



# 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 =  $434 \pm 15$  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 to 18 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  $60.1 \pm 1.8\%$  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  $80.6 \pm 2.0\%$ , 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

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 (Spiels 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

## INTRODUCTION

In a study by B. E. Meyer, N. A. Cole, and J. C. MacDonald (unpublished data), 3 lipid levels of

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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 in-

cubation 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-

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