Effects of different sources and levels of dietary gossypol on gossypol residues in plasma and milk of lactating cows

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ABSTRACT

Free gossypol residues in tissues or milk from feeding whole cottonseed and cottonseed meal were measured for their effect on health of dairy cows and humans. Forty lactating cows were randomly assigned to 5 treatments in a 60-d experiment to investigate the effects of sources and dietary level of gossypol on plasma and milk gossypol concentrations in lactating cows. Five experimental diets had identical net energy for lactation and crude protein content on a dry matter (DM) basis. Soybean meal was the main protein ingredient used in the control diet. Cottonseed meal (CSM) or whole cottonseed (WCS) substituted for part of the soybean meal in the other 4 diets. Gossypol levels in the 5 diets were 0 (control), 91.15 mg/kg of DM in CSM1, 117.31 mg/kg of DM in CSM2, 385.43 mg/kg of DM in WCS1, and 611.13 mg/kg in WCS2. Yields of 3.5% fat-corrected milk were significantly higher for cows in the WCS2 group; cows in the CSM1 and WCS1 groups showed no differences but both were numerically higher than the control and CSM2 groups. Milk protein concentration was lower for cows consuming WCS1 compared with the control group. Lactose concentration was lower for cows in the CSM2 group compared with the WCS2 group, but no differences were observed among other diets. Aspartate aminotransferase in serum was significantly higher for the WCS2 group compared with the control and WCS1 groups, but no difference was observed with the CSM1 and CSM2 groups. Concentrations of gossypol in plasma and milk of cows in the WCS1 and WCS2 groups were both higher than those of the other groups. No adverse effects were observed on cows fed diets containing 12.0% CSM, and no gossypol was found in plasma and milk. When WCS comprised 15% of the diet DM, yields of 3.5% fat-corrected milk were increased in cows and gossypol was detected in plasma and milk but not at harmful levels.

Key words: cottonseed, cottonseed meal, gossypol, dairy cow

INTRODUCTION

Whole cottonseed (WCS) and cottonseed meal (CSM) are extensively used as energy and protein sources in dairy cattle diets. However, both contain gossypol, a polyphenolic binaphthyl dialdehyde that can produce toxic effects in animals. Gossypol exists in free and bound forms. Free gossypol (FG) is toxic to animals, whereas the bound form is considered nontoxic because it cannot be absorbed in the digestive tract. Some researchers have found that bound gossypol may be released as FG during the digestion process, which can be absorbed by the digestive tract (Blackwelder et al., 1998; Noftsker et al., 2000; Mena et al., 2001). Absorbed gossypol either is retained in organs such as the kidney and in muscle, or it is excreted via feces, urine, or milk (Lin et al., 1992; Gamboa et al., 2001a,b; Wan et al., 2003). If the contaminated tissues or milk are consumed by humans, they could have detrimental effects. Thus, it is necessary to pay close attention to gossypol concentrations in animal products, but as yet, little information has been made available on gossypol residues in milk. Only 2 studies have reported milk gossypol concentrations of dairy cows, but used only limited numbers of animals (Zhong, 2007) or inappropriate detection methods (Wan et al., 2003). Objectives of this study were to feed varying amounts of gossypol from WCS or CSM over a long period of time and determine effects on plasma and milk gossypol concentrations in lactating dairy cows.

MATERIALS AND METHODS

Experimental Design

Design and Treatments. The experimental design was a randomized complete block. Cows were blocked according to parity and milk yield or age at calving, and randomly assigned to 1 of 5 diets. Forty early lacta-

Received August 25, 2011.

Accepted May 17, 2012.

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tion Holstein cows (86 ± 25 DIM) were assigned to 1 of 5 treatment diets (8 cows per diet) on the basis of parity, DIM, daily milk yield, and age at calving. The study period lasted 70 d. During a 10-d pretreatment period, all cows were fed a gossypol-free diet, which was also the control diet during the treatment period (control; Table 1). Experimental diets were fed as TMR and varied in their amounts of forage and concentrate to accommodate different amounts of CSM and WCS. Diets were formulated to be isonitrogenous and isoenergetic, and met the requirements of feeding standards of dairy cows [China NongYe HangYe BiaoZhun/Tuijian-34 (China NY/t34, 2004)]. Detailed descriptions of dietary ingredients and nutrient composition are presented in Table 1. Cows were assigned to 1 of 5 treatment groups: no gossypol (control group), 91.15 mg of FG/kg from CSM (CSM1 group), 117.31 mg of FG/kg from CSM (CSM2 group), 385.43 mg of FG/kg from WCS (WCS1 group), or 611.13 mg of FG/kg from WCS (WCS2 group).

Animals and Housing. Cows were housed in open pens equipped with a neck rail. When feeding, all cows were tied alone to enable taking measurements of daily feed intakes from individual cows. All pens were identical in design, size, and location. Diets were fed twice daily at 0500 to 0830 and 1700 to 2030 h. Cows were milked twice daily at 0500 and 1700 h. Water was available ad libitum.

Measurements and Data

Feed Intake and Feed. Whole cottonseed and CSM were both from Yongqing, Hebei Province, China. Individual feed intakes were recorded daily during the trial period, and the TMR composition is presented in Table 1.

Milk Yield and Composition. Individual milk samples were collected on d 0, 15, 30, 45, and 60 of the experimental period, milk yields recorded, and milk analyzed for fat, protein, lactose, and SNF by infrared

Table 1. Ingredient and nutrient composition of diets

Composition	Treatment ¹				
	Control	CSM1	CSM2	WCS1	WCS2
Ingredient, g/100 g of DM					
Alfalfa hay	14.2	15.0	14.8	14.5	14.2
Corn straw	25.8	25.4	25.2	26.3	26.7
Corn	30.2	32.7	32.7	24.2	21.7
Wheat bran	6.4	1.4	1.4	6.6	6.7
Soybean meal	19.8	12.3	10.3	14.8	12.1
Cottonseed meal ²	0.0	9.6	12.0	0.0	0.0
Whole cottonseed ³	0.0	0.0	0.0	10.0	15.0
5% Premix ⁴	3.0	3.0	3.0	3.0	3.0
Salt	0.6	0.6	0.6	0.6	0.6
Chemical, g/100 g of DM					
DM	90.34	90.68	90.71	90.30	90.27
NE _L , ⁵ Mcal/kg of DM	1.54	1.54	1.54	1.54	1.54
NDF	39.20	38.92	39.06	43.39	45.60
ADF	23.16	23.92	24.09	26.90	28.84
Fat	4.21	3.91	3.88	5.51	6.47
CP	15.70	15.70	15.70	15.70	15.70
Calcium	0.73	0.71	0.70	0.73	0.74
Phosphorus	0.81	0.85	0.86	0.84	0.86
TG, mg/kg of DM	0.00	651.97	839.08	816.66	1,294.88
FG, mg/kg of DM	0.00	91.15	117.31	385.43	611.13

¹Cows were assigned to 1 of 5 treatment groups: no gossypol (control group), 91.15 mg of free gossypol (FG)/kg free from cottonseed meal (CSM, CSM1 group), 117.31 mg of FG/kg from CSM (CSM2 group), 385.43 mg of FG/kg from whole cottonseed (WCS, WCS1 group), or 611.13 mg of FG/kg FG WCS (WCS2 group).

²Consisted of DM (90.98%), CP (39.08%), ether extract (EE; 0.29%), ADF (22.76%), NDF (36.00%), FG (902.27 mg/kg), and total gossypol (TG; 6,453.66 mg/kg).

³Consisted of DM (90.38%), CP (21.22%), EE (18.39%), ADF (22.76%), NDF (36.00%), FG (3,666.89 mg/kg), and TG (7,769.52 mg/kg).

⁴Premix composition: vitamin A, 12,240 IU/kg; vitamin D₃, 3,670 IU/kg; vitamin E, 67,600 IU/kg; Fe, 33.65 mg/kg; Cu, 25.1 mg/kg; Mn, 30.59 mg/kg; Zn, 107.6 mg/kg; I, 1.10 mg/kg; Se, 0.67 mg/kg; Co, 0.54 mg/kg; Ca, 3.06–4.59 mg/kg; P, 0.92–1.99 mg/kg; and salt, 4.59–7.34 mg/kg.

⁵Calculated value (based on Ministry of Agriculture of the People's Republic of China, 2004).

Table 2. Effects of dietary supplementation with cottonseed meal (CSM) or whole cottonseed (WCS) on lactation performance in lactating cows

Item	Treatment ¹					SEM ²
	Control	CSM1	CSM2	WCS1	WCS2	
DMI, kg/d	22.1	21.8	22.3	21.6	21.3	0.30
FGI, ³ g/d	0.00 ^e	1.98 ^d	2.62 ^c	8.32 ^b	13.02 ^a	0.07
3.5% FCM, kg/d	14.1 ^c	16.0 ^b	14.9 ^c	16.1 ^b	16.9 ^a	0.62
Milk protein, %	3.58 ^{ab}	3.68 ^a	3.55 ^{ab}	3.34 ^c	3.41 ^{bc}	0.04
Milk fat, %	3.31	3.54	3.51	3.21	3.36	0.08
Lactose, %	4.43 ^{ab}	4.47 ^{ab}	4.31 ^b	4.43 ^{ab}	4.58 ^a	0.04
SNF, %	8.84	9.00	8.67	8.64	8.81	0.05

^{a-e}Values with different superscripts in the same row indicate significant statistical differences ($P < 0.05$).

¹Cows were assigned to 1 of 5 treatment groups: no gossypol (control group), 91.15 mg of free gossypol (FG)/kg from CSM (CSM1 group), 117.31 mg of FG/kg from CSM (CSM2 group), 385.43 mg of FG/kg from WCS (WCS1 group), or 611.13 mg of FG/kg from WCS (WCS2 group). The original date of each replicate is an average of dates collecting at 15, 30, 45, and 60 d before analysis.

²Standard error of least squares means.

³Free gossypol intake.

analysis (Foss MilkoScan; Foss Food Technology Corp., Eden Prairie, MN).

Plasma and Serum Parameters. Blood samples (10 mL) were collected from 15 cows (3 cows per treatment) on d 0, 30, and 60 of the experimental period by coccygeal artery puncture using tubes containing anticoagulant (sodium heparin; Greiner Bio-One GmbH, Stonehouse, UK) or no anticoagulant agent for separation of plasma and serum, respectively. Blood samples were immediately placed on ice and transported to the laboratory within 3 h of collection. After blood coagulated, blood tubes were centrifuged at $3,000 \times g$ for 10 min in a refrigerated centrifuge at about 10°C for plasma or serum separation. Plasma was frozen at -20°C and later analyzed for urea N and gossypol. Serum samples were also frozen at -20°C and later analyzed for their concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Free Gossypol and Total Gossypol. The FG and total gossypol in CSM and WCS (Wu and Xu, 2009), plasma samples (Kim and Calhoun, 1995), and milk samples (Zhong, 2007) were determined by HPLC.

Statistical Analysis. The data were analyzed as a completely randomized design with repeated measures using the MIXED procedures of SAS (SAS Institute Inc., Cary, NC). Fixed effects included treatment, day, and treatment $\times$ day interaction. The variance for cow nested within treatment was used as the random error term to test the main effect of treatment. The day of collection was a repeated measure (compound symmetry covariance structure). Data are presented as covariate adjusted least squares means. The significance level was declared at $P < 0.05$.

RESULTS

Lactation Performance

Dry matter intake was not affected by gossypol intake from CSM or WCS, and averaged 21.74 kg/d (Table 2). Compared with the control group, treatments had greater 3.5% FCM ($P < 0.05$), except for the CSM2 group ($P > 0.05$). Milk protein concentration was lower for cows consuming WCS1 compared with the control group ($P < 0.05$). Lactose concentrations were lower for cows in the CSM2 group compared with the WCS2 group ($P < 0.05$), but no differences were observed among other diets ($P > 0.05$).

Plasma and Serum Parameters

No differences (Table 3) were observed in ALT and urea N among control and treatment groups ($P > 0.05$). Cows of the WCS2 group had higher AST concentrations than the control and WCS1 groups ($P < 0.05$), but no difference was observed between the CSM1 and CSM2 groups ($P > 0.05$).

Plasma Gossypol and Milk Gossypol

Gossypol was not transferred to plasma and milk in cows fed CSM (Figure 1). However, plasma and milk gossypol concentrations in cows consuming WCS were both greater than other treatment groups ($P < 0.05$). The concentration of plasma gossypol in WCS2 group at 60 d was greater than that in the WCS1 group ($P < 0.05$). And the concentration of milk gossypol in the

Table 3. Concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum and urea N in plasma of lactating dairy cows fed varying amounts of gossypol from cottonseed meal (CSM) and whole cottonseed (WCS)

Item	Treatment ¹					SEM ²
	Control	CSM1	CSM2	WCS1	WCS2	
ALT, U/L	35.8	32.6	33.4	33.6	36.0	1.77
AST, U/L	67.6 ^{bc}	74.8 ^{ab}	69.4 ^{abc}	65.4 ^c	77.8 ^a	2.05
Urea N, mmol/L	4.81	4.57	4.66	4.20	4.77	0.11

^{a-c}Values with different superscripts in the same row indicate significant statistical differences ($P < 0.05$).

¹Cows were assigned to 1 of 5 treatment groups: no gossypol (control group), 91.15 mg of free gossypol (FG)/kg from CSM (CSM1 group), 117.31 mg of FG/kg from CSM (CSM2 group), 385.43 mg of FG/kg from WCS (WCS1 group), or 611.13 mg of FG/kg from WCS (WCS2 group). The original date of each replicate is an average of dates collecting at 30 and 60 d before analysis.

²Standard error of least squares means.

WCS2 group at 30 and 60 d was also higher than that in the WCS1 group ($P < 0.05$).

DISCUSSION

Cottonseed byproduct did not affect DMI when CSM or WCS replaced wheat bran and soybean meal in diets for lactating dairy cows containing 12% of DM as CSM or 15% of DM as WCS, and the total intake of FG was up to 13.02 g/d. Prieto et al. (2003) indicated that substitution of cracked Pima cottonseed with whole linted Upland cottonseed had no effect on DMI of primiparous and multiparous cows receiving 12% of DM. Thus, available data indicated no effect on DMI when CSM or WCS partially replaced other ingredients in diets of dairy cows.

Yield of 3.5% FCM may be improved by adding WCS or CSM. But WCS was more effective ($P < 0.05$) than other diets in increasing 3.5% FCM. This was probably because of higher fat concentration (Table 1) in those diets containing WCS, resulting in increased energy content. Others have observed similar responses (Coppock et al., 1987; Mena et al., 2001). Milk protein concentrations were lower for diets with WCS, which is supported by previous work by Smith et al. (1981) and Mena et al. (2001). Although responsible for greater milk production, the higher fat content in diets with 15% WCS probably caused the depression in milk protein content. Preferential accumulation of gossypol in the liver has been studied in several livestock animal species, such as sheep (Morgan, 1990), pigs (Sharma et al., 1966), and broiler chickens (Gamboa et al., 2001a,b). To confirm that hepatic function was not affected by dietary gossypol, we evaluated activity of urea N, AST, and ALT in serum as associated with hepatic cell viability. These indexes occur in most cells, but are usually increased in serum when hepatocyte cells have suffered cellular damage (Barraza et al., 1991; Kreutzer et al.,

2008). Concentrations of ALT in serum and urea N in plasma were both unaffected by diets, which agree with the results of other studies (Higginbotham et al., 2004; Mena et al., 2004; McCaughey et al., 2005). Compared with control and CSM2 groups, concentrations of AST in WCS2 fed cows were higher, which was similar to findings in other studies (Mena et al., 2004). Although

Figure 1. Plasma and milk gossypol concentrations of lactating dairy cows fed varying levels of gossypol from cottonseed meal (CSM) and whole cottonseed (WCS): no gossypol (control), 91.15 mg of free gossypol (FG)/kg from CSM (CSM1), 117.31 mg of FG/kg from CSM (CSM2), 385.43 mg of FG/kg from WCS (WCS1), or 611.13 mg of FG/kg from WCS (WCS2). The concentrations of plasma and milk gossypol were detected only in WCS1 and WCS2 groups. ND = not detected (control, CSM1, and CSM2). Results represent mean $\pm$ standard error. * $P < 0.05$, WCS1 versus WCS2.

15% WCS increased the AST concentration in serum, values observed were within the normal range for cows (43–127 IU/L; Kreutzer et al., 2008).

No gossypol was transferred to blood in cows consuming less than 3 g of FG/d (CSM1 and CSM2), but cows receiving more than 8 g of FG/d (WCS1 and WCS2) had higher plasma gossypol concentrations. Plasma concentration is a marker for gossypol bioavailability and may be used to assess the amount of cottonseed products that can be used safely (Blackwelder et al., 1998; Santos et al., 2002, 2003). Noftsker et al., (2000) found that no signs of clinical gossypol toxicity were observed when cows consumed 21.6 and 30.9 g of FG/d from WCS. Santos et al., (2003) observed that lactating cows consuming 22.8 g of FG/d from WCS for a period of 170 d had marked increases in plasma gossypol, but this did not affect health, culling, or mortality of cows. Visible signs of gossypol toxicity including dyspnea, anorexia, decreased milk production, weakness, and sudden death were not observed in any of the cows in a study by Mena et al. (2004). Therefore, based on data and findings from other researchers, Millard (2002) suggested that 5 µg/mL be considered the safe upper limit for plasma gossypol in dairy cattle fed cotton feed products for extended periods.

Gossypol is transferred to edible parts, muscle and offal of ruminants and poultry, and is probably transferred to cow's milk, as it is transferred to milk in rats (Lin et al., 1992). However, very little quantitative information exists on the transfer of gossypol in feed to milk of cows. Only few studies reported milk gossypol concentrations of lactating dairy cows, but used either limited numbers of animals (Zhong, 2007) or colorimetry to determine gossypol concentration, which is considered an unreliable technique (Wan et al., 2003). Extracted gossypol is determined spectrophotometrically (at 440 nm) as adduct (Schiff base) with aniline (i.e., dianilino-gossypol; European Commission, 1972). Specificity of this method is questionable, as compounds other than gossypol can contribute to formation of UV adsorption at 440 nm. However, some gossypol-like compounds (e.g., gossypol methyl ethers of presumably similar toxicity as gossypol) will be codetermined with gossypol in spectrophotometric methods. High-performance liquid chromatography determination of gossypol is preferred, as it offers a higher degree of specificity (Chamkasem, 1988; Hron et al., 1990; Jaroszewski et al., 1992).

We used HPLC to detect gossypol in milk and found no gossypol in milk of cows fed cottonseed meal (CSM1 and CSM2), whereas milk gossypol levels in cows receiving 8 g of FG/d originating from cottonseed were 0.133 and 0.221 µg/mL for WCS groups at 60 d. Wan et al., (2003) fed 18 lactating Holstein cows a diet with 2.8% WCS and 16.5% CSM, which provided 5.1 g of

total gossypol/d, for 104 d, using colorimetry to determine the gossypol in milk, and found no gossypol. Zhong (2007) fed 8 Holstein cows with 0, 5, 10, and 15% WCS, which provided 0, 6.3, 12.93, and 20.34 g of FG/d, respectively, for 120 d, in a replicated 4 × 4 Latin square design. Zhong (2007) used HPLC and found that residual levels of gossypol in milk were 0, 0.2, 0.24, and 0.32 mg/kg, respectively. Maximum residue limits of FG in food determined by the Food and Agriculture Organization (FAO) and US Food and Drug Administration (FDA) were 0.06 and 0.045%, respectively. According to these data, we found that milk gossypol concentration was lower than the maximum residue limits of FG.

CONCLUSIONS

When cows were fed up to 117.31 or 611.13 mg of dietary FG/kg from cottonseed meal or whole cottonseed, respectively, plasma and milk free gossypol concentrations were below the safe upper limit for dairy cows.

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