Physiological Responses of Lactating Cows to Gossypol from Cottonseed Meal Rations

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ABSTRACT

We evaluated the potential for gossypol intoxication and resulting effects of feeding large amounts of cottonseed meal to dairy cows in early lactation. Twenty-four Holstein cows were grouped by age, prior production, and days postpartum and randomly assigned to one of three diets. After 14-day standardization cows individually were fed a blended corn-corn silage ration supplemented with screw-pressed cottonseed meal, direct solvent extracted cottonseed meal, or soybean meal during a 14-wk comparison.

Packed cell volume, copper in plasma, activities of transaminases, and gross composition of milk were not affected by ration. Hemoglobin was depressed, and total protein of plasma was elevated by the 9th wk in cows fed the solvent meal. Erythrocyte fragility was detected in those cows by the 7th wk and appeared later in cows fed the pressed meal. Gossypol was identified and quantitated in plasma and liver but was not detected in erythrocytes or milk from cows fed cottonseed meal. Elevated ambient temperatures precipitated increased respiration rates in the cows fed solvent meal.

Physiological changes and gossypol in tissues of cows suggest that intoxication is possible in mature ruminants consuming cottonseed meal containing high free gossypol.

INTRODUCTION

Cottonseed meal (CSM), a by-product of the cotton fiber and cottonseed oil industries, is an important protein supplement in livestock rations in cotton producing states. This product contains 40 to 50% crude protein and is an excellent source of protein for cattle. However, the use of CSM in nonruminant rations is limited in many instances by several physiologically active compounds. The yellow polyphenolic pigment, gossypol, is a major concern in nonruminant rations due to its abundance in CSM and its potential toxicity.

In the early 1900's, ruminants also were considered subject to gossypol toxicity in the form of "cottonseed meal injury". However, by 1930 it had been demonstrated (17, 20) that CSM injury in ruminants was attributable to vitamin A deficiency rather than gossypol in the ration. Subsequently, gossypol toxicity has not been reported in mature ruminants consuming cottonseed or CSM. Ruminants, however, are susceptible to gossypol intoxication upon intravenous administration of gossypol and display symptoms of gossypol toxicity similar to those in nonruminants (12). Therefore, the absence of gossypol toxicity in ruminants consuming CSM has been attributed to traditionally low levels of protein supplement provided in ruminant rations and detoxification of free gossypol by its binding to soluble proteins in the rumen (31).

Recent trends toward increasing protein in dairy rations (16), as well as increased feed intake by high-producing dairy cows consuming rations containing CSM, conceivably could tax the protective function of the rumen in preventing gossypol toxicity. To evaluate this possibility, our feeding study was a) to investigate the physiological effects of feeding high CSM to high-producing dairy cows in early lactation, and b) to determine if gossypol

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toxicity can be demonstrated in mature ruminants consuming CSM.

MATERIALS AND METHODS

Twenty-four Holstein cows from the Auburn University herd were grouped into eight replicates on age, previous production, and day of lactation. Among replicates, ages ranged from 40 to 100 mo, and the interval from parturition to the beginning of standardization ranged from 9 to 30 days. In their previous lactations all cows had reached a peak production of >30 kg milk/day. Within replicates, cows were assigned randomly to one of three treatment groups receiving as the protein supplement either soybean meal (SBM), screw-pressed CSM (SPCSM), or direct solvent-extracted CSM (SOLCSM). The SOLCSM contained more free gossypol than SPCSM (.225% vs. .061%), but total gossypol content of the CSM were similar. The SBM supplement served as a control since it does not contain gossypol. After receiving a standardization ration for 2 wk, the cows were maintained on their respective treatments for 14 wk.

The experimental rations were formulated to contain 24% crude protein with 82% of the protein derived from the SBM and CSM supplements (Table 1). The 24% protein was to obtain high intakes of gossypol by cows fed the CSM rations. Rations were balanced to contain equal and adequate fiber, net energy for lactation, vitamin A, calcium, and phosphorous to meet NRC (National Research Council) requirements (26) for lactating cows. The standardization ration contained both SPCSM and SBM to allow the cows to become acclimated to the high protein ration and to low daily intakes of free (1.31 ± .03 g) and total (51.05 ± 1.17 g) gossypol.

Concentrates containing the protein supplements, corn, mineral supplements, molasses, and vitamin A were premixed and blended with fresh corn silage at feeding. The protein concentrates and corn silage were sampled periodically for determination of proximate composition (3), free and total gossypol (30), iron (14), and copper (29). By analysis the crude protein contents of the standardization, SBM, SPCSM, and SOLCSM-blended rations were 25.2, 25.4, 24.6, and 25.2%. Free and total gossypol, iron, and copper content of the protein concentrates and blended rations are in Table 2.

The cows were housed in a conventional barn in individual pens bedded with wood shavings approximately 16 h daily. After the two daily milkings, at 0100 and 1300 h, the cows were allowed to loaf in a concrete paddock.

Twice daily the cows were fed individually enough of the assigned ration to assure a 5 to 10% weighback with intakes determined and recorded. They had access to the assigned

TABLE 1. Experimental ration formulations[a]

Ingredients	Int. ref. no.	Ration designations[b] Std	SBM	SPCSM	SOLCSM
		(% DM)			
Corn silage	3–08–153	42.0	51.3	37.5	37.5
SBM	5–04–604	22.5	38.7	...	...
SPCSM	5–01–617	22.5	...	45.0	...
SOLCSM	5–01–621	...	...	...	45.0
Corn, ground	4–02–931	7.5	4.0	11.5	11.5
Calcium carbonate	6–02–632	.7	...	1.5	1.5
Dicalcium phosphate	6–01–080	...	1.5	...	...
Salt (trace mineralized)[c]		.5	.5	.5	.5
Molasses	4–04–696	4.3	4.0	4.0	4.0

[a]All rations contained 3200 IU of vitamin A/kg dry matter as vitamin A palmitate.

[b]Std – standardization; SBM – soybean meal; SPCSM – screw-pressed cottonseed meal; SOLCSM – direct solvent-extracted cottonseed meal.

[c]Diamond Crystal Salt Company, St. Clair, MI 48079.

TABLE 2. Free and total gossypol, iron, and copper content of protein-concentrate mixtures and blended rations, dry matter basis.

Components	Standardization		SBM		SPCSM		SOLCSM	
	Ration designations[a]							
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Free gossypol, %	.011	.001[b] (.006)[c]		0(0)	.026	.002(.016)	.151	.045[d] (.094)
Total gossypol, %	.439	.017 (.255)		0(0)	.986	.029(.616)	1.112	.016 (.695)
Iron, ppm	267	52 (207)	652	31(381)	180	15 (159)	207	22 (176)
Copper, ppm	75	2 (61)	69	6(55)	64	2 (56)	82	5 (67)

[a]SBM – soybean meal; SPCSM – screw-pressed cottonseed meal; SOLCSM – direct solvent-extracted cottonseed meal.

[b]Mean concentration of component in the protein concentrates ± SE.

[c]Numbers in parentheses are mean concentrations of components in the respective blended rations.

[d]The high variation in free gossypol content of the SOLCSM ration was due to utilization of two sources of SOLCSM.

ration 8 h after each feeding and to water continuously.

The health of the cows was monitored daily and those showing abnormality were examined by a veterinarian.

Cows were weighed and samples of blood and 24-h composite milk were collected at the end of the standardization period and on days 7, 21, 35, 49, 63, 77, 91, and 98 of the comparison period. Bleeding was by jugular puncture at 0800 h following a 7-h fast. Milk production was recorded at the two daily milkings. Liver samples were obtained from each cow at the end of the comparison period by punch biopsy.

Heparinized blood was used to determine hemoglobin (Hb) concentrations (9) and packed cell volume (PCV) in micohematocrit tubes. Plasma, obtained by centrifugation of heparinized blood at 1600 × *g* for 15 min, was used in determination of glutamic-oxalacetic transaminase (GOT, EC 2•6•1•1) and glutamic-pyruvic transaminase (GPT, EC 2•6•1•2) activity as described by Sigma (2). The biuret reaction was used to quantitate total proteins and albumin of plasma after globulin precipitation with sodium sulfite buffer (4). Iron, copper, and total iron-binding capacity (TIBC) of plasma were measured colorimetrically (8, 29).

Severity of red blood cell hemolysis was evaluated qualitatively in plasma with a scale of: 0, indicating no hemolysis; 1, slight hemolysis; 2, moderate hemolysis; and 3, severe hemolysis. Erythrocyte fragility in hypotonic buffers was determined by the method of Dacie and Lewis (11) on washed fresh red blood cells obtained on days 49 and 98 of the comparison. Mean corpuscular fragility (MCF) was estimated by the best-fitting line through NaCl concentrations near the initiation and completion of hemolysis.

Free and bound gossypol contents of plasma, milk, and liver were determined by the procedure of Smith (36). Reduced reagent volumes appropriate to sample size were used. Milk and liver samples were lyopholized before gossypol was determined. The primary gossypol standard to quantitate all gossypol analyses was purified from gossypol acetate (22), and the purity was verified by molar extinction coefficient measurements in cyclohexane at 358 nm. Recovery of gossypol in "spiked" samples ranged from 86 to 100%. The identity of the dianilinogossypol derived from plasma and liver samples was verified by characterization of the absorption spectra between 350 and 500 nm with a Beckman Model 25 spectrophotometer.

Milk fat was determined by the Babcock method (3). Total milk solids were measured by drying aliquots of whole milk to a constant weight at 60 C. Small quantities of liver remaining after gossypol analysis were ashed for iron and copper analyses (14, 29).

Variance was analyzed (38) as a 3 (treatment) × 8 (replicate) × 9 (time) factorial arrangement, split plot. Treatment and time effects were tested by treatment × replicate and time × replicate interactions, respectively. Correlation coefficients (38) estimated the mutual relationship between variables.

RESULTS AND DISCUSSION

Mean daily intake of dry matter and free and total gossypol per cow by ration groups during the comparison are in Table 3. The SBM-fed cows received no gossypol during this period. Cows on the two CSM rations consumed an average of 10.1 kg of the respective meals daily. Total gossypol intake/kg body weight per day was similar for cows fed the two CSM rations. Cows fed the SOLCSM ration ingested 6.5 times more free gossypol/kg body weight per day than SPCSM-fed cows, and at the peak (4th wk) their intake of free gossypol was 54.5 mg/kg body weight per day.

Although the free gossypol intake by cows in the SPCSM group was consistently low, the average daily free gossypol intake of SOLCSM-fed cows declined 42% over the 98-day comparison. The declining gossypol intake was primarily a result of decreased ration consumption and changes in sources of direct solvent-extracted CSM.

The patterns of dry matter intake on the two CSM rations were similar (Figure 1), and, therefore, it is doubtful that the general decline in dry matter consumption in the SOLCSM-fed cows during comparison was due to ingestion of free gossypol. A drop in intake of dry matter was precipitous in several SOLCSM-fed cows during the 5th and 6th wk of comparison. Since anorexia is a symptom of gossypol intoxication in nonruminants (1, 5, 33), the abrupt decrease in ration consumption by SOLCSM-fed cows may have been related to gossypol.

Total milk production, percent milk fat, and total solids were not affected by ration (Table 4). A significant treatment × week interaction for milk production indicates that the high intake of free gossypol by cows fed the SOLCSM ration may have decreased the persistency of milk production. From the peak of lactation through the 14th wk of the comparison, milk yields by cows fed SPCSM, SOLCSM, and SBM rations declined by 29, 42, and 34%.

TABLE 3. Mean daily dry matter and free and total gossypol intakes of cows by treatment during the comparison period.

	Ration designations[a]		
	SBM	SPCSM	SOLCSM
Dry matter intake			
kg/day	20.8	22.4	22.5
kg/100 kg body weight per day	3.44	4.05	3.92
Gossypol intakes[b]			
Free			
g/day	0	3.6	24.2
mg/kg body weight per day	0	6.6	42.7
Total			
g/day	0	138.1	156.7
mg/kg body weight per day	0	251.0	273.3

[a]SBM – soybean meal; SPCSM – screw-pressed cottonseed meal; SOLCSM – direct solvent extracted cottonseed meal.

[b]Calculated weekly on an individual cow basis using gossypol content of the protein concentrate premix being fed at that time.

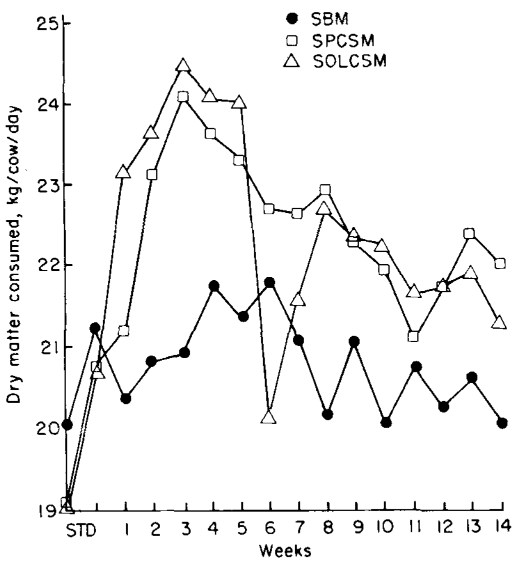

Figure 1. Mean daily consumption of standardization (STD) and experimental rations on a dry basis for the soybean meal (SBM), screw-pressed (SPCSM), or solvent-extracted (SOLCSM) cottonseed meal treatment groups.

A particularly abrupt 12% decrease in milk production by the SOLCSM-fed cows during the 5th wk corresponded to the decreased consumption of dry matter by these cows at that time.

Mean hemoglobin (Hb) concentrations (Figure 2) were within the normal range (27) throughout the study. The SBM and SPCSM-fed cows maintained uniform Hb over the comparison with SPCSM-fed cows showing consistently lower Hb than SBM-fed cows. In contrast, during the 9th wk Hb of cows fed the SOLCSM ration was lower than that of cows fed the SBM ration ($P<.07$) and approached the lower limit of the normal range for lactating dairy cows (27). Packed cell volume (PCV) followed the same trends as Hb. However, its variation prevented significant differences in PCV among treatments at any sampling time.

Decreased Hb and PCV are symptomatic of gossypol toxicity in nonruminants (1, 5, 6, 21) and calves (32). These variables also can be depressed by iron and copper deficiency; however, iron and copper in the rations (Table 2) met NRC recommendations for dairy cattle (26). Dietary gossypol reduces iron absorption and retention (18), and there is a possibility that the gossypol in the CSM rations precipitated iron deficiency anemia by binding dietary iron. However, the normal liver iron (Table 5) in several cows at the end of the experiment are indicative of normal iron status in the SPCSM and SOLCSM cows.

To evaluate further possible effects of gossypol on iron metabolism in dairy cows, all samples of plasma collected during comparison were to be analyzed for iron and total iron-binding capacity of plasma. However, erythrocyte hemolysis resulted in hemoglobin contamination of a large number of plasma samples and prevented a meaningful study of these variables. Since gossypol has had hemolytic activity on sheep erythrocytes in vitro (24), Hb in plasma was rated qualitatively by visual observation as none, slight, moderate, or severe to determine a treatment effect (Figure 3). The Hb in plasma did not not vary significantly during the first 7 wk of treatment. However, by the 9th wk, hemolysis of erythrocytes in blood samples from cows in the SOLCSM group was greater ($P<.05$) than in samples from cows in the SPCSM and SBM groups. Erythrocyte hemolysis peaked by the 11th wk in samples from cows fed the two CSM rations at which time Hb in plasma in all treatment groups were different ($P<.05$). Although Hb of plasma declined in cows from the SOLCSM and SPCSM groups from wk 11 through 14, amounts for cows in the SBM and SOLCSM groups differed ($P<.05$).

Hematuria has been observed in calves succumbing to gossypol toxicity (32). Since hematuria was not assessed critically and no hematuria was observed in cows fed the two CSM rations, we propose that the Hb in plasma was a result of extravascular erythrocyte hemolysis.

The elevated Hb in plasma of cows fed the two CSM rations is indicative of erythrocyte fragility. Mean corpuscular fragility (MCF) or the susceptibility of erythrocytes to hypotonic lysis was used to quantitate the apparent fragility of erythrocytes from the experimental cows (Table 6). The MCF of erythrocytes from cows fed the SBM ration was similar to the .505% NaCl reported by Perk et al. (28) for MCF in mature cattle. On day 49 of comparison, erythrocytes from SOLCSM-fed cows were more fragile than erythrocytes from those fed SBM ($P<.05$). By the end of the study, the MCF in cows fed the SOLCSM and SPCSM

TABLE 4. Mean total and daily milk production and milk composition during the comparison period by treatment groups.

	Treatment designations[a]					
	SBM		SPCSM		SOLCSM	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Total milk production, kg[b]	2406.5		2758.4		2350.6	
Daily milk production, kg	24.6	.7[c]	28.1	.5	24.2	.9
Milk fat, %	3.5	.1	3.2	.1	3.5	.1
Milk total solids, %[d]	12.3	.1	12.0	.1	12.2	.1
Milk solids non-fat, %[d]	8.9	.1	8.8	.1	8.7	.1

[a]SBM – soybean meal; SPCSM – screw-pressed cottonseed meal; SOLCSM – direct solvent-extracted cottonseed meal.

[b]Values for two cows which were unable to complete comparison period were predicted from regression equations defining their individual persistencies.

[c]Mean of 8 cows over the 14 week comparison period ± SE.

[d]Average of 8 determinations on each of 8 cows over the comparison period.

rations was greater ($P<.05$) than in cows receiving the SBM ration. Similar increases in MCF have been reported in gossypol-fed rats (39). Generally erythrocyte fragility is increased in acquired hemolytic anemias while fragility is decreased in iron deficiency anemia (13). Since erythrocyte fragility was increased in cows consuming gossypol, it seems probable that the decreased hemoglobin in the SOLCSM-fed cows was caused by the hemolytic activity of gossypol rather than a gossypol-induced iron deficiency. This does not exclude iron deficiency anemia as a possible problem in mature ruminants, but this mode of gossypol action was not apparent.

The lowered hemoglobin in SOLCSM-fed cows, the increased in vitro erythrocyte fragility, and the increased extravascular hemolysis in the blood of cows receiving both CSM rations suggest that ingestion of gossypol adversely affects erythrocytes of lactating dairy cows.

Copper concentrations in plasma monitored during comparison were normal for mature cattle and were not affected by treatment. At the end of comparison, copper in liver tissue from cows fed the two CSM rations (Table 5) were within the normal range of from 200 to 600 μg/g of dry tissue reported by Cunningham (10). Copper deficiency can cause reduced Hb, and clinical symptoms of gossypol toxicity are similar to copper toxicity (32). However, the normal plasma and liver copper in the cows indicate that their copper status was not a factor in the effects.

An increase in total protein concentration of plasma of SOLCSM-fed cows over that of SBM-fed cows ($P<.05$) was noted during the 9th wk of comparison (Figure 4). This elevated protein in plasma returned to normal by wk 13. The hyperproteinemia was characterized by a

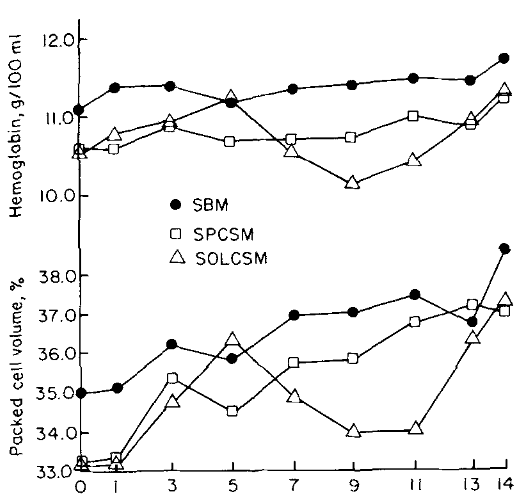

Figure 2. Hemoglobin and packed cell volume of cows fed soybean meal (SBM), screw-pressed (SPCSM), or solvent-extracted (SOLCSM) cottonseed meal during the comparison period.

TABLE 5. Mean free, bound, and total gossypol and iron and copper concentrations in liver of cows at the end of the comparison period in relation to ration fed.[a]

	Ration fed[b]				
	SBM	SPCSM		SOLCSM	
	(μg/g dry tissue)				
		$\bar{X}$	SE	$\bar{X}$	SE
Free gossypol[c]	0(7)[d]	59	5(7)	94	2(6)
Bound gossypol[c]	0(7)[d]	80	7(7)	136	7(6)
Total gossypol[c]	0(7)[d]	139	12(7)	230	8(6)
Iron	292(1)	236	41(4)	204	38(5)
Copper	. . .	470	13(4)	309	54(5)

[a]Mean ± SE; Numbers in parentheses are the number of observations associated with each mean.

[b]SBM – soybean meal ration; SPCSM – screw-pressed cottonseed meal ration; SOLCSM – direct solvent-extracted cottonseed meal ration.

[c]Within rows, all means are different among treatments ($P<.01$).

[d]Apparent free, bound, and total gossypol were 21 ± 3, 16 ± 2, and 37 ± 4, respectively. See discussion in text.

disproportionate increase in globulin of plasma. Hypoproteinemia with hypoalbuminemia has been observed in nonruminants consuming gossypol (21, 25), but the extensive liver degeneration (37) necessary to precipitate hypoproteinemia was not in cows consuming either of the CSM rations.

Increased GOT and GPT activity in plasma of gossypol-fed swine (6) and rats (5) are indicative of gossypol-induced liver damage. However, plasma GOT and GPT activities of cows were not affected ($P>.05$) by treatment. Therefore, intake of 251 to 273 mg of total gossypol/kg body weight per day for 14 wk was not adequate to damage liver tissue.

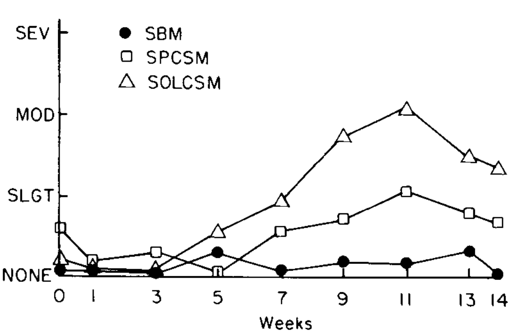

Figure 3. Qualitative assessment of hemoglobin in plasma of cows fed soybean meal (SBM), screw-pressed (SPCSM), or solvent-extracted (SOLCSM) cottonseed meal during the comparison period.

The apparent effects of gossypol ingestion on Hb and PCV of blood and plasma and plasma proteins of CSM-fed cows was most prominent during the 9th through the 11th wk of comparison with a return of the variables toward normal at the end of the study. In nonruminant species gossypol is metabolized by the liver and eliminated in bile (1) with toxicity when the intake of gossypol exceeds its detoxification and elimination. In our study, gossypol consumed by the SOLCSM-fed cows during the first 9 wk of the comparison appeared adequate to cause physiological disturbances. The return toward normal of blood variables of cows fed the two CSM rations near the end of the experiment could have been the result of lower intakes of free and total gossypol over that phase.

Low levels of gossypol were characterized in the plasma of cows consuming the CSM rations, and all of the gossypol in these samples was in the bound form. Since gossypol readily binds to bovine serum albumin (23) and bound gossypol is the predominant form isolated from swine plasma (34), the presence of bound gossypol in bovine plasma is consistent with these observations.

Detectable gossypol in plasma was in all treatment groups at the initiation of the comparison (Figure 5), indicating that the standard-

TABLE 6. Mean corpuscular fragility of erythrocytes from cows fed soybean meal (SBM), screw-pressed (SPCSM), or direct solvent-extracted (SOLCSM) cottonseed meal rations[a].

Days on experimental rations	SBM		SPCSM		SOLCSM	
	(% NaCl at which 50% hemolysis occurs)					
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
49	.53	.014[b]	.55	.015[bc]	.61	.011[c]
98	.51	.016[b]	.57	.013[c]	.59	.012[c]

[a]Mean ± SE. Increasing concentrations of NaCl indicate increasing mean corpuscular fragility.

[b,c]Within rows treatment means with unlike superscripts are different ($P<.05$).

ization ration which contained SPCSM provided adequate gossypol to allow some by-pass of the rumen detoxification mechanism (31). The slow decline in plasma gossypol of SBM-fed cows from the initial sampling through the 7th wk is indicative of the slow elimination of gossypol from these cows. The mean gossypol concentrations in plasma of cows fed SPCSM and SOLCSM rations increased from the initial

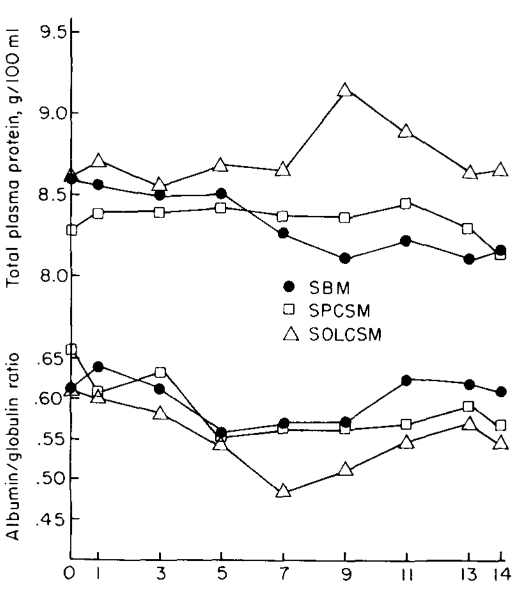

Figure 4. Total plasma protein and albumin to globulin ratio in plasma of cows fed soybean meal (SBM), screw-pressed (SPCSM), or solvent-extracted (SOLCSM) cottonseed meal during the comparison period.

sampling to approximately 2 μg/ml by the 7th wk (Figure 5) and were essentially the same in both groups even though the SPCSM-fed cows were consuming considerably less free gossypol than the SOLCSM-fed cows. After the 7th wk, plasma gossypol in cows fed the CSM rations remained consistent although free and total gossypol consumption dropped. Two factors which may have contributed to this lack of variation in gossypol concentration of plasma were time of sampling and persistency of free gossypol in rumen contents. Since all plasma samples were collected before the morning feeding following a 7-h fast, gossypol at this time may have been a residual quantity which was not cleared from the plasma by the liver and other tissues during the preceding fast. Also, low free gossypol may have been continuously available from rumen digesta since free gossypol was found in rumen contents of fistulated cows fed both CSM rations at all sampling times from 1 h prefeeding (7 h fast) through 12 h postfeeding.

Quantities of free, bound, and total gossypol in liver tissue of cows are in Table 5. Absorption spectra for dianilinogossypol from liver samples of SBM-fed cows were atypical; therefore, the apparent liver gossypol reported for SBM-fed cows was probably due to nonspecific colorimetric interference (7, 36). Free and bound gossypol isolated from liver of SPCSM and SOLCSM-fed cows gave absorption spectra characteristic of dianilinogossypol. The levels of free, bound, and total gossypol in liver differed significantly among the treatments with the cows receiving the SOLCSM ration yielding the

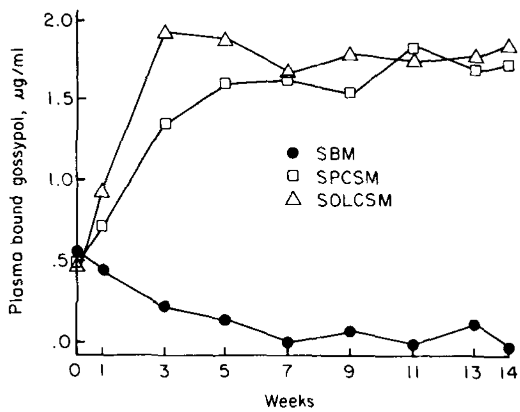

Figure 5. Bound gossypol in plasma of cows fed soybean meal (SBM), screw-pressed (SPCSM), or solvent-extracted (SOLCSM) cottonseed meal during the comparison period.

highest concentrations of liver gossypol. Total liver gossypol was correlated (r = .87, $P<.01$) with average free gossypol consumption per kilogram of body weight per day.

No gossypol was in any of the milk samples collected during comparison. The sensitivity of the gossypol assay was .5 μg of gossypol/ml of milk with a recovery of from 92 to 93%. The absence of gossypol in milk agrees with the findings of F. H. Smith (personal communication) that no gossypol was in the milk of gossypol-fed goats.

No suitable assay could be developed to isolate gossypol from erythrocytes. Although the presence of gossypol in plasma and liver and erythrocyte anomalies suggest that gossypol may be in erythrocyte of cows consuming gossypol, the high iron in erythrocytes probably prevented its detection.

Since the feeding study extended over 10 mo, different replicates of cows were exposed to varying climatic conditions. During a period of hot weather with mean daily temperatures above 32 C, cows receiving the SOLCSM ration were panting heavily during much of the day and early evening. In contrast, cows consuming SBM and SPCSM rations showed only a slight increase in respiratory rate. Dyspnea in gossypol intoxication of nonruminants (37) is attributed generally to severely depressed blood Hb. However, low blood Hb was not apparent in all SOLCSM-fed cows displaying dyspnea. It is possible that gossypol-induced erythrocyte alterations discussed earlier could have reduced the oxygen-carrying or releasing capacity of the blood. The reduced oxygen releasing capacity following in vitro gossypol treatment of ovine erythrocytes (24) and the bright red blood in calves dying from gossypol intoxication (19, 40) may indicate that gossypol interferes with the normal oxygen exchange in ruminant erythrocytes.

During hot weather, a 7-yr-old cow, which had been consuming the SOLCSM ration for 81 days, died. The cow collapsed while eating and was dead within minutes. During the 81 days this cow was fed the SOLCSM ration, she consumed 8 mg of free and 222 mg of total gossypol/kg body weight per day. The physical and hematological changes in this cow at 2 wk and at 4 days prior to death are in Table 7. The major lesion during necropsy was focal fatty areas of subcapsular necrosis, yellowish-white in color. Histopathological examination revealed severe fatty degeneration of hepatocytes in these areas. Fatty degeneration of liver tissue previously has been noted in calves succumbing to gossypol intoxication (32) and increased liver fat has been observed in gossypol-fed swine (21). Analysis of liver from this cow showed 78, 186, and 264 μg of free, bound, and total gossypol/g of dry tissue and was the highest concentration of total gossypol noted in any cow on the experiment.

From the foregoing we concluded the cow died of factors induced by increased gossypol ingestion. The onset of symptoms and course of the intoxication appeared to be enhanced by the age of the animal and the combined stress of high milk production (peaked at 42.8 kg/day during 6th wk) and high ambient temperatures. Several workers (15, 33) have suggested that environmental and physiological stress enhance gossypol intoxication in animals.

The feeding of high CSM to high-producing dairy cows resulted in several observations which can be attributed to increased gossypol consumption. Possibly the most significant observation was the presence of gossypol in the tissues of these mature ruminants indicating that the capacity of the rumen to detoxify dietary gossypol is limited. Since gossypol toxicity has been demonstrated in mature ruminants when the protective function of the rumen was bypassed via intravenous administration of gossypol (12), gossypol intoxication is possible in mature ruminants consuming CSM

TABLE 7. Physical and hematological observations prior to death of a cow fed the SOLCSM ration.

Observations	Interval prior to death	
	2 weeks	4 days
Ration consumption	Normal	Normal
Milk production	Slightly depressed	Slightly depressed
Respiration rate[a]	Normal	Increased
Hemoglobin, g/100 ml	7.9 (-5.4)[b]	8.5 (-4.3)
Packed cell volume, %	26.5 (-6.2)	27.5 (-5.1)
Glutamic-oxalacetic transaminase, IU/l	94.0 (+2.8)	88.0(<2.0)
Total plasma protein, g/100 ml	9.8 (+3.2)	8.4(<2.0)
Erythrocyte hemolysis[a]	Severe	Severe

[a]Subjective evaluations.

[b]Numbers in parentheses are the number of standard deviations which given means deviate from normal means. Normals were means of the variables obtained from all cows fed soybean meal during the same sampling period.

provided the free gossypol content of the meal is high and intake of the CSM is elevated. The high-producing dairy cow would appear liable to gossypol intoxication in a practical feeding situation in which the high requirements for dietary protein were met by CSM containing high free gossypol.

Since low gossypol can be tolerated by most animal species, the presence of gossypol in the tissues of animals consuming CSM is not necessarily indicative of gossypol intoxication. For dietary gossypol to be considered toxic to mature ruminants, detrimental physiological changes attributable to gossypol must be observed. In our study, physical changes attributable to CSM consumption were not readily apparent. However, persistency of milk production was reduced in cows fed the ration (SOLCSM) containing high free gossypol, and dyspnea was observed in these cows during hot weather.

Alterations in normal erythrocyte structure, metabolism, and/or function which decreased Hb, erythrocyte fragility, and susceptibility to extravascular lysis appear to be the major physiological effects of gossypol in lactating dairy cows. The absence of literature reporting hemolysis in nonruminants indicates that erythrocyte fragility is not a predominant feature of gossypol toxicity in nonruminant species and suggests that the etiology of gossypol intoxication in ruminants may be different from nonruminants, with erythrocyte involvement more prominent in ruminants.

The lack of physical symptoms, the high free gossypol consumption necessary to produce a response, and the rapid recovery of hematological variables when free gossypol consumption declines make it clear why gossypol toxicity is not a common problem in ruminants. In addition, the gossypol-induced aberrations in bovine erythrocytes do not appear to be detrimental unless the animal is subjected to physiological, nutritional, and/or environmental stress.

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