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ORIGINAL ARTICLE

Site and extent of digestion of nitrogen compounds and organic matter in steers fed a finishing diet with dried distillers grains plus solubles supplemented with urea

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ABSTRACT. Four Holstein steers (266 ± 13 kg) with ruminal and duodenal cannulae were used to evaluate the effect of inclusion of different levels of urea (0%, 0.4%, 0.8% and 1.2%) in a steam-flaked, corn-based finishing diet containing dried distillers grains plus solubles (DDGS) on the site and extent of N compounds and organic matter (OM) digestion, and on the ruminal N-NH3 and blood concentrations of urea nitrogen. Increasing the urea levels linearly decreased the OM flow to the duodenum ($P<0.04$) and increased the total N and non-ammoniacal N flow to the duodenum ($P<0.01$), which resulted from linear increases in the flow of microbial N and NH_3-N ($P<0.01$) without affecting the feed residual N flow ($P\geq0.47$). Increasing the urea levels linearly increased ruminal digestion of OM and dietary N ($P\leq0.03$), and decreased protein efficiency ($P<0.01$) without affecting microbial efficiency. The urea supplementation did not affect post-ruminal digestion. Urea supplementation linearly increased ($P<0.01$) total tract digestion of N compounds without affecting total tract digestion of OM. Ruminal pH averaged 6.09 ± 0.03 and was not affected ($P\geq0.97$) by the inclusion of urea. Ruminal NH_3-N concentration increased with urea supplementation (linear component, $P<0.01$). The same effect was observed for blood urea concentrations and plasma urea ($P<0.01$). On the basis of the results observed here, urea can be incorporated in finishing corn-based diets that include DDGS. However, this must done carefully to avoid exceeding the RDP concentration in the diets in order to optimise ruminal fermentation and reduce the risk of high N excretion in the faeces. This is relevant when higher levels of DDGS are included in the diets (i.e. 30%).

Key words: fermentation, ruminal nitrogen solubility, DDGS, plasmatic ureic nitrogen.

INTRODUCTION

As a result of the increased use of cereals for biofuels production, the availability of dried distillers grains plus solubles (DDGS) has increased; thus, their use in livestock diets has become increasingly popular in North America the past few years (Rosentrater 2012). This is due to the competitive price of DDGS when compared to cereals as well as the fact that its energy content is comparable to that of corn. Likewise, as a result of removing the starch fraction from the grain during distillation, the concentration of CP (~30%) in DDGS is 3-fold that of corn (Rosentrater 2006, Klopfenstein *et al* 2008). Since DDGS is a good source of protein its protein ruminal solubility is an important component on the feeding value of DDGS. But the data generated in experiments about this topic are scarce and quite different from current standards. Based on initials reports (Klopfenstein *et al* 1978) the current standard (NASEM 2016, former NRC which is widely used North America as reference to formulate diets for livestock in industry and experimentation), indicates that corn DDGS protein has 32% rumen degradability. However, recent reports (Carrasco *et al* 2013, Castro-Pérez *et al* 2013) indicate an average of 61% RDP in DDGS. Because the reference value of rumen degradability of DDGS is 25% lower than steam-flaked corn, when steam-flaked corn is replaced by DDGS at moderate (10-15%) to high levels (>30%) in finishing diets, the inclusion of urea in the diets is considered necessary to meet the minimum requirement of degradable protein in the rumen to achieve optimal microbial synthesis and ruminal fermentation efficiency (Zinn and Shen 1998, Ceconi *et al* 2015[a]). Thus, the apparent underestimation of RDP in DDGS becomes critical when formulating diets, since a greater quantity of RDP in the diets would lead to possible waste of N, resulting in greater energetic requirements for its elimination and a negative effect on the environment through contamination via the greater accumulation of nitrogen in manure and urine.

Although DDGS is one of the most studied ingredients in the last decade, the evaluation of the effect of urea level supplementation in finishing diets contained a moderate level of DDGS on utilization of dietary N and OM has not been poured and discussed in a single manuscript. Therefore, the objective of the present experiment was to evaluate the effect of inclusion different levels of urea in a steam-flaked, corn-based finishing diet containing dried distillers grains plus solubles (DDGS) on the site and extent of nitrogen and organic matter (OM) digestion and on the ruminal ammoniacal nitrogen (NH_3-N) and blood urea concentrations.

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MATERIAL AND METHODS

The trial was conducted at the Ruminant Metabolism Experimental Unit of the Instituto de Investigaciones en Ciencias Veterinarias of the Universidad Autónoma de Baja California, 10 km south of Mexicali City in north-western México (32°40'7" N and 115°28'10" W). The area is about 10 m above sea level and has Sonoran desert conditions (BWh classification according to Köppen).

All animal management procedures were conducted within the following guidelines of locally approved techniques for animal use and care: NOM-051-ZOO-1995[1]: Humanitarian care of animals during their mobilization; NOM-062-ZOO-1995[2]: Technical specifications for the care and use of laboratory animals, livestock, farms, production, breeding and breeding centres, zoos and exhibition halls must comply with the basic principles of animal welfare; and NOM-024-ZOO-1995[3]: Stipulations and characteristics of animal health during transport. These regulations are in accordance with the specific principles and guidelines presented in IACUC-290-30 and by the Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS 2010).

CHARACTERISTICS OF THE EXPERIMENTAL UNITS, EXPERIMENTAL DIET AND SAMPLING

Four Holstein steers (266 ± 13 kg live weight) with ruminal ("T" tygon cannula with 3.8 cm i.d.) and duodenal ("T" tygon cannula 1.9 cm i.d. placed within 15 cm of the pyloric sphincter; Zinn and Plascencia 1993) were used in a 4 × 4 Latin square design with the aim of evaluating the effect of the treatments on the site and the extent of N and OM digestion, as well as the ruminal ammoniacal nitrogen (NH_3-N) and blood urea concentrations.

The steers were housed in individual pens (12.6 m^2) in an indoor facility, with a concrete floor covered with a neoprene mat, automatic waterers and individual feed bunks. Chromic oxide (3.0 g/kg diet, air-dry basis) was used as an indigestible marker to estimate nutrient flow and digestibility. Chromic oxide was mixed in a 2.5-m^3 capacity concrete mixer (model 30910-7, León Weill, SA, Coyoacán, México) for 5 min with minor ingredients (mineral supplement composed of limestone and trace mineral salt) before being mixed with the rest of the ingredients (with the exception of supplemental urea, which was offered as a dressing at feeding time). The treatments consisted of basal diet (table 1) supplemented with the equivalent of 0%, 0.4%, 0.8% or 1.2% urea in dry matter (DM) base. The amount of urea supplemented for each treatment was weighed using a precision balance (Ohaus, model AS612, Pine Brook, NJ) and added at feeding time (top-dressed) by mixing carefully with equal proportions of basal diet. Feed was offered twice a day at 08:00 and 20:00 h. To avoid feed refusal, DM intake was restricted during the experiment to 5.77 kg/d (90% of observed DM intake during a 14-d preliminary period before the start of the trial). The experimental period was 21 days long with 17 days for dietary treatment adjustment and 4 days for sample collection. During the collection period, duodenal and faecal samples were obtained from all steers twice daily as follows: d 1, 07:50 and 13:50 h; d 2, 09:00 and 15:00 h; d 3, 10:50 and 16:50 h; and d 4, 12:00 and 18:00 h. Individual samples consisted of approximately 500 mL duodenal chyme and 200 g (wet basis) faecal material. Feed, duodenal and faecal samples from each steer and within each collection period were prepared for analysis as follows: Samples were oven-dried at 70°C and then ground in a laboratory mill (Micro-Mill, Bell-Art, Pequannock, NJ). The samples were

Table 1. Ingredients and chemical composition of basal diet used in fed steers.

Ingredient composition (% Dry matter basis)	
Steam flaked corn	65
Dried distillers grains plus solubles	15
Sudan grass hay	12
Yellow grease	2.5
Molasses	3.5
Z2016M1 supplement	1.7
Chromic oxide	0.3
Chemical composition (% Dry matter basis)[1]	
Dry matter	87.92
Crude Protein	11.91
Neutral Detergent Fiber	21.57
Ether extract	7.23
Ash	5.42
RDP[2]	37.95
Estimated NE (Mcal/kg)[3]	
Maintenance	2.15
Gain	1.47

[1]The chemical composition of the diet for CP, EE, ashes and neutral detergent fiber (NDF, tested with amylase and expressed exclusively from residual ash) was analysed using subsamples collected and composed throughout the experiment.
[2]Rumen degradable intake protein (RDP), expressed as proportion of total CP in the ration, was calculated based on tabular RDP values for individual ingredients (NRC 2000).
[3]Net energy was calculated on the basis of tabular net energy (NE) values for individual ingredients (NRC 2007).

[1] NOM-051-ZOO-1995. 1995. Trato humanitario en la movilización de animales. http://www.dof.gob.mx/nota_detalle.php?codigo=4870842&fecha=23/03/1998 (03.11.2015).

[2] NOM-062-ZOO-1999. 1999. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. http://www.ibt.unam.mx/computo/pdfs/bioterio.NOM-062.pdf (03.11.2015).

[3] NOM-024-ZOO-1995. 1995. Estipulaciones de salud animal y características zoosanitarias durante el transporte de animales. http://dof.gob.mx/nota_detalle.php?codigo=4883147&fecha=16/10/1995 (03.11.2015).

then oven-dried at 105°C until a constant weight and stored in sealed glass jars. During the last day of each period, a sample of ruminal content (approximately 350 mL) and blood content (10 mL) was obtained from each steer before the morning feed (08:00 h) and at 4 h and 8 h after the morning feed. The ruminal samples were obtained from the rumen ventral sac through the ruminal cannula with the aid of a portable vacuum pump (Cole-Parmer, Vernon Hills, IL) connected to a Tygon tube (1.8 m length × 1.90 cm diameter; USP, Lima, OH). The ruminal fluid pH (Orion 261S potentiometer, Thermo Fisher Scientific, Pittsburgh, PA, USA) was determined from fresh samples. The samples were then strained through four layers of cheesecloth until approximately 100 mL ruminal fluid was obtained to measure the ruminal NH_3-N. Blood samples from each steer were obtained by jugular vein venopuncture into vacutainer tubes without additive (Vacuette, Greiner bio-one, México). The blood samples were immediately centrifuged at 2000 ×*g* for 15 min at 5°C, and the plasma was stored at –20°C until plasma urea concentrations were analysed. Both, ruminal and blood samples were composited to obtain one sample of each by animal and period. On the final day of the experiment, a composite sample of ruminal content (500 mL per steer) was obtained from all steers to isolate ruminal bacteria by differential centrifugation (Bergen *et al* 1968). The microbial isolate served as a source of purines to be used as a N reference for estimating the contribution of microbial N and microbial OM in duodenal chyme (Zinn and Owens 1986).

SAMPLE ANALYSES AND CALCULATIONS

The basal diet, duodenal and faecal samples were analysed as follows (Association of Official Analytical Chemists [AOAC] 2000): DM (oven-drying at 105°C to constant weight, method 930.15); ash (method 942.05), Kjeldahl N (method 984.13) and chromic oxide (Hill and Anderson 1958). Ammoniacal N (NH_3-N; method 941.04, AOAC 2000) and purines (Zinn and Owens 1986) were determined from the duodenal samples. In addition, concentration of NDF (Van Soest *et al* 1991, corrected for NDF-ash, incorporating heat stable α-amylase (Ankom Technology, Macedon, NY) at 1 mL per 100 mL of NDF solution (Midland Scientific, Omaha, NE)); starch (Zinn 1990), and ether extract (method 920.39; AOAC, 2000) were determined in DDGS and diets. The basal diet, duodenal and faecal OM was estimated as the difference of the DM minus the ash content. OM fermented in the rumen (OMFR) was considered equal to OM consumption minus the difference between the amount of total OM that reached the duodenum and the MOM that reached the duodenum. The feed residual N that reached the small intestine was considered equal to the total N that left the abomasum minus the NH_3-N and MN; therefore, this estimate includes any endogenous contribution of N. Plasma urea was determined by spectrophotometry (SPIN 200E automated spectrophotometer, Spinreact, Barcelona, Spain) at 340 nm wavelength at 37°C using a set of Spinreact commercial reagents.

STATISTICAL ANALYSIS

The effects of the treatments on the digestion characteristics, ruminal fermentation, and blood urea level were analysed as a 4 × 4 Latin square design using the MIXED procedure (SAS 2007). The fixed effect was assigned to the treatment, and the random effects were assigned to the steer and the period. The statistical model used was as follows:

$Yijk = \mu + Si + Pj + Tk + Eijk$, where Yijk = the response variable, μ = the common experimental effect, Si = the effect of the steer, Pj = the effect of the period, Tk = the effect of the treatment and Eijk = the residual error.

The effects of the treatments were tested as follows: 1) linear effect of the urea level; 2) quadratic effect of the urea level; and 3) cubic effect of the urea level. The coefficients for the orthogonal contrasts (linear, quadratic and cubic effects of the urea level) with equal increments (0%, 0.4%, 0.8% and 1.2% urea in DM base) were determined according to SAS statistical program (SAS 2007).

RESULTS

Physicochemical composition of DDGS was: CP, 29.2%; NDF, 44.2%; ether extract, 10.3%, and 5.5% ash. Compared to the physicochemical composition values assigned to corn DDGS by NASEM (2016), the relative values of CP, neutral detergent fibre (NDF), ether extract and ash in the present study were 0.98, 0.96, 0.96 and 1.07, respectively.

The effects of the different levels of urea on OM and N compound digestion are shown in table 2. N intake was increased (linear component, $P<0.01$) as the urea level increased. Increasing urea levels increased the duodenal flow of non-ammonia N (NAN) ($P<0.01$) via linear increases of NM and NH3-N flow ($P<0.01$) without affecting the flow of feed residual N ($P\geq0.47$). Increasing the urea levels linearly increased ruminal digestion of OM and dietary N ($P\leq0.03$) without affecting microbial efficiency. The urea supplementation did not affect post-ruminal digestion. Urea supplementation linearly increased ($P<0.01$) total tract digestion of N compounds without affecting total tract digestion of OM.

The effects of the treatments on the average (taken at 0 h, 4 h and 8 h post-feeding) ruminal pH, ruminal NH3-N concentration, and urea and blood urea concentrations are shown in table 3. The average ruminal pH was 6.09 ± 0.03 and was not affected by the treatments ($P\geq0.97$). Ruminal NH3-N concentration increased with urea supplementation (linear component, $P<0.01$). The same effect was observed for blood urea concentrations and plasma urea ($P<0.01$).

Table 2. Influence of urea supplementation level on characteristics of OM and N utilization in Holstein steers.

	Urea supplementation level (%)				SEM	Contrast *P*-value		
	0	0.4	0.8	1.2		Linear	Cuadratic	Cubic
Feed (g/d)								
Dry matter	5,772	5,771	5,771	5,772	1.05	0.911	0.211	0.617
Organic matter	5,459	5,443	5,442	5,444	7.08	0.207	0.262	0.708
Nitrogen	110	121	131	141	0.44	<0.001	0.855	0.938
Duodenal Flux (g/d)								
Dry matter	3,091	3,031	2,955	2,827	85.87	0.065	0.707	0.934
Organic matter	2,645	2,598	2,524	2,377	77.36	0.044	0.540	0.896
Total N	119	125	122	133	2.01	0.006	0.255	0.049
$N\text{-}NH_3$	2.31	2.56	2.77	3.32	0.14	0.002	0.333	0.581
Non ammoniacal N	117	122	122	129	2.08	0.010	0.298	0.059
Microbial N	66.3	71.7	74.3	81.2	3.77	0.031	0.847	0.683
Feed residual N	50.6	50.6	47.7	48.2	4.20	0.528	0.715	0.471
Ruminal Digestión (%)								
Dry matter	46.4	47.4	48.7	51.0	0.01	0.072	0.667	0.936
Organic matter	60.2	61.5	63.4	67.2	0.01	0.005	0.343	0.801
Feed N	54.3	57.8	63.6	66.0	0.03	0.028	0.662	0.469
Microbial efficiency[1]	19.1	20.1	20.4	21	1.09	0.270	0.891	0.859
Post-ruminal digestion (%)								
Dry matter	60.7	59.2	59.2	59.4	0.02	0.714	0.735	0.902
Organic matter	59.3	57.8	57.9	57.9	0.03	0.742	0.774	0.874
Nitrogen	76.5	77.3	76.0	78.5	0.63	0.141	0.224	0.086
Faecal Excretion (g/d)								
Dry matter	1,213	1,238	1,119	1,152	50.13	0.356	0.501	0.815
Organic matter	1,076	1,098	1,058	1,002	49.83	0.288	0.467	0.844
Nitrogen	27.9	28.2	29.2	28.5	0.92	0.512	0.577	0.568
Total tract digestion (%)								
Dry matter	78.9	78.6	79.3	80.1	0.01	0.322	0.492	0.803
Organic matter	80.3	79.8	80.6	81.6	0.01	0.279	0.452	0.810
Nitrogen	74.7	76.6	77.7	79.8	0.01	0.003	0.827	0.592

[1]The microbial efficiency was estimated as MN of the flow to the duodenum, g kg-1 minus the true OM fermented in the rumen.

Table 3. Influence of urea supplementation level on ruminal pH, $N\text{-}NH_3$, and urea and ureic nitrogen in blood[1].

Concept	Urea supplementation level (%)				SEM	Contrast *P*-value		
	0	0.4	0.8	1.2		Linear	Cuadratic	Cubic
pH	6.11	6.13	6.05	6.12	0.07	0.974	0.672	0.589
Ammonia N in rumen (mg/dL)	2.22	2.53	3.19	4.12	0.23	0.003	0.217	0.933
Urea in blood (mg/dL)	8.89	10.46	11.53	13.64	0.64	0.002	0.689	0.612
Ureic N in blood (mg/dL)	4.15	4.88	5.38	6.37	0.30	0.002	0.689	0.612

[1]Average values of the samples taken before the morning feed (0800 h), and at 4 and 8 hours after the morning feed.

DISCUSSION

Considering only energy intake, Burroughs *et al* (1975) proposed that the N microbial flow to the small intestine is equivalent to 0.0166 total digestible nutrients (TDN) intake (0.0166 × TDN × DM intake, kg/d). Then, considering that the expected TDN value of the basal diet is 85.90% (NASEM 2016) and considering the average DM intake of the experimental diets (table 2), the predicted flow of microbial N to the small intestine

was 78 g/d. The observed flow of MN in the present experiment was 66.3 g/d, 71.7 g/d, 74.3 g/d and 81.8 g/d. Consequently, with supplementation of 0%, 0.4%, 0.8% and 1.2% urea, the observed flow of MN to the small intestine was 85%, 92%, 95% and 105%, respectively, of the expected flow. This increase in net microbial synthesis in response to increased urea levels is consistent with May *et al* (2014), who observed that the MN flow to the small intestine decreases when the RDP content in the diet is <100 g/kg digestible OM in the total tract. In the present experiment, the RDP averaged 85 g/kg, 100 g/kg, 123 g/kg and 130 g/kg digested OM following 0%, 0.4%, 0.8% and 1.2% urea supplementation, respectively. Therefore, it is apparent that when the RDP intake is <100 g/kg digestible OM, there is insufficient compensation in ruminal N recycling to maintain optimal microbial growth, and as microbial growth decreases, it also decreases ruminal OM digestion. This effect can be corroborated by the increase observed in ruminal OM digestion ($P<0.01$) as the urea level in the diet was increased.

The similarities between the control diet and urea treatments on the dietary N flow to duodenum can be explained by the complete ruminal degradability of urea. Based on RUP values (NASEM 2016) of each ingredient, as well as on its inclusion level in the control basal diet and level of intake, the expected ruminal degradation of feed N based on NASEM (2016) Level 1 is 58% (63 g/d of dietary N reaching duodenum). This value is 21% greater than the average dietary N reaching duodenum observed in this trial and could be an indicative of a greater RDP value assigned for DDGS. Previous reports (Carrasco *et al* 2013, Castro-Pérez *et al* 2013), indicate an average DDGS RDP of 39% as measured in sheep and cannulated steers fed finishing diets containing 15-30% DDGS. Using an *in situ* technique, Corrigan *et al* (2009) determined 28% RUP for DDGS. However, using cannulated steers, Castillo-López *et al* (2013) determined 63% RUP for DDGS supplemented in a forage-based diet. The current standards (NASEM 2016) indicate 68% RUP for DDGS. Much of the variation in the solubility of corn DDGS proteins can be attributed to plant-to-plant differences in the proportions of soluble distillers added during processing (Kim *et al* 2008, Cao *et al* 2009), the type of processing to which they are exposed during starch extraction (Liu 2011) and to the method of CP degradability determination (NASEM 2016).

The absence of the effect of increasing urea levels in post-ruminal OM digestion is consistent with previous studies (Willms *et al* 1991, Cameron *et al* 1991, Zinn 1994). However, results in relation to post-ruminal N digestion have been inconsistent. In some reports, increasing urea in diets increased post-ruminal nitrogen digestibility (Zinn *et al* 1994, Kozloski *et al* 2000); in others, as in the present experiment, increasing urea levels in the diet had no effect on post-ruminal N compounds digestion (Zinn *et al* 2003, May *et al* 2014). The difference on post-ruminal N digestion between reports is mainly due to the method of substitution of protein ingredients by urea. In our experiment, due the method of supplementation, urea did not substitute a particular ingredient.

Consistent with previous studies, the urea level did not affect total tract OM digestion (Kolzoski *et al* 2000, May *et al* 2014, Ceconi *et al* 2015[a]). As expected, the apparent total tract N digestion increased linearly ($P<0.01$) as the urea levels increased. This increase is partly due to the digestibility of urea and partly due to the increasing concentration of CP in the diet as the urea level increased (Holter and Reid 1959). Adjusting for the faecal loss of metabolic protein (Swanson 1977, NRC 1985), the true digestion of protein in the present study was 91.84%, similar to the average of the previous measurements summarised by the National Research Council (NRC 1985).

The ruminal pH observed in the present study is similar to that predicted based on diet formulation (5.90, NRC 2000, Level 1). The alkalizing potential of urea is well-known (Vedharathinam and Botte 2014). This potential to increase ruminal pH in the first hour post-feeding has been reported (Zinn *et al* 2003); however, at later readings (≥4 h), the effect is diluted (May *et al* 2014), possibly by the curve of the rate of urea degradation and by the ruminal dilution rate. We observed increases in ruminal NH3-N at higher urea levels (linear component, $P<0.01$). Increases in soluble protein in the rumen, regardless of the source (protein N or NPN), increase the concentration of ruminal NH3-N (May *et al* 2014, Ceconi *et al* 2015[a,b]). The linear increase in the ruminal NH3-N ($P<0.01$) observed here was reflected in the increased NH3-N flow to the duodenum and the increased plasma urea in the blood. A simple diffusion mechanism regulates NH3-N uptake at the ruminal epithelial level, so it is subject to the concentration differential, while ruminal pH mediates the absorption rate (Orskov 1992); the higher the ruminal NH3-N concentration, the greater the passage of blood through the portal system, and the higher the pH (vgr.>6.5), the greater the speed of NH3-N absorption to the blood system. According to the NRC (1985), the relationship between ruminal NH3-N concentration and plasma urea concentration is predicted using the following equation: $Y = (79 + 14.5X)/10$, where Y = plasma urea (mg/dL) and X = ruminal NH3-N (mg/dL). Applying this equation, the predicted average concentration is 12.27 mg urea/dL plasma, which represents a value close to the average of 11.13 mg urea/dL plasma observed in the present experiment.

On the basis of the results observed here, urea can be incorporated in finishing corn-based diets that include DDGS. However, this must be done carefully to avoid exceeding the RDP concentration in the diets in order to optimising ruminal fermentation and reducing the risk of high N excretion in the faeces. This is relevant when higher levels of DDGS are included in the diets (i.e. 30%).

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