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Original Article

Effect of supplemental nitrogen from urea on digestibility, rumen fermentation pattern, microbial populations and nitrogen balance in growing goats

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Abstract

For this study, four Thai Native (TN) x Anglo Nubian (AN) crossbred growing goats with an average liveweight of 19.0±1 kg were randomly used in a 4x4 Latin square design to determine the effect of supplemental nitrogen from urea on digestibility, rumen fermentation pattern, microbial populations and nitrogen balance in growing goats. Fresh elephant grass (FEG) was offered *ad libitum* as the roughage. Four dietary treatments with supplemental nitrogen from urea were T_1 = urea at 0% cassava chip, (CC = 30%), T_2 = urea at 1% (CC = 40%), T_3 = urea at 2% (CC = 50%) and T_4 = urea at 3% (CC = 60%), respectively. Based on this experiment, it was found that there was no significant difference ($p>0.05$) among treatment groups regarding nutrient intake (OMI, CPI, NDFI and ADFI) and digestion coefficients of nutrients (DM, OM, CP, NDF and ADF), while digestible nutrient intake of CP (g/d) was affected by increasing urea levels. Ruminal volatile fatty acid profiles were similar among treatments. Moreover, rumen microorganism populations were not affected ($p>0.05$) by increasing urea levels. The amount of N absorption and retention were similar among treatments, except for T_4 which tended to be slightly lower in N absorption as compared to control diet, but higher N output retained (% of N intake) than the control-fed goats. From the overall results, it can be concluded that a higher level of urea (3%) could be used with a high level of CC (60%) in concentrate when fed with FEG and it was found to be a good approach to exploiting the use of local feed resources for goat production.

Keywords: Urea, cassava chip, growing goat, digestibility, nitrogen blance.

1. Introduction

Poor quality roughages are characterized by their high contents of lignocellulose and low level of nitrogen (N). Consequently, these roughages are poorly digested and often unable to support the maintenance requirements of ruminant animals. Low intake and poor utilization of this feedstuff can be partly attributed to an inefficient rumen ecosystem and an imbalance in the products of rumen fermentation (Bird, 1999). Providing supplementations with a high concentration of true protein to ruminants fed low-quality roughage stimulates roughage intake, digestion, and performance (Petersen, 1987; McColum and Horn, 1990). However, substituting non-protein nitrogen (NPN) such as urea has been shown to increase voluntary feed intake (McAllen, 1991; Huntingto and Archibeque, 1999), which is generally attributed to an improvement of nutrients digestibility and an increase passage from the rumen. Because fibrolytic bacteria use ammonia as a chief N source (Russell *et al.*, 1992), NPN should be able to substitute for at least a portion of the ruminally degradable protein (RDP); as recommended 60 to 65% of CP as RDP, and roughly 50% of the RDP as soluble protein (NRC, 1989). It was postulated that supplementation of readily degraded energy will enhance the utilization of

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available N in the rumen and further improve the productivity of goats through an increased efficiency of utilization of ammonia-N for microbial protein synthesis (Rode and Satter, 1988; McCarthy *et al*., 1989; Cameron *et al*., 1991; Castillo *et al*., 2001). Our objective was to determine the effect of supplemental nitrogen from urea on digestibility, rumen fermentation pattern, microbial populations and nitrogen balance in growing goats.

2. Materials and Methods

2.1 Animals and experimental diets

Four, Thai Native (TN) x Anglo Nubian (AN) crossbred growing goats (approximately 8 months old) averaging 191 kg (mean SD) (initial BW) were randomly assigned to dietary treatments according to a 4x4 Latin square design experiment to study the effect of supplemental nitrogen from urea on digestibility, rumen fermentation pattern, microbial populations and nitrogen balance. Four isonitrogenous-isocaloric experimental diets were given as shown in Table 1. The dietary treatments were; Control T_1 = urea at 0% (CC = 30%); T_2 = urea at 1% (CC = 40%); T_3 = urea at 2% (CC = 50%) and T_4 = urea at 3% (CC = 60%), of dietary dry matter (DM), respectively.

All goats were drenched for internal worms and injected with vitamins A, D_3 and E prior to commencing the experiment. Each goat was kept individually in a ventilated metabolism crate in well-ventilated sheds where water and mineral salt were available at all times. During each period, all animals received a concentrate diet at 2% BW (DM basis) and were allowed to consume chopped (3-5 cm) fresh elephant grass (FEG, *Pennisetum purpureum*) on an *ad libitum*, allowing for 10% refusals. Feeds were provided twice daily in two equal portions at 0800 and 1600 daily. Feed refusals were weighed and recorded daily at 0700. Fresh orts samples were bulked by pen and subsamples, dried at 60°C, were used for dry matter determinations. This information was used to calculate fresh elephant grass intake. Feed samples were obtained each time and the experimental diets were oven dried at 60°C for 72 h and ground to pass through a 1-mm sieve, and composited by period on equal weight basis for further analysis. Goats were weighed at the beginning of each experimental period before the 0800 feeding.

Table 1. Ingredients and chemical composition of goat rations (% DM basis).

Composition	Dietary treatment (% urea) [1]			
	T1(0)	T2(1)	T3(2)	T4(3)
Ingredients, %				
Cassava chip, CC	30.00	40.00	50.00	60.00
Palm cake kernel, PCK	10.00	10.00	10.00	10.00
Soybean meal, SBM	21.35	14.00	6.65	0.00
Broken rice, BR	14.65	11.00	7.35	3.00
Rice bran, RB	20.00	20.00	20.00	20.00
Urea	0.00	1.00	2.00	3.00
Molasses	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00
Dicalcium	1.00	1.00	1.00	1.00
Sulfur	0.50	0.50	0.50	0.50
Mineral mix [a]	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0
Estimated nutrients (%)				
TDN, %	77.89	76.81	75.74	74.65
CP	14.00	14.00	14.00	14.00
ME, Mcal/kg DM[2]	2.81	2.77	2.73	2.70
Concentrate cost, US $/kg[3]	0.18	0.16	0.14	0.12
Reduction cost, %	0.00	11.11	22.22	33.33

[1] T1 = Level of urea 0%, T2 = Level of urea 1%, T3 = Level of urea 2%, T4 = Level of urea 3%,

[a] Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

[2] Metabolizable energy (ME) = TDN*0.04409*0.82, [3] Official rate of exchange: 34 Baht = US $1

2.2 Sampling techniques

Each experimental period lasted for 21 days; 15 days were used to measure feed intake and the last 6 days were used to measure digestibility using total collection method. This comprised of 5 days with a total collection of feces and urine, followed by 1 day of rumen fluid and blood collection. At the end of each period, rumen fluid samples were collected from a stomach tube at 0 and 4 h-post feeding. Then, the pH of the rumen samples were measured immediately by pH meter (Orion Research portable meter 200 series, USA). Rumen fluid samples were then strained through two layers of cheesecloth and divided into two portions. One portion was used for NH_3-N and VFA analysis where 3 ml of H_2SO_4 solution (1M) was added to 30 ml of rumen fluid. The mixture was centrifuged at 16000 x *g* for 15 min and supernatant stored at -20°C prior to NH_3-N and VFA analysis. Another portion was fixed with 10% formalin solution in normal saline (0.9% NaCl) (Galyean, 1989) and cultured groups of bacteria using the roll-tube technique the method described by Hungate (1969), for identifying the bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

2.3 Laboratory analyses

Feed, refusal and feces were analyzed in duplicate for DM, ash, CF, ether extract and Kjeldahl N using AOAC (1990) procedures. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) fractions were determined with the procedure of Goering and Van Soest (1970). Hemicellulose is the difference between NDF and ADF, and cellulose is the difference between ADF and ADL, respectively. Digestion coefficients were calculated by using the formula given by Schneider and Flatt (1977).

2.4 Statistical analyses:

Statistical analysis was performed using the General Linear Models (GLM) procedure of the Statistical Analysis System Institute (SAS, 1990). Data were analyzed using the model

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

Where Y_{ijk} observation from animal j, receiving diet i, in period k; μ, the overall of mean, M_i, the mean effect of level urea (i = 1, 2, 3, 4), A_j the effect of animal (j = 1, 2, 3, 4), P_k, the effect of period (k = 1, 2, 3, 4), e_{ijk}, the residual effect. Treatment means were statistically compared using Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1980).

3. Results and Discussion

3.1 Chemical composition of feeds

The chemical composition of experimental diets and roughage are presented in Table 2. The four experimental diets contained similar concentrations of DM, ash, OM, CP, EE, CF, ADF and ADL. Diets containing high levels of urea and cassava-based diets had a slightly higher NFE and nonstructural carbohydrate (NSC) and varied NDF among

Table 2. Chemical composition of the experimental diets and elephant grass.

Chemical composition on Dry matter basis, %	Dietary treatment (% urea) [1/]				
	T1(0)	T2(1)	T3(2)	T4(3)	Elephant grass
DM [2/]	90.01	89.90	89.38	89.76	91.61
Ash	8.72	8.79	8.66	8.58	10.62
OM	91.28	91.21	91.34	91.42	89.38
CP	14.10	14.04	14.07	14.01	10.64
EE	5.83	5.32	5.23	5.31	3.89
NSC [3/]	48.98	45.65	46.24	49.82	9.87
NDF	21.52	26.20	25.17	22.28	64.98
ADF	11.49	11.58	11.76	11.56	37.75
ADL	2.15	2.25	2.38	2.63	3.43
Hemicellulose [4/]	10.03	14.62	13.41	10.72	27.23
Cellulose [5/]	9.34	9.33	9.38	8.93	34.32

[1/] T1 = Level of urea 0%, T2 = Level of urea 1%, T3 = Level of urea 2%, T4 = Level of urea 3%,

[2/] DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NSC: nonstructural carbohydrate; NDF: neutral detergent fiber; ADF: acid detergent fiber and ADL: acid detergent lignin.

[3/] Estimated: NSC = 100-(CP+NDF+EE+Ash).

[4/] Estimated: Hemicellulose = NDF-ADF, [5/] Estimated: Hemicellulose = ADF-ADL.

those diets. NSC was dramatically increased as the level of cassava chip increased in the diets. The difference among concentrate mixed diets in NSC, NDF and fiber components can be related to the difference in the ingredients used in diet formulation (Table 1).

Chemical composition of fresh elephant grass (FEG) is presented in Table 2. Elephant grass contained 10.64% CP (1.7% N). Similar values for FEG have been previously reported by Kabi *et al*. (2005); Chanjula *et al.* (2007). The relatively high levels of CP and low level of ADL in FEG suggest its suitability for goat, in terms of feed intake and digestibility, which have a limited rumen capacity to use highly lignified feeds. Nevertheless, the nutritive value of FEG may depend on cultivar, age of plant, plant density, the plant part, soil fertility, harvesting frequency, season and climate.

3.2 Effect on nutrient intake and apparent digestibility

The effects of supplemental nitrogen from urea on nutrient intake of growing goats are presented in Table 3. Nutrient intake in terms of OMI, CPI, NDFI and ADFI were seen to be statistically unaffected by dietary treatments when comparing the experimental diets (1-3% urea) with the control diet. Likewise, apparent digestibilities of DM, OM, CP, NDF and ADF were similar ($p>0.05$) for all diets, whilst digestible nutrient intake of CP (g/d) was affected by increasing urea levels (Table 3) as compared with control diet. The slightly lower digestible CP intake with increasing urea may have been a result of lower CP intake of concentrate that contained slightly lower true protein (soy bean meal, SBM) in the diet (Table 1). Saxena *et al.* (1971) indicated that supplementation of true protein was more effective than that of NPN. Similarly, McAllan (1991); Huntington and Archibeque (1999) reported that protein digestion in animals supplemented with true protein was greater than tn those supplemented with urea or NPN. However, feeding control fed-goat tended to be greater in ADF digestibility as compared to increasing urea levels in the diets. Previous reports (Hoover, 1986) have suggested that providing a source of more degradable NSC can result in a substantial decrease in ruminal pH and fiber digestibility because of reduced cellulytic activity of ruminal bacteria (Chesson *et al*., 1982). Furthermore, it is possible that low digestibility could have been attributed to the high fibrous fraction (ADL) (Hart and Wanapat, 1992). Lignin interferes with the digestion of cell

Table 3. Effect of supplemental nitrogen from urea in concentrate on apparent digestibility and digestible nutrient intake in growing goats fed on elephant grass as roughage.

Item	Dietary treatment (% urea)[1/]				SEM
	T1(0)	T2(1)	T3(2)	T4(3)	
Total OM intake, g/d	667.06	629.26	631.09	626.57	17.88
Total CP intake, g/d	93.41	86.58	88.73	85.93	2.50
Total NDF intake, g/d	302.52	306.39	300.39	293.22	8.01
Total ADF intake, g/d	169.65	161.44	161.56	163.38	4.93
Apparent digestibility, %					
DM	74.98	74.07	74.64	74.07	0.65
OM	78.00	77.23	77.64	77.28	0.47
CP	71.69	72.32	73.25	72.74	1.05
NDF	62.08	62.52	61.96	60.54	1.35
ADF	56.78	53.59	54.29	54.37	1.87
Digestible nutrient intake, g/d					
OM	521.64	486.13	490.06	485.29	11.38
CP	67.52[a]	62.71[b]	65.40[ab]	62.94[b]	1.09*
NDF	189.30	191.95	187.40	177.97	6.60
ADF	97.40	86.46	88.94	89.06	4.65
Estimated energy intake [a]					
ME Mcal/d	1.98	1.84	1.86	1.84	0.04
ME Mcal/kg DM	2.68	2.65	2.66	2.65	0.01

[1/] T1 = Level of urea 0%, T2 = Level of urea 1%, T3 = Level of urea 2%, T4 = Level of urea 3%,

[a-c] Means within the same row not sharing a common superscript are significantly different ($p<.05$)

* $p<.05$, ** $p<.001$, SEM = Standard error of the mean (n = 4)

[a] 1 kg DOM = 3.8 Mcal ME/kg (Kearl, 1982).

wall components in goats (Narjisse *et al*., 1995). The three diets with increased urea were slightly more lignified than control diet (Table 2). Mertens (1977) concluded that changes of composition of cell wall involving lignin and possibly silica limited the potential extent of digestion whereas the rate of digestion is limited by the chemical entities other than by the crystalline or physical nature of fiber. Nevertheless, ME (Mcal/d) and ME (Mcal/kgDM) of host energy metabolism were not affected ($p>0.05$) by increasing urea levels.

3.3 Volatile fatty acid profiles

The effects of supplemental nitrogen from urea on pH and production of total VFA concentrate, acetic acid proportion, propionic and butyric acid concentrations and acetic to propionic ratio are shown in Table 4. Overall means of pH and total VFAs, acetic, propionic and butyric concentrations in the rumen were not affected by dietary treatments. In this study, the pH values were within the normal range (6.0-7.0) (Hoover, 1986) and also the total VFA concentration in all diets ranged from 70 to 130 m*M*, similar to that reported by France and Siddons (1993). Thus, although the acetate to propionate ratio tended to be slightly lower by increasing urea levels, it is known that high-fiber digestion elevate acetate at the expense of propionate production goats (Van Soest, 1994).

3.4 Rumen micro-organisms

Table 5 presents rumen microorganism populations. Total viable bacterial counts, proteolytic, cellulolytic and amylolytic bacteria did not change ($p>0.05$) with increasing urea levels as compared with control diet. But overall populations tended to be slightly greater from 0 to 4 h post feeding for goats fed at the highest urea level (3% urea) as compared with other treatments. In this study, overall means of total viable bacterial counts, proteolytic, cellulolytic and amylolytic bacteria populations in all treatments ranging from 3.4-5.0 $x10^{10}$, 4.6-6.3$x10^{7}$, 4.4-5.2$x10^{9}$ and 3.5-5.2$x10^{8}$ CFU/ml, respectively, were similar to what was reported by

Table 4. Effect of supplemental nitrogen from urea in concentrate on volatile fatty acid profiles in growing goats fed on elephant grass as roughage.

Item	Dietary treatment (% urea)				SEM
	T1(0)	T2(1)	T3(2)	T4(3)	
Ruminal pH	6.77	6.72	6.67	6.72	0.04
Total VFA (mmol/l)					
0 h-post feeding	70.45	59.54	71.92	68.85	4.87
4	96.83	102.20	101.21	102.88	2.56
Mean	83.64	80.87	86.57	85.87	3.03
Molar proportion of VFA mol/ 100 mol					
Acetate (A), C_2					
0 h-post feeding	68.98	68.54	71.39	69.49	1.42
4	68.75	65.97	66.63	67.06	1.25
Mean	68.87	67.26	69.01	68.27	1.05
Propionate (P), C_3					
0 h-post feeding	20.12	20.01	17.45	19.71	1.38
4	19.76	22.52	22.41	22.64	0.81
Mean	19.94	21.27	19.93	21.17	0.84
Butyrate, C_4					
0 h-post feeding	10.88	11.43	11.14	10.78	0.37
4	11.48	11.49	10.95	10.29	0.49
Mean	11.18	11.46	11.04	10.54	0.36
A: P ratio					
0 h-post feeding	3.43	3.43	4.09	3.53	0.43
4	3.48	2.93	2.97	2.96	0.32
Mean	3.45	3.16	3.46	3.22	0.29

[1/] T1 = Level of urea 0%, T2 = Level of urea 1%, T3 = Level of urea 2%, T4 = Level of urea 3%,

[a-c] Means within the same row not sharing a common superscript are significantly different ($p<.05$)

* $p<.05$, ** $p<.001$, SEM = Standard error of the mean (n = 4)

Table 5. Effect of supplemental nitrogen from urea in concentrate on population of rumen bacteria in growing goats fed on elephant grass as roughage.

Item	Dietary treatment (% urea)				SEM
	T1(0)	T2(1)	T3(2)	T4(3)	
Total viable bacteria (x10^{10} CFU/ml)					
0 h-post feeding	5.2	3.5	4.0	4.0	1.43
4	2.8	3.2	3.1	5.9	1.86
Mean	4.0	3.4	3.6	5.0	1.15
Proteolytic bacteria (x10^{7} CFU/ ml)					
0 h-post feeding	5.0	4.5	5.6	6.0	2.30
4	4.8	6.6	3.5	6.5	3.00
Mean	4.9	5.6	4.6	6.3	2.30
Amylolytic bacteria(x10^{8} CFU/ ml)					
0 h-post feeding	5.3	5.6	4.4	4.4	2.30
4	2.8	3.2	2.6	5.9	1.90
Mean	4.1	4.4	3.5	5.2	1.70
Cellulolytic bacteria (x10^{9} CFU/ ml)					
0 h-post feeding	4.2	4.9	4.2	6.8	0.96
4	4.6	5.4	4.9	3.6	1.38
Mean	4.4	5.2	4.6	5.2	0.68

[1/] T1 = Level of urea 0%, T2 = Level of urea 1%, T3 = Level of urea 2%, T4 = Level of urea 3%,

[a-c] Means within the same row not sharing a common superscript are significantly different ($p<.05$)

* $p<.05$, ** $p<.001$, SEM = Standard error of the mean (n = 4)

Hungate (1966).

3.5 Nitrogen utilization

Whole body N balance is presented in Table 6. Total N intake in this study in terms of N-roughage and total N intake were similar ($p>0.05$) between control diet and urea inclusion in diets even though there was a trend for intake of N-concentrate to be lower for increasing urea levels than control diet. This trend may be related to the lower DMI and CP digestibility of goats fed diets containing urea. Likewise, total excretion of fecal N was not significantly different, while the decline in urinary N, total N excretion and N absorption were evident after urea inclusion exceeded 3%. This pattern of fecal and urine excretion is indicative of the extremely low N intake for goats fed diets containing 3% urea and the extremely high intake for other treatments. This could be explained by the fact that excess ruminal NH_3-N is absorbed and excreted in the urine in the form of urea (Nolan, 1993).

The amount of N retention was similar ($p>0.05$) among treatments. It is now well established that nitrogen retention depends on the intake of nitrogen, amount of fermentable carbohydrate of the diet (Sarwar *et al.*, 2003). In this regard, However the positive N balance observed in this study indicates the positive influence of increasing proportions of urea with FEG based feeding of goats. The differences in the quantity and routes of N excretion with consequent influences on N retention could reflect treatment feed differences in N metabolism, in which N retention is considered as the most common index of the protein nutrition status of ruminants (Owens and Zinn, 1988).

4. Conclusions

Isonitrogenous substitution of urea for true protein (SBM) did not affect nutrient intake and digestibility. Based on this experiment, it could be concluded that higher levels of urea (3%) could be used with high levels of CC in concentrate without altering nutrient intake, digestion coefficients of nutrients, ruminal volatile fatty acid profiles, rumen microorganism populations, amount of N absorption and retention when compared with control diet, except for T_4 which tended to be slightly lower of N absorption as compared to control diet, but higher N output retained (% of N intake) than control-fed goats. Based on these data, it can be assumed that this is a potential approach to exploiting the use of local feed resources such as cassava chip and urea for goats. However, It would be desirable to conduct further research on the use of urea in practical rations for small ruminant feeding systems

Table 6. Effect of supplemental nitrogen from urea in concentrate on nitrogen utilization in growing goats fed on elephant grass as roughage.

Item	Dietary treatment (% urea)				SEM
	T1(0)	T2(1)	T3(2)	T4(3)	
N balance, g/d					
N-concentrate	9.14	8.60	8.67	8.24	0.54
N-roughage	5.81	5.42	5.64	5.51	0.28
Total N intake	14.94	13.85	14.19	13.75	0.40
N excretion, g/d					
Fecal N	4.14	3.82	3.73	3.67	0.24
Urinary N	3.80 [a]	3.00 [ab]	3.27 [ab]	2.79 [b]	0.22*
Total N excretion	7.94[a]	6.82[b]	7.01[ab]	6.46[b]	0.28*
Absorbed N	10.80 [a]	10.03 [b]	10.46 [ab]	10.07 [b]	0.17*
Retained N	7.00	7.03	7.18	7.28	0.32
N output (% of N intake)					
Absorbed	71.69	72.31	73.24	72.75	1.05
Retained	46.65[b]	50.65[ab]	50.45 [ab]	52.15 [a]	1.42*

[1/] T1 = Level of urea 0%, T2 = Level of urea 1%, T3 = Level of urea 2%, T4 = Level of urea 3%,

[a-c] Means within the same row not sharing a common superscript are significantly different (p<.05)

* p<.05, ** p<.001, SEM = Standard error of the mean (n = 4)

as well as to use this approach for on-farm research to explore more relevant findings on animal performance and palatabilty.

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