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Nutrient Utilisation in Ram Lambs Fed with Coated Slow Release Non-protein Nitrogen Sources

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ABSTRACT

Background: Urea is commonly used as source of non protein nitrogen for ruminants. However, the fact that the rate of release of NH_3-N must coincide with the rate of digestion of carbohydrates or else lead to toxicity, this has led the industry to seek the development of compounds with a slow release urea (SRU), which have less risk of toxicity in animals.

Methods: A study was conducted on 30 ram lambs (1-2 month old; 14.73±0.49 kg body weight) randomly allotted to 5 dietary treatments with 6 animals in each group in a completely randomized design to evaluate the effect of supplementation of SRNPN products on nitrogen balance, plane of nutrition and nutrient utilisation. Five maize stover based complete diets (2190kcal ME/kg and 14.6% CP) were prepared *viz.* control diet with SBM as major protein source (45% CP) and without any NPN supplement, positive control diet, having urea (46% N) around 1% of dietary DM and in other 3 diets, urea was totally replaced with FCU, CFCU and HMCCU.

Result: Supplementation of SRNPN products did not affect the intake of nutrients *viz.* DM, OM, CP, CF, NFE, NDF, ADF, hemicellulose and cellulose and the digestibilities of DM, OM, NFE and ADF. While, CP digestibility (%) reduced (P<0.01) with feeding of urea (78.52) based diet compared to control diet (84.34) and replacement of urea with FCU (85.24) improved the CP digestibility (%) and was comparable to control. Incorporation of urea in the diets decreased (P<0.01) the CF and cellulose digestibility compared to control, while incorporation of FCU or CFCU in diets improved (P<0.01) CF, NDF and hemicellulose digestibilities. The N intake, excretion and balance (g/d) was though comparable among the dietary groups, the retention in lambs fed urea diets was lower by 14.71% compared to control group, while in FCU, CFCU and HMCCU fed lambs, N retention improved by 18.17, 10.05 and 7.84%, respectively compared to urea diet fed lambs.

Key words: Lambs, Nitrogen balance, Nutrient digestibility, Plane of nutrition, Slow release urea products.

INTRODUCTION

Urea is commonly used as source of non protein nitrogen (NPN) for ruminants. In the rumen, urea is hydrolyzed into ammoniacal nitrogen (N-NH3), which is later incorporated by ruminal microorganisms and transformed into amino acids and proteins of great importance to ruminants. Any excess of ammonia is absorbed by the rumen wall and can become toxic to the animal, because the liver is unable to metabolize it. Furthermore, the metabolism of ammonia into urea involves energy expenditure (Bourg *et al.,* 2012). According to Castaneda *et al.* (2009), the fact that the rate of release of NH_3-N must coincide with the rate of digestion of carbohydrates, has led the industry to seek the development of compounds with a slow release urea (SRU), which have less risk of poisoning animals. However, the literature reports different results on the use of SRU in the nutrition of sheep and other ruminants. According to Lizarazo *et al.* (2014), the use of protected urea does not affect the digestibility of dry matter (DM) and dietary neutral detergent fiber (NDF), or microbial synthesis in lambs. Further, in a study by Alves *et al.* (2014), the substitution of conventional urea by SRU (Optigen®) in sheep feed altered the nitrogen intake (NI) and the plasma urea-N concentration, but not the excretion of urinary nitrogen. Similarly in a study conducted by Geron *et al.* (2016) to evaluate the inclusion of 0.0%, 0.4%, 0.8% and 1.2% SRU in sheep feed on intake and digestibility of nutrients and ruminal parameters, showed that inclusion of SRU did not alter nutrient intake and ruminal parameters however, it caused a change in the digestibility coefficient of DM and CP. Thus, the present study was designed to investigate the effect of incorporating

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three coated slow-release urea products (SRUP) on plane of nutrition, digestibility of nutrients and nitrogen balance in lambs.

MATERIALS AND METHODS

This study was conducted at Animal Experimentation Unit, Department of Animal Nutrition, Veterinary College, Shivamogga, Karnataka, India with approval of Institutional Animal Ethics Committee (No. VCS/IAEC/LA-27/2019-20; Dated: 15.09.2020) in the year 2020.

Source of slow release urea products and other feed ingredients

Slow release urea products (SRUP) having 40% nitrogen under the investigation were *fat coated urea* (FCU) procured from M/s. Alltech Biotechnology Private Limited, Bangalore, India; Calcium salts of fatty acid coated urea (CFCU) and Hydroxypropyl methylcellulose coated urea (HMCCU) procured from Kemin Agri foods, Chennai, India. All the products contained 40% nitrogen.

Experimental diets

The complete diet for control group was formulated with Maize stover, maize grain and SBM as major protein source (45% CP) and without any supplement of non protein nitrogen sources (Table 1). The complete diet in positive control group was prepared by partially replacing SBM nitrogen in the basal complete feed with urea @ 1% of DMI having 46% nitrogen. In the other three experimental complete diets, the urea in the positive control was totally replaced FCU, CFCU and HMCCU. All the total mixture rations were iso-nitrogenous and iso-caloric and contained 14.6% CP and 2190 kcal ME/kg, respectively.

Experimental animals and management

Thirty Tumkur breed male lambs (3-4 months of age) with average body weight of 14.73±0.49 kg were purchased from a shepherd of Tumkur district of Karnataka, India. After initial quarantine and adaptation period for 15 days, they were randomly divided into 5 groups consisting of 6 animals each in a completely randomized design. Lambs were fed with respective rations to meet the nutrient requirement for maintenance and growth (100 g/day) as per ICAR (2013) twice in a day at 9.00 AM and 3.00 PM in equal proportions. All lambs were adapted to urea containing complete diet gradually for about 7 days prior to feeding experimental rations.

Metabolic trial and analytical procedure

A metabolic trial with 5 day collection period was conducted after 120 days of feeding with experimental diets by shifting them to metabolic cages (40 inches length, 26 inches width) with facilities for individual feeding and watering two days prior to the start of the collection period. The feed, faecal and urine samples were analysed for proximate constituents (AOAC, 2016).

Statistical analysis

The data was subjected to one-way analysis of variance under a completely randomized design as per the procedure of Snedecor and Cochran (1980) by using SPSS 20. The difference between means was tested by significance using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of experimental diets

The chemical composition of dietary treatments were similar (Table 2) as only the SBM in the control diet was replaced with either urea or coated SRNPN sources and the rations were formulated to be iso-nitrogenous and iso-caloric.

Nutritive value and plane of nutrition

The DCP content was significantly (P<0.01) higher in FCU diet than urea based diet and was comparable to control

Table 1: Ingredient composition (%) of complete rations containing various slow release non protein nitrogen products fed to growing lambs.

Ingredient	Control	Urea[1]	FCU[2]	CFCU[3]	HMCCU[4]
Maize stover	48.4	50.7	50.6	50.6	50.6
Maize grain	32.3	36.1	36.0	36.0	36.0
Soybean meal	18.4	11.4	11.4	11.4	11.4
Mineral and vitamin mixture*	0.3	0.3	0.3	0.3	0.3
Salt	0.6	0.6	0.6	0.6	0.6
Non protein nitrogen source	0.0	0.9[1]	1.1[2]	1.1[3]	1.1[4]
Total	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	14.6	14.6	14.6	14.6	14.6
ME (Mcal/kg)	2.19	2.18	2.18	2.18	2.18

*Mineral mixture provided per kg diet: Calcium 0.72 g, Phosphorus 0.36 g, Magnesium 0.18 g, Iron 0.018 g, Zinc 6.6 mg, Copper 12 mg, Iodine 0.6 mg, Cobalt 0.3 mg, Vitamin B_1 3.9 mg, Vitamin B_6 0.39 mg, Vitamin B_{12} 9 mg, Vitamin A 3300IU, Vitamin D_3 600IU and Vitamin E 0.6 IU.

Sodium sulphate was added over and above to urea, FCU, CFCU and HMCCU ration at the rate of 0.4%

[1]Urea contained 46% N and all other coated non protein nitrogen products contain 40% N.

[2]Fat coated urea.

[3]Calcium salts of fatty acids coated urea.

[4]Hydroxypropyl methylcellulose coated urea.

Table 2: Chemical composition of complete diet (% dry matter basis) containing various slow release non protein nitrogen products fed to growing lambs.

Constituent	Control	Urea[1]	FCU[2]	CFCU[3]	HMCCU[4]
Dry matter	91.20	91.20	90.63	90.21	91.25
Organic Matter	92.56	93.43	93.13	93.25	92.53
Crude protein	14.58	14.64	14.66	14.68	14.61
Ether extract	1.32	1.88	2.02	2.09	1.77
Crude fibre	36.46	41.88	40.68	41.34	42.09
Nitrogen free extract	40.2	35.03	35.77	35.14	34.06
Total ash	7.44	6.57	6.87	6.75	7.47
Neutral detergent fibre	60.02	61.34	59.89	61.23	60.21
Acid detergent fibre	33.43	34.23	33.54	34.43	34.45
Hemicellulose	26.59	27.11	26.35	26.8	25.76
Cellulose	27.44	28.11	27.63	28.33	28.78
Lignin	3.88	4.02	3.81	4.12	3.82

Each value is the average of duplicate analysis.

[1]Urea contained 46% N and all other coated non protein nitrogen products contain 40% N.

[2]Fat coated urea.

[3]Calcium salts of fatty acids coated urea.

[4]Hydroxypropyl methylcellulose coated urea.

diet (12.30%). While, replacement of urea with CFCU (11.58%) and HMCCU (11.40%) had no effect (P>0.05) on DCP content of the diets and was lower (P<0.05) than control group. The TDN and ME contents of the diets were similar among the group, since the diets were prepared to be iso-caloric as well as the NFE intake and digestibility was similar among the dietary groups (Table 3).

Dry matter, digestible protein and energy intake *viz.* DM, DCP, TDN and ME in terms of g or Mcal per kg/day were similar among different dietary treatments (Table 3). Palatability and digestibility are the main factors affecting the intake of nutrients (Ribeiro *et al*., 2014). In the present study, the intake values have clearly indicated that, SRNPN sources used in present study were acceptable to the animals. Further, intake of DCP, TDN and ME are well within the range of recommendation by ICAR (2013) for a 20 kg growing lamb with ADG of 100 g (DMI - 680 g; TDN- 455 g; ME - 1638 kcal; CP –109 g; DCP 72 g). Raouf *et al*. (2017) reported no effect (P>0.05) in TDN, DCP, DE, ME and NE_l in lactating Holstein cows with replacement of 1 and 0.5 kg soybean meal by 125 and 62.5 g Optigen™. Similarly, Sevim and Onol (2019) studied the affect of supplementing slow-release urea (polymer coated - Optigen) with or without non-structural carbohydrates in sheep and goats fed groundnut straw based diet and reported no effect (P>0.05) on the plane of nutrition.

Nutrient digestibility

The digestibility of DM, OM, NFE were not affected by replacing a part of SBM - N with either urea or various SRNPN sources in growing lambs (Table 3). While, the CP digestibility reduced with incorporation of urea compared to SBM based diet (control) which could be the result of faster rate of degradation of urea to ammonia leading to decreased utilisation and increased excretion of N (Table 4). Gonçalves *et al*. (2015) also reported decreased CP digestibility in beef steers fed urea based diet compared to those fed SBM based control diet when 40% of protein requirements were met through various NPN sources (feed grade urea, polymer coated slow release urea). In the present study, replacement of urea with CFCU and HMCCU had no effect (P>0.05) on CP digestibility and was comparable to urea, but replacement of urea with FCU improved (P<0.01) the CP digestibility and was comparable to control. The higher CP digestibility observed in lambs fed FCU diet compared to other urea based diets was due to lower fecal nitrogen excretion (Table 4). Gardinal *et al.* (2017) also reported increase (P<0.01) in CP digestibility in steers fed diets containing 2% polymer coated urea compared with that fed feed grade urea at similar level of inclusion.

Incorporation of urea had no effect (P>0.05) on EE digestibility and was comparable to control. Similar observations were made by Cherdthong *et al.* (2011) in cross bred beef steers with feeding of soybean meal based control diet, urea, urea $CaCl_2$ mixture and urea $CaSO_4$ mixture diets. Replacement of urea with various SRNPN sources reduced (P<0.01) EE digestibility in present study, which could be due to higher CF digestibility observed in these groups and result of higher number of fiber degrading bacteria compared to lypolytic bacteria (Noel Samantha, 2013).

The digestibility of CF, NDF, hemicellulose and cellulose differed significantly among the dietary treatments (Table 3). The CF digestibility was significantly (P<0.01) lower in urea diet fed lambs compared to control lambs. Wahrmund *et al*. (2007) also reported decreased CF, NDF and ADF digestibility in urea fed cows (urea supplying 25% of protein requirement) compared to control group (no urea). In the present study, the FCU and CFCU diet fed lambs had higher (P<0.01)

CF digestibility than urea and was comparable to that of control group indicating better synchrony in utilisation of NH_3-N released from the these SRNPN sources and enhancing the fibre degradation by rumen microbes. On the other hand, the digestibility of CF in HMCCU group was lower (P<0.01) compared to control as well as other two SRNPN based groups, which may be due to lower digestibility of CP observed. Interestingly, the digestibility

Table 3: Nutrient intake, digestibility and plane of nutrition in growing lambs fed diets containing various slow release non protein nitrogen products.

Attributes	Control	Urea[1]	FCU[2]	CFCU[3]	HMCCU[4]	SEM	P value
Nutritive value of total ration (%)							
DCP (%)	12.30^{a}±0.20	11.50^{b}±0.30	12.50^{a}±0.11	11.58^{b}±0.11	11.40^{b}±0.20	0.117	0.001
TDN (%)	61.90±0.98	59.14±1.63	62.58±1.15	62.72±1.02	59.94±1.19	0.574	0.167
ME (Mcal/kg DM)[5]	2.23±0.035	2.13±0.059	2.25±0.04	2.26±0.03	2.16±0.04	0.020	0.168
Nutrient intake, g/day							
Dry matter Intake (g)	755.37±56.28	749.44±64.74	788.84±49.76	734.54±36.85	752.47±38.61	21.180	0.96
Crude protein intake (g)	110.13±8.20	109.72±9.47	115.64±7.29	107.83±5.41	109.94±5.64	3.098	0.96
Digestible crude protein (g)	92.41±5.82	86.18±8.18	98.72±6.72	85.06±4.20	85.86±5.07	2.735	0.47
Total digestible nutrients (g)	478.69±38.56	451.22±49.66	505.57±43.02	466.28±22.14	457.53±34.24	16.44	0.87
Metabolisable energy (Mcal)	1.73±0.14	1.63±0.18	1.82±0.16	1.68±0.08	1.65±0.12	0.062	0.87
Nutrient digestibility (%)							
Dry matter	63.89±1.08	58.83±2.14	63.46±1.22	63.63±1.01	61.52±1.26	0.682	0.09
Organic matter	65.53±1.05	60.61±2.11	65.37±1.21	65.34±1.08	62.94±1.28	0.683	0.08
Crude protein	84.34^{a}±1.37	78.52^{b}±2.08	85.24^{a}± 0.72	78.91^{b}±0.76	78.02^{b}±1.39	0.808	0.001
Ether extract	75.48^{a}±0.73	77.64^{a}±1.16	67.33^{c}±1.10	68.45^{c}±0.88	71.29^{b}±0.94	0.840	0.001
Crude fibre	53.37^{a}±1.92	40.79^{b}±2.79	53.42^{a}±1.36	53.39^{a}±1.38	43.74^{b}±2.65	1.350	0.001
Nitrogen free extract	71.05±1.13	71.81±1.35	68.63±2.29	70.53±2.40	73.18±0.82	0.766	0.45
Neutral detergent fibre	60.88^{c}±1.17	62.19^{bc}±1.96	64.01^{bc}±1.21	69.24^{a}±0.85	65.17^{b}±1.14	0.765	0.001
Acid detergent fibre	67.41±0.82	65.66±1.75	68.25±1.25	68.43±0.93	66.51±1.36	0.569	0.50
Hemicellulose	82.80^{b}±0.79	84.88^{b}±0.96	81.91^{b}±0.61	89.01^{a}±0.37	88.07^{a}±1.68	0.662	0.001
Cellulose	64.61^{a}±1.07	56.56^{b}±2.04	63.94^{a}±1.57	63.89^{a}±0.78	58.02^{b}±0.94	0.659	0.001

Each mean value is an average of 6 values.

[1]Urea contained 46% N and all other coated non protein nitrogen products contain 40% N.

[2]Fat coated urea.

[3] Calcium salts of fatty acids coated urea.

[4]Hydroxypropyl methylcellulose coated urea.

[5]1 kg TDN = 3.6 Mcal ME per kg (NRC on Beef Cattle, 2016).

Table 4: Nitrogen balance in growing lambs fed diets containing various slow release non protein nitrogen products.

Attribute	Control	Urea[1]	FCU[2]	CFCU[3]	HMCCU[4]	SEM	P value
Metabolic body weight	12.49±0.52	11.77±0.65	11.95±0.83	12.29±0.55	12.24±0.63	0.271	0.94
N intake (g/d)	17.62±1.31	17.55±1.52	18.50±1.16	17.25±0.86	17.59±0.90	0.496	0.96
N excretion (g/d)							
Faecal	2.82±0.42	3.75±0.49	2.71±0.16	3.63±0.24	3.83±0.27	0.166	0.06
Urinary	2.09±0.37	2.96±0.73	2.99±0.25	1.69±0.21	2.07±0.49	0.211	0.19
Total	4.91±0.68	6.71±0.93	5.70±0.32	5.32±0.43	5.90±0.60	0.284	0.35
Nitrogen balance							
g/day	12.71±0.80	10.84±1.34	12.81±0.89	11.93±0.59	11.69±1.073	0.426	0.61
g/kg metabolic body weight	1.03±0.07	0.91±0.07	1.09±.08	0.97±.024	0.95±.049	0.028	0.34
% of intake	72.63±2.22	61.74±4.54	69.11±.93	69.27±1.45	66.06±3.83	1.392	0.13

Each mean value is an average of 6 values.

[1]Urea contained 46% N and all other coated non protein nitrogen products contain 40% N.

[2]Fat coated urea.

[3]Calcium salts of fatty acids coated urea.

[4]Hydroxypropyl methylcellulose coated urea.

of NDF, hemicellulose and cellulose was higher (P<0.01) in lambs fed various SRNPN diets compared to control and urea based group indicating better utilisation of cell wall contents with the supplementation of SRNPN sources at around 1% of dietary DM. Inclusion of SRU sources in the poor roughage based diet is reported to increase the fiber degrading bacterial count and further enhanced the fiber degradation (Noel Samantha, 2013). In agreement with the present study findings, Mentz *et al.* (2015) also observed higher CP and CF digestibility in wethers fed with poor-quality roughage diet supplemented with urea and Optigen® II in ratio of 0: 100, 25: 75, 50: 50, 75: 25 and 100: 0. Geron *et al.* (2016) evaluated the effect of inclusion of 0.0, 0.4, 0.8 and 1.2% Optigen in sheep diets and reported higher digestibility of DM, CP and NDF with dietary inclusion of Optigen at 0.4 and 0.8%.

Nitrogen balance

Partial replacement of SBM in the control diet with either urea or SRNPN sources did not significantly (P>0.05) affect nitrogen intake, excretion and retention in the growing lambs (Table 4). The N intake (g/d) was comparable among the different dietary treatments as the diets were iso nitrogenous, animals had similar DMI and urea or SRNPN sources were included at around 1% in the diet. Geron *et al.* (2018) also observed that CP intake was not affected in sheep when urea or SRNPN sources inclusion were around 1% of diet. Excretion of N through faeces and urine was although statistically comparable, excretion of N through faeces and urine was higher in urea diet fed lambs compared to control lambs by 32.98 and 41.63%, respectively which could be due to higher rate of urea degradation to ammonia and consequently increased N excretion without being incorporated to microbial protein. Replacement of urea with SRNPN sources non-significantly (P>0.05) reduced the excretion of N in the urine or faeces compared to urea based diets. The total N excretion was lower in FCU, CFCU and HMCCU fed lambs by 15.05, 20.71 and 12.07%, respectively compared to urea diet fed lambs. Lower N excretion in the SRNPN groups suggests better turn over and utilisation of NH_3-N from SRNPN sources compared to urea as N source. Alves *et al.* (2014) also reported reduction in the total N excretion in polymer coated slow release urea fed sheep compared to urea fed group in a study involving feeding of polymer coated urea as a replacement to urea at 0, 20, 40, 60 and 80% levels. In the present study, even though N retention was statistically comparable, the N retention in the urea diet fed lambs was lower by 14.71% compared to that of control lambs while, in the FCU, CFCU and HMCCU fed lambs the N retention improved by 18.17, 10.05 and 7.84% respectively, compared to urea diet fed lambs. Geron *et al.* (2018) observed that, with dietary inclusion of SRU at 0.6 to 0.8% of total DM resulted in lowest fecal and urinary nitrogen losses and higher nitrogen balance in sheep. Comparable N retention was reported by various researchers in response to dietary SRU supplementation *viz.* Galina *et al.* (2004a) in goats, Galina *et al.* (2004b) in sheep and Cherdthong *et al.* (2013) in beef cattle. Sevim and Onol (2019) also reported no effect (P>0.05) on the fecal and urinary N excretion and N retention among the treatments in sheep and goats fed 10 g/d Optigen with or without molasses along with basal diet (containing groundnut straw). While, Gardinal *et al.* (2017) reported decreased (P≤0.01) N intake, fecal and urinary N excretion and increased N retention when SRU was supplemented in the diet of Nellore steers @ 20 g/kg DM in place of feed grade urea.

CONCLUSION

Feeding of urea as such found to reduce the nutrient intake, plane of nutrition, digestibility of nutrients and increased the nitrogen excretion compared to the control, while replacement of urea with coated urea products enhanced the intake, digestibility and nitrogen retention in growing lambs.

Conflict of interest

All authors declare that they have no conflict of interest.

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