

EFFECTS OF DIETS CONTAINING GOSSYPOL ON SPERMATOGENIC TISSUES OF YOUNG BULLS¹

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Summary

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Eighteen yearling beef bulls were used to determine the effects of diets containing gossypol on spermatogenic tissues, Sertoli cells and Leydig cells. Bulls were randomly assigned (six each) to diets formulated using alfalfa hay and corn (gossypol-free control), whole cottonseed or cottonseed meal as a source of gossypol (3% BW). Testicular tissues collected from bulls receiving dietary gossypol were examined histologically and compared to tissues from similar bulls fed gossypol-free diets. Following a 2-mo period (P₁) when bulls were fed diets containing gossypol, one-half of the bulls were placed on a gossypol-free diet for an additional 2 mo (P₂) to determine if gossypol effects were reversible. At the end of P₁, bulls fed whole cottonseed (WCS) and cottonseed meal (CSM) had larger (P<.01) lumens (LU) in their seminiferous tubules (ST), decreased (P<.001) wall thickness (WT) in their ST, and a reduced (P<.0001) number of cell layers in their ST, when compared with bulls fed a gossypol-free diet. These histological changes indicate detrimental effects to the spermatogenic tissues and associated cells. Following the initial 2-mo of receiving feedstuffs containing gossypol, herd mates were fed a gossypol-free diet for 2-mo (P₂). Micrograph examination of testicular tissues at the end of P₂ showed improvement in histological appearance, indicating gossypol-induced effects were reversible.

Introduction

Gossypol, a specific toxic substance present in the pigment glands of cottonseed, is a yellow polyphenolic compound of empirical formula C₃₀H₂₈O₈ (Myers and Throneberry, 1966). This chemical agent is regarded as a male contraceptive drug, which directly or indirectly disturbs development of spermatozoa. The contraceptive effect of gossypol has been attributed to an impairment of spermatogenesis, an accompanying reduction in sperm output, and the presence of morphological abnormal, immotile spermatozoa in the duct systems of humans, bulls, hamsters, rats and rabbits (Chang et al., 1980; Stahnke, 1986; Sotelo et al., 1982). Ultrastructure studies of testis have demonstrated that late and mid-spermatids are more sensitive to, and suffer most from, gossypol administration (Yanwan et al., 1985). A diet high in gossypol content may result in temporary sterility. A report from China (National Coordinating Group on Male Antifertility Agents, 1978) indicates low dosage levels of gossypol is an effective and reversible oral contraceptive for use in the human male. The potential effects of gossypol on reproduction among farm animals that consume cotton products is an area that needs investigation. The objectives of this study were to examine the effects of gossypol on spermatogenic tissues of young bulls, to determine

the mode of possible damage by histological examination of tissues, and to ascertain if damage was reversible.

Materials and Methods

Eighteen crossbred bulls (1 yr old) were randomly assigned to one of three treatment groups for a period of 4 mo. Diets consisted of (1) alfalfa hay and ground corn (control), (2) control diet and cottonseed meal (CSM) (3% of body weight), and (3) control diet and whole cottonseed (WCS) (3% of body weight) fed once a day for 2 mo (P₁). At the end of this period, three bulls from each group were slaughtered and one testicle was removed from each bull for a separate histological study. The remaining bulls were fed the control ration (P₂) for 2 additional mo to determine if any gossypol-induced effects were reversible. At the end of P₂, the remaining bulls were slaughtered and one testicle was removed from each bull for histological study.

Testicular tissue samples approximately 10 x 10 x 5 mm were removed immediately after slaughter from each animal, numbered and placed in 10% buffered formalin solution for 4 d. Following fixation, tissues were dehydrated, infiltrated and embedded according to Luna (1968). Tissues were sectioned at 2 microns and stained with Hematoxylin and Eosin for histological examination.

For quantitative evaluation, 20 randomly chosen, circular, seminiferous tubules from each bull were examined. Diameter of each seminiferous tubule (ST), lumen (LU) of the seminiferous tubule, Sertoli cell (SC), Leydig cell (LC) and wall thickness (WT) was measured in microns by the use of a calibrated micrometer eyepiece. The number of layers (NL) of spermatogenic cells in each tubule was counted.

Data were analyzed as a completely randomized design with a 3 x 2 factorial arrangement of treatments, using the General Linear Models procedure of the Statistical Analysis System (SAS, 1985; Steel and Torrie, 1980). When significant F-values were noted, means were separated using contrasts.

RESULTS

There were no significant differences in the diameter of ST and LC when compared with the control group during P₁ and P₂. During P₁, when bulls were fed CSM and WCS (Table 1), histological examination showed an increase in lumen diameter (P<.01) and a decrease in the number of cell layers (P<.0001), ST wall thickness (P<.001) and Sertoli cell size (P<.01) in comparison to tissues from bulls fed the control diet. Following P₂, when all

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bulls had been fed the control diet (Table 2), tissues from bulls fed either CSM or WCS during P₁ still had increased LU diameters (P<.01), decreased ST wall thickness (P<.001), decreased Sertoli cell size (P<.001) and a reduced number of cell layers (P<.0001) when compared with the control groups.

Discussion

Light microscopic examination showed the fine structure of control animal testicular tissue similar to that described by Fawcett and Burgos (1956) and Carmon and Green (1952). Light microscopic examination of ST from CSM-treated bulls revealed ruptured basement membranes, with presence of spermatocytes in the lumen and interstitial tissues because of destruction of the seminiferous tubules and interruption of spermatogenic tissue.

Seminiferous tubules from WCS-treated bulls had only a single layer of cells, consisting of the Sertoli cells and spermatogonia, in the tubule wall. This finding agrees with Shepu et al. (1980), who found a single layer of cells containing Sertoli cells and spermatogonia in the tubules of rats treated with gossypol. Presence of spermatocytes cells in the lumen indicates degeneration of the tubule structure.

The structure of Sertoli cells observed in samples from control bulls appeared to be essentially identical to that described by Bawa (1963) and Banks (1974). Sertoli cells are normally triangular, with a triangle-shaped nucleus located centrally in the cell. Sertoli cells from CSM and WCS-fed bulls were rounded with an abnormal shaped nucleus and dark spots in the cytoplasm. Size of sertoli cells were smaller (P<.01) in bulls receiving gossypol, suggesting impairment of normal cellular activity. However, because of the irregular shape of the sertoli cell and difficulty in measurement, further study may be needed to more clearly define gossypol effects. Kerr et al. (1979) and Nakamura and Hall (1980) reported that testis treated with heat or during cryptorchidism showed depolarization of the ST, and an increase in membrane permeability. Consequently, spermatogenesis was inhibited and the ultrastructural of Sertoli cells was damaged. In addition, Yanwan et al. (1985) found that dysfunction of the Sertoli cells disturbs the physiological environment for developing spermatocytes.

The structure of control bulls' Leydig cells was observed to be basically similar to that described by Banks (1974) and Maximow and Bloom (1953). The elongated Leydig cells are located in the interstitial tissue with large spherical nuclei. Leydig cells from CSM- and WCS-fed bulls did not differ (P>.01) in size when compared with Leydig cells of control bulls. Shepu et al. (1980), reported no detectable damage in the Leydig cells from rats treated with gossypol, which agrees with findings of this study. Continho (1982) and Shandilya and Clarkson (1982) found no decrease in plasma levels of testosterone in humans and monkeys fed gossypol, which suggests no effect on Leydig cells.

In the present study, number of layers and wall thickness of ST in the two groups of bulls fed CSM and WCS during the first period were higher in comparison to the same groups of bulls fed gossypol-free diet during the second period. This effect was attributed to the short length of recovery time during the second period. However, light micrographic observation of ST of animals fed a gossypol-

free diet in P₂ showed more circular arrangement of spermatocytes in comparison to that seen in tissues from bulls fed CSM and WCS during the first period. The improved histological characteristics of spermatogenic tissues following the second period indicates gossypol-induced effects are reversible.

Conclusions

Histological and structural examination of tissues from bulls fed diets containing gossypol revealed numerous morphological changes. The morphology of spermatogenic tissues of the seminiferous tubules was altered by feeding cottonseed meal and whole cottonseed. Partial recovery and regeneration of testicular tissue was observed at the end of the last period when animals were fed a gossypol-free diet. Results of this study suggest the possibility that feeding gossypol-containing rations to breeding bulls may reduce their fertility and be detrimental to their reproductive performance, indicating alternate protein sources may be desirable for breeding males.

References

- Banks, W. J. 1974. Histology and Comparative Organology. Williams and Wilkins Co. Baltimore.
- Bawa, S. R. 1963. Fine structure of the Sertoli cell of the testis. J. Ultrastructure Res. 9:459.
- Carmon, J. L. and W. W. Green. 1952. Histological study of the development of the testis of ram. J. Anim. Sci. 11:674.
- Chang, M. C., Z. Gu and S. K. Saksena. 1980. Effects of gossypol on the fertility of male rats, hamsters and rabbits. Contraception. 21:461.
- Continho, E. M. 1982. Clinical studies with gossypol. Arch. Androl. 9: 37.
- Fawcett, D. W. and M. H. Burgos. 1956. Observations on the cytomorphosis of the germinal and interstitial cells of the human testis. In: Wolstenholme, G. E. W. and E. C. P. Millar. (Eds.). A cila Foundation Colloguia on Aging. Little, Brown and Co., Boston. 2:86.
- Kerr, J. B., K. A. Rich and D. M. de Kretser. 1979. Effects of experimental cryptorchidism on the ultrastructure and function of the Sertoli cell and peritubular tissue of the rat testis. Biol. Reprod. 21:823.
- Luna, L. G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Co., New York.
- Maximow, A. A. and W. Bloom. 1953. Textbook of Histology. Saunders, W. B. Co., Philadelphia.
- Myers, B. D. and G. O. Throneberry. 1966. Effect of gossypol on some oxidative respiratory enzymes. Plant Physiol. 41:787.
- Nakamura, M. and P. F. Hall. 1980. The mechanism by which body temperature inhibits protein biosynthesis in spermatids of rat testes. J. Biol. Chem. 255:2907.
- National Coordinating Group On Male Antifertility

- Agents. 1978. Gossypol- a new antifertility agent for males. Chinese Med. J. 6:417.
- SAS. 1985. SAS Users' Guide: Statistics, version 5 Edition. Cary, NC: SAS Institute Inc.
- Shandilya, L. H. and T. B. Clarkson. 1982. Antireproductive and hypolipidemic effects of gossypol in male cynomolgus monkeys (*Macaca Fascicularis*). Arch. Androl. 9:32.
- Shepu, X., Z. Shudong, S. Shuyun, W. Yanwan, L. Yi, Z. Zengbua and M. Xixin. 1980. Antispermato-genic effect of gossypol on the germinal epithelium of the rat testes. Scientia Sinica. 23:647.
- Sotelo, A., I. Montalva, M. de la L. Crail and M. Gonzalez-Garza. 1982. Infertility in male rats induced by diets containing whole cottonseed flour. J. Nutr. 112:2052.
- Stahnke, C. R. 1986. Effects of gossypol on scrotal circumference and semen quality of crossbred bulls. M.S. Thesis. New Mexico State University.
- Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York.
- Yanwan, W., T. Dashan, Y. Dong, W. Naiying, Z. Shudong and X. Yikuan. 1985. Effect of gossypol on the potential difference of rat seminiferous tubules. Scientia Sinica. 28:402.

Table 1. Histological characteristics of bovine testis from young bulls fed cottonseed meal (CSM), whole cottonseed (WCS) or a gossypol-free diet during the first 2 mo period.

Treatment	No. bulls	Seminiferous tubule means				Cell diameter	
		Diameter (μ)	Lumen diameter (μ)	Layers (no.)	Wall thickness (μ)	Sertoli (μ)	Leydig (μ)
CSM	3	35.7	25.3**	3.0**	10.2**	11.9**	11.7
WCS	3	34.9	20.8**	3.2**	14.0**	12.3**	10.6
Control	3	35.5	17.9	6.3	17.4	13.1	11.4
Pooled SEM	-	0.9	0.9	0.4	0.8	0.6	0.6

** = P<.01

Table 2. Histological characteristics of bovine testis from young bulls fed a gossypol-free diet for 2 mo following feeding of cottonseed meal (CSM) or whole cottonseed (WCS).

Treatment	No. bulls	Seminiferous tubule means				Cell diameter	
		Diameter (μ)	Lumen diameter (μ)	Layers (no.)	Wall thickness (μ)	Sertoli (μ)	Leydig (μ)
CSM	3	35.3	25.6**	2.8**	9.7**	12.2**	11.2
WCS	3	34.3	24.2**	2.7**	10.1**	12.1**	10.5
Control	3	34.6	19.5	5.4	15.1	13.4	10.8
Pooled SEM	-	0.9	0.8	0.5	0.8	0.5	0.6

** = P<.01