

Nutrient Supplement to Enhance the Rumen Fermentation and Milk Production of Cows

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Abstract

In this paper it is 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. Eight animals were randomly allotted to 8 dietary treatments according to a 4 × 4 factorial arrangement in 8 × 8 Latin square design. The treatments were: 400 g DM of DFPP + 200 g of urea (T1), 400 g DM of DFPP + 300 g of urea (T2), 500 g DM of DFPP + 200 g of urea (T3), and 500 g DM of DFPP + 300 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 digestibility, but increased the apparent digestibility 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 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. The plasma antioxidant activity was increased ($P > 0.05$) with the addition of DFPP. The DFPP improved ($P < 0.01$) microbial protein synthesis. According to above, addition of DFPP at 380 g/animal per day with urea at 80 g/animal per day improved rumen fermentation, plasma antioxidant activity, milk yield and milk fat %.

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I. 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 phytochemicals (PTN) sources (Ibrahim et al., 2017). Phytochemicals 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. Furthermore, these compounds exert impacts on antioxidant activity which can prevent the availability of free radicals. Free radicals and reactive oxygen species are continuously formed in the body of animal. 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. 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.

Dragon fruit (*Hylocereus undatus*) peel is one of the alternative sources of plant containing antioxidants, especially PC that is non-toxic and biologically safe. A current study has shown that antioxidant addition in the ruminant diet could alleviate oxidative stress status) and increase plasma antioxidant capacity. Moreover, these plants contain phytochemicals such as CT and SP, with the specific effect of improving milk profile, nitrogen efficiency in dairy cows, enhancing rumen fermentation, 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. However, dietary non-protein-nitrogen (NPN) supplementation has been shown to enhance 76 the crude protein level of low quality agro77 industrial by-products. 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 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.

Care of Animal and its management

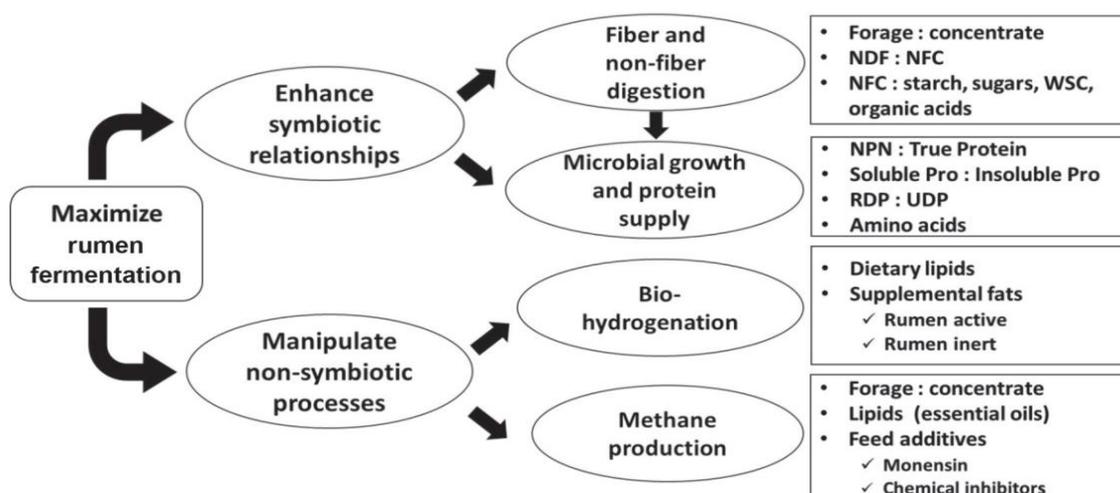
All Holstein-Friesian crossbred cows were approved by the Institute of Animals for Scientific Purpose Development. Dairy cows were injected with vitamin AD3E and drenched with Ivermectin (1 mL per 50 kg live weight) before imposing the respective treatments. Animals were housed in a ventilated barn and in individual pens (3 m × 4 m). Mineral blocks and clean drinking water were available freely.

Preparation of Dragon fruit peel pellet

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).

II. PROCEDURE

Four Holstein-Friesian crossbred cows were used in 99 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 hours.

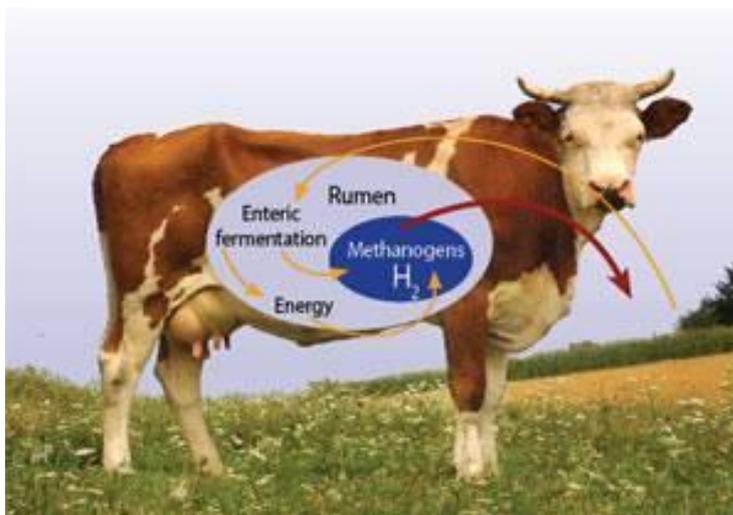


Treatments

The dietary treatments were two factors of dragon fruit peel pellet, 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 topdressed on the concentrate, manually mixed and fed to animals.

III. FEEDS COMPOSITION

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, and fiber contents were determined using Ankom A200i Fibre Analyser. Furthermore, the DFPP was analyzed for SP content by using reflux distillation method, and for CT following the modified method of vanillin-HCl as described by Wanapat and Pongchompu. Total phenolic content (TPC) was determined by the modification of the 123 Folin-Ciocalteu spectrometric method.



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 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 concentration of microbial purine derivatives (PD) absorption (X; mmol/d) corresponding to the PD excretion (Y; mmol/d) was calculated based on their relationship: $Y = 0.85X + (0.385\text{BW}^{0.75})$. The microbial N supply (MNS) was estimated by using the method of Chen and Gomes (1992): $\text{MNS (g/d)} = (X \times 70) / (0.116 \times 0.83 \times 1,000) = 0.727 \times X$, 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 148 and Gomes (1992): $\text{EMNS} = \text{microbial nitrogen (g/d)} / \text{DOMR}$, where DOMR is digestible organic matter fermented in the rumen (the ruminal digestion was 650 g/kg OM of digestion in total tract). $\text{DOMR} = \text{DOMI} \times 0.65$, 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 ($\text{K}_2\text{Cr}_2\text{O}_7$) 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. 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. Samples (45-mL) were mixed with 1 mol/L sulphuric acid (5-mL) to stop the microbe activity and then centrifuged ($3,000 \times g$ at 4°C for 10 min) and stored at -20°C until analysis concentration of volatile fatty acids (VFA); using high-performance liquid chromatography (HPLC) (Model Water 600; UV detector, Millipore Crop) and ammonia-nitrogen ($\text{NH}_3\text{-N}$); using Kjeldahl process. Rumen samples (1-mL) and 10% formalin (9 mL) were mixed well for the total direct counts of bacteria and protozoa, which were calculated using a Haemocytometer. The blood samples were centrifuged at $500 \times g$ for 10 min at 4°C to separate plasma and stored at -20°C for blood urea nitrogen (BUN) analysis. Furthermore, the lipid oxidation of plasma was determined with thiobarbituric acid reactive substances (TBARS). Briefly, 173 supernatants were measured with absorbance at 532 nm via UV absorption spectrophotometry. The results were calculated as TBARS concentration expressed in $\mu\text{mol/L}$ of malondialdehyde (MDA) using the molar extinction coefficient of the pink TBA chromagen as $1.56 \times 10^{-5} \text{M/cm}$.

IV. DISCUSSION

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. Additionally, when exceeding a CT concentration 245 (approximately > 6% DM in the diet), a reduction in feed consumption is commonly observed. Under this experiment, it was shown that moderate amounts of CT and SP in the DFPP might enhance digestion without affecting DM 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 phytochemicals 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. 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.

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. Rumen pH was not influenced by level 270 of CT in lactating dairy cows. Rumen pH is one of the most important functions of fermentation variables instantly affecting microbial activity and growth. The rumen NH₃-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 non-protein-nitrogen, resulting in enhancement of the NH₃-N content. The concentrate, protein, and roughage sources in the diet that can affect the animals is based on the knowledge of which NH₃-N is the major-product of rumen protein digestion. Most of the nitrogen used by rumen microorganisms was provided from the available NH₃-N pool in the rumen. Accordingly, adding dragon fruit peel pellet at 400 g/animal per day to Holstein crossbred bulls resulted in increased rumen NH₃-N concentration.

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. The concentration of BUN was significantly improved in dairy cows given DFPP and urea. This may be because BUN correlated with the NH₃-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.

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 phytochemicals 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. Addition of rambutan peel powder at 4% dry matter intake subsequently increased rumen propionate production in beef cattle. PTN was effectively improving propionate and reducing acetate concentration in dairy cows.

Plasma antioxidant activity and oxidative stress

The levels of DPPH scavenging activity were greater in animals fed with DFPP at 400g/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. In agreement with these observations, phenolic compounds 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. 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. MDA is the end-product of lipid peroxidation, thus is used as an index of oxidative stress. In this recent study, MDA was not influenced by DFPP supplementation. This could be due to PC 320 impacting on antioxidant status, thus MDA could be maintained at a normal range (1.0 to 1.5). Similarly, the plasma concentration of MDA was not influenced by pomegranate by-products in transition dairy cows.

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. 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. 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. Furthermore, MNS and EMNS were significantly increased with DFPP and urea addition. Similarly, lactating dairy cows that using Flemingia could improve MNS and EMNS. Production of microbial protein synthesis (MPS) using NH₃-N released from rumen protein degradation would support the outflow of protein into the small intestine.

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. 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 and rambutan peel powder. 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.

V. CONCLUSIONS

DFPP was an alternative by-product resource containing phytochemicals 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.

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