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Effect of soybean meal replacement by slow-release urea on ruminal parameter, blood metabolites, and microbial protein synthesis in Zel ram

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ABSTRACT. The objective of this study was to determine the effect of soybean meal replacement by slow-release urea on feed intake, ruminal parameters, blood metabolites, dry matter, nutrients digestion, and microbial protein synthesis (MPS) in ram. Rams were used for four 21-day periods. The experimental design was a 4×4 Latin square design. The experiment has consisted of the following four rations: 1) basic diet with soybean meal as a protein source, 2) basic diet with soybean meal plus 0.5 % of DM slow-release urea (SRU), 3) basic diet with soybean meal plus 1 % of DM SRU, and 4) basic diet with soybean meal plus 1.5 % of SRU, respectively. Soybean meal replacement by SRU decreased ruminal ammonia and blood urea. SRU increase in diets significantly increased acetic acid 3 hours after morning feeding, blood glucose, total purine, uric acid excretion, microbial nitrogen, and microbial protein. The results showed that soybean meal replacement by SRU sources had no negative effect on rams performance.

Keywords: microbial protein; purine derivative; ruminal ammonia; nutrients digestion.

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Introduction

Urea is rapidly hydrolyzed to NH_3 in the rumen in the first-hour post-ingestion to improve the utilization of ammonia released from urea. Urea is cheap and its can improve the utilization rate of carbohydrates and the feed conversion rate (Ding, Lascano, & Heinrichs, 2015). Slow-release sources have been designed to promote the constant availability of ammonia (NH_3-N) over long periods (Taylor-Edwards et al., 2009). Rumen bacteria break down true protein to peptide, amino acids, and often NH_3-N. Ruminants can utilize non-protein nitrogen (NPN) sources such as urea and biuret and synthesize microbial protein (Hristov & Jouany, 2005). Urea can produce NH_3-N which exceeds the ruminal bacterial capacity (Galo, Emanuele, Sniffen, White, & Knapp, 2003). The NH_3-N/urea high concentrations in blood have multiple negative effects on ruminants including reduced feed consumption, changes in liver metabolism, reduced performance, induced NH_3-N toxicity which may all result in the animal's death (Huntington, Harmon, Kristensen, Hanson, & Spears, 2006). The SRU resources can synchronize the availability of N and carbohydrate fermentation (Highstreet, Robinson, Robison, & Garrett, 2010; Huntington et al., 2006), which improves microbial protein production and meet the amino acid requirements for animals. Microbial growth depends on protein degradation rate and NH_3-N availability in rumen (Brooks, Harvey, Johnson, & Kerley, 2012). SRU in the ruminant diet can decrease N pollution and diets cost, therefore, the objective of this research was to investigate the effect of slow-releasing NPN replacement by SBM on feed intake, ruminal pH and volatile fatty acid (VFA), blood metabolites, dry matter and nutrients digestion, and MPS.

Material and methods

Ethics statement

All experimental procedures described in this study were reviewed and approved by the research ethical committee of University of Sari Agriculture and Neutral Science.

Rams, diets and experimental design

The research was conducted at the Ruminant Research Center of Sari Agricultural Sciences and Natural Resources University (SANRU). Samples analyses were performed in the Animal Nutrition Laboratory at the Department of Animal Science. The experimental design was a 4×4 Latin square design with four mixed ration treatments, four Zel rams (average body weight 30 ± 2.0 kg), and four 21 days' period (14 days for adaptation and 7 days as collection period). Rams were placed in individual pens, with a bucket of tap water and separate manger. After each period, lambs were redistributed in the pens (1.5×1.5 m) to account for the effects of pens location. The lambs were fed with four total mixed rations (TMR) as treatments including 1) basic diet with soybean meal as a protein source, 2) basic diet with soybean meal plus 0.5% of DMSRU, 3) basic diet with soybean meal plus 1% of DM SRU, and 4) basic diet with soybean meal plus 1.5% of DM SRU, respectively (Table 1). The constituents of SRU consisted of 85% urea and 15% bentonite, which were pellets. DM of SRU was 96%. Lambs were housed in individual pens and fed ad libitum, twice daily at 0900 AM and 2100h with TMR, allowing for at least 10% residuals.

Table 1. Ingredients and chemical composition (g kg^{-1} of DM or as stated) of the diets containing different levels of slow release urea (SRU).

	Treatments			
Item	1	2	3	4
Ingredient (%DM)				
Barley grain	40.06	40.06	40.06	40.06
Beet pulp	7.65	7.65	7.65	7.65
Corn silage	20.5	22.50	23.50	25.50
Limestone	1.30	1.30	1.30	1.30
SBM[1]	8.24	5.95	3.40	0.85
Wheat bran	14.00	13.00	12.00	11.00
Wheat straw	8.25	9.04	11.09	12.14
SRU[2]	00	0.50	1.00	1.50
Chemical composition (%DM)				
DM	67.58	65.02	63.96	69.64
CP	14.10	14.00	14.30	14.40
NDF	38.20	39.30	40.70	43.60
EE[3]	2.40	2.50	2.50	2.50
Ash	7.10	7.10	7.30	7.10
NFC[4]	40.2	39.50	38.70	43.60
ME[5] (Mcal kg^{-1} DM)	2.89	2.87	2.85	2.85

[1]SBM = soybean meal; [2]SRU = slow release urea; [3]EE = ether extraction; [4]NFC=100 - (% Neutral Detergent Fiber+ % Crude Protein+ % Ether Extraction + Ash); [5]ME = Metabolism Energy.

Sampling

Lambs were weighed biweekly and dry matter intake (DMI) was measured daily. Total feces of all lambs from day 14 to 20 (5 days) were collected, weighed and 100 g of the fecal samples were frozen for further analysis. On day 20 the frozen samples were mixed and a 100 g final fecal sample was submitted to the lab for further analysis.

On day 21 of each period, rumen fluid samples (50 mL) were collected from the ventral sac at 0 (before the morning feeding), 3, 6, and 12h after the morning feeding using the rumenocentesis technique to measure pH, NH3-N and VFA. Ruminal pH was measured immediately after ruminal fluid collection using a pH meter (model 632, Metrohm, Herisau, Switzerland), and samples were frozen at -20°C to analyze NH3-N and VFA. Urine volume was measured daily between days 15 and 20. To prevent purine derivatives (PD) precipitation in urine during storage, 10% of the daily amount was sampled, diluted 5-fold with distilled water, and then stored at -20°C to estimate PD and N.

Laboratory analyses

Feeds, feces and orts samples were dried at 55°C and ground through a Wiley mill (1-mm screen). Samples were analyzed for DM, Kjeldahl nitrogen, ether extract, organic matter and ash at 605°C for 3h (Association of Official Analytical Chemists [AOAC], 2002), NDF and ADF (Van Soest, Robertson, & Lewis, 1991); with α-amylase being added and without sodium sulfite. The NDF was expressed without residual ash. The NFC was calculated using following formula: 100-(% CP+ %NDF+ %Ash+ %EE).

The chemical components of TMR and feces were used to calculate intake and nutrients digestibility. Table 1 displays a feed analysis. The NH3-N concentration was measured using Kjeltec Auto Analyser (Model 1030, Tecator Co. Sweden; Fenner, 1965).

Blood samples were collected from the jugular vein on day 21 in each period. Samples were collected just before the morning feeding (time 0). Approximately 10 mL of blood was collected by evacuated collection tubes containing Ethylenediaminetetra-Acetic Acid, transferred to the laboratory on ice, and centrifuged (3,500 × g at 4°C for 10 min), and plasma was stored at -20°C until analysis. The concentrations of glucose, triglycerides, cholesterol, Low-Density Lipoprotein, High-Density Lipoprotein, and blood urea were measured using the auto analyzer system (270 - CA FURUNO).

Urinary PD was estimated by spectrophotometric methods (Chen & Gomes, 1995). Allantoin was measured in urine using a calorimetric method at 522 nm after its conversion to phenylhydrazine. The sum of xanthine and hypoxanthine was calculated by conversion to uric acid with xanthine oxidase (X-1875; Sigma Aldrich, St. Louis, MO) and measuring subsequent optical density at 293 nm. Uric acid was measured from the reduction in optical density at 293 nm after degradation of uric acid to allantoin with urease (U-9375; Sigma-Aldrich). Total PD excretion per day was calculated as the sum of all 4 compounds (allantoin, uric acid, and xanthine plus hypoxanthine). The daily absorbed exogenous purines were estimated, and MPS was predicted. The urinary N concentration was estimated using the Kjeldahl method (AOAC, 2002), and N retention was calculated as daily N excretion (urinary N plus fecal N) subtracted from the daily N intake

Calculations and statistical analysis

Estimated MPS and urinary purine derivative excretion were calculated using the equations of Chen and Gomes (Chen & Gomes, 1995).

$$Y = 0.84x + 0.150BW^{0.75}e^{-0.25x};$$

where: Y (mmol d^{-1}) = PD excreted in urine; X (mmol d^{-1}) = duodenal absorption of microbial purines; e (mmol/$BW^{0.75}$ daily) = endogenous PD excretion.

$$\text{microbialN}\left(\frac{\text{gN}}{\text{d}}\right) = \frac{\text{X(mmol d}^{-1}) \times 70}{(\text{purineN: microbialN}) \times 0.83 \times 1000}$$

Data were analysed using the 4 × 4 Latin Square design by Glimmix procedure of (Hermenegildo & Puebla, 2002), based on following model:

$$Y_{(i)jk} = \mu + T_{(i)} + P_j + A_K + e_{(i)jk}$$

where, Y(i)jk was the dependent variable of each treatment in period j of rams; μ, was the overall mean; T(i) was the effect of treatment i (i=1, ..., 4); Pj was the effect of period j (j = 1, ..., 4); Ak was the effect of rams k (k = 1, ..., 4); and e(i)jk was a random error associated with each observation. The P-value was set at 0.05.

Results

Nutrients intake and digestibility

Dry matter, nutrients intake and digestibility are shown in Table 2. The SBM replacement by SRU did not affect DMI, CPI, EEI, NDFI, NFCI, Ash ($p \geqslant 0.11$).

Ruminal pH and ammonia

The rumen fluid pH did not vary for different levels of SRU in the ration at 0, 3, 6, 12 h ($p \geqslant 0.10$; Table3). There were significant effects between ruminal ammonia before feeding at 0 time (linear p = 0.02; quadratic p = 0.01; p = 0.02; Table3). When SRU increased in diet NH_3-N decreased. Treatment 4 showed the lowest NH_3-N concentration. Concentration of ruminal NH_3-N did not differ at 3, 6, and 12h (p = 0.09), after feeding.

Blood metabolite

The blood glucose increased by SBM replacement with SRU in the diet (linear p = 0.02; quadratic p = 0.01; p =0.02; Table 4), which had no effect on cholesterol, triglycerides, high-density lipoprotein, low-density

lipoprotein (p ⩾ 0. 07; Table4), There were significant differences among treatments on plasma urea concentration (linear p = 0.01; quadratic p = 0.01; p = 0.01). The results were achieved due to the increase in the SRU level of the diet which lowered blood urea.

Table 2. Effects of slow releasing urea replacement by soybean meal on dry matter and nutrients intake and digestibility in lambs fed experimental rations.

	Treatments[1] Experimental						contrasts	
Item	1	2	3	4	SEM	p-value	Linear	Quadratic
				Intake (g d^{-1})				
DM	1775.4	1861.2	1796.6	1927.9	41.24	0.59	0.75	0.74
CP	291.25	288.56	291.04	307.30	8.54	0.86	0.59	0.58
EE	49.57	50.80	50.88	53.35	1.48	0.84	0.48	0.46
NDF	789.06	802.68	828.33	930.42	24.59	0.27	0.11	0.11
NFC	830.38	802.69	787.63	783.18	22.98	0.88	0.53	0.55
Ash	146.66	146.31	148.57	151.51	4.30	0.97	0.72	0.74
				Apparent Digestibility (%)				
DM	81.215	82.310	84.021	82.183	0.43	0.24	0.41	0.58
OM	83.11	82.19	84.97	82.96	0.45	0.27	0.70	0.93
CP	73.35	72.03	74.44	70.86	3.47	0.98	0.96	0.84
EE	54.36	54.43	55.44	58.01	3.00	0.97	0.56	0.58
NDF	79.04	77.71	79.05	77.27	0.46	0.82	0.68	0.56
NFC	94.06	93.26	97.42	95.66	1.43	0.75	0.40	0.63

EE = ether extraction; NFC=100 - (% neutral detergent fiber+ % crude protein+ % ether extraction + ash); OM = organic matter. [1]Four experimental rations as treatments including: 1) basal diet contained soybean meal, 2, 3, and 4) basal diet contained soy bean meal plus 0.5, 1, and 1.5 % of DM slow release urea.[1]Footnote explaining heading. The SRU level in diets did not affect the apparent DM, OM, CP, EE, NDF and NFC digestibility (p ⩾ 0.24).

Table 3. Effect of slow releasing urea replacement by soybean meal on ruminal pH and ruminal ammonia concentration (mg dL^{-1}) at different time after feeding in lambs fed experimental rations.

Time (after feeding)	Treatments[1]						contrasts	
	1	2	3	4	SEM	p-value	Linear	Quadratic
				Ruminal pH				
0	6.59	6.85	6.99	6.63	0.05	0.10	0.54	0.77
3	6.35	6.28	6.56	6.26	0.08	0.61	0.98	0.59
6	6.29	6.22	6.48	6.27	0.12	0.87	0.83	0.96
12	6.88	6.57	6.96	6.86	0.09	0.49	0.72	0.93
Ruminal ammonia								
0	20.03[a]	17.06[ab]	17.06[ab]	14.84[b]	0.49	0.02	0.02	0.01
3	20.03	19.66	18.92	22.63	0.57	0.22	0.29	0.22
6	19.66	18.92	18.17	23.74	0.85	0.20	0.12	0.09
12	21.14	20.03	18.55	18.55	0.84	0.66	0.35	0.41

[1]Four experimental rations as treatments including: 1) basal diet contained soybean meal, 2, 3, and 4) basal diet contained soy bean meal plus 0.5, 1, and 1.5 % of DM slow release urea.[1]Footnote explaining heading. [a,b] Values within a row with different superscripts differ significantly at p < 0.05.

Microbial protein

The SRU addition had significant effects on total purine derivatives (linear p = 0.01; quadratic p = 0.01; p = 0.04), microbial nitrogen (linear p = 0.01; quadrati, p = 0.001; p = 0.04), microbial protein (linear p = 0.01; quadratic p = 0.01; p = 0.04), and uric acid (linear p = 0.10; quadratic p = 0.04; p = 0.04, Table 5). The amount of allantoin excreted in urine was greater in treatment 3 and 4 in comparison to treatments 1 and 2 (linear p = 0.04; quadratic p = 0.08; p = 0.10). The amount of uric acid excreted in urine significantly increased by SBM replacement with SRU Treatment 4 showed the greatest amount of excreted uric acid which was significantly different from others (linear p = 0.10; quadratic p = 0.02; p = 0.04).

Volatile fatty acid

The SBM replacement with SRU did not have significant effects on ruminal VFA patterns or concentrations at 0 h (TVFA , acetic acid, propionic acid, Isobutyric acid, N-Butyric acid, Isovaleric acid, Valeric acid, AC:AP)(p ⩾ 0.07), 3 h (TVFA ,propionic acid, Isobutyric acid, N-Butyric acid, Isovaleric acid, AC:AP) (p ⩾ 0.15) and Valeric aci (linear p = 0.04) ; 6 h (TVFA , acetic acid, propionic acid, Isobutyric acid, N-Butyric acid, Isovaleric acid, AC:AP)(p ⩾ 0.07) and Valeric acid (linear p = 02); 12h (TVFA, acetic acid, propionic acid, Isobutyric acid, N-Butyric acid, Isovaleric acid, AC:AP)(p ⩾ 0.06) and Valeric acid (linear p = 0.04); the only significance increasing was encountered on acetate concentration at 3h after feeding (acetic acid, linear p = 0.05; quadratic p = 0.06; p = 0.02; Table 6).

Table 4. Effect of slow releasing urea replacement by soybean meal on blood metabolites in lambs fed experimental rations.

Item	Treatments[1]						contrasts	
	1	2	3	4	SEM	p-value	Linear	Quadratic
	blood metabolites (mg dL^{-1})							
Glucose	66.5[b]	78.25[a]	78.00[a]	70.00[b]	0.99	0.02	0.45	0.42
Cholesterol	64.50	57.50	59.00	63.50	1.90	0.54	0.97	0.94
Triglycerides	35.00	30.00	34.50	36.75	2.33	0.77	0.69	0.82
HDL	29.00	24.50	25.00	28.50	0.58	0.07	0.91	0.86
LDL	28.50	27.25	26.00	28.75	1.26	0.86	0.97	0.96
Urea	25.38[a]	24.76[a]	23.48[a]	19.86[b]	0.41	0.01	0.01	0.01

HDL = high-density lipoprotein; LDL = low-Density lipoprotein. [1]Four experimental rations as treatments including: 1) basal diet contained soybean meal, 2, 3, and 4) basal diet contained soy bean meal plus 0.5, 1, and 1.5 % of DM slow release urea.1 Footnote explaining heading. [a,b,c] Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 5. Effect of replacement of slow releasing urea by soybean meal on total purine derivatives, microbial nitrogen, and microbial protein in lambs that fed experimental rations.

Item mmol d^{-1}	Treatments[1]						contrasts	
	1	2	3	4	SEM	p-value	Linear	Quadratic
Allantoin,	4.82	4.25	8.14	8.99	0.66	0.10	0.04	0.08
Uric acid,	1.01[b]	2.31[a]	0.852[b]	2.42[a]	0.18	0.04	0.10	0.02
Total purine derivatives,	6.24[b]	7.14[b]	10.02[ab]	12.85[a]	0.66	0.04	0.01	0.01
Microbial N, g d^{-1}	4.53[b]	5.19[b]	7.28[ab]	9.34[a]	0.48	0.04	0.01	0.01
Microbial protein, g d^{-1}	28.36[b]	32.48[b]	45.53[ab]	58.39[a]	3.01	0.04	0.01	0.01

[1]Four experimental rations as treatments including: 1) basal diet contained soybean meal, 2, 3, and 4) basal diet contained soy bean meal plus 0.5, 1, and 1.5 % of DM slow release urea.1 Footnote explaining heading. [a,b] Values within a row with different superscripts differ significantly at $p < 0.05$.

Discussion

Dry matter intake and digestibility

In this experiment, it was hypothesized that SBM replacement with SRU in sheep diets would not affect the synchrony between N and energy in the ruminal environment and ruminal MPS. Consequently, SBM replacement with SRU would maintain the diet digestibility and DM intake.

The DMI and total track digestibility were not significantly affected by experimental diets (Table 2). The absence of the effects on DMI can be explained by the fact that the diets were isoproteic and that the SRU has no effect on diet palatability. Treatment 4 had the highest SRU percentage of SRU and DMI. Some studies showed that SRU in the ruminant diet did not demonstrate significant differences in DMI (Eweedah, Bassuony, EL-Razik, & El-Baky, 2016; Pinos-Rodríguez, Peña, González-Muñoz, Bárcena, & Salem, 2010; Taylor-Edwards et al., 2009). Previous studies showed that the absence of the effects on DMI as a consequence of urea plus SRU supplementation has been observed in finishing lambs fed with a 50:50 forage and concentrated diet and in steers fed a finishing diet with 70% concentrate (Castañeda-Serrano, Ferriani-Branco, Teixeira, Garcia-Diaz, & Diego-Sofiati, 2013; Pinos-Rodríguez et al., 2010). The SRU replacement may improve the DMI and nutrients due to a higher fibrolytic bacteria activity. The rumen ability to hold rumen contents and the microbial biomass increase results in the higher digestion rate and more post-ruminal amino acids flow. The SRU in rations had no effect on DM, NDF, and ADF digestibility (Valério Geron et al., 2016; Xin, Schaefer, Liu, Axe, & Meng, 2010). Besides, Alves et al. (2014) showed that SBM replacement by SRU did not change the CP intake. The SRU improves nutrient imbalance for rumen bacteria by increasing the energy availability from carbohydrates. Also, the hepatic urea cycle may indirectly trigger the oxidative metabolism in the liver, which would increase the production of ATP affecting the animal feed intake (Santiago et al., 2015). Changes in productivity and/or energy efficiency can be partially be explained by improvements in nutrient synchrony between N and carbohydrate compounds in the rumen and greater N retention (decreases in ruminal ammonia concentration and increases in the flow of microbial N to the duodenum). However, this is not to be confused with the popular notion that the rate of soluble feed N release to the rumen be in synchrony with carbohydrate fermentation. The provision of adequate ruminal available N, regardless of the rate at which it is degraded or solubilized within the rumen, is the relevant factor affecting microbial protein synthesis. The above researchers have argued that their results can be partially explained by the possible synchrony of ruminal degradation rates between urea and starch.

Table 6. Effect of slow releasing urea replacement by soybean meal in different hour after feeding on volatile fatty acids in lambs fed experimental rations.

	Treatments[1]						contrasts	
Item	1	2	3	4	SEM	p-value	Linear	Quadratic
			At feeding time					
TVFA, mmol L^{-1}	64.88	63.19	51.00	48.17	4.50	0.50	0.13	0.20
Acetic acid	60.93	62.11	63.80	62.77	0.44	0.23	0.09	0.16
Propionic acid	21.26	17.67	19.29	21.66	0.72	0.27	0.77	0.90
Isobutyric acid	0.57	0.48	0.58	0.65	0.06	0.78	0.47	0.58
N-Butyric acid	14.78	17.62	14.04	12.10	0.58	0.07	0.15	0.29
Isovaleric acid	1.27	1.07	1.32	1.69	0.09	0.23	0.14	0.19
Valeric acid	1.17	1.04	0.94	1.10	0.03	0.10	0.36	0.50
AC:AP	3.01	3.52	3.41	3.05	0.12	0.39	1.00	0.94
			3 hour after feeding					
TVFA, mmol L^{-1}	74.08	67.24	65.73	63.62	4.40	0.85	0.37	0.36
Acetic acid	60.65[b]	62.45[a]	63.01[a]	63.31[a]	0.21	0.02	0.05	0.06
Propionic acid	24.18	21.19	22.75	23.67	0.64	0.44	1.00	0.84
Isobutyric acid	0.33	0.32	0.33	0.30	0.02	0.92	0.71	0.66
N-Butyric acid	12.78	13.96	11.64	10.98	0.55	0.32	0.15	0.28
Isovaleric acid	0.89	87.40	0.90	1.026	0.08	0.89	0.62	0.66
Valeric acid	1.15	1.18	0.98	0.99	0.04	0.24	0.04	0.10
AC:AP	2.55	2.94	2.81	2.77	0.08	0.42	0.59	0.48
			6 hour after feeding					
TVFA, mmol L^{-1}	66.13	72.98	54.86	58.41	4.55	0.54	0.33	0.56
Acetic acid	52.13	62.84	63.13	63.36	0.38	0.70	0.32	0.33
Propionic acid	22.34	19.99	21.88	23.09	0.51	0.28	0.61	0.77
Isobutyric acid	0.28	0.31	0.21	0.32	0.02	0.36	0.99	0.73
N-Butyric acid	13.36	14.89	12.82	11.12	0.39	0.07	0.16	0.25
Isovaleric acid	0.84	0.82	0.97	1.20	0.09	0.48	0.30	0.33
Valeric acid	1.02	1.12	0.96	0.89	0.03	0.11	0.02	0.07
AC:AP	2.82	3.16	2.93	2.87	0.09	0.56	0.94	0.90
			12 hour after feeding					
TVFA, mmol L^{-1}	55.51	50.90	64.66	52.12	4.24	0.67	0.94	0.83
Acetic acid	61.44	63.49	63.94	66.22	0.56	0.11	0.09	0.06
Propionic acid	20.91	18.40	19.02	18.13	0.83	0.66	0.25	0.19
Isobutyric acid	0.55	0.63	0.58	0.44	0.06	0.72	0.45	0.49
N-Butyric acid	14.66	15.34	14.37	12.71	0.48	0.34	0.17	0.21
Isovaleric acid	1.19	1.12	1.14	1.55	0.09	0.35	0.17	0.16
Valeric acid	1.23	1.00	0.92	0.91	0.06	0.29	0.04	0.05
AC:AP	3.02	3.52	3.40	3.68	0.16	0.56	0.16	0.12

TVFA = Total volatile fatty acids. [1] Four experimental rations as treatments including: 1) basal diet contained soybean meal, 2, 3, and 4) basal diet contained soy bean meal plus 0.5, 1, and 1.5 % of DM slow release urea.1 Footnote explaining heading. [a,b] Values within a row with different superscripts differ significantly at $p < 0.05$.

pH and NH3-N

The SBM replacement by the SRU did not affected on ruminal pH and ammonia concentration in ruminal environment (Table 3) reported no difference in the rumen pH in sheep and goat fed NPN. Van Soest (1994) reported that cellulolytic organisms grow optimally at pH 6.7 and pH level below 6.2 inhibits the digestion, decreases acetic acid, and depresses cellulolytic activity. Ruminal fermentation products and, rumen pH are highly affected by factors such as digestibility, DMI, forage, diet concentration, feed physical structure and feeding method (Van Soest, 1994). In this study, there was no difference in sheep ruminal pH and feeding SRU had no detrimental effect on ruminal pH and metabolism. In addition, ruminal pH range of 6.22 to 6.99 was suitable for cellulolytic bacteria and fiber digestion.

After feeding there was a significant effect between different treatments and NH_3-N concentration (Table 3). Treatment 4 had the lowest NH_3-N concentration (14.46 mg dL^{-1}). Treatments with SRU had significant effect on 'control' treatment. The ruminal NH_3-N concentrations range from 14.84 to 23.74 mg dL^{-1} and were close to the optimal ruminal NH_3-N range (15 to 30 mg dL^{-1}) (Anantasook, Wanapat, Cherdthong, & Gunun, 2013). The NH_3-N was absorbed across the rumen wall into the blood as ammonium. When rumen pH increased, especially above pH 7.0, NH_3-N absorption increased. The high concentrate rations typically result in lower ruminal pH, hence, decreasing the rate of NH_3-N absorption into the blood and consequently the risk of toxicity. Furthermore, there is more energy available for NH_3-N conversion to MPS in the high fermentable concentrate, especially when the urea and carbohydrate degradation rate were

synchronized. There are 2 ways N losses can be managed; either by decreasing protein degradation or by increasing the N use by the rumen microbes (Bach, Calsamiglia, & Stern, 2005). The SRU utilization results in a better synchrony between the urea hydrolysis and ammonia utilization by ruminal bacteria. (Holder et al., 2013), demonstrated higher N and microbial CP values for diets with SRU. The ruminal NH_3-N concentration and blood urea nitrogen were similar among sheep fed different SRU levels. However, the NH_3-N concentration in treatment 4 was greater at 3 and 6 h after feeding. It could be inferred that SRU diets prolong microbial utilization of additional N sources during ruminal fermentation. Thus, the synchronization between rumen NH_3-N release and carbohydrate availability may be improved, therefore resulting in higher microbial protein synthesis. Future research is required to 1) investigate the N balance effect on animal performance; 2) to ensure the SRU inclusion level and replacement by SBM 3) to improve tissue deposition and animal performance.

Blood metabolites

Glucose was significantly different between treatments including the SRU and 'control'. Control group had the lowest glucose concentration among treatments (Table 4). Increase in glucose concentration may be due to more protein bypass and increased availability of glucogenic amino acid for gluconeogenesis. This is probably due to increased blood glucose in diets including the SRU, the deamination and glucose synthesis from amino acids has decreased, and consequently, since the amine group of amino acids has not been released, urea production has been reduced by the liver, hence resulting in decreased urea plasma concentration. (Huntington et al., 2006) suggested that feeding urea, decreases insulin and increases plasma glucose concentration due to increased gluconeogenesis in the liver which this result was agree with our results. This experiment showed that feeding high rumen undegradable protein resulted in increased blood glucose concentration due to more glucogenic amino acids availability for gluconeogenesis.

Replacement the SBM by SRU had significant effect on blood urea (Table 4). Treatment 4 had the lowest blood urea. Other studies have reported that the SRU reduced blood urea concentration when compared to feed grade urea (Cherdthong, Wanapat, & Wachirapakorn, 2011; Taylor-Edwards et al., 2009). Huntington et al. (2006) reported no difference in blood urea concentration between the feed grade urea and a slow release urea-calcium. However, the blood urea nitrogen concentration was lower for lactating dairy cows fed by the SRU and SBM rather than regular urea (Gonçalves et al., 2015). Probably, more urea was produced in the liver and then excreted in urine for cows consuming urea (Xin et al., 2010). The excess NH_3-N in the rumen is absorbed into blood and transported into liver, where it is converted to urea, but if there is excess NH_3-N in the rumen, the liver is unable to convert it all, therefore increase in in peripheral blood ammonia results in toxicity. This endogenous urea is then excreted into urine or milk, around 10 to 40% of N consumed in the feed is recycled back to the digestive tract as urea from saliva where it can be used again for microbial synthesis or if the needs are already met, the urea is excreted into urine (Bach et al., 2005). In this study, degradable protein intake from the SBM in the diets was effectively replaced by up to 1.5% SRU without any adverse effects on intake or production. In addition, 1.5% urea inclusion in the diet did not increase blood urea and had no negative effect on blood metabolism. The SBM replacement by SRU allows better urea recovery in liver, saliva and rumen. The SRU provides higher urea concentrations that SBM.

Microbial protein synthesis

There was a significant difference among uric acid, excreted purine derivatives, microbial N and microbial protein (Table 5). Ttreatment 1 and treatment 2 had the lowest levels of purine derivatives, microbial N and microbial protein. The SRU replacement at higher levels increased those levels in treatments 3 and 4. Furthermore, excreted purine derivatives in treatment 1 (without SRU) were lower than treatments 2, 3 and 4. Treatment 4 had the highest percentage of excreted purine derivatives, microbial N, and microbial protein.

(Herrera-Saldana, Gomez-Alarcon, Torabi, & Huber, 1990) reported that microbial growth and microbial protein increased by energy and nitrogen synchronization in diet. Excreted purine derivative in urine is an index to measure synthesized rumen microbial protein (Chen & Gomes, 1995) which increased in sheep urine in treatment 4. Experimental rations increased microbial nitrogen and protein. Treatment 4 had the most microbial nitrogen and protein. This research showed that carbohydrate fermentation synchronization with non-protein nitrogen is essential for more efficient microbial protein synthesis. When energy is available, the final products of protein breakdown (peptide, amino acid and NH_3-N) can be used to make the

microbial protein (Bach et al., 2005). It seemed that microbial protein increase in treatment 4, is due to slow and continuous release of nitrogen from SRU (Corte et al., 2018). Therefore, the ruminal NH_3-N concentration has an impact on MPS, and a synchrony of these processes would improve the efficiency of NPN incorporation into microbial protein and thereby improving the overall efficiency of N use. Hence, the excretion of nitrogen into the environment is reduced and the environmental pollution caused by it is decreased. Lastly, SBM replacement with SRU in the diet can also reduce the final price of the diet, which would be more cost effective than true protein.

Volatile fatty acids

This study demonstrated that acetic acid concentration significantly increased 3 h after feeding, and continued up to 12 hours. It is worth noting that the diet did not play a role on other VFA components. This might be explained by a possible selective effect of urea sources on ruminal microorganisms. Several ruminal microorganisms, especially the fibrolytic bacteria, hold a greater relationship with NPN. Fiber digesting bacteria in the rumen have a preference for NH3-N while producing more acetate than propionate (Van Soest, 1994). Therefore, the supplementation with NPN sources may cause bacteria to pick additional NPN. Volatile fatty acids are the end product of rumen microbial fermentation and represent energy supply for ruminants (Van Soest, 1994). Gardinal et al. (2017) suggested that SRU have no negative effect on ruminal fermentation. Additionally, no change in the total VFA or VFA proportions were reported using urea compared to the SRU. Researchers observed comparable urea effects and recommended that greater acetate and lower butyrate concentrations in diets containing urea produced a lower acetate transformation in conformity with ruminal butyrate (Gardinal et al., 2016; Xin et al., 2010). Khattab, Salem, Abdel-Wahed, &and Kewan (2013) observed greater acetate concentration after feeding urea, and an expansion in microbial protein synthesis. It is possible that adding SRU to high-forage diets provides the NH3-N for fiber digesting bacteria, and induces their growth and population. The results showed that partial SBM replacement by SRU in rations, did not have any negative effect on VFA concentration but, increased the levels of acetic acid.

Conclusion

Replacement of slow-release products has no negative effects on sheep. In addition, in many cases, SRU functioned like SBM, and the released nitrogen increased ruminal microbial protein synthesis compared to SBM The SBM replacement with SRU resulted in the improvement in total purine, allantoin and uric acid excretion, microbial nitrogen, and microbial protein, blood glucose, acetic acid and reduced blood urea. Treatment 4 appeared to perform better but still needs further investigation. Slow release sources of N perform similarly to protein meal and therefore will reduce costs without affecting the livestock production performance.

References

Alves, E. M., Magalhães, D. R., Freitas, M. A., Santos, E. d. J. d., Pereira, M. L. A., & Pedreira, M. d. S. (2014). Nitrogen metabolism and microbial synthesis in sheep fed diets containing slow release urea to replace the conventional urea. *Acta Scientiarum. Animal Sciences, 36*(1), 55-62. doi: 10.4025/actascianimsci.v36i1.21377

Anantasook, N., Wanapat, M., Cherdthong, A., & Gunun, P. (2013). Effect of plants containing secondary compounds with palm oil on feed intake, digestibility, microbial protein synthesis and microbial population in dairy cows. *Asian-Australasian Journal of Animal Sciences, 26*(6), 820-826. doi: 10.5713/ajas.2012.12689

Association of Official Analytical Chemists [AOAC]. (2002). *Official Methods of Analysis* (20th ed.). Arlington, VA: AOAC International.

Bach, A., Calsamiglia, S., & Stern, M. (2005). Nitrogen metabolism in the rumen. *Journal of Dairy Science, 88*, E9-E21. doi: 10.3168/jds.S0022-0302(05)73133-7

Brooks, M. A., Harvey, R., Johnson, N., & Kerley, M. (2012). Rumen degradable protein supply affects microbial efficiency in continuous culture and growth in steers. *Journal of Animal Science, 90*(13), 4985-4994. doi: 10.2527/jas.2011-4107

Castañeda-Serrano, R. D., Ferriani-Branco, A., Teixeira, S., Garcia-Diaz, T., & Diego-Sofiati, A. (2013). Urea de liberación lenta en dietas para bovinos productores de carne: digestibilidad, síntesis microbiana y cinética ruminal. *Agrociencia, 47*(1), 13-24.

Chen, X. B., & Gomes, M. (1995). *Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details*: Aberdeen, UK: International Feed Resources Unit.

Cherdthong, A., Wanapat, M., & Wachirapakorn, C. (2011). Effects of urea–calcium mixture in concentrate containing high cassava chip on feed intake, rumen fermentation and performance of lactating dairy cows fed on rice straw. *Livestock Science, 136*(2), 76-84. doi: 10.1016/j.livsci.2010.08.002

Corte, R., Brito, F., Leme, P. R., Pereira, A. S. C., Freitas, J., Rennó, F. P., ... Nogueira Filho, J. C. M. (2018). The effects of partial substitution of soybean with urea or slow-release urea on finishing performance, meat quality, and digestion parameters of Nellore steers. *Animal Production Science, 58*(12), 2242-2248. doi: 10.1071/AN16609

Ding, L., Lascano, G., & Heinrichs, A. J. (2015). Effect of precision feeding high-and low-quality forage with different rumen protein degradability levels on nutrient utilization by dairy heifers. *Journal of Animal Science, 93*(6), 3066-3075. doi: 10.2527/jas.2014-8260

Eweedah, N., Bassuony, M., EL-Razik, A. A., & El-Baky, M. A. (2016). Effect of supplementing different levels of opteigen as a replacer to soybean on the steers'performance. *Journal of Agricultural Research. Kafr El-Shaikh University, 42*(2), 129-143. doi: 10.21608/JSAS.2016.2878

Fenner, H. (1965). Method for determining total volatile bases in rumen fluid by steam distillation. *Journal of Dairy Science*, *48,* 249-251. doi: 10.3168/jds.S0022-0302(65)88206-6

Galo, E., Emanuele, S., Sniffen, C., White, J., & Knapp, J. (2003). Effects of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. *Journal of Dairy Science, 86*(6), 2154-2162. doi: 10.3168/jds.S0022-0302(03)73805-3

Gardinal, R., Calomeni, G., Cônsolo, N., Takiya, C., Freitas Jr, J., Gandra, J., ... Rennó, F. (2017). Influence of polymer-coated slow-release urea on total tract apparent digestibility, ruminal fermentation and performance of Nellore steers. *Asian-Australasian Journal of Animal Sciences, 30*(1), 34. doi: 10.5713/ajas.16.0058

Gardinal, R., Gandra, J. R., Calomeni, G. D., Vendramini, T. H. A., Takiya, C. S., Freitas Júnior, J. E., ... Rennó, F. P. (2016). Effects of polymer coated slow-release urea on ruminal fermentation and nutrient total tract digestion of beef steers. *Revista Brasileira de Zootecnia, 45*(2), 63-70. doi: 10.1590/S1806-92902016000200004.

Gonçalves, A. P., Nascimento, C. F. M. d., Ferreira, F. A., Gomes, R. C., Manella, M. Q., Marino, C. T., ... Rodrigues, P. H. M. (2015). Slow-release urea in supplement fed to beef steers. *Brazilian Archives of Biology and Technology, 58*(1), 22-30. doi: 10.1590/S1516-8913201502162.

Hermenegildo, M., & Puebla, G. (2002). Static Analysis. In *9th International Symposium, SAS 2002*. Madrid, ES: Springer Science & Business Media.

Herrera-Saldana, R., Gomez-Alarcon, R., Torabi, M., & Huber, J. (1990). Influence of Synchronizing Protein and Starch Degradation in the Rumen on Nutrient Utilization and Microbial Protein Synthesis1. *Journal of Dairy Science, 73*(1), 142-148. doi: 10.3168/jds.S0022-0302(90)78657-2

Highstreet, A., Robinson, P., Robison, J., & Garrett, J. (2010). Response of Holstein cows to replacing urea with with a slowly rumen released urea in a diet high in soluble crude protein. *Livestock Science, 129*(1), 179-185. doi: 10.1016/j.livsci.2010.01.022

Holder, V. B., El-Kadi, S. W., Tricarico, J. M., Vanzant, E. S., McLeod, K. R., & Harmon, D. L. (2013). The effects of crude protein concentration and slow release urea on nitrogen metabolism in Holstein steers. *Archives of Animal Nutrition, 67*(2), 93-103. doi: 10.1080/1745039X.2013.773647

Hristov, A., & Jouany, J. (2005). *Factors affecting the efficiency of nitrogen utilization in the rumen Nitrogen and phosphorus nutrition of cattle and environment.* Wallingford, UK: CAB International. doi: 10.1079/9780851990132.0117

Huntington, G., Harmon, D., Kristensen, N. B., Hanson, K., & Spears, J. (2006). Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. *Animal Feed Science and Technology, 130*(3), 225-241. doi: 10.1016/j.anifeedsci.2006.01.012

Khattab, I., Salem, A., Abdel-Wahed, A., & Kewan, K. (2013). Effects of urea supplementation on nutrient digestibility, nitrogen utilisation and rumen fermentation in sheep fed diets containing dates. *Livestock Science, 155*(2), 223-229. doi: 10.1016/j.livsci.2013.05.024

Pinos-Rodríguez, J. M., Peña, L. Y., González-Muñoz, S. S., Bárcena, R., & Salem, A. (2010). Effects of a slow-release coated urea product on growth performance and ruminal fermentation in beef steers. *Italian Journal of Animal Science, 9*(4), e4. doi: 10.4081/10.4081/ijas.2010.e4

Santiago, B. T., Villela, S. D. J., Leonel, F. d. P., Zervoudakis, J. T., Araújo, R. P., Machado, H. V. N., ... Oliveira, T. S. d. (2015). Slow-release urea in diets for lactating crossbred cows. *Revista Brasileira de Zootecnia, 44*(5), 193-199. doi: 10.1590/S1806-92902015000500005.

Sevim, Ö., & Önol, A. G. (2019). Supplemental slow-release urea and non-structural carbohydrates: effect on digestibility and some rumen parameters of sheep and goats. *Journal of Animal and Plant Sciences, 29*(1), 1-7.

Taylor-Edwards, C. C., Hibbard, G., Kitts, S. E., McLeod, K. R., Axe, D. E., Vanzant, E. S., ... Harmon, D. L. (2009). Effects of slow-release urea on ruminal digesta characteristics and growth performance in beef steers. *Journal of Animal Science, 87*(1), 200-208. doi: 10.2527/jas.2008-0912

Valério Geron, L. J., Aguiar, S. C., Carvalho, J. T. H., Juffo, G. D., Silva, A. P., Sousa Neto, E. L., ... Paula, E. J. H. (2016). Effect of slow release urea in sheep feed on intake, nutrient digestibility, and ruminal parameters. *Semina: Ciências Agrárias, 37*(4). doi: 10.5433/1679-0359.2016v37n4Supl1p2793

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant*: Ithaca, NY: Cornell University Press.

Van Soest, P. J., Robertson, J., & Lewis, B. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Xin, H., Schaefer, D., Liu, Q., Axe, D., & Meng, Q. (2010). Effects of polyurethane coated urea supplement on in vitro ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. *Asian-Australasian Journal of Animal Sciences, 23*, 491-500. doi: 10.5713/ajas.2010.90153