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Performance and Ruminal Fermentation of Dairy Cows Fed Whole Cottonseed with Elevated Concentrations of Free Fatty Acids in the Oil

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ABSTRACT

Twenty-four lactating Holstein cows were used in an 8-wk completely randomized design trial to examine the effects of feeding whole cottonseed (WCS) with elevated concentrations of free fatty acids (FFA) in the oil on intake and performance. Treatments included WCS with normal concentrations of FFA (6.8%, control) and 2 sources of WCS with elevated FFA [HFFA1 (24.1%) or HFFA2 (22.3%)]. The 2 sources of WCS with elevated FFA differed in that HFFA2 were discolored from being initially stored with excess moisture, which led to heating and deterioration during storage, whereas HFFA1 were normal in appearance and the increase in FFA occurred without heating and visible damage to the WCS. Nutrient concentrations were similar among WCS treatments, which provided 14% of the total dietary dry matter. Dry matter intake tended to be higher for cows fed HFFA2 compared with control and HFFA1. Yield of milk and components was similar among treatments, but milk fat percentage was lower for HFFA1 and HFFA2 compared with control. In a concurrent 3 × 3 Latin square trial with 6 ruminally canulated Holstein cows, molar proportions of isobutyrate were higher for HFFA2 than control and HFFA1, but no differences were observed in acetate or propionate. Results of these trials indicate that feeding WCS with high concentrations of FFA decreases milk fat percentage but does not alter dry matter intake, milk yield, or concentrations of other components. The minor changes in ruminal fermentation that were observed do not account for the decrease in milk fat percentage.
Key words: cottonseed, free fatty acid

INTRODUCTION

Whole cottonseed (**WCS**) is commonly used in rations for lactating dairy cows as a source of energy, fiber, and protein. The high oil content of WCS makes it an attractive energy-dense feed for animals with high energy requirements, such as lactating dairy cattle. The high fiber concentrations provided by the lint and the hulls are desirable for maintaining effective fiber levels in the diet.

Under certain conditions, WCS will have elevated concentrations of FFA in the oil. This typically occurs following a period of heavy rainfall from a tropical storm that delays harvest. The warm, humid conditions that normally persist following such a storm result in the enzymatic hydrolysis of fatty acids from the triglyceride, resulting in elevated FFA concentrations (Karon and Altschul, 1944). In other situations, the quality of the oil deteriorates during storage because of higher moisture concentrations in the WCS that lead to heating and enzymatic breakdown (Karon and Altschul, 1946). In extreme cases, the heat may be sufficient to reduce protein quality as well. As defined by the National Cottonseed Products Association (1997), WCS with greater than 12% FFA are considered to be off-quality and are typically sold as livestock feed. Sullivan et al. (2004) noted that feeding diets with WCS containing 12% FFA did not alter fiber digestibility, milk yield, or composition. When diets containing WCS with 18% FFA were fed to Holstein steers, increases in the molar proportion of acetate and acetate to propionate ratio were observed, but no negative impact on nutrient intake was observed (Sullivan et al., 2005). The potential effects of feeding diets containing WCS with even higher concentrations of FFA have not been examined. Feeding supplemental fats with elevated concentrations of FFA to cows was reported to reduce DMI and fiber digestibility (Eastridge and Firkins, 1991). Increased levels of unsaturated FFA in the rumen may have a toxic effect on the rumen microflora, resulting in changes in normal rumen fermentation patterns (Jenkins, 1993). This author also suggested that including high levels of polyunsaturated fatty acids in the diet could also physically coat feed particles, preventing cellulolytic bacterial attachment, which would also depress fiber digestibility. The oil in WCS is 70% unsaturated (Keele et al., 1989), and inclusion of high levels

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of WCS in the diet of lactating dairy cows (>15% of dietary DM) has been shown to negatively affect fiber digestion (DePeters and Cant, 1992). The objective of this study was to determine the effect of feeding WCS with elevated concentrations of FFA on nutrient intake, milk yield and composition, and ruminal fermentation of lactating dairy cows.

MATERIALS AND METHODS

Three sources of WCS differing in FFA concentrations were obtained from warehouses in South Georgia and transported to the University of Georgia Dairy Research Center in Tifton, Georgia. One source represented normal WCS with an average FFA content of 6.8% in the oil (control). Two additional sources of WCS with elevated concentrations of FFA in the oil were identified: 24.1 (**HFFA1**) or 22.3% (**HFFA2**). Both sources of WCS with elevated FFA contained similar concentrations of nutrients and FFA but differed in the reason for the FFA. The HFFA1 was normal in appearance and had been properly stored. The HFFA2 were discolored, indicating that those WCS had initially been stored with more moisture than desired, resulting in heating during storage. The HFFA2 had 2.2% bound nitrogen (AOAC, 1990) compared with 2.1% for control and HFFA1, indicating that the heating was not sufficient to alter nitrogen availability.

Production Trial

Twenty-four lactating Holstein cows were used in an 8-wk completely randomized design trial at the Dairy Research Center in Tifton, Georgia. All protocols were reviewed and approved by the University of Georgia Institute of Animal Care and Use Committee. The trial consisted of a 2-wk standardization period followed by a 6-wk experimental period. Cows were housed in a free stall barn and averaged 159 ± 97 DIM and 33.1 ± 4.8 kg/d of milk at the beginning of the trial. Cows were trained to eat behind Calan doors (American Calan Inc., Northwood, NH) before the beginning of the trial. All cows received the control diet (Table 1) during the 2-wk standardization period. Cows were individually fed a TMR once daily (0800) in amounts to provide approximately 10% orts for ad libitum consumption. At the end of the standardization period, cows were assigned randomly to 1 of the 3 treatments. Treatments included TMR containing control, HFFA1, or HFFA2 at approximately 14% of the ration DM (Table 1). Amounts of feed offered and refused were recorded daily. Cows were milked twice daily at approximately 0400 and 1500 h. Individual milk yield was recorded electronically (Alpro, DeLaval, Kansas City, MO) at each milking and summed for each day.

Table 1. Ingredient composition of experimental diets containing whole cottonseed with increasing concentrations of FFA in the oil

Ingredient	% of DM
Corn silage	39.87
Alfalfa hay	5.48
Wet brewers grains	12.38
Steam-flaked corn	12.96
Whole cottonseed	13.96
Concentrate[1]	15.35

[1]Concentrate contained (DM basis) 52.8% soybean meal, 48% CP; 13.2% calcium salts of long-chain fatty acids; 13.2% Prolak (H. J. Baker & Bro. Inc., Stamford, CT); 4.8% limestone; 2.6% dicalcium phosphate; 1.0% magnesium oxide; 1.8% white salt; 4.0% sodium bicarbonate; 0.8% potassium-magnesium-sulfate; 3.2% potassium carbonate; 1.6% yeast culture; and 1.6% trace mineral-vitamin premix.

Sample Collection and Analysis

Milk samples were collected from 2 consecutive p.m. and a.m. milkings each week. Samples were shipped to Dairy Farmers of America (Knoxville, TN) for analyses of fat, protein, lactose, SNF, and MUN concentrations (AOAC, 1990).

Samples of WCS, experimental diets, and orts were collected 3 times each week and dried in a forced-air oven at 55°C for 48 h. Samples were composited each week of the trial and ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Samples were shipped to Cumberland Valley Analytical Laboratories (Hagerstown, MD) for chemical analysis of DM (AOAC, 1990), CP (Leco FP-528 Nitrogen Analyzer, St. Joseph, MI), ADF (AOAC, 1990), NDF, and lignin (Van Soest et al., 1991), ash, and minerals (AOAC, 1990). An additional set of composite samples of WCS was shipped to MidContinent Laboratories Inc. (Jackson, MS) for analysis of moisture, oil, FFA, and protein according to the procedures outlined by National Cottonseed Products Association (1997).

Body weights were recorded on 2 consecutive days during the standardization period and wk 6 of the experimental period. To minimize variation, all BW were recorded after the p.m. milking and before animals had access to feed or water.

Ruminal Fermentation Trial

Six lactating Holstein cows previously fitted with ruminal cannulas were used in a concurrent replicated 3 × 3 Latin square design trial with 2-wk periods to evaluate the effect of FFA in WCS on ruminal fermentation. Cows were housed in a free stall barn and trained to eat behind Calan doors (American Calan Inc.) before beginning the trial. Cows averaged 282.5 ± 5.9 DIM and 21.7 ± 7.7 kg/d of milk at the beginning of the trial. Treatments and experimental diets were the same as

those used in the production trial. Feeding schedules and management were the same as those described for the production trial.

On the last day of the experimental period, ruminal fluid samples were collected at 0, 2, 4, 6, and 8 h postfeeding. Approximately 50 mL of ruminal fluid was collected and strained through 3 layers of cheesecloth. A 40-mL subsample was strained through 3 layers of cheesecloth and immediately mixed with 10 mL of metaphosphoric acid (25% wt/vol). The sample was frozen for later analyses of VFA (Erwin et al., 1961). These samples were later thawed and centrifuged at 10,000 $\times g$ for 10 min and the supernatant collected for VFA analysis using a Hewlett-Packard 2890A gas chromatograph (Hewlett-Packard Company, Avondale, PA) fitted with a nitroterephthalic acid modified polyethylene glycol megabore column (30 m $\times$ 0.53 mm i.d. with 1-μm film; J & W Scientific, Folsom, CA). Initial oven temperature was 130°C for 7 min, and helium flow was 7 mL/min. The oven temperature was increased at the rate of 2.9°C/min over 7 min to a final temperature of 150°C. Helium flow was increased to 9 mL/min. Airflow was 400 mL/min, and hydrogen flow was 29 mL/min. Heptanoic acid was used as an internal standard. The remaining sample was analyzed for pH.

Statistical Analysis

Data from the production trial were subjected to analysis of covariance using PROC MIXED procedures of SAS (2001). The model included covariate, treatment, week, treatment $\times$ week, and error. The corresponding data from standardization period were used as a covariate. Cow within treatment was included as a random effect and week as a repeated effect. Ruminal pH and VFA data were subjected to ANOVA using PROC MIXED procedures of SAS (2001). The model included cow, hour, period, treatment, hour $\times$ treatment, and error. Hour was included as a repeated effect. When a significant F-test was determined ($P < 0.10$), the PDIFF option was used for treatment mean separation.

RESULTS AND DISCUSSION

Chemical Composition of WCS and TMR

The chemical composition of WCS treatments is presented in Table 2. The DM content averaged 89.5% for all WCS treatments. The FFA content of the WCS averaged 6.8, 24.1, and 22.3% for control, HFFA1, and HFFA2. Both lots of WCS with elevated FFA had slightly higher concentrations of moisture and CP but lower concentrations of oil and ADF compared with control. The experimental diets were similar in nutrient content (Table 3).

Table 2. Chemical composition of whole cottonseed (WCS) differing in concentrations of FFA in the oil[1]

Item	Control	HFFA1[2]	HFFA2
Moisture,[3] %	9.4 ± 0.4	10.6 ± 0.5	11.9 ± 0.7
Oil,[3] % of DM	18.4 ± 0.6	17.1 ± 0.3	15.9 ± 0.8
FFA,[3] % of oil	6.8 ± 1.0	24.1 ± 2.0	22.3 ± 3.9
Protein,[3] % of DM	19.6 ± 0.4	20.7 ± 0.3	21.0 ± 0.4
	%		
DM	90.7 ± 0.7	89.4 ± 0.4	88.6 ± 0.9
	% of DM		
CP	20.2 ± 0.6	21.9 ± 0.4	22.5 ± 0.8
NDF	51.3 ± 2.4	50.1 ± 1.4	50.1 ± 1.9
ADF	42.1 ± 1.6	39.8 ± 0.6	39.6 ± 1.2
Ash	4.0 ± 0.4	4.2 ± 0.2	4.1 ± 0.4
NFC[4]	23.6 ± 2.8	22.3 ± 2.4	22.3 ± 2.8
Ca	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
P	0.57 ± 0.03	0.56 ± 0.01	0.59 ± 0.04
Mg	0.35 ± 0.01	0.36 ± 0.01	0.37 ± 0.02
K	1.14 ± 0.04	1.14 ± 0.04	1.16 ± 0.04
	ppm of DM		
Mn	16.8 ± 1.0	19.0 ± 0.09	17.5 ± 1.8
Zn	28.0 ± 2.8	30.8 ± 1.2	36.5 ± 2.3
Cu	5.7 ± 2.0	6.7 ± 2.3	7.7 ± 2.0

[1]All data are presented as mean ± SD.

[2]HFFA1 = WCS with 24.1% FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

[3]Results of National Cottonseed Products Association (1997) analysis.

[4]NFC = 100 − (ash + CP + EE + NDF).

Production Trial

Cows fed diets containing HFFA2 tended to have higher (P = 0.07) DMI compared with control and HFFA1 (Table 4). No differences were observed in DMI when lactating cows were fed diets with WCS containing 12% FFA or steers fed diets with WCS containing 18% FFA (Sullivan et al., 2004, 2005). Plascencia et al. (1999) reported increased DMI when feeding Holstein steers yellow grease with up to 42% FFA. Bock et al. (1991) also noted increased DMI in steers supple-

Table 3. Chemical composition of experimental diets containing whole cottonseed (WCS) with increasing concentrations of FFA in the oil[1]

Item	Control	HFFA1[2]	HFFA2
DM, %	44.6 ± 0.8	43.9 ± 0.9	44.4 ± 0.8
	% of DM		
CP	17.2 ± 0.6	18.8 ± 1.0	17.7 ± 1.2
NDF	32.6 ± 3.3	31.2 ± 3.3	32.1 ± 4.1
ADF	22.6 ± 2.8	21.6 ± 2.5	22.4 ± 3.1
Ash	7.1 ± 0.7	7.0 ± 1.2	6.7 ± 0.8
NFC[3]	40.1 ± 2.4	40.5 ± 3.8	40.5 ± 3.2

[1]All data are presented as mean ± SD.

[2]HFFA1 = WCS with 24.1% FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

[3]NFC = 100 − (ash + CP + ether extract + NDF).

Table 4. Dry matter intake, milk yield, and milk composition of cows fed diets containing whole cottonseed (WCS) with increasing concentrations of FFA in the oil

Item	Control	HFFA1[1]	HFFA2	SE	*P*-value
DMI, kg/d	21.6[d]	22.0[d]	23.5[c]	0.6	0.07
Milk, kg/d	35.0	34.0	35.1	1.0	0.39
Fat, %	4.22[a]	3.64[b]	3.58[b]	0.14	0.008
Fat, kg/d	1.38	1.25	1.29	0.05	0.11
Protein, %	3.15	3.08	3.06	0.05	0.34
Protein, kg/d	1.07	1.05	1.05	0.03	0.75
Lactose, %	4.71	4.80	4.73	0.04	0.54
Lactose, kg/d	1.67	1.64	1.65	0.04	0.78
SNF, %	8.74	8.67	8.64	0.07	0.63
SNF, kg/d	3.05	2.97	3.00	0.08	0.65
ECM, kg/d	36.5	34.5	35.7	1.0	0.24
MUN, mg/dL	8.86	11.11	9.39	0.78	0.19
Efficiency					
Milk/DMI	1.58	1.58	1.50	0.08	0.88
ECM/DMI	1.66	1.57	1.52	0.07	0.23

[a,b]Means in the same row with superscripts differ ($P < 0.01$).

[c,d]Means in the same row with superscripts differ ($P < 0.10$).

[1]HFFA1 = WCS with 24.1% FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

mented with soybean oil soapstocks with 50% FFA and tallow with 15% FFA at 3.5% of the diet.

Yields of milk fat and other milk components were similar among treatments (Table 4). However, milk fat percentage was lower ($P = 0.008$) for cows fed diets containing HFFA1 or HFFA2 compared with control: 4.22, 3.64, and 3.58% for control, HFFA1, and HFFA2, respectively. Concentrations of protein, lactose, and SNF were not affected by FFA in WCS. Because DMI and ECM were similar among treatments, efficiency of milk production was similar among all treatments.

These results are consistent with those reported by Bernard et al. (2007) in which cows fed diets containing WCS with 23.1 or 35.5% FFA produced similar quantities of milk with reduced milk fat percentage compared with those fed diets containing WCS with 10.7% FFA. In contrast with the results of the current trial, Sullivan et al. (2004) did not observe any change in milk fat percentage when cows were fed diets containing low-FFA WCS or WCS with 12% FFA.

Ruminal Fermentation Analysis

No interaction of treatment × sampling time was observed; therefore, treatment means across all sampling times are reported in Table 5. Ruminal pH was similar among all treatments of WCS. Total VFA concentrations tended to be slightly higher ($P = 0.09$) for HFFA2 compared with control or HFFA1. There were no differences among treatments for molar proportions of acetate or propionate, but molar proportions of butyrate tended to be higher ($P = 0.08$) and molar proportions of isobutyrate were higher ($P = 0.0004$) for HFFA2 compared with control and HFFA1.

Sullivan et al. (2005) noted a cubic response in total ruminal VFA concentrations when WCS containing 8, 11.3, 14.7, or 18% FFA were fed to Holstein steers. The highest concentration of total VFA was associated with the 11.3% FFA treatment, intermediate for 8 and 18% FFA, and lowest for 14.7% FFA. These authors also reported a linear decrease in molar proportions of isobutyrate as FFA concentrations in WCS increased. Decreased molar proportions of butyrate have been reported as dietary concentrations of FFA increase (Keele et al., 1989; Avila et al., 2000). Noftsger et al. (2003) reported increased concentrations of isobutyrate and valerate when fermentors were supplemented with 2-hydroxy-4(methylthio) butanoic acid. These authors suggested that the increase was due to reduced utiliza-

Table 5. Effect of increasing levels of FFA in the oil of whole cottonseed (WCS) on rumen VFA concentrations[1]

Item	Control	HFFA1[2]	HFFA2	SE	*P*-value
pH	6.07	6.14	6.21	0.06	0.28
Total VFA, m*M*	84.77[d]	85.88[d]	89.13[c]	1.44	0.09
	% Molar proportion				
Acetate (A)	60.51	59.82	59.56	0.50	0.38
Propionate (P)	24.82	24.96	24.62	0.50	0.89
Butyrate	10.16[d]	10.06[d]	10.60[c]	0.17	0.08
Isobutyrate	0.47[b]	0.52[b]	1.08[a]	0.11	0.0004
Isovalerate	2.06	2.16	2.05	0.05	0.22
Valerate	1.99	1.89	2.10	0.07	0.14
A:P	2.49	2.44	2.46	0.07	0.87

[a,b]Means in the same row with superscripts differ ($P < 0.01$).

[c,d]Means in the same row with superscripts differ ($P < 0.10$).

[1]Data represent least squares means across all sampling times. No interaction of treatment × sampling time ($P > 0.10$) was observed.

[2]HFFA1 = WCS with 24.1% FFA and normal color; HFFA2 = WCS with 22.3% FFA and discolored.

tion of these branched-chain VFA for synthesis of the corresponding AA because ammonia concentrations were similar across treatments. Milk urea nitrogen concentrations increased when cows were fed WCS with more than 30% FFA, suggesting increased degradation of protein provided by WCS (Bernard et al., 2007). Isobutyrate is produced when valine is catabolized, which is more likely the reason for the observed increase in the current trial. The HFFA2 had undergone heating during storage as evidence from the discoloration of the lint, which would potentially alter the degradability of the protein. The changes in rumen VFA concentrations observed in this trial do not indicate major changes in rumen microbial fermentation patterns that would explain the reduced milk fat percentage.

In general, oils and oilseeds with a high degree of unsaturation disturb rumen fermentation and fiber digestibility, leading to lower acetate production and milk fat synthesis (Coppock and Wilks, 1991). The oil in WCS is 70% unsaturated and is completely hydrolyzed to fatty acids in the rumen (Keele et al., 1989) normally, but when WCS are processed and the oil is readily available, ruminal fermentation is altered and milk fat is depressed (Bernard, 1999). However, ruminal fermentation analysis for this trial does not indicate a shift in VFA production that would account for the decreased milk fat percentage observed with HFFA1 and HFFA2. Sullivan et al. (2005) did not observe a change in FA profile of WCS oil containing 8 or 18% FFA, so reduced milk fat percentage is not likely due to changes in supply of dietary fatty acids that would alter the transfer of fatty acids into the mammary gland.

Increasing supplemental dietary fat has generally decreased de novo synthesis of fatty acids in the mammary gland (Grummer, 1991; Palmquist et al., 1993) because fatty acids from the supplemental dietary fat are incorporated directly into milk fat. Litherland et al. (2005) recently reported a decrease in milk fat percentage and short- and medium-chain milk fatty acid concentrations as the amount of highly unsaturated fatty acids from soy oil infused into the abomasum increased from 0 to 600 g/d.

Bauman and Griinari (2001) indicated that multiple factors can cause milk fat depressions related to nutrition including the production of specific *trans* fatty acids. These authors suggested that altered rumem microbial processes and the presence of unsaturated fatty acids alter the biohydrogenation of fatty acids, resulting in the production of *trans*-10, *cis*-12 conjugated linoleic acid, which reduces milk fat synthesis in the mammary gland. More recent work by this group has demonstrated a similar reduction in de novo synthesis and incorporation of dietary fatty acids into milk fat when cows were fed a high-concentrate, low-forage diet (Peterson et al., 2003). These researchers measured a corresponding reduction in mRNA for milk fat synthesis enzymes without any change in the concentrations of mRNA for enzymes required for milk protein synthesis. In our previous trial in which WCS with 12% FFA were fed, there was a slight reduction in the proportion of C4:0 to C12:0 FA and a slight increase in C14:0 to C17:0, but the proportion of long-chain fatty acids was similar (Sullivan et al., 2004). However, milk fat percentage was not decreased in that trial. Because milk fatty acids were not measured in the current trial, it is not possible to determine if there were changes in ruminal biohydrogenation that would explain the reduced milk fat percentage.

CONCLUSIONS

Feeding diets containing WCS with elevated FFA did not alter DMI, milk yield, or yield of milk components, but did reduce milk fat percentage. Ruminal fermentation was not altered by the increased concentration of FFA in WCS other than an increase in isobutyrate for HFFA2, which had undergone heating during storage. The reason(s) for the decline in milk fat percentage is not apparent from our results but appears to be related to changes in milk fat synthesis by the mammary gland.

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