ABSTRACT: As a result of the cost of grains, the replacement of grains by co-products (i.e. DDGS) in feedlot diets is a common practice. This change produces diets that contain a lower amount of starch and greater amount of fibre. Hypothetically, combining feed grade urea (U) with slow release urea (Optigen) in this type of diet should elicit a better synchrony between starch (high-rate of digestion) and fibre (low-rate of digestion) promoting a better microbial protein synthesis and ruminal digestion with increasing the digestible energy of the diet. Four cannulated Holstein steers (213±4 kg) were used in a 4×4 Latin square design to examine the combination of Optigen and U in a finishing diet containing different starch:acid detergent fibre ratios (S:F) on the characteristics of digestive function. Three S:F ratios (3.0, 4.5, and 6.0) were tested using a combination of U (0.80%) and Optigen (1.0%). Additionally, a treatment of 4.5 S:F ratio with urea (0.80% in ration) as the sole source of non-protein nitrogen was used to compare the effect of urea combination at same S:F ratio. The S:F ratio of the diet was manipulated by replacing the corn grain by dried distillers grain with solubles and roughage. Urea combination did not affect ruminal pH. The S:F ratio did not affect ruminal pH at 0 and 2 h post-feeding but, at 4 and 6 h, the ruminal pH decreased as the S:F ratio increased (linear, p<0.05). Ruminal digestion of OM, starch and feed N were not affected by urea combination or S:F ratio. The urea combination did not affect ADF ruminal digestion. ADF ruminal digestion decreased linearly (p = 0.02) as the S:F ratio increased. Compared to the urea treatment (p<0.05) and within the urea combination treatment (quadratic, p<0.01), the flow of microbial nitrogen (MN) to the small intestine and ruminal microbial efficiency were greater for the urea combination at a S:F ratio of 4.5. Irrespective of the S:F ratio, the urea combination improved (2.8%, p = 0.02) postruminal N digestion. As S:F ratio increased, OM digestion increased, but ADF total tract digestion decreased. The combination of urea at 4.5 S:F improved (2%, p = 0.04) the digestible energy (DE) more than expected. Combining urea and Optigen resulted in positive effects on the MN flow and DE of the diet, but apparently these advantages are observed only when there is a certain proportion of starch:ADF in the diet. (Key Words: Slow-release Urea, Finishing Diets, Steers, Digestion, Microbial Nitrogen)
ruminal degradation rates between urea and starch. Currently, as a result of the cost of corn grain, the replacement of corn grain by dried distillers grain with solubles (DDGS) in feedlot diets is a common practice (Klopfenstein et al., 2008). Although the energy value of DDGS is similar to steam flaked corn (NRC, 1996), DDGS are lower in starch content (<6%) and higher in their content (>30%) of digestible fibre (Rosentraer, 2012; Carrasco et al., 2013). Therefore, depending on the replacement level, the fibre content in finishing diets can be increased up to two-fold (from 8% to 15% of total acid detergent fibre [ADF]) and the total starch content can be decreased by 20% (from 50% to 40%). Hypothetically, combining feed grade urea with slow-release urea in finishing diets should promote the synchrony between starch (high-rate of digestion) and fibre (low-rate of digestion), promoting better microbial protein synthesis and increases in ruminal digestion and the digestible energy of the diet. The beneficial effects of the supplementation of slow-release urea has been extensively studied in cattle that were fed a high-forage diets, i.e. rations for dairy and growing cattle (Inostroza et al., 2010; Alvarez et al., 2011); however, no research has examined the role of the starch:fibre ratio of the finishing diets on the effects of the combination of both sources of urea. Therefore, the purpose of this study was to evaluate the effects of combining feed grade urea and a slow-release urea product (OPT) on the characteristics of digestion, microbial protein synthesis and digestible energy in steers fed diets with different starch:ADF ratios.

MATERIALS 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 located 10 km south of Mexicali City in northwestern México (32° 40’ 7”N and 115° 28’ 6”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 guidelines of locally-approved techniques for animal use and care (NOM-051-ZOO-1995: humanitarian care of animals during mobilization of animals; NOM-062-ZOO-1995: technical specifications for the care and use of laboratory animals. Livestock farms, farms, centres of production, reproduction and breeding, zoos and exhibition hall, must meet the basic principles of animal welfare; NOM-024-ZOO-1995: animal health stipulations and characteristics during transportation of animals. These regulations are in accordance with the principles and specific guidelines presented in the Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animals, diets and sampling

Four Holstein steers (213±4 kg) with ruminal and duodenal cannulas (Zinn and Plascencia, 1993) were used in a 4×4 Latin square design to examine the effect of the combination of Optigen (a polymer-coated urea; Optigen, Alltech Mexico, Guadalajara, Jalisco) and U in a finishing diet containing different starch:acid detergent fibre ratios (S:F) on the characteristics of digestion, microbial protein synthesis and digestible energy of diet. Three S:F ratios (3.0, 4.5, and 6.0) were tested using a combination of U (0.80%) and Optigen (1.0%). Additionally, a treatment of 4 S:F ratio with urea (0.80% in ration) as sole source of NPN was used to compare the effect of urea combination at same S:F ratio. For the above, four dietary treatments were compared:

- Diet formulated to supply 2.14 Mcal/kg of NE\text{\textsubscript{\text{\%}}} with a S:F ratio of 4.5, containing 0.80% conventional urea as the sole source of NPN (U-4.5)
- Diet formulated to supply 2.14 Mcal/kg of NE\text{\textsubscript{\text{\%}}} with a S:F ratio of 6.0, containing 0.80% conventional urea plus 1.00% OPT (OPT-6)
- Diet formulated to supply 2.07 Mcal/kg of NE\text{\textsubscript{\text{\%}}} with a S:F ratio of 4.5, containing 0.80% conventional urea plus 1.00% OPT (OPT-4.5)
- Diet formulated to supply 2.00 Mcal/kg of NE\text{\textsubscript{\text{\%}}} with a S:F ratio of 3.0, containing 0.80% conventional urea plus 1.00% of OPT (OPT-3)

The S:F ratio in the diet was manipulated by replacing the steam-flaked corn grain by dried distillers grain with solubles (DDGS) and forage (sudangrass hay and wheat straw) to reach S:F ratios of 3.0, 4.5, or 6.0 (Table 1). Chromic oxide (used as a source of chromium to estimate nutrient flow and coefficient of digestion) was added to the diets (3.5 g/kg of diet on an air dry basis) and it was premixed with minor ingredients (urea and mineral supplement composed of limestone and trace mineral salts) before incorporation into complete mixed diets. All steers received ad libitum access to the control diet (U-4.5) for 14 days before initiation of the trial. To avoid refusals once the experiment started, dry matter intake was restricted to 4.55 kg/d (90% of observed ad libitum intake of steers during the 14-d preliminary period). The slow-release urea product was hand-weighted using a precision balance (Ohaus, mod AS612, Pine Brook, NJ), and was added (top-dressed) in equal proportions to the diet at time of feeding. Diets were fed in two equal portions at 0800 and 2000 h daily. Animals were housed in individual pens (3.9 m\textsuperscript{2}) in an indoor facility with a concrete floor covered by a neoprene carpet, automatic waterers and individual feed bunks. Experimental periods consisted of a 17-d diet adjustment period followed
Table 1. Ingredients and composition of experimental diets

| Item                        | Treatment U-4.5 | Treatment OPT-6.0 | Treatment OPT-4.5 | Treatment OPT-3.0 |
|-----------------------------|-----------------|-------------------|-------------------|-------------------|
| Ingredient composition (%) DMB |                 |                   |                   |                   |
| Steam flaked corn           | 58.00           | 68.00             | 60.00             | 55.00             |
| DDGS                        | 15.00           | 4.00              | 6.50              | 7.00              |
| Sudangrass hay              | 6.00            | 6.00              | 10.00             | 12.00             |
| Wheat straw                 | 4.00            | 4.00              | 5.50              | 8.00              |
| Urea                        | 0.80            | 0.80              | 0.80              | 0.80              |
| Optigen 1200¹                | -               | 1.00              | 1.00              | 1.00              |
| Bakery waste                | 8.00            | 8.00              | 8.00              | 8.00              |
| Yellow grease               | 3.50            | 3.50              | 3.50              | 3.50              |
| Trace mineral salt ²        | 2.00            | 2.00              | 2.00              | 2.00              |
| Limestone                   | 1.80            | 1.80              | 1.80              | 1.80              |
| Chromic oxide               | 0.35            | 0.35              | 0.35              | 0.35              |
| NE concentration² (Mcal/kg of DM basis) |     |                   |                   |                   |
| ENₚ (Mcal/kg)               | 2.14            | 2.14              | 2.07              | 2.00              |
| ENₜ (Mcal/kg)               | 1.48            | 1.48              | 1.41              | 1.34              |
| Nutrient composition (%) of DM³ |     |                   |                   |                   |
| Crude protein               | 12.48           | 13.50             | 13.70             | 13.53             |
| Starch                      | 42.92           | 48.20             | 44.90             | 39.40             |
| ADF                         | 9.63            | 8.03              | 10.10             | 13.27             |
| Calcium                     | 0.73            | 0.78              | 0.80              | 0.73              |
| Phosphorus                  | 0.43            | 0.31              | 0.33              | 0.33              |

¹Optigen-1200. Alltech de México, Guadalajara Jalisco.
²Trace mineral salt contained: CoSO₄, 0.068%; CuSO₄, 1.04%; FeSO₄, 3.57%; ZnO, 1.24%; MnSO₄, 1.07%; KI, 0.052%; NaCl, 92.96%.
³Based on tabular net energy (NE) values for individual feed ingredients (NRC, 2000) with the exception of supplemental fat, which was assigned NEₚ and NEₜ values of 6.03 and 4.79, respectively (Zinn, 1988).
⁴Dietary composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.

Sample analysis and calculations

Feed, duodenal and faecal samples were subjected to the following analysis: DM (oven drying at 105°C until no further weight loss; method 930.15; AOAC, 2000); ash (method 942.05; AOAC, 2000); Kjeldahl N (method 984.13; AOAC, 2000); ADF (Van Soest et al., 1991); chromic oxide (Hill and Anderson, 1958); and starch (Zinn, 1990). In addition, gross energy (GE, using the adiabatic bomb model 1271; Parr Instrument Co., Moline, IL, USA) was determined for feed and faecal samples. Ammonia N (method 941.04; AOAC, 2000) and purines (Zinn and Owens, 1986) were determined in duodenal samples. Ether extract (method 920.39; AOAC, 2000), calcium (method 927.02; AOAC, 2000) and phosphorus (method 964.06; AOAC, 2000) were determined in feed samples.

Microbial organic matter (MOM) and microbial nitrogen (MN) leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). Organic matter fermented in the rumen (OMF) was considered equal to the OM intake minus the difference between the amount of total OM reaching the duodenum and the MOM reaching the duodenum. Feed N escaping to
the small intestine was considered equal to the total N leaving the abomasum minus ammonia-N and MN and, thus, includes any endogenous contributions.

Statistical design and analysis

The effects of the treatments on the characteristics of digestion in the cattle were analysed as a 4×4 Latin square design using the GLM procedure (SAS Inst., Inc., Cary, NC). The statistical model for the trial was as follows:

\[ Y_{ijk} = \mu + S_i + P_j + T_k + E_{ijk}, \]

Where, \( Y_{ijk} \) is the response variable, \( \mu \) is the common experimental effect, \( S_i \) is the steer effect, \( P_j \) is the period effect, \( T_k \) is the treatment effect and \( E_{ijk} \) is the residual error. The response curves of the S:F ratio in OPT treatments were analysed with orthogonal polynomials. In addition, the urea combination (urea vs OPT) at same S:F ratio (4.5) was compared using the Student’s t-test. The analysis was carried out using SAS (SAS Inst., Inc., Cary, NC; Version 9.1). The ruminal pH values, which were recorded at 0, 2, 4, and 6 h post-feeding, were analysed with a linear mixed model for repeated measures in a Latin square design according to SAS (SAS Inst., Inc., Cary, NC; Version 9.1), with evaluation of the covariance structures: A, CS, AR (1), and the animal as random components.

RESULTS AND DISCUSSIONS

The ratios of starch:ADF determined in the laboratory reached at least 98% of the planned for each treatment (Table 1). The effects of treatment on ruminal pH are shown in Table 2. The ruminal pH observed for each treatment in the present study was slightly greater (3.7) than predicted based on the diet formulation (NRC, 1996, Level 1). This may be because of the incorporation of 1.80% limestone in the diet (Table 1), which has a high ruminal buffering capacity (Russell et al., 1980; Haaland et al., 1982). Ruminal pH was not affected by urea combination. The S:F ratio did not affect ruminal pH at 0 and 2 h post-feeding but, at 4 and 6 h post-feeding, the ruminal pH decreased as the S:F ratio increased (linear, \( p<0.05 \)). As consequence of the rapid ruminal fermentation of starch, diets that contain increasing amounts of starch (grains) tend to promote a lower ruminal pH than those that contain lower quantities of starch (Huntington, 1997). In this sense, the S:F ratio of 6.0 contained 6.8% and 8.2% more starch than S:F ratios 4.5 and 3.0, respectively. At equal S:F ratios, the ruminal pH was very similar (\( p = 0.98 \)) between the urea and OPT treatments, averaging 6.26 vs 6.22 for U-4.5 and OPT-4.5, respectively. This indicates that, although U-4.5 contained 3.3% more energy than OPT-4.5 (2.14 vs 2.07 Mcal EN\(_{\text{ad}}$/kg), the S:F is the most important factor, rather than the energy level per se, that impacts the ruminal pH.

Treatment effects on the characteristics of ruminal and total tract digestion are summarized in Table 3. Urea combination did not modify ruminal digestion of OM, starch, ADF, and feed N. The S:F ratio did not affect the ruminal digestion of OM, starch and feed N, however, ruminal ADF digestion decreased linearly (\( p = 0.02 \)) as the S:F ratio increased. It has been observed that the depression in fibre digestion is largely a function of ruminal anion concentration (Peters et al., 1992). Russell and Wilson (1996) observed that fibre digestion decreases because cellulolytic bacteria cannot adequately control their intracellular anion gradient as pH declines. Grant and Weidner (1992) further verified this concept in vitro by fermenting various forages in cultures of ruminal fluid wherein pH was controlled experimentally by means of citrate buffers. They observed that both the lag phase and the rate of fibre digestion were sensitive to sustained culture pH below 6.2. The ruminal pH with the OPT-6 treatment was consistently maintained below 6.0, while in the rest of the treatments ruminal pH remained above 6.2 (Table 2).

The flow of microbial N to the small intestine (quadratic effect, \( p<0.01 \)) and ruminal microbial efficiency (flow of microbial N to the small intestine as a proportion of fermented OM; quadratic effect, \( p = 0.03 \)) were greater than the 4.5 S:F ratio within OPT treatments. In the same manner, compared to the control diet, at an equal S:F ratio (4.5), the

### Table 2. Effects of treatments on ruminal pH taken at 0, 2, 4, and 6 h post-feeding

| Item      | U-4.5 | OPT-6 | OPT-4.5 | OPT-3 | S:F ratio | EEM | U-4.5 vs OPT-4.5 | Linear | Quadratic |
|-----------|-------|-------|---------|-------|-----------|-----|------------------|--------|-----------|
| Ruminal pH|       |       |         |       |           |     |                  |        |           |
| 0 h       | 6.60  | 6.47  | 6.56    | 6.76  | 0.12      | 0.19| 0.06             | 0.19   |           |
| 2 h       | 5.82  | 5.84  | 5.89    | 6.05  | 0.07      | 0.67| 0.09             | 0.63   |           |
| 4 h       | 6.20  | 5.80  | 6.11    | 6.15  | 0.09      | 0.48| 0.04             | 0.32   |           |
| 6 h       | 6.42  | 6.06  | 6.41    | 6.51  | 0.09      | 0.98| 0.02             | 0.31   |           |

\(^1\) U-4.5 = Diet formulated to supply 2.14 Mcal/kg of NE\(_{\text{ad}}\) with a ratio of starch:FDA of 4.5, containing 0.80% of conventional urea as the sole source of NNP. OPT-6 = Diet formulated to supply 2.14 Mcal/kg of NE\(_{\text{ad}}\) with a ratio of starch:FDA of 4.0, containing 0.80% of conventional urea plus 1.00% of Optigen; OPT-4.5 = Diet formulated to supply 2.07 Mcal/kg of NE\(_{\text{ad}}\) with a ratio of starch:FDA of 4.5, containing 0.80% of conventional urea plus 1.00% of Optigen, and OPT-3 = Diet formulated to supply 2.00 Mcal/kg of NE\(_{\text{ad}}\) with a ratio of starch:FDA of 3.0, containing 0.80% of conventional urea plus 1.00% of Optigen (OPT-3).
urea combination increased (p ≤0.02) the MN flow to the duodenum by 7.1% and the MN efficiency by 7.3%. Burroughs et al. (1975) proposed that microbial N flow to the small intestine was equivalent to 0.0166TDN, estimated as follows: 0.0166×TDN×DMI, kg/d. According to the expected TDN values (NRC, 1996) and DM intakes of the experimental diets (Table 3), the predicted flow of microbial N to the small intestine would be 62.3, 65.02, 63.06, and 59.25 g/d with U-4.5, OPT-6, OPT-4.5 and OPT-3, respectively. The MN flows of the U-4.5 and OPT-3 treatments were very close to those expected (98%); however, surprisingly, the MN flow of treatment OPT-6 was 5.6% less than expected. The decreased response observed for OPT-6 can be explained, in part, by the reduced ADF ruminal digestion observed for this S:F ratio. In contrast, the urea combination for an S:F ratio of 4.5 produced a flow of N 4.2% greater than expected, reflecting a positive synchrony for this proportion of starch and ADF in the diet. It has been observed that MN production, among others, is a result of synchronization between the rate of hydrolysis of carbohydrates, and the rate at which N-NH₃ is produced during the hydrolysis of N compounds in the rumen (NRC, 1985; Orskov, 1992). The difference in microbial production observed between U-4.5 and OPT-4.5 could be
partially explained by the improved synchrony for the combination of the two urea sources in OPT-4.5.

The S:F ratio did not affect post-ruminal digestion of OM, starch, ADF or N, although the digestion of organic matter tended (linear effect, $p = 0.09$) to decrease as the S:F ratio in the diet decreased. Irrespective of the proportion of starch and fibre in the diets, the combination of two urea sources improved post-ruminal N digestion by an average of 2.8% ($p = 0.02$) with no difference in the digestion of other components evaluated.

The S:F ratio affected OM and ADF total tract digestion. As the ratio increased, OM digestion increased (linear effect, $p<0.01$), but ADF total tract digestion decreased ($p = 0.03$). The reduction in total tract OM digestion as the S:F ratio decreased was expected, and was largely attributable to relative differences in the total tract digestion of ADF (36%) versus starch (99%). Compared to the control diet, the inclusion of OPT in the diets increased the total tract apparent N digestion (2.8%, $p<0.05$). However, this effect may be more a function of the increased N content of the diet brought about by the replacements (Holter and Reid, 1959).

Consistent with the effects on total tract OM digestion, the S:F ratio affected the digestibility of GE. As the ratio decreased, the digestibility of GE also decreased (linear effect, $p<0.01$). According to the expected DE values (NRC, 1996), the predicted DE were 1.00, 1.00, 1.02, and 1.00 times the expected values for U-4.5, OPT-6, OPT-4.5 and OPT-3, respectively. Thus, at a ratio of 4.5, the digestive energy was improved by 2% over the expected ($p = 0.04$) level. This improvement represents an increase of 0.077 Mcal/kg of digestible energy. If we consider that: i) the contribution of digestible energy content in diets is mainly due to changes in the participation of corn and DDGS; ii) that the DDGS contains a similar energy concentration as corn (Depenbusch et al., 2008; Uwitze et al., 2010); and iii) the difference in the content of DDG plus corn between U-4.5 and OPT-4.5 represents 6.5% (the sum of corn and DDGS participation in U-4.5 is 73%, whereas for diet OPT-4.5 it is 66.5%, Table 1), then the 2% of energy improvement in OPT-4.5 treatment represents the equivalent increase of 4.3% $[(0.117-0.04)/(0.117/6.5)]$ corn grain in the diet.

It is concluded that combining feed grade urea and Optigen resulted in positive effects on the MN flow and digestible energy of the diet, but apparently these advantages are observed only when there is a certain proportion (4.5) of starch:ADF in the diet. Either a higher or lower S:F ratio than 4.5 failed to offer advantages over any of the parameters evaluated. It is necessary to continue research on the conditions of the finishing diet so that it is possible to get the most out of it with the use of slow release urea.

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