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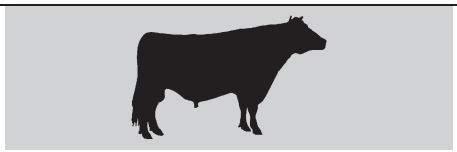

Case Study: In situ determination of protein digestibility of dried distillers grains containing 3 lipid concentrations using a mobile bag method

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ABSTRACT

Ethanol producers remove lipid from distillers grains (DG) for applications such as biodiesel production. The effects of the lipid removal on ruminal protein degradability and total-tract CP digestibility of DG are not known. Five ruminally and duodenally cannulated Angus-cross steers (BW = 434 ± 15 kg) were used to incubate in situ bags for determination of protein digestibility of low-lipid (5.54%) DG, medium-lipid (8.40%) DG, high-lipid (12.46%) DG, and cottonseed meal. Ingredients were weighed into individual in situ bags and incubated in the ventral sac of the rumen for 16 h. After ruminal incubation and simulated abomasal digestion, bags were inserted into the duodenal cannula of corresponding steers and collected from feces approximately 12 to18 h later. Bags were washed, dried, and analyzed for CP. The CP concentration in DG increased with decreasing lipid concentrations, and the RUP fraction of the CP in DG decreased with decreasing lipid concentration (54.5, 54.8, and 60.1 ± 1.8% RUP for low-, medium-, and high-lipid DG, respectively) suggesting that lipid extraction increased rumen protein degradability. The total-tract indigestible protein and postruminal digestibility of RUP were not different among the varying lipid concentrations in DG. The RUP digestibility of the low-, medium-, and high-lipid DG (79.5, 80.4, and 80.6 ± 2.0%, respectively) was consistent with the commonly used NRC model value of 80%. These data suggest the extraction of lipid from DG may alter ruminal degradability of CP but does not change the postruminal digestibility of the RUP.

Key words: dried distillers grains, lipid extraction, protein digestibility

INTRODUCTION

In a study by B. E. Meyer, N. A. Cole, and J. C. MacDonald (unpublished data), 3 lipid levels of dried distillers grains plus solubles (**DDGS**) were included in steam-flaked corn–based diets to determine the energy value of the lipid within the DDGS. During the course of the experiment, questions arose over the quality and digestibility of the protein in the DDGS because of the varying coloration between the 3 lipid concentrations (also observed by Saunders and Rosentrater, 2009). It is known that the nutrient profiles of DDGS are variable and may, in part, be due to location, fermentation process, drying temperature, drying time, chemical effects, and other factors (Spiehs et al., 2002). The effects of lipid removal on protein degradability and digestibility are unknown in DDGS but have been shown to influence protein characteristics in cottonseed meal (Goetsch and Owens, 1985). The objective of this experiment was to determine CP as a percentage of DM, RUP and RDP as a percentage of CP and DM, total-tract indigestible protein as a percentage of CP (**TTIDP**), and postruminal digestible RUP as a percentage of RUP (**RUPDIG**) of DDGS that

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contained 3 lipid concentrations using the nylon mobile bag method.

MATERIALS AND METHODS

Animals and Diet

All procedures involving the management and use of live animals were approved by the Amarillo Area Cooperative Research, Education and Extension Team Animal Care and Use committee. Five Angus-cross ruminally and duodenally cannulated steers (BW = 434 ± 15 kg) were fed a steam-flaked corn–based diet with 30% (DM basis) medium-lipid-level (8.4% solvent-extracted lipid) DDGS (POET Nutrition, Sioux Falls, SD), 53.5% steam-flaked corn, 10.0% alfalfa hay, 4.0% molasses, and 2.5% supplement that was formulated to provide a dietary DM inclusion of 0.30% salt, 60 mg/kg Fe, 40 mg/kg Zn, 30 mg/kg Mg, 25 mg/kg Mn, 10 mg/kg Cu,1 mg/kg I, 0.15 mg/kg Co, 0.1 mg/kg Se, 1.5 IU/g vitamin A, 0.15 IU/g vitamin D, 8.81 IU/kg vitamin E, 33 mg/kg monensin (Elanco Animal Health, Indianapolis, IN), and 8.7 mg/kg tylosin (Elanco Animal Health).

Rumen Incubations and Mobile Bag Technique

The mobile bag procedure used has been described by Haugen et al. (2006). Steers were used to incubate 5 × 10 cm in situ bags with 50-μm pore size (Ankom Technologies, Macedon, NY). Approximately 1.25 g of each dry ingredient was added to individual in situ bags and heat sealed before being placed into 1 of 5 mesh bags, for a total of 10 in situ bags of each ingredient per mesh bag. Ingredients added to the bags included low-lipid (5.54% fat) DDGS, medium-lipid (8.40% fat) DDGS, high-lipid (12.46% fat) DDGS (POET Nutrition) and a cottonseed-meal (**CSM**; Hi-Pro Feeds, Friona, TX) control. Lipid content of the DDGS was determined gravimetrically using a biphasic extraction method with diethyl ether and hexane as solvents (Bremer et al., 2010). Of the 10 in situ bags of each sample incubated per steer, 4 were used for determination of RUP (% of CP) and 6 for determination of RUPDIG. One mesh bag containing 40 in situ bags (10 of each ingredient so that every ingredient was incubated in every steer) was placed in the ventral sac of the rumen of each steer and incubated for 16 h. An incubation of 16 h has been commonly used for determination of RUP of concentrate feeds (Kopečný et al., 1998; MacDonald et al., 2007). After incubation, mesh bags were lightly rinsed in water to remove excess ruminal fluid. Four in situ bags (for RUP determination) per ingredient per steer were separated, placed in separate bags, and stored at 0°C until washing. The remaining 6 in situ bags (for RUPDIG determination) per ingredient per steer were agitated in a pepsin and HCl (1 g of pepsin/L and 0.01 *N* HCl; 62.5 mL/bag) solution for 3 h at 37°C, to mimic abomasal digestion, then stored at 0°C until duodenal insertion. After thawing, one bag was placed through the duodenal cannula every 10 min (up to 20 bags inserted per day) and collected from feces approximately 12 to 18 h later. The frozen bags from the rumen were thawed and added to the bags collected from the feces. Bags were washed with water (0.375 L/bag) in a washing machine with 5 cycles consisting of a 1-min agitation and 2-min spin each. After rinsing, bags were dried in a forced-air oven for 48 h at 60°C and then weighed. Sample residues from each in situ bag (4 for RUP and 6 for RUPDIG) within steer were composited and shipped to Foundation Analytical Laboratory (Cherokee, IA) for CP analysis (AOAC, 2000; method 2001.11).

Calculations

Rumen undegradable protein was obtained by determining the grams of CP remaining in each bag after ruminal fermentation of each sample as a percentage of the CP added to the bag. Total-tract indigestible protein was determined by dividing the grams of CP in each bag after total-tract incubation by the initial grams of CP in the bag. The percentage of RUP digested postruminally was determined by subtracting the quotient of TTIDP and RUP from one according to the procedures of Haugen et al. (2006). Ruminally undegradable protein as a percentage of DM was calculated by multiplying the CP (% of DM) by the RUP (% of CP). Ruminally degradable protein as a percentage of DM was calculated similarly but with 1-RUP (% of CP) instead of RUP.

Statistical Analysis

Data were analyzed using the Mixed procedure with a completely randomized design model (SAS Institute Inc., Cary, NC) with steer as the experimental unit. Preplanned contrasts of CSM versus DDGS and linear and quadratic relationships between the 3 lipid levels of DDGS were analyzed using orthogonal polynomials. Significance was determined at $P \leq 0.05$, with tendencies reported at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Table 1 provides values of CP, RUP, TTIDP, and RUPDIG for each feed ingredient. Crude protein concentrations of the 3 DDGS ingredients were determined to be less than that of CSM. Measurements of CP in DDGS were similar to the NRC (2001) value of 30.4%, and values for CSM were slightly greater than the NRC (2001) value of 46.1%. Starch removal from corn during the ethanol production process produces a product containing greater percentages of fiber, fat, and protein than in prefermented corn (Klopfenstein et al., 2008). If fat is also removed, the remaining product would be assumed to have greater percentages of fiber and protein than the preextracted product. This assumption is supported by Table 1 in which CP concentration increased as lipid levels in the DDGS decreased.

Rumen undegradable protein (% of CP) for DDGS samples (54.5, 54.8, and 60.1 ± 1.8% for low, medium and high lipid concentrations, respective-

Table 1. Protein characteristics of selected feed ingredients

	Dietary treatment[1]					*P*-value[3]		
Item	Low	Med	High	CSM	SE[2]	CSM vs. DDGS	Lin	Quad
CP, % of DM	31.8	30.6	29.2	47.7	—	—	—	—
RDP, % of DM	14.5	13.8	11.7	27.3	0.6	<0.01	0.01	0.03
RUP, % of DM	17.2	16.8	17.6	20.4	0.6	<0.01	0.32	0.85
RUP, % of CP	54.5	54.8	60.1	42.7	1.8	<0.01	0.03	0.21
TTIDP,[4] % of CP	11.2	10.8	11.5	9.9	1.1	0.20	0.55	0.90
RUPDIG,[5] % of RUP	79.5	80.4	80.6	76.9	2.0	0.09	0.91	0.62

[1]Low = low-lipid dried distillers grains; Med = medium-lipid dried distillers grains; High = high-lipid dried distillers grains; CSM = cottonseed meal.

[2]n = 5.

[3]CSM vs. DDGS = preplanned contrast of CSM and dried distillers grains; Lin = linear effect of fat concentration in dried distillers grains; Quad = quadratic effect of fat concentration in dried distillers grains.

[4]Total-tract indigestible protein.

[5]RUP digestibility.

ly) were greater than the NRC (2001) estimated value of 52%, whereas the RUP of CSM (42.7%) was similar to the estimate from NRC (2001) of 43%. The NRC (1985) stated that the CSM RUP value was 46% determined by studies using dry-rolled or ground corn diets with a minimum of 20% forage. Zinn et al. (1981) reported that CSM RUP values varied from 24 to 61% of dietary CP. Similarly, estimates of CSM RUP in our laboratory have varied from 30.2 to 48.7% of diet CP (J. C. MacDonald, 2012, Texas AgriLife Research, 6500 Amarillo Blvd. W., Amarillo, TX, personal communication). Because CSM is also the product of a lipid-extraction process, it is possible that a portion of the variability in RUP values for CSM is due to variation in lipid-extraction procedures as well as variability in drying procedures, which may result in a change in the solubility characteristics of the inherent protein (Goetsch and Owens, 1985) in some cases. Additionally, variation in experimental conditions such as incubation times, extent of nylon bag washing, sample size, sample processing, and animal diet can affect estimates of ruminal degradability (Vanzant et al., 1998). Goetsch and Owens (1985) demonstrated that both lipid-extraction methods and experimental conditions affected the site of protein digestion of CSM.

Ruminal undegradable protein (% of CP) was greater ($P < 0.01$) in DDGS samples than in CSM and increased linearly ($P = 0.03$) as the lipid concentration increased in DDGS. As a percentage of DM, RUP was greater ($P < 0.01$) in CSM than in DDGS and was not affected ($P = 0.32$) by lipid concentration; however, RDP (% of DM) increased with decreasing lipid concentration. This suggests that as lipid is removed from the DDGS, the increased concentration of CP is ruminally degradable. As a result, RUP (% of CP) decreases and RUP (% of DM) is not affected. These data suggest that using DDGS with varying lipid concentrations may not alter diet formulations for RUP, but may change RDP formulation strategies. It is important to note that all steers in the current experiment consumed diets containing the medium-lipid DDGS. It is possible the different DDGS products may influence the microbial population, which in turn may influence ruminal protein degradability. Nevertheless, the current data set provides evidence that RDP may vary with lipid concentration in DDGS products.

Zinn and Owens (1983) suggested that protein digestibility in the rumen can be predicted based on protein characteristics, environment within the rumen, and ruminal retention time. Because the steers in this study were fed the same diet and bags containing ingredients were incubated in a single experiment for equal lengths of time, it can be assumed that the protein characteristics, specifically protein digestibility, between CSM and the DDGS ingredients differ as did the protein digestibility among the 3 DDGS lipid concentrations. It is possible that the change in rumen protein degradability among the DDGS samples in this case study is due to the process of removing the lipid rather than the lowered lipid content per se. For example, if lipid was removed from the solubles stream after the fermentation and centrifugation (Majoni et al., 2011), it can be assumed that the concentration of soluble CP in the solubles fraction increased, leading to a concomitant increase in the CP concentration of the DDGS when the solubles fraction was added back to the solid grains. Because soluble protein is thought to be rapidly degraded in the rumen (Schwab et al., 2003; Kelzer et al., 2010), it is possible that the corresponding decrease in RUP with decreasing lipid content in the DDGS reported in Table 1 resulted from

the greater concentration of soluble protein in the lipid-extracted solubles fraction. Additionally, the drying process is suspected of affecting the protein quality and ruminal degradability because of the application of heat (Van Soest, 1994; NRC, 2001). Because all samples in the current data set were derived from the same source, we have assumed that the drying procedures were similar across the 3 DDGS samples and, therefore, would not interact with the effects of lipid removal.

Although there were differences observed in RDP (% DM), TTIDP between the 3 lipid concentrations of DDGS did not differ ($P = 0.55$), nor did TTIDP differ between DDGS and CSM ($P = 0.20$). The digestible portion of the RUP tended to be greater ($P = 0.09$) in DDGS ingredients than in CSM. The RUPDIG of the low-, medium-, and high-lipid DDGS (79.5, 80.4, and 80.6%, respectively) are consistent with the NRC (1996) metabolizable protein model, which assumes all RUP is 80% digestible. Cottonseed meal RUPDIG was determined to be 76.9%, which is lower when compared with other protein digestibility studies. In other unpublished experiments by J. C. MacDonald (2012, Texas AgriLife Research, Amarillo, TX, personal communication), values of the RUPDIG of CSM have been determined to be 94% in diets including wet distillers grains and 85.6% in diets with DDGS included. This indicates variability in experimental conditions or the CSM itself as supported by Goetsch and Owens (1985). MacDonald et al. (2007) obtained CP values of DDGS that support the current study's values, but RUP (51.3%) and TTIDP (57.0%) were lower and RUPDIG (88.8) was higher than in the current study. As demonstrated in this study, RDP (% of DM) in DDGS can be affected by lipid level, and the differences in TTIDP and RUPDIG observed in the current experiment compared with others may be due to variability of DDGS explained by Spiehs et al. (2002) and Klopfenstein et al. (2008).

IMPLICATIONS

Dried distillers grains at the 3 lipid levels tested had greater RDP and RUPDIG than did CSM. Lipid extraction appears to increase the availability of RDP based on the increased RDP as a percentage of CP or DM but does not affect total-tract digestibility or the digestibility of RUP. Accurate estimates of RDP for DDGS with different lipid concentrations may be necessary for accurate metabolizable protein formulations. Further research regarding the effects of lipid extraction on RDP will be useful in selecting distillers by-products for individual production needs.

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