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SUPPLEMENTAL SLOW-RELEASE UREA AND NON-STRUCTURAL CARBOHYDRATES: EFFECT ON DIGESTIBILITY AND SOME RUMEN PARAMETERS OF SHEEP AND GOATS

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ABSTRACT

A study was conducted to evaluate the effect of slow-release urea (SRU) and/or non-structural carbohydrates (NSC) supplementation to groundnut straw (*Arachis hypogaea*) on digestibility and some rumen parameters in sheep and goats. A total of four male sheep (43±2.6 kg BW) and four male goats (37±1.8 kg BW) were used in a 4 × 4 Latin square design. Treatments consisted of four experimental groups; control (C) group fed basal diet, T1 fed basal diet + 10 g/day SRU, T2 fed basal diet +10 g/day SRU + molasses at 10% of ration on dry matter (DM) basis, and T3 fed basal diet +10 g/day SRU + starch at 5% of ration on DM basis. SRU and/or NSC supplementation significantly enhanced DM and organic matter (OM) digestibility, nitrogen (N) intake, urinary N, N retention, and N digestibility in sheep whereas OM digestibility, N intake, fecal and urinary N output, and N digestibility in goats. Only ammonia-N (NH_3-N) levels among the rumen fluid parameters were significantly different in both sheep ($P \leq 0.01$) and goats ($P \leq 0.05$) at 2 h. In sheep and goats, rumen pH and NH_3-N ($P \leq 0.001$), as well as propionate levels ($P \leq 0.001$) in goats were different at 2 h compared to 6 h. In conclusion, SRU and/or NSC supplementation improved the digestibility and weight gain, especially with corn starch under the conditions of present study.

Keywords: Slow-release urea, non-structural carbohydrate, goat, sheep, digestibility.

INTRODUCTION

The inclusion level of non-protein nitrogen (NPN) in ruminant diets is limited because of rapid hydrolysis of the nitrogen (N) in NPN sources to ammonia (NH_3). There are several solutions to control the release of NH_3 in rumen such as binding urea to calcium chloride (Huntington *et al.,* 2006), encapsulating urea with oil (Garrett *et al.,* 2005), or polymer (Lizarazo *et al.,* 2014). Coating urea with polymer is an effective way to reduce NPN degradation in rumen (Lizarazo *et al.,* 2014). The use of polymer-coated urea may increase dry matter intake (DMI) (Ribeiro *et al.,* 2011), dry matter (DM) digestibility (Chegeni *et al.,* 2013), and N availability (Hristov, 2012) in ruminants.

Synchrony of carbohydrate (CHO) degradation rate and N availability is one of the several factors governing the NH_3 utilization in rumen. This synchrony limits microbial protein synthesis (Heldt *et al.,* 1999) and ruminal NH_3 utilization (Hristov *et al.,* 1997). Therefore, ruminal CHO degradation is a critical factor for efficiency of NH_3 utilization (Hristov *et al.,* 2005). Supplementation of non-structural carbohydrates (NSC) to rations containing slow release urea (SRU) as an NPN source is one of the possibilities that may improve N utilization in the rumen. Lizarazo *et al.* (2014) reported that molasses supplementation along with SRU in rations had no effect on N utilization in lambs. To date, no other study has been reported that describes the effect of source of NSC on N utilization derived from SRU. Therefore, we hypothesized that corn starch as a source of NSC may be effective to increase the N utilization of SRU in small ruminants. The aim of experiment was to determine the influence of dietary SRU and/or two different NSC sources on digestibility in general and rumen N utilization in particular in sheep and goats fed low quality forage.

MATERIALS AND METHODS

This experiment was carried out at the experiment station in Faculty of Veterinary Medicine, Adnan Menderes University with prior approval from Animal Research Ethics Committee of the university.

Animals and Treatments: Four crossbred rams (43±2.6 kg body weight) and four crossbred male goats (37±1.8 kg body weight) were randomly placed in 4 × 4 Latin square design, separately, to measure the effects of SRU (Optigen II, Alltech Biotechnology Corporation, USA) and NSC (beet molasses and corn-starch). Each experimental period lasted 15 days; 10 days of adaptation and 5 days of sampling. The basal diet consisted of 99.4% chopped groundnut straw, 0.5% salt and 0.1% vitamin-mineral premix (Kavimix VM 602 from Kartal Chemistry Co. Kocaeli, Turkey). Each kilogram premix

contained 10,000,000 IU vitamin A; 2,000,000 IU vitamin D_3; 30,000 mg vitamin E; 50,000 mg Mg; 50,000 mg Fe; 50;000 mg Zn; 10,000 mg Cu; 800 mg I; 150 mg Co; and 150 mg Se. Treatments consisted of four experimental groups; control (C) group fed basal diet, T1 fed basal diet + 10 g/day SRU, T2 fed basal diet +10 g/day SRU + molasses at 10% ration on DM basis, and T3 fed basal diet +10 g/day SRU + starch at 5% ration on DM basis. The chemical composition of ingredients has been shown in Table 1. Animals were kept in metabolic cages (1.2 × 0.6 m) and fed twice daily at 08:30 and 16:30. Leftover feed from the previous day was weighed before feeding at 08:30.

Sample Collection and Analyses: Groundnut straw samples were collected once at beginning of each experimental period and refusal were collected daily and dried in an air-forced oven at 60 ºC for 48 h. Collected refusal within each treatment were mixed before sampling. Samples were ground to pass through a 1 mm screen using a mill (Retsch, Germany). Samples were analyzed for DM, crude protein (CP), ash, ether extract (EE) (AOAC, 2012), and NDF and ADF (Van Soest *et al.,* 1991).

Fecal bags were placed on animals for collection of feces used for the determination of digestibility coefficients. Fecal bags were emptied, feces weighed, mixed well by hand, and subsamples were collected (10% wet weight) for all animals twice daily before feeding. Fecal samples were dried at 60 ºC until completely dry in an air-forced oven and stored for further analysis. All samples were ground in a mill to pass through 1 mm screen and analyzed for DM, CP, NDF and ADF as described previously.

Urine output was collected into plastic bottles that contained 5 ml H_2SO_4 (50%) to minimize bacterial growth and N loss. Before feeding in the morning, daily urine output was measured and the representative sample was collected in 50 ml aliquot. Urine samples were stored at 4 ºC. Samples were analyzed for N as described by AOAC (2012).

Rumen fluid was sampled approximately 2 and 6 h post-feeding via an esophageal tube on last day of each sampling period. Ruminal pH was measured immediately using a portable pH meter (Orion Model 720, Thermo Electron Corporation, Beverly, MA, US) with an electrode. A 10 ml of ruminal fluid was acidified with 1 ml H_2SO_4 (50%) for ruminal NH_3-N analysis and 10 ml of ruminal fluid was mixed with 2 ml of 25% (wt/vol) metaphosphoric acid for volatile fatty acid (VFA) analysis. Ruminal fluids were stored at -20 ºC. Frozen rumen fluid samples were thawed and centrifuged (3000 × g for 15 min.), and the supernatant was collected for analysis of NH_3-N and VFA. NH_3-N concentrations were determined colorimetrically (HI 96733 ion selective meter, Hanna Instruments). Ruminal VFA concentrations were measured using a gas chromatograph (Agilent 7890B) as described by Erwin *et al.* (1961).

Statistical Analyses: All statistical analyses were performed with a statistical software package SPSS 17.0. Parameters depending on time were analyzed with paired T test. Data involving the effect of SRU and/or NSC supplementation on body weight gain (BWG) were analyzed with one-way ANOVA test. The other data were analyzed with GLM procedure for 4 × 4 Latin square design. Differences among means were evaluated according to Duncan's multiple range test ($P \leq 0.05$):

$Y_{ijk} = \mu + a_i + b_j + c_k + d_{ijk}$

where Y_{ijk} is the analyzed parameter (DM, organic matter, NDF, ADF digestibility etc.), μ the general mean effect; a_i the ith effect of the row (animal); b_j the jth effect of column (period); c_k the treatment effect that appears in the jth row/column; d_{ijk} the random error with experimental unit row/column (ij).

RESULTS

SRU and/or NSC supplementation to groundnut straw had a positive effect on BWG on both sheep and goats ($P \leq 0.001$) (Table 2)

The daily DMI and nutrient digestibility pattern are presented in Table 4. For sheep, there was an increased DM (46.53%) and organic matter (OM) (54.81%) digestibilities in T3 ($P \leq 0.01$ and $P \leq 0.001$, respectively) compared to other dietary treatments. OM digestibility was significantly higher ($P \leq 0.05$) in T3 (58.66%) in goats than other groups whereas SRU and/or NSC supplementation had no significant effect on DMI, DM, NDF, and ADF digestibilities in goats. Daily N intake was highest in T2 group (sheep 19.74 and goat 21.75 g/day) in both sheep ($P \leq 0.01$) and goat ($P \leq 0.001$). Despite the fact that there were no significant differences in fecal N in sheep, urinary N ($P \leq 0.001$), N retention ($P \leq 0.01$) and N digestibility ($P \leq 0.001$) had significant differences among the experimental groups. N retention was found higher in T2 (1.46 g/day) and T3 (1.12 g/day) compared to C and T1 groups. SRU supplementation improved N digestibility in sheep in T1, T2, and T3 groups in comparison with those in C group ($P \leq 0.001$). In goats, fecal and urinary N excretion values had significant differences ($P \leq 0.05$ and $P \leq 0.001$, respectively). N retention was found approximately two times more at SRU supplemented groups than C, however, interestingly no significant effect was observed. N digestibility levels was found significantly higher ($P \leq 0.001$) in T2 in comparison with other dietary treatments.

There were no differences in both 2 and 6 h rumen parameters (Table 4) except 2 h NH_3-N ($P \leq 0.01$) in sheep. At 2 h, T1 (207.29 mmol/L) and T2 (219.61 mmol/L) NH_3-N levels were determined higher than C

(126.88 mmol/L) and T3 (163.74 mmol/L). At 6 h, NH_3-N levels were similar among the groups. In goats, the data were similar to sheep except rumen NH_3-N that was significantly different in T1 and T2 than C at 2 h.

Rumen parameters changing between 2 and 6 h in sheep and goats have been demonstrated in Table 5. Rumen fluid pH was increased (P≤0.001) at 6 h in both sheep (7.00) and goats (6.92). Rumen NH_3-N levels (P≤0.001) were lower at 6 h in sheep (114.46 mg/L) and goats (102.51 mg/L). Moreover, propionate level at 2 h (9.88 mmol/L) was found higher for goats (P≤0.001) than 6 h (6.70 mmol/L). Statistically, in both sheep and goats, acetate and butyrate values, and propionate value in sheep had no significant differences between 2 and 6 h.

Table 1. Nutrient composition of ingredients, % DM.

Ingredients	DM	OM	CP	EE	CF	NSC	Ash	NDF	ADF
Groundnut straw	87.08	92.63	7.53	1.67	32.35	51.08	7.37	54.33	46.18
SRU	-	-	277.46	-	-	-	-	-	-
Molasses	61.30	70.69	34.24	-	-	36.45	29.31	-	-
Cornstarch	89.06	99.93	-	-	-	-	0.07	-	-

Table 2. Effects of SRU and/or NSC supplementation on BWG in sheep and goats, g/day.

	C	T1	T2	T3	P
Sheep	62.95^{d}±0.24	71.15^{c}±0.42	110.03^{b}±1.28	111.43^{a}±0.92	***
Goat	83.78^{c}±0.35	92.28^{b}±0.25	120.90^{a}±0.31	121.83^{a}±0.43	***

n: 4 for each specie

a, b, c, d: Means bearing different superscripts within the same row are significantly different.

***: P≤0.001

Table 3. DM intake, digestibility and N balance in sheep and goats.

	Sheep						Goat					
Groups	C	T1	T2	T3	SEM	P	C	T1	T2	T3	SEM	P
DMI, g/day/kg $BW^{0.75}$	62.01	60.75	58.62	64.83	2.23	ns	68.50	68.50	73.50	72.25	2.45	ns
DM digestibility, %	41.75^{b}	41.11^{b}	43.57^{b}	46.53^{a}	0.72	**	46.75	48.25	49.50	50.00	0.84	ns
OM digestibility, %	49.27^{c}	50.75^{b}	51.52^{ab}	54.81^{a}	0.55	***	$53,82^{b}$	54.75^{b}	55.52^{b}	58.66^{a}	0.78	*
NDF digestibility, %	38.76	38.87	38.89	40.67	1.03	ns	41.16	44.49	41.97	42.64	1.43	ns
ADF digestibility, %	34.15	34.45	32.59	34.73	0.82	ns	37.88	39.33	38.03	36.53	0.72	ns
N intake, g/day	13.45^{c}	17.82^{ab}	19.74^{a}	17.57^{b}	0.58	**	13.27^{c}	18.07^{b}	21.75^{a}	17.60^{b}	0.40	***
N feces, g/day	8.94	9.14	8.64	9.08	0.41	ns	7.30^{b}	7.95^{ab}	8.38^{a}	8.06^{a}	0.19	*
N urine, g/day	4.61^{d}	8.18^{b}	9.64^{a}	7.38^{c}	0.18	***	5.29^{d}	9.01^{b}	12.03^{a}	8.09^{c}	0.22	***
N retained, g/day	-0.10^{c}	0.51^{b}	1.46^{a}	1.12^{a}	0.16	**	0.67	1.12	1.35	1.45	0.20	ns
N digestibility, %	34.03^{c}	49.02^{b}	56.39^{a}	48.36^{b}	1.28	***	44.85^{c}	55.96^{b}	61.39^{a}	54.22^{b}	0.78	***

a, b, c, d: Means bearing different superscripts within the same row are significantly different.

ns: not significant, *: P≤0.05, **: P≤0.01, ***: P≤0.001

Table 4. Rumen pH, NH_3-N and VFA values in sheep and goats at 2 and 6 hours.

		2 h						6 h					
Groups		C	T1	T2	T3	SEM	P	C	T1	T2	T3	SEM	P
pH	Sheep	6.75	6.78	6.93	6.74	0.05	ns	6.97	6.93	7.04	7.08	0.06	ns
	Goat	6.64	6.74	6.87	6.70	0.06	ns	6.87	6.91	7.03	6.87	0.08	ns
NH_3-N, mg/L	Sheep	126.88^{c}	207.29^{a}	219.61^{a}	163.74^{b}	7.87	**	106.12	128.82	104.57	118.34	9.37	ns
	Goat	151.80^{b}	225.72^{a}	250.84^{a}	199.04^{ab}	17.64	*	93.70	108.35	108.64	99.33	6.54	ns
Acetate,	Sheep	45.40	65.71	65.57	59.50	9.50	ns	57.64	73.21	59.16	62.35	14.15	ns
mmol/L	Goat	52.80	66.31	52.33	59.80	8.78	ns	60.50	49.71	48.27	47.20	6.32	ns
Propionate,	Sheep	7.79	7.53	9.31	9.72	1.06	ns	8.37	8.28	7.36	6.76	1.04	ns
mmol/L	Goat	10.62	10.04	9.51	9.35	1.15	ns	6.76	6.05	7.02	6.97	0.66	ns
Butyrate,	Sheep	3.45	3.51	2.88	3.98	0.74	ns	4.18	5.22	3.13	3.53	0.83	ns
mmol/L	Goat	3.51	2.90	3.13	3.23	0.39	ns	2.92	2.94	2.72	3.69	0.35	ns

a, b, c: Means bearing different superscripts within the same row are significantly different.
ns: not significant, *: P≤0.05, **: P≤0.01

Table 5. Rumen kinetics between 2 and 6 h in sheep and goats.

Time	pH	NH_3-N, mg/L	Acetate, mmol/L	Propionate, mmol/L	Butyrate, mmol/L
			Sheep		
2	6.80	179.38	59.04	8.59	3.46
6	7.00	114.46	63.09	7.69	4.01
t	5.92	5.32	0.50	1.14	1.08
P	***	***	ns	ns	ns
			Goats		
2	6.74	206.85	57.81	9.88	3.19
6	6.92	102.51	51.42	6.70	3.07
t	5.35	10.22	1.38	5.16	0.45
P	***	***	ns	***	ns

ns: not significant, ***: P≤0.001

DISCUSSION

Many studies in sheep and goats suggested similar results regarding SRU supplementation to low-quality forage increased the BWG (Galina *et al.,* 2004b; Ahmed *et al.,* 2017). Galina *et al.* (2007) reported that SRU supplementation to sugar cane tops improved the BWG (70 g/d in control vs 135 g/d in treatment) in lambs. Similarly, Galina *et al.* (2004b) reported that Alpine kids fed corn stubble gained 83.4 g/d and SRU supplemented group gained 101.6 g/d.

The SRU and/or NSC supplementation had no effect on DMI. Similarly, some researchers reported no differences in DMI with using SRU (Estrada-Angulo *et al.,* 2016). However, increase in DMI has been established in some studies when supplied SRU (Ribeiro *et al.,* 2011). Lizarazo *et al.* (2014) determined no effect on DMI following the molasses addition in diets containing SRU. This has been explained by Mertens (1985; 1994) who stated that DMI is maximized when daily NDF intake is approximately 12.5 g/kg BW. Later, this was confirmed by Bohnert *et al.* (2002) who suggested that the lack of an increase in forage intake is attributed to NDF intake.

Highest DM and OM digestibility was found in T3 in both sheep (46.53% and 54.81%) and goats (50.00% and 58.66%), respectively. However, digestibility of NDF and ADF was not improved by SRU and/or NSC supplementation. SRU supplementation had no effect on DM digestibility but had effect on OM digestibility. In goats SRU and/or NSC had no significant effect on DM digestibility. Different results have been established about SRU supplementation. Galina *et al.* (2004a) reported an increase in DM and OM digestibility with SRU supplementation; however, Galina *et al.* (2004b) determined no effect on respective parameters in goats. In sheep, Galina *et al.* (2004c) recorded SRU supplementation had significant effect on DM digestibility but no effect on OM digestibility. Abdel-Raouf *et al.* (2017) reported no differences in DM, OM, CP, NDF and ADF digestibility in response to the supplementation of SRU source. These differences may be depending on variation of animals and SRU sources. Molasses supplementation in diets containing SRU had no effect on DM and OM digestibility in both species. However, starch supplementation significantly affected DM ($P \leq 0.01$) and OM digestibility ($P \leq 0.001$) in sheep, and starch improved OM digestibility ($P \leq 0.05$) in goats. Nothing has been reported on starch supplementation on diets containing SRU. Lizarazo *et al.* (2014) established no differences in DM and NDF digestibility by adding molasses to diets containing SRU.

In sheep, N intake increased according to SRU and/or molasses supplementation. Highest N intake value ($P \leq 0.01$) was found in T2 (19.74 g/d) because of the N content of the molasses (5.48% DM). Lowest fecal N excretion (8.64 g/d) was determined in same group parallel to the highest N retention ($P \leq 0.01$) and N digestibility ($P \leq 0.001$). Similar N intake and N digestibility results were found in sheep and goats. For both sheep and goat, highest urinary N excretion was found in T2 (9.64 and 12.03 g/d, respectively).

In the present study, N retention and digestibility were affected positively with SRU and NSC supplementation for both species. Similar results were reported by many researchers in response to dietary SRU supplementation such as Galina *et al.* (2004c) in sheep, and by Galina *et al.* (2004a) in goats, and by Cherdthong *et al.* (2014) in beef cattle. Lizarazo *et al.* (2014) reported molasses supplementation in diet containing SRU had no effect on N retention and microbial N. However, in the present study, we found that molasses supplementation had positive effect on N retention and N digestibility in sheep, and N digestibility in goats. Data on the N digestibility have shown no differences in both species by starch supplementation to diet containing SRU.

All rumen parameters did not differ significantly among treatments either in sheep or in goats except NH_3-N. For sheep and goats, highest NH_3-N values ($P \leq 0.01$ and $P \leq 0.05$, respectively) were established in T1 and T2 at 2 h that decreased with time and were similar at 6 h. Starch supplementation reduced the rumen NH_3-N level. It may be due to the fact that protein and carbohydrate have different degradation rate in the rumen. It is well-known that molasses gets degraded to VFA quickly in the rumen but microbial protein synthesis couldn't reach this rate. So, excessive NH_3-N passes from rumen to blood and converts to urea that is excreted via urine to some extent. In T3, more synchronized protein and carbohydrate degradation rate might have happened because of corn-starch degradation at a slower rate than molasses. This notion is also supported by the decreased urinary N values. Similarly, Calomeni *et al.* (2015) reported that rumen pH and NH_3-N levels increased with SRU supplementation, not observing any significant differences in acetate, propionate and butyrate levels. Likewise, Galina *et al.* (2004a) reported that SRU supplementation increased the rumen NH_3 level without any significant change in VFA levels in goats. Rumen pH increased and rumen NH_3-N decreased with time in the present study. Similar results were reported by Galina *et al.* (2004b) and Gonçalves *et al.* (2015) in response to dietary SRU supplementation. However, Geron *et al.* (2016) reported that SRU supplementation increased both pH and NH_3-N levels between 2 and 6 h in sheep, but decreased NH_3-N at 8 h.

This study indicated that SRU and/or NSC supplementation on low-quality forage based diets increase digestibility and BWG. Corn starch addition to rations contain SRU is more effective than molasses on N utilization.

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