Type of Cottonseed and Level of Gossypol in Diets of Lactating Dairy Cows: Plasma Gossypol, Health, and Reproductive Performance

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ABSTRACT

Objectives were to determine effects of altering gossypol intake by feeding whole linted Upland (WUP) or a 1:2 blend of WUP and cracked Pima (BUPCP) cottonseed on plasma gossypol (PG) concentrations, reproduction, and health of Holstein cows. Cows, 813, on three dairy farms were assigned to one of two diets starting at 13 ± 11 d in milk for a 170-d experiment. Diets contained 717 and 951 mg of free gossypol/kg of dry matter from WUP and BUPCP, respectively. Concentrations of PG, as well as the proportion of total gossypol (TG) as the minus isomer were higher for cows fed BUPCP vs cows fed WUP. Conception rate at the first postpartum artificial insemination did not differ between treatments. However, cows consuming the higher gossypol diet had reduced subsequent conception rates and a lower pregnancy rate. Incidence of abortions increased in the higher gossypol diet, and cows that aborted or remained open had higher PG concentrations. Increasing PG concentrations resulted in reduced conception rates and extended days open. The probability of conception after the first artificial insemination declined at a decreasing rate as the plasma TG increased. Incidence of health disorders were unaffected by gossypol intake and PG concentrations. Similarly, gossypol intake and PG concentrations had no effect on culling or mortality. Six cows died in each diet, and none had postmortem signs compatible with gossypol toxicity. Consumption of a high gossypol diet for 170 d had no effect on health of lactating dairy cows, but it increased PG concentrations and impaired reproductive performance.

(**Key words:** cottonseed, gossypol, reproduction, dairy cow)

Abbreviation key: BUPCP = blend of whole linted Upland and cracked Pima cottonseed; FG = free gossypol;

PG = plasma gossypol; TG = total gossypol; WUP = whole linted Upland cottonseed.

INTRODUCTION

The two major types of cotton grown in the southwestern USA are Pima and Upland. Pima is a long-staple cotton that after ginning yields lint-free seeds, and Upland is a short-staple cotton that yields seeds with lint after ginning. In the southwestern USA, availability of Pima cottonseed for feeding cattle has increased in recent years, while production of whole linted Upland (WUP) cottonseed has remained unchanged.

Cottonseed is extensively used as a source of energy, protein, and fiber in diets of dairy cattle. Because of its lower fiber content, Pima contains more fat and protein than WUP cottonseed (Sullivan et al., 1993; Santos et al., 2002), which might be favorable for high levels of milk production. Pima cottonseed is often processed prior to feeding to cattle by cracking or grinding to improve nutrient utilization and animal performance (Sullivan et al., 1993).

Pima cottonseed contains more gossypol than the WUP, and more of the gossypol in Pima is in the minus (–) isomer form (Sullivan et al., 1993; Prieto et al., 2003; Santos et al., 2002). Gossypol is a toxic, polyphenolic binaphthyl dialdehyde, yellow pigment found primarily in the pigment glands of the seed. Two distinct stereoisomer forms of gossypol occur in cottonseed, the plus (+) and the minus (–) isomer. The (–) isomer has been shown to have greater biological activity, to be retained in the body for a longer period of time, and appears to be more detrimental to fertility of males (Wang et al., 1986). Gossypol intake depressed fertility in bulls (Chenoweth et al., 2000) and reduced viability of gametes in cattle (Zirkle et al., 1988; Lin et al., 1994; Brocas et al., 1997; Chenoweth et al., 2000). In an extensive review of literature, Randel et al. (1992) observed that gossypol might be detrimental to fertility of ruminants. However, most of the data available were associated with male fertility. Gossypol causes degeneration of seminiferous tubules in

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the parenchyma of the testicles, but similar effects have not been demonstrated in the gonads of female ruminants *in vivo*. In studies with heifers (Randel et al., 1996), feeding up to 15 g/d of free gossypol (FG) for 65 d had no effect on ovarian function and embryo characteristics. Similarly, Gray et al. (1993) observed no effect of feeding a high FG diet to lactating cows for 33 wk on ovarian function and blood concentrations of hormones. Nevertheless, neither of those studies reported plasma gossypol (PG) concentrations.

The female ruminant might be relatively insensitive to the antifertility effect of gossypol (Randel et al., 1992; Gray et al., 1993), but *in vitro* data indicate some inhibition of embryonic development (Zirkle et al., 1988) and ovarian steroidogenesis (Gu et al., 1990; Lin et al., 1994). Recently, Brocas et al. (1997) observed a negative effect of gossypol on gametes and embryos *in vitro*, but not when it was fed in the diet of cows. Feeding cottonseed meal to dairy cows did not affect the number of oocytes collected per cow, cleavage rate after *in vitro* maturation and fertilization, or the proportion of *in vitro* fertilized oocytes or embryos that developed to blastocysts. When oocytes were exposed *in vitro* to low levels of gossypol, similar to those observed in plasma of cows fed diets with 10% WUP cottonseed (Mena et al., 2001; Santos et al., 2002), cleavage rates and subsequent development were not affected. In contrast, increasing gossypol concentration in the media, similar to that observed for cows fed diets containing cracked Pima cottonseed (Prieto et al., 2003; Santos et al., 2002), reduced embryo cleavage rate. Based on these data, the authors concluded that developing embryos are sensitive to gossypol and diets that increase PG concentrations might negatively impact fertility of cows.

A primary concern with feeding large amounts of cottonseed is the possibility of gossypol toxicity and a potential depression in fertility in dairy cows (Lindsey et al., 1980; Arieli, 1998). We have recently shown (Prieto et al., 2003; Santos et al., 2002) that feeding blends of WUP and cracked Pima cottonseed raises plasma total gossypol (TG) concentrations above 10 $\mu\text{g}/\text{ml}$, which is similar to the levels found to negatively impact oocytes, embryos, and luteal and follicular cells *in vitro* (Zirkle et al., 1988; Gu et al., 1990; Lin et al., 1994; Brocas et al., 1997). The objectives of this study were to determine the effects of feeding two amounts of gossypol from WUP or a blend of WUP and cracked Pima cottonseed (BUPCP) on PG concentrations, reproductive performance, and health of lactating high producing Holstein dairy cows during the first 183 d in lactation. To our knowledge no research information exists in the literature evaluating the relationship of gossypol intake and PG concentrations on reproductive performance and health of lactating dairy cows.

MATERIALS AND METHODS

Animals, Housing, and Feeding

Eight-hundred and thirteen early lactation Holstein cows (206 primiparous and 607 multiparous; 13 ± 11 DIM) on three dairy farms in the San Joaquin Valley of California were assigned to one of the two diets in a randomized complete block design on the basis of parity, 305-d mature equivalent milk yield during the previous lactation, and age at calving. The study period lasted 170 d, and cows ended the experimental period at 183 ± 11 DIM. During the prepartum period, all cows on all dairies received a gossypol-free diet. Within each dairy, experimental diets varied only in the type of cottonseed and amount of gossypol fed (Table 1). Detailed description of the dietary ingredients, TMR, and feeding management was reported previously (Santos et al., 2002). Within each dairy, diets were isonitrogenous and isocaloric, and met the nutrient requirements for lactating Holstein cows weighing 650 kg and producing 40 kg of 3.5% FCM (NRC, 2001). Diets contained 10% cottonseed either as WUP or a 1:2 blend of WUP and cracked Pima (BUPCP) cottonseed and were formulated to contain 700 and 950 mg/kg of FG for WUP and BUPCP, respectively. Group consumption of FG was higher ($P < 0.01$) for cows fed BUPCP vs WUP, and it averaged 22.8 and 17.5 g/d, respectively (Santos et al., 2002).

The experiment was conducted from July 2000 to March 2001. Cows were housed in 10 free-stall barns (five pens/treatment). Within each dairy, all pens were identical in design, size, and location, with similar number of animals housed and similar milking and feeding times. At dairy 2, only multiparous cows were utilized and all cows received 500 mg of exogenous bST (Posilac, Monsanto Co., St. Louis, MO) every 14 d, starting between 60 and 70 d postpartum. Body condition of all cows was scored (Ferguson et al., 1994) on 2 consecutive d prior to the beginning and at the end of the 170-d experimental period and again at d 61 and 91 postpartum.

Blood Sample Collection and Analyses

Blood samples (10 ml) were collected from all cows on d 61 and 91 postpartum, or study d 48 ± 11 and 78 ± 11 , respectively, by puncture of the coccygeal vein or artery using heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). A total of 793 cows were sampled, 188 cows on dairy 1, 302 cows on dairy 2, and 303 cows on dairy 3. Blood 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

Table 1. Ingredient and nutrient composition of diets.

Ingredients	Diets ¹					
	Dairy 1		Dairy 2		Dairy 3	
	WUP	BUPCP	WUP	BUPCP	WUP	BUPCP
	(% of DM)					
Corn silage	16.4	16.4	19.7	19.7	19.5	19.5
Alfalfa hay	14.8	14.8	19.3	19.3	15.4	15.4
Alfalfa silage	13.9	13.9	...	...	7.0	7.0
Steam-rolled corn grain	12.9	12.9	23.7	23.7	5.6	5.6
Steam-rolled barley grain	12.9	12.9	...	...	14.0	14.0
Almond hulls	...	...	9.0	9.0	...	...
Citrus pulp	6.9	6.9	...	...	...	...
Beet pulp shreds	...	...	...	...	3.2	3.2
Soybean hulls	...	...	...	...	7.8	7.8
Soybean meal, 47% CP solvent extract	4.1	4.1	6.7	6.7	3.0	3.0
Canola meal, 38% CP solvent extract	...	...	3.6	3.6	3.0	3.0
Dried distillers grains, corn	...	...	...	...	5.5	5.5
Animal-marine protein blend ²	3.6	3.6	...	...	...	...
Lignosulfonate treated soybean meal ³	...	...	3.3	3.3	...	...
Tallow	1.4	1.4	1.3	1.3	0.7	0.7
Calcium salts of long chain fatty acids ⁴	...	...	...	...	0.8	0.8
Molasses, liquid	0.6	0.6	...	...	2.3	2.3
Minerals & vitamins ⁵	2.5	2.5	3.4	3.4	2.2	2.2
Whole Upland cottonseed	10.0	3.1	10.0	3.1	10.0	3.1
Cracked Pima cottonseed	...	6.9	...	6.9	...	6.9
Nutrient composition						
NE _L , ⁶ Mcal/kg	1.72	1.73	1.70	1.71	1.68	1.68
DM, %	54.1	53.3	62.3	62.8	60.5	60.1
OM, %	90.0	89.8	92.1	92.0	91.3	92.2
CP, %	18.9	18.8	18.0	18.1	18.2	18.1
Fat, %	7.1	7.0	6.4	6.6	7.4	7.1
NDF, %	33.8	33.3	33.5	32.0	34.4	33.2
ADF, %	22.1	21.3	20.5	21.7	23.1	22.9
NFC, ⁷ %	31.6	32.0	35.5	36.8	32.9	35.3
Ca, %	1.00	1.01	0.81	0.82	1.05	1.07
P, %	0.53	0.52	0.54	0.56	0.54	0.54
Mg, %	0.40	0.36	0.35	0.37	0.37	0.36
K, %	1.65	1.60	1.46	1.48	1.50	1.51
Na, %	0.53	0.52	0.40	0.42	0.46	0.45
Fe, mg/kg	678	642	356	398	364	342
Zn, mg/kg	70	63	67	70	74	74
Cu, mg/kg	24	23	21	21	23	21
Mn, mg/kg	69	61	67	68	71	70
Total gossypol, mg/kg	720	1003	740	1016	770	984
Free gossypol, mg/kg	710	965	710	938	730	951
(-) isomer, mg/kg	309.6	523.6	308.6	535.4	326.5	513.7
(+) isomer, mg/kg	412.6	479.4	431.4	484.6	445.1	472.3

¹WUP = whole linted Upland cottonseed diet; BUPCP = blend of whole linted Upland and cracked Pima cottonseed.

²Animal marine protein blend; Pro-Lak (blend of marine and animal by-products; H. J. Baker & Bro., Inc., Stamford, CT).

³Lignosulfonate treated soybean meal; Amino Plus (Ag Processing, Inc., Emmetsburg, IA).

⁴Calcium salts of long chain fatty acids; EnerGII (Bioproducts, Inc., Fairlawn, OH).

⁵Dairy 1: Niacin, 1.4%; Zinpro, 2.1%; Ca, 5.7%; P, 3.2%; K, 5.1%; Mg, 5.1%; Na, 8.2%; Cl, 3.2%; S, 1.2%; and (per kg) 850 mg of Zn, 540 mg of Mn, 220 mg of Cu, 20 mg of I, 15 mg of Co, 10 mg of Se, 150 KIU of vitamin A, 35 KIU of vitamin D, and 1400 IU of vitamin E; Dairy 2: Zinpro 4-plex, 2.0%; Ca, 4.5%; P, 2.2%; K, 4.2%; Mg, 2.5%; Na, 5.6%; Cl, 2.1%; S, 0.9%; and (per kg) 890 mg of Zn, 580 mg of Mn, 200 mg of Cu, 15 mg of I, 14 mg of Co, 8 mg of Se, 135 KIU of vitamin A, 30 KIU of vitamin D, and 980 IU of vitamin E; Dairy 3: Zinpro, 1.8%; Diamond V, 9%; Ca, 4.9%; P, 2.6%; K, 3.6%; Mg, 4.6%; Na, 6.9%; Cl, 2.3%; S, 1.1%; and (per kg) 940 mg of Zn, 790 mg of Mn, 220 mg of Cu, 15 mg of I, 17 mg of Co, 10 mg of Se, 145 KIU of vitamin A, 33 KIU of vitamin D, and 890 IU of vitamin E.

⁶NE_L calculated from NRC (2001) based on the energy content of feedstuffs for a cow consuming at 3 × maintenance and the energy values of cottonseed obtained in the current study.

⁷NFC = Nonfibrous carbohydrates; NFC = OM – [CP + Fat + (NDF – NDF insoluble CP)].

separation. Plasma was frozen at -25°C and later analyzed for gossypol (Mena et al., 2001).

Characterization and Incidence of Health Disorders

Incidence of health disorders were recorded daily for individual cows by herd personnel. In addition, two of the authors visited each site at least once weekly and each individual cow was evaluated for signs of clinical disease. All cows were examined for clinical signs of mastitis by herd personnel during milking twice daily in sites 1 and 3, and three times daily in site 2. Clinical mastitis cases were characterized by the presence of abnormal milk, or by signs of inflammation in one or more quarters, and were treated by intramammary infusion of antibiotics according to treatment protocols established by the herd veterinarian. Prior to the first intramammary infusion of antibiotics, when mastitis was diagnosed, an aseptic fore sample of milk was collected, immediately frozen, and later taken to the Milk Quality Laboratory (VMTRC in Tulare, CA) for microbiological culture in blood agar, brain and heart infusion, and Mycoplasma medium. A new case of mastitis was defined for the same cow when a different quarter was affected or a period of 21 d had elapsed since the previous clinical mastitis diagnosis.

Diagnosis of left displacement of abomasum was based upon clinical signs presented by the affected cow, which included reduced milk production, rumen atony, ketonuria (Ketostix[®], Bayer Co., Pittsburgh, PA), diarrhea, and presence of an acute ping sound at auscultation and percussion on the left side of the abdomen. The diagnosis was performed either by trained dairy personnel or by the herd veterinarian. When an animal with displaced abomasum was diagnosed, the affected cow was treated by surgical correction in site 1 or by toggle pin suture in sites 2 and 3 (Bartlett et al., 1995).

The diagnosis of lameness was based on weekly visual observation of cows when walking from the milking parlor back to the free-stall barns, and further confirmed by visual inspection of the hoof on a trimming table by the hoof trimmer. In sites 2 and 3, a trained dairy personnel performed all the hoof trimming, and in site 1 a commercial hoof trimmer visited the dairy weekly.

All cows that were sold or died during the study were recorded. Dead cows were subjected to a post-mortem examination within the first 24 h of death to determine whether the cause of death was related to gossypol consumption. A macroscopic examination was performed by an experienced pathologist (C.A. Holmberg) and further histologic examination was performed as needed.

Reproductive Management

Reproductive data were collected for the entire experimental period. On $d\ 33 \pm 3$, and again on $d\ 47 \pm 3$ postpartum,

all cows received an injection of 25 mg of $\text{PGF}_{2\alpha}$ i.m. (Lutalyse, Dinoprost Tromethamine; Pharmacia & Upjohn, Kalamazoo, MI). Following the second $\text{PGF}_{2\alpha}$ treatment, estrus was detected once daily by visual observation and by tail chalking using paintsticks (All-weather Paintstik, LA-CO Industries, Chicago). Cows observed in estrus during the 7 d following the second $\text{PGF}_{2\alpha}$ treatment were used for the analysis of estrus detection. Artificial insemination was performed in all cows after a postpartum voluntary waiting period of 47 ± 3 d. Within each dairy AI was performed by the same technician, except for 1 d each week when a relief technician inseminated the cows. Those cows not found in estrus up to 14 d of the second $\text{PGF}_{2\alpha}$ treatment were then enrolled in a timed AI protocol (Pursley et al., 1995).

Pregnancy diagnosis was performed by rectal palpation between 35 and 45 d after AI, and pregnant cows had their pregnancy reconfirmed immediately prior to completing the study period, between 172 and 194 DIM. Cows that were not diagnosed as pregnant during the rectal exam were enrolled in a timed AI protocol to assure that all animals would be reinseminated in the following 10 d of the pregnancy exam. Estrus detection rate after two $\text{PGF}_{2\alpha}$ treatments, DIM at first postpartum AI, conception rate at first and subsequent inseminations, conception rate for all AI during the study, overall pregnancy rate at the end of the study, number of services per conception, days open for pregnant cows, days open for all cows, incidence of abortions, and incidence of cystic ovarian disease were all evaluated.

Days open were defined as the number of days from calving to conception for those cows diagnosed as pregnant, in addition to the DIM when a cow left or completed the study (183 DIM) for any cow diagnosed as not pregnant at the end of the study. Conception rate was defined as the proportion of pregnant animals relative to the number of artificially inseminated cows in each treatment for each postpartum AI. Pregnancy rate was defined as the number of pregnant animals out of the total number of cows on each diet. A cystic ovary was determined by the presence in one ovary of a soft, follicle-like structure with an estimated size of more than 25 mm in the absence of a corpus luteum. In sites 2 and 3, cystic ovaries were diagnosed by rectal palpation, and in site 1, diagnosis was performed by ultrasonography.

Experimental Design and Statistical Analyses

The experimental design was a randomized complete block design (Kuehl, 1994). Within each dairy, cows were blocked according to parity and previous lactation 305-d mature equivalent milk yield (multiparous) or age at calving (primiparous), and randomly assigned to one of the two diets.

Continuous variables such as DIM when a health disorder was diagnosed were analyzed by the GLM procedure of SAS (1999) using a model that included the observed mean, diet treatment effects, parity effects, dairy effects, interaction between diet and parity effects, interaction between diet and dairy effects, and random experimental error.

Plasma gossypol concentrations were analyzed by ANOVA for repeated measures by the PROC MIXED procedure of SAS (Littell et al., 1998) using a model that included the observed mean, diet effects, parity effects, dairy effects, period effects (61 vs 91 DIM), interaction between diet and parity effects, interaction between diet and dairy effects, interaction between diet and period effects, and interaction between diet and parity and period effects, with cow nested within diet as the random experimental error.

Reproductive data with dichotomous outcomes such as conception rate, incidence of abortion, and pregnancy rate were all analyzed by logistic regression (Stokes et al., 1995) using the LOGISTIC procedure of the SAS (1999) program according to a model that included the observed outcome, diet effects, parity effects, dairy effects, BCS at 61 DIM effects, milk production effects, TG in plasma effects, and higher order interactions. To determine if an interaction between diet and postpartum service number (i.e., first, second, third, and fourth postpartum AI) occurred on conception rates, conception rate for the first 4 postpartum AI in all cows was analyzed by logistic regression with the effects of diet, parity, dairy, and the interaction between diet and postpartum AI number.

Health data with dichotomous outcomes such as mortality and culling rates, incidence of cows that left the study, and incidence of health disorders were all analyzed by logistic regression (Stokes et al., 1995) using the LOGISTIC procedure of the SAS (1999) program according to a model that included the observed outcome, diet effects, parity effects, dairy effects, TG in plasma effects, interaction between diet and parity effects, interaction between diet and dairy effects, and the interaction between diet and plasma TG effects. Because mortality rate was low and only 12 cows died during the course of the experiment, mortality rate was analyzed by Chi-square with only the effect of diet in the model.

In order to characterize PG concentrations in cows that experienced or did not experience any health problem, in those that were pregnant or were open during the study, and in those that experienced abortion, plasma TG concentration was analyzed with a model including diet, the variable evaluated, the interaction between diet and the variable evaluated, and random experimental error.

To determine the effect of diet on DIM when a cow was first artificially inseminated, left the study, and con-

ceived (days open), the product limit method of the Kaplan-Meier model (Kaplan and Meier, 1958) for the survival analysis procedure of the Minitab (MINITAB, 1996) program was utilized. For both variables, an event was defined as inseminated, left the study, and conceived, respectively. Cows that did not experience an event at the end of the study were censored at 183 DIM. Univariate differences in respective cumulative proportion between WUP and BUPCP were assessed using the log-rank nonparametric method.

Diet differences with $P \leq 0.05$ were considered significant and $P > 0.05$ and $P \leq 0.15$ were considered a tendency.

RESULTS AND DISCUSSION

Plasma Gossypol

Feeding BUPCP increased mean plasma TG, as well as concentrations of the (+) and the (–) isomers ($P < 0.0001$; Table 2). Although the TG and FG content of the diet increased only 32% when cracked Pima replaced a portion of the WUP cottonseed, mean plasma TG more than tripled in cows fed BUPCP compared with those fed WUP (8.33 vs 2.65 $\mu\text{g/ml}$; $P < 0.001$). Gossypol in the free form is available for absorption, and a relationship between FG intake and plasma gossypol concentrations was established for lactating Holstein cows fed combinations of WUP cottonseed and cottonseed meal (Mena et al., 2001). However, the increase in plasma TG and gossypol isomers when cows were fed BUPCP cannot be explained solely by the gossypol content of the diet and the FG intake. In a companion study, Santos et al. (2002) observed that cows consuming a blend of WUP and cracked Pima cottonseed had considerably higher PG concentrations during the first 152 DIM than those fed WUP (3.07 vs 7.76 $\mu\text{g/ml}$; $P < 0.001$), despite the fact that FG intake only increased 32%. Mena et al. (1997) fed Holstein steers various combinations of cottonseeds and observed that cracking of WUP and Pima cottonseeds increased plasma concentrations of TG, although TG intake measured as g/d or as mg/kg of BW per day did not differ. Recently, Prieto et al. (2003) observed that when cracked Pima replaced all the WUP cottonseed in the diet, TG intake increased 57% and 41% for primiparous and multiparous cows, respectively, while plasma TG concentrations more than doubled in primiparous and multiparous cows.

The increase in plasma concentrations of gossypol is related to gossypol intake as well as the extent of gossypol detoxification in the reticulum-rumen. Santos et al. (2002) suggested that the lack of lint, decreased particle size after cracking, and increased density of cracked Pima compared with WUP cottonseed might affect the mean rumen retention time of the seed. Mena et al.

Table 2. Effect of type of cottonseed and dietary gossypol on plasma gossypol concentrations in dairy cows.

	Treatment ¹				SEM	<i>P</i> <		
	WUP		BUPCP			TRT	Parity	TRT × Parity
	Primiparous	Multiparous	Primiparous	Multiparous				
Plasma gossypol, µg/ml								
Total ^{ab}	2.41	2.90	7.63	9.03	0.36	0.0001	0.0001	0.002
(+) isomer ^{ab}	0.94	1.13	2.76	3.12	0.13	0.0001	0.0001	0.10
(−) isomer ^{ab}	1.47	1.77	4.86	5.89	0.24	0.0001	0.0001	0.0002
Isomer, % TG								
(+) isomer ^{ab}	39.6	39.5	35.9	34.6	0.002	0.0001	0.005	0.005
(−) isomer ^{ab}	60.4	60.5	64.1	65.4	0.002	0.0001	0.005	0.005

^aSignificant effect of period (61 vs 91 DIM) when blood was collected ($P < 0.001$).

^bSignificant interaction between treatment and period ($P < 0.001$).

¹WUP = whole Upland cottonseed diet containing 717 mg of free gossypol/kg of DM, BUPCP = blend of whole Upland and cracked Pima cottonseed diet containing 951 mg of free gossypol/kg of diet DM.

(2001) proposed that gossypol detoxification within the rumen might be reduced when mean rumen retention time of gossypol containing feeds is reduced. Therefore, the increase PG concentration associated with feeding processed Pima cottonseed may be a consequence of its higher gossypol content and its faster rate of passage compared to WUP. Prieto et al. (2003) observed that cows fed cracked Pima cottonseed produced milk with increased concentration of C18:2 compared with WUP, which supports the proposed increased rumen passage rate. In primiparous cows, total replacement of WUP with cracked Pima cottonseed increased milk linoleic acid from 2.36 to 2.77% of the total milk fatty acids. Similarly, in multiparous cows, total replacement of WUP with cracked Pima cottonseed increased milk linoleic acid from 2.61 to 3.38% of the total milk fatty acids. Such changes in milk fatty acid composition might reflect reduced biohydrogenation of α -linoleic acid within the rumen and greater incorporation into milk fat, which reinforces the idea that rumen retention time of cracked Pima cottonseed is probably reduced compared with WUP cottonseed. The reduced rumen retention of Pima cottonseed would increase gossypol availability for absorption, probably by reducing detoxification within the rumen.

The proportion of TG in plasma represented by the (–) isomer increased ($P < 0.0001$) when cows were fed the higher gossypol diet. This response was anticipated because cracked Pima cottonseed contained more (–) isomer as percentage of TG than WUP cottonseed (Santos et al., 2002). In addition, an interaction between treatment and parity on the plasma concentration of (–) isomer was observed since the BUPCP diet resulted in a greater increase in plasma (–) gossypol isomer in multiparous than in primiparous cows. Similarly, the ratio of (+) and (–) isomers varied with diets, and cows fed the BUPCP diet had a higher proportion of TG as the (–) isomer (P

< 0.0001). Furthermore, a treatment by period interaction on the concentrations of plasma TG and (–) isomer was observed ($P < 0.0001$), and cows fed BUPCP had a greater increase in PG from 61 to 91 DIM than cows fed WUP (Figure 1). Santos et al. (2002) collected blood samples from 56 cows monthly, during the first 5 mo in lactation, for measurement of PG concentrations. Plasma TG concentrations increased 24% from 91 to 152 DIM (1.74 $\mu\text{g/ml}$), and it had not plateaued by 152 DIM. These data suggest that higher PG values could be expected had samples been collected later in lactation. The effect of parity on plasma concentrations of gossypol is probably caused by the higher intakes of DM, and consequently

Figure 1. Least square means of plasma gossypol ($\mu\text{g/ml}$) concentrations in lactating dairy cows fed varying amounts of gossypol from whole linted Upland cottonseed (WUP) or a blend of WUP and cracked Pima cottonseed (BUPCP) during the first 183 DIM. Total gossypol: dashed line (---; SEM = 0.34); (–) gossypol isomer: solid line (—; SEM = 0.23); WUP: solid square (■); BUPCP: solid triangle (▲). Significant effects: treatment ($P < 0.0001$); period ($P < 0.0001$); and treatment by period ($P < 0.0001$).

FG. Increasing FG intake has directly increased PG concentrations.

Culling and Mortality of Dairy Cows

Culling rates of dairy cows did not differ between the two diets (Table 3). Thirty-one and 27 cows were sold during the study in WUP and BUPCP groups, respectively. Plasma gossypol concentration had no effect on culling rates, and cows with plasma TG above or below the mean PG had culling rates of 4.1 and 5.9%, respectively.

Concurrent with culling rates, mortality of cows was similar between both groups and the same number of cows (6) died per diet. Of the cows that died during the study, two died because of lymphosarcoma of the heart and abomasum, two because of reticuloperitonitis caused by a perforating foreign object in the forestomach, two because of septicemia caused by *Salmonella dublin*, one trapped under the loop in the stall overnight, three died during the first 3 wk postpartum of a combination of uterine infection and ketosis with increased fatty infiltration in the liver, and two died of coliform mastitis with signs of septicemia. Tissue samples were collected from a number of the cows for histologic examinations to determine whether gossypol toxicity was involved in the cause of death. None of the cows had lesions in the heart, liver, kidneys and gastrointestinal tract that would be compatible with gossypol toxicosis (Holmberg et al., 1988). Fatty degeneration of hepatocytes has been demonstrated in calves that died from gossypol toxicity (Holmberg et al., 1988). The three cows with signs of hepatic lipidosis died during the first 3 wk postpartum and had been diagnosed with fever, uterine infection, and clinical ketosis, which explain the hepatic changes observed histologically. In addition, 3 wk of feeding cottonseed during early postpartum, when DM intakes are low, is extremely unlikely to cause gossypol toxicosis.

For those cows that left the study either dead or culled prior to the end of the 170-d experimental period, the

interval from calving to removal from the study did not differ between the two diets ($P < 0.91$; Table 3). Furthermore, cows that left the study had similar plasma TG concentrations at 61 DIM to those that remained in the study for the 170-d period (Table 4). When survival analysis of DIM when cows left the study was performed, feeding diets with different gossypol concentrations had no impact on cow survivability ($P < 0.51$; Figure 2).

The lack of an association between plasma TG concentrations and culling rates indicates that the concentrations of PG observed here do not increase the likelihood of cows leaving the herd during the first 183 DIM. Generally, cows leave a herd during lactation because of health problems that impair milk production (Gröhn et al., 1998). Since dietary gossypol concentrations had no effect on milk yield (Santos et al., 2002) or on incidence of health disorders, it is unlikely that feeding diets with up to 950 and 1000 mg/kg of FG and TG, respectively, affects culling rate of dairy cows.

Lindsey et al. (1980) reported that a cow fed a diet high in gossypol from solvent-extract cottonseed meal died with clinical signs and lesions suggestive of gossypol toxicity. This cow had been consuming the diet for 81 d, and the daily intakes of FG and TG were 8 and 222 mg/kg of body weight/d. For an average Holstein cow weighing 650 kg, the respective daily intakes of FG and TG would be 5.2 and 144 g/d. The mean daily FG and TG intakes for cows fed the BUPCP diet were 22.8 and 24.0 g/d (Santos et al., 2002). The discrepancy between FG and TG intakes between the two studies is because cottonseed meal is high in TG, but most of gossypol is in the bound form. In contrast, whole cottonseed has lower TG content than cottonseed meal with 95% or more of the gossypol in the free form. Although cows in the study by Lindsey et al. (1980) were consuming extremely high TG diets, very little was in the free form and it is unlikely that the 5.2 g/d of FG ingested by that cow would elevate PG concentrations above those observed for the current study. Indeed, PG concentrations in Lindsey et

Table 3. Effect of type of cottonseed and dietary gossypol on mortality and culling rates of dairy cows (LSM $\pm$ SEM).

	Treatment ¹		$P <$
	WUP	BUPCP	
Culling rate, %	7.65	6.37	0.47
DIM when culled, d	84.4 ($\pm$ 11.4)	90.5 ($\pm$ 17.2)	0.77
Mortality rate, %	1.48	1.47	0.99
DIM when dead, d	70.0 ($\pm$ 23.1)	65.8 ($\pm$ 26.8)	0.91
Left study, ² %	9.14	7.84	0.51
DIM when left study, d	81.2 ($\pm$ 10.2)	79.2 ($\pm$ 14.3)	0.91

¹WUP = whole Upland cottonseed diet containing 717 mg of free gossypol/kg of DM; BUPCP = blend of whole Upland and cracked Pima cottonseed diet containing 951 mg of free gossypol/kg of diet DM.

²Cows that died or were culled prior to the end of the 170-d experimental period.

Table 4. Association between plasma total gossypol (TG) on d 61 postpartum in cows that experienced (Yes) or did not experience (No) health disorders (LSM $\pm$ SEM).

Health disorder	Plasma TG ¹ , $\mu\text{g/ml}$		<i>P</i> < ²	
	Yes	No	TG	TRT $\times$ TG
Death	5.73 ($\pm$ 0.85)	6.00 ($\pm$ 0.09)	0.75	0.79
Culled	5.57 ($\pm$ 0.38)	6.01 ($\pm$ 0.09)	0.25	0.34
Left study ³	5.60 ($\pm$ 0.34)	6.02 ($\pm$ 0.09)	0.24	0.44
Mastitis	5.80 ($\pm$ 0.24)	6.02 ($\pm$ 0.09)	0.39	0.58
Lameness	6.01 ($\pm$ 0.23)	5.97 ($\pm$ 0.09)	0.68	0.28
Any health disorder ⁴	5.91 ($\pm$ 0.16)	6.03 ($\pm$ 0.10)	0.52	0.83

¹Plasma total gossypol (TG) in cows that experienced (Yes) or not (No) the health disorder.

²TG = effect of total gossypol; TRT $\times$ TG = interaction between treatment and total gossypol.

³Left study = cows that left the study prior to their 170-d experimental period.

⁴Any health disorder = includes death, mastitis, lameness, left displacement of abomasums, and miscellaneous problems.

al. (1980) study were low and usually below 2 $\mu\text{g/ml}$ throughout the 14-wk feeding period.

In a study with growing Holstein heifers, feeding up to 30% of the diet DM as whole cottonseed did not have a substantial impact on the biochemical parameters measured (Collin-Negrete et al., 1996). The most consistent response to increasing intake of FG from cottonseed was an increase in plasma lipid concentration. This response would be expected since feeding more cottonseed increased the intake of dietary fat. One heifer receiving the high cottonseed diet consumed approximately 2000 mg of gossypol per kg of TMR and died after 396 d on the study. The authors reported that diets containing 15% whole cottonseed could be fed safely to growing heifers, but additional information is required on the safety

of feeding a diet with 30% whole cottonseed. Nevertheless, PG concentrations were not reported and the study population was limited to five animals per treatment group.

Contrary to the findings of Lindsey et al. (1980), the present study demonstrated that feeding diets with up to 950 mg/kg of FG from a blend of WUP and cracked Pima cottonseed to lactating dairy cows for a period of 170 d had no effect on mortality and culling rates, even though PG concentrations were 4 to 5-fold greater. Our results agree with recommendations by Collin-Negrete et al. (1996) and Mena et al. (2001) in which diets with up to 1000 mg/kg of FG from cottonseed can be fed to dairy cattle with no increased risk for gossypol toxicosis and death.

Figure 2. Proportion of cows fed whole Upland cottonseed (—; LSMean = 159.1; SEM = 1.28) and of cows fed a blend of whole Upland and cracked Pima cottonseed (---; LSMean = 161.7; SEM = 1.23) remaining in the study. Cows were censored at 183 DIM. Treatment effect ($P < 0.51$).

Incidence of Health Problems

Type of cottonseed and dietary gossypol intake had no effect on incidence of health disorders (Table 5). Incidence of clinical mastitis did not differ between cows fed WUP and BUPCP, and it averaged 13.4%. Plasma gossypol concentrations had no effect on mastitis incidence, and no association between plasma TG and mastitis was observed (Table 4). Similarly, no association between the (–) gossypol isomer and health disorders were observed. Incidence of mastitis in cows with plasma concentrations of TG above the mean was 10.1% compared with 16.0% for those with plasma TG below the mean gossypol concentrations ($P = 0.16$). The interval from the beginning of the study to the diagnosis of the first mastitis case was similar ($P < 0.28$) for both treatment groups, and it averaged 79.5 d.

Incidence of lameness and DIM at the diagnosis of the first lameness case did not differ for cows fed the WUP and BUPCP diets. Incidence of miscellaneous health problems or any clinical health problem was similar for

Table 5. Effect of type of cottonseed and dietary gossypol on incidence of health disorders in dairy cows (least squares means $\pm$ SEM).

Health disorder	Treatment ¹		<i>P</i> <
	WUP	BUPCP	
Mastitis, %	16.3	10.5	0.22
DIM at first mastitis, d	73.4 ($\pm$ 7.4)	85.5 ($\pm$ 8.3)	0.28
Lameness, %	11.1	16.2	0.57
DIM at lameness diagnosis, d	94.9 ($\pm$ 10.1)	109.4 ($\pm$ 8.5)	0.27
Miscellaneous health problems ²	7.7	7.8	0.96
Any health problem, %	30.9	31.6	0.89

¹WUP = whole Upland cottonseed diet containing 717 mg of free gossypol/kg of DM, BUPCP = blend of whole Upland and cracked Pima cottonseed diet containing 951 mg of free gossypol/kg of diet DM.

²Miscellaneous health problems = diarrhea, left displacement of abomasum, pneumonia, undetermined fever.

both diets. Overall, 31.3% of all cows suffered from one or more health problem during the course of the study.

In general, a greater proportion of cows with low PG concentrations suffered from health disorders. After categorizing cows with plasma TG as above or below the mean plasma concentration, 29.7% of the cows fed WUP and 43.1% of those fed BUPCP with plasma TG below the mean experienced at least one health problem compared with 16.7 and 28.6% of cows fed WUP and BUPCP, respectively, but with PG above the mean ($P < 0.09$). Although there was a tendency for a greater proportion of cows with plasma TG below the mean PG concentration to experience health problems in both treatment diets, cows with health disorders had similar plasma TG concentrations at d 61 postpartum to those that did not experience any disease (Table 4), as well as at 91 d postpartum ($P > 0.15$).

It has been suggested that the (–) gossypol isomer is more biologically active than the (+) isomer (Wang et al., 1986). When PG (–) isomer concentrations were included in the analyses of health data in addition to plasma TG concentrations, no effect of (–) isomer concentration was observed for any of the health problems evaluated ($P > 0.15$).

In some studies, feeding diets with a high FG content resulted in increased erythrocyte fragility in dairy cows (Lindsey et al., 1980; Mena et al., 2001), as well as growing dairy heifers (Collin-Negrete et al., 1996). Alterations in red blood cell structure and metabolism could potentially predispose cows to diseases. Thus, diets that result in higher PG concentrations might be expected to disturb cell membrane metabolism and increase susceptibility of cows to health problems. However, our data indicated that feeding diets containing up to 950 and 1000 mg/kg of FG and TG, respectively, from a blend of WUP and cracked Pima cottonseed for the first 6 mo of lactation had no effect on incidence of the commonly observed diseases of lactating dairy cows when compared with cows fed a diet with lower gossypol content from WUP.

Indeed, no association between plasma concentrations of TG and gossypol isomers with incidence of commonly observed health disorders in lactating dairy cows was found. Furthermore, cows that suffered from any health disorder had similar PG concentrations to those that did not experience any health problem (Table 4). Therefore, increasing dietary gossypol concentrations from 720 up to 1000 mg/kg of DM had no effect on health of dairy cows.

Reproductive Performance

Twenty-one cows were excluded from the statistical analyses of reproductive performance because they either died or were culled before d 60 of the study. Of the remaining 792 cows, 395 cows received the WUP diet and 397 received the BUPCP diet.

After receiving two injections of PGF_{2 α} for estrus synchronization, estrus detection rate in the following 7 d of the second PGF_{2 α} tended ($P = 0.10$) to be higher for cows consuming the higher gossypol diet (Table 6). In fact, cows with a higher plasma TG had a higher estrus detection rate ($P < 0.01$). When plasma TG was divided into quartiles, estrus detection rate was highest for cows in the fourth (i.e., highest plasma TG concentrations) quartile, and they were 47.7%, 55.4%, 49.5%, and 65.5% for plasma TG quartiles Q1, Q2, Q3, and Q4, respectively. These data suggest that PG did not interfere with estrus expression after synchronization of estrus with PGF_{2 α} . Plasma gossypol concentrations are correlated with intake of FG (Mena et al., 2001). It is possible that cows consuming more diet DM were more likely to be cycling and so would respond to PGF_{2 α} by displaying signs of estrus. This may explain the positive association between higher plasma TG and estrus detection rate.

Postpartum cyclicity is affected by energy balance in dairy cows (Butler and Smith, 1989), and most of the variation in postpartum energy balance in early lactation dairy cows was due to DM intake. Cows in better energy balance tend to be at a higher BCS and are more likely

Table 6. Effect of type of cottonseed and dietary gossypol on reproductive parameters in dairy cows (LSM $\pm$ SEM).

Reproductive parameter	Treatment ¹		<i>P</i> <
	WUP	BUPCP	
Estrus detection rate, %	51.5	57.0	0.10
Cystic ovarian disease, %	5.6	7.4	0.45
DIM at first AI, ² d	58.9 (± 0.63)	58.7 (± 0.56)	0.99
Conception at first AI, %	28.2	29.3	0.73
Conception at >1 st AI, %	36.8	30.7	0.04
Conception at all AI, %	33.4	30.3	0.03
SPC ³	2.22 (± 0.15)	2.58 (± 0.15)	0.14
Abortion incidence, %	3.3	7.9	0.01
Pregnancy rate, ⁴ %	79.1	70.6	0.01

¹WUP = whole Upland cottonseed diet containing 717 mg of free gossypol/kg of DM, BUPCP = blend of whole Upland and cracked Pima cottonseed diet containing 951 mg of free gossypol/kg of diet DM.

²AI = artificial insemination.

³SPC = services per conception.

⁴Pregnancy rate = proportion of pregnant cows at the end of the 170-d experiment.

to be cycling during the first 68 DIM (Stevenson, 2001). In fact, cows with a BCS equal to or lower than 2.75 at 61 DIM had lower estrus detection rate than those with a BCS equal to or greater than 3.0 (38.0 vs 56.8%; $P < 0.01$). Similarly, cows with a BCS equal to or lower than 2.75 at 61 DIM had lower plasma TG (5.4 vs 6.9; $P < 0.01$) and (–) isomer (3.7 vs 4.1; $P < 0.01$) than cows with a BCS equal to or higher than 3.0, and this effect was detected regardless of treatment because no diet by BCS interaction was observed ($P = 0.20$). Therefore, it is possible that more cows with higher PG displayed signs of estrus because they had higher DM intakes and were in better energy status.

Incidence of cystic ovaries averaged 6.5% in lactating cows, and no effect of treatment ($P < 0.45$) or PG concentrations ($P < 0.20$) were observed for cystic ovarian disease. Incidence of cystic ovaries was higher in multiparous than in primiparous cows (8.0 vs 2.0 %; $P < 0.01$), and higher producing cows had a higher incidence of cystic ovarian disease. When milk production was categorized as above or below the mean milk yield, cows with production above the mean had a higher ($P < 0.01$) incidence of cystic ovaries than those with milk production below the mean (9.14 vs 3.71%).

The effects of type of cottonseed and PG concentrations on fertility of dairy cows varied throughout the study. The interval from calving to first postpartum AI was similar between the two treatment diets (Figure 3) and it averaged 58.8 d. Type of cottonseed and dietary gossypol had no effect on first postpartum AI conception rates (Table 6), but fertility decreased after the first AI for cows fed the BUPCP diet (Figure 4). An interaction between diet and postpartum AI number on conception rate was observed ($P < 0.04$; Figure 4), which reinforces the proposal that the effects of dietary gossypol on conception varied as the lactation progressed.

It was suggested that the female ruminant might be relatively insensitive to the antifertility effects of gossypol (Randel et al., 1992; Gray et al., 1993); but in vitro data indicate inhibition of embryonic development and ovarian steroidogenesis (Brocas et al., 1997; Zirkle et al., 1988). Recently, Brocas et al. (1997) observed that oocyte cleavage rate and subsequent development were not affected by in vitro concentrations of gossypol below 5 $\mu\text{g}/\text{ml}$. In contrast, increasing gossypol concentration in the media to 10 $\mu\text{g}/\text{ml}$, which is similar to the gossypol concentration observed in the plasma of cows fed BUPCP cottonseed after 91 DIM (Santos et al., 2002), reduced embryo cleavage rate. Brocas et al. (1997) concluded that

Figure 3. Proportion of cows fed whole Upland cottonseed (—; LSMeans = 58.9; SEM = 0.63) and of cows fed a blend of whole Upland and cracked Pima cottonseed (---; LSMeans = 58.7; SEM = 0.56) remaining non-inseminated over the course of the study period. Cows were censored at 183 d in milk. Effect of treatment ($P < 0.99$).

Figure 4. Effect of type of cottonseed and gossypol intake on conception rates (CR) of dairy cows during the first four postpartum artificial inseminations (AI). Solid black bars: whole linted Upland cottonseed diet; White bars with vertical shading: blend of whole linted Upland and cracked Pima cottonseed. Effect of treatment: * $P < 0.10$; ** $P < 0.05$. An interaction between treatment and service number was detected on conception rates ($P < 0.04$).

developing embryos are sensitive to gossypol and diets that increase PG concentrations above $5 \mu\text{g/ml}$ might negatively impact fertility of cows. However, it is not known if PG concentrations also reflect uterine concentrations of gossypol, but if this situation occurs, PG concentrations above $5 \mu\text{g/ml}$ would be expected to suppress fertility of cows.

The distinct effects of dietary gossypol and PG concentrations on conception rates at the first and subsequent AI might be related to concentrations of PG and the period of time that cows were fed gossypol. The mean days from calving to first postpartum AI was 58.8 d (95% CI = 57.9 to 59.7) and the median was 53 d, and they did not differ between treatments. Therefore, the first AI was performed in most cows prior to 61 d postpartum, when plasma TG concentrations were 2.42 and $7.13 \mu\text{g/ml}$ for cows fed the low (WUP) and high gossypol (BUPCP) diets, respectively. In addition, the period of time that cows were fed gossypol was lower for the first compared with the second and subsequent postpartum AI ($P < 0.001$). When the second and subsequent postpartum AI were performed, plasma TG concentrations were above $10 \mu\text{g/ml}$ for cows fed the higher gossypol diet. Therefore, it is possible that the combination of time exposed to gossypol with increased PG concentrations later in lactation might have resulted in reduced conception rates in cows fed the higher gossypol containing diet. When days open were analyzed by survival analyses, it is clear that cows fed BUPCP had decreased rate of pregnancy after approximately 70 to 80 DIM compared with those fed WUP (Figure 5). Collectively, these data suggest that gossypol intake only affected fertility after cows were exposed to it for 70 to 80 d, which was the

required time to increase plasma TG above $10 \mu\text{g/ml}$ in cows fed BUPCP.

Incidence of abortions was higher for cows fed BUPCP compared with WUP (7.9 vs 3.3%; $P < 0.01$). The interval from AI to diagnosis of abortion was 92.6 and 89.6 d for cows fed WUP and BUPCP ($P < 0.75$). Similarly, the interval from pregnancy diagnosis to diagnosis of abortion did not differ between the two treatments ($P < 0.44$), and it averaged 49.4 d.

Figure 5. Proportion of cows fed whole Upland cottonseed (—; LS Mean = 111.2; SEM = 2.35) and of cows fed a blend of whole Upland and cracked Pima cottonseed (---; LS Mean = 119.1; SEM = 2.59) remaining non-pregnant over the course of the study period. Cows were censored at 183 d in milk. Significant effect of treatment ($P < 0.01$).

Figure 6. Proportion of cows remaining non-pregnant over the course of the study period according to plasma gossypol concentrations. Cows were categorized into quartiles based upon the mean plasma total gossypol concentrations: Quartile 1 (Q1) = 0.92 to 3.64 $\mu\text{g/ml}$; Quartile 2 (Q2) = 3.65 to 5.48 $\mu\text{g/ml}$; Quartile 3 (Q3) = 5.49 to 9.04 $\mu\text{g/ml}$; and Quartile 4 (Q4) = 9.06 to 18.93 $\mu\text{g/ml}$. Least square means $\pm$ SEM for days open were: Q1 = 107.9 ± 3.03 ; Q2 = 111.3 ± 2.99 ; Q3 = 113.3 ± 3.21 ; and Q4 = 115.6 ± 3.28 ($P < 0.05$). Cows were censored at 183 d in milk.

The decrease in conception rate after first postpartum AI and the increase in incidence of abortions for cows fed BUPCP resulted in a reduced proportion of pregnant cows at the end of the 170-d experiment (79.1 vs 70.6; $P < 0.01$) and extended the period from calving to conception ($P < 0.01$; Figure 5). The effect of diet on days open was associated with PG concentrations. When TG concentrations in plasma were divided into quartiles, cows with higher PG also had increased interval from calving to conception ($P < 0.05$; Figure 6). The negative effect of BUPCP compared with WUP on days open was probably not caused by the type of cottonseed itself, but because of the higher PG concentrations in those cows fed BUPCP. For those cows diagnosed as non-pregnant after the first postpartum AI, a greater proportion of cows fed BUPCP were within the high plasma gossypol quartiles (Q3 and Q4) compared with a greater proportion of cows fed WUP within the low gossypol quartiles (Q1 and Q2). For plasma TG quartiles Q1, Q2, Q3 and Q4, the proportion of cows fed BUPCP within each quartile was 0.7, 12.9, 84.2, and 100%, respectively. Nevertheless, as plasma TG concentrations in cows increased, conception rates for the inseminations performed subsequent to the first postpartum AI during the study decreased ($P < 0.04$; Figure 7).

Cows that were not pregnant at the conclusion of the study had higher plasma TG concentrations than those that were diagnosed pregnant during the study ($P < 0.01$;

Figure 7. Effect of plasma gossypol (PG) concentrations on conception rates (CR) of dairy cows after the first postpartum artificial insemination. Quartile 1 (Q1) = 0.92 to 3.64 $\mu\text{g/ml}$; Quartile 2 (Q2) = 3.65 to 5.48 $\mu\text{g/ml}$; Quartile 3 (Q3) = 5.49 to 9.04 $\mu\text{g/ml}$; and Quartile 4 (Q4) = 9.06 to 18.93 $\mu\text{g/ml}$. Effect of PG quartile on conception rate ($P < 0.04$).

Figure 8). Similarly, cows that experienced pregnancy loss as the study progressed also had higher plasma TG concentrations than those that did not abort their fetus ($P < 0.04$; Figure 9). Interestingly, the probability of a cow conceiving after the first postpartum AI decreased at a decreasing rate as the plasma TG concentration increased ($P < 0.006$; Figure 10). This suggests that fertility in dairy cows was suppressed as plasma TG concentrations increased, but no further suppression was observed in conception after plasma TG reached approximately 15 $\mu\text{g/ml}$. Furthermore, the relationship between plasma TG and the probability of conception (Figure 10) indicates that even at plasma TG concentrations below 5 $\mu\text{g/ml}$, the probability of a cow conceiving after the

Figure 8. Plasma total gossypol (TG) concentrations ($\mu\text{g/ml}$) in pregnant (black solid bar; SEM = 0.17) and open (white bar with vertical shading; SEM = 0.28) cows at the end of the 170-d experimental period. Values differ ($P < 0.05$).

Figure 9. Plasma total gossypol (TG) concentrations ($\mu\text{g/ml}$) in cows that did not experience (black solid bar; SEM = 0.17) and those that experienced (white bar with vertical shading; SEM = 0.63) abortion during the 170-d experimental period. Values differ ($P < 0.05$).

first postpartum AI is decreased as plasma TG increases. Perhaps, even low plasma TG concentrations impact fertility of lactating dairy cows by disrupting fertilization or establishment of pregnancy. However, this concept warrants further investigation with studies that include diets containing no gossypol.

In this study, evidence is presented for the first time that consumption of a diet high in gossypol resulting in increased PG concentrations impaired fertility of lactating dairy cows. The exact mechanisms by which gossypol might disturb establishment and maintenance of pregnancy in cattle is not known. Studies with cultured bo-

vine luteal cells suggest that steroidogenic activity of the corpus luteum may be impaired at gossypol concentrations similar to those observed for cows fed a high gossypol diet (Gu et al., 1990). Plasma progesterone concentrations affect conception in dairy cows and conceptuses from cows with higher plasma progesterone secrete more interferon Tau (Mann et al., 1999), which blocks the luteolytic mechanism that regresses the corpus luteum and signals the uterus to maintain pregnancy. It is possible that the higher PG concentration in cows fed BUPCP might retard embryo development (Zirkle et al., 1988; Brocas et al., 1997) and impairs steroidogenic activity of follicular (Lin et al., 1994) and luteal cells (Gu et al., 1990). Such effects could reduce embryo survivability, thereby decreasing conception rates and increasing the incidence of abortions.

Some of the discrepancy between in vivo and in vitro data on the effects of gossypol on luteal function and embryo development might be related to the ability of ruminant animals to detoxify gossypol and minimize its absorption. However, when diets increase PG to concentrations above 5 to 10 $\mu\text{g/ml}$, reproductive functions might be compromised and fertility depressed.

CONCLUSIONS

When cottonseed comprised 10% of the diet DM, replacement of whole linted Upland cottonseed with a 1:2 blend of whole linted Upland and cracked Pima cottonseed increased dietary FG content from 717 to 951 mg/kg of diet DM. The higher gossypol diet resulted in higher plasma gossypol concentrations at 61 and 91 d postpartum. Dietary gossypol concentration had no effects on incidence of diseases, or on culling and mortality rates. No association was observed between plasma concentrations of gossypol and incidence of health disorders. Although dietary gossypol did not suppress estrus detection rate and conception rate at first postpartum artificial insemination, cows consuming the high gossypol diet had impaired reproductive performance. The negative effects of gossypol on fertility of lactating dairy cows were only detected after approximately 70 to 80 d postpartum and it resulted in decreased conception rates and increased incidence of abortions, which resulted in extended days open. Cows that experienced abortion or were open at the end of the 170-d study had higher plasma gossypol concentrations. These data suggest that feeding a diet high in gossypol content for extended periods of time results in increased plasma gossypol concentrations, which impairs fertility of lactating dairy cows.

Figure 10. Probability of conception after the first postpartum artificial insemination (AI) according to plasma total gossypol (TG) concentrations ($\mu\text{g/ml}$). Conception = $0.505586 - 0.0301448 \text{ TG} + 0.0009661 \text{ TG}^2$, where TG = plasma TG concentration ($\mu\text{g/ml}$). Adjusted $r^2 = 0.88$. Quadratic effect of plasma TG on the probability of conceiving after the first postpartum AI was significant ($P < 0.006$).

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