	13.7[b]	13.8[b]	0.34	0.03	0.40	0.37
3.5% FCM	12.1[a]	13.3[a]	13.9[b]	14.0[b]	0.43	0.01	0.16	0.25
Milk composition, %								
Fat	3.3[a]	3.5[ab]	3.6[b]	3.6[b]	0.05	0.02	0.03	0.51
Protein	3.5	3.5	3.5	3.7	0.14	0.47	0.50	0.48
Solids-not-fat	9.0	8.8	9.0	9.2	0.23	0.32	0.95	0.32
Total solids	12.3	11.3	11.8	12.1	0.83	0.76	0.58	0.33
Lactose	4.6	4.4	4.6	4.6	0.12	0.37	0.47	0.42
Galactose	0.24	0.24	0.28	0.27	0.13	0.63	0.98	0.98
Glucose	0.23	0.25	0.22	0.21	0.05	0.59	0.93	0.77
Casein	2.71	2.71	2.71	2.85	0.11	0.54	0.56	0.53
MUN, mg/dL	12.0	12.5	12.7	13.3	0.05	0.06	0.14	0.26

DFPP = dragon fruit peel pellet; SEM = standard error of the mean; FCM = fat collected milk; MUN = milk urea nitrogen.
[a,b] Mean within rows with different letters differ significantly ($P < 0.05$).

solid not fat, total solids, lactose, galactose, glucose, and casein were not significantly affected by the treatments. Moreover, no difference in MUN was observed in the group fed DFPP and urea.

4. Discussion

4.1. Dry matter consumption and nutrients digestibility

One important limitation in feeding CT and SP in ruminant feeds is the low palatability, which may limit the DM consumption of feeds below the levels expected (Wang et al., 2018). Additionally, when exceeding a CT concentration (approximately >6% DM in the diet), a reduction in feed consumption is commonly observed (Peng et al., 2016). Under this experiment, it was shown that moderate amounts of CT and SP in the DFPP might enhance digestion without affecting DM intake. These results agree with Avila et al. (2020) who stated that *Acacia mearnsii* fed at 20 g/kg of DM in the diet had no reduction in the dry matter intake.

In the present experiment, the addition of DFPP and urea to the diet clearly improved DM, OM, and CP digestibilities. This could be attributed to the increase of microbial population which would degrade more feeds. Moreover, this finding could be due to DFPP which contained CT and SP. The presence of phytonutrients improved the digestibilities of DM and OM, which could be due to the concentration used causing a fluctuating shift in the rumen microorganisms, thus did not impact feed digestion. The CP

digestibility was significantly increased by the DFPP addition; this change could be attributed to the binding of CT with protein which was able to increase rumen by-pass protein. This finding agrees with Wang et al. (2018) who reported that adding CT and SP often entails adverse effects on nutrient digestibility, especially protein digestion where a decreased rumen protein digestion is desirable, provided the tannin-CP bonds are later separated and the protein can be digested in the lower-gut. Ampapon et al. (2019) evaluated the use of phytonutrients based on using both mangosteen peel powder and banana flower powder and found the enhancement of fiber digestibility with increasing the rumen bacteria population.

4.2. Rumen fermentation and blood metabolite

The rumen pH of dairy cows was unaffected by the DFPP and urea levels, ranging from 6.6 to 6.7. This is a notable range that was suitable for fibrolytic bacteria population to breakdown of fiber in the rumen and with no impact on rumen acidosis, as demonstrated by Wanapat et al. (2014) and Abdela (2016). Accordingly, Dschaak et al. (2011) revealed that rumen pH was not influenced by level of CT in lactating dairy cows. Rumen pH is one of the most important functions of fermentation variables instantly affecting microbial activity and growth (Li et al., 2009).

The rumen NH_3–N concentration was improved by the DFPP and urea supplementation and was higher in DFPP supplemented with 200 g/animal per day of urea. This could be a positive effect of the DFPP which contained 5.4% CP and NPN, resulting in enhancement ofing the NH_3–N content. Supapong et al. (2017) explained that the concentrate, protein, and roughage sources in the diet that can affect the animals is based on the knowledge of which NH_3–N is the major product of rumen protein digestion. Most of the nitrogen used by rumen microorganisms was provided from the available NH_3–N pool in the rumen. Accordingly, adding DPFF at 400 g/animal per day to Holstein crossbred bulls resulted in increased rumen NH_3–N concentration (Matra et al., 2020).

The bacterial population was increased by the DFPP and urea levels, while the protozoal population was decreased. It is well known that PTN presents a broad range of anti-microbial effects against microbial populations, especially protozoa and PTN, attributed to the hydrophobicity of their active compounds (Calsamiglia et al., 2007; Patra, 2012). Perna Junior et al. (2017) used *A. mearnsii* containing CT at 6 g/kg in dry cows, which resulted in a decrease in the protozoal population. Moreover, SP-containing plants or extracts have inhibitory effects on protozoa as shown by Patra and Saxena (2009). In addition, Fagundes et al. (2020) reported that tannin-rich forage increased the rumen bacteria population.

The concentration of BUN was significantly improved in dairy cows given DFPP and urea. This may be because BUN correlated with the NH_3–N concentration availability in the rumen. BUN concentration could be employed to detect nitrogen metabolism in ruminants, as higher BUN can reflect higher protein degradation in the rumen (Broderick and Clayton, 1997).

4.3. Rumen volatile fatty acid

Under this investigation, the molar proportion of propionate and total VFA were significantly improved by DFPP supplementation, whereas the acetate proportion was decreased. This may be related to the effect of phytonutrients on bacterial population in the rumen. The increase in bacterial population in this trial could improve the rumen fermentation, thus yielding higher total VFA and propionate production. Accordingly, Ampapon and Wanapat (2020) stated that addition of rambutan peel powder at 4% dry matter intake subsequently increased rumen propionate production in beef cattle. Furthermore, Khiaosa-Ard and Zebeli (2013) showed that PTN was effectively improving propionate and reducing acetate concentration in dairy cows.

4.4. Plasma antioxidant activity and oxidative stress

The levels of DPPH scavenging activity were greater in animals fed with DFPP at 400 g/animal per day. This might be a positive effect of the DFPP containing PC. These compounds are effective as dietary antioxidants in animal feeding which has been receiving more attention. PC is a natural antioxidant that is ingested by animals and is believed to contribute towards an enhanced antioxidant activity (Vasta and Luciano, 2011). In agreement with these observations, López-Andrés et al. (2014) stated that PC present in ryegrass could contribute to enhancing the antioxidant activity of the plasma of grazing lambs. This important mediator of cellular macromolecule damage must be continuously eliminated and controlled by antioxidant mechanisms to prevent various diseases especially mastitis (Konvičná et al., 2015). Antioxidants play an essential role against the deteriorating action of free radicals in the organisms. Insufficiency of antioxidants in living organisms leads to oxidative stress (Pourreza, 2013).

MDA is the end-product of lipid peroxidation, thus is used as an index of oxidative stress (Esterbauer et al., 1991). In this recent study, MDA was not influenced by DFPP supplementation. This could be due to PC impacting on antioxidant status, thus MDA could be maintained at a normal range (1.0 to 1.5). Similarly, Safari et al. (2018) showed that the plasma concentration of MDA was not influenced by pomegranate by-products in transition dairy cows.

4.5. Urinary purine derivatives and microbial protein synthesis

In the present study, estimated urinary purine derivatives were significantly increased with DFPP in combination with urea. Accordingly, lower urine allantoin excretion has been reported in cows fed a diet with lower CP percentage than those of present study Cutrignelli et al. (2007). This could be due to a higher level of DFPP supplementation, hence higher N intake, together with its effect on forming of protein complex. The high dietary CP intake increased the flow of protein from the rumen to the lower gut (Giang et al., 2016). The rumen microbial protein synthesis additionally plays an essential role in ruminants since it provides a high level of protein resources for host animals. Microbial protein synthesis efficiency depends on DM intake, rumen carbohydrate and the protein rate of fermentation, and rumen dilution rate (Stern et al., 1997). Furthermore, MNS and EMNS were significantly increased with DFPP and urea addition. Similarly, Viennasay and Wanapat (2020) found in lactating dairy cows that using *Flemingia* could improve MNS and EMNS. Production of microbial protein synthesis (MPS) using NH_3–N released from rumen protein degradation would support the outflow of protein into the small intestine (Russell and Rychlik, 2001).

4.6. Milk yield and composition

Milk yield and milk fat were increased when supplemented with DFPP. This could be due to the CT and SP present in the DFPP. Accordingly, Benchaar et al. (2008) reiterated that CT and SP when contained at moderate concentration could be beneficial to rumen fermentation end-products and production. Several studies have shown positive effects from tropical fruit peel by-products on rumen fermentation, as well as milk yield; using mangosteen peel pellet (Norrapoke et al., 2012) and rambutan peel powder (Ampapon and Wanapat, 2020). Importantly, high levels of propionate concentration would be converted into glucose via

gluconeogenesis which could produce more NADPH via the pathway and be utilized later for the synthesis of fatty acids (Isobe et al., 2011).

5. Conclusions

DFPP was an alternative by-product resource containing phytonutrients namely PC, CT, and SP. Based on the findings, supplementation of DFPP at 400 g/animal per day with urea at 100 g/animal per day enhanced rumen fermentation characteristics, microbial protein synthesis, milk yield and milk fat, as well as increased plasma antioxidant activity. Therefore, DFPP could be a promising dietary rumen enhancer to replace chemicals and antibiotics for ruminant feeding.

Author contributions

Maharach Matra: Conceptualization, Methodology, Data duration, Formal analysis, Writing – Original draft preparation. **Metha Wanapat:** Conceptualization, Methodology, Writing – Original draft preparation, Reviewing and Editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, Ishtiaq A, Hussain S, Suleria HAR. Natural polyphenols: an overview. Int J Food Prop 2017;20:1689–99.
Abdela N. Sub-acute ruminal acidosis (SARA) and its consequence in dairy cattle: a review of past and recent research at global prospective. Achiev Life Sci 2016;10:187–96.
Ampapon T, Phesatcha K, Wanapat M. Effects of phytonutrients on ruminal fermentation, digestibility, and microorganisms in swamp buffaloes. Animals 2019;9(9):671.
Ampapon T, Wanapat M. Dietary rambutan peel powder as a rumen modifier in beef cattle. Asian-Australas J Anim Sci 2020;33(5):763.
AOAC. Official methods of analysis. 19th ed. Gaithersburg, MD: Association of Official Analytical Chemists; 2012.
ARC. The nutrient requirements of ruminant livestock, Suppl. No.1. Slough: Commonwealth Agricultural Bureaux; 1984.
Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc 1998;75:199–212.
Avila AS, Zambom MA, Faccenda A, Fischer ML, Anschau FA, Venturini T, Tinini RCR, Dessbesell JG, Faciola AP. Effects of black wattle (*Acacia mearnsii*) condensed tannins on intake, protozoa population, ruminal fermentation, and nutrient digestibility in Jersey steers. Animals 2020;10(6):1011.
Benchaar C, McAllister TA, Chouinard PY. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extracts. J Dairy Sci 2008;91(12):4765–77.
Borges G, Mullen W, Crozier A. Comparison of the polyphenolic composition and antioxidant activity of European commercial fruit juices. Food Funct 2010;1:73–83.
Broderick GA, Clayton MK. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J Dairy Sci 1997;80:2964–71.
Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A. Invited review: essential oils as modifiers of rumen microbial fermentation. J Dairy Sci 2007;90(6):2580–95.
Chen XB, Kyle DJ, Ørskov ER. Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization. J Chromatogr 1993;617:241–7.
Chen XB, Gomes MJ. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details. In: International feed resources unit. Aberdeen, UK: Rowel Research Institute; 1992.
Cherdthong A, Prachumchai R, Wanapat M, Foiklang S, Chanjula P. Effects of supplementation with royal poinciana seed meal (*Delonix regia*) on ruminal fermentation pattern, microbial protein synthesis, blood metabolites and mitigation of methane emissions in native Thai beef cattle. Animals 2019;9(9):625.
Croker CL. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am J Med Technol 1967;33:361–5.
Cutrignelli MI, Piccolo G, D'Urso S, Calabrò S, Bovera F, Tudisco R, Infascelli F. Urinary excretion of purine derivatives in dry buffalo and Fresian cows. Ital J Anim Sci 2007;6:563–6.
Dschaak CM, Williams CM, Holt MS, Eun JS, Young AJ, Min BR. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. J Dairy Sci 2011;94(5):2508–19.
Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radical Bio Med 1991;11:81–128.
Fagundes GM, Benetel G, Carriero MM, Sousa RL, Muir JP, Macedo RO, Bueno ICS. Tannin-rich forage as a methane mitigation strategy for cattle and the implications for rumen microbiota. Anim Prod Sci 2020;61(1):26–37.
Foiklang S, Wanapat M, Norrapoke T. *In vitro* rumen fermentation and digestibility of buffaloes as influenced by grape pomace powder and urea treated rice straw supplementation. Anim Sci J 2016;87(3):370–7.
Frutos P, Hervás G, Giráldez FJ, Mantecón AR. Review: tannins and ruminant nutrition. Spanish J Agric Res 2004;2:191–202.
Galyean M. Laboratory procedure in animal nutrition research. USA: Department of Animal and Range Science, New Mexico State University; 1989.
Giang NTT, Wanapat M, Phesatcha K, Kang S. Level of *Leucaena leucocephala* silage feeding on intake, rumen fermentation, and nutrient digestibility in dairy steers. Trop Anim Health Prod 2016;48:1057–64.
Ibrahim UK, Kamarrudin N, Suzihaque MUH, Abd HS. Local fruit wastes as a potential source of natural antioxidant: an overview. In: IOP conference series: materials science and engineering, vol. 206. IOP Publishing; 2017. p. 12040. No. 1.
Isobe N, Kubota H, Yamasaki A, Yoshimura Y. Lactoperoxidase activity in milk is correlated with somatic cell count in dairy cows. J Dairy Sci 2011;94:3868–74.
Kang S, Wanapat M, Cherdthorng A. Effect of banana flower powder supplementation as a rumen buffer on rumen fermentation efficiency and nutrient digestibility in dairy steers fed a high-concentrate diet. Anim Feed Sci Technol 2014;196:32–41.
Khiaosa-Ard R, Zebeli Q. Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. J Anim Sci 2013;91(4):1819–30.
Konvičná J, Vargová M, Paulíková I, Kováč G, Kostecká Z. Oxidative stress and antioxidant status in dairy cows during prepartal and postpartal periods. Acta Vet 2015;84(2):133–40.
Li M, Penner GB, Hernandez Sanabria E, Oba M, Guan LL. Effects of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. J Appl Microbiol 2009;107(6):1924–34.
Lim YY, Lim TT, Tee JJ. Antioxidant properties of guava fruit: comparison with some local fruits. Sunway Academic Journal 2006;3:9–20.
López-Andrés P, Luciano G, Vasta V, Gibson TM, Scerra M, Biondi LA, Priolo A, Mueller-Harvey I. Antioxidant effects of ryegrass phenolics in lamb liver and plasma. Animal: an International Journal of Animal Bioscience 2014;8(1):51.
Lykkesfeldt J, Svendsen O. Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet J 2007;173:502–11.
Martinez S, Valek L, Rešetić J, Ružić DF. Cyclic voltammetry study of plasma antioxidant capacity–comparison with the DPPH and TAS spectrophotometric methods. J Electroanal Chem 2006;88(1):68–73.
Matra M, Totakul P, Viennasay B, Phesatcha B, Wanapat M. Dragon fruit (*Hylocereus undatus*) peel pellet as a rumen enhancer in Holstein crossbred bulls. Anim Biosci 2020;34(4):594–602.
Norrapoke T, Wanapat M, Wanapat S. Effects of protein level and mangosteen peel pellets (Mago-pel) in concentrate diets on rumen fermentation and milk production in lactating dairy crossbreds. Asian-Australas J Anim Sci 2012;25(7):971.
Patra AK, Saxena J. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutr Res Rev 2009;22(2):204–19.
Patra AK. Dietary phytochemicals and microbes. New York, NY: Springer; 2012.
Peng K, Shirley DC, Xu Z, Huang Q, McAllister TA, Chaves AV, Acharya S, Liu C, Wang S, Wang Y. Effect of purple prairie clover (*Dalea purpurea* Vent.) hay and its condensed tannins on growth performance, wool growth, nutrient

digestibility, blood metabolites and ruminal fermentation in lambs fed total mixed rations. Anim Feed Sci Technol 2016;222:100–10.
Perna Junior F, Cassiano ECO, Martins MF, Romero LA, Zapata DCV, Pinedo LA, Marino CT, Rodrigues PHM. Effect of tannins-rich extract from *Acacia mearnsii* or monensin as feed additives on ruminal fermentation efficiency in cattle. Livest Sci 2017;203:21–9.
Pourreza N. Phenolic compounds as potential antioxidant. Jundishapur J Nat Pharm Prod 2013;8(4):149.
Rizzo A, Ceci E, Pantaleo M, Mutinati M, Spedicato M, Minoia G, Sciorsci RL. Evaluation of blood and milk oxidative status during early postpartum of dairy cows. Animal 2013;7:118–23.
Russell JB, Rychlik JL. Factors that alter rumen microbial ecology. Sci 2001;292:1119–22.
Safari M, Ghasemi E, Alikhani M, Ansari-Mahyari S. Supplementation effects of pomegranate by-products on oxidative status, metabolic profile, and performance in transition dairy cows. J Dairy Sci 2018;101(12):11297–309.
Salem AZ, Kholif AE, Elghandour MM, Buendía G, Mariezcurrena MD, Hernandez SR, Camacho LM. Influence of oral administration of *Salix babylonica* extract on milk production and composition in dairy cows. Ital J Anim Sci 2014;13(1):2978.
Salles MSV, Zanetti MA, Salles FA, Titto EAL, Conti RMC. Changes in ruminal fermentation and mineral serum level in animals kept in high temperature environments. Rev Bras Zootec 2010;39:883–90.
Samuel M, Sagathewan S, Thomas J, Mathen G. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian J Anim Sci 1997;67:805–11.
SAS. User's guide: statistic, version 9. 4th ed. Cary, NC: SAS Inst. Inc.; 2013.
Stern MD, Bach A, Calsamiglia S. Alternative techniques for measuring nutrient digestion in ruminants. J Anim Sci 1997;75:2256–76.
Supapong C, Cherdthong A, Seankamsorn A, Khonkhaeng B, Wanapat M, Gunun N, Gunun P, Chanjula P, Polyorach S. Effect of *Delonix regia* seed meal supplementation in Thai native beef cattle on feed intake, rumen fermentation characteristics and methane production. Anim Feed Sci Technol 2017;232:40–8.
Tian X, Xin H, Paengkoum P, Paengkoum S, Ban C, Sorasak T. Effects of anthocyanin-rich purple corn (*Zea mays* L.) stover silage on nutrient utilization, rumen fermentation, plasma antioxidant capacity, and mammary gland gene expression in dairy goats. J Anim Sci 2019;97(3):1384–97.
Toaldo IM, Cruz FA, de Lima Alves T, de Gois JS, Borges DL, Cunha HP, da Silva EL, Bordignon-Luiza MT. Bioactive potential of *Vitis labrusca* L. grape juices from the southern region of Brazil: phenolic and elemental composition and effect on lipid peroxidation in healthy subjects. Food Chem 2015;173:527–35.
Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.
Vasta V, Luciano G. The effect of dietary consumption of plant secondary compounds on small ruminants' product quality. Small Rumin Res 2011;101:150–9.
Viennasay B, Wanapat M. Enhancing lactating dairy cow rumen fermentation and production with *Flemingia* silage containing phytonutrients. Livest Sci 2020;241:104201.
Wanapat M, Gunun P, Anantasook N, Kang S. Changes of rumen pH, fermentation and microbial population as influenced by different ratios of roughage (rice straw) to concentrate in dairy steers. J Agric Sci 2014;152:675–85.
Wanapat M, Poungchompu O. Method for estimation of tannin by Vanillin-HCL method (A modified method of Burns, 1971). Thailand: Department of Animal Science, Khon Kaen University, Khon Kaen; 2001. p. 40002.
Wang S, Terranova M, Kreuzer M, Marquardt S, Eggerschwiler L, Schwarm A. Supplementation of pelleted hazel (*Corylus avellana*) leaves decreases methane and urinary nitrogen emissions by sheep at unchanged forage intake. Sci Rep 2018;8(1):1–10.
Wang Z, Fang YZ. Analysis of sugars in traditional Chinese drugs. J Chromatogr B 2004;812:309–24.