

Original Research Article

Reproductive Characteristics of Growing Boars Fed Diets Containing Raw or Fermented Cotton Seed Cake

Abstract

This experiment was conducted to investigate the effect of dietary Raw or Fermented Cotton Seed Cake (RCSC or FCSC) on reproductive characteristics of growing boars. Thirty (30) weanling boars (n=6 per treatment) were randomly assigned to five dietary treatments arranged in a 2x2 factorial with a control within a Completely Randomized Design (CRD). Solid state fermentation (SSF), using *Aspergillus niger*, was employed to ferment the CSC. The RCSC and FCSC were separately included in diets at 0 (control), 10 and 20% levels. At the end of the 12 weeks feeding trial, three (3) animals per treatment were slaughtered and reproductive organs were carefully dissected out, measured and processed for sperm evaluation. The reproductive parameters evaluated include testicular and epididymal morphometrics, Daily Sperm Production (DSP), Daily Sperm Production per gram testis (DSP/g), Gonadal Sperm Reserve (GSR), sperm count and motility. The results showed that Cotton Seed Cake (CSC) level resulted in significant ($p<0.05$) reduction in testicular weights, lengths and volume. The epididymal weight was significantly ($p<0.05$) lower for boars that were fed CSC based diets compared to the control group. Significant ($p<0.05$) reduction in epididymal length was recorded for the boars fed 20% FCSC compared to those fed the control diet. The DSP/g was significantly ($p<0.05$) higher in boars fed 10% RCSC than those fed other diets. Sperm count and motility, normal and life sperm, GSR, DSP and DSP/g were significantly ($p<0.05$) lower for boars that were fed FCSC-based diets compared to those fed the control diet and 10% RCSC. It was concluded from this study that feeding RCSC - or FCSC - based diets to growing boar adversely affected the testicular and epididymal morphometrics; however, 10% RCSC in diet did not compromise gonadal sperm characteristics.

Key Words: Boars, Cottonseed cake, Fermentation, Testis and Sperm characteristics

Introduction

Cotton seed cake (CSC) is a by-product of the cotton processing industry (Apata, 2010). This cake is rich in protein (42%) but contains gossypol which has been recognized to be toxic to animals (Risco and Chase, 1997).

In monogastrics, gossypol interfered with protein digestion, depressed appetite, caused reproductive impairment and intestinal and other internal organ abnormalities (Francis *et al.*, 2001). Earlier, Eisele (1986) had reported that feeding sows and gilts diets containing free gossypol either reduced conception rates or caused conception failure. Gossypol has been shown to exert anti-fertility effects in males (Randel *et al.*, 1992). It has direct damaging effects on the epididymides, testes and the developing germ cells (Frick and Danner, 1985).

Different methods have been used either to completely or partially remove the gossypol from cottonseed cake meant for animal feeding. These include ferrous sulphate treatments (Tabataba *et al.*, 2002) and microbial fermentation (Shi and Noblet, 1993). Microbial fermentation has been proved to be more effective in the detoxification of CSC (Shi and Noblet, 1993). This study was designed to investigate the effect of raw or fermented CSC-based diets on the reproductive characteristics of growing boars.

Materials and Methods

Thirty (30) growing boars, aged between 9-10 weeks, were randomly divided into five (5) groups (n = 6 animals per treatment) arranged in a 2x2 factorial with a control within a completely randomized design (CRD). Each animal served as a replicate. Solid State Fermentation (SSF) using *Aspergillus niger* was employed to ferment the CSC.

Isolated and purified culture of the fungus *Aspergillus niger* which had been grown on Potato Dextrose Agar (PDA); supplemented with 20% sucrose at pH 5.5, temperature of 30°C and preserved at 4°C was obtained from the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. A loopful of the mycelium of *Aspergillus niger* was inoculated into Kirks' basal inoculum medium, thus, an aqueous suspension of *Aspergillus niger* was prepared. The suspension was collected and stored in a refrigerator until it was used. Moisture content of dry CSC was raised to 60%. The CSC was then sterilized in a locally fabricated 24 kg-capacity fermentor. Sterilization was done by autoclaving at 121°C for 3 hours and the CSC was allowed to cool. The CSC was later inoculated and left to ferment for 7 days at 30±1°C. Prior to autoclaving, each plate containing CSC was covered with muslin cloth and aluminium foil.

The boars were acclimatized for a period of one week during which soybean meal-based diet was offered with clean water "containing anti-stress (stress strok, at 5g/10 litres) and antibiotics (Anicilin)". The boars were supplied with feed and water *ad-libitum*. Animals were fed the experimental diets for 12 weeks. The diets were prepared to contain 18% crude protein such that soybean meal (SBM) was the main protein source for the control diet (T₁). In diet 2 (T₂) and diet 3 (T₃), 10% and 20% of raw cotton seed cake (RCSC), respectively, were included to replace SBM; while 10% and 20% fermented cotton seed cake (FCSC) were included in diet 4 (T₄) and diet 5 (T₅), respectively. The gross composition of the experimental diets is presented in Table 1.

At the end of the feeding trial, 3 animals were slaughtered per treatment and their reproductive organs were carefully dissected out and measured. The testes and epididymides of each animal were removed and separated from each other. Data were obtained for testicular and epididymal morphometrics, sperm characteristics, Gonadal Sperm Production (GSP), Gonadal Sperm Reserve (GSR) and Daily Sperm Production (DSP).

Vernier Caliper was used to measure the length and width of the testes, while the volume of testis was determined by Archimede's principles of water displacement, using a measuring cylinder. Right and left testis weight; paired testis weight; paired testis volume; right and left

testis length; right and left testis width were measured; right and left epididymal weight and length were measured.

The GSP and GSR were determined haemocytometrically by homogenate technique using a modification of the method of Igboeli and Rakha (1971), Egbunike (1981) as described by Adejumo (2006) and Amao *et al.* (2012).

Daily sperm production (DSP) was determined from the GSR. The DSP was obtained using the formula proposed by Amann (1970).

$$\text{DSP} = \frac{\text{Testis sperm count (GSR)}}{\text{Time divisor}}$$

The value of time divisor for boars = 4.37 (Amann, 1970). Daily sperm production per gram parenchyma (testis) per animal was estimated by the formula:

$$\text{DSP/g parenchyma} = \frac{\text{Gonadal Sperm Reserve}}{\text{Gross testis wt} - \text{Tunica albuginea wt}} \times \frac{1}{4.37}$$

Sperm morphology was determined according to the method of Zemjanis (1970).

Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) appropriate for a 2x2 factorial and one-way using SAS (2002). Means were separated by Duncan's option of the same statistical package.

Results

The Results of this study are presented in Tables 3 to 8. The testicular parameters of growing boars fed raw or fermented cottonseed cake are presented in Table 3. All parameters, except the testis width, were significantly ($p < 0.05$) affected by the treatments. Left testis weight, right testis weight, paired testis weight, left testis length, right testis length, mean testis length, left testis volume, right testis volume and paired testis volume were significantly ($p < 0.05$) reduced in boars fed CSC based diets compared to those fed the control diet.

The effect of CSC level and processing on the testicular characteristics of growing boars are shown in Table 4. All the testicular characteristics measured were not significantly ($p > 0.05$) affected by the CSC levels and processing. The epididymal characteristics of growing boars fed raw or fermented CSC based diets are presented in Table 5. The left, right and paired epididymal weights; left and right epididymal lengths were significantly ($p < 0.05$) affected by the dietary treatments. The epididymal weights (left, right, paired and mean) were significantly ($p < 0.05$) lower for the boars that were fed cottonseed cake based diets compared to the control group. The epididymal lengths (left, right and mean) of boars offered 20%

FCSC were shorter than those fed the control. The effects of CSC level and processing on epididymal characteristics of growing boars are shown in Table 6. The left, the right and mean epididymal weights and lengths were not significantly ($p>0.05$) affected by CSC level or processing.

Table 7 shows the effect of different levels of raw or fermented cottonseed cake on sperm characteristics of growing boars. All sperm parameters were significantly ($p<0.05$) affected by the treatments. Sperm count was significantly ($p<0.05$) reduced in boars fed either raw or fermented CSC. Both live and normal sperm were similar for boars fed 10% RCSC and the control. Sperm motility was reduced in boars fed either fermented CSC based diet or 20% RCSC. Daily sperm production per gram testis was significantly ($p<0.05$) higher for boars fed 10% RCSC than those that consumed other diets. Gonadal sperm reserve and daily sperm production per gram testis were significantly ($p<0.05$) lower for the boars that were fed FCSC compared to the control and RCSC groups.

The effects of CSC levels and processing on sperm characteristics of growing boars are presented in Table 8. The sperm characteristics namely: sperm count, sperm motility, normal sperm, live sperm, GSR, DSP and daily sperm production per gram testis were significantly ($p<0.05$) affected by processing. Fermentation significantly ($p<0.05$) reduced all the sperm characteristics except GSR. Cotton seed cake level had no significant ($p>0.05$) effect on sperm characteristics.

Discussion

The significant reduction observed in most of the testicular parameters (testis weights, lengths and volume) of boars fed either raw or fermented CSC-based diets suggests that gossypol compromised the growth of the testes and fermentation was not able to completely ameliorate the adverse effect of gossypol contained in the fermented CSC-based diets. Perry and Peterson, (2001) reported that testis size reflects both the present and future sperm production capacity, as well as the breeding quality of a male animal. Hence, reduced testicular size as indicated by reduction in the weight, length and volume of the testis of boars fed CSC-based diets suggests that the testes of these boars could have a reduced sperm production capacity and the boars may not perform maximally for breeding purpose.

Although most testicular parameters were significantly reduced, few exceptions were recorded for the width of the testis. The width of the testis was not significantly affected by the treatment. This observation therefore makes the results of this study to be in partial agreement with the report of Amao and Showumi (2016) on rabbits and Velasquez-Perreria *et al.*, (1998) on bulls that were both fed cottonseed cake-based diets. The two reports submitted that testicular morphometrics may not reveal the adverse effect of diets, most especially when the period of dietary treatment is short.

The significant reduction in the epididymal weights and lengths of boars fed either raw or fermented CSC-based diets suggests that these boars would have a low sperm storage capacity. Observations on the epididymal weights in this study are contrary to that reported by Amao and Showumi (2016) which stated that the epididymal weight of rabbit bucks fed raw CSC-based diets was heavier than the control. Significant reduction in the epididymal weight in this study is however justifiable as gossypol contained in the raw CSC could have adversely affected the growth of the epididymides.

The observation that there was significant reduction in sperm count and motility; normal and live sperm; GSR, DSP and DSP/g of boars fed either raw or fermented CSC-based diets is an indication of the antispermatogenic effect of gossypol contained in CSC-based diets and the inability of fermentation to completely get rid of gossypol toxicity. Antispermatogenic effect of gossypol had been linked with the tendency of gossypol to damage the germinal epithelium of seminiferous tubules (Babashani *et al.*, 2014). This was similar to the report of Shandilya *et al.*, (1982) which stated that gossypol inhibits sperm motility and decreased sperm concentration in male cynomolgus monkeys (*Macaca fascicularis*).

The inability of fermentation to ameliorate the deleterious effect of gossypol contained in CSC observed in this study contradicts the report of Amao and Showumi (2016) which stated that antispermatogenic effect of gossypol was neutralized by fermentation when rabbits are used. This could probably be due to the differences in the species of animal used for the experiments. Furthermore, possibly fermentation by *Aspergillus niger* degraded the nutrients in the cottonseed cake such that the protein quality of the FCSC could not support the testicular and epididymal growth in the boars.

It was concluded from this study that feeding RCSC - or FCSC - based diets to growing boar adversely affected the testicular and epididymal morphometrics; however, 10% RCSC in diet did not compromise gonadal sperm characteristics. Further studies to investigate the use of other micro-organisms apart from *Aspergillus niger* to ferment CSC are recommended.

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Table 1: Gross composition of experimental diets for weaner pigs

Ingredients	T₁(Control)	T₂(10% RCSC)	T₃(20% RCSC)	T₄ (10% FCSC)	T₅ (20% FCSC)
Maize	52.21	46.99	42.43	47.46	42.61
Soybean Meal	22.54	17.76	12.32	17.29	12.14
Cotton Seed Cake	-	10.00	20.00	10.00	20.00
Fish Meal	2.00	2.00	2.00	2.00	2.00
Wheat Offal	15.00	15.00	15.00	15.00	15.00
Bone Meal	3.00	3.00	3.00	3.00	3.00
Blood Meal	3.00	3.00	3.00	3.00	3.00
Oyster Shell	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Premix*	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
<u>Calculated nutrients</u>					
Crude Protein (%)	21.00	21.00	21.00	21.00	21.00
Metabolizable	2796.98	2703.27	2614.40	2706.76	2615.72
Energy(Kcal/kg)					
Crude Fibre (%)	3.91	5.93	7.99	5.91	7.98

***Premix composition:** Each feed contained Vit A, 1500IU; Vit D₃, 2500IU, Vit E, 11IU, Vit B₂, 10mg; Vit B₃, 40mg; Vit B₆, 20mg; choline chloride, 400mg; Mn, 120mg; Fe, 70mg; Cu, 10mg; Zn, 2.2mg; Se, 0.2mg; Co., 0.02mg.

Table 2: Gross composition of experimental diets for grower pigs

Ingredients	T₁(Control)	T₂ (10% RCSC)	T₃(20% RCSC)	T₄(10% FCSC)	T₅ (20% FCSC)
Maize	57.21	53.99	49.43	53.46	50.61
Soybean Meal	17.54	10.76	5.32	11.29	4.14
Cotton Seed Cake	-	10.00	20.00	10.00	20.00
Fish Meal	1.00	1.00	1.00	1.00	1.00
Wheat Offal	16.00	16.00	16.00	16.00	16.00
Bone Meal	2.00	2.00	2.00	2.00	2.00
Blood Meal	3.00	3.00	3.00	3.00	3.00
Oyster Shell	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Premix*	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
Crude Protein (%)	18.00	18.00	18.00	18.00	18.00
Metabolizable	2,823.78	2,737.41	2,655.88	2,740.86	2,664.54
Energy (Kcal/kg)					
Crude Fibre (%)	3.71	5.73	7.75	5.71	7.69

***Premix composition:** Each feed contained Vit A, 1500IU; Vit D₃, 2500IU, Vit E, 11IU, Vit B₂, 10mg; Vit B₃, 40mg; Vit B₆, 20mg; choline chloride, 400mg; Mn, 120mg; Fe, 70mg; Cu, 10mg; Zn, 2.2mg; Se, 0.2mg; Zn, 45mg; Co., 0.02mg.

Table 3: Effect of different levels of raw or fermented cottonseed cake on Testicular characteristics of growing boars.

Parameter	T1 (Control)	T2 (10% RCSC)	T3 (20% RCSC)	T4 (10% FCSC)	T5 (20% FCSC)	SEM
Left Testis Weight (g)	31.23 ^a	16.53 ^b	19.60 ^b	16.73 ^b	9.50 ^c	3.48
Right Testis Weight (g)	29.83 ^a	16.86 ^b	19.06 ^b	16.20 ^b	11.40 ^c	3.33
Paired Testis Weight (g)	61.06 ^a	33.40 ^b	38.66 ^b	32.93 ^b	20.90 ^c	6.78
Left Testis length (cm)	5.66 ^a	4.03 ^b	4.66 ^b	4.80 ^b	4.70 ^b	0.31
Right Testis Length (cm)	5.80 ^a	4.30 ^b	4.96 ^b	4.56 ^b	4.30 ^b	0.32
Mean Testis Length (cm)	5.73 ^a	4.16 ^b	4.81 ^b	4.68 ^b	4.50 ^b	0.31
Left Testis Width (cm)	3.03	2.50	2.63	3.23	2.66	0.17
Right Testis Width (cm)	3.46	2.76	3.16	2.90	2.53	0.18
Mean Testis Width (cm)	3.25	2.63	2.90	3.06	2.60	0.17
Left Testis Volume (cm)	33.33 ^a	17.00 ^{bc}	20.00 ^{bc}	18.66 ^{bc}	13.33 ^c	3.63
Right Testis Volume (cm)	32.00 ^a	17.66 ^b	19.50 ^{bc}	18.43 ^b	12.33 ^c	3.59
Paired Testis Volume (cm)	65.33 ^a	34.66 ^{bc}	39.50 ^{bc}	37.10 ^{bc}	25.66 ^c	7.22

^{a, b, c:} Means within the same row with different superscripts differ significantly (p<0.05)

Table 4: Effect of CSC level and processing on the Testicular characteristics of growing boars.

Parameter	CSC level			Processing		
	10%	20%	SEM	RCSC	FCSC	SEM
Left Testis Weight(g)	16.63	14.55	5.05	18.07	13.12	4.85
Right Testis Weight(g)	16.53	15.23	4.81	17.97	13.80	4.64
Paired Testis Weight(g)	33.17	19.78	9.81	36.03	26.92	9.43
Left Testis Length(cm)	4.42	4.68	0.49	4.35	4.75	0.50
Right Testis Length(cm)	4.43	4.63	0.46	4.63	4.43	0.45
Mean Testis Length(cm)	4.43	4.65	0.46	4.49	4.59	0.47
Left Testis Width(cm)	2.87	2.65	0.28	2.57	2.95	0.29
Right Testis Width(cm)	2.83	2.85	0.29	2.97	2.72	0.28
Mean Testis Width(cm)	2.85	2.75	0.27	2.77	2.83	0.28
Left Testis Volume(cl)	18.50	16.00	5.04	18.50	16.00	5.04
Right Testis Volume(cl)	18.05	15.92	5.06	18.58	15.38	5.01
Pair Testis Volume(cl)	35.88	32.58	10.19	37.08	31.38	10.04

Table 5: Effect of different levels of raw or fermented cottonseed cake on epididymal characteristics of growing boars

Parameter	T1 (Control)	T2 (10% RCSC)	T3 (20% RCSC)	T4 (10% FCSC)	T5 (20% FCSC)	SEM
Left Epididymal weight (g)	15.10 ^a	8.96 ^b	6.50 ^b	6.83 ^b	3.96 ^c	1.50
Right Epididymal Weight (g)	13.70 ^a	9.03 ^b	6.66 ^{bc}	7.33 ^{bc}	5.50 ^c	1.33
Paired Epididymal Weight (g)	28.80 ^a	18.00 ^b	13.16 ^{bc}	14.16 ^{bc}	9.46 ^c	2.83
Mean Epididymal Weight (g)	14.40 ^a	9.00 ^b	6.58 ^{bc}	7.08 ^{bc}	4.73 ^c	1.41
Left Epididymal Length (cm)	10.90 ^a	9.40 ^{ab}	8.46 ^{ab}	8.56 ^{ab}	7.63 ^b	0.45
Right Epididymal Length (cm)	10.36 ^a	8.40 ^{ab}	7.90 ^{ab}	8.50 ^{ab}	7.30 ^b	0.42
Mean Epididymal Length (cm)	21.27 ^a	17.80 ^{ab}	16.37 ^{ab}	17.07 ^{ab}	14.93 ^b	0.85

^{a, b, c}: Means within the same row with different superscripts differ significantly (p<0.05)

Table 6: Effect of CSC level and processing on epididymal characteristics of growing boars

Parameter	CSC	Level	SEM	Processing		SEM
	10%	20%		RCSC	FCSC	
Left Epididymal weight (g)	7.90	5.23	1.80	7.73	5.40	1.80
Right Epididymal Weight (g)	8.18	6.08	1.79	7.85	6.42	1.81
Mean Epididymal Weight (g)	8.04	5.66	1.78	7.79	5.91	1.79
Left Epididymal Length(cm)	8.98	8.05	0.66	8.93	8.10	0.67
Right Epididymal Length(cm)	8.45	7.60	0.58	8.15	7.90	0.62
Mean Epididymal length (cm)	17.43	15.65	0.98	17.08	16.00	1.26

Table 7: Effect of different levels of raw or fermented cottonseed cake on sperm characteristics of growing boars.

Parameter	T1(Control)	T2(10% RCSC)	T3(20% RCSC)	T4(10% FCSC)	T5(20% FCSC)	SEM
Sperm count ($\times 10^6/g$)	48.50 ^a	36.30 ^b	30.97 ^b	19.20 ^c	15.77 ^c	4.47
Sperm motility (%)	68.01 ^a	68.55 ^a	61.45 ^b	56.05 ^b	57.10 ^b	2.05
Normal sperm (%)	71.34 ^a	70.90 ^a	61.87 ^b	52.76 ^c	54.61 ^c	2.72
Live sperm (%)	74.76 ^{ab}	77.29 ^a	67.98 ^{bc}	61.54 ^c	64.41 ^c	2.13
GSR ($\times 10^6$)	1928.31 ^a	1451.56 ^a	1199.18 ^{ab}	748.50 ^b	610.81 ^b	17.53
DSP ($\times 10^6$)	335.26 ^a	332.16 ^a	274.41 ^b	171.28 ^c	139.77 ^c	40.12
DSP/g ($\times 10^6$)	17.54 ^b	40.44 ^a	20.21 ^b	16.02 ^b	13.53 ^b	4.41

^{a,b,c}: Means within the same row with different superscripts differ significantly ($p < 0.05$); GSR= Gonadal Sperm Reserve; DSP= Daily Sperm Production; DSP/g = Daily Sperm Production per gram testis.

Table 8: Effect of CSC level and processing on the sperm characteristics of growing boars

Parameter	CSC	Level	SEM	Processing		SEM
	10%	20%		RCSC	FCSC	
Sperm Count ($\times 10^6/\text{g}$)	27.75	23.37	1.48	33.63 ^a	17.48 ^b	2.71
Sperm Motility (%)	62.30	59.28	2.25	65.00 ^a	56.58 ^b	1.33
Normal Sperm (%)	61.83	58.24	3.55	66.39 ^a	53.69 ^b	2.43
Live Sperm (%)	69.42	66.20	2.96	72.63 ^a	62.98 ^b	2.12
GSR ($\times 10^6$)	1014.03	1004.99	79.88	1325.37	1679.65	13.17
DSP ($\times 10^6$)	251.72	207.09	41.16	303.29 ^a	155.53 ^b	25.90
DSP/g ($\times 10^6$)	28.23	16.87	7.17	30.33 ^a	14.77 ^b	6.34

^{a, b}: Means within the same row with different superscripts differ significantly ($p < 0.05$)