The Effects of Varying Gossypol Intake from Whole Cottonseed and Cottonseed Meal on Lactation and Blood Parameters in Lactating Dairy Cows

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ABSTRACT

Effects of varying amounts of gossypol from whole Upland cottonseed (WCS) and cottonseed meal (CSM) were evaluated in 40 midlactation Holstein cows. After 14 d of pretreatment, cows were assigned to 1 of the 5 treatments for 84 d: control (no gossypol), 931 mg/kg total gossypol (TG) and 850 mg/kg free gossypol (FG) from WCS (moderate TG and high FG); 924 mg/kg TG and 91 mg/kg FG from CSM (moderate TG and low FG), 945 mg/kg TG and 479 mg/kg FG with equal amounts of TG from WCS and CSM (moderate TG and FG), or 1894 mg/kg TG and 960 mg/kg FG with equal amounts of TG from WCS and CSM (high TG and FG). Concentrations of plasma gossypol (PG) and its isomers were directly proportional to FG intake. Concentrations of PG reached a plateau after 28 d on treatment, and they were highest in cows receiving a diet with high TG and FG. Erythrocyte fragility differed among treatments and increased with increasing FG intake. Plasma gossypol returned to negligible concentrations 28 d after withdrawal of cottonseed products from the high TG and FG diet. Serum vitamin A was similar among treatments, but vitamin E increased with increasing FG intake. Serum enzymes were generally unaffected by treatments, but urea N increased in diets higher in TG and FG. Intake of dry matter was higher for the diet high in TG and FG than for the control diet, but was similar for other treatments. Cows receiving the high TG and FG diet produced more milk and 3.5% fat-corrected milk, with no changes in milk composition. Feeding a diet containing 1894 mg/kg TG and 960 mg/kg FG for 84 d increased PG concentrations and erythrocyte fragility and resulted in minor changes in blood metabo-

lites and enzymes, but no detrimental effect on lactation performance was observed. Indicators of liver, kidney, and muscle cell viability suggest that the higher amounts of gossypol consumed in this study had only minor effects on those tissues in lactating dairy cows. (**Key words:** gossypol, whole cottonseed, cottonseed meal, dairy cow)

Abbreviation key: BG = bound gossypol, CSM = cottonseed meal, FG = free gossypol, PG = plasma gossypol, TG = total gossypol, WCS = whole cottonseed.

INTRODUCTION

Whole cottonseed (WCS) is a product of the cotton fiber industry that is extensively used as an energy and protein source in dairy cattle diets. Cottonseed meal (CSM) is a product of the oil extraction from WCS and is used as a protein supplement in diets for ruminants. Both WCS and CSM contain gossypol, a yellow polyphenolic compound found primarily in the pigment glands of the cotton plant. Gossypol exists in the free and bound forms. In the intact whole seed, gossypol is mostly found in the free form. However, when cottonseed is processed, gossypol binds to proteins, possibly to the epsilon-amino group of lysine (Calhoun et al., 1995). When in the bound form, gossypol is considered nontoxic to ruminants because it cannot be absorbed in the digestive tract. However, some of the gossypol that is bound may be released as free gossypol (FG) during digestion, which then can be absorbed by the digestive tract. This phenomenon has been suggested with bound gossypol (BG) from processed WCS (Noftsker et al., 2000) and CSM (Wan et al., 1995; Blackwelder et al., 1998). In addition to the free and bound forms, 2 distinct stereoisomers of gossypol occur in WCS and CSM, the plus (+) and the minus (–) isomer.

In cows producing >40 kg of milk/d, feeding diets with 12% WCS and 16% CSM did not adversely affect lactation performance but did increase plasma gossypol (PG) concentrations (Blackwelder et al., 1998). Similar findings were reported by Mena et al. (2001), in which

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Table 1. Ingredient and nutrient composition of diets.

	Diet ¹				
	A	B	C	D	E
Ingredient	(% of DM)				
Alfalfa hay	48.0	52.0	45.5	48.7	38.0
Steam-flaked corn	43.5	31.5	44.5	38.0	38.5
Soybean meal	5.5	0.0	0.5	0.5	0.0
Whole cottonseed	0.0	13.5	0.0	6.8	13.5
Cottonseed meal	0.0	0.0	7.0	3.5	7.0
Buffer ²	1.2	1.2	1.2	1.2	1.2
Vitamins and minerals ³	1.8	1.8	1.8	1.8	1.8
Nutrient composition					
DM, %	91.35	91.97	91.58	91.44	92.13
	DM basis				
NE _L , ⁴ Mcal/kg	1.63	1.62	1.62	1.62	1.67
OM, %	91.17	91.97	91.58	91.44	92.13
CP, %	16.42	16.28	15.43	14.84	16.64
RUP, ⁴ %	5.30	4.20	5.70	4.90	5.60
Fat, %	3.39	5.61	3.37	4.49	5.59
NDF, %	41.59	42.30	40.28	38.90	40.31
ADF, %	24.64	31.39	27.53	25.29	30.16
Total gossypol, mg/kg	0.00	931.50	924.00	944.70	1894.30
Free gossypol, mg/kg	0.00	850.50	91.00	479.30	960.40
Bound gossypol, mg/kg	0.00	81.00	833.00	465.40	933.90
Iron, mg/kg	245.00	223.00	252.00	351.00	185.00

¹A = Control, no gossypol; B = 950 mg/kg total gossypol (TG) from whole cottonseed (WCS); C = 950 mg/kg TG from cottonseed meal (CSM); D = 950 mg/kg TG, one-half from WCS and one-half from CSM; and E = 1900 mg/kg TG, one-half from WCS and one-half from CSM.

²66% NaCO₃ and 34% MgO.

³Composition: niacin, 1.6%; Zinpro 100 (Zinpro Corp., Eden Prairie, MN), 0.81%; Ca, 7.8%; P, 6.9%, Na, 4.3%; Cl, 6.5%; S, 4.1%; and (per kg) 800 mg of Zn, 1600 mg of Mn, 30 mg of I, 600 mg of Cu, 10 mg of Co, 10 mg of Se, 67,000 IU of vitamin A, 700 IU of vitamin E, and 6700 IU of vitamin D.

⁴Calculated from NRC (2001) and adjusted for DMI for each treatment diet.

the diet highest in cottonseed improved milk yield in dairy cows, but resulted in higher concentration of PG. A primary concern associated with feeding large amounts of WCS is the possibility of gossypol toxicity (Calhoun et al., 1995; Arieli, 1998). Ruminants with a well-developed ruminal microbial population are able to detoxify gossypol by converting the FG to bound gossypol within the rumen, thereby impeding its absorption into the blood (Calhoun et al., 1995). However, it is possible that feeding excessive amounts of gossypol in the free form may exceed this protective mechanism and impair animal performance.

In a short-term study, PG concentrations were more correlated with FG than with total gossypol (TG) intake (Mena et al., 2001). Feeding recommendations for cottonseed products have been based on nutrient content, but only a few studies have evaluated the effects of FG and TG on PG concentrations, blood parameters, and lactation performance in dairy cows. Barraza et al. (1991) fed lactating cows diets containing different gossypol concentrations from WCS during an 8-wk period and found negative effects on lactation or blood parameters. Lindsey et al. (1980) fed lactating cows high amounts of FG from CSM during a 14-wk study and

observed only a depression in hemoglobin and elevation in plasma total protein of cows. In a recent large field trial, feeding a blend of WCS and cracked Pima cottonseed increased PG concentrations, but it did not affect lactation (Santos et al., 2002) or health (Santos et al., 2003) of dairy cows. We demonstrated that 42 d of feeding varying amounts of gossypol from WCS and CSM had minor effects on lactation performance despite changes in PG concentrations and osmotic fragility of erythrocytes (Mena et al., 2001). In that study, 4 to 5 wk of gossypol feeding raised PG concentrations to a plateau in midlactation cows. However, the short-term duration of the study might have negated possible negative effects of gossypol intake on performance of dairy cows. Because PG reached a plateau after 4 to 5 wk of feeding and because of the cumulative nature of gossypol in tissues (Velasquez-Pereira et al., 2002), the 42-d study might not have been long enough to detect possible negative effects of gossypol accumulation on blood parameters and lactation performance.

The objective of this study was to determine the effect of feeding varying amounts of TG and FG from linted Upland WCS, CSM, or a combination of both feeds on PG concentrations, erythrocyte fragility, certain blood

parameters, and lactation performance in lactating dairy cows for an extended period of time. We hypothesized that PG concentrations would reach a plateau at 4 to 5 wk of feeding (Mena et al., 2001), and doubling the study period was expected to be sufficient for cows to display signs of gossypol toxicosis or to suppress performance if the levels fed were indeed detrimental to lactating cows.

MATERIALS AND METHODS

Animals and Feeding

Forty multiparous Holstein cows in midlactation (127 DIM and 31.8 kg/d milk yield at the beginning of the study) were selected from the University of Arizona Dairy Research Center for an 84-d experiment in which varying amounts of gossypol from linted Upland WCS or CSM were fed. Cows were assigned to 5 treatment diets (8 cows per diet), and the study was conducted from July to October, a period of heat stress for dairy cattle in Arizona.

During the 14-d pretreatment period, all cows were fed a gossypol-free diet, which was also the control diet during the treatment period (Treatment A; Table 1). Experimental diets were fed as TMR and varied in their amounts of forage and concentrate to accommodate the different amounts of WCS and CSM. Diets were formulated to be isonitrogenous and met the requirements for lactating Holstein cows weighing 650 kg and producing 35 kg of 3.5% FCM (NRC, 2001). Diets contained 0 mg/kg gossypol (control, no gossypol; Diet A), 931 mg/kg TG and 850 mg/kg FG from WCS (moderate TG and high FG; Diet B), 924 mg/kg TG and 91 mg/kg FG from CSM (moderate TG and low FG; Diet C); 945 mg/kg TG and 479 mg/kg FG with equal amounts of TG from WCS and CSM (moderate TG and FG, Diet D), or 1894 mg/kg TG and 960 mg/kg FG with equal amounts of TG from WCS and CSM (high TG and FG; Diet E). Because diets varied in their contents of WCS, the fat content of the diets varied, and diets containing more WCS also had higher fat. All diets were mixed weekly as a TMR in an auger-type mixing wagon (Kirby Inc., Tulare, CA). Diet ingredients and TMR were sampled weekly, dried at 55°C for 48 h, and ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass through a 2-mm screen then in a cyclone mill (Udy Co., Fort Collins, CO) to pass through a 1-mm screen. Samples were then stored at -20°C for later analyses of nutrients. Weekly samples of TMR were composited, and a subsample was collected for analysis of DM, OM, CP, ether extract (AOAC, 1990), ADF and NDF (Van Soest et al., 1991), and iron. Samples of WCS and CSM also were collected weekly for analysis of TG and FG.

Cows were housed in open pens equipped with Calan gates (American Calan Inc., Northwood, NH) to enable measurement of daily feed intake from individual cows. Diets were fed twice daily for ad libitum intake, and the amount offered was adjusted daily so thatorts were at least 5% of the total offered. Cows were milked twice daily at 0400 and 1600 h, and milk yields were recorded daily. Individual milk samples were collected from consecutive milkings (a.m. and p.m.) once weekly and analyzed for SCC, fat, protein, lactose, and total solids with an infrared spectrophotometer (Foss 303 Milk-O-Scan; Foss Foods, Inc., Eden Prairie, MN) (AOAC, 1990) and SNF by difference (SNF = total solids - fat) at the Arizona DHI Laboratory in Phoenix.

Cows were weighed on 2 consecutive d, immediately after the morning milking, at the beginning and end of the 84-d experiment and again every time blood was sampled; measurements were taken after treatments were implemented and were used to determine the mean BW of each individual cow. Body temperatures and respiration rates were determined early in the morning, between 0700 and 0830 h, immediately after milking on d 28, 48, 56, 75, and 84 of the study.

Plasma Metabolites, Serum Proteins, Enzymes, and Vitamins

Blood samples (10 mL) were collected on the 1st day of the pretreatment period and again on d 0, 28, 56, and 84 of the treatment period by puncture of the coccygeal vein or artery using Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin or no anticoagulant agent for separation of plasma and serum, respectively. Samples were immediately placed on ice, transported to the laboratory within 3 h of collection, and centrifuged at 2000 × *g* for 15 min in a refrigerated centrifuge at about 10°C. Plasma samples were analyzed for concentrations of glucose by direct measurement using the YSI model 2700 SELECT Biochemistry Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Plasma urea N was analyzed using an N autoanalyzer (Bran and Luebbe, Analyzing Technologies, Elmsford, NY). Serum samples were analyzed for their concentrations of vitamins A and E at the toxicology and clinical chemistry laboratory of the Arizona Veterinary Diagnostic Laboratory in Tucson by HPLC. Serum total protein, albumin, globulin, enzyme activity (gamma glutamyltransferase, aspartate aminotransferase, creatinine phosphokinase, alkaline phosphatase, and lactate dehydrogenase), and plasma total bilirubin and creatinine were also determined at the Arizona Veterinary Diagnostic Laboratory using a clinical photospectrometric analyzer.

Gossypol Analyses and Erythrocyte Fragility

All cottonseed products were analyzed for FG and TG content in decorticated seeds as described previously (Mena et al., 2001) following the method of Hron et al. (1999).

Blood samples (10 mL) were collected on d 0 of the pretreatment period and on d 7, 14, 28, 42, 56, 70, and 84 of the treatment period by venipuncture of the coccygeal vein or artery using heparinized Vacutainer tubes (Becton Dickinson). Samples were immediately placed on ice and transported to the laboratory within 3 h of collection. Blood tubes were centrifuged at $2000 \times g$ for 15 min in a refrigerated centrifuge at about 10°C for plasma separation. Plasma was frozen at -20°C and later analyzed for gossypol. Plasma gossypol was analyzed by HPLC, which involves the simultaneous determination of TG and (+) and (-) gossypol isomers (Kim and Calhoun, 1995). Additional blood samples collected with evacuated tubes containing sodium heparin on the same days were kept at 4°C for 3 h, and whole blood was used for analysis of erythrocyte osmotic fragility as described by Nelson (1979) by measuring the percentage of hemolyzed red blood cells in a solution of 0.6% buffered saline. In addition to the blood samples collected from all cows for PG measurements, cows fed diet E (highest dietary gossypol) were switched to diet A (no gossypol) immediately after the end of the 84-d study, and blood samples were collected the day immediately prior to diet change and again at 1, 3, 7, 14, and 28 after diet change to determine changes in PG concentrations. Samples were collected and analyzed exactly as described previously.

Experimental Design and Statistical Analyses

The experimental design was randomized with complete blocks (Kuehl, 1994). Cows were blocked according to milk yield and DIM during the 14-d pretreatment period and, within each block, randomly assigned to 1 of the 5 treatment diets. Data from the pretreatment period were used as covariates in the statistical analyses of DMI, milk yield, FCM yield, gross feed efficiency, milk composition, BW, plasma TG, and erythrocyte fragility, and values of covariates for plasma TG and erythrocyte fragility are indicated in the figures. Repeated measurements were evaluated by ANOVA for repeated measures (Littell et al., 2002) using the PROC MIXED procedure of SAS (2001) with a statistical model that included the effects of block, pretreatment covariate, treatment, week of measurement, interaction between treatments and week of measurement, and cow nested within treatment as the random error. The covariance structure for the data was tested, and the structure that best fitted the data was chosen (Littell et al., 2002).

Orthogonal contrasts were performed to test the effects of TG intake (diet A vs. diets B, C, D, and E) and FG intake (diets B and E vs. diets C and D) on all outcomes evaluated (Kuehl, 1994). For the effect of FG intake on the outcomes evaluated, we used diets B and E vs. diets C and D because they were all fed gossypol, but different quantities of FG (Table 1), which resulted in different intakes of FG. To determine whether PG concentrations reached a plateau at d 42, concentrations of PG after d 56 were analyzed by ANOVA for repeated measures using the same model described previously. Least square means are reported for all parameters evaluated.

During the post-trial period, the effect of day of gossypol withdrawal from diet E was analyzed by ANOVA (Littell et al., 2002) using the PROC GLM procedure of SAS (2001) to evaluate the effect of day after diet change on PG concentrations. Orthogonal polynomials were performed to determine the relationship (linear, quadratic, or cubic) between PG concentrations and day of dietary gossypol withdrawal.

Multiple regression analyses utilizing the best subset regression procedure of MINITAB (MINITAB, 2000) were performed to determine the best predictors for PG concentrations using TG, FG, and BG intakes as the predictor variables. Treatment differences with $P < 0.05$ were considered significant and $P \leq 0.10$ were considered a tendency.

RESULTS AND DISCUSSION

Diets, Gossypol Intake, PG, and Erythrocyte Fragility

Diets were formulated to be isonitrogenous with 16% CP and to differ in their content of TG and FG from WCS and CSM or a combination of both. Small differences among treatments were observed in CP and RUP content (Table 1). Because of the experimental design, differences in concentrations of crude fat were observed; diets containing WCS had higher crude fat content. Concentrations ($\pm$ SD) of TG and FG in WCS were 0.69% (± 0.04) [65.7% + isomer and 34.3% - isomer] and 0.63% (± 0.03), respectively, and, in CSM, concentrations were 1.32% (± 0.10) [59.7% + isomer and 40.3% - isomer] and 0.13% (± 0.04), respectively. In WCS, FG represented approximately 91% of the TG, which is similar to values observed by Mena et al. (2001) and Santos et al. (2002) for linted Upland WCS. In CSM, FG was only 9.9% of the TG, which is similar to the 7% observed for Mena et al. (2001) and indicates that most of the gossypol in CSM is present in the bound form.

Mean BW of cows was similar for all 5 diets and averaged 648 kg (Table 2). As expected, TG and FG

Table 2. Body weights, gossypol intake, mean plasma total gossypol (TG) concentrations, and erythrocyte fragility in cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal.

Parameter	Diet ¹					SEM
	A	B	C	D	E	
BW, kg	652.0	645.0	649.0	636.0	658.0	18.2
Total gossypol intake						
g/d ^{X,Y}	0.00 ^c	20.08 ^b	20.74 ^b	20.13 ^b	42.03 ^a	0.92
mg/kg of BW per d ^{X,Y}	0.00 ^c	31.16 ^b	31.96 ^b	31.65 ^b	63.88 ^a	2.84
Free gossypol intake						
G/d ^{X,Y}	0.00 ^c	18.34 ^a	2.04 ^c	10.21 ^b	21.32 ^a	0.54
mg/kg of BW per d ^{X,Y}	0.00 ^c	28.43 ^a	3.14 ^c	16.05 ^b	32.40 ^a	1.50
Bound gossypol intake						
g/d ^{X,Y}	0.00 ^c	1.74 ^c	18.70 ^a	9.91 ^b	20.70 ^a	0.52
mg/kg of BW per d ^{X,Y}	0.00 ^c	2.70 ^c	28.81 ^a	15.58 ^b	31.46 ^a	1.46

^{a,b,c}Means in the same row without a common superscript are different ($P < 0.05$).

^XControl vs. gossypol ($P < 0.01$).

^YHigh vs. low free gossypol ($P < 0.01$).

¹A = control, no gossypol; B = 950 mg/kg total gossypol (TG) from whole cottonseed (WCS); C = 950 mg/kg TG from cottonseed meal (CSM); D = 950 mg/kg TG, one-half from WCS and one-half from CSM; and E = 1900 mg/kg TG, one-half from WCS and one-half from CSM.

intakes differed among dietary treatments, reflecting the different concentrations of FG and TG in the diets. The amount of TG consumed by cows increased as the amount of TG from WCS and CSM in the diet increased, but FG intake increased with the addition of more WCS to the diet. Cows receiving diet E had the highest TG intake, and those receiving diets B and E had the highest FG intake as measured in g/d or mg/kg of BW per d ($P < 0.001$). Diets in the current experiment were formulated based on a previous short-term feeding study (Mena et al., 2001).

Visible signs of gossypol toxicity including dyspnea, anorexia, decreased milk production, weakness, and sudden death, were not observed in any of the cows in the current study during the 84-d feeding period, although diets higher in FG increased PG and erythrocyte osmotic fragility (Figures 1, 3, and 5). These findings agree with those by Barraza et al. (1991) in which lactating Holstein cows consuming a diet with 15% WCS and 15% CSM, which provided 23 g/d of FG and 58 g/d of TG, for 8 wk showed no signs of gossypol toxicity. Similarly, Mena et al. (2001) observed that 42 d of feeding a high TG and FG diet resulted in no signs of gossypol toxicosis. A recent large field study evaluated the effects of feeding diets containing either 10% WCS or the same amount of cottonseed, but from a blend of 2:1 cracked Pima cottonseed and WCS on health of dairy cows (Santos et al., 2003). Cracked Pima cottonseed contained more TG and FG than whole Upland cottonseed, and the diet with a blend of both types of cottonseed resulted in intake of FG (22.8 g/d) similar to that observed in the current study for diets with WCS. Santos et al. (2003) observed that lactating cows consuming 22.8 g/d of FG from a blend of WCS and

cracked Pima cottonseed for a period of 170 d had marked increases in PG, but neither gossypol intake nor PG concentrations affected health, culling, or mortality of cows, although reproductive performance was compromised. Additional evidence of the ability of lactating cows to consume large quantities of TG and FG without displaying clinical signs of gossypol toxicity is supported by recent data from Noftsker et al. (2000) in which no signs of clinical gossypol toxicity were observed when primiparous and multiparous cows consumed 21.6 and 30.9 g/d of FG from WCS, respectively, from 30 to 120 DIM.

Prior to the beginning of the study, cows were being fed the University herd diet that contained little WCS and no CSM. Plasma gossypol concentrations at the beginning of the pretreatment period averaged 0.83 $\mu\text{g/mL}$ for all cows and dropped to 0.09 $\mu\text{g/mL}$ after 3 wk of feeding a gossypol-free diet (diet A), which demonstrates the ability of ruminants to metabolize and eliminate the compound from blood plasma. At 7 d after the initiation of treatments, PG concentrations already differed among treatments ($P < 0.001$); cows receiving the higher FG diets (diets B and E) had the highest PG concentrations (Figure 1), which increased sharply for the first 28 d of treatment, but cows receiving diets low in FG content (diets C and D) exhibited fewer changes in PG. Plasma gossypol concentrations reached a plateau in cows receiving all diets after about 28 d of treatment, averaging 1.79, 0.75, 1.10, and 3.23 $\mu\text{g/mL}$ for diets B, C, D, and E, respectively, and changed little between d 28 and 84 of treatment.

After cows receiving the highest TG and FG diet for 84 d (diet E) were fed a gossypol-free diet (diet A), PG concentrations declined sharply, in a quadratic man-

Figure 1. Least square means and SEM of plasma total gossypol (TG) concentrations ($\mu\text{g/mL}$) in lactating dairy cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal. Diet A (free gossypol [FG] intake = 0.0 g/d; plasma TG = 0 $\mu\text{g/mL}$), $\blacktriangle$; diet B (FG intake = 18.3 g/d; plasma TG = 1.70 $\mu\text{g/mL}$), $\square$; diet C (FG intake = 2.0 g/d; plasma TG = 0.79 $\mu\text{g/mL}$), $\circ$; diet D (FG intake = 10.2 g/d; plasma TG = 0.86 $\mu\text{g/mL}$), $\bullet$; and diet E (FG intake = 21.3 g/d; plasma TG = 3.61 $\mu\text{g/mL}$), $\blacksquare$. Significant effects: treatment ($P < 0.001$), day of blood collection ($P < 0.001$), and treatment by day interaction ($P < 0.001$). Contrast between control and gossypol diets (diet A vs. diets B, C, D, and E; $P < 0.0001$) and contrast between high and low free gossypol (diets E and B vs. diets C and D; $P < 0.0001$). Cov = pretreatment covariate value for plasma TG.

ner, and were only 10% of the maximal concentrations at 28 d post-treatment (Figure 2), declining from >4.0 $\mu\text{g/mL}$ to <0.4 $\mu\text{g/mL}$. When early postpartum cows were fed diets containing gossypol from WCS or a blend of WCS and cracked Pima cottonseed, PG concentrations increased linearly from 10 to 152 d postpartum

Figure 2. Least square means and SEM of plasma total gossypol (TG) concentrations ($\mu\text{g/mL}$) in cows fed diet E after withdrawal of cottonseed and cottonseed meal from the diet, with cows then fed diet A. Plasma TG concentrations differed between blood sampling days after d 1 ($P < 0.05$). A quadratic relationship between cottonseed withdrawal day and plasma TG concentration was observed ($P < 0.0001$). $\text{TG} = 4.11218 - 0.264777X + 0.0046404X^2$, where X is the withdrawal day ($r^2 = 0.90$).

(Santos et al., 2002). This prolonged increase was observed regardless of source of cottonseed or parity of cows. Risco et al. (2002) observed that PG concentrations increased steadily in the first 96 DIM and then reached a plateau when cows were fed a diet with 15% WCS. Because cows in the current study commenced treatment later in lactation, when DM and gossypol intake were constant as observed for the lack of week of study effect on intake ($P > 0.10$), PG concentrations reached a plateau more rapidly, whereas the early lactation cows in the study by Santos et al. (2002) and Risco et al. (2002) were probably increasing in DM and gossypol intakes during the entire treatment period. Mena et al. (2001) observed that concentrations of PG reached a plateau at 35 d in cows consuming 21 to 45 g/d of TG, supporting the finding that PG concentrations in midlactation cows reached a plateau after 4 to 5 wk of consuming diets relatively high in TG.

Plasma gossypol concentrations differed according to treatments, and gossypol intake increased mean PG concentrations during the 84-d study (Figure 1; $P < 0.01$). Similarly, increasing FG intake increased concentrations of plasma TG and both stereoisomers ($P < 0.01$), although the ratios of (+) to (−) isomer were similar among treatment diets. These results are similar to those observed by Mena et al. (2001) and indicate that midlactation cows consuming up to 32 mg of FG/kg of BW per d, when most of the FG is provided by WCS, will not exceed PG concentrations of 5 $\mu\text{g/mL}$. Contrary to the work by Lindsey et al. (1980), PG concentrations were not the same for cows fed diets similar in FG, but different in TG. Cows consuming Diets B and E had similar FG, but different TG intakes. In those cows, the mean plasma TG over the entire 84-d study almost doubled when TG was increased by adding CSM ($P < 0.05$; Figure 1). Two possible explanations are the release of gossypol from its bound form during digestion of CSM as suggested by Blackwelder et al. (1998) or that FG in CSM undergoes less detoxification in the rumen and, therefore, is more available for absorption than FG in WCS (Wan et al., 1995; Blackwelder et al., 1998; Mena et al., 2001). Noftsgger et al. (2000) fed lactating dairy cows diets containing either 14% regular WCS or a processed WCS (expanded-ex-pelled WCS) at varying amounts (14, 21, and 28% of dietary DM). Although FG intake in cows fed the expanded-ex-pelled WCS diets was $<30\%$ of that in cows fed WCS, PG concentrations at 60 and 90 d after the beginning of the study were higher for cows fed the expanded-ex-pelled WCS diets than those fed WCS. Santos et al. (2002) suggested that retention time of gossypol in the rumen might affect availability of FG in cotton products. Those researchers demonstrated that partial replacement of WCS with cracked Pima cottonseed,

Figure 3. Least square means and SEM of plasma (–) gossypol isomer concentrations ($\mu\text{g/mL}$) in lactating dairy cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal. Diet A (free gossypol [FG] intake = 0.0 g/d; (–) isomer = 0 $\mu\text{g/mL}$), $\blacktriangle$; diet B (FG intake = 18.3 g/d; (–) isomer = 0.83 $\mu\text{g/mL}$), $\square$; diet C (FG intake = 2.0 g/d; (–) isomer = 0.36 $\mu\text{g/mL}$), $\circ$; diet D (FG intake = 10.2 g/d; (–) isomer = 0.49 $\mu\text{g/mL}$), $\bullet$; and diet E (FG intake = 21.3 g/d; (–) isomer = 1.62 $\mu\text{g/mL}$), $\blacksquare$. Significant effects: treatment ($P < 0.001$), day of blood collection ($P < 0.001$), and treatment by day interaction ($P < 0.001$). Contrast between control and gossypol diets (diet A vs. diets B, C, D, and E; $P < 0.01$) and contrast between high and low free gossypol (diets E and B vs. diets C and D; $P < 0.01$). Cov = pretreatment covariate value for plasma (–) isomer gossypol.

which was of higher density and of smaller particle size, increased dietary FG intake by only 30%, but PG concentrations more than doubled.

The major site for FG detoxification is the forestomachs of ruminants. To be detoxified, gossypol in cotton products needs to be retained within the rumen for a sufficient time for binding to bacterial and feed proteins. As suggested by Mena et al. (2001), it is possible that rumen retention time of CSM is shorter than that for WCS, which might be associated with differences in particle size and specific gravity, as well as the presence of lint in WCS, which is thought to increase the

retention of WCS within the rumen fibrous mat. Based on increased fecal excretion of seeds, Coppock et al. (1985) suggested that rumen retention time of linted WCS is greater than that of acid-delinted WCS. The lint in WCS might help to retain the seed within the rumen fibrous mat, which would slow the passage rate of the seed through the forestomachs. When using the Cornell Net Carbohydrate and Protein System of the Cornell, Penn, Miner model (CPM-Dairy; version 3.0.4a, 2003), the rumen passage rate (Kp, %/h) for the average cow fed Diets B, D, and E were, respectively, 4.61, 4.62, 4.65%/h for WCS, and for Diets C, D, and E, the Kp of CSM were 5.84, 6.02, and 6.05%/h, respectively. Therefore, our data reinforce the idea that FG in CSM is more available than that in WCS because of the increased rumen passage rate, resulting in less extensive detoxification of FG.

Concentrations of the (–) isomer in plasma are depicted in Figure 3, and responses to dietary gossypol intake were similar to those observed for plasma TG. Mean plasma concentrations of (+) and (–) gossypol isomers were increased with dietary intake of gossypol ($P < 0.01$) and with higher intake of FG ($P < 0.01$). Similarly, concentrations of PG and gossypol isomers at the end of the study increased as FG intake increased (Table 3). Regression analyses to determine factors influencing PG concentrations showed that FG intake had the highest correlation coefficient ($r^2 = 0.75$) compared with TG ($r^2 = 0.67$) or bound gossypol intakes ($r^2 = 0.18$). As observed by Mena et al. (2001) and depicted in Figure 4, plasma TG concentrations on d 84 of the study were increased linearly with FG intake ($P < 0.0001$; $r^2 = 0.75$).

Erythrocyte fragilities followed a pattern similar to PG, in that at the beginning of pretreatment, the average percentage of hemolyzed cells was higher (33.8%) than that 2 wk after cows were fed the gossypol-free diet (22.1%; Figure 5). Time on diet affected erythrocyte fragility ($P < 0.001$), and it depended upon dietary treat-

Table 3. Plasma gossypol concentrations and erythrocyte fragilities on d 84 in cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal.

Parameter	Diet ¹					SEM
	A	B	C	D	E	
Plasma gossypol, $\text{X,Y} \mu\text{g/mL}$	0.00 ^d	1.70 ^b	0.79 ^c	0.86 ^c	3.61 ^a	0.18
Plasma (+) isomer, $\text{X,Y} \mu\text{g/mL}$	0.00 ^d	0.71 ^b	0.33 ^{cd}	0.36 ^c	1.52 ^a	0.10
Plasma (–) isomer, $\text{X,Y} \mu\text{g/mL}$	0.00 ^d	1.00 ^b	0.46 ^{cd}	0.50 ^c	2.10 ^a	0.19
(+):(–) isomers	—	0.71	0.72	0.72	0.73	—
Erythrocyte fragility, $\text{X,Y} \%$	17.73 ^c	39.49 ^a	24.55 ^{bc}	31.87 ^{ab}	42.43 ^a	4.50

^{a,b,c,d}Means in the same row without a common superscript are different ($P < 0.05$).

^XControl vs. gossypol ($P < 0.01$).

^YHigh vs. low free gossypol ($P < 0.01$).

¹A = Control, no gossypol; B = 950 mg/kg total gossypol (TG) from whole cottonseed (WCS); C = 950 mg/kg TG from cottonseed meal (CSM); D = 950 mg/kg TG, one-half from WCS and one-half from CSM; and E = 1900 mg/kg TG, one-half from WCS and one-half from CSM.

Figure 4. Relationship between free gossypol (FG) intake (g/d) and plasma gossypol (PG) concentration ($\mu\text{g/mL}$) in lactating dairy cows on d 84 (all cows included). Plasma gossypol = $-0.0623553 + 0.139486$ FG intake, where PG is determined in $\mu\text{g/mL}$, and FG intake is expressed in g/d ($P < 0.0001$; $r^2 = 0.75$).

ments as indicated by the interaction between day of blood collection and treatment ($P < 0.01$). In cows fed diet A and the low FG diet (diet C), erythrocyte fragility initially declined and reached a plateau after 42 d. Cows

Figure 5. Least square means and SEM for erythrocyte fragility (EF; %) in lactating dairy cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal. Diet A (free gossypol [FG] intake = 0.0 g/d; EF = 18.4%), $\blacktriangle$; diet B (FG intake = 18.3 g/d; EF = 31.4%), $\square$; diet C (FG intake = 2.0 g/d; EF = 21.9%), $\circ$; diet D (FG intake = 10.2 g/d; EF = 26.7%), $\bullet$; and diet E (FG intake = 21.3 g/d; EF = 28.7%), $\blacksquare$. Significant effects: treatment ($P = 0.04$), day of blood collection ($P < 0.001$), interaction between treatment and day of blood collection ($P < 0.01$). Contrast between control and gossypol diets (diet A vs. diets B, C, D, and E; $P = 0.01$) and contrast between high and low free gossypol (diets E and B vs. diets C and D; $P = 0.09$). Cov = pretreatment covariate value for erythrocyte fragility.

fed diets B, D, and E, which contained 500 to 950 mg/kg of FG, had increased erythrocyte fragility until d 28 of treatment, showed decreased fragility on d 42, and then increased fragility again from d 42 to 84 (Figure 5). Orthogonal contrasts indicated that consumption of gossypol increased erythrocyte fragility ($P < 0.01$), and cows consuming more FG tended to have higher erythrocyte fragility ($P = 0.09$) throughout the study. On d 84 of the study, FG intake increased erythrocyte fragility ($P < 0.01$; Table 3), which supports previous observations that erythrocyte fragility increases in cows fed high FG diets (Mena et al., 2001).

When diets differing in TG, but low in FG were compared, TG intake had no effect on erythrocyte fragility. However, TG intake increased erythrocyte fragility when diets differed in TG, but had a similar and high FG content (Mena et al., 2001). Those researchers suggested that this mixed effect might have been associated with the concentrations of gossypol in plasma. We evaluated the relationship between plasma TG concentrations and erythrocyte fragility on d 84 of the study. Although a linear relationship was observed ($P < 0.01$), it was weak, and plasma TG had only a small influence on the variation in erythrocyte fragility ($r^2 = 0.17$). Regardless, higher PG concentrations corresponded with higher erythrocyte fragilities on d 84 of treatment. Because of the interaction of treatment and day of blood collection ($P < 0.01$; Figure 5), which showed increased erythrocyte fragility with time that cows were exposed to high FG diets, we suggest that the full impact of PG on erythrocyte fragility may be a delayed reaction. Mena et al. (2001) indicated that PG concentrations $< 3.0 \mu\text{g/mL}$ do not affect erythrocyte fragility, but, on d 84, we observed that cows with $\text{PG} \geq 0.86 \mu\text{g/mL}$ had higher erythrocyte fragility than did control cows. Our findings agree with results observed in other studies that have suggested that erythrocyte fragility is sensitive to amounts of gossypol consumed (Lindsey et al., 1980; Colin-Negrete et al., 1996; Risco et al., 2002) and that small concentrations of PG can disrupt integrity of erythrocyte membranes when exposed to a hypo-osmotic saline solution.

Blood Parameters

Concentrations of vitamin A in serum were unaffected by treatment (Table 4), although they were numerically higher for cows receiving diet E. Cows in the current study were consuming approximately 27,000 IU/d of supplemental vitamin A in the mineral-vitamin premix added to the diet (Table 1). Weiss (1998) indicated that lactating cows supplemented with 50,000 to 80,000 IU of vitamin A have plasma concentrations of retinol of 0.4 to 0.5 $\mu\text{g/mL}$.

Table 4. Concentrations of vitamins, proteins, enzymes, and metabolites in blood plasma or serum of lactating dairy cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal.

Parameter ²	Diet ¹					SEM
	A	B	C	D	E	
Vitamin						
A, $\mu\text{g/mL}$	0.352	0.395	0.363	0.366	0.589	0.101
E, $\mu\text{g/mL}$	9.855 ^c	14.991 ^b	10.947 ^c	13.634 ^b	17.420 ^a	0.722
Protein						
Total, g/dL	7.82	8.11	8.03	7.94	8.20	0.19
Albumin (A), g/dL	4.11	4.09	4.06	4.10	4.10	0.08
Globulin (G), g/dL	3.71	4.03	3.96	3.85	4.10	0.22
A:G	1.12	1.05	1.08	1.08	1.02	0.07
Enzyme ³						
GGT, U/L	20.92	23.63	25.08	22.96	27.33	2.23
AST, U/L	58.00 ^b	65.58 ^b	60.67 ^b	63.50 ^b	82.33 ^a	4.90
CPK, U/L	138.87	167.79	172.58	134.12	177.17	23.93
ALP, U/L	48.17	52.75	46.08	49.29	60.45	6.33
LDH, U/L	948.46	918.21	922.33	940.83	1040.08	43.97
Bilirubin, mg/dL	0.133	0.196	0.150	0.154	0.192	0.022
Creatinine, mg/dL	0.75	0.78	0.82	0.83	0.86	0.06
Glucose, mg/dL	71.46 ^a	67.50 ^b	72.00 ^a	69.46 ^{ab}	69.29 ^{ab}	1.27
Urea N, mg/dL	13.08 ^c	15.00 ^b	12.33 ^c	14.50 ^b	17.96 ^a	0.57

^{a,b,c}Means in the same row without a common superscript are different ($P < 0.05$).

^xControl vs. gossypol ($P < 0.01$).

^yHigh vs. low free gossypol ($P < 0.05$).

^zHigh vs. low free gossypol ($P < 0.001$).

¹A = Control, no gossypol; B = 950 mg/kg total gossypol (TG) from whole cottonseed (WCS); C = 950 mg/kg TG from cottonseed meal (CSM); D = 950 mg/kg TG, one-half from WCS and one-half from CSM; and E = 1900 mg/kg TG, one-half from WCS and one-half from CSM.

²Means of samples collected throughout the entire 84-d experimental period.

³GGT = Gamma glutamyltransferase, AST = aspartate aminotransferase, CPK = creatinine phosphokinase, ALP = alkaline phosphatase, and LDH = lactate dehydrogenase.

Vitamin E concentrations in serum were highest for cows consuming diet E, whereas diets B and D were higher than diets A and C ($P < 0.05$). Orthogonal contrasts show that cows fed diets high in FG showed higher in vitamin E concentrations than those fed diets low in FG ($P < 0.001$). Lactating cows fed 15% of the diet as WCS had increased concentrations of vitamin E in plasma throughout the lactation (Risco et al., 2002). The concentration of vitamin E in WCS in the study by Risco et al. (2002) was 59.4 $\mu\text{g/g}$ of seed. Using this value for vitamin E, cows in the current study would be consuming an additional 169.2, 87.7, and 182.0 mg/d of α -tocopherol in diets B, D, and E from WCS, which is expected to increase the vitamin E intake by 64, 33, and 69%, respectively. These higher vitamin E intakes are probably responsible for the higher serum vitamin E concentrations observed for cows consuming WCS.

In a Florida study, heifers were fed soybean meal or CSM in diets containing 30 or 4000 IU of supplemental vitamin E. Feeding CSM increased plasma α -tocopherol in a time-dependent response (Velasquez-Pereira et al., 2002). Those researchers (Velasquez-Pereira et al., 2002) suggested that gossypol might be slowly replacing vitamin E in the cell membranes of erythrocytes and

thereby increasing plasma α -tocopherol concentration. However, cotton products also contain high amounts of vitamin E (Risco et al., 2002), which could have increased blood concentrations because of higher dietary intake when CSM was fed. Weiss (1998) indicated that a minimum of 3 $\mu\text{g/mL}$ of vitamin E in serum is required for optimum immune function in lactating dairy cows. Cows in the current study had concentrations 3 to 5 times higher, suggesting that vitamin E status was adequate in all treatment groups. Risco et al. (2002) also observed that cows consuming WCS had plasma vitamin E concentrations 3 times higher than those considered adequate. The high vitamin E concentrations in serum observed in the current study when cows were fed diets containing FG are likely to be related to the higher vitamin E intake.

Plasma glucose concentrations were higher for diets A and C compared with diet B ($P < 0.05$). This effect might be related to more ruminally degradable starch from steam-flaked corn in those diets. Feeding more ruminally degradable starch has been shown to increase plasma glucose concentrations (Santos et al., 2000) because of the greater output of glucose by the liver (Theurer et al., 1999). Similarly, cows fed diets A and C also had lower plasma urea N concentrations

than those fed diets B, D, and E ($P < 0.05$). Orthogonal contrasts indicate that FG intake increased plasma urea N concentrations in lactating dairy cows ($P < 0.01$). These effects might also be associated with more ruminally fermentable starch and OM in diets with more steam-flaked corn (Theurer et al., 1999).

Serum concentrations of total proteins, albumin, and globulin were not influenced by diets (Table 4). Furthermore, the ratio of albumin to globulin was similar among diets. Albumin concentrations in serum were slightly higher than those indicated as reference values for normal cows (3.0 to 3.6 g/dL; Turk and Casteel, 1997). Albumin is produced by the liver as are all serum proteins except for the immunoglobulins, and lack of a treatment effect indicates that hepatic function might not be altered by amount or source of gossypol.

Furthermore, to support that hepatic function was not altered by dietary gossypol, we evaluated activity of enzymes in serum associated with hepatic or muscle cell viability and observed that the values were not affected by TG or FG intake (Table 4). Serum gamma glutamyltransferase, creatinine phosphokinase, alkaline phosphatase, and lactate dehydrogenase did not differ among treatments. These enzymes occur in most cells, but are usually increased in serum when hepatocytes or muscle cells have suffered cellular damage (Turk and Casteel, 1997). These increases are usually associated with leakage from the cytoplasm from injured cells, but are also because of increased synthesis, as with gamma glutamyltransferase. The number of cows with serum gamma glutamyltransferase higher than the upper normal level (>39 IU/L) was 0, 0, 0, 0, and 2 for diets A, B, C, D, and E, respectively. For creatinine phosphokinase, 1 cow fed each diet had elevated activity in serum (>409 IU/L), and for alkaline phosphatase and lactate dehydrogenase, only 1 and 2 cows, respectively, fed diet E had elevated activity in serum.

The only enzyme in serum affected by diet was aspartate aminotransferase, which tended to be increased by gossypol intake ($P = 0.07$) and was increased by FG intake ($P < 0.05$). However, all of the FG effect on enzyme activity was caused by diet E, which contained the highest TG and FG. Although diet E increased aspartate aminotransferase concentration in serum, values observed were within the normal range for cows (43 to 127 IU/L; Turk and Casteel, 1997). However, 2 cows fed diet E were above the upper level considered normal (>127 IU/L).

Total bilirubin and creatinine concentrations in plasma were not affected by diet. Bilirubin, derived from the catabolism of erythrocytes, is incorporated into bile and excreted in the feces. Bile salts that are reabsorbed by the entero-hepatic circulation increase

plasma bilirubin, which, under normal levels, is excreted in the urine. However, when bile ducts are damaged, bilirubin accumulates in blood and can be used as an indicator of hepatic disease. Creatinine in plasma is often increased in the presence of abnormal kidney function and is often associated with reduced glomerular filtration (Finco, 1997). Therefore, the findings of the current study suggest that diets with up to 960 mg/kg of FG and 1894 mg/kg of TG had only minor effects on integrity and function of hepatic cells and kidney function as indicated by changes in enzyme activity in serum.

Lactation Performance, Respiration Rate, and Body Temperature

Intake of DM averaged 21.9 kg/d and was higher for cows consuming diet E than for controls ($P < 0.05$; Table 5), but no differences were observed among other diets. Usually, the addition of WCS to the diet has minimal effects on DMI as concluded by Coppock et al. (1987), who reviewed several studies in which up to 25% WCS was fed to lactating dairy cows. Cows consuming varying amounts of either soybean meal or CSM had similar DMI (Blackwelder et al., 1998), and Mena et al. (2001) also observed no effect of different amounts of CSM and WCS on DMI of lactating dairy cows. The difference in DMI for cows fed diets A and E might be related to differences in forage content of diets.

Yields of milk and 3.5% FCM followed a pattern similar to that observed for DMI, with cows fed diet E having higher yields of milk and 3.5% FCM than those fed diet A ($P < 0.05$). The higher milk yield for cows consuming diet E was probably because of the higher NE_L concentration in that diet originating from the higher fat content. Cows fed diet E had higher NE_L intakes than those fed diet A ($P < 0.05$) because of both a greater DMI and a higher NE_L content of the diet. Inclusion of WCS usually increases the energy content of diets because of its high fat content and has been shown to improve milk yield of dairy cows (Coppock et al., 1987; Wu and Huber, 1994; Mena et al., 2001). Efficiency of conversion of DM consumed into 3.5% FCM was similar for all diets, and cows produced 1.34 kg of 3.5% FCM for every kg of DM consumed.

Milk composition and yields of milk components were not affected by diets (Table 5). Arieli (1998) reported that, in most studies with WCS supplementation, milk fat content is not altered. Mena et al. (2001) also observed no effect of diets on content of fat in milk when different amounts of WCS and CSM were fed. However, because of the higher milk yield in cows receiving 15% WCS, milk fat yield increased compared with those receiving only CSM (Mena et al., 2001). Addition of fat

Table 5. Lactation performance of dairy cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal.

Parameter	Diet ¹					SEM
	A	B	C	D	E	
DMI, kg/d	20.7 ^b	21.1 ^{ab}	21.4 ^{ab}	21.7 ^{ab}	22.7 ^a	0.58
NE _L intake, Mca/d	34.5 ^b	34.9 ^{ab}	36.4 ^{ab}	35.1 ^{ab}	37.8 ^a	1.15
Milk yield, kg/d	28.6 ^b	30.8 ^{ab}	30.8 ^{ab}	31.1 ^{ab}	32.6 ^a	0.87
3.5% FCM, kg/d	27.5 ^b	29.8 ^{ab}	28.6 ^b	29.7 ^{ab}	31.0 ^a	0.80
3.5% FCM/DMI	1.33	1.36	1.25	1.38	1.35	0.05
Milk protein						
%	3.33	3.19	3.25	3.20	3.15	0.11
kg/d	0.97	0.96	0.99	0.99	1.00	0.04
Milk fat						
%	3.27	3.31	3.08	3.28	3.24	0.15
kg/d	0.96	0.99	0.94	1.02	1.03	0.05
Lactose						
%	4.95	4.91	5.01	5.01	5.12	0.10
kg/d	1.45	1.50	1.55	1.57	1.64	0.08
Solids nonfat						
%	8.88	8.70	8.82	8.86	8.93	0.13
kg/d	2.60	2.63	2.70	2.76	2.84	0.12

^{a,b,c}Means in the same row without a common superscript are different ($P < 0.05$).

¹A = Control, no gossypol; B = 950 mg/kg total gossypol (TG) from whole cottonseed (WCS); C = 950 mg/kg TG from cottonseed meal (CSM); D = 950 mg/kg TG, one-half from WCS and one-half from CSM; and E = 1900 mg/kg TG, one-half from WCS and one-half from CSM.

to the diet often decreases milk protein concentration, although it might increase milk protein yield (Wu and Huber, 1994). Others have observed a decrease in milk protein with addition of WCS to the diet (Smith et al., 1981; Coppock et al., 1987; Arieli, 1998; Mena et al., 2001). In the current study, neither concentration nor yield of milk protein differed with addition of varying amounts of WCS or CSM to diets. Moreover, content of lactose and SNF did not differ among treatments.

Respiration rates were not affected by diets (Figure 6), although a tendency was observed for FG intake to increase respiration rates in lactating cows ($P = 0.10$). Cows receiving diets B and E averaged 71.2 breaths/min, whereas cows fed diets C and D averaged 68.3 breaths/min. The current study was conducted during periods of heat stress in Arizona (July to October), when the maximum daily ambient temperatures are usually $>38^{\circ}\text{C}$, which was probably the reason for the high respiratory frequency. Because erythrocyte fragility tended to be higher for cows receiving the high FG diets throughout the study, and higher on d 84, it is possible that the slight increase in respiratory frequency might have been a compensatory effect to improve oxygen transport to tissues. We did not evaluate hematocrit in these cows, but the increased erythrocyte fragility and respiratory frequency in cows consuming more FG might indicate reduced oxygen transport to tissues because of compromised erythrocyte integrity and function. Lindsey et al. (1980) observed that cows fed solvent-extracted CSM had an increased respiration rate when exposed to elevated ambient temperatures. Al-

though a tendency for higher respiratory frequency was observed, the slight increase with cows fed higher FG might be of limited biological impact. Others (Coppock et al., 1985) evaluated respiration rates in lactating cows fed 0, 15, or 30% of the dietary DM as WCS and observed a linear decrease in number of breaths per minute (68.1 vs. 64.6 vs. 60.0, respectively) as WCS and FG in the diet increased.

Figure 6. Least square means and SEM for respiration rate (breaths/min) in lactating dairy cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal. Diet A (free gossypol [FG] intake = 0.0 g/d), Δ ; diet B (FG intake = 18.3 g/d), $\square$; diet C (FG intake = 2.0 g/d), $\circ$; diet D (FG intake = 10.2 g/d), $\bullet$; and diet E (FG intake = 21.3 g/d), $\blacksquare$. Significant effects: day ($P < 0.001$). Contrast between high and low free gossypol (diets E and B vs. diets C and D; $P = 0.10$).

Figure 7. Least square means and SEM for rectal temperature ($^{\circ}\text{C}$) in lactating dairy cows fed varying amounts of gossypol from whole cottonseed and cottonseed meal. Diet A (free gossypol [FG] intake = 0.0 g/d), $\blacktriangle$; diet B (FG intake = 18.3 g/d), $\square$; diet C (FG intake = 2.0 g/d), $\circ$; diet D (FG intake = 10.2 g/d), $\bullet$; and diet E (FG intake = 21.3 g/d), $\blacksquare$. Significant effects: day ($P < 0.001$).

Rectal temperatures decreased with time in the study ($P < 0.0001$; Figure 7) because of ambient temperatures, but they were not affected by diets. Coppock et al. (1985) also observed that consumption of FG from WCS did not affect body temperature in cows. Therefore, consumption of varying amounts of TG and FG from WCS and CSM does not seem to alter body temperature.

In the study reported herein, a number of treatment effects were observed, but feeding up to 960 mg/kg of FG and 1894 mg/kg of TG from a combination of WCS and CSM in the diet for 84 d showed no clear evidence of gossypol toxicity in lactating dairy cows. Although PG concentrations and erythrocyte fragilities increased with increased FG intake, lactation performance was not compromised. In fact, yields of milk and 3.5% FCM were higher for cows consuming the higher gossypol diet. Although high intakes of gossypol and PG concentrations reduce reproductive performance of lactating dairy cows (Santos et al., 2003), this study supports previous findings (Mena et al., 2001) that midlactation dairy cows can consume up to 21 and 42 g/d of FG and TG, respectively, with no adverse effect on lactation performance, even when fed for an extended period.

CONCLUSIONS

Cows fed diets containing more FG from WCS had higher PG concentrations than those fed CSM. The increase in PG from diets with WCS was caused by the higher content of FG in those diets. In fact, a linear relationship was observed between FG intake and plasma TG concentrations in lactating dairy cows. Plasma gossypol concentrations in cows fed CSM were

higher than expected, suggesting that either some bound gossypol from CSM might have been released as FG during digestion and absorbed by the digestive tract or that FG detoxification in CSM is less extensive than that in WCS, as suggested by other studies. Therefore, when determining guidelines for the safe feeding of gossypol containing products to dairy cows, the amounts of FG, as well as the source of TG should be considered. Plasma gossypol concentrations seem to reach a plateau after 28 d of feeding gossypol in the diet of midlactation dairy cows, which agrees with our previous finding in a short-term study. Similar to PG concentrations, gossypol isomers in plasma increased as the intake of FG increased, but no change in the proportion of (+) and (−) isomer was observed. Furthermore, 28 d of withdrawal from high dietary gossypol is sufficient for PG concentrations to return to almost undetectable values.

Serum vitamin E increased with WCS, which might have been associated with higher FG, but also a higher dietary fat content for diets with WCS. Small changes in blood concentrations of metabolites were observed, and glucose and urea N increased and decreased, respectively, in diets with no WCS. Serum enzymes were usually unaffected by treatment. Only aspartate aminotransferase was increased with higher FG in the diet, although the levels observed were within those considered as normal for cows. Plasma bilirubin, creatinine, and serum proteins were not influenced by treatments. These results suggest that liver and kidney function and muscle cell integrity are not affected by amount of gossypol normally consumed by lactating dairy cows.

In the current study, the only sign of gossypol toxicity observed was an increase in erythrocyte fragility for cows on treatments with higher FG content. The lack of changes in indicators of hepatic and kidney function and muscle cell integrity demonstrates that the amounts of TG and FG used in the current study did not affect integrity of cells in those tissues.

Yields of milk and 3.5% FCM were increased in cows receiving the diet with highest TG, which was probably related to the higher dietary energy content and higher NE_L intake, but no changes were observed in concentrations or yields of milk components. From these data, we conclude that a combination of WCS and CSM can furnish up to 1894 and 960 mg/kg of TG and FG, respectively, for lactating dairy cows during 84 d with no adverse effects on lactation or blood parameters.

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