



Short communication

Effects of slow-release urea and molasses on ruminal metabolism of lambs fed with low-quality tropical forage

A.C. Lizarazo^a, G.D. Mendoza^{a,*}, J. Kú^b, L.M. Melgoza^a, M. Crosby^c^a Doctorado en Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana Xochimilco, México, D.F., 04960 Mexico^b Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Yucatán 97315, Mexico^c Campus Montecillo, Colegio de Postgraduados, Estado de México 56230, Mexico

ARTICLE INFO

Article history:

Received 10 July 2013

Received in revised form 7 October 2013

Accepted 8 October 2013

Available online 25 October 2013

Keywords:

Urea

Molasses

Ruminal fermentation

Protein

Slow-release urea

ABSTRACT

The effects of two sources of slow-release urea (SRU) with a source of soluble carbohydrates on ruminal fermentation in lambs fed with a low-quality forage hay were evaluated. Optigen is a commercial source of slow-release urea, whereas Surelease is an ethyl cellulose-coated urea prepared in the Laboratorio de Farmacotecnia at the Metropolitan Autonomous University. Five Pelibuey lambs cannulated in the rumen and duodenum (24.8 ± 0.4 kg BW) were used in a Latin Square design. Lambs were fed a basal diet that consisted of *Brachiaria brizantha* hay and concentrate (ratio 67:33) with the following treatments: (1) feed-grade urea; (2) Surelease-coated urea (SRU-S); (3) SRU-S + molasses; (4) SRU Optigen (OPT); and (5) OPT + molasses. All sources of urea were dosed daily intra-ruminally (0.6 g/kg/BW), and molasses was fed at 1.2 g/kg BW. Compared to feed-grade urea, both sources of SRU decreased ruminal pH between 3 and 6 h after dosing ($P < 0.05$). At 3 and 9 h after dosing and at 15 and 21 h, both sources of slow-release urea reduced the rumen ammonia compared to urea ($P < 0.05$). The two sources of slow-release urea did not improve the total tract or rumen digestibility of dry matter (DM) and neutral detergent fiber (NDF) or the rate of microbial protein synthesis in growing lambs fed low quality forage.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Urea is rapidly hydrolyzed to NH_3 in the rumen in the first hour post-ingestion, and when fed in excess, it may be partially responsible for the low efficiency of N capture in the rumen by ruminal bacteria (Johnson and Clemens, 1973; Calsamiglia et al., 2010). This excess NH_3 may be detrimental to the animal (Bartley et al., 1981) and can contribute to environmental pollution (Broderick et al., 2009). To improve the utilization of ammonia released from urea, slow-release sources have been designed to promote the constant availability of N- NH_3 over long periods of time (Taylor-Edwards et al., 2009a). The combination of urea

with soluble carbohydrates has also been recognized as an important factor in the utilization of ammonia by ruminal microbes (Hristov and Ropp, 2003).

Polymer-coated urea is effective at reducing the ammonia concentration compared to urea. However, its use does not necessarily reduce N excretion or improve the performance of steers (Taylor-Edwards et al., 2009b). The potential benefits of slow-release urea (SRU) sources could be manifested in low-quality diets such as those based on tropical forages. Steers grazing tropical pastures supplemented with slow rumen degradation rate protein sources have shown better productive performance than those supplemented with urea (Ramos et al., 1998). Ribeiro et al. (2011) were able to increase dry matter intake in cattle fed low-quality hay and given a slow-release polymer-coated urea source.

Given that most evaluations of SRU have been conducted in intensive beef production systems based on

* Corresponding author. Tel.: +52 5554837000; fax: +52 54837238.

E-mail addresses: gmendoza@correo.xoc.uam.mx, gmendoza581@hotmail.com (G.D. Mendoza).

temperate forages (Kononoff et al., 2006), corn silage (Taylor-Edwards et al., 2009b), 50% concentrate diets (Galo et al., 2003; Golombeski et al., 2006) with conventional urea and have shown no response, the aim of this experiment was to evaluate the effect of two SRU sources and urea mixed with molasses on N metabolism and ruminal digestibility in lambs fed a low-quality tropical forage.

2. Materials and methods

This experiment was conducted under the supervision and approval of the Ethics and Animal Welfare Committee of the Autonomous University of Yucatán, Mérida, México.

2.1. Slow-release urea sources

Two sources of SRU were evaluated. The first was Optigen® II (Alltech, Inc.), a commercial blended urea product that involves coating urea pills with vegetable oil. The second source consisted of urea cores (Fermex®) with a size fraction between 2 and 2.38 mm that were coated with a polymer of ethyl cellulose (Surelease® Colorcon de México). The coating was carried on an aqueous dispersion of Surelease® prepared at 15% as described by Melgoza et al. (2007). The Surelease-coated urea was prepared in the Laboratorio de Farmacotenia from the Universidad Autónoma Metropolitana, México.

2.2. Animals and treatments

Five Pelibuey lambs (24.8 ± 0.4 kg BW) cannulated in the rumen and duodenum were used in a Latin Square design (sheep and periods) and fed a basal diet that consisted of *Brachiaria brizantha* hay and concentrate (67:33) with the following additions: (1) feed-grade urea; (2) ethyl cellulose Surelease®-coated urea (SRU-S); (3) SRU-S + cane molasses; (4) SRU Optigen (SRU-O); and (5) SRU-O + cane molasses. The concentrate was elaborated from Nutrimentos Peninsulares S.A. de C.V (Yucatán, México). All sources of urea were dosed daily intraruminally (0.6 g/kg BW), and molasses was fed at 1.2 g/kg BW. Forage, concentrate, molasses and minerals were mixed as a total mixed ration. Sheep were housed in metabolic crates supplied with a feeder and water was available at all times. Feed was offered in the morning at 3.7% of BW, and minerals were added at 0.5 g/kg BW daily. Each period consisted of 10 days of adaptation and 5 sample collection days. The composition (%) of the forage and concentrate was 91.6 dry matter (DM), 3.4 crude protein (CP) and 76.5 neutral detergent fiber (NDF) 41.4 acid detergent fiber (ADF) and 12.0 lignin, and 88.0 DM, 15.3 CP, 24.2 NDF, 10.9 FDA and 1.5 lignin, respectively. Mineral premix (Fogysal Ovino®) contained the following per kg: Ca 60 g, P 40 g, Mg 20 g, Se 3 mg, Co 5 mg, Mn 1000 mg, Cu 2 mg, I 25 mg, Zn 1000 mg, vitamin A 60 IU, vitamin D2 1 IU and vitamin E 120 IU.

2.3. Sample collection and analyses

Feed, feces and orts were collected daily during the collection period, rumen fluid (60 mL) was sampled on day 4 at 0, 1.5, 3, 6, 9, 12, 15, 18, 21 and 24 h after feeding and pH was measured immediately. Samples were acidified with 1 mL of sulfuric acid (30%) and then frozen at -20°C until laboratory analyses. Ten milliliters of rumen fluid were prepared with metaphosphoric acid and centrifuged ($40,000 \times g \times 10$ min), and the supernatant was used in measurements of the proportion of volatile fatty acids (VFA) via gas chromatography (Erwin et al., 1961). Ammonia N was measured using the indophenol method (McCullough, 1967). Duodenal samples were collected according to Stock et al. (1987) from day 1 to day 5 of the collection period to estimate ruminal digestion and N duodenal flow. Feed and duodenal samples were analyzed according to AOAC (1990) for dry matter (DM, method number 981.10), crude protein (CP, method number 967.03) and NDF and ADF fractions according to Van Soest et al. (1991) with a heat-stable amylase and expressed include residual ash. Feed, orts, feces and duodenal contents were used to determine acid insoluble ash as an internal marker to estimate digestibility (Van Keulen and Young, 1977). The amount of microbial protein synthesis was measured using purines (Zinn and Owens, 1986).

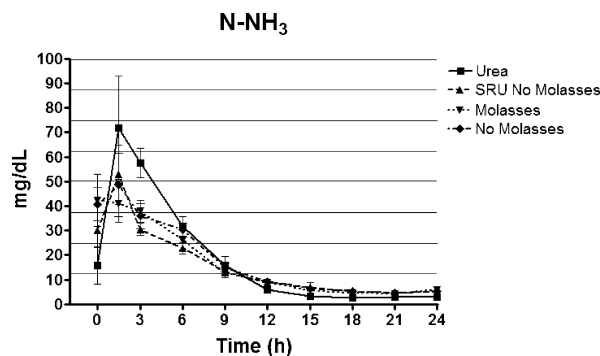


Fig. 1. Effect of slow release urea (SRU) and cane molasses on ruminal ammonia nitrogen in lambs fed tropical forage. Contrast I: urea vs. SRU sources (without molasses); Contrast II: molasses vs. no molasses.

2.4. Statistical analyses

The data were analyzed as a 5×5 Latin Square design using the GLM procedure SAS Inc. (2007). If the response variable was measured more than once, data were analyzed using the repeated analyses from GLM (SAS, 2007), and the following contrasts were tested: CI. urea vs. slow-release urea sources (without molasses) and CII. molasses vs. no molasses.

3. Results and discussion

3.1. Ammonia N and ruminal pH

Rumen ammonia N concentrations from the contrasts tested are presented in Fig. 1. Between 3 and 6 h after dosing and at 15 and 21 h, both sources of slow-urea release reduced the rumen ammonia compared to urea (Contrast I: $P < 0.05$). There was a time \times treatment interaction ($P < 0.018$). There were no effects of molasses on ammonia N concentration in all sampling times. The mean value of rumen N-NH₃ was 18.8 mg/dL, a value that allows fibrolytic activity in the rumen (Satter and Slyter, 1974). As shown in this study, other authors have also reported an increase in rumen N-NH₃ concentration within 1–3 h post-ingestion and its late gradual decline with slow-release urea sources (Pinos-Rodríguez et al., 2010; Xin et al., 2010). Tikofsky and Harrison (2006) evaluated the effect of two levels of non-protein nitrogen (urea or Optigen II) in an in vitro experiment with single-flow rumen-simulating fermenters and found no effect on pH or ammonia. In agreement with our results, no significant differences were found between polymer-coated urea and feed-grade urea in terms of N-NH₃ release (Galo et al., 2003) in most of the incubation times.

Ruminal pH readings from the contrasts tested are presented in Fig. 2. Compared to feed-grade urea, both sources of SRU decreased the ruminal pH between 3 and 6 h after dosing (Contrast II: $P < 0.05$). In hour 3, the ruminal pH was reduced by molasses; however, between 15 and 21 h ruminal pH was higher with molasses (Contrast II: $P < 0.05$). Most of the time, ruminal pH values were above 6.2 (Fig. 2). As observed in other studies, there were no major differences in ruminal pH between different sources of urea (Taylor-Edwards et al., 2009b; Tedeschi et al., 2002). Puga et al. (2001) found no differences in the pH and ammonia concentration between treatments in a ruminal

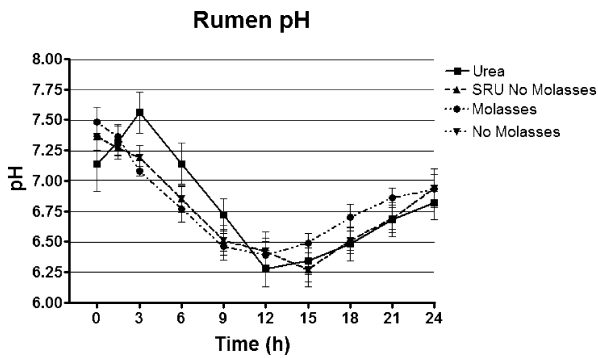


Fig. 2. Effect of slow release urea (SRU) and cane molasses on ruminal pH in lambs fed tropical forage. Contrast I: urea vs. SRU sources (without molasses); Contrast II: molasses vs. no molasses.

fermentation study evaluating the addition of a controlled-release urea supplement. As observed in this study, the increase in pH lasted for up to 6 h; however, it was higher in the SRU sources, perhaps due to the greater amount of N. The mean pH in this experiment (6.8) was considered optimum for cellulose degradation and is consistent with the results observed in experiments with low-quality forage diets (Bohrer et al., 2010).

Other slow-release urea sources have also been assessed (urea–calcium), with no differences in pH between treatments in steers fed an all forage diet (Huntington et al., 2006). SRU sources could provide continuous $\text{NH}_3\text{-N}$ for microbial growth, over the minimum of 15–30 mg $\text{NH}_3\text{-N/dL}$ found in rumen fluid, thus maximizing microbial growth (Leng and Nolan, 1984).

3.2. Intake, digestibility, nitrogen balance and ruminal fermentation

The use of SRU or molasses had no effect on DM intake or total tract digestibility (Table 1). Intake was on average 3.1% of BW. No differences in DM intake have been reported in previous studies using coated urea (Galo et al.,

2003, Pinos-Rodriguez et al., 2010, Highstreet et al., 2010), or urea–calcium sources (Cherdthong et al., 2010). However, Ribeiro et al. (2011) recorded an increase in DM intake in cattle fed low-quality hay when they supplied a slow-release polymer-coated urea source intraruminally. One difference that may explain why there was positive response in the experiment of Ribeiro et al. (2011) is because their forage had a limited amount of nitrogen compared with our forage. Regarding digestibility measurements, Tikofsky and Harrison (2006) did not find effects in *in vitro* experiments using Optigen and others (Xin et al., 2010; Highstreet et al., 2010) did not find responses in *in vivo* experiments when using coated urea and found no differences in DM digestibility.

The use of coated urea or the addition of cane molasses did not affect the urinary and fecal N excretion or the N retained (Table 1). There were no changes in the efficiency utilization of the N body among treatments. Furthermore, N can be recycled by ruminants to compensate for differences in the release time of N in the rumen (Reynolds and Kristensen, 2008).

In the current study, the pattern and level of VFA production did not differ significantly between SRU sources or following the addition of molasses in the diet (Table 1). In this context, Garret et al. (2005) showed that total VFA production in continuous culture fermenters was not affected by the source of urea (urea or oil-coated SRU). Xin et al. (2010) evaluated the effects of polyurethane-coated urea on the ruminal VFA concentration of Holstein dairy cows fed with a steam-flaked corn-based diet and found no significant differences in total VFA. Ruminal VFAs are derived mainly from dietary carbohydrate fermentation; consequently, the results of the present study suggest that the source of urea or the addition of molasses at the supplemented level has no effect on the ruminal fermentation.

3.3. Microbial protein synthesis

Data on the microbial nitrogen supply to the duodenum showed no difference between treatments (Table 1). Rihani

Table 1
Effect of slow-release urea and molasses on intake, N balance, and digestion of lambs fed a tropical forage.

	Urea	SRU	SRU-S + molasses	Optigen	Optigen + molasses	SEM	CI	CII
DM intake, g/d	766	779	758	779	776	8.26	0.23	0.18
N intake, g/d	14.7	14.8	14.7	14.8	14.8	0.04	0.14	0.06
N feces, g/d	10.4	10.2	10.8	9.04	10.16	0.63	0.31	0.19
N urine, g/d	1.0	1.1	0.8	1.05	0.99	0.13	0.79	0.17
N retained, g/d	3.3	3.5	3.1	4.62	3.60	0.68	0.35	0.32
Microbial N, g/d	3.3	3.1	3.5	3.3	3.3	0.10	0.64	0.14
Rumen digestibility, %								
DM	25.40	29.20	22.40	29.30	25.30	2.46	0.22	0.06
NDF	37.32	34.28	33.83	36.73	37.40	1.22	0.25	0.93
Total tract digestibility, %								
DM	51.06	47.64	45.72	45.72	52.46	2.05	0.39	0.97
NDF	44.09	43.91	42.42	44.93	44.92	1.03	0.79	0.48
VFA, mM/L	54.03	49.43	55.82	46.64	57.84	8.86	0.64	0.65
VFA, mol/100 mol								
Acetate	73.34	74.72	74.89	73.88	75.08	1.35	0.57	0.59
Propionate	17.9	17.15	17.64	18.52	15.98	0.91	0.95	0.29
Butyrate	8.76	8.12	7.45	7.6	8.93	0.99	0.92	0.76

SEM: standard error of the mean; SRU-S: slow-release urea Surelease; Optigen: commercial source of SRU; Probability of contrasts: CI, urea vs. slow-release urea sources; CII, molasses vs. no molasses.

et al. (1993) evaluated the effect of high levels of ruminal ammonia and continuous N release in high fiber diets in sheep, and found no differences in microbial net synthesis. In agreement with these results, Galo et al. (2003) reported that feeding polymer-coated urea (Optigen 1200®) to dairy cows did not alter the rumen microbial crude protein synthesis.

4. Conclusions

The results showed that the use of slow-release urea, regardless of its source (Surelease or Optigen), did not improve the total tract or rumen digestibility of nutrients or microbial protein synthesis in growing sheep fed low-quality hay. There was no clear improvement in nitrogen utilization by the ruminants, and the addition of fermentable sugars in combination with a slow-release urea source did not demonstrate any synergistic response.

Acknowledgments

This research was partially supported by grant no. 216470 CONACYT. The authors also thank Alltech Inc. México for supplying the Optigen for this experiment. We also thank the support personnel at the Department of Animal Nutrition on the Biological Science Campus at the Autonomous University of Yucatán, and the Nutrition Laboratory of Colegio de Postgraduados for supporting the laboratory analysis.

References

- AOAC, 1990. *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Bartley, E.E., Avery, T.B., Nagaraja, T.G., Watt, B.R., Davidovich, A., Galitzer, S., Lassman, B., 1981. Ammonia toxicity in cattle. V. Ammonia concentration of lymph and portal, carotid and jugular blood after ingestion of urea. *J. Anim. Sci.* 53, 494–498.
- Bohrer, E., Ospina, H., Finkler, A.L., López, J., Nörnberg, J.L., Brüning, G., 2010. Suplementação nitrogenada com ureia comum ou encapsulada sobre parâmetros ruminais de novilhos alimentados com feno de baixa qualidade. *Ciênc. Rural* 40, 622–627.
- Broderick, G.A., Stevenson, M.J., Patton, R.A., 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. *J. Dairy Sci.* 92, 2719–2728.
- Calsamiglia, S., Ferret, A., Reynolds, C.K., Kristensen, N.B., van Vuuren, A.M., 2010. Strategies for optimizing nitrogen use by ruminants. *Animal* 4, 1184–1196.
- Cherdthong, A., Wanapt, M., Wachirapakorn, Ch., 2010. 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. *Anim. Feed Sci. Technol.* 163, 43–51.
- Erwin, E., Marco, G., Emery, E., 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44, 1768–1771.
- Galo, E., Emmanuele, S.M., Sniffen, C.J., White, J.H., Knapp, J.R., 2003. Effects of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. *J. Dairy Sci.* 86, 2154–2162.
- Garret, J., Miller-Webster, T., Hoover, W., Sniffen, C., Putnam, D., 2005. Encapsulated slow release urea in lactating dairy cow diets impacts microbial efficiency and metabolism in continuous culture. *J. Anim. Sci.* 83 (Suppl. 1), 321.
- Golombeski, G.L., Kalscheur, K.F., Hippen, A.R., Shingoethe, D.J., 2006. Slow release urea and highly fermentable sugars in diets fed to lactating dairy cows. *J. Dairy Sci.* 89, 4395–4403.
- Highstreet, A., Robinson, P.H., Robinson, J., Garret, J.C., 2010. Response of Holstein cows to replacing urea with a slow rumen released urea in a diet high in soluble crude protein. *Liv. Sci.* 129, 179–185.
- Hristov, A.N., Ropp, J.K., 2003. Effect of dietary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. *J. Dairy Sci.* 86, 2416–2427.
- Huntington, G.B., Harmon, D.L., Kristensen, N.B., Hanson, K.C., Spears, J.W., 2006. Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. *Anim. Feed Sci. Technol.* 130, 225–241.
- Johnson, R.R., Clemens, E.T., 1973. Adaptation of rumen microorganisms to biuret as an NPN supplement to low quality roughage rations for cattle and sheep. *J. Nutr.* 103, 494–502.
- Kononoff, P.J., Heinrichs, A.J., Gabler, M.T., 2006. The effects of nitrogen and forage source on feed efficiency and structural growth of prepubertal Holstein heifers. *Prof. Anim. Scientist* 22, 84–88.
- Leng, R.A., Nolan, J.V., 1984. Nitrogen metabolism in the rumen. *J. Dairy Sci.* 67, 1072–1089.
- McCullough, H., 1967. The determination of ammonia in whole blood by direct colorimetric method. *Clin. Chem.* 17, 297–304.
- Melgoza, C.L.M., Rocha, A., Plata, P.F., Mendoza, M.G.D., Sandoval, H.T., 2007. Recubrimiento pelicular de comprimidos matriciales de alta densidad y pellets para la liberación modificada de urea en rumiantes. *Rev. Mex. Cienc. Farmac.* 38, 15–23.
- Pinos-Rodríguez, J.M., Peña, L.Y., González-Muñoz, S.S., Bárcena, R., Salem, A., 2010. Effects of slow-release coated urea product on growth performance and ruminal fermentation in beef steers. *Italian J. Anim. Sci.* 9, e4.
- Puga, D.C., Galina, H.M., Pérez-Gil, R.F., Sangines, G.L., Aguilera, B.A., Haenlein, G.F.W., Barajas, C.R., Herrera, H.J.G., 2001. Effect of a controlled-release urea supplementation on feed intake, digestibility, nitrogen balance and ruminal kinetics of sheep fed low quality tropical forage. *Small Rumin. Res.* 41, 9–18.
- Ramos, J.A., Mendoza, G.D., Aranda, E., García-Bojalil, C., Bárcena, R., Alanís, J., 1998. Escape protein supplementation of growing steers grazing stargrass. *Anim. Feed Sci. Technol.* 70, 249–256.
- Reynolds, S.M., Kristensen, N.B., 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. *J. Anim. Sci.* 86 (E. Suppl.), E293–E305.
- Ribeiro, S.S., Vasconcelos, J.T., Morais, M.G., Itavo, C.B.C.F., Franco, G.L., 2011. Effects of ruminal infusion of a slow-release polymer-coated urea or conventional urea on apparent nutrient digestibility, *in situ* degradability, and rumen parameters in cattle fed low-quality hay. *Anim. Feed Sci. Technol.* 164, 53–61.
- Rihani, N., Garret, W.N., Zinn, R.A., 1993. Influence of level of urea and method of supplementation on characteristics of digestion of high-fiber diets by sheep. *J. Anim. Sci.* 71, 1657–1665.
- SAS, 2007. *User's Guide: Statistics Version 9*, 6th ed. SAS Institute Inc., Cary, NC.
- Satter, L.D., Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Brit. J. Nutr.* 32, 199–208.
- Stock, R.A., Brink, D.R., Britton, R.A., Goedecken, F.K., Sindt, M.H., Kreikemeier, K.K., Bauer, M.L., Smith, K.K., 1987. Feeding combinations of high moisture corn and dry-rolled grain sorghum to finishing steers. *J. Anim. Sci.* 65, 290–302.
- Tikofsky, J., Harrison, G.A., 2006. Optigen II: improving the efficiency of nitrogen utilization in the dairy cow. In: *Proceedings of Alltech's 22nd Annual Symposium*, Lexington, Kentucky, USA, pp. 373–380.
- Taylor-Edwards, C.C., Elam, N.A., Kitts, S.E., McLeod, K.R., Axé, D.E., Vanzant, E.S., Kristensen, N.B., Harmon, D.L., 2009a. Influence of slow-release urea on nitrogen balance and portal-drained visceral nutrient flux in beef steers. *J. Anim. Sci.* 87, 209–221.
- Taylor-Edwards, C.C., Hibbard, G., Kitts, S.E., McLeod, K.R., Axé, D.E., Vanzant, E.S., 2009b. Effects of slow-release urea on ruminal digesta characteristics and growth performance in beef steers. *J. Anim. Sci.* 87, 200–208.
- Tedeschi, L.O., Baker, M.J., Ketchen, D.J., Fox, D.G., 2002. Performance of growing and finishing cattle supplement with a slow release urea product and urea. *Can. J. Anim. Sci.* 82, 567–573.
- Van Keulen, J., Young, B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44, 282–287.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Xin, H.S., Schaefer, D.M., Liu, Q.P., Axe, D.E., Meng, Q.X., 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-Aust. J. Anim. Sci.* 23, 491–500.
- Zinn, R.A., Owens, F.N., 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.* 66, 157–166.