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Response of sheep fed urea-treated corncob and supplemented with cassava leaf meal

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Abstract

The objective of this study was to evaluate the effects of feeding untreated or urea-treated ground corncob and supplementing with cassava leaf meal (CLM) in a total mixed ration on growth, feed intake, nutrient digestibility, nitrogen (N) utilization and rumen fermentation of sheep. Five diet treatments with five replications consisted of untreated corncob + concentrate (CC); urea-treated corncob + concentrate (UCC); and CC and UCC supplemented with CLM and designated as CC+CLM and UCC+CLM, respectively. A diet formulated with a mixture of elephant grass and concentrate was used as control (EG). There were no significant differences in dry matter intake (DMI) between treatments. The average DMI was 4.17% bodyweight. Average daily gain (ADG) of sheep fed the treatment diets was between 146.3 and 176.2 g/h/day, and was higher than EG (89.1 g/head/day). Thus, the treatments improved feed conversion ratio (FCR). Nitrogen retention was lowest for EG and highest for UCC and UCC+CLM. The addition of CLM had no effect on growth, feed intake, nutrient digestibility, nitrogen utilization and rumen fermentation characteristics. It was concluded that corncob could be used as a replacement for elephant grass in postweaning diets for sheep.

Keywords: digestibility, growth, nitrogen utilization, total mixed ration

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Introduction

From 2016 to 2018, Indonesia was among the top ten maize producing countries in the world (OECD/FAO, 2019). Maize is one of the strategic food crop commodities that were targeted by the Indonesian government for domestic self-sufficiency to support the food and feed industry. The rise in maize grain production would result in a large increase in by-products, especially corncobs, which can be used as a fibre source in ruminant feed, particularly in a dry season when the availability of grass is limited. However, corncob is generally characterized as having low crude protein (CP) and digestibility, whereas its fibre content is high (Khan *et al*., 2004; Yulistiani *et al*., 2012). Urea treatment was an effective method of increasing CP content and in vitro digestibility of corncob (Yulistiani *et al*., 2012). In a total mixed ration, ground corncob could replace rice straw as a fibre source for crossbred Holstein dairy cows without affecting milk production (Wachirapacorn *et al*., 2016). Previous studies have evaluated mixtures of corncob and cowpea husk as a basal diet of West African dwarf sheep (Ososanya *et al*., 2013), and urea-treated corncobs have been used to feed Arsi-Bale sheep (Negewo *et al*., 2018).

Improving feed intake, feed digestibility, and productivity of ruminants fed low-quality forage can be achieved through supplementing with a natural protein source or non-protein nitrogen (NPN) (McGuire *et al*., 2013). However, natural protein supplementation is superior to NPN because of its amino acid and metabolizable protein content. Natural protein, particularly rumen degradable protein, provides amino acids that are catabolized into branched-chain volatile fatty acids, which serve as food for fibre-degrading bacteria in the rumen (Arroquy *et al*., 2004). Increased activity by cellulolytic bacteria in response to protein supplementation enhanced fibre digestibility and provided high-quality rumen microbial protein for the host animal (May *et al*., 2003). A sufficient supply of RDP optimized rumen microbial growth and increased the inflow of metabolizable protein to the intestine (Naves *et al*., 2015). Cassava leaves contained 19 - 25% CP, with almost 85% as true protein (Ravindran, 1993; Oni *et al*., 2010; Jaswandi & Jhon, 2019) and had a higher concentration of amino acids compared with alfalfa hay (Wanapat *et al*., 2000). Thus cassava leaves

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could be used as a natural protein supplement to increase microbial protein synthesis and fibre digestibility in the rumen. Various forms of cassava leaf – fresh cassava foliage, cassava leaf hay, CLM, and cassava top silage – have been used as protein sources to supplement low-quality forage (Hue *et al*., 2008; Ampapon *et al*., 2016; Odusanya *et al*., 2016; Wanapat *et al*., 2018). Fresh cassava foliage supplementation was able to replace concentrate in lambs fed urea-treated rice straw (Hue *et al*., 2008). Cassava hay was used as a concentrate for dairy cattle fed lime-treated rice straw supplemented with urea (Lunsin *et al*., 2012). West African dwarf sheep were fed cassava hay as a supplement to fresh chopped *Megathyrsus maximus* (Jacq.) (Odusonya *et al*., 2016). Likewise, cassava hay was used with urea-treated rice straw as feed for lactating dairy cattle (Wanapat *et al*., 2000). Cassava top silage could be used as a supplement to ammoniated rice straw for dairy cattle (Wanapat *et al*., 2018) and to low-quality grass fed to goats (Phengvichith & Ledin, 2007). Cassava hay meal was also used as rumen enhancer in a rice straw basal diet for dairy steers (Phesatcha *et al.*, 2016).

Previous studies focused on cassava leaf supplementation of rice straw or grass basal diets, and few reported the effects of supplementation on diets containing corncob. It was expected that cassava leaf would increase rumen fermentation, which would enhance sheep performance. Therefore, the objective of the study was to assess growth performance, feed intake, feed efficiency, nutrient digestibility, nitrogen utilization, and rumen fermentation of sheep fed diets that contained untreated or urea-treated ground corncob with and without CLM.

Materials and Methods

About 500 kg of dry corncob was obtained from maize fields and ground to pass through a 5-mm sieve. The urea-corncob was sprayed with 3% urea solution (1 L/kg dry matter (DM) straw), mixed thoroughly, and put in black plastic bags (5 kg cob/bag), which were pressed carefully to remove the entrapped air, sealed tightly, and stored for a minimum of three weeks. After incubation, the excess ammonia in the corncob was evaporated by spreading it evenly on a concrete floor for a day before offering it to the sheep.

Cassava leaf meal was prepared by harvesting cassava foliage at a cassava farm in Bogor District after the cassava tubers had been gathered at about 10 months old. The foliage consisted of the young stem, leaf, and the tip of the stem from the top down with a length of approximately 50 cm and was chopped to about 5 cm pieces, followed by sun-drying for three days. After drying, the DM content of the foliage was approximately 90%. It was ground to pass through a 2-mm sieve and kept for use in the experiment.

The animal experimentation was conducted after a review by the Institutional Animal Care and Use Committee of the Ministry of Agriculture Indonesia based on the Guide for the Care and Use of Laboratory Animals (NRC 2011). The growth trial was carried out at the Research Station of Indonesian Research Institute for Animal Production (IRIAP), Bogor, Indonesia. The sheep that were used in the study were of the Compass Agrinak breed, which is a composite of 50% local Sumatera, 25% St Croix and 25% Barbados Blackbelly. In total, 25 weaned lambs were used, with an average BW of 15.6 ± 3.28 kg. Each sheep was kept in an individual pen during the 12 weeks of the experiment. The sheep were stratified by initial bodyweight and assigned to one of the five diet treatments.

The experiment was conducted in a completely randomized block design with five replications in each treatment. The treatments consisted of experimental diets that were offered as total mixed rations containing 40% untreated corncob or 40% urea-treated corncob and 60% concentrate (Table 1). In the diets that were supplemented with CLM, the corncob was reduced to 38% and the concentrate to 57%. The control diet was formulated with 40% elephant grass and 60% concentrate. The concentrate mixture contained 12% coconut meal, 30% rice bran, 20% soybean meal, 24% ground maize grain, 20% soybean meal molasses, 1% urea, 1.5% salt, and 1.5% mineral supplement.

Feed consumption were measured daily by subtracting the amount of orts from the amount of feed offered. Then orts from the previous day were weighed before feed was offered in the morning. Data on ADG during the 12 weeks of the experiment was calculated from the weights of the sheep obtained weekly in the morning before feeding. Feed conversion ratio was calculated by dividing daily DMI by ADG. At the end of the growth trial, the sheep were moved to metabolic crates for a digestibility trial.

The digestibility trial consisted of a 14-day period for adaptation to the metabolic crates, followed by seven days of sample collection with rumen fluid collected on a subsequent day. Feed intake, orts, and the amounts of excreted faeces and urine of individual sheep were measured daily during the collection period. A sample consisting of 10% of the daily total faecal production was oven-dried at 60 °C for 48 hours prior to analysis of chemical composition. At the end of the seven-day collection period, the faeces samples from each sheep were pooled to provide a single sample for each animal and a 10% sub-sample was taken, ground to pass through 1 mm sieve, and kept frozen until it was analysed. Similar to the faeces collection, the total daily urine excretion of each sheep was collected every morning in a bucket containing 100 mL of

10% sulfuric acid to maintain pH below 3 to inhibit microbial activity and N losses. The volume of urine was recorded daily, mixed thoroughly, and a representative sample of 10% was taken and kept in the freezer. After the collection period, the daily samples were pooled for each sheep and 10% sub-samples were taken to measure urinary N. On the last day of the digestibility trial, rumen fluid was collected from each sheep four hours after morning feeding with a stomach tube. After sampling, the ruminal fluid pH was measured immediately using a portable pH temperature meter (LaMotte pH 5 PLUS, Maryland, USA). One drop of concentrated sulfuric acid was added to this ruminal fluid (to halt microbial activity) and then centrifuged at 3000 g for 10 min. After centrifugation, approximately 10 mL of the supernatant was removed, kept in an airtight container, and stored at -20 °C until analysed for ammonia (NH3-N) content and VFAs.

Table 1 Experimental diets and their chemical composition to evaluate corncob and cassava meal as feedstuffs for sheep

Treatments	Proportion feed ingredients (%)				
	Elephant grass	Corncob	Urea-treated corncob	Concentrate	Cassava leaf meal
EG	40	-	-	60	-
CC	-	40	-	60	-
CC+CLM	-	38	-	57	5
UCC	-	-	40	60	-
UCC+CLM	-	-	38	57	5
Chemical composition feed ingredients (g/kg)					
Organic matter	893.9	955.3	944.7	894.0	900.2
Crude protein	78.5	56.0	60.9	193.0	231.0
Neutral detergent fibre	683.0	781.5	816.6	23.8	397.5
Acid detergent fibre	439.8	386.2	486.3	14.2	319.5
Hemicellulose	243.2	395.2	330.3	9.6	78.0
Cellulose	341.6	319.2	385.0	91.4	248.0

EG: elephant grass (control diet), CC: untreated ground corncob diet, CC+CLM: ground corncob diet supplemented with cassava leaf meal, UCC: urea-treated ground corncob diet, UCC+CLM urea-treated ground corncob diet supplemented with cassava leaf meal

Chemical analysis for feeds, residues and faeces included DM, organic matter (OM), and CP contents according to the procedures of AOAC (2012). The methods of Van Soest et al. (1991) were applied to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF). Rumen NH3-N concentrations were analysed using the micro-diffusion technique of Conway (1962). Total and individual VFA concentrations were assessed with gas chromatography (Chrompack CP-9002, Chrompack, Inc., Raritan, New Jersey, USA). Microbial population referred to the number of protozoa, which was determined with a haemocytometer and the bacterial population was recorded by the roll tube method (Ogimoto & Imai, 1981).

An analysis of variance was implemented for the randomized complete block design using SAS version 9.1 (SAS Inc., Cary, North Carolina, USA). Duncan's multiple range tests were applied to differentiate the treatment means at a probability of 5%.

Results and Discussion

In this study, the CP content of corncob was 56 g/kg (Table 1), which was higher than results reported by previous researchers, which varied from 23 to 44 g/kg (Negewo *et al.*, 2018; Ososanya *et al.*, 2013; Wachirapacorn *et al.*, 2016; Yulistiani *et al.*, 2012), but may have been affected by different maize cultivars. The urea treatment of the corncob increased its CP content by 20% (Table 1), which is much lower than in previous studies, which varied from 73% to 190% (Oji *et al.*, 2007; Kayastha *et al.*, 2012; Negewo *et al.*, 2018). This increase resulted from the rates at which urea was applied. Oji *et al*. (2007) used 5.3% urea to increase the CP content of corncob from 26 to 45 g/kg. Negewo *et al.* (2018) used 4% urea to increase its CP content from 44 to 93 g/kg. Other factors that affected the CP content included ambient temperature, moisture content, treatment period, sealing, and quality of the forage (Chenost & Kayouli 1997; Oji *et al.,* 2007).

The CP content of cassava leaf in the current study was 231 g/kg (Table 1), which falls within the range of 160 to 250 g/kg reported by other researchers (Ravindran, 1993; Hue *et al.*, 2008; Ampapon *et al.*, 2016; Wanapat *et al*., 2018; 2000). The variability of CP content is affected by the leaf to stem ratio, with leaves being higher in CP than stems. Likewise, harvesting age and cutting interval affect the CP content, with the younger leaves having higher CP (Kang *et al*., 2005).

Feed consumption was expressed in g/head/day, as a percentage of bodyweight, and in g/kg metabolic weight. There were no significant treatment effects (*P* >0.05) on feed consumption. Thus, the use of ground corncob did not reduce palatability of these diets, which implied that it could be used as a fibre source to replace elephant grass in a basal diet. Ground corncob was also used in place of rice straw as a fibre source in diets for dairy cows without affecting milk production (Wachirapacorn *et al.*, 2016). Supplementation with 5% CLM did not increase DMI. Phesatcha *et al.* (2016) observed that 6% cassava hay meal supplementation had no effect on DMI. Even levels of more than 20% cassava leaf did not increase DMI (Wanapat *et al.*, 2000; Ampapon *et al.*, 2016; Wanapat *et al.*, 2018; Viennasay *et al.*, 2018). However, Odusanya *et al.* (2017) found that inclusion of 20% CLM in the concentrate diet produced higher DM intake by West African dwarf rams compared with those fed 0%, 10%, and 30% CLM. Feed consumption, ADG, and feed conversion from the growth trial are presented in Table 2.

Table 2 Treatment effects on dry matter intake, average daily gain, and feed conversion ratio of sheep

Variables	Treatment					SE	*P*-value
	EG	CC	CC+CLM	UCC	UCC+CLM		
DMI, g/head/day	1049	1092	1149	1111	1240	90	0.64
DMI, % BW	4.32	4.07	3.97	4.14	4.37	0.25	0.76
DMI, gr/BW$^{0.75}$	95.9	92.1	92.0	93.6	100.6	5.3	0.59
ADG, g/day	89.1^{b}	146.3^{a}	176.2^{a}	164.8^{a}	169.0^{a}	3.5	<0.01
FCR	12.14^{a}	7.54^{b}	6.62^{b}	6.77^{b}	7.43^{b}	0.71	<0.01

DMI: dry matter intake, BW: bodyweight, ADG: average daily gain, FCR: feed conversion ratio, EG: elephant grass control diet, CC: untreated ground corncob diet, CC+CLM: ground corncob diet supplemented with cassava leaf meal, UCC: urea-treated ground corncob diet, UCC+CLM urea-treated ground corncob diet supplemented with cassava leaf meal

^{a,b} Means with a common superscript did not differ with probability *P* =0.05

The ADG of sheep fed EG was lower (*P* <0.05) than those fed the CC, UCC, CC+CLM and UCC+CLM diets, which produced similar levels of performance (Table 2). Similarly, FCR was improved with these diets compared with EG, which indicated that corncob untreated or treated with urea could be used as a fibre source to increase bodyweight gain of sheep after weaning. Negewo *et al*. (2018) used urea-treated corncob as a fibre source to study the effects of supplemental Noug seed cake and wheat bran in Arsi-Bale sheep. Ososanya *et al.* (2013) observed that the highest weight gain and most efficient utilization were achieved by feeding a diet of 66.7% cowpea husk and 33.3% corncob to West African dwarf sheep. The ADG achieved in the current study was higher than that reported by Ososanya *et al.* (2013). This disparity in response may be because of differences in the level of cassava leaf supplementation, in diet composition and in the breed of the sheep. In contrast, Yulistiani and Puastuti (2012) reported that sheep fed a diet containing urea-treated corncob had similar ADG to those fed a diet containing grass. The higher ADG in the current study than in Yulistiani and Puastuti (2012) may be attributed to the ratios of corncob and concentrate. In the previous study, the ratio was 50% to 50%, whereas in the present study it was 40% to 60%. In addition, the corncob in the current study was of better quality, as was revealed by its higher CP content. The higher ADG of sheep fed the treatment diets and the similarity in feed consumption in the current study resulted in better (*P* <0.05), the responses in FCR corresponding to those in ADG (Table 2). Lunsin *et al*. (2012) also reported that cassava hay supplementation to a diet consisting of concentrate and urea-lime-treated rice straw basal diet did not increase milk production, but it affected milk composition in lactating dairy cows. However, Wanapat *et al*. (2018) reported that cassava top silage supplementation to total mixed ration that included rice straw did not affect DMI but increased milk production. On the other hand, Odusanya *et al*. (2017) reported that supplementation of CLM at 20% in the *Panicum maximum* basal diet increased ADG by 13.5%. In the current study, the lack of response in ADG to cassava leaf supplementation may have resulted from the unsupplemented rations being sufficient to meet the nutritional requirements of the animals.

There was no significant difference ($P > 0.05$) in nutrient digestibility among treatments except for CP. The CP digestibility of UCC+CLM was the lowest ($P < 0.05$) among the treatments, but did not differ from EG ($P > 0.05$). In contrast, the effect of urea treatments on corncob (Oji *et al*., 2007), rice straw (Gunun *et al*., 2013; Zhang *et al*., 2019) and wheat straw (Yadete, 2014) increased nutrient digestibility. The difference between this study and those just referenced could be because of the diets. In the current study, the ration was formulated to meet the nutrient requirements (Kearl, 1982) of growing sheep weighing 15 kg, which provided sufficient nitrogen and fermentable carbohydrates to create optimal rumen function for microbial protein synthesis and for fibre degradation in all treatments. The effects of the treatments on nutrient digestibility are presented in Table 3.

Table 3 Treatment effects on nutrient digestibility (%) by sheep

Nutrient	Treatments					SE	*P*-value
	EG	CC	CC+CLM	UCC	UCC+CLM		
Dry matter	59.7	61.6	57.1	59.0	56.2	1.96	0.34
Organic matter	65.9	66.9	61.8	67.6	61.1	2.24	0.17
Crude protein	76.8^{ab}	78.4^{a}	78.6^{a}	79.8^{a}	73.8^{b}	1.20	0.03
NDF	47.8	47.2	50.4	46.0	51.8	1.81	0.26
ADF	40.0	42.0	46.9	46.0	47.4	2.24	0.15
Cellulose	51.7	50.6	54.2	60.4	53.5	1.95	0.06
Hemicellulose	60.3	54.2	58.6	57.5	59.9	2.38	0.50

NDF: neutral detergent fibre, ADF: acid detergent fibre, EG: elephant grass control diet, CC: untreated ground corncob diet, CC+CLM: ground corncob diet supplemented with cassava leaf meal, UCC: urea-treated ground corncob diet, UCC+CLM urea-treated ground corncob diet supplemented with cassava leaf meal

^{a,b} Means with a common superscript did not differ with probability $P = 0.05$

The addition of cassava leaves did not increase nutrient digestibility (Table 3). Currently, there appears to be no information about the supplementation of diets that incorporate corncob as a fibre source with cassava leaves. However, wilted cassava leaf supplementation of low-quality gamba grass (*Andropogon gayanus*) increased CP, NDF, and ADF digestibility (Phengvichith & Ledin, 2007). Lunsin *et al*. (2012) also reported that the supplementation of cassava hay to urea-lime-treated rice straw increased NDF digestibility. The lack of effects on digestibility in the present study might be because of the lower level of cassava that was fed.

The N intake in CC+CLM, UCC, and UCC+CLM did not differ ($P > 0.05$). Sheep fed the UCC and UCC+CLM diets consumed more N ($P < 0.05$) than EG and CC. Sheep that were fed the UCC, CC+CLM and UCC+CLM diets excreted more N in faeces than EG and CC. Higher faecal N because of urea treatment of feedstuffs was reported for rice straw (Yulistiani *et al*., 2003; Gunun *et al*., 2013; Zhang *et al*., 2019) and wheat straw (Nurfeta *et al*., 2009; Yadete, 2013). Ammonia bound to the cell wall of urea-treated roughages is more readily absorbed in the hindgut and could account for the higher level of faecal nitrogen with an ammoniated diet (Rath *et al*., 2001). The higher bacterial counts in sheep fed ammoniated straw compared with those fed untreated rice straw may contribute to higher faecal N (Cann *et al*., 1991). Faecal nitrogen is produced from bacterial nitrogen and from nitrogen bound to the undegraded cell wall. Hassen and Chenost (1992) reported more nitrogen bound to the cell wall of ammoniated straw than with untreated straw. The cell wall that was not degraded in the rumen would be fermented in the large intestine and that partly undegraded cell wall would pass the large intestine, resulting in the higher faecal N excretion in the UCC diet. Treatment effects on nitrogen utilization of sheep are presented in Table 4.

Table 4 Treatment effects on nitrogen utilization by sheep

Variables	Treatments					SE	P-values
	EG	CC	CC+CLM	UCC	UCC+CLM		
N intake, g/day	19.6^{c}	25.1^{bc}	32.2^{ab}	34.7^{a}	38.3^{a}	2.39	<0.01
N excretion, g/day							
Feses	4.56^{c}	5.29^{c}	6.83^{b}	7.05^{b}	10.04^{a}	0.50	<0.0
Urine	1.57^{ab}	1.26^{b}	1.61^{ab}	1.22^{b}	1.83^{a}	1.14	0.05
N retention, g/day	13.5^{c}	18.5^{bc}	23.8^{ab}	26.5^{a}	26.4^{a}	1.90	<0.01
N absorption, g/day	15.1^{c}	19.8^{bc}	25.4^{ab}	27.7^{a}	28.2^{a}	2.02	<0.01
N absorption/intake, %	76.8^{ab}	78.4^{a}	78.6^{a}	79.8^{a}	73.8^{b}	1.20	0.03
N retention/intake, %	68.8^{b}	73.4^{a}	73.6^{a}	76.2^{a}	69.0^{b}	1.19	<0.01
N retained/N absorption, %	89.4^{b}	93.4^{a}	93.7^{a}	95.6^{a}	93.6^{a}	0.01	<0.01

EG: elephant grass control diet, CC: untreated ground corncob diet, CC+CLM: ground corncob diet supplemented with cassava leaf meal, UCC: urea-treated ground corncob diet, UCC+CLM urea-treated ground corncob diet supplemented with cassava leaf meal

^{a,b,c} Means with a common superscript did not differ with probability *P* =0.05

Cassava leaf meal supplementation of CC and UCC increased faecal N excretion (*P* <0.05) by 29.1 and 42.4 %. The UCC+CLM diet produced the highest (*P* <0.05) N excretion among the treatments, which was consistent with it having the highest CP content and the lower observed digestibility of CP. in the past, the effects of feeding cassava leaves on N excretion in faeces were inconsistent. Ukanwoko *et al*. (2009) and Thang *et al.* (2010) reported that cassava leaf supplementation increased N excretion in the faeces when feeding cassava peel and rice straw, respectively. On the other hand, cassava leaf supplementation of urea-treated rice straw that was fed with molasses (Hue *et al*., 2008) and increasing the level of cassava leaf supplementation from 10% to 30% on a *Panicum maximum* diet Odusonya *et al*., 2016) did not affect faecal N excretion. Therefore, these inconsistent effects might be attributed to the difference in rate and form of feeding cassava leaf and composition of the diets.

Urinary N from sheep fed UCC+CLM, EG and CC+CLM diets did not differ (*P* >0.05). However, only the urinary N of sheep fed UCC+CLM was significantly higher than those fed the CC and UCC diets. Nitrogen excretion in the urine of sheep fed the CC and UCC diets was similar. Cassava leaf meal supplementation increased N excretion in the urine produced by the sheep that were fed CC+CLM and UCC+CLM by 27.7% and 50%, respectively (Table 4). According to Hristov *et al*. (2004), protein in the rumen was degraded into amino acid, peptide and ruminal NH_3-N. The N excreted in urine resulted when ruminal NH_3-N concentration was in excess of the needs of the rumen microbes and was not incorporated in microbial protein. This excess ruminal NH_3-N can be converted to urea in the liver and excreted in the urine (Hristov *et al*., 2004).

The N retained by sheep fed the UCC and UCC+CLM diets was similar (*P* >0.05) and higher (*P* <0.05) than EG and CC. Nitrogen retention by sheep fed CC+CLM differed only (*P* <0.05) from that the EG diet. The proportion of N retained relative to N intake of sheep fed the UCC+CLM diet was the lowest (*P* <0.05) but did not differ from EG (*P* >0.05). In the present study, the efficiency of N utilization relative to intake was high (69 - 79 %) for all diets (Table 4) and more than 90% of digested N was retained in the body. These results suggested that CLM supplementation is not needed in diets that contain about 40% corncob.

The optimal rumen function because of the treatments was indicated by the similarity in ruminal ammonia concentration and bacterial population (Table 5). Rumen pH was between 6.54 and 6.72, which reflected ideal conditions for rumen microbial activity and the digestion of these diets. Wanapat and Cherdong (2009) reported that optimum ruminal pH for digestion of fibre was 6.5 - 7.0 when feeding high roughage diets. Huhtanen *et al*. (2006) indicated that increasing the proportion of concentrate in the diet from 25% to 50% lowered rumen pH from 6.43 to 6.21 and resulted in a reduction NDF digestibility. Wachirapakorn *et al*. (2016) reported that a diet with 40% ground corncob and 60% concentrate fed to crossbred dairy cows produced a ruminal pH of 6.8 - 6.9. In the current study, CLM supplementation did not affect ruminal pH. For lactating dairy cows, similar results were reported by Lunsin *et al*. (2012) and Wanapat *et al*. (2018) using cassava hay and cassava top silage as supplements, respectively.

Table 5 Treatment effects on rumen fermentation of sheep

Parameter	Treatments					SE	P-value
	EG	CC	CC+CLM	UCC	UCC+CLM		
pH	6.72	6.54	6.63	6.68	6.67	0.08	0.49
Ammonia, mg/dL	25.9^{a}	13.4^{b}	15.2^{b}	15.9^{b}	19.5^{b}	1.90	0.01
Total VFA, mMol	101.2^{c}	142.0^{a}	114.4^{bc}	120.2^{bc}	127.5^{ab}	6.61	0.01
VFA, %							
Acetic acid	74.39^{b}	73.85^{b}	74.48^{b}	77.08^{a}	76.91^{a}	0.77	0.01
Propionic acid	15.27^{ab}	17.56^{a}	16.26^{a}	13.19^{b}	13.04^{b}	0.92	0.01
Butyric acid	7.67	8.25	7.17	7.51	8.20	0.10	0.59
Iso-butyric acid	1.35^{a}	0.83^{b}	0.81^{b}	1.01^{b}	0.92^{b}	0.55	0.01
Iso-valeric acid	1.38^{a}	0.83^{b}	0.95^{b}	1.22^{ab}	0.89^{b}	0.13	0.04
Acetic acid /propionic acid	4.87^{ab}	4.20^{b}	4.5^{b}	5.84^{a}	5.08^{a}	0.31	<0.01
Bacteria, x10^{9} CFU/mL	1.50	2.12	2.70	2.70	2.66	0.67	0.74
Protozoa, x10^{5} cell/mL	9.50	16.48	19.25	19.33	13.66	2.69	0.15

VFA: volatile fatty acids, EG: elephant grass control diet, CC: untreated ground corncob diet, CC+CLM: ground corncob diet supplemented with cassava leaf meal, UCC: urea-treated ground corncob diet, UCC+CLM urea-treated ground corncob diet supplemented with cassava leaf meal

^{a,b,c} Means with a common superscript did not differ with probability *P* =0.05

Ruminal NH_3-N concentration in EG was significantly higher (*P* <0.05) than in any other diet. Ruminal NH_3-N supplied from degradation of rumen-degradable protein or NPN is an important nutrient in the rumen for protein microbial synthesis and for stimulating ruminal fermentation efficiency (Satter & Slyter, 1974; Cherdthong *et al*., 2011). Satter and Slyter (1974) suggested for optimum microbial fermentation, NH_3-N concentration should be at least 5 mg/dl. Whereas, Erdman *et al*. (1986) suggested a higher level of NH_3-N would be needed for maximum rate of fermentation and Preston and Leng (1987) found that a rumen NH_3-N level of 15 - 20 mg/dl was needed to increase intake and digestibility of cattle fed low-quality roughage such as straw. The higher rumen NH_3-N in EG may have been because fresh forage was fed, and mastication solubilized its protein by up to 56 - 65% (Ulyatt *et al*., 1975). Yulistiani (2016) reported that ruminal NH_3-N of sheep fed fresh chopped elephant grass was higher than a corncob silage-based diet. In this study, ruminal NH_3-N concentration was 13.4 - 19.5 mg/dL which was more than adequate to promote increased feed intake and fibre digestibility.

Total volatile fatty acid concentration in CC was higher (*P* <0.05) than for the other diets, but did not differ (*P* >0.05) from UCC+CLM. In contrast, the proportion of propionic acid was significantly lower in the UCC and UCC+CLM diets compared with the CC and CC+CLM, but did not differ from EG. Butyric acid was similar (*P* >0.05) among treatments. The observation that cassava leaf supplementation did not increase VFA production was not reported in sheep. However, it was reported in dairy cows when cassava top silage replaced rice straw (Viennassay *et al*., 2018), supplemented rice straw (Wanapat *et al*., 2018), and supplemented urea-lime-treated rice straw (Lunsin *et al*., 2012). The higher VFA production and more efficient use of N with the CC diet composed of about 40% corncob compared with EG could be viewed as a positive outcome for sheep feeding and further the finding that it was not necessary to use urea to increase corncob quality was favourable because this method is more practical and cheaper.

Ruminal cellulolytic bacteria play a crucial role in fermenting fibrous forage diets. In the current study, CLM supplementation was expected to provide additional non-ammonia N to improve fibre digestibility in particular and to increase microbial synthesis and animal performance. However, digestibility of NDF, ADF, cellulose and hemicellulose was not increased with CLM supplementation. Thus, the current findings contradict previous studies, which could be associated with different N sources, deriving from ruminal NH_3-N and non-ammonia nitrogen. Russell *et al*. (1992) stated that bacterial fermentation of structural carbohydrates used only ruminal NH_3-N as the source of N. However, Carro and Miller (1999) used non-ammonia N (protein, peptide or amino acid) isolated from soybean meal with sufficient energy availability in in vitro and in vivo studies and demonstrated increased fibre degradability, total VFA production, and microbial protein synthesis. Zhang *et al*. (2012) also used non-ammonia N in an in vitro study in the form of valine, leucine and iso-leucine to increase VFA and NDF degradability from a wheat straw substrate. Zain *et al*. (2008) used ammoniated palm press fibre supplemented with amino acids (valine, leucine, and iso-leucine) and methionine hydroxyl analogue in a total mixed ration and reported increased feed digestibility, total VFA production, bacterial counts and live weight gain in sheep.

In the current study, microbial protein synthesis of sheep fed CC+CLM and UCC+CLM was not different from other diets, as indicated by similar bacterial counts. Ruminal NH_3-N derived from cassava leaves was not used for rumen microbial synthesis, as was revealed by the higher N excretion in the urine, which could be related to availability of fermentable energy in the rumen providing energy and carbon skeletons in support of the synthesis of ruminant microbial protein (Hoover & Stokes, 1991). Thus, it was rationalized that NH_3-N derived from the N in cassava leaves was not balanced by available energy. Second, fermentation of cassava leaves did not improve fibre digestibility and increase VFA production because of the lack of effect on the microbial population. Similarly, Phesatcha *et al*. (2016) found that cassava leaf hay, which made up 6% of the diet, did not enhance rumen fermentation. In contrast, studies using non-ammonia nitrogen sources increased fibre digestibility and rumen fermentation because they used N supplementation from pure branch-chain amino acids and isolated branch-chain amino acids (BCAA) from soybean (Carro & Miller, 1999; Zain *et al.*, 2008; Zhang *et al*., 2012).

Volatile fatty acids are products of fermentation of feedstuffs by rumen microbes. The lack of effects on the populations of bacteria and protozoa in the sheep fed all treatments, except for EG, may have resulted in the similarity of their VFA production. In contrast, Wanapat *et al*. (2009) reported that VFA production of a urea-treated rice straw basal diet was higher than untreated rice straw because of increased nutrient digestibility. The higher VFA production in CC in the current study was because of the lower ADF content of the corncob, with most of the cell wall component being rumen-fermentable hemicellulose (Yulistiani *et al*., 2012). Gunun *et al*. (2013) observed that lactating cows had similar VFA production when fed diets containing untreated and urea-treated rice straw. Supplementation with branch-chain amino acids (BCAA) increased VFA production because of accelerated substrate degradation and BCAA metabolism (Zhang *et al*., 2012). However, the lack of effect on VFA production in the current study might be because BCAA could not stimulate microbial growth. The acetic acid proportion was higher in sheep fed the urea enhanced diets than in EG, CC and CC+CLM because of the tendency (P <0.06) towards higher digestibility of cellulose. Fermentation of nonstructural carbohydrates produces acetic acid (Firkin *et al*., 2006). Iso-butyric and iso-valeric acids are the products of fermentation of BCAA that are essential growth factors for most fibre-degrading microorganisms in the rumen (Yang, 2002). The results of the current study indicated that CLM supplementation was insufficient to increase supply of BCAA for the cellulolytic bacteria.

Conclusion

It was not necessary to apply urea to increase the quality of a diet consisting of 40% corncob. Use of CLM to supplement these diets also did not increase sheep performance. Sheep fed corncob as a fibre source had better growth rate and feed efficiency than those fed a diet that incorporated elephant grass, which suggested the viability of corncob as an alternative fibre source.

Author’s Contributions

DY and WP were in charge of project design and project implementation. DY, WP and EW analysed data, interpreted the data, and wrote the manuscript. This manuscript was read and approved by all of the authors

Conflict of Interest Declaration

There is no conflict of interest related to this manuscript

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