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REVERSIBILITY OF THE REPRODUCTIVE TOXICITY OF DIETARY GOSSYPOL IN GUINEA-PIG (*Cavia porcellus*)

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ABSTRACT

In order to study the effects of gossypol and the reversibility of its reproductive toxicity, forty- eight male guinea pigs were divided into four groups of 12 each. Each group received one of four levels of cottonseed cake corresponding to four doses of dietary gossypol: 0.00; 7.78; 15.56; 23.34 mg.kg^{-1} body weight day^{-1}. At the end of a two month-treatment period, six guinea-pigs per group were sacrificed per lot. The other six were fed for two additional months without cottonseed cake before being sacrificed in turn. The weight of the vas deferens and accessory glands were reduced significantly ($P<0.05$) in guinea-pigs receiving gossypol at 23.3 mg kg^{-1} body weight. There were no significant differences ($P>0.05$) among treatments in testis weight and volume and the weight of the epididymis. The reaction time of a male to the presence of a female was significantly reduced by increasing levels of gossypol, however there were no changes in testosterone concentration. Consistent increases in the weight and volume of reproductive organs, testosterone concentration, and epididymal sperm count and motility after removal of cotton cake from the diet suggested that there had been a reversal of any adverse effects of gossypol. Thus up to 23 mg kg^{-1} body weight day^{-1} of gossypol in cottonseed cake can be fed to guinea-pigs without any risk of a definitive alteration of their fertility.

Keywords: cottonseed cake, gossypol, male guinea pig, reproduction, toxicity, reversibility.

INTRODUCTION

To address the gap between available resources and population growth, non-conventional livestock practices like caviaculture, the breeding of guinea-pigs, are gaining attention. In an extensive system, guinea-pigs are fed with forages only; hence their poor growth performance (Manjeli *et al*., 1998). Optimisation of production requires a more sophisticated approach that better meets their dietary needs. Achieving this goal will require feeds that contain higher levels of nutrients than found in forages.

In Cameroon, cottonseed cake, a by-product of oil extraction from cottonseed (Mena *et al*., 2004; EPSA, 2008), is commonly used in the formulation of animal feed and is an important source of proteins (Velasquez-Pereira *et al*. 1998; Mena *et al*., 2004; El-Mokadem *et al*., 2012). During food shortages, or increases in the price of competing protein sources like soybean, it may serve as the main protein source in animal feed. As cotton products or by-products contain gossypol, this is not desirable.

At certain concentrations, gossypol is known to cause reproductive toxicity in domestic animals. The most reported effects are the reductions in ejaculate volume, sperm motility, spermatogenesis, semen fructose concentration and serum concentration of testosterone, and an increase of morphologically abnormal spermatozoa (Velasquez-Pereira *et al*., 1998; Amini and Kamkar, 2005; El-Mokadem *et al*., 2012). Non-ruminant animals are particularly sensitive (Randel *et al*., 1992). In China in the 1970s, gossypol was tested as a human-male antifertility drug (Waites *et al*., 1998, Romualdo *et al*., 2013); in some instances this led to lowered fertility and sterility (Waites *et al*., 1998). Nevertheless, the toxic effects can be reversed following the withdrawal of gossypol as has been demonstrated in cattle (Hassan *et al*., 2004), rabbit (Taha *et al*., 2005) and man (Waites *et al*., 1998). Failure to recover is attributed to elevated doses, duration of exposure, animal species and testicular volume (Waites *et al*., 1998). To the best of our knowledge, a similar study has not been done in guinea-pigs.

In this study, we determined the reproductive toxic effects of gossypol on guinea-pigs and whether they can be reversed after removal of its source from the rations commonly offered.

METHODS

Animals and feeding

Forty eight adult male guinea-pigs raised at Dschang university farm were used. Mean weight was 510±53 g at the start of the assay. Sexually mature females were also used, but only at the end of gossypol- and gossypol-free feeding periods.

Animals were fed with elephant grass-based rations and a supplement of provender feed. Four formulations of feeds were used (Table-1). The control was cotton cake-free (R0) and the three others (R1 to R3) contained increasing per cent quantities of cotton cake. As cotton-cake quantity increased, the per cent quantities of corn, wheat bran, soya bean meal and palm kernel cake were decreased in order to maintain constant crude protein levels among feeds (Table-1).

Assay

Guinea-pigs were allocated randomly to four identical groups of 12 each, at the same time ensuring that

ARPN Journal of Agricultural and Biological Science

the distribution of body weight was similarly represented in each group. Each group was assigned to one level of cottonseed cake (0; 10; 20; 30% of the supplement). The corresponding calculated doses of gossypol were 0, 7.8, 15.6, or 23.3 mg kg^{-1} body weight day^{-1}. The quantity of the supplement given was sufficient to deliver the required concentration of gossypol to each animal. Sixty days after the treatments commenced, half (6) the guinea-pigs in each feeding group were selected randomly and sacrificed for analysis. The remainder were fed for an additional 60 days without cottonseed cake and then sacrificed in turn.

Table-1. Composition and chemical characteristics of the supplement in the rations.

Composition of the supplement				
Ingredients	**R0**	**R1**	**R2**	**R3**
Corn	35	37	43	57
Wheat bran	25	28	25	8
Cotton cake	0	10	20	30
Soybean meal	13	4	3	1
Palm kernel cake	14	8	3.5	1
Fishmeal	1	1	0.5	0.5
Bone powder	1	1	1	1
NaCl	1	1	1	1
CAMV	10	10	3	0.5
Chemical characteristics of the supplement				
Gossypol content (% DM)	0.00	0.014	0.028	0.042
Crude protein (% DM)	19.20	19.16	19.15	19.54
Digestible energy (kcal.kg^{-1}DM)	2328.30	2458.90	2843.10	3151.10
Lysine (% DM)	0.65	0.58	0.69	0.73
Methionine (% DM)	0.19	0.19	0.24	0.29

R0 is the control without cotton cake. DM is dry weight.

Data collection

A day before each sacrifice, the female guinea pigs were presented to the males. Sexual activity was recorded as the percentage of males reacting to the presence of a female, and the elapsed time until intercourse occurred. After sacrifice of the males, the weights of their testes, epididymis, vas deferens and accessory glands, the volume of their testicles, the characteristics of cauda epididymal sperm and the concentration of serum testosterone were recorded. Testis volume was measured by displacement of water in a graduated tube. The characteristics of cauda epididymal sperm were assessed following the methods described by WHO (2010). The concentration of serum testosterone was measured using a Diagnostic Automation/Cortez Diagnostic Inc. testosterone ELYSA kit (AccuDiag^{TM}).

Statistical analysis

All data, except the proportion of males reacting to the presence of females, were expressed as mean ± S.D and compared with one way ANOVA and Duncan's Multiple Range Test ($P<0.05$).

RESULTS

Reproductive organ weight and volume

At the end of the 60-day gossypol feeding period, there was a significant reduction ($P<0.05$) in the weight of the vas deferens and accessory glands in guinea-pigs receiving the highest level of gossypol as compared to the control (no gossypol) and the lowest gossypol treatment (Table-2). There were no significant differences ($P>0.05$) in the volume and the weights of testes and epididymis between treatments.

ARPN Journal of Agricultural and Biological Science

Table-2. The effects of gossypol on the genital organ weight and volume in guinea-pig 60 days after the treatments with cottonseed cake commenced, and 60 days after these treatments were stopped.

Organ	**Doses of gossypol** (mg/kg/day)			
	0.0	**7.8**	**15.6**	**23.3**
Testis (g)				
-At the end of treatment	1.18±0.36aα	1.14±0.16aα	1.01±0.44aα	1.00±0.37aα
-60 days after treatment	1.13±0.15aα	1.34±0.11bα	1.07±0.13aα	1.08±0.20aα
Testis (ml)				
-At the end of treatment	1.10±0.22aα	1.12±0.22aα	0.96±0.35aα	1.20±0.45aα
-60 days after treatment	1.04±0.09aα	1.52±0.36bα	1.20±0.33aα	1.45±0.33aα
Epididymis (g)				
-At the end of treatment	0.29±0.20aα	0.21±0.03aα	0.23±0.16aα	0.25±0.13aα
-60 days after treatment	0.26±0.05abα	0.30±0.02aα	0.22±0.05bα	0.25±0.05abα
Vas deferens (g)				
-At the end of treatment	0.30±0.09aα	0.28±0.07aα	0.25±0.09abα	0.16±0.04bα
-60 days after treatment	0.34±0.07abα	0.36±0.07aα	0.24±0.04cα	0.26±0.06bcα
Accessory glands (g)				
-At the end of treatment	1.32±0.68aα	1.45±0.54aα	0.90±0.77abα	0.40±0.20bα
-60 days after treatment	1.54±0.34abβ	2.21±0.97aα	1.02±0.29bα	1.52±0.19abβ

Different letters indicate significant differences among groups (p<0.05) in the same line (a, b,,,) and column (α, β…).

Sixty days after the cessation of the gossypol treatments, the weight and/or volume of reproductive organs were the highest in animals submitted to 7.8 mg kg^{-1} day^{-1} when compared to other treatments (Table-2). For the testis weight and volume, this difference was significant (P<0.05). For the weight of epididymis, vas deferens and accessory glands, the difference were respectively significant (P<0.05) compared to the 15.6, 15.6 and 23.3, and 15.6 mg Kg^{-1} day^{-1} treatments.

Within gossypol treatments, the weight of accessory glands 60 days after exposure was significantly greater (P<0.05) than at the termination of treatment in the controls and guinea pigs receiving 23.3 mg kg^{-1} day^{-1}. Others reproductive organs followed a similar tendency, but without any significant difference (P>0.05).

Characteristics of cauda epididymal sperm

At the end of the gossypol feeding period, the cauda epididymal sperm count and motility decreased not significantly (P>0.05) with increasing dose of gossypol (Table-3). Sixty days after the cessation of the gossypol feeding, differences between doses remained non-significant with one exception; there was a significantly greater number of sperm (P>0.05) in guinea pigs fed 7.8 mg kg^{-1} day^{-1} gossypol compared to the other treatments. Gross motility was significantly greater 60 days after gossypol feeding than at the end of treatment in guinea pigs receiving 23.3 mg kg^{-1} day^{-1} (Table-3).

Table-3. Cauda epididymal sperm characteristics.

Caudal Epididymal sperm characteristics	**Doses of gossypol** (mg/animal/day)			
	0.00	**7.8**	**15.6**	**23.4**
Number ($x10^6$)/tail				
-At the end of treatment	113.1±76.2aα	102.8±18.1aα	92.9±64.5aα	131,3±4.7aα
-60 days after treatment	104.2±21.7aα	159.5±38.9bα	119.4±34.6abα	109.2±4.08abα
Gross motility				
-At the end of treatment	3.20±0.84aα	2.17±0.98aα	1.80±1.30aα	1.75±0.96aα
-60 days after treatment	2.60±1.14aα	2.80±0.84aα	2.60±1.14aα	2.25±1.26aβ
Individual motility (%)				
-At the end of treatment	60.4±18.7aα	60.7±9.0aα	56.8±17.1aα	57.6±9.4aα
-60 days after treatment	61.8±9.2aα	75.5±5.0aα	71.3±8.5aα	66.6±20.1aα

Different letters indicate significant differences among groups (p<0.05) in the same line and column.

Serum testosterone and male guinea-pig sexual behaviour

There were no significant differences between treatments (p>0.05) in serum testosterone concentrations, both at the suspension of gossypol ingestion and 60 days later (Table-4). Within each lot of guinea-pigs, serum testosterone levels were consistently greater 60 days after gossypol feeding period of treatment than at the end of treatment, though differences were not significant (P<0.05).

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There was a significant reduction (P<0.05) in the percentage of males that reacted to the presence of a female with increasing levels of gossypol, both at the end of treatment and 60 days later (Table-4). However, no significant difference in the reaction time existed among gossypol doses. Within each lot, there was no significant difference in the percentage of males that reacted (P>0.05) at the end of treatment and 60 days later. As for the reaction time, guinea-pigs receiving 7.8 mg kg^{-1} day^{-1} gossypol reacted significantly less rapidly (P<0.05) at the end of treatment than 60 days after the termination of gossypol exposure.

Table-4. Effects of dietary gossypol on the serum testosterone and guinea pig libido.

Parameters	**Doses of gossypol** (mg/animal/day)			
	0.00	**7.8**	**15.6**	**23.4**
Serum testosterone (ng/ml)				
-At the end of treatment	2.37±1.55aα	1.91±1.66aα	2.05±1.06aα	1.96±0.69aα
-60 days after the treatment	3.09±0.47aα	2.60±0.17aα	2.77±0.18aα	2.96±0.57aα
Percentage of reaction				
-At the end of treatment	91.00aα	63.64bα	40.00cα	30.00cα
-60 days after the treatment	75.00aα	67.00aα	40.00bα	25.00cα
Reaction time				
-At the end of treatment	179.6±112.2aα	142.1±117.8aα	300.0±00.0aα	260.0±69.3aα
-60 days after the treatment	130.0±148.0aα	202.5±113.3aβ	75.0±21.21aα	60.00±00.0aα

Different letters indicate significant differences among groups (p<0.05) in the same line and column.

DISCUSSIONS

Feeding gossypol to guinea-pigs over a 60-day period resulted in a tendency, but not significant decrease of the weight and volume of the testis. Thus higher doses of gossypol than used in our study (> 23.3 mg.kg^{-1}) are likely to be required for deleterious effects to occur (Waites *et al*., 1998). Since the development of the male reproductive organs is under the hormonal control of the testis, the weight of the epididymis was affected accordingly. However, the fact that the highest dose of gossypol fed to guinea pig, 23.3 mg kg^{-1} day^{-1}, led to a significant reduction in the weight of the vas deferens and accessory glands remained unexplained. The production of testosterone by the testes also controls their development (Singer, 2005). However, testosterone production was unaffected by gossypol treatments.

At the termination of the gossypol feeding period, cauda epididymal sperm count and motility decreased not significantly by increasing doses of dietary gossypol. In mice (Amini and Kamkar, 2005), rabbit (Roychoudhury *et al*., 2009), cattle (Chenoweth *et al*., 1994), and sheep (Cunha *et al*., 2012), sperm quality decreased and cauda epididymal sperm characteristics were prejudiced when gossypol was ingested. As testicular size is a good estimator of sperm number and motility (Senger, 2005), the epididymal sperm quality obtained in our study is inherent to the testis development. Guinea pig appears to be more tolerant to the potential effects of gossypol on sperm quality than other animals, including non-ruminants, examined to date.

Serum testosterone concentration remained unchanged with increasing levels of gossypol. A previous study using 13.43 mg kg^{-1} day^{-1} gossypol resulted in inhibition of testicular steroidogenesis in mice (Ali *et al*., 2010); however sensitivity to gossypol in this respect is known to differ among species (Amini and Kamkar, 2005; D'Cruz *et al*., 2010). Although the histology of the testis was not examined in this study, it is likely that the interstitial cells would not have been affected either structurally or functionally, or at least sufficiently to significantly decrease testosterone production.

The percentage of males reacting to the presence of a female was significantly reduced by increasing levels of gossypol. As there were no treatment differences in serum testosterone concentration, this result was unexpected because libido is considered to reflect levels of circulating testosterone. However another manifestation of libido, the elapsed time until intercourse occurred, was not affected by treatment. This result differs from that of Valesquez-Pereira *et al*. (1998) who reported that bulls offered 14 mg kg^{-1} day^{-1} gossypol exhibited less sexual activity. Thus in guinea pigs, male sexual behaviour appears not to be compromised at the levels of gossypol used in this experiment.

Although usually not significant, increases in the weight and volume of reproductive organs, blood testosterone concentration, and epididymal sperm count and motility were consistently recorded sixty days after cessation of treatments that included cottonseed cake. This suggests that there had been a reversal of adverse effects of gossypol on these organs. Another possible explanation is that reproductive organs were still developing during this period of the study. However the lack of significant differences in all variables in the controls between the end of treatment and 60 days after the cessation of treatment suggests that this was not the case. The loss of the regular trend to the diminution in weight and volume of reproductive organs at the cessation of gossypol-free feeding period among treatments is another expression of the reversibility of gossypol in guinea-pig.

CONCLUSIONS

The inclusion of cotton cake in their diet caused deleterious effects on guinea-pig reproductive organs and in particular an apparent loss of some aspects of their libido and the weight of the vas deferent and accessory glands. However there was no effect on testosterone concentration and an indication of reversal of any deleterious effects 60 days after the removal of cottonseed cake from the ration. Thus a quantity of cotton product or by-product that delivers up to 23 mg kg^{-1} body weight day^{-1} can be fed to guinea-pigs without any risk of a definitive alteration of their fertility.

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