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Ruminal Fermentation and Amino Acid Flow in Holstein Steers Fed Whole Cottonseed with Elevated Concentrations of Free Fatty Acids in the Oil

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ABSTRACT

The influence of feeding whole cottonseed (WCS) containing elevated concentrations of free fatty acids (FFA) in the oil on ruminal fermentation and amino acid (AA) flow to the abomasum was evaluated in a 4 × 4 Latin square trial. Four ruminally and abomasally cannulated Holstein steers were fed diets containing 12.5% of dry matter as WCS with concentrations of 8.0, 11.3, 14.7, or 18.0% FFA in the oil. Intake, ruminal digestibility, and flow to the abomasum of dry matter, organic matter, and acid detergent fiber were not affected by FFA level of WCS. Intake of neutral detergent fiber and total kilograms of neutral detergent fiber digested in the rumen were similar for all treatments. Ruminal neutral detergent fiber digestibility was lower for 8 and 14.7% FFA, resulting in a cubic effect on flow to the abomasum. Ruminal pH, molar proportions of isobutyrate, and total branched-chain volatile fatty acids (VFA) decreased linearly, whereas molar proportions of acetate and acetate:propionate ratio increased linearly as FFA in WCS increased. Total VFA were lower, and molar proportions of propionate were higher, for 8 and 14.7% FFA, resulting in a cubic effect. Intake of N, total N flow, and nonmicrobial N flow to the abomasum were similar among treatments. Flow of microbial N was lower for the 11.3% FFA treatment, resulting in a quadratic response. Only nonsignificant differences were observed in AA flow to the abomasum. Results of this trial indicate that WCS with FFA up to 18% may result in small changes in rumen fermentation.
(**Key words:** whole cottonseed, free fatty acid, ruminal fermentation, amino acid flow)

Abbreviation key: FA = fatty acid, **IADF** = indigestible ADF, **MN** = microbial N, **WCS** = whole cottonseed.

INTRODUCTION

In normal years, the concentration of FFA in the oil of whole cottonseed (**WCS**) rarely exceeds 12%. However, when tropical storms delay cotton harvest, the hot, humid conditions often lower the quality of cotton fiber and seed. Whole cottonseed with >12% FFA are considered to be off-quality as defined by the National Cottonseed Products Association (1997) and are typically sold as livestock feed. Limited data exist on the implications of feeding WCS with higher than normal concentrations of FFA, but recent research indicated that feeding WCS with up to 12% FFA in the oil to high-producing dairy cattle does not significantly alter nutrient intake, milk yield, or milk composition (Sullivan et al., 2004).

High levels of dietary unsaturated fatty acids (**FA**) can be toxic to certain rumen microorganisms and can coat fiber particles, preventing fibrolytic bacteria from attaching, which subsequently depresses fiber digestion (MacLeod and Buchanan-Smith, 1972; Eastridge and Firkins, 1991). Jenkins (1993) reported that the level of unsaturated FFA in the rumen might be the determining factor in disruption of normal rumen fermentation. Because oilseeds contain high concentrations of unsaturated FA, increased concentrations of FFA in WCS could negatively affect fiber digestion by increasing the rate of FFA release from the seed (Martinez et al., 1991; DePeters and Cant, 1992). Previous research has demonstrated that increasing the rate of FFA release in soybeans by extrusion reduced NDF and ADF digestibility in mixed culture in vitro ruminal fermentations (Reddy et al., 1994).

It is well documented that feeding oilseed, such as WCS, tends to reduce milk protein percentage (Anderson et al; 1979; Smith, et al., 1981; DePeters et al., 1985). This decrease has been attributed to reduced microbial protein synthesis, which is a consequence of replacing ruminally fermentable carbohydrates with fat that does not provide energy in support of microbial protein synthesis; reduced microbial efficiency caused by growth uncoupling; and/or reduced protozoal and fibrolytic bacterial populations (Dunkley et al., 1977). High FFA WCS could also negatively affect membrane

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function and alter protozoal and fibrolytic bacterial populations. The objective of this trial was to determine the effects of feeding WCS with elevated concentrations of FFA in the oil on reticuloruminal fermentation and flow of nutrients to the abomasum in cannulated Holstein steers.

MATERIALS AND METHODS

Two lots of WCS containing 8 or 12% FFA were obtained from WCS warehouses in South Georgia and transported to Dairy Cattle Research Center at The University of Georgia in Athens. The FFA content of the WCS containing 12% FFA in the oil was elevated by increasing the moisture content to 20% for 72 h before air drying to approximately 90% DM. The FFA concentration in the WCS was determined according to NCPA (1997).

Four 350-kg Holstein steers fitted with ruminal (10.2 cm; Bar Diamond, Inc., Parma, ID) and abomasal (2.5 cm) rubber cannulae were used in a 4×4 Latin squares to evaluate the effects of increasing FFA levels in WCS. Steers were cannulated and managed according to procedures approved by The University of Georgia Institutional Animal Care and Use Committee. Experimental periods were 14 d in length with 10 d for ration adjustment and 4 d for sample collection. Steers were housed in a tie stall barn and allowed to exercise for 2 h/d in a dry lot.

Diets were formulated (Table 1) as described by Sullivan et al. (2004). The 2 lots of WCS were blended to provide 8, 11.3, 14.7, and 18% FFA as outlined in Table 1. The experimental diets were fed as a TMR twice daily at 110% of the previous day's intake. The amount of TMR offered and refused was recorded daily, and nutrient intake was calculated for d 7 to 14 of each period. Samples of experimental diets and orts were collected daily on d 10 to 14 of each experimental period and composited by steer within period.

Ruminal and abomasal samples were collected on d 10 to 14 at 12-h intervals. The sampling schedule was advanced 3 h each day. Ruminal fluid was collected and strained through 4 layers of cheesecloth, analyzed for pH, and frozen for analysis. Rumen fluid samples were analyzed for VFA concentrations using a Varian 3400 gas chromatograph (Varian, Walnut Creek, CA). A composite ruminal fluid sample was formed by combining 25 mL from each collection time. Ruminal bacteria were isolated by centrifugation at $27{,}000 \times g$ using a KSB continuous flow system (Kendro Laboratory Products, Newton, CT). Bacterial isolates were lyophilized and stored for analyses. Abomasal samples were frozen for storage and later composited by steer within treatment and lyophilized.

Table 1. Ingredient and partial chemical composition of total mixed rations containing whole cottonseed (WCS) with increasing concentrations of free fatty acids (FFA) in the oil.

	FFA in oil of WCS			
	8%	11.3%	14.7%	18%
	(% of DM)			
Ingredient				
Wheat silage	44.9	44.9	44.9	44.9
Coarse ground corn	24.5	24.5	24.5	24.5
Soybean hulls	3.5	3.5	3.5	3.5
8% FFA WCS	12.5	8.3	4.2	—
18% FFA WCS	—	4.2	8.3	12.5
Soybean meal (48% CP)	9.2	9.2	9.2	9.2
Protein supplement[1]	3.8	3.8	3.8	3.8
Mineral premix[2]	2.1	2.1	2.1	2.1
Chemical composition				
DM, %	58.5	60.9	58.5	57.9
	(% of DM)			
CP	19.9	19.4	19.6	19.4
NDF	48.8	48.2	48.3	48.9
ADF	28.7	28.9	29.8	29.3
EE[3]	6.1	6.0	5.9	6.0
NE_L,[4] Mcal/kg of DM	1.7	1.7	1.7	1.7

[1]Protein supplement was composed of 60% menhaden fish meal and 40% distillers grains with solubles.

[2]Premix contained 34.4% CP from urea, 86.7% ash, 24.50% Ca, 3.68% P, 1.27% Mg, 0.08% K, 3.03% Na, 4.60% Cl, 0.31% S, 11.67 ppm Co, 665 ppm Cu, 4622 ppm Fe, 58 ppm I, 2039 ppm Mn, 14.69 ppm Se, 293,370 IU/kg vitamin A, 117,350 IU/kg vitamin D, and 1466 IU/kg vitamin E.

[3]Ether extract.

[4]Calculated using NRC (2000).

Diet, orts, and abomasal samples were ground to pass through a 1-mm screen using a Wiley mill (Authur H. Thomas, Philadelphia, PA) and analyzed for DM and ash (AOAC, 1984), NDF and ADF (Robertson and Van Soest, 1981), and total N (FP-528; Protein/Nitrogen Analyzer, Leco Corp. St. Joseph, MI). Ether extract of diet and ort samples was determined using Soxhlet extraction with petroleum ether (AOAC, 1984). Reticuloruminal nutrient digestibility and abomasal flow was calculated using indigestible ADF (**IADF**) as a marker (Henderson et al., 1985). The IADF of diet, ort, and abomasal samples was determined using a Daisy II 200 Rumen Fermentor (Ankom Technology Co., Fairport, NY).

Diet and abomasal samples were refluxed in 6 *N* HCl under 99.9% pure N_2 atmosphere for 24 h and then dried under reduced pressure (Amos et al., 1976). Hydrolysates were resuspended in a diluting buffer and analyzed for AA using a Beckman 1600 automated AA analyzer (Beckman Instruments, Inc., Fullerton, CA) with norleucine as an internal standard. Purine concentrations in the abomasal and bacterial isolate samples were determined using the procedure of Zinn and Owens (1986). The ratio of purines to microbial N (**MN**)

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