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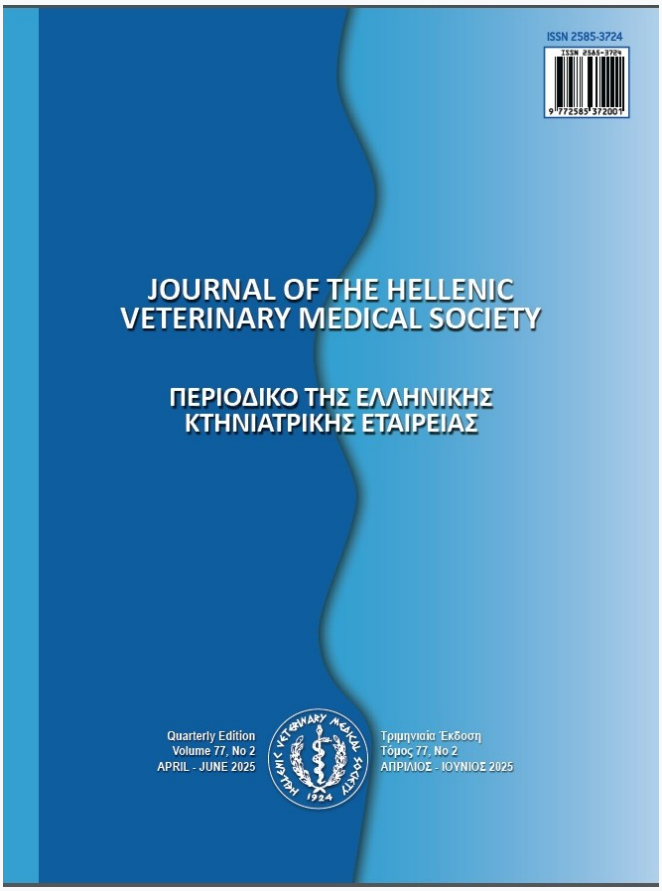


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# Effects of Slow-release Urea vs conventional urea and Molasses on Digestibility, Rumen Fermentation, Nitrogen Balance and Microbial Protein yield in Sheep

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**ABSTRACT:** The objectives of this study were to evaluate the effects of using a type of slow- release urea (SRU) and conventional feed grade urea (CFU) with or without molasses (MO) on the diets of growing sheep. An experiment was conducted by using 4 mature-male Lori sheep (24.7 ± 0.9 kg BW) cannulated in the rumen in a- 4×4 Latin-square design with a factorial arrangement of 2×2 to evaluate the effects of two main factors of urea sources (US) (feed-grade urea versus slow- release urea) and level of molasses (M) (0% versus 20%) on intake, nutrient digestibility, nitrogen retention, rumen fermentation, and microbial nitrogen in sheep. In addition, an *in situ* experiment was conducted to determine N disappearance of urea sources from polyester bags. The lambs were fed with 4 isonitrogenous and isocaloric diets consisting 70% concentrate and 30% sugar cane tops (DM basis) that were offered in two equal meals (08.00 and 20.00; 5 to 10 percent orts on an as-fed basis). The following treatments implicative of (UM0) feed-grade urea (16 g/kg DM) without molasses, (UM20) feed-grade urea (16 g/kg DM) with molasses (200 g/kg), (SM0) slow-release urea (18 g/kg DM) without molasses, and (SM20) slow-release urea (18 g/kg DM) with molasses (200 g/kg). Nutrient digestibility, nitrogen balance, total purine derivatives (PD( and estimated microbial protein synthesis were not different between the treatment groups although digestibility of OM tended to increase in 20% M supplemented groups (P= 0.057). Overall, the means of total VFA concentration and its proportions were not different (P> 0.05), but with the addition of molasses to the diets, the concentrations of propionate (P= 0.016) and butyrate (P= 0.024) decreased and increased, respectively. Ruminal pH, NH3–N, and plasma metabolites were not affected by the addition of US or M (P> 0.05). Significant effects of the sampling time on ruminal pH (P= 0.002), ruminal NH3–N (P<0.001), BUN, and plasma glucose (P<0.001) were observed. It could be concluded that the inclusion of M or US did not affect the feed intake, digestibility, blood metabolites, and generally, most of the ruminal fermentation parameters after evaluation; but, more research is required to evaluate their uses in diets.

***Keyword:*** slow- release urea; molasses; rumen fermentation; microbial protein; nitrogen balance.

***Abbreviations:*** NPN, non-protein nitrogen; SRU, slow-release urea; M, molasses; US, urea sources; CU, common urea; NDFom, ash-free neutral detergent fiber; ADFom, ash-free acid detergent fiber; CP, crude protein; DM, dry matter; OM, organic matter; EE, ether extract; NFC, non-fiber carbohydrates; VFA, volatile fatty acids; BUN, blood urea-N; CP, crude protein; PD, purine derivatives; TPD, total purine derivatives; MPS, microbial protein synthesis.

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## INTRODUCTION

Due to their low costs and sufficiently good sources of N, than vegetable proteins as well as the abilities of ruminants for N utilization Non-Protein Nitrogen (NPN) sources are being attractively included in ruminant diets (Jooste, 2012, Salami et al. 2021). Fiber-digesting rumen bacteria need ammonia for protein synthesis (NRC, 2001). Depending on the diet, microbial protein can contain 50-80% of the total absorbable protein (Salami et al. 2020). Urea is quickly hydrolyzed into NH3 in the rumen during the first hour after ingestion. Thus, ruminal bacteria may less efficiently capture N in the rumen when urea is excessively used in animal diets (Calsamiglia et al. 2010). This rapid breakdown to ammonia can occur at a much more quickly than microbial growth, as well as ruminal carbohydrate degradation and ammonia utilization by the rumen bacteria, which result in the accumulation and escape of ammonia from the rumen ( Satter and Roffler, 1975, Campos et al. 2021). This implies subsequently potential N waste from NPN sources that has not been used by rumen bacteria. Hence, excessive utilization of NH3 may have detrimental impacts on the animals (Bartley et al. 1981) and lead to environmental pollution (Broderick et al. 2009). Alternatively, N-NH3 constant availability over long periods of time can be provided by using slow-release sources of NPN instead of using urea-released ammonia (Taylor-Edwards et al. 2009a). Applications of these sources depend on their costs with regard to their urea and vegetable protein efficiencies and effectiveness on microbial growth, as well as animal performance (Sinclair et al. 2008). The protection techniques can be efficacious if allowing urea to be constantly available for hydrolysis in the rumen by avoiding it's too tight binding, (Johnson and Clemens, 1973). These compounds have not proven as beneficial as urea because a significant portion of the NPN they contain may exit the rumen without being converted to ammonia. Consequently, this reduces its incorporation into microbial protein (Henning et al. 1993). Yet, urea is not as effective as polymer-coated urea in terms of lowering ammonia concentration. Nevertheless, reduced N excretion or improved steer performance would not be always achieved by its application (Taylor-Edwards et al. 2009b). Therefore, a form of more slowly degradable urea would be required to be applied to the rumen.

An important alternative can be providing a coordination between the production rate of ammonia in the rumen and the digestion rate of carbohydrates since the produced ammonia in the rumen contributes to microbial growth when sufficient energy is available. It has been understood that urea combined with soluble carbohydrates can serve as an important source of ammonia for ruminal microbes (Hristov and Ropp, 2003). Fluctuated ammonia concentrations may occur to the rumen after feeding along with the unsynchronized production and digestion rates of rumen ammonia and carbohydrates, respectively (Henning et al. 1993). Thus, high fermentation without microbial growth and subsequently more N losses and less efficient ruminal fermentation can be resulted from asynchronous N and energy supplies (Reynolds and Kristensen, 2008). Sugars can be more quickly fermented to produce energy in the rumen compared to starch. Hence, molasses that is rich in sucrose can be regarded as a useful feed supplement capable of being synchronized with the high levels of degradable proteins available in the rumen (Soder et al. 2010). Molasses is a sugar-containing liquid feed that can enhance the ruminal fermentability of dietary carbohydrates, while stimulating DMI (Firkins et al. 2008). Incorporating a sugar-based product into the diets can result in the altered patterns of ruminal fermentation, as well as decreased and increased ruminal NH3 and butyrate concentrations, respectively (DeFrain et al. 2006). Sugars undergo rapid fermentation in the rumen, theoretically leading to lactic acid production and decreasing ruminal pH, which could potentially depress fiber digestibility (Oelker et al. 2009). However, Broderick and Radloff (2004) reported that replacing high-moisture corn with molasses resulted in improved fiber digestibility, likely reflecting the stimulatory effect of molasses on fiber-digesting ruminal bacteria. Rumen fermentation can be optimized by feeding NPN sources together with molasses, which is generally N-deficient and often needs an improved N status (Preston et al. 1986).

So, The aim of this experiment was to evaluate the effect of using SRU or CFR with or without molasses on ruminal fermentation, microbial protein supply, nitrogen balance, nutrient digestibility and blood parameters of lambs in diets containing low-quality forage (sugar cane tops).

## MATERIALS AND METHODS

This experiment was conducted under the supervision and approval of the Ethics Committee of Animal Welfare of Ramin Agriculture and Natural Resources University of Khouzestan, Ahwaz, Iran.

**Slow-release urea sources**

Two sources of SRU were evaluated. The first was a type of slow- release urea containing 40% nitrogen and 250% equivalent crude protein, manufactured by Danesh Bahavar Shaya Co. (www.parsa78.ir.) in Iran. The second source consisted of Optigen® II (Alltech, Inc.) as a commercial product urea pills coated with vegetable oil.

**Experiment 1**

***Animal study***

This study was conducted in an animal farm located at Safiabad Agricultural Research Center in Dezful, Iran in January 2017.

Four mature-male Lori sheep (24.7 ± 0.9 kg BW) cannulated in the rumen were randomly assigned to 1 of 4 dietary treatments in a 4×4 Latin-square design (sheep and periods) with 4 trial periods. A factorial arrangement of 2×2 was allocated individually in metabolic cages to allow the total collection of feces and urine. The two main factors of (US) urea sources (feed-grade urea versus slow-release urea) and (M) level of molasses (0% versus 20%) were assessed.

The diets were ad libitum and provided in two equal meals at 08:00 and 20:00 h with free access to clean water every day. The body weights of the animals were individually recorded at the beginning and end of each experimental period.

Each period lasted 21 days (d) with 14 d of diet adaptation and 7 d of sampling (5 d for digestibility, 1 d of rest before rumen fluid sampling to prevent interference with the digestibility trial, and 1 d for rumen fluid collection).

Four isonitrogenous and isocaloric dietary treatments composed of 30% sugar corn tops (DM basis) and 70% concentrate (DM basis) were formulated according to NRC (2007) as shown in Table 1. The dietary treatments (DM basis) were (UM0) feed-grade urea (16 g/kg DM) without molasses, (UM20) feed-grade urea (16 g/kg DM) with molasses (200g/kg), (SM0) slow-release urea (18 g/kg DM) without molasses, and (SM20) slow-release urea (18 g/kg DM) with molasses (200 g/kg). During the last week of the experiment, the samples of feeds and feces from each sheep related to each treatment were weighed, while 10% of the representative samples were frozen for later analysis. The total apparent digestibilities of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), ash-free Neutral Detergent Fiber (NDFom), ash-free Acid Detergent Fiber (ADFom) and Ether Extract (EE) were measured using the total fecal collection method described by (Givens et al. 2000).

Urine samples were simultaneously collected in a bucket consisting of a solution of 1M sulphuric acid (100 ml) to maintain a final pH of <3. The collected samples were individually examined every morning to prevent the precipitations of Purine Derivatives (PDs), particularly uric acid, in them during storage. The PD compositions of uric acid, allantoin, and xanthin+hypoxanthin were estimated by preparing a 4-times diluted sub-sample of 20% urine and storing it at −20◦C (Chen and Gomes, 1995). One composite sample was finally prepared for each sheep for analysis after 5 days of pooling the representative samples collected from them.

***Rumen fluid and blood samples***

The samples of ruminal contents were collected on Day 21 of each period at 0, 3, 6, and 9 h post-feeding. The pH values were determined by using a portable pH meter (315i/SET, WTW Co. Germany) immediately after sampling. Approximately 0.2 L of the ruminal contents was obtained from several sites within the rumen and rained through 4 layers of cheesecloth. Ten milliliters of filtrate subsamples were preserved with sulfuric acid concentration of 7.2 N (0.1 ml) (Atkinson et al. 2007) and HCL concentration of 0.2 N (10 ml) for determining VFA and NH3–N concentrations, respectively. The samples were stored at -20°C until analyzed.

Blood samples (10 ml) were collected from all the animals by jugular venepuncture on Day 21 of each period just before the morning feeding and 3, 6, and 9 h after feeding. After centrifugation at 1500×g at room temperature for 15 min, plasma samples were stored at −20◦C until analyzed.

***Chemical analysis***

The DM, ash, N, and EE were analyzed based on the AOAC (1990) procedure numbers of 930.15, 924.05, 984.13, and 954.02, respectively. OM was calculated as the difference between 100 and the ash percentage. The NDFom and ADFom were determined without sodium sulphite and amylase treatment, while being expressed exclusive of residual ash according to the Ankom A200 (Ankom Technology Corp. Fairport, NY) filter bag technique. Before determining NDF and ADF, pepsin (P7000, Sigma-Aldrich Co. LLC. USA), and heat-stable α-amylase (A4551, Sigma-Aldrich Co. LLC. USA) were pre-treated due to the presence of high protein and starch concentrations in some feed ingredients (Van Soest et al. 1991).

**Table 1.** Ingredients and chemical composition (g/kg DM) of the experimental diets

| | Treatment[1] | | | |
|---|---|---|---|---|
| | **UM0** | **SM0** | **UM20** | **SM20** |
| Ingredient | | | | |
| Sugar cane tops | 300 | 300 | 300 | 300 |
| Corn grain | 440 | 440 | 240 | 240 |
| Wheat bran | 174 | 172 | 150 | 148 |
| Soy bean meal | 50 | 50 | 74 | 74 |
| Cane molasses | 0 | 0 | 200 | 200 |
| Urea | 16 | 0 | 16 | 0 |
| Slow Release urea (Nitroza)[2] | 0 | 18 | 0 | 18 |
| Ca carbonate | 9.4 | 9.4 | 9.4 | 9.4 |
| Sodium sulfate | 2.6 | 2.6 | 2.6 | 2.6 |
| Salt | 2 | 2 | 2 | 2 |
| Mineral and vitamin permix[3] | 6 | 6 | 6 | 6 |
| Nutrient composition | | | | |
| ME(Mcal/kgDM)[3] | 2.45 | 2.45 | 2.43 | 2.43 |
| CP | 151 | 151 | 151 | 151 |
| RDP(% DM)[4] | 9.1 | 9.1 | 9.5 | 9.5 |
| RDP(% CP)[4] | 60.3 | 60.3 | 62.9 | 62.9 |
| NDF | 381 | 380 | 354 | 353 |
| ADF | 188 | 187 | 179 | 179 |
| NFC[5] | 432 | 432 | 441 | 441 |
| Hemi cellulose | 194 | 193 | 175 | 174 |
| EE | 39.5 | 39.4 | 30.2 | 30.1 |
| Ca | 7 | 7 | 9 | 9 |
| P | 5 | 5 | 4 | 4 |

[1] UM0: feed-grade urea (16 g/kg DM) without molasses; UM20: feed-grade urea (16 g/kg DM) with molasses (200 g/kg); SM0: slow-release urea (18 g/kg DM) without molasses; SM20- slow-release urea (18 g/kg DM) with molasses (200g/kg)

[2] Slow-release urea containing 40% nitrogen and 250% equivalent crude protein manufactured by Danesh Bahavar Shaya Co. (www.parsa78.ir)

[3] Premix containing Na (60 g), P (90 g), Ca (180 g), Mg (20 g), Fe (3 g), Zn (3 g), Mn (2 g), Se (1 mg), Cu (300 mg), Co (100 mg), I2 (100 mg), vitamin E (100 mg), vitamin A (500000 IU), and vitamin D3 (100000 IU) manufactured by Science Livestock Supplement, Tehran, Iran

[4] Nutrient requirements of small ruminants 2007.

[5] NFC= 100-(NDF+CP+EE+Ash)

The calcium contents of the feeds were determined through Atomic Absorption Spectrometry (AAS) (AOAC, 1990; method 968.08D). The P concentrations of the feeds were measured via the colorimetric assay (AOAC, 1990; method 965.17).

The concentration of NH3–N in the ruminal fluid was determined by centrifuging the supernatant at 10,000×g for 10 min and analyzed for ammonia-N through a phenol-hypochlorite assay according to Broderick and Kang (1980).

Urinary PD concentrations, including allantoin, uric acid, xanthine, and hypoxanthine were estimated by using spectrophotometric methods (Chen and Gomes, 1992). A colorimetric method was employed at 522 nm to measure allantoin in urine by converting it into phenylhydrazone. Xanthine oxidase (Sigma; Catalog No. X-1875, 5 Units, Germany) was utilized with its subsequent optical density at 293 nm to calculate the sum of xanthine and hypoxanthine through their conversion into uric acid. Uricase (Sigma; Product No. U-9375, Germany) was used to measure uric acid by degrading it to allantoin and estimate its reduced optical density at 293 nm. Finally, all the 4 compounds of xanthine, hypoxanthine, uric acid, and allantoin were summed up to

calculate the total PD excretion per day. Then, the daily absorbed exogenous purines and MNS were estimated and predicted, respectively.

Based on Chen and Gomes (1992) technique, the non-linear equation for describing the quantitative relationship between the absorption of microbial purines and excretion of PD in urine can be expressed as follows:

$$Y = 0.84\,X + (\,0.15\,W^{0.75}e^{-0.25X}\,)$$

where Y is the daily urinary PD excretion in mmol/d; X is the daily absorbed exogenous purines in mmol/d; and $W^{0.75}$ stands for the metabolic body weights (kg) of animals.

Calculation of X from Y based on the above equation can be performed by means of the Newton–Raphson iteration process as given below:

$$X(n+1) = Xn - \frac{f(Xn)}{f'(Xn)}$$

Where

$f(X) = 0.84X + 0.150W^{0.75}$ and the derivatives of $f'(X) = (0.84 - .038W^{0.75}\,e^{-0.25X}\,)$

Finally, the produced microbial nitrogen was estimated through the following equation:

$$\textit{Microbial N (g/d)} = \frac{X(mmol/d)\times 70}{0.116\times 0.83\times 1000} = 727X$$

The concentration of urinary N was estimated by the Kjeldahl method (AOAC, 1990). Nitrogen retention was calculated as daily N excretion (urinary N plus fecal N) subtracted from daily N intake.

After thawing, the strained rumen fluid samples were centrifuged (14,000 rpm, 15 min) and VFA were determined via gas chromatography (Philips Pu4410, U.S.A.) by using 4-methyl-valeric acid as the internal standard according to the procedure described by Ottenstein and Bartley (1971).

Plasma was analyzed for blood urea-N (BUN), triglyceride, cholesterol, and glucose using a spectrophotometer.

***Statistical analyses***

The data were analyzed through the GLM procedure of SAS (SAS Institute, 2003), according to the 4×4 Latin square design with the 2×2 factorial arrangement of the treatments. The following model was fitted for all the variables:

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

where $Y_{ijk}$ represents observation from animal j together with receiving diet i in period k; μ indicates the overall mean; $T_i$ demonstrates the main effect of the two (US) urea sources of feed-grade urea or slow-release urea and the two (M) levels of molasses (0% or 20%), as well as the interaction between them; $A_j$ stands for the effect of animal (j=1, 2, 3, and 4); $P_k$ shows the effect of period (k=1, 2, 3, and 4); and $e_{ijk}$ displays the residual error. The data of ruminal pH, NH3–N, and blood parameters were analyzed using the MIXED procedure of SAS (SAS Institute, 2003), for repeated measures. Sampling time was considered as the repeated variable. The model included the fixed effects of treatment and sampling time and the interaction between treatment and sampling time. The best covariance structure was selected for the final analysis of each dependent variable according to the lowest value of Akaike's Information Criterion (AIC). All the values were reported as least-squares means. The results were presented as the treatment means with an SEM at the significance level of $P< 0.05$, and a trend when $P< 0.10$. The treatment means were statistically compared by Tukey's test.

**Experiment 2**

***In situ degradability of the urea sources***

Three steers (BW=350±15 kg) were arranged in a randomized complete block design after fitting them with ruminal cannula and individually housing them in pens. The steers were then adapted to the dietary treatment of no urea sources with 30% forage and 70% concentrate from Day 1 to 5 and fed ad libitum from Day 6 to 8. Steers were fed once daily at 0900 h. On d 8, N-free polyester bags (5×10 cm, 50-μm porosity) containing 0 (blank) or 5 g DM basis of Urea, Optigen II, or Nitroza were introduced in the ventral sac of the rumen in each steer at 0900 h and removed at 0.5, 1, 2, 4, 6, 8, 12, and 24 h after incubation/feed delivery. The urea samples were oven-dried at 60°C for 48 h to determine their DM contents. At each time of incubation in the rumen, the polyester bags were placed in a water bath at 39°C to simulate insalivation for 30 min and then put into some weighted mesh bags that were attached to some strings whose ends were left outside of the rumen to facilitate their removal. The bags were rinsed with cold water immediately after their retrieval. Upon clearance of the rinsing water, they were dried in a forced-air oven at 60°C for 48 h. The bags corresponding to 0 h after feeding were only rinsed and

dried after removing them from the warm bath and were not thus incubated in the rumen. Duplicate bags were prepared for each combination of urea source (blank) and sampling time.

N disappearance from the polyester bags was estimated as this:

$$(N_{in}\,(g)- N_{out}\,(g))/(N_{out}\,(g)) \times 100$$

where 1 $N_{in}$ and $N_{out}$ represent the amount of N before and after incubation, respectively. Nitrogen contents were analyzed based on the AOAC (1990) procedure number of 984.13.

### *Statistical analyses*

The data were analyzed using the MIXED procedure of SAS (SAS Institute, 2003), while animal (block) was considered a random effect. The level of significance was set and separation of the treatment means was performed as described in Exp. 1.

## RESULTS

### Digestibility of nutrients and nitrogen balance

Table 2 presents DMI, total tract digestibilies of DM, OM, NDFom, ADFom, CP, and EE, and nitrogen balance. DMI and the total tract digestibilities of DM, NDFom, ADFom, CP, and EE were not affected by the treatments although OM digestibility tended to increase with the treatments of 20% molasses (P= 0.057). Total nitrogen intake, urinary nitrogen excretion, fecal nitrogen excretion, and nitrogen retention were not significantly different between the treatment groups.

### Purine derivatives and MPS

The total urinary PD excretion together with each PD component and microbial nitrogen yield are presented in Table 3. No differences were found in the urinary allantoin, uric acid, and excreted xanthin+hypoxanthin of the treatments. Therefore, the total PD and estimated microbial protein synthesis were not affected by the treatments.

### Ruminal parameters and plasma metabolites

The ruminal VFA values are shown in Table 4. The total ruminal VFA concentrations and the ratios of acetate, isovalerate, valerate, and acetate to propionate were not different between the treatments containing US, while propionate and butyrate concentrations decreased (P=0.016) and increased (P=0.024)

**Table 2.** Least square means for total digestibility and nitrogen balance by the sheep fed the experimental diets

| Item[1] | Treatments.,[2] | | | | SEM | P-Value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | UM0 | UM20 | SM0 | SM20 | | US | M | US× M |
| DMI(gr) | 904 | 897 | 890 | 892 | 18.1 | 0.841 | 0.948 | 0.906 |
| Apparent digestibility (%) | | | | | | | | |
| DM | 64.0 | 64.6 | 65.6 | 65.7 | 0.576 | 0.173 | 0.699 | 0.770 |
| OM | 68.4 | 71.5 | 70.0 | 71.4 | 0.580 | 0.429 | 0.057 | 0.364 |
| NDFom | 46.2 | 48.7 | 46.6 | 50.7 | 1.10 | 0.580 | 0.176 | 0.717 |
| ADFom | 35.6 | 38.1 | 34.9 | 36.2 | 0.916 | 0.470 | 0.282 | 0.721 |
| CP | 72.4 | 75.3 | 73.4 | 75.4 | 0.700 | 0.641 | 0.137 | 0.729 |
| EE | 65.4 | 62.3 | 65.8 | 63.1 | 1.17 | 0.832 | 0.309 | 0.940 |
| N.g/d | | | | | | | | |
| Intake | 21.8 | 21.7 | 21.5 | 21.6 | 0.437 | 0.814 | 0.848 | 0.906 |
| Urine excretion | 11.9 | 11.3 | 10.9 | 11.4 | 0.224 | 0.485 | 0.918 | 0.442 |
| Fecal excretion | 6.03 | 5.36 | 5.29 | 5.67 | 0.181 | 0.558 | 0.692 | 0.182 |
| Retention | 3.9 | 5.0 | 5.3 | 4.5 | 0.406 | 0.661 | 0.882 | 0.381 |

1- DM: dry matter; OM: organic matter; NDFom: ash-free neutral detergent fiber; ADFom: ash-free acid detergent fiber; CP: crude protein; EE: ether extract.

2- UM0: feed-grade urea (16 g/kg DM) without molasses; UM20: feed-grade urea (16 g/kg DM) with molasses (200g/kg); SM0: slow-release urea (18 g/kg DM) without molasses; SM20: slow-release urea (18 g/kg DM) with molasses (200 g/kg); SEM: standard error of the means.

3- Probabilities of the main effects of US (Urea Sources: Urea vs SRU); Probability of M main effect (molasses 0% vs 20% ); Probability of US×M interaction.

**Table 3.** Least square means for purine derivatives excretion and microbial protein supply (MPS) by the sheep fed the experimental diets

| Item[1] | Treatments[2] | | | | SEM | P-Value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | UM0 | UM20 | SM0 | SM20 | | US | M | US× M |
| Purine derivatives (mmol/d) | | | | | | | | |
| Alantoin | 5.73 | 5.65 | 5.53 | 5.43 | 0.219 | 0.674 | 0.861 | 0.980 |
| Uric acid | 1.28 | 1.19 | 1.22 | 1.28 | 0.032 | 0.652 | 0.652 | 0.045 |
| X+H | 0.758 | 0.700 | 0.750 | 0.745 | 0.016 | 0.436 | 0.214 | 0.287 |
| TPD excreted | 7.76 | 7.54 | 7.49 | 7.45 | 0.231 | 0.728 | 0.794 | 0.855 |
| TPD absorbed | 8.79 | 8.53 | 8.48 | 8.42 | 0.276 | 0.734 | 0.797 | 0.864 |
| MPS (g/d) | 39.9 | 38.7 | 38.5 | 38.3 | 1.253 | 0.734 | 0.797 | 0.864 |

1- X+H: xanthine+hypoxanthine; TPD: totale purine derivatives

2- UM0: feed-grade urea (16 g/kg DM) without molasses; UM20: feed-grade urea (16 g/kg DM) with molasses (200 g/kg); SM0: slow-release urea (18 g/kg DM) without molasses; SM20: slow-release urea (18 g/kg DM) with molasses (200 g/kg); SEM: standard error of the means.

3- Probabilities of the main effects of US (Urea Sources: Urea vs SRU); Probability of M main effect (molasses 0% vs 20% ); Probability of US×M interaction.

after including molasses in the diets, respectively. The ruminal pH values, NH3–N concentrations, and plasma metabolites are shown in Table 5. The ruminal NH3–N and BUN concentrations at different sampling times are present in Figs. 1 and 2. The overall means of the ruminal NH3–N concentrations and pH values were not affected by the additions of US or molasses ($p>0.05$). Significant effects of sampling time were observed for the ruminal pH values ($p=0.002$) and NH3–N concentrations ($p<0.001$) (Table 5). Ruminal pH and NH3–N concentration values decreased and increased at 3, 6, and 9 h and 3 h post-feeding as compared with their values before feeding, respectively, while the latter values gradually decreased at 6 and 9 h afterwards. There were no effects of treatment groups and treatment and sampling time interactions on the mean concentrations of any measured plasma metabolites. The effects of sampling time on BUN and plasma glucose ($P<0.001$) were observed to be significant, whereas the post-feeding concentrations of plasma glucose showed a gradual enhancement after several hours (Table 5). Maximum concentrations of BUN were observed at 3, 6, and 9 h post-feeding (Fig. 2).

### In situ degradability of the urea sources

N disappearance from the polyester bags was complete at 0 h of incubation for common urea (CU),

**Table 4.** Least square means for ruminal VFA by the sheep fed the experimental diets

| Item | Treatments[1] | | | | SEM | P-Value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | UM0 | UM20 | SM0 | SM20 | | US | M | US× M |
| Total VFA (mmol) | 72.9 | 74.1 | 72.6 | 72.0 | 0.588 | 0.545 | 0.742 | 0.555 |
| Acetate (%) | 61.4 | 61.3 | 60.5 | 61.5 | 0.538 | 0.773 | 0.715 | 0.609 |
| Propionate (%) | 20.4 | 17.9 | 20.2 | 17.7 | 0.436 | 0.745 | 0.016 | 0.964 |
| Butyrate (%) | 15.2 | 18.1 | 16.4 | 17.8 | 0.417 | 0.548 | 0.024 | 0.312 |
| Isovalerate (%) | 1.64 | 1.63 | 1.70 | 1.77 | 0.107 | 0.584 | 0.865 | 0.802 |
| Valerate (%) | 1.30 | 1.11 | 1.24 | 1.25 | 0.045 | 0.730 | 0.441 | 0.398 |
| Acetate: propionate | 3.01 | 3.43 | 3.03 | 3.49 | 0.588 | 0.545 | 0.842 | 0.555 |

1- UM0- feed-grade urea (16 g/kg DM) without molasses; UM20- feed-grade urea (16 g/kg DM) with molasses (200g/kg); SM0- slow-release urea (18 g/kg DM) without molasses; SM20- slow-release urea (18 g/kg DM) with molasses (200g/kg). SEM, standard error of the means.

2- Probability of US (Urea Sources) main effect (Urea vs SRU); Probability of M main effect (molasses 0% vs 20% ); Probability of US× M interaction..

**Table 5.** Least square means for ruminal parameters and plasma metabolites by the sheep fed the experimental diets

| Item | Treatments[1] | | | | SEM | P-Value[2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | UM0 | UM20 | SM0 | SM20 | | US | M | Time | Diet× Time |
| Ruminal parameters | | | | | | | | | |
| pH | 6.58 | 6.75 | 6.83 | 6.69 | 0.040 | 0.531 | 0.917 | 0.002 | 0.422 |
| Ammonia (mg/dl) | 21.5 | 20.9 | 21.8 | 20.3 | 0.389 | 0.779 | 0.138 | <0.001 | 0.584 |
| Plasma metabolites | | | | | | | | | |
| Blood urea-N (mg/dl) | 12.1 | 11.9 | 12.2 | 12.1 | 0.216 | 0.632 | 0.732 | <0.001 | 0.490 |
| Cholesterol (mg/dl) | 55.4 | 53.9 | 55.8 | 54.8 | 0.526 | 0.608 | 0.347 | 0.513 | 0.239 |
| Triglycerides (mg/dl) | 22.1 | 22.9 | 22.6 | 21.9 | 0.270 | 0.681 | 0.918 | 0.226 | 0.711 |
| Glucose (mg/dl) | 55.4 | 54.6 | 55.8 | 55.4 | 0.526 | 0.592 | 0.629 | <0.001 | 0.058 |

1-UM0: feed-grade urea (16 g/kg DM) without molasses; UM20: feed-grade urea (16 g/kg DM) with molasses (200 g/kg); SM0: slow-release urea (18 g/kg DM) without molasses; SM20: slow-release urea (18 g/kg DM) with molasses (200 g/kg); SEM: standard error of the means

2- Probabilities of the main effects of US (Urea Sources: Urea vs SRU); probability of M main effect (molasses 0% vs 20% ); probability of diet×time interaction

while it rapidly increased from 7.67% at 0 h to 80.8% at 2 h after incubation and continued to augment almost at a constant rate afterwards until it reached 88.0% at 24 h after incubation for Optigen II (P<0.05; Fig. 3). N disappearance for Nitroza was intermediate between CU and Optigen II, whereas showing a quick enhancement from 79.3% at 0 h to 92.5% at 0.5 h after incubation and continuing to elevate almost at a constant rate afterwards to finally reach 93.2 at 24 h after incubation (P<0.05; Fig. 3).

## DISCUSSION

### Digestibility of nutrients and nitrogen balance

In agreement with our findings, no differences in DMI and nutrient digestibility have been reported in the previous studies using polymer- coated urea and molasses (Lizarazo et al. 2014) or urea–calcium sources (Cherdthong et al. 2011). This result obtained for DMI in our study is also in line with the previous findings of Galo et al. (2003), who observed no DMI disruptions in cows fed with polymer-coated urea. In this study, N and energy synchrony for the efficient productions of ruminal microbial proteins in the ruminal environment were not affected by of Soy Bean Meal (SBM) replacement with SRU in the sheep diets. Consequently, SBM replacement with SRU could maintain the animal's diet digestibility and DM intake. Microbial protein can meet 100% of the metabolizable protein requirements of beef cattle (NRC 2000). This might explain ineffectiveness of including SRU in diets on total nutrient digestibility. The lack of differences in the estimated microbial protein among the treatments (Table 3) and nitrogen balance (Table 2) support the observed data of total apparent digestibility.

In the current study, total OM digestibility tended to increase by adding 20% molasses (P=0.057), which might be due to sucrose availability as a result of its more rapid fermentability than starch in the treatments (Chamberlain et al. 1993). In agreement with these results, Broderick and Radloff (2004) reported enhancement of OM digestibility when molasses was replaced by corn in the rations of dairy cows. In contrast, the digestibility coefficients of DM, OM, NDF, and ADF were seen to linearly enhance when different amounts of molasses (0, 40, 80, or 120 g/kg of DM diet) were added at the expense of corn in their rations (Broderick and Radloff, 2004). The differences in the used amounts of molasses may possibly explain the differences between our results and other findings.

In this study, there were no changes in the efficiency utilization of the N body among the treatments. In the similar works, the use of SRU (Geron et al. 2018) or SRU and addition of cane molasses did not affect the urinary and fecal N excretions or retainments (Lizarazo et al. 2014). Furthermore, N can be recycled in ruminants to compensate for the differences in its release times in the rumen (Reynolds and Kristensen, 2008). The results are consistent with those reported by Alves et al. (2014) when working with isonitrogenous diets fed to sheep with no differences in the ingested amounts of N. The lack

of any differences in the amounts of nitrogen excreted in the feces might be due to the lack of treatment effects on CP digestibility (Alves et al. 2014). The urinary N was not influenced by the treatments. According to Van Soest (1994), there is a relationship between the amount of excreted N into the urine and the diet CP content, which can augment urea excretion into the urine when there is an enhanced N intake this behavior is associated with a more urea production of in the liver. On the other hand, a low N intake leads to decreased urea excretion in the urine so to maintain serum urea pool under the physiological control of homeostasis. The similaritis of the N amounts lost through urine could be explained by the ineffectiveness of the applied isonitrogenous diets on N intakes in this study. The difference between N intake and excretion into urine and feces can be the reason for N retention. According to the consistent findings of Bourg et al. (2012) in the current study, designing isonitrogenous diets and having similar DMI intakes in the treatments could be the probable reasons for similar N efficiencies (Table 2).

**Purine derivatives and microbial protein synthesis (MPS)**

In the current study, total PD and estimated MPS were not affected by the treatments. As described by Chen and Gomes (1992), allantoin, uric acid, and xanthine + hypoxanthine, which were in the normal ranges of 60-80, 10-30, and 5-10, respectively, could be expressed as percentages of the total excreted purine derivatives in sheep (Table 3). These findings are in agreement with the results reported by Lizarazo et al. (2014), who evaluated the two sources of SRU and molasses in the feeding of growing sheep. Additionally, Galo et al. (2003) and Chegeni et al. (2013) reported that feeding polymer-coated urea (Optigen 1200®) to dairy cows and growing sheep did not alter urinary PD excretion and rumen MPS. Galo et al. (2003) reported that the main route for N excretion in cows is urine followed by feces. Due to a protein surplus or an unbalanced amino acid profile, the urinary excretion of N can be enhanced by ruminal ammonia accumulation or high deamination levels in the body. Ammonia-N can be converted into microbial protein in the presence of adequate sources of energy in the rumen (Bach et al. 2005, Geron et al. 2018). Great amounts of N can be converted into ammonia when protein degradation rate occurs more rapidly than carbohydrate fermentation. Likewise, inefficient MPS can be resulted from the higher rate of carbohydrate fermentation than protein degradation (Bach et al. 2005). However, in our study, PD and MPS in the sheep fed with SU with or without molasses displayed similar results. This may be caused by ameliorated nutrient synchrony for N and energy supplies to rumen microorganisms in the SU and SBM fed sheep. According to Yu et al. (2002), dietary protein and energy sources, feed additives, animal species, and their body weights are the main effective factors on xanthine, hypoxanthine, uric acid, and allantoin excretions. This information would provide useful explanation to our results though the the excretions of purine derivatives were not found to be affected by the mentioned factors, which was probably due to the uses of isonitrogenous and isocaloric diets and equal animal weights in the experiments.

**Ruminal parameters and plasma metabolites**

In agreement with our results, Lizarazo et al. (2014) reported that total VFA production or individual VFA proportions did not significantly differ between the mentioned urea sources in growing sheep diet. Likewise, Xin et al. (2010) showed the unchanged total VFA concentration and its individual proportions in the diets of slow-releasing compared to conventional urea, as well as isolated soybean protein. The unaltered total VFA produced from slow-releasing urea was also reported by Cherdthong et al. (2011) though it was seen to enhance propionate proportion. In our experiments, total VFA levels in the different treatments were indicative of no adverse effects on rumen fermentation after SU replacement with SBM. Since fermentation of dietary carbohydrates mainly results in ruminal VFAs (Firkins, 1996), ruminal fermentation was suggestive of the inefficiencies of urea sources in the current study.

In the current study, similar to the findings of Lizarazo et al. (2014), the addition of molasses did not affect TVFA production and the proportions of acetate, isovalerate, valerate, and acetate to propionate ratio, but decreased and increased the proportions of propionate and butyrate, respectively (Table 4). Varied ruminal VFA patterns depending on the included amounts of molasses in the diets have been shown by the researchers. Araba et al. (2002) reported reduced concentrations of total VFA, acetate, and propionate after replacing barley with 0, 200, 400, and 600 g of sugar beet molasses. Similar to the previous studies (Araba et al. 2002), a higher concentration of butyrate was obtained by replacing molasses with corn in the present research. The increase in butyrate concentration in a molasses diet is likely attributable to the stimulation of a large population of small,

significant protozoa that primarily produce butyrate as their main fermentation end product, compared to other microbes (Araba et al. 2002). Pate (1983) concluded that replacing cereal grain with molasses results in an increased molar proportion of butyrate relative to propionate.

The overall mean ruminal pH values in all the treatment groups were not different as being within the range of 6.5-7.0, which was considered optimal for microbial digestion of fiber and protein (Wanapat and Cherdthong, 2009) (Table 5). This finding is similar to the results of Pinos-Rodríguez et al. (2010), who found no influences of different urea sources in ruminal pH. Other slow-release urea sources like urea–calcium have been also assessed to have no differences in the pH values between the treatments of steers fed with an all-forage diet (Huntington et al. 2006). Ruminal pH is a key factor that limits rumen fiber digestion (Ørskov, 1995). Researchers have reported the different effects of molasses addition on ruminal pH. The reduction in rumen pH observed in sheep fed increasing levels of molasses, compared to those on a control diet, may be attributed to decreased salivary secretion due to the physical and chemical properties of molasses (Benavides and Rodriguez, 1971) and the higher sugar content fermented in the rumen. However, Ruminal pH increased when ground barley was replaced with varying levels of molasses in the diet of bulls (Araba et al. 2002). This enhancement may be attributed to the higher amounts of cations (like $K^+$, $Ca^{2+}$, and $Mg^{2+}$) present in molasses. It seems that the applied levels of molasses and compositions of diets are the reasons for these differences. Similar to our findings, the results obtained by Lizarazo et al. (2014) revealed the significant effect of time sampling on ruminal pH as ruminal pH gradually decreased after several hours of post feeding.

In all the experimental groups, urea sources did not change the overall means of ruminal $NH_3$-N and BUN concentrations (Table 5). As shown in this study, Almora et al. (2012) compared Optigen II and Ruma Pro with feed-grade urea and reported that the concentrations of rumen fluid ammonia-N and plasma urea-N were similar in the N supplements. In agreement with our results, Galo et al. (2003) reported that N-NH3 release during most of the incubation times were not affected by the diet supplemented with polymer-coated urea vs feed-grade urea. These findings suggested that recycling N may effectively change SRU effect on ruminal N (Tedeschi et al. 2002) or SRU does not exhibit slow-release properties as demonstrated by Huntington et al. (2006). Another reason for this can be the too fast formations of ammonia from these compounds in rumen to allow rumen bacteria to optimize microbial protein production though its formation is already slower than urea (Henning et al. 1993).

In agreement with our findings, Lizarazo et al. (2014) did not detect any effects of molasses on ammonia N concentration during all their sampling times of feeding growing lambs. Feeding diets high in sugar may reduce ammonia-N concentration in rumen fluid if readily fermentable carbohydrates limit microbial protein production. However, the incremental effects of adding sugar on ammonia-N concentration may be minimal if basal diets already exhibit high rumen fermentability (Oba, 2011). Finally, Oba (2011) suggested that replacing dietary starch with sugars does not necessarily lead to improved nitrogen utilization. The reasons for the difference between the results of the present experiment and those of other studies are probably the different levels of molasses addition (Oba, 2011) and properties of the applied diets or recycled urea-N.

By evaluating $NH_3$–N curves (Fig. 1), we noticed that the $NH_3$–N levels in all the experimental groups continuously increased until they reached their highest levels at 3 h and then decreased at 6 and 9 h after feeding. In the current study, the mean value of $NH_3$–N was 21.1 mg/dL, which was a value that could allow fibrolytic activity in the rumen (Satter and Slyter, 1974). As shown in this study, other researchers have also reported an increase in rumen $NH_3$–N concentration within 1–3 h of post-ingestion and its late gradual decline (Pinos-Rodríguez et al. 2010; Xin et al. 2010).

It has been reported a positive association between BUN concentrations and ruminal ammonia concentrations (DePeters and Ferguson, 1992). In our research, in all the animals, BUN concentration showed a parallel pattern to the ruminal ammonia concentration during the first 3 h after feeding, whereby the BUN levels gradually increased to reach their highest levels at about 3, 6, and 9 h after feeding (Fig. 2). Similarly, Alves et al. (2014) indicated that in diets with a higher share of conventional urea, the supply of ammonia-N and BUN peak might have occurred shortly after its consumption and nearly 4 hours after eating.

In the present study, the overall means of BUN (12.1 mg/dL) are in agreement with those reported by Alves et al. (2014), who evaluated the diets

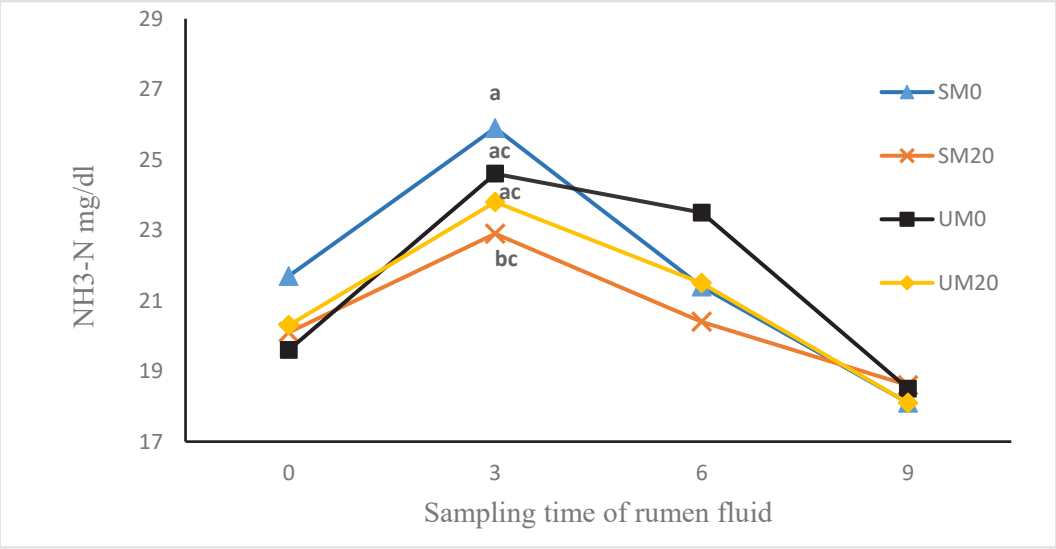


**Figure 1.** Ruminal ammonia concentration at different sampling times in the ruminal fluid of the sheep. The treatments included: UM0: feed-grade urea (16 g/kg DM) without molasses; UM20: feed-grade urea (16 g/kg DM) with molasses (200 g/kg); SM0: slow-release urea (18 g/kg DM) without molasses; SM20: slow-release urea (18 g/kg DM) with molasses (200g/kg).
Significant difference of means with uncommon superscripts ($P<0.05$) (Time, $P<0.001$).

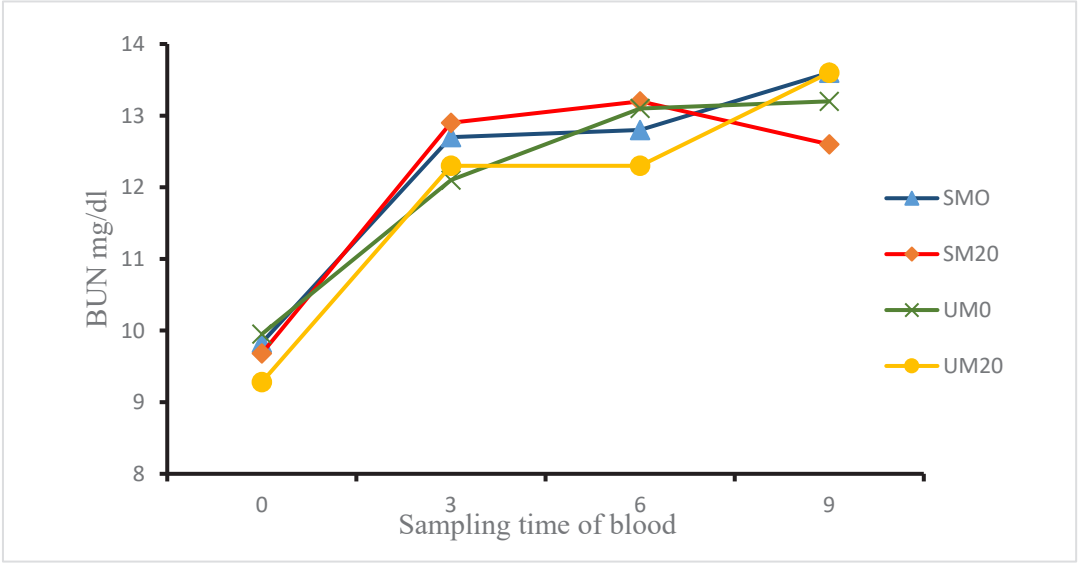


**Figure 2.** Blood urea concentration at different sampling times in the sheep fed with the experimental rations. The treatments included: UM0: feed-grade urea (16 g/kg DM) without molasses; UM20: feed-grade urea (16 g/kg DM) with molasses (200 g/kg); SM0: slow-release urea (18 g/kg DM) without molasses; SM20: slow-release urea (18 g/kg DM) with molasses (200 g/kg)
Significant difference of means with uncommon superscripts ($P<0.05$) (Time, $P<0.001$).

containing slow-release urea replaced with conventional urea in sheep feeding. BUN concentration in animals may vary depending on animal species or category; thus, further studies are required to establish an ideal range for any situations. Too high BUN concentration may indicate N waste and energy expenditure, while its too low concentration may display N deficiency in diets (Alves et al. 2014). BUN concentration can be also related to the contents of Non-Fiber Carbohydrates (NFC) in diets since this type of carbohydrate can quickly provide energy to be then used by rumen microorganisms. According to Valadares et al. (1999), When the NFC content of the diet is below 35%, the efficiency of ammonia-N utilization is reduced due to increased absorption through the rumen wall and elevated nitrogen concentration in the bloodstream. In the current study, the NFC contents of the diets were similar (>35%), while this variable did not interfere with the concentration of plasma urea-N.

Plasma glucose concentration was not affected by the diets, which might be due to the occurrence of a similar concentration of ruminal propionate as the main glucose precursor obtained by all the animals (Brockman, 1993). Similar to our data, DePeters and Ferguson (1992) reported that the plasma concentrations of glucose and triglyceride were not affected by the diets in the same way as propionate concentration in the rumen was not altered, while the dry matter intakes among their groups were similar.

**Degradability of the urea sources**

In agreement with our findings, Ceconi et al. (2015) reported that urea disappearance from polyester bags was complete after a 15-min exposure to warm water (0 h incubation) for CU, while N disappearance for Optigen II rapidly enhanced from 27.8% at 0 h to 63.0% at 1 h after incubation and continued to increase almost at a constant rate afterwards to ultimately reach 93.2% at 24 h after incubation. In contrast, Holder (2012) reported that urea disappearance from polyester bags for CU was complete at 1 h after incubation in the rumen, while for Optigen II, it increased from 0% to about 20% within 1 h and augmented thereafter up to 60% at 24 h. The fact that polyester bags were not soaked in warm water before incubation in Holder's study (2012) may explain the slower disappearance of Optigen II compared with that in the present experiment. By evaluating the degradability curves (Fig. 3), as well as the results

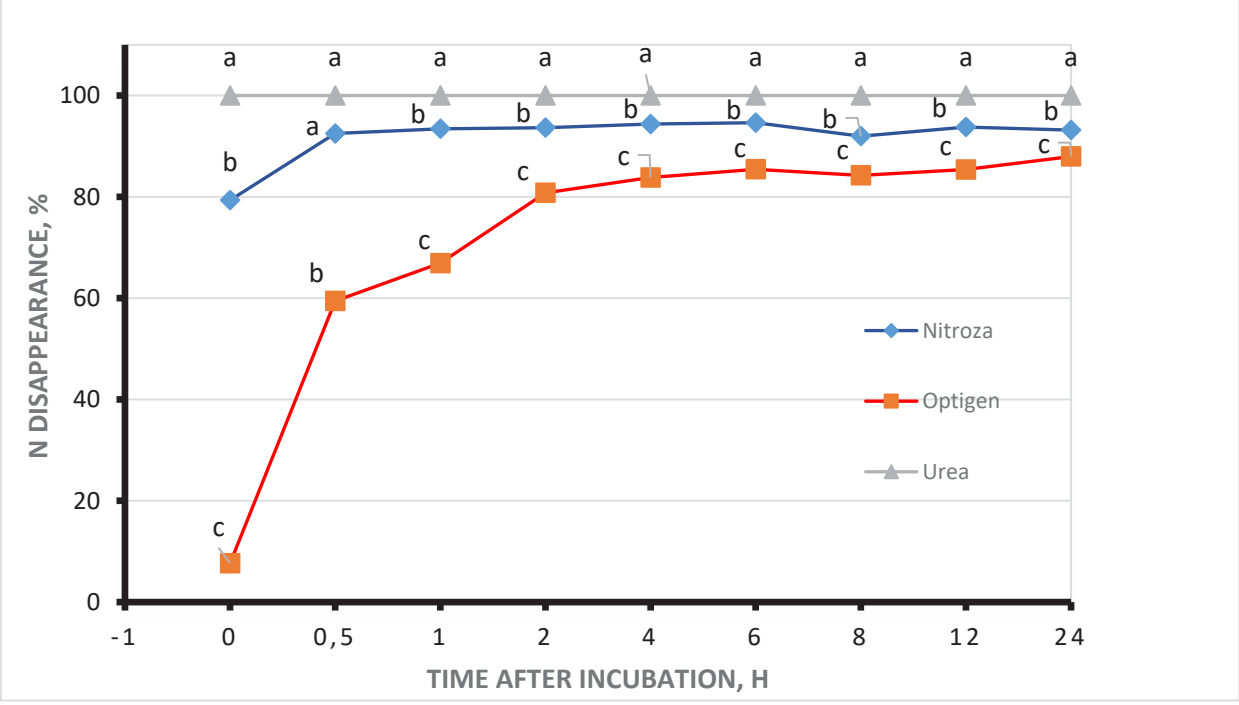


**Figure 3.** Degradabilities of urea and the 2 slow-release urea sources of Optigen and Nitroza affected by hours after incubation in the rumen (Exp. 2). Significant difference of means with uncommon superscripts within hours after incubation (P<0.05)
SED at each averaged time point (4.4%).

of apparent digestibility, nitrogen balance, and microbial protein synthesis, we noticed that ammonia formation from Nitroza in the rumen was still too fast to optimize microbial protein production by the rumen though it was slower than common urea.

## CONCLUSION

Generally, use of slow-release urea vs conventional urea with or without molasses in high-concentrate diets did not improve nutrient digestibility, nitrogen balance, microbial protein synthesis, rumen fermentation, and blood metabolites in the growing sheep. Molasses addition influenced some parameters of rumen fermentation, but these changes were not great enough to ameliorate the animal performance. No synergistic responses were observed by the additions of fermentable sugars (molasses) combined with a slow-release urea source. So, further studies with more animals (as replicate) are needed to investigate the effects of slow-release urea product on sheep or other animals.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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