Effect of replacing soybean meal protein with protein from upland cottonseed, Pima cottonseed, or extruded Pima cottonseed on production of lactating dairy cows¹

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ABSTRACT

Pima cotton production is increasing in the United States, but Pima cottonseed generally contains higher concentrations of the antinutritive pigment gossypol than conventional upland cottonseed. Heating promotes the reaction of gossypol with protein, reducing gossypol absorption and toxicity. The objective of this study was to assess the nutritional value for dairy cattle of Pima cottonseed cake (PCSC) that was heated and oil largely removed by an experimental extrusion process, compared with upland cottonseed (UCS) and Pima cottonseed (PCS). The PCS had greater crude protein (CP) and ether extract, less neutral detergent fiber (NDF) and acid detergent fiber (ADF), similar total gossypol, but higher (–)-gossypol isomer compared with UCS. Extrusion reduced lipid content by 73%, increased concentrations of CP, NDF, and ADF, and reduced total gossypol, (+)-gossypol, and (–)-gossypol in PCSC versus PCS. Forty lactating Holsteins (8 with ruminal cannulas) were blocked by days in milk into 5 squares in a replicated, incomplete 8 × 8 Latin square, and were fed diets containing, on a dry matter (DM) basis, 30% alfalfa silage, 31% corn silage, 21 to 25% high-moisture corn, and about 15% CP. Diets were fed as total mixed rations for ad libitum intake. Supplemental CP was from (1) solvent soybean meal (SSBM) only or 50% from SSBM plus 50% from (2) UCS, (3) PCS, (4) PCSC, (5) UCS plus PCS, and (6) UCS plus PCSC, or (7) 50% from expeller soybean meal (ESBM) plus 50% from PCS, and (8) 50% from ESBM plus 50% from PCSC. Periods were 4 wk long (total of 16

wk); production data were collected over the last 2 wk and blood and ruminal samples were taken on d 28 of each period. Data were analyzed using Proc Mixed of SAS (SAS Institute Inc., Cary, NC). Diet affected dry matter intake, with greatest intake on diet 6 and lowest intake on diets 1 and 3. The highest milk fat content was observed on diet 5 and the greatest fat yield on diet 7; fat content and yield were lowest on diet 1 (soybean meal control). Milk fat secretion was proportional to dietary fat content, indicating that cottonseed oil was used effectively for milk fat synthesis. We observed a trend for an effect on milk protein yield with the greatest protein secretion occurring on diet 7. Milk urea was lowest on diets 3, 7, and 8. Ruminal concentrations of branched-chain volatile fatty acids were lower, or tended to be lower, when PCSC replaced either UCS or PCS in the diet, suggesting reduced degradation and increased escape of PCSC protein. Among cottonseed-containing diets, total gossypol intake was lowest on PCSC, intermediate on PCS, and highest on UCS. Total gossypol and both (+)- and (–)-isomers of gossypol were higher in blood plasma on PCS and lower on PCSC than on the corresponding diets containing UCS, indicating that the extrusion process reduced gossypol absorption. In this trial, production on diets supplemented with UCS, PCS, or PCSC was comparable to that on diets containing soybean meal.

Key words: upland cottonseed, Pima cottonseed, extrusion, milk production

INTRODUCTION

Although still accounting for only 5.5% of total US ginnings, production of Pima cotton (*Gossypium barbadense*) increased by 69% in 2011 over 2010 compared with a 16% decline for upland cotton (*Gossypium hirsutum*; USDA-NASS, 2011). This greater production increased the supply of Pima cottonseed, which contains greater concentrations of CP and oil, but also generally higher concentrations of the antinutritive pigment gos-

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sympol, than upland cottonseed (Sullivan et al., 1993). Elevated gossypol concentration lowers the commercial value of Pima cottonseed, but biofuel production from Pima cottonseed oil would improve the economics of Pima cotton production. However, Pima cottonseed is often fed directly to livestock without removal of oil. One of the authors (T. M. K.) developed an extrusion process that removes 70 to 75% of the oil from cottonseed. The heat generated in this process could reduce total and free gossypol content, which would decrease gossypol absorption (Mena et al., 2001) and improve protein utilization by increasing the ruminal escape of cottonseed protein (Pena et al., 1986). Craig and Broderick (1981) found that optimal heating of cold-extracted cottonseed meal increased intestinally digestible RUP substantially more than it reduced nutritionally available Lys, thus increasing overall supply of MP and metabolizable Lys.

In commercial practice, cottonseed is fed to dairy cows to provide effective fiber as well as energy and protein and is used to replace a portion of other CP sources in the diet (Coppock et al., 1987). Cottonseed protein is lower in Lys, but higher in Met and total sulfur-AA (Met plus Cys) than soybean meal (**SBM**), thus making the 2 proteins complementary in AA pattern (NRC, 2001). Replacing 50% of RUP from SBM with RUP from cottonseed would reduce ratios of Lys:Met and Lys:(Met plus Cys) in supplemental protein from 4.4 and 2.1 to, respectively, 3.5 and 1.7, based on AA compositions reported in NRC (2001) tables. Cottonseed protein also is higher in His and Arg than soybean protein (NRC, 2001). Limited His content in microbial protein formed in the rumen may make this essential AA critical in circumstances where there is little RUP and microbial protein supplies nearly all of the MP (Vanhatalo et al., 1999).

Therefore, the objectives of this experiment were to determine whether (1) upland or Pima cottonseed could replace 50% of the CP from supplemental soybean meal; (2) extrusion of Pima cottonseed using a proprietary process to remove oil and heat-treat the protein would reduce blood gossypol concentrations when the extruded cake is fed to dairy cattle; and (3) feeding any of these 3 cottonseed sources to supplement soybean meal would improve milk production.

MATERIALS AND METHODS

Cottonseed Supplements

Conventional upland cottonseed (**UCS**) and Pima cottonseed (**PCS**) were obtained from Mesa Farmers Cooperative Gin (Vado, NM). About 6,000 kg of Pima cottonseed was processed using a modified Conex 5500

Extruder (Continental Agra Equipment, Newton, KS). This proprietary process heat-treated the PCS and removed 73% of the oil, exiting the extruder in the form of a Pima cottonseed cake (**PCSC**). Nine large containers totaling about 5,000 kg of each cottonseed material were shipped to the US Dairy Forage Center Research Farm in Prairie du Sac, Wisconsin. Two samples, taken from each container, were analyzed for total N (Leco 2000, Leco Instruments Inc., St. Joseph, MI) and for DM after 24 h at 105°C (method 967.03; AOAC, 1990) and results were used in formulating diets. Before mixing into the TMR, the PCS was cracked by rolling through a roller mill (Buffalo KwikCracker, Henke Farm Equipment, Norfolk, NE) with rollers set at 1.0 to 2.0 mm. The PCSC was processed through the same machine except with rollers set at about 2.5 mm. Mean compositions of the 3 cottonseed sources, based on analysis of samples collected during wk 3 and 4 of each period during the lactation trial, are reported in Table 1.

Thirty-two multiparous Holstein cows, 8 of which were fitted with permanent 10-cm ruminal cannulas (Bar Diamond Inc., Parma, ID) with mean (SD) 2.6 (0.9) parity, 91 (69) DIM, 46 (8) kg of milk/d, and 616 (70) kg of BW, plus 8 primiparous cows with mean (SD) 101 (50) DIM, 37 (4) kg of milk/d, and 510 (46) kg of BW were used in the trial. Cows were grouped into five 8 × 8 Latin squares to give 1 square of primiparous cows, 1 square of cannulated cows, and 3 squares of multiparous cows blocked by DIM. Cows were randomly assigned within squares to 1 of 5 dietary treatment sequences as described in the Statistical Analysis section. Diets were fed as TMR and contained alfalfa silage, corn silage, and high-moisture corn, solvent-extracted soybean meal (**SSBM**) or expeller soybean meal (**ESBM**; SoyPlus, West Central Coop, Ralston, IA), plus 1 or 2 different cottonseeds. Compositions of the other major feed ingredients are in Table 2. Thus, supplemental CP was provided from 8 sources: (1) all from SSBM, or 50% from SSBM plus 50% from (2) UCS, (3) PCS, or (4) PCSC; 50% from SSBM plus 25% from each of (5) UCS and PCS, or (6) UCS and PCSC; or 50% from ESBM plus 50% from (7) PCS or (8) PCSC. These combinations of supplements provided between 31 and 33% of total dietary CP. Composition of experimental diets actually fed during the trial (based on daily mean weights and weekly mean DM contents of each ingredient mixed in the TMR) are in Table 3. All cows were injected every other week with bST (500 mg of Posilac, Monsanto, St. Louis, MO) beginning about 60 DIM; injections were synchronized such that animals received a full dose on d 1, followed by subsequent bST doses at 14-d intervals throughout the trial. Cows were housed in tie stalls and had free access to water. The Animal Care

Table 1. Composition of cottonseed supplements¹

Component	UCS		PCS		PCSC	
	Mean	SEM	Mean	SEM	Mean	SEM
DM, %	93.4	0.5	93.6	0.3	94.9	0.1
CP, % of DM	20.2	1.3	21.5	0.1	27.7	0.2
OM, % of DM	95.9	0.1	95.2	0.1	94.1	0.1
Ether extract, % of DM	21.2	2.4	23.0	0.3	6.2	0.2
NDF, % of DM	48.8	1.3	44.6	0.5	46.6	0.7
ADF, % of DM	32.0	1.1	27.6	0.3	31.6	0.3
Total gossypol, µg/g of DM	8.75	1.26	8.83	0.16	6.55	0.23
(+)-Gossypol, µg/g of DM	5.05	0.72	4.09	0.08	3.05	0.11
(+)-Gossypol, % of total	57.7	<0.1	46.3	0.1	46.6	0.7
(-)-Gossypol, µg/g of DM	3.71	0.53	4.74	0.08	3.50	0.12
(-)-Gossypol, % of total	42.4	<0.1	53.6	0.1	53.5	0.1
NDIN, % of DM	11.4	1.0	12.1	0.3	9.7	0.4
ADIN, % of DM	7.2	0.5	7.5	0.3	6.9	0.2
B3, ² % of total N	4.2	0.7	4.6	0.6	2.8	0.3

¹UCS = upland cottonseed, PCS = Pima cottonseed, PCSC = Pima cottonseed cake (extruded).

²B3 = NDIN – ADIN.

and Use Committee of the College of Agricultural and Life Sciences of the University of Wisconsin-Madison approved all procedures involving the animals.

Each of the 4 experimental periods lasted 28 d and consisted of 14 d for adaptation after diet reversal and 14 d for collection of intake and production data. Diets were offered once daily at 1000 h; orts were collected and weights recorded at 0900 h. Feeding rate was adjusted daily to yield orts equivalent to about 5 to 10% of intake. The mean refusal rate observed over the trial was 8.1% of feed DM offered. Weekly composites of alfalfa silage, corn silage, high-moisture shelled corn, and the 8 different TMR and orts, were obtained from daily subsamples of about 0.5 kg of each material that were stored at –20°C. Weekly samples of soyhulls, SSBM, ESBM, UCS, PCS, and PCSC were also collected and

stored at room temperature. Dry matter was determined in weekly composites of corn silage, alfalfa silage, and high-moisture shelled corn by drying at 60°C for 48 h and in weekly samples of soyhulls, SSBM, ESBM, UCS, PCS, and PCSC by drying for 24 h at 105°C (method 967.03; AOAC, 1990). These DM contents were used to adjust DM composition of TMR over the trial. Intake of DM was computed based on 60°C DM determinations of weekly composites of TMR and orts. Dried (60°C) samples of corn silage, alfalfa silage and high-moisture shelled corn and undried samples of soyhulls, SSBM, ESBM, UCS, PCS and PCSC from wk 3 and 4 of each period (8 samples of each ingredient over the trial) were ground to pass a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). These were analyzed for total N, DM (method 967.03; AOAC, 1990), ash and

Table 2. Composition of principal dietary ingredients¹

Component	Alfalfa silage		Corn silage		HMSC		SSBM		ESBM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	42.1	4.3	31.4	0.8	73.6	0.9	90.0	0.4	91.5	0.8
CP, % of DM	21.0	0.9	6.5	1.1	7.7	0.6	52.3	1.5	45.8	2.0
OM, % of DM	90.2	0.8	95.8	0.3	98.6	0.2	92.5	0.4	93.2	0.3
NDF, % of DM	41.3	2.3	37.9	3.9	7.9	0.7	8.1	0.7	25.3	0.6
ADF, % of DM	31.8	2.2	20.2	2.2	1.9	0.1	4.8	0.4	8.0	0.5
NDIN, % of total N	1.9	0.3	2.3	0.3	2.1	0.4	0.5	0.1	29.4	0.5
ADIN, % of total N	0.8	0.1	0.7	0.1	0.1	0.2	0.2	0.0	2.4	0.4
B3, ² % of total N	1.1	0.3	1.6	0.2	2.0	0.3	0.4	0.1	27.0	0.6
NPN, % of total N	49.5	9.25	69.1	16.0	—	—	—	—	—	—
NH ₃ , % of total N	1.94	0.32	4.39	1.55	—	—	—	—	—	—
Total AA-N, ³ % of total N	22.0	3.3	18.4	5.4	—	—	—	—	—	—
pH	4.48	0.08	4.26	1.09	—	—	—	—	—	—

¹HMSC = high-moisture shelled corn, SSBM = solvent soybean meal, ESBM = expeller soybean meal (SoyPlus, West Central Coop, Ralston, IA).

²B3 = NDIN – ADIN.

³Computed assuming 40.3 µmol of total free AA/mg of N in alfalfa and corn silage (Broderick, 1987).

Table 3. Composition (% of DM unless otherwise noted) of experimental diets

Item	Supplemental protein ¹							
	SSBM	SSBM + UCS	SSBM + PCS	SSBM + PCSC	SSBM + UCS + PCS	SSBM + UCS + PCSC	ESBM + PCS	ESBM + PCSC
Ingredient,								
Alfalfa silage	29.9	29.8	29.9	30.0	29.8	29.9	29.8	30.0
Corn silage	30.8	30.7	30.8	30.9	30.7	30.8	30.7	30.9
High-moisture shelled corn	25.3	20.5	21.3	24.1	21.1	22.4	20.9	23.3
Solvent soybean meal	6.8	4.6	4.7	4.7	4.6	4.6	0	0
Expeller soybean meal	0	0	0	0	0	0	5.4	5.4
Upland cottonseed	0	11.9	0	0	5.9	5.9	0	0
Pima cottonseed ²	0	0	10.8	0	5.3	0	10.7	0
Pima cottonseed cake ³	0	0	0	7.9	0	3.9	0	7.9
Soyhulls	4.6	0	0	0	0	0	0	0
Calcium sulfate	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42
Biophos	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Sodium chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Magnesium oxide/sulfate	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53
Vitamins-trace minerals ⁴	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Composition								
CP	14.8	14.7	14.8	14.8	14.7	14.7	14.7	14.7
Soybean CP, % of total	31	16	17	17	16	16	17	17
Cottonseed CP, % of total	0	17	16	15	16	16	16	15
Ash	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.5
NDF	27.3	30.5	29.7	28.7	30.0	29.5	30.1	29.2
Fat	3.0	5.1	5.2	3.3	5.1	4.2	5.4	3.5
NFC	1.5	1.6	1.6	1.8	1.6	1.7	2.0	2.3
Total gossypol, g/kg of DM	0	1.04	0.95	0.52	0.98	0.77	0.94	0.52
NE _L , ⁵ Mcal/kg of DM	1.50	1.55	1.56	1.50	1.55	1.52	1.57	1.50
NE _L -allowable milk, ⁵ kg/d	42	44	44	42	44	43	45	42
MP-allowable milk, ⁵ kg/d	36	33	34	33	33	33	37	37
MP, ⁵ g/d	2,506	2,407	2,421	2,397	2,411	2,399	2,588	2,567
Lys:Met ratio in MP ⁵	3.6	3.5	3.5	3.5	3.5	3.5	3.6	3.5

¹Sources of supplemental CP: SSBM = solvent soybean meal, ESBM = expeller soybean meal (SoyPlus, West Central Coop, Ralston, IA), UCS = upland cottonseed, PCS = Pima cottonseed, PCSC = Pima cottonseed cake (extruded).

²Pima cottonseed was cracked by rolling before incorporation into the TMR.

³Pima cottonseed cake, prepared by experimental extrusion process, was rolled to reduce mean particle size before incorporation into the TMR.

⁴Provided (per kilogram of DM) 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6,440 IU of vitamin A, 2,000 IU of vitamin D, 16 IU of vitamin E, and 12 mg of monensin.

⁵Computed according to NRC (2001) model using mean observed DMI (26.3 kg/d) and measured fat contents of cottonseed materials, and assuming that the MP contribution from CP in PCS and PCSC was the same as that from UCS. This computation assumed no effect of gossypol on bioavailability of Lys.

OM (AOAC, 1980), sequentially for NDF, ADF, and ADIN using heat stable α -amylase and Na_2SO_3 (Van Soest et al., 1991; Hintz et al., 1996), and for NDIN omitting α -amylase and Na_2SO_3 during extraction (Licitra et al., 1996). The TMR samples collected during wk 4 of each period were also analyzed for indigestible ADF (ADF remaining after 288 h of in situ incubation) by the method of Huhtanen et al. (1994). The 8 samples each of UCS, PCS, and PCSC were analyzed for total lipids according to AOAC International (1997; method 920.39; Dairyland Laboratories, Arcadia, WI) and for gossypol [total gossypol, (+)-gossypol, and (–)-gossypol] by methods based on AOCS Recommended Practice Ba 8a-99 (AOCS, 1998) as modified by Pettigrew and Dowd (2011). Frozen composites of alfalfa and corn silages were thawed and analyzed for NPN (Muck, 1987; Leco FP-2000 N Analyzer).

Cows were milked twice daily at 0500 and 1700 h, and milk yield was recorded at each milking in all experimental periods. Milk samples from a.m. and p.m. milkings were collected on d 17 to 18 and 24 to 25 of each period and analyzed for fat, true protein, lactose, and SNF by infrared analysis (AgSource, Verona, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN) using AOAC (1990; method 972.16). For MUN determination, 5 mL of milk from each milking was treated with 5 mL of 25% (wt/vol) TCA. Samples were vortexed and allowed to stand for 30 min at room temperature (22–24°C) before filtering through Whatman #1 filter paper. Filtrates were stored at –20°C until MUN analysis by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow-injection (Lachat Quik-Chem 8000 FIA, Lachat Instruments, Milwaukee, WI). Concentrations and yields of fat, true

protein, lactose, and SNF and concentration of MUN were computed as weighted means based on a.m. and p.m. milk yields on each test-day. Yields of 3.5% FCM (Sklan et al., 1992) and ECM (Krause and Combs, 2003) were also computed. Efficiency of conversion of feed DM was calculated for each cow over the last 2 wk of each period by dividing mean yield of actual milk, and of FCM and ECM, by mean DMI. Apparent N efficiency (assuming no retention or mobilization of body N) was computed for each cow by dividing the period mean for milk N secretion (milk true protein/6.38) by mean N intake. For computation of BW change, BW was measured on 3 consecutive days at the beginning of the experiment and at the end of each period.

On d 28 of each period, blood samples were collected from a coccygeal vessel into evacuated heparinized tubes at approximately 4 h postfeeding and placed on ice until transported to the laboratory. Blood was centrifuged ($1,160 \times g$, 15 min, 4°C) and the resulting plasma was stored at -20°C until analyzed for total gossypol, (+)-gossypol, and (–)-gossypol as described for cottonseed samples except scaled to the lower concentrations found in plasma as described by Bullock et al. (2010). Spot urine and fecal samples were also collected on d 28 of each period at 6 h before and 6 h after feeding. Urine was immediately diluted by mixing 15 mL of each sample with 60 mL of 0.072 *N* H_2SO_4 and storing at -20°C until analysis. Fecal samples were dried for 72 h at 60°C , ground through a 1-mm screen (Wiley mill), and composited on an equal DM basis to obtain 1 fecal sample/cow per period. All fecal samples were analyzed for DM, ash, OM, NDF, ADF, total N, and indigestible ADF using the assays described above. Indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal output based on computations used by Cochran et al. (1986). Urine samples were thawed and analyzed for total N by elemental analysis (Leco FP-2000 N Analyzer), for urea using the colorimetric assay used for MUN, and for creatinine (Valadares et al., 1999). Daily urine volume and excretion of urea N and total N were estimated from mean urinary concentrations in each period assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

On d 27 to 28 of each period, about 100 to 200 mL of fluid digesta was collected from 4 locations in the ventral rumen at 0 (just before feeding), 1, 2, 4, 6, 8, 12, 18, and 24 h after feeding from the 8 lactating Holstein cows fitted with ruminal cannulas using the probe described by Olmos Colmenero and Broderick (2006). At each sampling, mixed fluid digesta was strained through 2 layers of cheesecloth and pH measured immediately in strained fluid using a glass

electrode. Two 10-mL aliquots of ruminal fluid were then preserved in scintillation vials by addition of 0.2 mL of 50% H_2SO_4 and stored at -20°C . The remaining fluid and digesta were returned to the rumen. Just before analysis, samples were thawed and centrifuged ($15,300 \times g$ for 20 min at 4°C) and flow-injection analyses (Lachat QuikChem 8000) applied to supernatants to determine ammonia, using a phenol-hypochlorite method (Lachat method 18-107-06-1-A), and total AA using a fluorimetric procedure based on the reaction with α -phthalaldehyde (Roth, 1971). Leucine was the standard in the α -phthalaldehyde assay and total AA values were reported in Leu equivalents. Samples were also thawed and centrifuged ($28,000 \times g$ for 30 min at 4°C) before VFA determination using a modification of the gas-liquid chromatography method for free fatty acids described in Supelco Bulletin 855B (Supelco Inc., Supelco Park, Bellefonte, PA) with flame-ionization detection. Standards or supernatants (0.5 or 1 μL) were injected onto a ZB-FFAP capillary column (30 m $\times$ 0.53 mm $\times$ 1.0 μm ; 7HK-G009-22; Phenomenex Inc., Torrance, CA) with helium carrier gas at 100 kPa and a flow rate of 20 mL/min. Column oven temperature was 100°C at injection; after 2 min, temperature was increased to 130°C at $10^{\circ}\text{C}/\text{min}$. Injector and detector temperatures were 230°C and 250°C . The method did not resolve isovalerate and 2-methyl butyrate.

Statistical Analysis

The lactation trial was conducted as an incomplete 8×8 Latin square, replicated 5 times. It was intended from the outset to run the trial for 4 periods only, rather than for the full 8 periods, to avoid confounding effects due to the decline in production that would have occurred later in the lactation curve. Five incomplete 8×8 Latin squares were constructed by fixing the first column of treatments, ordered A through H, as the diets fed in period 1, and then randomly assigning 3 of 7 possible columns of treatments (ordered B, C, ..., A; C, D, ..., B; ..., H, A, ..., G) as the subsequent series of diets in periods 2, 3, and 4. With this arrangement, columns represented periods and rows represented cows within squares. The 5 groups of cows, blocked as described above, were randomly assigned to 1 of these 5 incomplete 8×8 Latin squares. Within squares, cows were randomly assigned to 1 of the 8 diet sequences. Equal numbers of observations were made on all traits for each dietary treatment. Results from the lactation trial were analyzed using the mixed procedures of SAS Institute (1999–2000) for replicated Latin squares. The following model was used to fit production data to assess effects of dietary treatments:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl},$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $C_{k(i)}$ = effect of cow k (within square i), T_l = effect of dietary treatment l , ST_{il} = interaction of square i and treatment l , and E_{ijkl} = residual error. All terms were considered fixed, except for $C_{k(i)}$ and E_{ijkl} , which were considered random.

The following model was used for ruminal variables for which there were repeated measurements over time (pH, ammonia, total free AA, and individual and total VFA):

$$Y_{ijkl} = \mu + P_i + C_j + T_k + E_{ijk} + Z_l + ZT_{lk} + \omega_{ijkl},$$

where Y_{ijkl} = dependent variable, μ = overall mean, P_i = effect of period i , C_j = effect of cow j , T_k = effect of dietary treatment k , E_{ijk} = whole plot error, Z_l = effect of time l , ZT_{lk} = interaction of time l and treatment k , and ω_{ijkl} = subplot error. The spatial covariance structure SP(POW) was used for estimating covariance and the subject of the repeated measurements was defined as cow(period $\times$ treatment). All terms were considered fixed, except for C_j , E_{ijk} , and ω_{ijkl} , which were considered random. Differences between least squares means are reported only if the F -test for dietary treatment was significant at $\alpha = 0.05$. Trends were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Feed Quality and Diet Composition

Composition data of the 3 cottonseed sources fed in the trial are in Table 1. As is typically observed (Sullivan et al., 1993; Santos et al., 2002; 2005), PCS contained higher concentrations of CP and lipid and lower concentrations of NDF and ADF compared with UCS. However, the high-speed ginning process used to produce the PCS left more fiber in that material than was reported previously, which diluted the CP and ether extract. Sullivan et al. (1993) observed 6.1 and 3.1 percentage units more CP and ether extract, respectively, in PCS than UCS, whereas Santos et al. (2002) found 4.5 and 2.4 percentage units more CP and ether extract in PCS than UCS. The PCS and UCS fed in this trial had similar contents of total gossypol, which was unexpected. Although most reports indicated that Pima cottonseed has greater total gossypol than upland cottonseed (Sullivan et al., 1993; McCaughy et al., 2005; Santos et al., 2005), others have observed gossypol concentrations in some Pima

cultivars that were similar to those of upland cottonseed (Scheffler and Romano, 2008). In the current study, cottonseeds contained different proportions of the 2 gossypol isomers: UCS contained 57.7% (+)-gossypol, whereas PCS contained 53.6% (–)-gossypol. The experimental extrusion process reduced lipid content by 73% and total gossypol by 26% in PCSC compared with that present in the PCS starting material. Heat treatment of cottonseed products promotes reaction of free gossypol with Lys residues in cottonseed protein (Craig and Broderick, 1981), a process that may reduce gossypol absorption (Santos et al., 2005). Distribution of the gossypol isomers was the same in PCSC as in PCS. Despite the extrusion step, which presumably promoted reaction of gossypol with protein in PCSC, this material contained a lower proportion of total N as NDIN. Heating of proteins generally increases NDIN concentration and proportion of total N (e.g., Coblenz et al., 2010).

Contents of DM, CP, NDF, and ADF in alfalfa silage, corn silage, high-moisture corn, and the 2 soybean meals (Table 2) indicated that these feedstuffs were of typical composition (NRC, 2001). The NPN in alfalfa silage was about 50% of total N; NPN normally accounts for 45 to 55% of total N in alfalfa silage (Broderick, 1995). Very low contents of ammonia-N in both alfalfa and corn silages indicated that these feeds were well preserved (McDonald et al., 1991). The 8 diets ranged from (DM basis) 27.3 to 30.5% NDF, 1.50 to 1.56 estimated Mcal of NE_L /kg (computed at $3 \times$ maintenance; NRC, 2001), and 3.0 to 5.2% total fat (Table 3). Supplemental soybean meal plus cottonseed ingredients supplied between 31 and 33% of total dietary CP; cottonseed from UCS, PCS, or PCSC provided about 50% of this CP in the 7 experimental diets to which they were added. Replacement of soybean meal CP with CP from UCS and PCS increased NDF, total fat, and, because of the added fat, estimated Mcal of NE_L /kg (NRC, 2001); substitution of all or half of either of these cottonseed CP sources with PCSC decreased NDF and fat to concentrations similar to that of the SSBM control diet (Table 3). Based on NRC (2001) computations at the overall mean DMI (26.3 kg/d), MP-allowable milk ranged from 33 to 37 kg/d, whereas NE_L -allowable milk ranged from 42 to 44 kg/d (Table 3). Moreover, all diets contained 14.7 to 14.8% CP; Broderick et al. (2008) found that yield of milk and milk components on a diet with 14.8% CP was lower than that on 16.1 and 17.3% CP. This suggested that MP or AA supplies limited production in the current study. Thus, production differences between the SSBM control diet and the other experimental diets may be attributed to effects on MP supply from replacing SSBM protein with that from other protein sources.

Animal Responses

Lactation performance was relatively high in this experiment, with DMI, milk yield, and protein yield averaging, respectively, 26.3, 44.4, and 1.26 kg/d (Table 4). Significant effects ($P \leq 0.04$) of diet were observed on DMI, milk fat content and yield, milk protein content, and MUN concentration. Intake was lowest on the SSBM + PCS diet and greatest on the SSBM + UCS + PCSC diet, with the other treatments falling in between. Replacing SSBM CP with PCSC rather than PCS increased DMI by 1.4 kg/d, implying that the experimental extrusion process directly affected feed intake. However, extrusion reduced oil content and, thus, dietary NE_L from 1.54 to 1.50 Mcal/kg of DM in the SSBM + PCSC diet (Table 3), suggesting that this difference was due to the lower energy density stimulating intake (NRC, 2001). It was noted that DMI on the SSBM control was similar to that on SSBM + PCS, despite the lower fat and NE_L in that diet.

Milk fat content and yield were influenced by diet (Table 4), with the greatest fat concentration occurring when cows were fed UCS + PCSC and the greatest fat yield when cows were fed ESBM + PCS; these traits were lowest on the SSBM control diet, with the other treatments being intermediate. Feeding the 2 PCSC diets with fat and NE_L contents similar to the SSBM control (SSBM + PCSC and SSBM + UCS + PCSC) gave rise to milk fat content and yield that were not different from the SSBM control. These results confirm the many observations indicating that cottonseed oil is an effective energy source for lactation with few or no negative effects on milk fat synthesis. In their review, Coppock et al. (1987) summarized data showing that increased milk fat content and yield were the most commonly reported effects when whole cottonseed was fed to lactating cows. Mohamed et al. (1988) observed similar milk and component yields on 16.5% CP diets when supplemental protein came from either SSBM or cottonseed meal; however, replacing cottonseed meal with whole cottonseed increased fat and protein yields, whereas replacing SSBM with whole soybeans depressed yields of fat and protein. Mena et al. (2001) fed isonitrogenous diets supplemented with combinations of SSBM, cottonseed meal, and whole UCS and observed that milk fat yields were proportional to dietary fat intake coming from whole cottonseed. We observed trends for an effect of diet on ECM yield but, as expected, the ECM trend followed the pattern of fat yield, with the numerically highest yield on ESBM + PCS and the lowest on SSBM control.

Milk protein content was influenced by diet, being highest on SSBM + PCSC and the SSBM control and lowest on SSBM + UCS + PCS (Table 4). The reduc-

tion of milk protein from 2.95 to 2.83% when ESBM replaced SSBM in the PCSC diet was surprising. Milk protein content is less important than, and often inversely related to, protein yield (NRC, 2001), and trends for protein secretion displayed some interesting differences in this trial. The numerically greatest protein yield occurred on ESBM + PCS, the diet with the numerically highest milk and ECM yields; however, protein yield was numerically lowest on the other 2 diets containing PCS (SSBM + PCS and SSBM + UCS + PCS). This pattern suggested that RUP (from ESBM or possibly PCSC) may have improved protein utilization, but the lesser production on PCSC + ESBM suggested that the combination impaired protein secretion because of inadequate RDP supply. The large number of dietary treatments prevented all cows from receiving all 8 experimental diets in this study, which probably reduced trial sensitivity and may have prevented our detecting the numeric differences as statistically significant. It is noted that each diet followed every other diet 2 or 3 times over the course of the study, which would be expected to balance carryover effects across treatments.

Several researchers have reported high levels of milk production when cottonseed was fed to lactating dairy cows. Mohamed et al. (1988) observed similar milk yield but greater protein yield when dietary CP from soybeans or SSBM was replaced with equal CP from upland cottonseed or cottonseed meal. However, Brito and Broderick (2007) reported similar milk yield and a trend for reduced protein yield when all supplemental CP was fed as cottonseed meal rather than SSBM. Santos et al. (2002) found no difference in most milk production traits but higher milk protein content when cracked Pima cottonseed replaced 69% of dietary upland cottonseed in a large-scale trial conducted on commercial dairies using early- to mid-lactation cows. Prieto et al. (2003) fed lactating cows diets containing 12.8% whole cottonseed in which Pima replaced 0, 33, 67, and 100% of dietary upland cottonseed and found no effect on yield of milk and milk components until the diet contained 12.8% Pima cottonseed, at which point DMI was reduced.

Altered MUN was also observed in this trial, with concentrations being lowest on SSBM + PCS and the 2 diets containing ESBM, highest on SSBM + UCS + PCSC, and intermediate on the other 4 diets (Table 4). However, MUN concentrations were low in this trial, <10 mg/dL on all treatments, reflecting the relatively low CP diets that were fed (Nousiainen et al., 2004) and the relatively high N-efficiencies (milk-N:N-intake) observed in this trial (Broderick and Clayton, 1997). These results indicated that MP utilization was efficient and implied that this MP had an effective AA pattern. Estimates of the Lys:Met ratio in MP made using the

Table 4. Effects of source of supplemental protein on intake and production and on concentrations of gossypol isomers in blood plasma¹

Item	Supplemental protein								SE	Diet ² <i>P</i> > <i>F</i>
	SSBM	SSBM + UCS	SSBM + PCS	SSBM + PCSC	SSBM + UCS + PCS	SSBM + UCS + PCSC	ESBM + PCS	ESBM + PCSC		
DMI, kg/d	25.7 ^{de}	26.3 ^{bcd}	25.5 ^e	26.9 ^{ab}	26.0 ^{cde}	27.2 ^a	26.5 ^{bc}	26.3 ^{bcd}	0.42	<0.01
BW gain, kg/d	0.21	0.37	0.13	0.31	0.03	0.29	0.34	0.15	0.109	0.28
Milk, kg/d	43.7	43.8	43.1	44.4	44.3	45.7	45.9	43.8	1.19	0.11
Milk/DMI	1.70	1.66	1.69	1.65	1.70	1.68	1.72	1.67	0.036	0.46
ECM, kg/d	40.3	42.3	41.6	41.4	42.5	43.4	44.3	41.0	1.23	0.08
ECM/DMI	1.57	1.60	1.62	1.54	1.63	1.59	1.66	1.56	0.038	0.13
Fat, %	3.65 ^c	3.94 ^{ab}	4.03 ^a	3.75 ^{bc}	4.02 ^a	3.95 ^{ab}	3.92 ^{ab}	3.76 ^{bc}	0.108	0.02
Fat, kg/d	1.57 ^c	1.72 ^{ab}	1.72 ^{ab}	1.64 ^{bc}	1.77 ^{ab}	1.77 ^{ab}	1.81 ^a	1.66 ^{bc}	0.063	0.02
True protein, %	2.94 ^a	2.86 ^{abc}	2.87 ^{abc}	2.95 ^a	2.80 ^c	2.87 ^{abc}	2.85 ^{bc}	2.83 ^c	0.048	0.04
True protein, kg/d	1.26	1.24	1.22	1.29	1.22	1.28	1.31	1.23	0.033	0.10
Lactose, %	4.97	4.97	4.95	4.93	4.94	4.97	4.94	4.87	0.053	0.78
Lactose, kg/d	2.17	2.19	2.12	2.17	2.17	2.24	2.29	2.12	0.067	0.21
SNF, %	8.73	8.68	8.67	8.75	8.59	8.70	8.66	8.53	0.081	0.43
SNF, kg/d	3.79	3.80	3.70	3.83	3.76	3.90	3.99	3.72	0.105	0.18
MUN, mg/dL	9.1 ^{bc}	8.9 ^{cd}	8.9 ^{cd}	9.3 ^{ab}	9.1 ^{bc}	9.8 ^a	8.6 ^c	9.5 ^{ab}	0.30	<0.01
Milk-N/N-intake, %	32.2	31.1	31.3	32.0	31.1	31.4	32.4	31.2	0.61	0.47
Gossypol intake, g/d	0 ^f	27.4 ^a	24.5 ^c	14.0 ^e	25.5 ^b	20.8 ^d	25.1 ^{bc}	13.6 ^e	0.32	<0.01
Gossypol intake, mg/kg of BW per day	0 ^f	44.5 ^a	40.1 ^c	22.4 ^e	42.0 ^b	33.9 ^d	40.8 ^{bc}	22.1 ^e	0.76	<0.01
Blood plasma gossypol										
Total gossypol (period 1), ³ µg/mL	0.15	1.61	4.36	0.82	2.83	1.11	3.00	0.66	—	—
Total gossypol, µg/mL	0.45 ^e	1.40 ^c	3.26 ^a	0.79 ^d	2.48 ^b	1.32 ^c	2.75 ^b	0.74 ^d	0.106	<0.01
(–)-Gossypol, µg/mL	0.29 ^e	0.86 ^c	2.24 ^a	0.52 ^d	1.62 ^b	0.84 ^c	1.89 ^b	0.49 ^d	0.075	<0.01
(+)-Gossypol, µg/mL	0.16 ^e	0.54 ^c	1.02 ^a	0.26 ^d	0.86 ^b	0.48 ^c	0.86 ^b	0.25 ^d	0.033	<0.01
(+)-Gossypol/total, %	38 ^a	39 ^a	32 ^c	34 ^b	35 ^b	37 ^a	31 ^c	35 ^b	1.0	<0.01

^{a–e}Means within a row with different superscripts differ ($P < 0.05$).¹Sources of supplemental CP: SSBM = solvent soybean meal, ESBM = expeller soybean meal (SoyPlus, West Central Coop, Ralston, IA), UCS = upland cottonseed, PCS = cracked Pima cottonseed, PCSC = Pima cottonseed cake (extruded).²Probability of a significant effect of diet.³Mean total gossypol concentration in blood plasma at the end of period 1 (5 cows/treatment).

NRC (2001) model, which does not discount Lys bioavailability due to gossypol binding, ranged from 3.5 to 3.6 on all diets (Table 3). However, cottonseed protein is low in Lys and was estimated to contribute only 20 to 23 g/d of absorbable Lys, assuming 23% RUP with 80% intestinal digestibility (NRC, 2001). Ratios of Lys:Met would be reduced to 3.0 to 3.1 if none of the Lys in the cottonseed materials were bioavailable. We expected that replacing SSBM with ESBM would have given measurable improvement in milk and protein yield. Although the ESBM + PCS treatment gave rise to the greatest fat yield as well as numerically highest yields of milk, ECM, and protein, production was lower on ESBM + PCSC despite similar DMI. Applying NRC (2001) assumptions to estimate Met supply suggested that absorbable Met was increased only from 46 to 48 g/d when ESBM replaced SSBM in the diets containing PCS and PCSC. If absorbable Met were the limiting nutrient, then the extra 2 g/d of Met supply may have been too small to produce consistent differences in production.

We observed large, significant effects observed for concentrations of total gossypol, and (+)- and (-)-isomers of gossypol, in blood plasma (Table 4). Lowest concentrations occurred, as expected, on the SSBM control diet, which contained no gossypol. Among diets containing one of the cottonseed treatments, the highest concentrations of total gossypol and gossypol isomers were observed when PCS was fed: SSBM + PCS > ESBM + PCS = SSBM + UCS + PCS. Plasma gossypol was lower on the 2 diets containing UCS than on any PCS diet. Diluting half of the UCS with PCSC did not significantly alter plasma gossypol; however, the lowest gossypol concentrations other than control were observed when cows were fed diets supplemented with only PCSC plus either SSBM or ESBM. Circulating gossypol concentration generally reflects gossypol content of the diet (Mena et al., 2001) and this also held true for the 2 gossypol isomers. Cows fed diets containing UCS had greater proportions of the (+)-isomer in their blood plasma as was found in that source; PCS and PCSC-containing diets gave rise to lower plasma (+)-gossypol and higher (-)-gossypol, as was found in those cottonseed materials (Table 1).

An interesting observation in the current study was that, although total gossypol intake was lower on SSBM + PCS (24.5 g/d; 40 mg/kg of BW) than on SSBM + UCS (27.4 g/d; 45 mg/kg of BW), feeding the PCS containing diet resulted in substantially greater total gossypol concentrations in the blood plasma (Table 4). McCaughey et al. (2005) found that a mean increase from 18 to 22 g/d of total gossypol intake, resulting when 67% of dietary UCS was replaced with PCS, approximately doubled total gossypol in plasma. Santos

et al. (2002) fed cows 18.3 g/d of total gossypol as 10% dietary UCS, or 24.0 g/d of total gossypol as 3.1% UCS (contributing 5.5 g total gossypol) plus 6.9% PCS (contributing 18.5 g total gossypol) in the diet, and observed mean plasma gossypol concentrations of 3.1 and 7.8 µg/mL over the first 150 d of lactation. These researchers also reported substantially higher plasma gossypol concentrations in multiparous cows than in primiparous cows and observed that gossypol continued to increase over the whole 150-d sampling period (Santos et al., 2002). Much lower blood plasma gossypol concentrations were observed in our trial, with total gossypol intakes ranging from 14 to 27 g/d (Table 4), but blood samples were collected after only 28 d on each experimental diet. Mena et al. (2001) reported that plasma gossypol reached a plateau after 35 d in cows consuming 22 to 25 g/d of free gossypol, results that were at variance with those of Santos et al. (2002). In a follow-up study, Mena et al. (2004) observed that, at gossypol intakes similar to their earlier trial, plasma gossypol reached a plateau after about 28 d on treatment; plasma gossypol decreased by 87%, from 4 to 0.5 µg/mL, after cows were withdrawn for 28 d from the highest gossypol intake (42 g/d of total gossypol, 21 g/d of free gossypol). Dowd and Calhoun (1996) found that the decline of gossypol concentrations in blood plasma and several tissues in lambs fed gossypol was described by 2 first-order rates; plasma total gossypol decreased by 75%, from 8 to about 2 µg/mL in 15 d, and then declined more slowly. Low plasma gossypol on the SSBM control in the present experiment reflected carryover from previous periods of the Latin square. Mean total gossypol concentration measured after period 1 (in which cows consumed no cottonseed material) was 0.15 µg/mL; plasma total gossypol on this treatment averaged 0.45 µg/mL over the whole trial (Table 4). However, total plasma gossypol concentrations at the end of period 1 were very similar to the overall means for the other 7 treatments (Table 4). The overall mean gossypol concentration on the 7 cottonseed-containing diets was 1.82 µg/mL. If we assume that this represents the mean plasma gossypol concentration before switching to the SSBM control treatment, which had mean 0.45 µg/mL, then total gossypol declined approximately 75% in 28 d, implying a slower rate of turnover of plasma gossypol than was reported by Mena et al. (2004). This also suggested that plasma gossypol remaining in cows fed the SSBM control was influenced at least in part by the second, slower turnover pool detected by Dowd and Calhoun (1996). High levels of feed intake and milk production indicated that, under the conditions of our short-term reversal trial, there were no adverse animal effects of gossypol. It is noted that Mena et al. (2004) reported

that, compared with a control with zero gossypol, red blood cell fragility was increased at any concentration of blood gossypol, ranging from 0.8 to 3.6 µg/mL of plasma; however, those researchers also observed little change in serum enzymes indicative of impaired liver, kidney, and muscle cell viability.

Excretion and Nutrient Digestibility

Fecal and urinary N excretion and nutrient digestibility, estimated using internal markers, are reported in Table 5. Fecal N-excretion ranged from 183 to 216 g/d and was influenced by diet ($P = 0.02$); however, there did not appear to be a specific pattern attributable to the source of supplemental CP. For example, the SSBM control and SSBM + PCSC diets both gave rise to about equal fecal N-excretion. Although urine volume was affected by treatment ($P = 0.02$), excretion of urea-N and total N, as well as proportion of urea-N in total-N excretion, were not affected by source of supplemental CP ($P \geq 0.21$). Reducing urinary urea-N excretion is important because it is the form most rapidly degraded to ammonia and then volatilized to the environment (Van Horn et al., 1994). The relatively high N-efficiencies (Table 4) observed in this trial were reflected in low proportions of urea-N, which ranged from 47 to 58% of total urinary N (Table 5). Olmos Colmenero and Broderick (2006) reported that urea increased from 55 to 82% of total urinary N when dietary CP increased from 13.5 to 19.4% CP. In the current study, apparent digestibility

of DM, OM, NDF, and ADF were also not affected by dietary source of supplemental CP. Overall mean NDF and ADF digestibility (about 48%) were comparable to those observed earlier on diets containing 60% total forage from similar proportions of alfalfa and corn silages (Chen et al., 2011). Similar fiber digestibility on diets with equivalent NDF and ADF from UCS, PCS, or PCSC indicated no advantage for upland versus Pima cottonseed in this trial. However, apparent N digestibility ranged from 65% (SSBM control and ESBM + PCSC) to 70% (SSBM + PCS). Differences in DMI (Table 4) may have partly influenced observed apparent N digestibilities; thus, true N digestibility was computed using the NRC (2001) estimate of 4.8 g of metabolic fecal N/kg of DMI (Table 5). A range of 5 percentage units was still observed after this adjustment. Among the 4 diets supplemented with SSBM or SSBM plus a single cottonseed, estimated true N digestibility was greater on SSBM + PCS, suggesting that N digestibility of PCS may have exceeded that of SSBM, UCS, and PCSC. It was noted that estimated true N digestibility on diets containing SSBM plus 2 different cottonseed materials was not different from that on SSBM + PCS, and that estimated true N digestibility on the 2 diets containing ESBM were not different from the SSBM control.

Ruminal Metabolites

Effects of experimental diets on mean concentrations of metabolites in the rumen are given in Table 6. We

Table 5. Effects of source of supplemental protein on excretion and apparent digestibility¹

Item	Supplemental protein								SE	Diet ² <i>P</i> > F
	SSBM	SSBM + UCS	SSBM + PCS	SSBM + PCSC	SSBM + UCS + PCS	SSBM + UCS + PCSC	ESBM + PCS	ESBM + PCSC		
Excretion ³										
Fecal-N, g/d	214.2 ^a	210.9 ^{ab}	182.9 ^c	216.1 ^a	194.2 ^{bc}	202.9 ^{abc}	212.2 ^{ab}	212.6 ^{ab}	8.17	0.02
Urine volume, L/d	26.4 ^b	26.0 ^b	30.7 ^a	26.7 ^{ab}	29.6 ^{ab}	25.0 ^b	29.6 ^{ab}	29.3 ^{ab}	1.48	0.02
Urinary urea-N, g/d	75.9	89.2	90.7	86.3	93.0	91.5	89.7	83.0	5.03	0.21
Total urinary-N, g/d	164.6	162.2	181.6	168.9	172.9	168.4	172.6	175.6	6.41	0.43
Urea-N/total-N, %	47.2	58.2	51.8	51.5	55.0	54.2	52.9	49.5	2.95	0.22
Digestibility, %										
Apparent DM	68.9	70.5	71.9	69.6	70.2	70.8	69.2	69.8	0.84	0.21
Apparent OM	70.5	72.0	73.2	71.1	71.7	72.4	70.7	71.3	0.79	0.21
Apparent NDF	47.0	51.2	49.7	47.4	47.6	48.7	47.7	48.3	1.32	0.26
Apparent ADF	47.5	50.7	48.8	47.3	46.1	48.0	45.5	47.1	1.32	0.14
Apparent N	64.9 ^c	66.4 ^{bc}	70.1 ^a	66.0 ^{bc}	68.3 ^{ab}	67.6 ^{abc}	66.0 ^{bc}	64.9 ^c	1.12	0.01
Estimated true N ⁴	85.2 ^d	86.8 ^{bcd}	90.4 ^a	86.3 ^{bcd}	88.7 ^{ab}	88.0 ^{abc}	86.4 ^{bcd}	85.3 ^d	0.70	<0.01

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹Sources of supplemental CP: SSBM = solvent soybean meal, ESBM = expeller soybean meal (SoyPlus, West Central Coop, Ralston, IA), UCS = upland cottonseed, PCS = cracked Pima cottonseed, PCSC = Pima cottonseed cake (extruded).

²Probability of a significant effect of diet.

³Fecal excretion estimated using indigestible ADF as an internal marker (Cochran et al., 1986). Urine volume and urinary N excretion estimated using creatinine as an internal marker assuming 29 mg of creatinine excreted/kg of BW per day (Valadares et al., 1999).

⁴True N digestibility estimated assuming 4.8 g of metabolic fecal N excreted/kg of DMI (NRC, 2001).

Table 6. Effects of source of supplemental protein on ruminal pH and metabolite concentration¹

Item	Supplemental protein								SE	Diet ² <i>P</i> > <i>F</i>
	SSBM	SSBM + UCS	SSBM + PCS	SSBM + PCSC	SSBM + UCS + PCS	SSBM + UCS + PCSC	ESBM + PCS	ESBM + PCSC		
pH	6.68	6.70	6.73	6.71	6.68	6.65	6.67	6.56	0.036	0.12
Ammonia-N, mg/dL	3.9	4.4	4.1	4.3	4.5	4.9	5.0	3.9	0.45	0.40
Total AA, mM	2.5	2.3	2.4	2.5	2.6	2.3	3.0	2.3	0.34	0.73
Total VFA, mM	114.6	114.5	115.1	109.9	115.0	113.9	110.2	117.3	3.10	0.51
Acetate, mM	69.0	69.7	69.9	67.9	69.6	69.7	68.1	70.6	1.62	0.91
Propionate, mM	26.6	26.7	26.7	24.1	27.2	26.3	24.8	27.8	1.20	0.20
Ac:Pr ratio	2.68	2.66	2.72	2.93	2.66	2.75	2.82	2.60	0.116	0.27
Butyrate, mM	13.8	12.5	12.7	12.8	12.8	12.5	12.1	13.5	0.72	0.63
Isobutyrate, mM	1.25	1.32	1.32	1.18	1.27	1.28	1.24	1.22	0.044	0.09
Isovalerate + 2-methyl butyrate, mM	1.93	2.40	2.34	2.14	2.24	2.25	2.27	2.02	0.179	0.07
BCVFA, ³ mM	3.18 ^b	3.71 ^a	3.67 ^a	3.32 ^b	3.51 ^{ab}	3.53 ^{ab}	3.51 ^{ab}	3.24 ^b	0.210	0.04
Valerate, mM	2.04	1.95	1.92	1.82	1.84	1.95	1.80	2.14	0.075	0.08

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Sources of supplemental CP: SSBM = solvent soybean meal, ESBM = expeller soybean meal (SoyPlus, West Central Coop, Ralston, IA), UCS = upland cottonseed, PCS = cracked Pima cottonseed, PCSC = Pima cottonseed cake (extruded).

²Probability of a significant effect of diet.

³Branched-chain VFA (isobutyrate and isovalerate + 2-methyl butyrate).

found no significant effects on most traits, with average pH of 6.67, and typical mean concentrations of total VFA (114 mM), acetate (69 mM), propionate (26 mM), and butyrate (13 mM), and acetate:propionate ratio (2.7). Low ruminal ammonia and total AA reflected the low CP diets fed in this study. However, we did observe trends ($P \leq 0.09$) for dietary effects on ruminal concentrations of isobutyrate and isovalerate + 2-methylbutyrate, and we detected significant effects of diet on the small differences in total branched-chain VFA (BCVFA). Lowest BCVFA were observed on the SSBM control and on the 2 diets in which 50% of supplemental CP was provided by PCSC. Highest BCVFA were found on SSBM + UCS and SSBM + PCS, with the other diets being intermediate. The BCVFA are formed from microbial catabolism of the branched-chain AA, and reduced concentrations of BCVFA reflect reduced ruminal protein degradation (Van Soest, 1994). Our results suggested that the experimental extrusion process decreased ruminal degradation of protein in the PCSC supplement. That BCVFA were not different due to soybean meal source on the PCSC diets (SSBM + PCSC vs. ESBM + PCSC) suggested that the lack of positive response with addition of PCSC to ESBM was not caused by inadequate RDP supply. Pena et al. (1986) found that extruding UCS (maximum temperature = 150°C, decreasing to 95°C in 30 s and to ambient temperature in 5–10 min) also decreased ruminal BCVFA and increased duodenal flow of nonammonia N by 20% in cannulated dairy cows. Tagari et al. (1986) and Stutts et al. (1988) both reported decreased ruminal

degradation of protein in extruded whole cottonseed as assessed by in situ methodology.

CONCLUSIONS

A lactation trial was conducted to assess the nutritional value for dairy cattle of UCS, PCS, and PCSC (i.e., PCS processed by an experimental extrusion process) as supplements to soybean meal. Extrusion reduced fat content by 73%, increased concentrations of CP, NDF, and ADF, and reduced concentration of total gossypol, (+)-gossypol, and (–)-gossypol in PCSC versus PCS. Replacing half of the supplemental dietary CP from SSBM with CP from UCS, PCS, PCSC, UCS plus PCS, or UCS plus PCSC, or replacing SSBM with CP from ESBM plus PCS or ESBM plus PCSC influenced DMI, and intake generally increased with addition of cottonseed materials. Feeding the greatest amounts of cottonseed oil increased milk fat content and yield in this trial. Milk protein content was also influenced by dietary treatment. The greatest fat yield and the numerically highest yields of milk, ECM, and milk protein occurred on the diet supplemented with ESBM plus PCS. Reduced concentrations of BCVFA in the rumen suggested reduced degradation of PCSC protein. Concentrations of total gossypol, and both (+)- and (–)-isomers of gossypol, were higher in blood plasma on PCS, and lower on PCSC, than on corresponding diets containing UCS, indicating that the extrusion process reduced gossypol absorption. In this trial, production of milk and milk components on diets

supplemented with UCS, PCS, or PCSC was comparable to that on diets containing supplemental CP as SSBM alone. The extrusion process appeared to reduce gossypol absorption from PCS and may have increased ruminal protein escape.

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