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PAPER

Supplemental effect of Chaya (*Cnidoscolus aconitifolius*) leaf pellet on rumen fermentation, nutrients digestibility and microbial protein synthesis in growing crossbred bulls

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ABSTRACT

This experiment was conducted to assess the effect of Chaya (*Cnidoscolus aconitifolius*) leaf pellet (CHYP) on rumen fermentation, nutrients digestibility and microbial protein synthesis in growing crossbred bulls. Four animals, with an average liveweight of 160 ± 10 kg were randomly arranged in a 4 × 4 Latin square design. There were four treatments: 0, 4, 6 and 8% CHYP supplementation of DMI. The study findings showed that increasing supplementation level of CHYP linearly enhanced ($p < 0.05$) DM, OM, and CP digestibilities. Rumen characteristics, namely NH_3–N concentration and bacterial population were increased ($p < 0.05$) while, protozoal and fungal population remained unchanged, as level of CHYP supplementation increased. Total rumen volatile fatty acids (VFA) and propionic acid (C_3) were enhanced ($p < 0.05$). Furthermore, N utilisation especially N absorption, N retention and efficiency of microbial nitrogen synthesis (EMNS) were significantly improved by increasing level of CHYP supplementation. Hence, CHYP supplementation is highly promising for ruminant feeding.

HIGHLIGHTS

- Chaya leaf pellet (CHYP) contains high level of crude protein, essential amino acids and minerals.
- CHYP supplementation increased nutrient digestibility and propionic acid (C_3) in rumen.
- CHYP enhanced N–balance, microbial nitrogen supply (MNS) and efficiency of microbial nitrogen synthesis (EMNS).

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KEYWORDS
Feed resource; fodder shrub; protein source; rumen fermentation; microbial protein synthesis

Introduction

Livestock have been an important component in the integrated crop–livestock farming systems especially in the developing countries. Essentially, the conversion of materials, inedible for humans, such as roughages and agricultural crop–residues into human protein–food by ruminants will continue to serve as an integral function of animal agriculture (Wanapat et al. 2013). Fodder trees or shrubs are important feed resources for livestock under the wide range of farming systems environment. Extension services and farmers have been developing and promoting fodder trees as multiple–function resources. Fodder trees have been demonstrated to thrive well in diverse environment and can contribute greatly to livestock productivity (Devendra et al. 2000). Importance of shrubs or fodder trees have been increasingly important to be used as supplementary feeds as they contain high protein, minerals, as well as plant secondary compounds containing condensed tannins and saponins (Wanapat et al. 2013).

Many shrubs and fodder trees have been reported to be successfully used as leaf meal, silage and/or fresh–cut. For examples, such feeds include the use of *Leucaena leucocephala* (Nguyen et al. 2016; Piñeiro–Vázquez et al. 2017), *Flemingia macrophylla* (Wanapat et al. 2013; Phesatcha and Wanapat 2017), *Moringa oleifera* (Mendieta–Araica et al. 2011) and *Tithonia diversifolia* (Ribeiro et al. 2016). An ethno

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Table 1. Feed ingredients and chemical composition of the feeds used in the experiment.

Items	Concentrate	Rice straw	CHYP
Ingredients (% as fed)			
Cassava chip	56		9
Rice bran	18		–
Palm kernel meal	20		–
Urea	2.5		–
Molasses	1		1
Sulphur	1		–
Mineral mixture[a]	1		–
Salt	0.5		–
Chaya leaf meal			90
Chemical compositions			
DM (%)	88.4	90.5	90.2
		% of dry matter	
OM	90.6	85.6	87.2
Ash	9.4	14.4	12.8
CP	14.3	2.7	23.6
NDF	19.7	81.7	19.3
ADF	11.1	55.3	16.2
CT			1.02
Mineral (% of DM)			
K			0.56
Ca			4.70
Mg			1.79
P			0.71
Na			0.09

CHYP: Chaya leaf meal pellet; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre, ADF: acid detergent fibre; CT: condensed tannins; K: potassium; Ca: calcium; Mg: magnesium; P: phosphorus; Na: sodium.
[a]Mineral mixture contains per kg: 4,000,000 IU vitamin A, 400,000 IU Vitamin D_3, 4,000 IU vitamin E, 0.002 g vitamin B_{12}, 16 g Mn, 24 g Fe, 10 g Zn, 2 g Cu, 0.05 g Se, 0.2 g Co, 0.5 g I.

botany study performed by Ross-Ibarra and Molina-Cruz (2002) on a vegetable called Chaya or *Cnidoscolus aconitifolius* found that the leafy green vegetable is commonly found in the Centro American regions of Guatemala, Belice, Southeast Mexica, and the Yucatan peninsula. Chaya belongs to the *Cnidoscolus* genus and the plants are either evergreen or drought-deciduous shrubs that can reach heights of up to 6 metres. Additionally, the Chaya plants possess large lobed leaves that can measure up to 32 cm long and 30 cm wide (Ross-Ibarra and Molina-Cruz 2002). It was reported that Chaya leaf contained significantly high level of crude protein (29.6% DM) with high level of essential amino acids (lysine, leucine and phenylalanine) as well as macro–minerals (Ca and K). Interestingly, the essential amino acids content was similar to those of soybean meal and alfalfa hay (Donkoh et al. 1990; Kuti and Kuti 1999). Nevertheless, limited information related to the use of Chaya as a feed in ruminants has been lacking. Importantly, the advantage of pellet feeds have several benefits: increased nutrient density, easy storage, enhanced palatability, less segregation, no sorting, enhanced feed intake and productivity (Hale and Theurer 1972).

Hence, this experiment aimed at evaluating Chaya leaf pellet (CHYP) on dry matter intake, nutrients digestibility, nitrogen utilisation efficiency in growing crossbred bulls.

Materials and methods

Ethical issue and place of experimentation

This experiment procedure was carried out by the Institute of Animals for Scientific Purpose Development (IAD), Thailand (record no. U1–06565–2526) and was done at the Ruminant Nutrition and Metabolism Research Centre, Tropical Feed Resources Research and Development Centre (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University.

Experimental animals and feeding

Four, crossbred dairy bulls 1.4 year olds with initial body weight (BW) of 160 ± 10 kg were arranged according to a 4 × 4 Latin square design to receive CHYP at 0, 4, 6 and 8% of DMI (concentrate and rice straw intake), (T1 = control or 0% CHYP, T2 = 4% CHYP, T3 = 6% CHYP and T4 = 8% CHYP). All animals were fed with concentrate (Table 1) at 0.5% BW with CHYP supplementation at the referred levels and were fed in two equal meals per day (07:00 and 16:00 h). Rice straw was fed ad libitum and clean water was available at all times. The animals were raised in individual pens (2 × 3 m). The experiment was conducted for 4 periods with 21 days per each. The first 14 days were for adaptation period and the last 7 days were

for samples collection when animals were moved to the metabolism crates.

Chaya leaf pellet preparation

Experimental Chaya leaf was harvested from the regrowth biomass of about 8 weeks. The leaves were chopped into 2–3 mm, sun–dried until achieving the dry matter of at least 90%. It was further ground to pass 1-mm screen mill in order to use it in the pellet. Chaya leaf pellet was prepared by combining 1% molasses, 9% cassava starch and 90% Chaya leaf. After mixing all ingredients well, then adding water with in the ratio of 5 kg of mixture ingredients with 800 ml of water and then, pelleted by using Ryuzo kun pelleting machine.

Data collection, sampling procedure and chemical analysis

The individual feed intake of concentrate, rice straw and CHYP were measured daily by weighing the offered and refused feeds before the morning feeding. The last 7 days the animals were moved to metabolism crates for total collection (faeces and urine). Faecal samples were collected via the total collection method when animals were in metabolism crates. The faecal samples were collected by sampling 5% of total fresh weight and were separated into two parts: the first part was for dry matter assessment daily. The second part was kept in the freezer and pooled by animal at the end of each period for chemical analysis and calculation for nutrient digestibility. The urine samples were corrected in the morning about 10% of total output, at 45 mL combining with 5 mL of H_2SO_4 solution (0.1 M) to inhibit microbial activity then kept in the freezer for analysis of purine derivatives (PD) according to the procedure of Chen and Gomes (1995). Microbial nitrogen supply was calculated form urinary purine derivatives absorption: MN (g/d) = 0.727 × absorption of PD (mmol/d). The efficiency of microbial nitrogen synthesis (EMNS) was calculated as followed: EMNS = microbial N (g/d)/DOMR; where DOMR = digestibility organic matter apparently fermented in the rumen (assuming that rumen digestion = 65% of digestion in total tract), DOMR = DOMI × 0.65; DOMI = digestion organic matter intake (Chen and Gomes 1995).

On the last day of each period (at 0 and 4 h–post–feeding), blood sample (approximately 10 mL) was taken from the jugular vein using a 21-ga needle and mixed with EDTA (12 mg) as anticoagulant in the tubes. The plasma was separated by centrifuged at 3000 × g for 10 minute at 4 °C and kept at −20 °C until used for blood urea nitrogen (BUN) analysis according to Crocker (1967). Rumen fluid sample was collected about 200 mL by using the stomach tube connected with suction pump. The rumen pH and temperature were immediately measured by using a portable pH metre (HANNA Instrument HI 8424 microcomputer, Singapore). The rumen fluid (1 mL) was combined with 9 mL (10% formalin solution) for total direct count (bacteria, protozoa and fungi zoospores) according to Galyean (1989); using Haemacytometer (Boeco, Singapore). The sample (45 mL) were filtered and mixed with 5 mL of H_2SO_4 solution (0.1 M) to stop fermentation process of microbial activity for analysis of ammonia nitrogen (NH_3–N) (Kjeltech Auto 1030 analyser, Tecator, Hoganiis, Sweden) and volatile fatty acids (VFA) using High Performance Liquid Chromatography (HPLC) (Model 600E; water model 484 UV detector; column novapack C18; column size 3.9 mm; mobile phase 10 mM H_2SO_4 [pH 2.5]) according to Sanmuel et al. (1997). Methane (CH_4) production was estimated by the equation of Moss et al. (2000).

Feeds and faeces were dried at 60 °C then were ground to pass through a 1–mm screen. Sample (feed and faeces) were chemically assessed for dry matter, ash, crude protein (AOAC 2012) and fibre fractions (Van Soest et al. 1991); using Ankom A200i Fibre Analyser (Ankom Technology Co., New York, NY, USA.). Chaya leaf meal pellet (CHYP) were analysed for condensed tannins contents using the vanillin–HCl method as modified by Wanapat and Poungchompu (2001) and minerals using Atomic absorption spectrometer (Model analytic jena nova 350).

Statistical analysis

All data were subjected to ANOVA according to a 4 × 4 Latin square design using the General Linear Models (GLM) procedures (SAS 2013). Data were statistically analysed using the model: $Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$; where Y_{ijk} = observation from animal j, receiving diet i, in the period k; μ = the overall mean; M_i = effect of treatment; A_j = effect of animal; P_k = effect of period and ε_{ijk} = residual effect. The data were shown as means with the standard error of the means. Difference among means with $p < 0.05$, $p < .01$ and $p < 0.001$ were tested as statistical differences. Treatment means were analysed according to Duncan's New Multiple Range Test (Steel and Torrie 1980). The orthogonal polynomial contrasts were performed to determine the effect of CHYP supplementation levels.

Results

Feed ingredients and chemical compositions

Feed ingredients and their chemical compositions are presented in the Table 1. CHYP contained 23.6% CP, 19.3% NDF and 16.2% ADF, respectively. In addition, it contained high minerals of 0.56% K, 4.70% Ca, 1.79% Mg, 0.71% P, 0.09% Na and 1.02% condensed tannin (CT). The CHYP appeared in good physical property and was readily consumed by the animals.

Table 2. Effect of supplementation of Chaya leaf meal pellet (CHYP) on dry matter intake (DMI) and nutrients digestibility.

	CHYP (% of DMI)[A]					Contrast		
Items	0	4	6	8	SEM	L	Q	C
DM intake								
Rice straw								
Kg/d	3.4	3.3	3.4	3.4	0.07	NS	NS	NS
%BW	1.9	1.9	1.9	1.9	0.44	NS	NS	NS
g/kg $BW^{0.75}$	70.6	70.5	71.1	71.3	1.34	NS	NS	NS
Concentrate								
Kg/d	1.3	1.3	1.3	1.3	–	–	–	–
%BW	0.5	0.5	0.5	0.5	–	–	–	–
g/kg $BW^{0.75}$	25.3	25.3	25.3	25.3	–	–	–	–
CHYP								
Kg/d	0.0^{a}	0.17^{b}	0.24^{c}	0.40^{d}	0.01	<0.001	NS	0.03
%BW	0.0^{a}	0.10^{b}	0.15^{c}	0.21^{d}	0.004	<0 .001	0.02	NS
g/kg $BW^{0.75}$	0.0^{a}	3.6^{b}	5.5^{c}	7.3^{d}	0.12	<0 .001	0.01	NS
Total intake								
Kg/d	4.7	4.8	4.9	5.0	0.11	NS	NS	NS
%BW	2.4^{a}	2.5^{ab}	2.6^{b}	2.6^{b}	0.04	<0.01	NS	NS
g/kg $BW^{0.75}$	95.8^{a}	99.4^{b}	101.0^{b}	103.9^{b}	1.75	<0.01	NS	NS
Nutrients digestibility (%)								
DM	57.1^{a}	57.8^{ab}	58.5^{ab}	62.5^{b}	0.67	<0.05	NS	NS
OM	61.0^{a}	62.3^{ab}	62.7^{ab}	65.2^{b}	0.59	<0.05	NS	NS
CP	44.3^{a}	53.8^{ab}	55.4^{ab}	59.6^{b}	1.59	<0.05	NS	NS
NDF	53.4	53.2	53.6	53.8	0.38	NS	NS	NS
ADF	43.9	43.2	44.0	44.1	0.23	NS	NS	NS

SEM: standard error of the mean; Contrast: L, linear; Q, quadratic; C, cubic; NS: non-significant; [a-d]Means in the same row with different superscripts differ ($p < 0.05$). DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre, ADF: acid detergent fibre.
[A]Animals were fed a diet with CHYP at 0, 4, 6 and 8% of DMI.
BW: Body weight.

Dry matter intake and nutrients digestibility

Dry matter intake and nutrients digestibility are shown in Table 2. CHYP supplementation in dairy bulls had no effect on rice straw intake ($p > 0.05$) and ranged from 3.3–3.4 kg/day. DM, OM and CP digestibilities were remarkably increased (linear) ($p < 0.05$) with enhancing level of CHYP supplementation, while the digestibilities of fibrous fractions were not altered ($p > 0.05$) among treatments.

Rumen fermentation characteristics and blood metabolites

The rumen fermentation and blood metabolites data are presented in Table 3. Supplementation of CHYP had no significant effect on rumen temperature, pH and blood urea nitrogen (BUN) ($p > 0.05$). However, rumen NH_3–N concentration was increased (linear) ($p < 0.05$) when enhancing level of CHYP in the concentrate. The findings of this experiment revealed that total VFA production and C_3 were enhanced with CHYP supplementation ($p < 0.05$). However, C_2, C_4, C_2:C_3 ratio and CH_4 were similar among dietary treatment groups ($p > 0.05$). Furthermore, CHYP supplementation had no effect ($p > 0.05$) on rumen protozoa and fungi population. Nevertheless, bacterial population was significantly increased as CHYP supplementation level increased.

N–balance and urinary purine derivatives

The results of nitrogen balance and purine derivatives in dairy bulls are shown in Table 4. N–intake was linearly increased ($p < 0.001$) by CHYP supplementation level which were 30.5 to 43.1 g/day. Faecal N

Table 3. Effect of supplementation of Chaya leaf meal pellet (CHYP) on rumen fermentation, ecology, and blood metabolites.

	CHYP (% of DMI)[A]					Contrast		
Items	0	4	6	8	SEM	L	Q	C
Temperature (°C)	39.1	38.8	39.3	39.1	0.09	NS	NS	NS
pH	6.8	6.7	6.8	6.8	0.01	NS	NS	NS
NH_3–N (mg/dL)	19.0^{a}	19.5^{ab}	20.2^{b}	20.2^{b}	0.16	<0.05	NS	NS
Total VFA (mmol/L)	100.2^{a}	101.4^{a}	100.8^{a}	103.7^{b}	0.27	<0.05	NS	NS
C_2 (mmol/L)	63.9	64.4	63.0	63.8	0.28	NS	NS	NS
C_3 (mmol/L)	22.0^{a}	22.4^{a}	23.1^{ab}	24.4^{b}	0.15	<0.005	NS	NS
C_4 (mmol/L)	14.3	14.6	14.6	15.4	0.18	NS	NS	NS
C_2:C_3 ratio	2.7	2.8	2.6	2.6	0.05	NS	NS	NS
CH_4[B] (mmol/L)	28.5	28.6	27.8	28.2	0.21	NS	NS	NS
Bacteria ($\times 10^9$ cells/mL)	5.6^{a}	6.7^{b}	7.8^{c}	7.6^{c}	0.09	<0.001	0.02	NS
Protozoa ($\times 10^6$ cells/mL)	8.4	5.2	7.9	5.6	0.49	NS	NS	NS
Fungi ($\times 10^5$ cells/mL)	4.4	4.8	4.5	4.8	0.10	NS	NS	NS
BUN (mg/dL)	14.0	13.7	14.1	14.3	0.09	NS	NS	NS

SEM: standard error of the mean; Contrast: L, linear; Q, quadratic; C, cubic; NS: non-significant; [a-d]Means in the same row with different superscripts differ ($p < 0.05$); NH_3-N: ammonia nitrogen concentration; C_2: acetic acid; C_3: propionic acid; C_4: butyric acid; C_2:C_3 ratio, C_2 to C_3 ratio; BUN: blood urea nitrogen concentration.
[A]Animals were fed a diet with CHYP at 0, 4, 6 and 8% of DMI.
[B]Methane production = $(0.45 \times C_2) - (0.275 \times C_3) + (0.40 \times C_4)$ (Moss et al. 2000).
VFA: volatile fatty acids.

Table 4. Effect of supplementation of Chaya leaf meal pellet (CHYP) on nitrogen (N) balance and urinary purine derivatives.

	CHYP (% of DMI)[A]					Contrast		
Items	0	4	6	8	SEM	L	Q	C
N intake (g/d)	30.5^{a}	37.4^{b}	40.8^{c}	43.1^{d}	0.13	<0.001	NS	NS
Faecal N excretion (g/d)	14.7^{a}	15.3^{a}	17.3^{b}	17.6^{b}	0.47	<0.05	NS	NS
Urinary N excretion (g/d)	6.9	5.8	5.5	6.0	0.17	NS	NS	NS
N balance (g/d)								
Absorption	15.8^{a}	22.0^{b}	23.5^{bc}	25.4^{c}	0.44	<0.001	NS	NS
Retention	9.0^{a}	16.3^{b}	18.0^{b}	19.5^{b}	0.58	<0.001	NS	NS
Urinary purine derivatives[B] (mmol/d)								
Allantoin (mmol/d)	121.3^{a}	133.9^{a}	134.4^{a}	175.1^{b}	2.79	<0.001	NS	NS
PD excretion (mmol/d)	133.5^{a}	152.5^{b}	159.7^{c}	193.8^{d}	0.30	<0.001	NS	NS
PD absorption (mmol/d)	117.4^{a}	139.8^{b}	148.1^{b}	168.3^{c}	1.77	<0.001	NS	NS
MNS (gN/d)	85.4^{a}	101.7^{b}	107.6^{b}	122.4^{b}	1.28	<0.001	NS	NS
EMNS (gN/kg DOMR)	33.9^{a}	39.3^{ab}	40.5^{ab}	44.8^{b}	0.95	<0.05	NS	NS

SEM: standard error of the mean; Contrast: L, linear; Q, quadratic; C, cubic; NS: non-significant; $^{a-d}$ Means in the same row with different superscripts differ ($p < 0.05$); N: nitrogen; PD: purine derivative; MNS: microbial nitrogen supply calculated form (PD absorption × 0.727); EMNS: efficiency of microbial nitrogen synthesis calculated from microbial N (g/d)/ DOMR, where DOMR = digestibility organic matter apparently fermented in the rumen (assuming that rumen digestion = 65% of digestion in total tract), DOMR = DOMI × 0.65; DOMI = digestion organic matter intake.
[A]Animals were fed a diet with CHYP at 0, 4, 6 and 8% of DMI.
[B]Urinary purine derivative contained allantoin 80–85%, Calculated from (PD excretion − 0.147 × $BW^{0.75}$)/0.85.

excretions was changed ($p < 0.05$) while urinary N excretion were similar ($p > 0.05$) with the supplementation of CHYP. Nevertheless, N–absorption was increased by increasing level of CHYP supplementation (0, 4, 6 and 8% of DMI). Treatments with CHYP at 8% DMI had higher N–absorption and N–retention (25.4 and 19.5 g/day) than in the non–supplementation groups (15.8 and 9.0 g/day). The parameters of allantoin, both purine absorption and excretion were found linearly increased ($p < 0.001$). While, the amount of microbial N supply (g/day) was linearly increased ($p < 0.001$) and the highest efficiency of microbial N synthesis (EMNS) was increased ($p < 0.05$) with 8% CHYP of DMI.

Discussion

Feed ingredients and chemical compositions

Chaya is a tropical shrub and its leaf has been used as a vegetable for human consumption (Munguía-Rosas et al. 2019). However, research work related to ruminant feeding has been very limited. While, Donkoh et al. (1990) reported that Chaya contained high level of CP and minerals. In this study, the amount of CP in CHYP was 23.6% DM and with lower ADF and NDF contents, while Sarmiento–Franco et al. (2003) found that Chaya leaf contained higher CP of 26.0% CP. The CP and macro–minerals found under this study were at high level and could be nutritionally used by the ruminants (Table 1). Furthermore, Chaya leaf was rich in minerals such as Ca, K, Mg and P, as well as vitamins (Jiménez-Arellanes et al. 2014). Under this study, the results of CP, macro–minerals, as well as phytonutrients (condensed tannin, CT) were relatively comparable to those reported by Kuri–Garcia et al. (2017). As reported by Wanapat et al. (2013) that plant phytonutrients were beneficial by enhancing rumen fermentation efficiency and increasing more rumen by-pass protein. Nevertheless, other factors such as soil, climate and growth stage can contribute to the variations of the chemical compositions of CHYP (Waterman and Mole 1994; Obichi et al. 2015).

Dry matter intake and nutrients digestibility

Dry matter intake can greatly contribute to the livestock productivity and could be influenced by many factors including chemical compositions, physical and chemical characteristic (Baumont 1996). In the current study, total intake was not significantly changed with CHYP supplementation. This finding agreed with Phesatcha and Wanapat (2016) who stated that using *Flemingia macrophylla* leaf was similar in total DMI. However, when feeds containing high rumen degradable protein, it would enhance the microbial growth in the rumen especially the cellulolytic bacteria. Thus, the degradability of feeds would be consequently increased by enzymatic activity of cellulolytic bacteria (Traore et al. 2010). Under this study, DM, OM and CP degradation were improved when increasing level of CHYP (0, 4, 6 and 8%) supplementation, especially at 8%. Similarly, Huyen et al. (2012); Khy et al. (2012) reported that using Mulberry leaf pellets and *Leucaena* leaf pellets, as a protein sources could enhance DM and CP digestibilities, respectively. Nevertheless, NDF and ADF digestibility were not further affected by the CHYP supplementation. Accordingly, Chanthakhoun et al. (2011) stated that the amount of crude protein presented in the diets did not alter the digestibility of fibre proportions when fed to cattle.

Rumen fermentation characteristics and blood metabolites

The ruminal temperature and pH were not affected by CHYP supplementation. This value of rumen pH (6.7 to 6.8) was in good range value which would support the optimal microbial activity and degradation of fibre. However, the ruminal NH_3–N concentration was improved (linear) when level of CHYP supplementation increased. It is well known that increasing of NH_3–N concentration in the rumen could promote ruminal nitrogen source for microbial protein synthesis (Lu et al. 2019). Similarly, Putnam et al. (1997) reported that ruminal NH_3–N increased when CP supplementation increased. Nevertheless, BUN concentration under this study was normal and was similar to the data of Bhatta et al. (2005) who used fodder tree leaf as a supplement. They found that BUN concentration was in the range 10.5–15.5 mg/dL. The higher BUN values in the supplemented group indicated the excess of nitrogen. CHYP supplementation showed no adversely affect the BUN. The efficiency of microbial growth and their activities in the rumen depend on rumen fermentation, involved in the production of VFA, as the energy source for maintenance and growth especially, propionic acid being a primary glycogenic precursor (Wanapat and Rowlinson 2007). In this current study, the proportion of C_2, C_4 and CH_4 were not changed by dietary treatment while total VFA and C_3 proportion were improved by CHYP supplementation. Similarly, Khy et al. (2012) reported that the increase of *Leucaena* pellet at 450 g/day can improve C_3 proportion in swamp buffalo. Kholif et al. (2015) showed interesting results when using *Moring Oleifera* leaf meal (24% CP), as a protein source in lactating goat could increase C_3 production and ultimately improved the productivity. Under this work, this result may be due to increases of nutrient digestibility. Wanapat and Rowlinson (2007) illustrated that efficient fermentation process in the rumen would yield higher VFA and increased C_3, as well as C_2:C_3 ratio, required to support better growth and lactation.

N–balance and urinary purine derivatives

Under this study, the increase in faecal N excretions is associated with an increase in N intake. This could be due to the CHYP supplementation. Similarly, Berends et al. (2014) reported that increasing of protein intake increased the faecal N excretions. However, the treatment inclusion with 8% CHYP were high in N–absorption and N–retention (25.4 and 19.5 g/day, respectively) than in other treatments. This could be due to the CHYP which contained high protein level (23.6%CP), and condensed tannins which would enhance more rumen by-pass protein. As stated by Firkins et al. (2007), that more rumen by-pass protein would provide more protein in the lower-gut. Furthermore, under this study, the urinary purine derivatives were higher with increased level of CHYP supplementation, especially at 8% CHYP of DMI. Accordingly, Luc et al. (2009) revealed that increasing dietary crude protein from fodder tree using *Sesbania grandiflora* foliage resulted in increased amount of urinary purine derivatives excretion in cattle. Furthermore, the microbial protein synthesis provides the CP supply through the small intestine in ruminants (Firkins et al. 2007). In addition, the rumen microbial protein will later provide a source of protein for ruminant in the lower-gut. Importantly, the rumen microbes are a major source of protein and microbial growth can affect amino acid available to the animals. The rumen microbial protein synthesis in this study, the values ranged from 133.5 to 193.8 g MN. The values of MNS was 85.4–22.4 gN/d and was higher than those reported in ARC (1984) (14.0–60.0 g MN). In the addition, Hung et al. (2013) showed the supplementation of *Leucaena* leaf pellet (24% CP) had the efficiency of microbial N synthesis of 35.0 gN/kg DOMR. In the current study, supplementation of CHYP at 8% DMI was the highest of microbial nitrogen synthesis (EMNS) (50.7 gN/kg DOMR). Under this work, the results may be due to supplementation of CHYP at 8% DM intake can provide crude protein for microbial growth. Similarly, Umunna et al. (1995) demonstrated that *Leucaena* and *Sesbania* (fodder trees) can increase the efficiency of microbial N synthesis when fed to sheep. Any improvement in microbial N synthesis was closely linked to enhance in rumen feed degradation (Cherdthong et al. 2011). Moreover, Wang et al. (2009) elucidated that the microbial N flow into the duodenum would support the animal host productivity. Therefore, provision of N in feeds to animals can increase rumen N degradation and microbial protein synthesis (Seo et al. 2010). Likewise, Pilgrim et al. (1970) elucidated that microbial nitrogen is derived from NH_3–N and preformed amino acid, with the latter would highly be dependent on the dietary nitrogen source. Buttery et al. (1977); Bal and Ozturk (2006) addressed that microbial protein synthesis in the rumen would require dietary sulphur for synthesis of methionine and cysteine by rumen bacteria. Chaya leaf was rich in sulphur containing amino acid, hence it would support the synthesis of S–containing amino acid (Sarmiento–Franco et al. 2003).

Conclusions

Based on this experiment, it could be concluded that the CHYP supplementation especially at 8% of DMI significantly improved CP digestibility, and nitrogen utilisation efficiency. CHYP is a promising alternative protein source shrub for ruminant feeding; however, further studies are required to elucidate more relevant information for implementation.

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Ethical approval

The experimental protocols were approved by the Institute of Animals for Scientific Purpose Development (IAD), Thailand (record no.U1–06565–2526).

Disclosure statement

No potential conflict of interest was reported by the authors.

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