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Effect of Graded Concentrations of Gossypol on Calf Performance: Toxicological and Pathological Considerations[1]

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ABSTRACT

The effects of feeding diets containing 0, 100, 200, 400, or 800 ppm of free gossypol were examined in 60 Holstein bull calves from 1 to 120 d of age. The concentrations of free gossypol were varied by varying amounts of cottonseed meals from three different sources. Cottonseed meal totaled 31% of each treatment ration. Feed consumption, BW, and blood parameters were collected on all calves at 30-d intervals throughout the trial. There were no significant differences between the groups for feed consumption, BW, or average daily gain. Changes in the group means for hematology and chemistry variables examined were modest and insufficient to distinguish diagnostically between safe and unsafe free gossypol concentrations for the different groups. Clinical evidence of disease was limited to the calves fed 400 or 800 ppm of free gossypol after 90 d of age. One calf in the group fed 400 ppm and 4 in the group fed 800 ppm died as a result of circulatory failure associated with gossypol consumption. We conclude from this study that a ration containing up to 200 ppm of free gossypol is safe, 400 ppm of free gossypol is toxic, and 800 ppm of free gossypol results in death losses. These results are compatible with previously reported naturally occurring toxicological outbreaks.
(**Key words**: cottonseed meal, free gossypol, nutrition, calves)

Abbreviation key: **CSM** = cottonseed meal, **SBM** = soybean meal, **FG** = free gossypol.

INTRODUCTION

Cottonseed meal (**CSM**) has long been recognized as an excellent and economical source of protein in starter and grower rations for dairy calves (3, 12). After the solvent extraction of oil from prepared cottonseeds, the resulting CSM is available for feeding. Gossypol, a polyphenolic binaphthyl aldehyde contained in the seed pigment glands, is released during the extraction process (1, 3, 21). The gossypol content of CSM is influenced by the species of cotton plant, growing conditions, and the method of oil extraction used (8, 22, 27). Because gossypol is toxic to animals, a limiting factor of CSM is its potential toxicological effect (8, 10, 16, 18). Gossypol in unprocessed seed is in the free form, not bound to proteins. During processing, some of the free gossypol (**FG**) complexes with proteins, primarily the ε-amino group of Lys, and becomes bound (8). Toxicity generally is attributed to FG; however, some recent evidence

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suggests that bound gossypol may be converted to FG in the gastrointestinal tract (6, 18).

Monogastric species, such as swine and poultry, are quite susceptible to gossypol toxicity. In contrast, ruminants detoxify gossypol in the rumen by binding it to soluble proteins or by dilution and slowed absorption. The gossypol-protein complex is less toxic. In monogastric species, this binding mechanism is less effective (26). Calves with undeveloped rumens (preruminants) function essentially as monogastrics, and gossypol cannot be efficiently detoxified. Despite years of favorable practical experiences with the feeding of CSM to calves, especially in California, recent reports (14, 16, 19) have described gossypol poisoning in calves and lambs. In those reports, the variability in calf responses to CSM feeding was due to different undefined dietary and management conditions, raising questions about the actual safe concentration of FG in the ration. In addition, there has been a paucity of research evaluating CSM with various concentrations of gossypol fed to preruminant calves. Therefore, the purpose of our study was to establish a safe concentration for FG in concentrate rations containing CSM fed to calves under commercial calf production conditions typical of large dairy herds.

MATERIALS AND METHODS

This investigation was conducted for 120 d beginning December 12, 1989 at a commercial calf ranch in southern California. Sixty 1-d-old Holstein bull calves from local dairy farms were assembled at the calf ranch and allocated randomly to five treatment groups of 12 calves each. Each treatment provided the calves with an 18% protein starter ration that was fed for ad libitum intake during the entire investigation from d 1 to 120. The final calculated FG content for each of the treatments was 0, 100, 200, 400, and 800 ppm. The control ration (0 ppm) lacked any cottonseed product. All rations were isocaloric and isonitrogenous. During the investigation, the calves were not fed any hay or ferrous salt supplementation. The total CSM content for rations containing 100, 200, 400, and 800 ppm of FG was 31%. The control ration contained soybean meal (SBM) as the main protein source (Table 1). Graded concentrations of FG for the treatment rations were obtained by mixing two sources of CSM that contained 3850 and 230 ppm as reported by the feed mill. A third source of a 250-ppm CSM was added to balance the total CSM percentage in the rations. The three sources of CSM were analyzed for FG content prior to ration mixing and before the meals were pelleted. Mixed rations containing the CSM pellets and the other feed ingredients were analyzed for FG content three times during the study. The composition of the trace mineral mix and vitamin supplement used in the rations is shown in Table 2. Feed consumption was measured at five intervals by measuring the total amount fed to all calves of each group. The average daily feed consumption per calf per each group for each time period was determined using the following formula (feed consumed per time period divided by calf days per time period) for each group. Using that formula, multiplied by the calculated FG in each group (0, 100, 200, 400, and 800 ppm), an estimate of the accumulated FG intake per group was determined. Feed consumption periods were 0 to 36, 37 to 59, 60 to 82, 83 to 93, and 94 to 120 d. Calf days per group was defined as the number of live calves times the number of days that each calf was alive. This calculation equalized and removed any bias of reduced calf numbers occurring during the study.

For 3 d after arrival, calves were fed 4 L of colostrum containing >80 mg/ml of Ig measured by colostrometer. At d 4 of age, all calves were switched to 4 L/d of a commercial milk replacer containing .454 kg of replacer in 4 L of water. The calves were fed 2 L of milk replacer twice a day in individual bottles until weaning at d 60. Calves were kept in individual hutches for 1 wk after weaning at which time they were moved by treatment groups to five separate 700-m^2 pens until the completion of the study at 120 d of age. All calves had access to their respective treatment ration from 1 d of age, which was the start of each calf's experimental period.

All calves were weighed, and blood was collected at 2, 30, 60, 90, and 120 d of age. Hematological (EDTA vacutainers) and serum chemistry (serum from plain vacutainers) tests were conducted on all samples collected. All chemistry tests were conducted on a commer-

TABLE 1. Ration composition.

Ingredient	Free gossypol				
	800 ppm	400 ppm	0 ppm	100 ppm	200 ppm
Rolled corn	28.3	28.3	30.4	27.8	28.0
Rolled barley	15.4	15.4	17.7	15.1	15.3
Rolled oats	15.4	15.4	17.7	15.1	15.3
CSM[1]	2.6	6.4	0	10.1	8.2
CSM Pellet A[2]	6.7	15.4	0	22.2	20.4
CSM Pellet B[3]	21.8	9.2	0	0	3.1
Total CSM	31.1	31.1	0	32.3	31.6
SBM[4] Pellet	0	0	24.3	0	.0
Sodium propionate	.19	.10	.10	.10	.10
Tallow	.77	.77	.76	.76	.76
Dicalcium phosphate	.00	.00	.51	.00	.00
Limestone flour	1.3	1.3	1.0	1.3	1.3
Salt	.8	.8	.8	.8	.8
Trace minerals	.2	.2	.2	.2	.2
Vitamins A, D, and E	.2	.2	.2	.2	.2
Molasses	6.4	6.4	6.3	6.3	6.4
Niacin (30 ppm)	.2	.2	.2	.2	.2
Total	100.0	100.0	100.0	100.0	100.0

[1]Cottonseed meal (CSM) = 250 ppm of free gossypol, feed mill results.

[2]CSM Pellets = 230 ppm of free gossypol, feed mill results.

[3]CSM Pellets = 3850 ppm of free gossypol, feed mill results.

[4]SBM = Soybean meal.

cial clinical photospectrometric analyzer (Cobas Mira, Roche Diagnostic-Systems, Nutley, NJ) and ion-selective electrode of cations (Corning 902, Medford, MA). Complete necropsies were performed, and a blood sample was collected on all calves that died or on moribund calves euthanatized during the investigation. Euthanasia was performed by lethal intravenous injection of a pentobarbital sodium and phenytoin sodium solution (Beuthanasia®-D Special, Schering-Plough Animal Health Corporation, Kenilworth, NJ). The necropsies consisted of a gross description of significant findings and tissue sample collection from all major organs for histopathology. Tissues were fixed in 10% neutral-buffered formalin and were processed for histological examinations by routine paraffin embedding procedures; 6-μm sections were stained with hematoxylin. Samples of stomach, ileum, and colon contents were collected for bacteriological examination and examination for *Cryptosporidium* sp. and fluorescent antibodies to coronavirus and to rotavirus. In addition, the entire liver and one kidney were collected for gossypol analysis.

During the investigation, sick calves were treated by calf ranch personnel according to therapeutic protocols established for the ranch. Treatment consisted of antibiotic administration to calves with respiratory problems and oral fluid electrolyte replacement therapy for calves with diarrhea. Individual records for treatments were kept for each calf until 30 d of age. Individual calf evaluations included 1) change in BW, 2) serum hematology and chemistry, 3) post-mortem examinations, 4)

TABLE 2. Composition of trace mineral mix and vitamin supplement.

Ingredients	Concentrations
	(ppm)
Mn	205.43
Zn	298.80
Cu	90.20
Co	18.65
Se	2.40
I	7.48
Mo	4.14
Vitamin A, $\times 10^3$ KIU	39.89×10^3
Vitamin D, $\times 10^3$ KIU	9.97×10^3
Vitamin E, IU	600
Niacin	30

TABLE 3. Feed group differences in treated day divided by days at risk.[1]

Feed group	Days at risk	Respiratory treatments	Diarrhea treatments	Total treated days
0 ppm of FG[2]	360	.035	.029	.064
100 ppm of FG	360	.023	.040	.063
200 ppm of FG	344	.017	.008	.025
400 ppm of FG	348	.028	.022	.050
800 ppm of FG	360	.036	.033	.069
Mean, all groups	354	.028	.027	.054

[1]Values for respiratory treatments, diarrhea treatments, and total treated days are number of treatment days required divided by days at risk for each group.

[2]FG = Free gossypol.

morbidity and mortality, and 5) tissue gossypol concentrations.

Gossypol Analyses

Gossypol content in feedstuffs was determined by aniline reaction procedures according to the American Oil Chemists Society methods Ba 7-58 and Ba 8-78 for FG and bound gossypol, respectively (4, 5). Gossypol analyses for the three CSM lots and the mixed ration were performed by Pope Laboratory (Dallas, TX).

Tissue assays for gossypol were performed by similar aniline reaction procedures according to the method described by Smith (25). Tissue aliquots were lyophilized to eliminate the need for ethanol dilution of tissue water and diatomaceous earth treatment called for in the original procedure. An aliquot without aniline was processed identically to correct for tissue products absorbing at 440 nm before calculation of "total" gossypol based on a purified gossypol standard.

Statistical Procedures

Quantitative results were analyzed by repeated measures ANOVA. Analyses were performed using a general linear modeling program (Super ANOVA, Abacus Concepts, Berkeley, CA). Missing values because of calf deaths were replaced by means for the treatment group at the time points for which values were missing. The variables examined included BW, daily BW gain, serum chemistry tests, and hematological test results.

RESULTS

Clinical Observations

Incidence of diarrhea or pneumonia, as measured by the number of calves treated per group and duration of the condition, was similar among treatments during the first 30 d of age (Table 3). All calves in the group fed 200 ppm of FG and the majority of calves in the other groups appeared to be healthy, were in good condition, and had good appetite during the study. However, appetite decreased, and the overall appearance was dull, at 90 d of age for 3 calves fed 800 ppm of FG and 1 calf in the group fed 400 ppm. At 100 d of age, those calves exhibited dyspnea and coughing and preferred a recumbent posture. Severe intermandibular swelling and jaundice in the sclera was noted after 100 d of age in 2 of the calves in the group fed 800 ppm of FG.

Feed Consumption and BW Gains

The group fed 200 ppm of FG consistently showed the best appetite through all periods. Control calves had decreased feed consumption during the last two periods. Feed consumption per calf decreased in the groups receiving 400 or 800 ppm of FG during the 83- to 93-d period but increased in the last 26-d period for the survivors in the group fed 800 ppm (Figure 1).

The mean BW (Figure 2) and daily BW gains (Figure 3) for the 120-d study are presented. The mean final BW varied from 133.4 kg for the group fed 400 ppm to 143.4 kg for the group fed 800 ppm. The daily BW

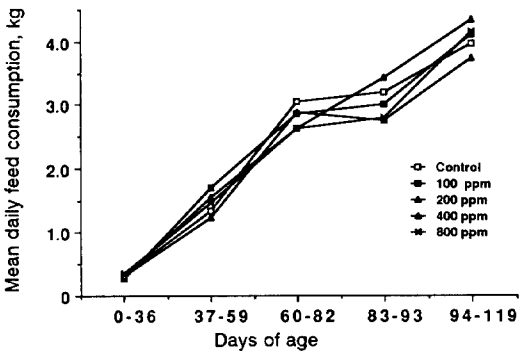

Figure 1. Average daily feed consumption for groups with different concentration of free gossypol in feed.

gain for the final 30 d varied from 1.18 kg for the group fed 400 ppm to 1.33 kg for the group fed 200 ppm. There were no significant differences with the repeated measures ANOVA or by Student's *t* test pairwise comparisons of each group fed FG with the control group (Table 4). Estimates of accumulative FG consumption per group is given in Figure 4.

Clinical Chemistry

The results for serum chemistry assays are summarized in Table 5. For each variable examined, the probability from the repeated measures ANOVA is listed in the first column. In the next two columns, each mean for the group fed FG that was statistically different from the control group mean ($P < .05$ from pairwise comparison by Student's *t* test) is listed according to the direction in which the mean varied from the control group mean.

Some groups fed FG had significant depression of albumen, globulin, and total protein. Individual group means at the sequential sample times were examined for patterns of change from the control groups. Serum albumen for the control group was slightly higher (3.4 to 3.5 g/dl) than that for all groups fed CSM (3.0 to 3.4 g/dl) for the 90- and 118-d samples without consistent changes at earlier sample times. Serum globulin for the group fed 800 ppm was lower (2.7 g/dl) only at the 118-d sample time; values for all other groups and sampling times were normal. Total protein reflected the changes in both albumen and globulin and had an FG dose-associated decrease for all groups fed CSM that was evident for only the 90- and 118-d samples. The decrease was quantitatively small, and all means were in the expected range of 6.7 to 7.6 g/dl for calves of this age except for the group fed 800 ppm with a concentration of 5.9 g/dl for the 118-d sample.

The glucose and cholesterol means of groups fed CSM lacked FG dose-associated change in magnitude, and all were within expected range for calves of these ages. No biologically important patterns of means were associated with treatments.

Of the serum elements examined, differences were significant for Ca, Cl, and Na. Calcium of control group was minimally higher than for groups fed CSM at 30 and 60 d, similar at 90 d, and lower than at 118 d in groups fed 400 and 800 ppm of FG. All group

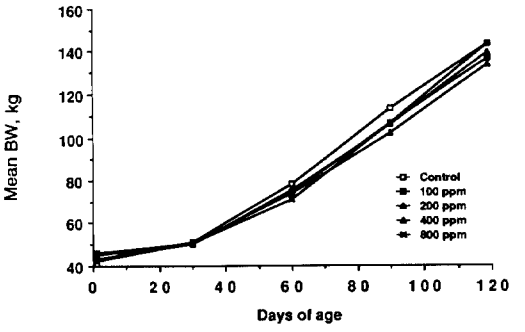

Figure 2. Mean BW for groups with different concentrations of free gossypol in feed.

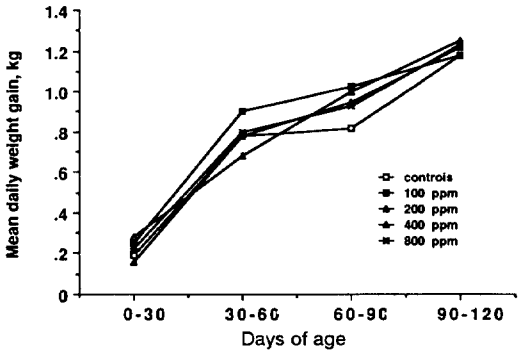

Figure 3. Mean daily weight gain for groups with different concentrations of free gossypol in feed.

TABLE 4. Body weight at 119 d of age and mean daily gain for 90 to 119 d of age.[1]

Feed group	119-d BW		P	90- to 119-d Gain		P
	(kg)			(kg/d)		
	$\bar{X}$	SD		$\bar{X}$	SD	
Control of FG[2]	143.15	17.66	. . .	1.18	.14	. . .
100 ppm of FG	136.46	15.06	.2715	1.22	.21	.2691
200 ppm of FG	139.08	13.48	.3401	1.33	.30	.7317
400 ppm of FG	133.41	21.09	.1162	1.18	.41	.0756
800 ppm of FG	143.39	11.95	.4397	1.24	.18	.5746
Repeated measures, ANOVA P			.612			.400

[1]Pairwise comparison of group means with control group (Student's *t* test).

[2]FG = Free gossypol.

means for serum Ca were within the expected range for normal calves of the respective ages (9.1 to 12.7 mg/dl). All means for serum Cl were in the expected range (110 to 119 mmol/L) without evidence of an FG dose-related change. The serum Na concentrations were similar and within the expected range for healthy calves for all groups at each sample time except for slight elevations (135 and 137 mmol/L) for the groups fed 400 and 800 ppm of FG at the 118-d sampling.

All group means for serum urea N and creatinine were within the expected range for calves (4 to 18 and .9 to 1.2 mg/dl, respectively) with normal renal function. The variation in group means for serum urea N resulting in the single group with significantly different values from the control was associated with differences in feed consumption rather than with evidence of renal disease.

No significant differences existed in group means for bilirubin or the various enzyme activities indicative of tissue necrosis. Sorbitol dehydrogenase and γ-glutamyltransferase are increased primarily in association with liver necrosis. Aspartate aminotransferase is increased primarily in association with liver and muscle necrosis. Creatine kinase is increased primarily in association with muscle necrosis. All these enzymes and total bilirubin (increased primarily in association with decreased hepatic secretory function or increased erythrocyte destruction) were within the expected ranges for normal calves.

The results for hematology assays are summarized in Table 6. For each variable examined, the probability from the repeated measures ANOVA is listed in the first column. In the next two columns, each group mean for calves fed FG that was different from the control group mean ($P < .05$, from pairwise comparison by Student's *t* test) is listed according to the direction in which the mean varied from the control group mean.

Significant differences existed between the groups fed FG and the control group for the three primary measures of circulating erythroid cell population: erythrocytes per microliter, hematocrit, and hemoglobin concentration. These all reflected a depression of the erythroid measures at 90 and 120 d. All groups

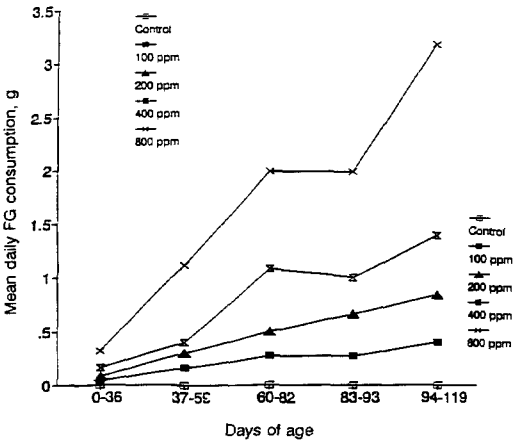

Figure 4. Estimate of accumulative free gossypol (FG) consumption for groups with different concentrations of free gossypol in feed.

TABLE 5. Statistical analysis results for serum chemistry parameters.

	Repeated measures ANOVA P	Groupwise comparisons with controls	
		$P \leq .05$, < control mean	$P \leq .05$, > control mean
Albumen, g/dl	.19	800, 100	. . .
Globulin, g/dl	.19	800	. . .
Total protein, g/dl	.00	200, 400, 800	. . .
Glucose, mg/dl	.10	400, 100	. . .
Cholesterol, mg/d	.04	. . .	00, 200
Ca, mg/dl	.00	100, 200	800
P, mg/dl	.94	. . .	. . .
M, mg/dl	.84	. . .	. . .
C, mmol/L	.03	. . .	100, 200
Na, mmol/L	.00	. . .	800
K, mmol/L	.47	. . .	. . .
Serum urea N, mg/dl	.02	. . .	. . .
Creatinine, mg/dl	.20	100	. . .
Total bilirubin, mg/d	.25	. . .	. . .
Sorbitol dehydrogenase, U/L	.76	. . .	. . .
α-Glutamyltransferase, U/L	.16	. . .	. . .
Aspartate aminotransferase, U/L	.66	. . .	. . .
Creatine kinase, U/L	.42	. . .	. . .

fed FG either did not exhibit the normal increase occurring at this age or showed a modest decrease at the 90-d sample followed by an increase in hemoglobin of each group at the 120-d sample. At both 90 and 120 d, all group means were within the expected normal range for this age of 9.0 to 12.5 g/dl. The pattern of change (modest quantitative depressions at 90 d and return toward normal at 120 d for groups fed FG) for hematocrit and erythrocyte concentration was the same as for hemoglobin. At 90 d, the control group mean hematocrit was 36.1%, and the range was 29.3 to 33.6% for the groups fed FG. At 90 d, the control group mean erythrocyte concentration was 8709/μl, and the range for the groups fed FG was 7300 to 7908/μl. The absence of significant differences with the red cell indices

TABLE 6. Statistical analysis results for hematology assays.

Item	Repeated measures ANOVA P	Groupwise comparisons with controls	
		$P \leq .05$ < control mean	$P \leq .05$ > control mean
Erythrocyte, /μl	0	400, 200	. . .
Hematocrit, %	.04	400	. . .
Hemoglobin, g/dl	.09	400	. . .
Mean cell hemoglobin, pg	.79	. . .	. . .
Mean cell hemoglobin concentration, g/dl	.51	. . .	. . .
Mean cell volume, fl	.96	. . .	. . .
White blood cells, /μl	.31	. . .	. . .
Band neutrophils, /μl	.73	. . .	. . .
Segmentor neutrophils, /μl	.65	. . .	. . .
Lymphocytes, /μl	.21	800	. . .
Monocytes, /μl	.24	800	. . .
Eosinophils, /μl	.02	200	. . .
Basophils, /μl	.44	. . .	. . .
Fibrinogen, mg/dl	.37	. . .	. . .

(mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) when the erythroid mass was depressed, and returning to normal indicates the mildness of physiological effect for the surviving calves. Mean corpuscular volume, as an indicator of changes in proportion of immature circulating erythrocytes, was essentially the same for all groups during the 60- to 120-d period.

Moderate, consistent elevation of the mean segmented neutrophil concentration of the group fed 400 ppm above the control group occurred throughout the study. This increase was interpreted as a result of the response of specific calves to inflammatory processes (including calf 19) rather than a direct effect of FG ingestion. The significant difference for eosinophils with the repeated measures ANOVA was because of differences between different groups fed FG with no pairwise differences between groups fed FG and the control group.

Mortality

The group fed 800 ppm of FG had deaths definitively related to FG. One calf in the group fed 400 ppm had clinical signs of pneumonia prior to euthanasia at 116 d of age, had poor BW gain compared with pen mates, and, on post-mortem examination, findings were suggestive of gossypol toxicity. The group fed 800 ppm had the highest mortality rate (33%; 4 of 12), and those fed 200 ppm had no deaths. Using Fisher's exact test, the groups fed 400 and 800 ppm, combined, had significantly ($P < .05$) higher FG-related death rates than the control group or groups fed 100 or 200 ppm of FG. Calf 46 in the group fed 100 ppm died at 12 d of age from bloat. Calf 30 in the control group died at 16 d and had a history of chronic diarrhea. Enteric disease was confirmed by post-mortem examination, histopathology, and culture. Because these calves died from causes unrelated to FG, they were excluded from the mortality analysis.

Calf 40 in the group fed 100 ppm died at 108 d; moderate pneumonia was the only remarkable finding on post-mortem examination. Liver gossypol concentrations were less than 50 μg/g. Calf 1 in the group fed 800 ppm was euthanatized at 110 d. This calf was recumbent, had difficulty breathing, and was unable to rise for 3 d prior to euthanasia. A severe fibrinopurulent pneumonia with an enlarged flabby heart was noted on necropsy. The liver was swollen and enlarged and had liver gossypol concentrations greater than 160 μg/g. Calf 19 in the group fed 400 ppm was euthanatized at 116 d. This calf exhibited severe respiratory distress, a purulent nasal discharge, rough haircoat, and small size compared with others in the group. On post-mortem examination, a severe fibrinopurulent pneumonia and approximately 30 cc of a straw-colored fluid were noted in the thoracic cavity. The abdomen was unremarkable except for approximately 42 cc of a straw-colored fluid. The heart and spleen were enlarged, and liver gossypol concentrations were greater than 120 μg/ml. The fluid transudate in the body cavities, increased heart and spleen weights, and high liver gossypol were interpreted as evidence of circulatory failure to which gossypol was a contributing factor (14). However, the calf lacked hepatic necrosis typical of acute deaths in gossypol toxicity, which is interpreted to be a result of euthanasia occurring before death occurred from the disease.

Calves 12, 11, and 8 in the group fed 800 ppm of FG died at 92, 97, and 112 d of age, respectively. They exhibited respiratory failure, intermandibular edema, jugular distention, and a pendulous abdomen for 1 wk prior to death. Post-mortem findings in these calves were compatible with symptoms of gossypol toxicity as described by Smith (26). These included moderate pneumonia, severe effusion of a straw-colored fluid into the body cavities, edema and icteric discoloration of the mesentery, and hepatomegaly with a nutmeg color on the cut surface of the liver. The most consistent histological finding was severe centrilobular hepatic necrosis. Calf 12 in this group had no evidence of inflammatory disease of significance. In contrast, calves 11 and 8 contained healed inflammatory foci in the lungs from a previous insult that occurred at least 2 wk prior to death. Liver gossypol in these calves was greater than 160 μg/g.

Bacteriological and viral isolations were unremarkable in all calves necropsied. This finding is expected because these calves had been administered antibiotic medications by the ranch personnel and had been vaccinated for viral respiratory agents.

TABLE 7. Free gossypol (FG) feed analysis.

Diet	Calculated	Laboratory analysis
	(ppm)	
800 ppm of FG	800	322
400 ppm of FG	400	202
0 ppm of FG	0	25
100 ppm of FG	100	70
200 ppm of FG	200	105
CSM[1]	250	250
CSM A[2]	230	230
CSM B[3]	3850	3600

[1]Cottonseed meal (CSM) = 250 ppm of FG, feed mill results.

[2]CSM Pellets = 230 ppm of FG, feed mill results.

[3]CSM Pellets = 3850 ppm of FG, feed mill results.

Gossypol Concentrations

Table 7 presents the analyzed FG content in the CSM prior to mixing with other ration ingredients at the beginning of the study, as well as the analyzed and calculated concentrations of FG for the mixed rations. These are averages of monthly analyses during the study. The level is a mathematical calculation based on the percentage of FG from the CSM sources mixed in the individual rations. The variation in values is discussed.

DISCUSSION

In general, calves fed CSM had good feed consumption, BW gains, and clinical health during the study. These parameters were not different compared with those of calves fed SBM. Disease was only obvious in those calves in the high FG group that eventually died or were euthanatized. In similar comparison trials using calves fed CSM, Claypool et al. (11) showed no differences in BW gains or performance when those calves were compared with calves fed SBM. The FG content in the CSM in this study (11) was not reported. Except for those calves that died in the group fed 800 ppm and 1 calf that was euthanatized from the group fed 400 ppm, there was no difference in the clinical appearance of all calves during the investigation. Of the calves that died in these groups, poor appetite and labored breathing were noted shortly before death. These findings are similar to those of Hollon et al. (13) involving 16 Holstein calves; FG intake had little effect on the appetite and growth response of calves prior to the onset of clinical signs. Similarly, in lambs fed varying amounts of FG, appetite and BW gains were not affected prior to the onset of clinical signs of toxicity (19). Also, in two field reports of gossypol toxicity outbreaks, most calves were found dead without any prior sign of clinical illness or history of poor feed consumption (13, 14). Those findings and those reported in the present study collectively suggest that FG intake has little effect on the appetite and growth response of calves prior to the manifestation of clinical symptoms of toxicity.

Literature attempts to define the maximum allowable nontoxic concentraiton of dietary FG are confusing in regard to young ruminants. This confusion can be attributed to two groups of variables. First, variation in the amount of FG is relative to amount and nature of other components of the diet, management factors, treatment of the gossypol in the cottonseed during processing and mixing in the feed (whole seed vs. CSM), limited feeding (sheep trials) versus free choice feeding (calves), whether the concentration of FG in feed was based on feed analysis or calculated from analysis values of the CSM before mixing, concentration and source of other protein in the diet (availability of amino groups for binding to gossypol), concentration of iron and other divalent cations in the diet, availability of hay, and length of time that milk is fed prior to weaning. Second, genetics or other ill-defined variables of animals, including natural disease and growth rate of the calf, confound results. Hollon et al. (13) reported high death losses in calves fed starters containing above 350 ppm of FG. No mortality was observed in their calves fed starter with 230 ppm of FG. In contrast, gossypol toxicity in housed calves occurred at concentrations above 840 ppm by Rogers et al. (24). In the study reported by Morgan et al. (19), 100% mortality occurred prior to 30 d of age in lambs administered 900 ppm of FG, and myocardial lesions were evident in euthanatized lambs consuming 100 ppm. However, in that investigation, FG was provided orally in gelatin capsules, which could have resulted in a more concentrated, undiluted dose of FG being made available for intestinal absorption within a very brief period.

The mortality rates from FG toxicity at all but the highest concentrations fed were surprisingly low in the present investigation, particularly in that protective measures, such as feeding hay or iron salts, were not used (ingredients reported to mitigate the effect of FG in ruminants) (28). Of the many factors affecting the response of calves to FG consumption, factors involved in the prevention of absorption, intake, and detoxification are of major importance. Diarrhea may speed transit time of the gossypol through the digestive tract and alter the functional mucosal surface area in a way that decreases gossypol absorption. Although we did not observe a difference in incidence of diarrhea among groups, the possibility that diarrhea reduces gossypol absorption in certain calves cannot be ruled out. The amount of feed intake and, thus, FG consumption could have been reduced in calves with clinical illness. However, clinical disease was similar for all groups and, therefore, was not a factor in group differences in early feed consumption and mortality. Furthermore, deaths in the group fed 800 ppm occurred in calves with both highest and lowest BW gains, indicating that feed intake was not a major factor in resistance to gossypol effects. Gossypol is detoxified in the rumen by being bound to soluble microbial proteins and by dilution and slowed absorption (23). It is not known precisely when the rumen becomes functional in growing calves, and evidence is lacking to identify factors that accelerate its development.

The syndrome of sudden death in calves that appeared to be healthy previously, reported elsewhere (14, 15) with gossypol toxicity, was not observed in the present study. Death of calves in the group fed 800 ppm occurred after clinical symptoms of respiratory distress were noted a few days prior to death. Post-mortem and histological findings in these calves were compatible with gossypol toxicity as reported by Holmberg et al. (14) and Smith (26). They included widespread edema and congestion, particularly in the lungs and body cavities. In addition, a straw-colored effusion was noted in the abdominal and thoracic cavities. In some calves, the heart was flabby and dilated. The livers in these calves were enlarged and congested; microscopically, there was central lobular degeneration of all but the most peripheral parts of hepatic cords. This change in the liver could be related both to anoxia from circulatory failure and to direct toxic effects of the gossypol on hepatocytes. Gossypol concentrations of all deaths in the group fed 800 ppm were greater than 160 μg/g of wet liver, whereas concentrations in calves fed nontoxic concentrations of FG were less than 50 μg/g.

Chemistry panels designed to monitor serum proteins, energy status, serum electrolytes, renal function, liver function, and necrosis did not prove useful to demonstrate impending toxic concentrations of FG when used as a group monitoring test. However, individual calf deviations existed in these parameters relative to clinical disease (C. A. Holmberg, 1991, University of California, Tulare, personal communication). This finding is in agreement with previously published reports and indicates that clinical chemistries may only be characteristic of liver failure during the terminal stages in affected calves.

Erythrocyte parameters were the only clinical pathology measures examined that varied from controls with an FG dose relationship. Decreased hemoglobin concentration, hematocrit, and erythrocyte numbers were most pronounced at 90 d of age and returned to normal by 120 d. Decreased hematocrit and hemoglobin concentrations have been reported in calves with gossypol toxicoses (18). Brahan et al. (9) reported that FG binds to iron when it is fed to pigs and is associated with decreased hematocrit. When ferrous ions were added to the diet containing gossypol, normal erythrocyte measures resulted, but toxicity resulted also (9). It has been suggested that iron bound to FG is unavailable for normal biosynthesis of hemoglobin and other processes in cattle (7). It is worth noting that iron deficiencies in the liver of calves with clinical, identifiable gossypol toxicity were not found (C. L. Holmberg, 1991, unpublished data). Decreased hematocrit could have resulted from a direct effect of FG on erythrocytes. Kuhlman et al. (17) demonstrated a linear decrease in erythrocyte fragility in cattle from 7.7 to 20% as FG intake increased from 5.9 to 18.5 g/d. Similarly, Wyse et al. (29) reported an increase in erythrocyte fragility in beef bulls consuming >2 g/d of FG. No signs of gossypol toxicity were observed in either of these studies.

Red blood cell indices were not different from controls and were in the normal range,

indicating that normal hematopoietic response was being maintained and supporting the observation that the degree of anemia at 120 d was mild in the survivors of even the group fed 800 ppm of FG. Overall changes in group means for hematology and serum chemistry variables were modest and insufficient to be used to distinguish, on a diagnostic basis, between safe and unsafe concentrations of FG in the diet in a monitoring program in situations in which young calves are consuming FG.

Death from gossypol toxicity is attributed to cardiac failure (8, 18). This accounts for the chronically labored breathing, fluid-filled lungs, hepatomegaly, and histopathological changes noted in the 4 dead calves consuming 800 ppm of FG. In swine and dogs, gossypol content of the liver was related directly to changes in electrical patterns of the heart (2, 20). Affected changes in the electrocardiogram were the T-wave amplitude, T-wave duration, and the isoelectric S-T segment. This pattern of changes is similar to humans with hyperkalemia (18). Elevated K were reported in two calves with gossypol toxicity (18). Electrical pattern alterations were prominent after prolonged gossypol feeding and may have led to a loss of electrical rhythm, predisposing calves to cardiac failure (2). However, no alterations in electrical patterns were found in other studies involving calves with gossypol toxicity (C. A. Holmberg, 1991, personal communications).

Laboratory analysis for total and FG concentration in the mixed rations fed was considerably lower than the calculated means. The standard analytical method used to determine FG concentrations in CSM is unsatisfactory when it is applied to mixed feeds (22). The problem with the standard method is that incomplete recovery of FG from feed mixtures and the extraction of other feed constituents interfere in subsequent colorimetric determinations. Pons and Hoffpauir (21) described a procedure using mixed isopropyl alcohol hexane water solvent containing a gossypol-complexing agent, 3-amino-1-propanol, which prevents interference by feed constituents and stabilizes FG during extraction. These compounds were not used in our methodology to determine FG and total gossypol in the mixed rations. Means for FG obtained from the pelleted and unpelleted CSM, using the procedures employed in this study, are reliable, and mathematical calculations were relied upon to give valid concentrations of FG in the mixed feeds. Barraza et al. (7) recently demonstrated that pelleted CSM reduced FG as much as 50%. However, in this study, the measured FG in the pellet was 3274 ppm compared with 3600 ppm (9% reduction) based on the FG value of the CSM after purchase. This reduction reflects both the effect of pelleting on the measurable FG and the effect of reduction that occurred during storage of the product prior to trial initiation. Therefore, calculated values closely reflect actual mixed feed levels. This observation has been made and commented on by other researchers (22).

In the study reported herein, we did not find any illness in calves consuming 100 or 200 ppm of FG (less than 1 g/d of FG intake) in a ration containing 31% CSM for the first 120 d of life. A single calf in the group fed 400 ppm of FG had, upon necropsy, evidence of circulatory failure contributing to the bacterial pneumonia and severe illness. Thus, we conclude that the feeding of 400 ppm of FG is mildly toxic for calves under 90 d of age. Feeding of 800 ppm of FG, however, resulted in significant death losses compatible with previous reports of gossypol toxicity. The relationship between respiratory disease and the chronic consumption of moderate concentrations of gossypol in the ration also needs to be defined.

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