

Reproductive and Hematological Effects of Feeding Diets Containing Gossypol to Mature Ewes

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ABSTRACT

The objectives were to determine what effects the short- or long-term feeding of diets containing gossypol to mature ewes had on reproductive and hematological variables. In Exps. 1 and 2, 29 Rambouillet ewes (avg wt, 128 ± 3.2 lb) and 35 Western white-face ewes (avg wt, 102.4 ± 3.6 lb), respectively, were randomly assigned to diets containing: (A) no cottonseed products (n = 10, 12); (B) cottonseed meal and cottonseed hulls (n = 9, 11); or (C) whole cottonseed and cottonseed hulls (n = 10, 12, respectively). Ewes were fed 3.3 lb hd⁻¹ d⁻¹ of their respective diet for either 87 ± 3.9 d (Exp. 1) or 126 ± 5 d (Exp. 2). Free gossypol (FG; Exp. 1) or total gossypol (TG; Exp. 2) intake for diets A, B, and C were: 0, 0; 0.95, 2.87; and 2.42, 3.14 g d⁻¹, respectively. Embryos were surgically flushed from the oviducts of superovulated ewes 5 d post-estrus (d 0 = estrus). Blood samples were taken from each ewe near the end of each experiment and serum hemoglobin and osmotic fragility of erythrocytes (RBC) were determined. Experimental data were analyzed by ANOVA and orthogonal polynomials. Controlled feeding of cottonseed products showed no effect (P > 0.05) on ADG, estrous cycle length, interval to onset of estrus after synchronization, number of ovulations after superovulation, number of embryos recovered, quality score of embryos, or percentage of abnormal embryos. Serum hemoglobin increased in a linear fashion (P < 0.005, Exp. 1) and a quadratic fashion (P < 0.001, Exp. 2) as FG or TG intake increased, respectively. Erythrocyte hemolysis increased in a linear manner (P < 0.001, Exp. 2) as TG intake increased. Feeding cottonseed products to mature ewes resulted in no detrimental effects on reproductive variables measured. However, hematological evidence indicated that fragility of RBC increased as gossypol intake increased.

KEYWORDS: embryo, erythrocyte fragility

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Gossypol, a yellowish phenolic compound, is a toxic constituent of pigments in the roots, stems, leaves, and seeds of cotton plants. In Texas and southern sections of the United States, cottonseed products represent an abundant source of feed ingredients for the diets of ruminant animals. During the fall and winter months when range forages are in short supply, animals used for reproductive purposes are often fed supplemental diets which may contain cottonseed hulls as a source of roughage and/or cottonseed meal or whole cottonseed as a source of protein.

Studies have indicated that gossypol has detrimental effects on humans (Qian and Wang, 1984), laboratory animals (Lin et al., 1980), non-ruminants (Skutches et al., 1974), and ruminants (Arshami and Ruttle, 1988, 1989). When fed in large quantities, signs of gossypol toxicity include anorexia, reduced growth rate, and labored breathing (Randel et al., 1992). More important in domestic farm animals is the antifertility effects of gossypol on males. Bulls and rams seem especially susceptible to gossypol, causing decreased fertility because of serious damage to the gonadal tissues. Lumen diameter, wall thickness, number of cell layers in the seminiferous tubules, and size of Leydig and Sertoli cells are reduced in males fed diets containing gossypol when compared to tissues from control males (Arshami and Ruttle, 1988, 1989; Kramer et al., 1989). Monogastric females are also sensitive to the antifertility effects of products containing gossypol. Estrous cycles, pregnancy, and early embryonic development all appear to be disrupted by gossypol (Randel, 1991). However, few studies have been reported that specifically investigate the effects of gossypol on female reproduction in ruminants. Using Rambouillet ewes, Kuhlmann et al. (1993) investigated the effects of feeding dietary supplements containing soybean meal, expander solvent (ES) cottonseed meal (CSM), and direct solvent (DS) CSM on selected reproductive characteristics. No differences were observed in ovulation rates, pregnancy rates, lambing rates, or birth weights of lambs from ewes consuming gossypol for a 16 wk period. In a study using Brangus heifers, Wyse et al. (1991a) found that the daily consumption of 5 g of free gossypol (FG) from CSM reduces ($P < 0.005$) the number of follicles > 4 mm in diameter when compared to heifers consuming 15 g FG daily from whole cottonseed (WCS) or control heifers consuming 0 g FG (32, 85, and 98 follicles, respectively). In a different experiment using the same treatments and similar animals, Wyse et al. (1991b) reported that heifers consuming CSM produce more ($P < 0.06$) degenerative embryos (33.3%) when compared to animals consuming WCS (13.9%). However, there were no observed differences in degenerative embryos when comparing control heifers to those consuming WCS. The objectives of the present experiments were to study the effects of short- or long-term feeding of diets containing gossypol on selected reproductive characteristics and hematology of red blood cells in mature ewes.

MATERIALS AND METHODS

Experiment 1

Twenty-nine Rambouillet ewes (avg wt, 128 ± 3.2 lb) were randomly assigned to one of three treatment diets containing: no cottonseed feed ingredients (control, $n = 10$), cottonseed meal and cottonseed hulls (CSM/CSH, $n = 9$), and whole cottonseed and cottonseed hulls (WCS/CSH, $n = 10$; Table 1). Treatment diets

contained 11.7% crude protein as determined by proximate analysis (AOAC, 1990) and were formulated to be isocaloric and isonitrogenous to meet or exceed NRC requirements (NRC, 1975). Ewes were offered water *ad libitum* and fed 3.3 lb per hd of their respective diet daily.

Table 1. Composition of diets.

Ingredient [†]	Diet		
	Control	CSM/CSH	WCS/CSH
	-----%-----		
Sorghum, dry rolled	26.78	26.78	26.78
Cottonseed hulls		48.15	39.94
Sudangrass hay [‡]	53.31		
Cottonseed meal		16.32	
Whole cottonseed			29.92
Soybean meal	10.50		
Fat, animal and(or) vegetable	6.05	5.39	
Urea	0.71	0.71	0.71
Salt	0.50	0.50	0.50
Ammonium Chloride	0.50	0.50	0.50
Dicalcium phosphate	0.89	0.89	0.89
Potassium chloride	0.07	0.07	0.07
Trace mineral premix [§]	0.50	0.50	0.50
Vitamin A, D, and E [¶]	0.19	0.19	0.19

[†]Dry matter bases, except whole cottonseed as fat source.

[‡]Ground with a tub grinder and mixed with other ingredients daily at feeding.

[§]Provides: 13.2 ppm Mn, 36 ppm Zn, 19.8 ppm Fe, 3.9 ppm Cu, 0.9 ppm I, 0.6 ppm Co, and 60 ppm Mg.

[¶]Provides: 540 IU per lb Vitamin A, 68 IU per lb Vitamin D, and 6 IU per lb Vitamin E.

Free gossypol percentage was determined by Pope Testing Laboratories, Dallas, TX. The results were: CSH, 0.045%; CSM, 0.258%; and WCS, 0.48% FG. By calculation, the ewes were offered the following amount of FG: control, 0 g d⁻¹; CSM/CSH, .95 g d⁻¹; and WCS/CSH, 2.42 g d⁻¹. Diets were fed for an average of 87 ± 3.9 d.

Estrous cycles were monitored daily using raddled rams equipped with marking harnesses. Weekly blood samples were obtained to monitor luteal function. Concentrations of serum progesterone were determined using a radioimmunoassay kit (Coat-A-Count TKPG5; Diagnostic Products Corp., Los Angeles).

After receiving their respective diet for an average of 68 d, ewes were synchronized by implanting one-half of a 6 mg Syncro-Mate-B (SMB) bovine implant (Sanofi Animal Health Corp., Overland Park, KS) subcutaneously in the ear as described by Ruttle et al. (1988). Synchronization implants were removed after 12 d.

Ewes were superovulated using a series of FSH-P injections (Schering Corp., Kenilworth, NJ) as follows: d 10 after implantation, 4 mg FSH-P b.i.d.; d 11, 3 mg FSH-P b.i.d.; and d 12, 2 mg FSH-P b.i.d. (total dose = 18 mg; Ruttle et al., 1988; Figure 1). After superovulation treatment and implant removal, the ewes were placed with rams equipped with a marking harness and mated naturally. A maximum of 3 ewes were placed with each ram during the breeding process. Breeding activity was observed at 12 hr intervals for 72 hr.

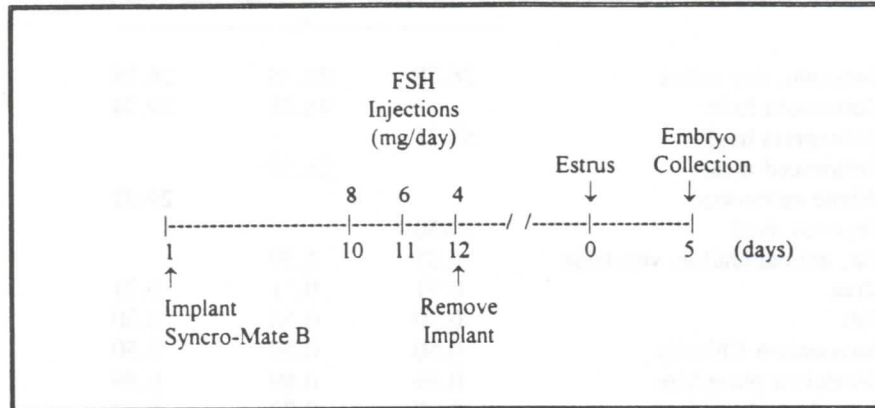


Figure 1. The synchronization and superovulation treatment plan for the ewes. The numbers below the dashed line refer to days of treatment. The dosages of FSH were divided equally between an a.m. and p.m. injection on d 10, 11, and 12.

Before breeding, the rams were fertility tested via electroejaculation and the semen evaluated for percent motility, normality, viability, live-dead, and concentration. Rams were fed diets that did not contain any cottonseed products and were not allowed access to any of the experimental diets.

Five days after estrus (estrus = d 0), the embryos were collected surgically via mid-ventral laparotomy. The number of corpora lutea (CL) on each ovary was counted and recorded. Embryos were flushed from each uterine horn using Dulbecco's phosphate buffered saline (American Embryo Systems, Grand Prairie, TX) containing 2% fetal calf serum (American Embryo Systems). Media flushed from each uterine horn was collected in a petri dish (Falcon Intergrid; Becton Dickinson & Co., Lincoln Park, NJ) and embryos were located under a stereomicroscope (Nikon, Japan). Evaluation of embryos was completed using a phase-contrast microscope (Leitz Fluovert, West Germany). Embryos were then classified on the basis of their morphological quality using a 5-point scale as follows: 1 = Excellent -- perfectly symmetrical with even granulation and no blastomere extrusion; 2 = Good -- occasionally slightly asymmetric in shape, even granulation

with some blastomere extrusion; 3 = Fair -- slightly asymmetrical in shape, blastomere extrusion and some degeneration; 4 = Poor -- uneven granulation, much blastomere extrusion and degeneration; and 5 = Unfertilized.

After the ewes were on their respective diets for an average of 82.5 d (pooled SE = .85), a blood sample was obtained for analysis of serum hemoglobin. Serum was obtained from the samples via centrifugation, frozen, and stored at -20°C until analysis. Serum hemoglobin was determined using a Co-Oximeter (Model 282, Instrumentation Laboratories, Lexington, MA).

End points evaluated by statistical analysis were: ADG, estrous cycle length, interval to onset of estrus after synchronization, number of CL after superovulation, number and quality of embryos collected per ewe, and serum hemoglobin. Data were analyzed by a completely randomized design analysis of variance (Steel and Torrie, 1980) using the General Linear Models procedure (SAS, 1985). The two degrees of freedom for treatment were resolved into a set of orthogonal polynomials (linear and quadratic) based on the grams of FG fed per day.

Experiment 2

Experimental materials and methods were the same for Exp. 2 with the following exceptions. Thirty-five Western white-faced ewes (avg wt, 102.4 ± 3.6 lb) were randomly assigned to the three treatment diets. By analytical determination, the percentages of FG and total gossypol (TG) in the cottonseed products were: CSH, 0.02, 0.05%; CSM, 0.16, 1.04%; and WCS, 0.57, 0.64%, respectively. The percentage of FG and TG was determined by Dr. M. C. Calhoun, Texas A&M Agricultural Experiment Station, San Angelo, TX. By calculation, the ewes were offered the following amount of FG and TG: control diet, 0, 0 g d⁻¹; CSM/CSH diet, 0.55, 2.87 g d⁻¹; and WCS/CSH diet, 2.7, 3.14 g d⁻¹, respectively. Experimental diets were fed for an average of 126 ± 4.6 d.

After receiving their respective diet for an average of 107 d, the same synchronization and superovulation scheme as Exp. 1 was implemented (Figure 1). Surgical procedures for collection and evaluation of embryos were also the same as previously described.

After evaluation, the embryos were incubated in DAPI (4'-6'-diamidino-2-phenylindole; Sigma Chemical Co., St. Louis, MO) stain in the dark for 15 min and then observed under a microscope using epifluorescence filters (excitation, 520 nm; barrier, 580 nm; Zeiss, West Germany). Subsequently, the nuclear membrane of embryos that were dead or degenerating was penetrated by the DAPI stain, emitting a brilliant yellow-colored fluorescence.

On d 155 of the experiment, a blood sample was obtained from each ewe for analysis of serum hemoglobin as previously described in Exp. 1. Serum was obtained from the samples via centrifugation, frozen, and stored at -80°C until analysis. In addition, on d 298 of the experiment, a sample of whole blood was obtained for determination of erythrocyte (RBC) hemolysis. Red blood cell hemolysis was performed by Dr. M. C. Calhoun according to the procedures of Nelson (1979).

Statistical analysis was the same as Exp. 1 with the following exceptions. Percentage of abnormal embryos was analyzed by Chi-Square, and the two degrees of freedom for treatment were resolved into a set of orthogonal polynomials (linear and quadratic) based on the grams of TG fed per day.

RESULTS AND DISCUSSION

Results from Exp. 1 indicated that mature ewes fed cottonseed products containing gossypol for an average of 87 d had no effect on ADG (0.27 lb), length of first (15.4 d) or second (16.3 d) estrous cycle, concentration of serum progesterone (2.0 ng mL⁻¹, range = 0.3 to 6.4 ng mL⁻¹, data not shown in tabular form), interval to onset of estrus after synchronization (26.8 h), number of ovulations after superovulation with FSH-P (10.6 CL), number of embryos recovered by surgical collection (4.6 embryos), or quality score of embryos (2.6; Table 2). Similar results were found in Exp. 2 after feeding cottonseed products for an average of 126 d. Mature ewes fed a diet containing gossypol showed no differences in ADG (0.24 lb), interval to onset of estrus after synchronization (33.9 h), number of ovulations after superovulation with FSH-P (14.1 CL), number of embryos recovered by surgical collection (5.8 embryos), quality score of embryos (3.7), or the percentage of abnormal embryos as determined by DAPI stain (57.6 %; Table 3).

Table 2. Least-squares means for performance and reproductive characteristics in ewes fed cottonseed products for 87 days (Experiment 1).

End point	Diet			Pooled	
	Control	CSM/CSH	WCS/CSH	SE	P [†]
Free gossypol fed, g d ⁻¹	0	0.95	2.42		
ADG, lb	0.29(10) [‡]	0.27(9)	0.24(10)	0.05	0.75
Estrous cycle length, d					
First cycle	15.4(10)	15.3(9)	15.4(10)	0.29	0.98
Second cycle	15.9(7)	16.2(6)	16.7(6)	0.52	0.57
Interval to onset of estrus after synchronization, h	31.2(10)	24.0(9)	25.2(10)	4.37	0.47
No. of ovulations after superovulation with FSH-P	9.3(8)	11.8(8)	10.7(10)	2.53	0.77
No. of embryos recovered	4.8(6)	5.4(7)	3.6(7)	1.54	0.68
Embryo quality score [§]	2.5(4)	2.8(3)	2.4(7)	0.55	0.89

[†]Probability of overall treatment difference.

[‡]Mean (No. of ewes).

[§]See Materials and Methods for explanation.

In agreement with these results, Kuhlmann et al. (1993) reported no differences in ovulation rates, pregnancy rates, lambing rates, or birth weights of lambs in Rambouillet ewes fed cottonseed supplements for 16 wk. With regard to embryo quality, Wyse et al. (1991b) found similar results in Brangus heifers where mean

quality grade, developmental stage score, and viability index of embryos recovered nonsurgically at 7 d postestrus were not affected by dietary FG.

Table 3. Least-squares means for performance and reproductive characteristics in ewes fed cottonseed products for 126 days (Experiment 2).

End point	Diet			Pooled SE	P [†]
	Control	CSM/CSH	WCS/CSH		
Total gossypol fed, g d ⁻¹	0	2.87	3.14		
ADG, lb	0.25(11) [‡]	0.22(11)	0.26(12)	0.04	0.79
Interval to onset of estrus after synchronization, h	33.3(9)	34.7(9)	33.8(11)	5.99	0.99
No. of ovulations after superovulation with FSH-P	11.8(8)	13.1(9)	17.3(11)	3.67	0.53
No. of embryos recovered	4.9(8)	4.2(9)	8.4(11)	2.36	0.39
Embryo quality score [§]	3.5(4)	4.1(4)	3.5(6)	0.54	0.68
Abnormal embryos, %	32.8(3)	75.0(4)	65.0(5)	18.06	0.32

[†]Probability of overall treatment difference.

[‡]Mean (No. of ewes).

[§]See Materials and Methods for explanation.

The only effects observed in these studies were the effects of feeding cottonseed diets on blood or serum characteristics. In Exp. 1, total hemoglobin ($P < 0.005$) increased in a linear fashion as the amount of FG fed per day increased (Figure 2). In Exp. 2, total hemoglobin ($P < 0.001$) increased in a quadratic manner as the amount of TG fed per day increased (Figure 3). Also in Exp. 2, hemolysis of RBC increased in a linear fashion ($P < 0.001$) as the amount of TG increased (Figure 4). Kuhlmann et al. (1993) also observed increased RBC osmotic fragility when feeding gossypol containing products to ewes. Erythrocyte fragility increased ($P < 0.01$) when the duration of feeding either ES or DS CSM increased from 6 to 16 wk, but RBC hemolysis was not affected by method of processing CSM (ES vs DS). These results are in agreement with those previously reported in cattle. Brangus heifers fed 5 g FG per day demonstrated an increased hemolysis of RBC at 42, 56, and 70 d of treatment when compared to heifers receiving less than 0.002 g FG per day (Wyse et al., 1991c). Increased concentration of plasma hemoglobin from ewes which consumed diets containing gossypol suggests that the feeding of cottonseed products increases the fragility of the outer membrane of red blood cells.

Therefore, based on the results of this study, diets containing cottonseed products fed for an average of 87 or 126 d in which the daily FG intake did not exceed 2.7 g or in which 65 to 70% of the diet contained cottonseed products had no detrimental effects on the reproductive characteristics observed.

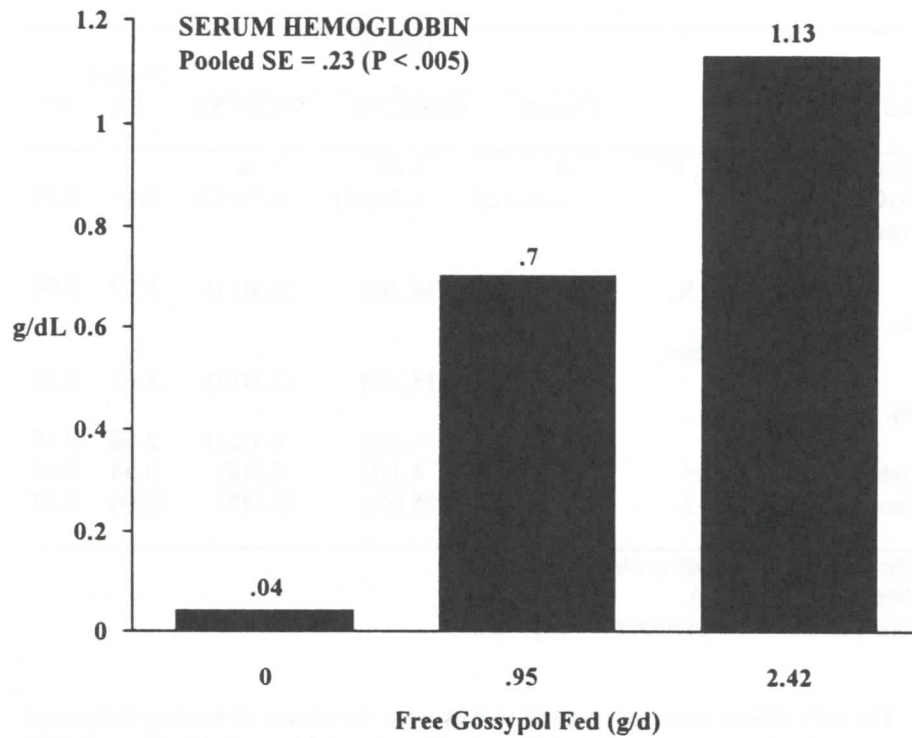


Figure 2. The effect of feeding cottonseed products (0 g FG, control diet; 0.95 g FG, CSM/CSH diet; and 2.42 g FG, WCS/CSH diet) to ewes on concentration of serum hemoglobin. The number of ewes that contributed to each mean were 8, 8, and 10, respectively.

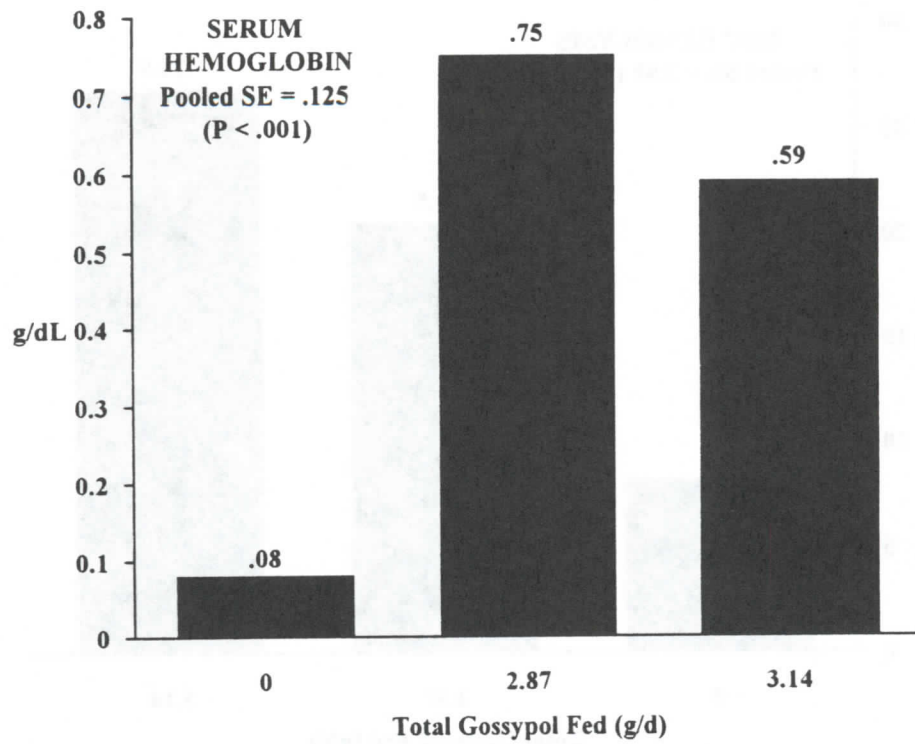


Figure 3. The effect of feeding cottonseed products (0 g TG, control diet; 2.87 g TG, CSM/CSH diet; and 3.14 g TG, WCS/CSH diet) to ewes on concentration of serum hemoglobin. The number of ewes that contributed to each mean were 10, 8, and 10, respectively.

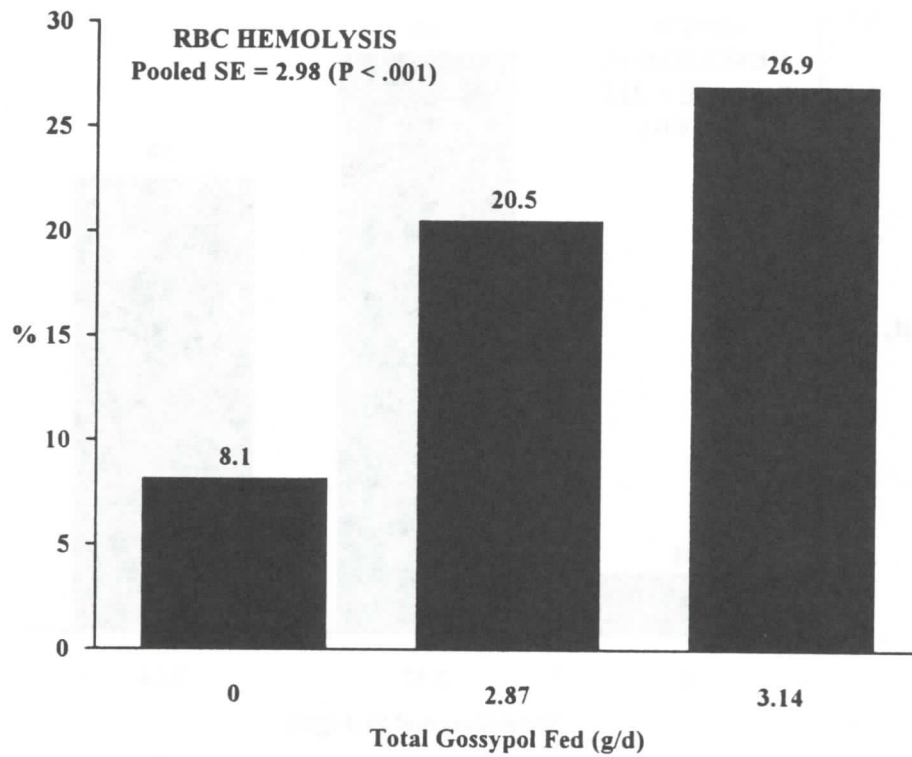


Figure 4. The effect of feeding cottonseed products (0 g TG, control diet; 2.87 g TG, CSM/CSH diet; and 3.14 g TG, WCS/CSH diet) to ewes on red blood cell hemolysis. The number of ewes that contributed to each mean were 10, 7, and 9, respectively.

REFERENCES

- AOAC. 1990. *Official Methods of Analysis* (15th Ed.). Association of Official Analytical Chemists, Washington, DC.
- Arshami, J., and J.L. Ruttle. 1988. Effects of diets containing gossypol on spermatogenic tissues of young bulls. *Theriogenology* 30:507.
- Arshami, J., and J.L. Ruttle. 1989. Effects of diets containing cottonseed meal on semen quality and testicular tissue in fine-wool rams. *J. Anim. Sci.* 67(Suppl. 2):195 (Abstr.).
- Kramer, R.Y., D.L. Garner, S.A. Ericson, D. Redelman, and T.W. Downing. 1989. The effect of dietary cottonseed on testicular development in pubertal rams. *Proc. Western Section Am. Soc. Anim. Sci.* 20:597.
- Kuhlmann, S.W., M.C. Calhoun, J.E. Huston, B.C. Baldwin, Jr., T.D. Willingham, and K.W. Bales. 1993. Reproductive performance of Rambouillet ewes supplemented with cottonseed meal. *J. Anim. Sci.* 71(Suppl. 1):30 (Abstr.).
- Lin, Y.C., M.A. Hadley, D. Klingener, and M. Dym. 1980. Effects of gossypol on the reproductive system of male rats. *Biol. Reprod.* 22(Suppl. 1):95A (Abstr.).
- Nelson, D.A. 1979. Erythrocyte disorders. p. 964. In J.B. Henry (ed.) *Todd, Sanford, and Davidsohn's Clinical Diagnosis and Management by Laboratory Methods* (16th Ed.). W.B. Saunders Co., Philadelphia, PA.
- NRC. 1975. *Nutrient Requirements of Sheep*. National Academy Press, Washington, DC.
- Qian, S.Z., and Z.G. Wang. 1984. Gossypol: A potential antifertility agent for males. *Annu. Rev. Pharmacol. Toxicol.* 24:329.
- Randel, R.D. 1991. Effects of gossypol on reproductive performance of domestic livestock. *J. Anim. Sci.* 69(Suppl. 1):536 (Abstr.).
- Randel, R.D., C.C. Chase, Jr., and S.J. Wyse. 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *J. Anim. Sci.* 70:1628.
- Ruttle, J., S. Lucero, S. Key, M. Daniels, F. Rodriguez, and H.S. Yim. 1988. Ovine estrus synchronization and superovulation using Norgestomet B and follicle stimulating hormone-pituitary. *Theriogenology* 30:421.
- SAS. 1985. *SAS User's Guide: Statistics*. SAS Inst., Cary, NC.
- Skutches, C.L., D.L. Herman, and F.H. Smith. 1974. Effect of dietary free gossypol on blood components and tissue iron in swine and rats. *J. Nutr.* 104:415.
- Steel, R.G.D., and J.H. Torrie. 1980. *Principles and Procedures of Statistics: A Biometrical Approach* (2nd Ed.). McGraw-Hill Book Co., New York.
- Wyse, S.J., J.M. Wilson, G.L. Williams, and R.D. Randel. 1991a. Effect of diets containing free gossypol on follicular development and function in bFSH treated Brangus heifers. *J. Anim. Sci.* 69(Suppl. 1):42 (Abstr.).
- Wyse, S.J., L.N. French, J.M. Wilson, and R.D. Randel. 1991b. Effect of diets containing free gossypol (FG) on embryo development, grade, and viability in bFSH treated Brangus heifers. *J. Anim. Sci.* 69(Suppl. 1):50 (Abstr.).
- Wyse, S.J., J.S. Velez, R.C. Stahringer, L.W. Greene, and R.D. Randel. 1991c. Effects of diets containing free gossypol on erythrocyte fragility and packed cell volume in cattle. *J. Anim. Sci.* 69(Suppl. 1):43 (Abstr.).

