

Testicular and Epididymal Characteristcs of Rabbit Bucks Fed Diets Containing Cottonseed Cake Supplemented with Carrot (Daucus carota) or Ginger (Zingiber officinale Roscoe)

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Abstract
This experiment was conducted to investigate the reproductive characteristics of rabbit bucks fed cottonseed cake-based diets supplemented with carrot or ginger. Twenty four (24) cross bred (New Zealand White X Chinchilla) 6-8 weeks old rabbit bucks were involved in the study. The bucks were balanced for weight and allocated to four dietary treatments T1, T2, T3 and T4. Treatment T1 (control) contained soyabean meal (SBM) as the main plant protein source denoted as (SBM or 0% CSC). T2 had 100% SBM replaced with cottonseed cake (CSC), while T3 and T4 had T2 supplemented with carrot and ginger respectively. Except for testicular width, testicular characteristics were not significantly ($P>0.05$) affected. Testicular width decreased from 2.20cm in the control (T1) to 1.63cm in T2 (100% CSC). Mean epididymal weight and mean epididymal length were significantly ($P<0.05$) affected with mean epididymal length decreasing from 13.72cm in the control to 8.32cm in T2 (100% CSC). Supplementing T2 with either carrot or ginger increased the epididymal characteristics significantly ($P<0.05$). It could be concluded from this study that CSC with or without carrot or ginger supplementation may not have adverse effect on testicular characteristics. However CSC depressed epididymal characteristics which were corrected by carrot or ginger supplementation with ginger proving more effective.
Keywords: Rabbit buck, Reproductive characteristics, Cottonseed cake, Carrot and Ginger.

Introduction

The increasing population of the world especially in the developing and underdeveloped countries where average income is low, standard of living is low and there is high rate of malnutrition from deficiency in essential nutrients, is a major concern. There is a great need for provision of cheap and affordable animal protein as most people in these countries cannot afford the cost of the conventional animal protein (beef). Rabbit (*Oryctolagus cunniculus*) described as a micro livestock species (Vietmeyer, 1985) with a short production cycle, relatively smaller space requirement, high fecundity, fast growth, genetic diversity, prolificacy and the ability to utilize forage and unconventional feed stuffs has a huge potential to meet this protein need.

Although rabbit can be raised on forage based diet, the poor and unbalanced quality of grasses, as well as seasonal availability of the forage are major constraints limiting the successful production of rabbit for consumption. Cheeke and Raharjo (1988) concluded in a review of rabbit production on tropical feed sources that tropical grasses were unsuitable as the sole feed for rabbits due to the low digestibility (less than 10%) of the forage. Due to their small size and high metabolic rate, high quality forage is needed by rabbit (Irlbeck, 2001). Rabbits can utilize low grain and high roughage diets (McNittel *et al.,* 1996). Because rabbits are able to utilize this type of diet, they are able to breed all year round.

The principal sources of plant protein in rabbit concentrate diet are soya bean meal (SBM) and groundnut cake (GNC). These plant protein sources also constitute major food items for man, especially where grain can only be justified for human use. Hence, stiff competition between man and animals. This competition has made the conventional feed stuffs too expensive thereby necessitating the use of locally and abundantly available unconventional feed stuffs as alternatives. Several oil seed cakes have been investigated as alternatives and cottonseed cake (CSC) a by-product of cotton processing industry is one of them. The cake is rich in protein (42% CP) but contains an anti-nutritional factor gossypol, the major pigment in cottonseed which is toxic to non ruminant animals and has limited the use of cottonseed meal as a dietary source of protein for monogastrics (Taha *et al.,* 2006). Gossypol is a toxic polyphenolic compound which interferes with protein digestion and causes deleterious effects in monogastric animals (Ikurior and Fetuga, 1984, Calhoun *et. al.,* 1990). Mature ruminants seem to have a large capacity to detoxify gossypol but in excessive quantities, it may overwhelm ruminal detoxification and become absorbed at potentially toxic concentrations (Randel *et al.,* 1992).

Signs of gossypol toxicities in non-ruminants, pre-ruminant and ruminants are similar and include laboured breathing, dyspnea, decreased growth rate and anorexia but are not pathognomonic. Post mortem findings include generalized oedema and congestion of lungs and liver, fluid-filled thoracic and peritoneal cavities and degeneration of heart fibres (Randel *et al.,* 1992). Gossypol is highly reactive and is capable of acting as a phenolic and aldehydic compounds. Therefore, gossypol has been implicated as a reproductive toxicant in anaimal feeding (Randel *et al.,* 1992).

Reproductive toxicity is seen particularly in males, where gossypol affects sperm motility, inhibits spermatogenesis and depresses sperm counts, cause sertoli cell toxicity and may also affect leydig cells (Randel *et al.*, 1992). Gossypol also seems to disrupt oestrous cycles, pregnancy and early embryo development, particularly in monogastric species (Abou-Donia, 1976; Berardi and Goldblatt, 1980; Randel *et al.*, 1992; Dodou 2005). At effective doses, gossypol causes males to be infertile because of sperm immotility and depressed sperm counts (Randel *et al*., 1992). Relatively small amount of gossypol fed to monogastric animals have detrimental effect on male reproduction. Epididymal and accessory sex glands weight were reduced when animals were fed high gossypol diets. Specific mitochondrial damage in the tails of spermatozoa seems to render them immotile and extensive damage to germinal epithelium may be responsible for depression in spermatogenesis (Randel *et al.*, 1992; Amao et al., 2012).

Carrot (*Daucus carota*) a root vegetable with 7.46% CP and 3% CF contains high amount of beta carotene (about 8285µg per 100g of carrot) which is an antioxidant which is converted to vitamin A. Ginger (*Zingiber officinale*) is a rhizome with 1.82% CP and 2% CF contains gingerol (6-gingerol), alpha tocopherol which is converted to vitamin E and vitamin A which are all antioxidants.

Bender *et al.* (1988) found that several antioxidants were reduced by feeding rat high concentration of gossypol. In dairy cattle, Lane and Stuart (1990) found that feeding high amount of gossypol decreased plasma alpha tocopherol concentrations, which indicates possible relationship between gossypol toxicity and antioxidant. The aim of this study was to was to investigate the reproductive toxicological effect of gossypol on rabbit bucks fed cottonseed cake based diets and an attempt to investigate the efficiency of using natural antioxidant sources to counteract gossypol toxicity in rabbit bucks fed cottonseed cake based diets supplemented with carrot or ginger.

Materials and Methhods

Experimental Site

The experiment was conducted at the Rabbit Research and Production unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria. The climatic condition of Ogbomoso as described by Oguntoyinbo (1978) is; Latitude 8° 15^{I}N and Longititude 4° 15^{I}, mean annual rainfall is 1,247mm and the relative humidity is between 75 and 95%, mean annual temperature is about 26.2°C and altitude is between 300m and 600m above sea level. It is in the derived savanna zone of Nigeria.

Experimental animals and management

Twenty four (24) weaned crossbred (New Zealand White X Chinchilla) rabbit bucks, aged 6-7 weeks old were balanced for weight and allocated to four treatment diets containing CSC with or without carrot or ginger supplementation. Animals were allowed an adaptation period of one week, during which they were fed with the control diet containing 16% crude protein (CP) and about 2600 kcalKg^{-1} ME; and treated prophylatically against endo- and ecto-parasites. After the adaptation period, the animals were subjected to nine weeks feeding trial. The rabbits were housed individually in wooden metabolic cages. Six bucks were allotted to each treatment in a Completely Randomized Design with each rabbit serving as a replicate. Table 1 shows the proximate composition of experimental diets.

Data collection

At the end of the feeding trial, 3 animals per treatment were sacrificed and their reproductive organs namely, testes and epididymides, were carefully dissected out and trimmed of adhering tissues. Testicular and epididymal morphometric characteristics; testis weight, testis length, testis width, testis volume and epididymal length were measured. The testis and epididymis were weighed using a sensitive electronic balance. Testis length, testis width and epididymal length were measured with the aid of a pair of vernier calipers, while the testis volume was measured by water displacement according to Archimedes principle (Bitto, 1989). Paired and mean testicular and epididymal parameters were computed from data for the left and right testes and epididymides.

Statistical Analysis

Data collected were subjected to one way analysis of variance (ANOVA) using SAS, (2000) analytical software. Means were separated by Duncan's option of the same statistical software.

Results

Testicular Characteristics

The results of this study are presented in Tables 3 and 4. Table 3 shows the effect of CSC-based diets supplemented with carrot or ginger on the testicular characteristics of rabbit bucks. All testicular parameters, except testis width, were not significantly (p>0.05) affected by the treatment diets. Although the paired and mean testis weights were not significantly affected by treatment, bucks that were fed the control diet had larger testis (1.00g) than those that were fed the CSC-based diets with those that fed on CSC + ginger having the smallest testis (0.52g). A similar trend was observed for the mean testis length. While paired testis volume was highest (2.07cm^3) for the control bucks, animals that were fed T3 (CSC + carrot) had the lowest volume (1.27cm^3). The mean testis width was significantly (P<0.05) higher for the control group (1.10cm) than those on CSC-based diets, which had similar values (0.82, 0.80 and 0.80 cm) for T2, T3 and T4 respectively.

Epididymal Characteristics

Table 4 shows the effect of CSC-based diets supplemented with carrot or ginger on epididymal characteristics of rabbit bucks. The paired epididymal weight, relative paired epididymal weight, relative mean epididymal weight and mean epididymal length were significantly (P<0.05) affected by the treatments. Rabbit bucks fed the control diet and those fed CSC + ginger diet had significantly (P<0.05) higher value (2.57g) for the paired epididymal weight than those on T2 (CSC) and T3 (CSC + carrot). The epididymal length for the control group (13.37 cm) was similar to that of CSC + ginger (11.45) but significantly (P<0.05) higher than that of T2 (CSC; 8.32 cm) and T3 (CSC + carrot; 10.79 cm).

Discussion

Testicular characteristics

It was observed from the study that supplementation of CSC based diet with either carrot or ginger did not have significant effect on testicular parameters except testicular width. Velasquez-Pereira *et al.* (1998) found similar trend for testicular weight and volume in which antioxidant supplementation (vitamin E) of CSC based diet did not have significant (p>0.05) effect on testicular parameters when fed to bulls. These observations were in agreement with the results of Chenoweth *et al.* (1994). These results were also consistent with the report that gossypol had no significant effect on testicular weight (Jimenez *et al.,*1989; Chase *et al.,* 1994). The significant (p<0.05) effect of CSC on testicular width indicates gossypol reduction of scrotal circumference. This is in contrast with the report of Chenoweth *et al.* (1994) that gossypol had no effect on scrotal circumference because gossypol caused apparent damage to spermatogenic epithelium by reducing its layers without corresponding changes in tubular diameter. The observation that T1 had the highest paired testicular weight suggests a high capacity for sperm production according to the report of Oyeyemi *et al.* (2002) that the higher the testicular weight (without any abnormality) the higher the capacity of the cells for spermatogenesis. This is in tandem with the report of Morris *et al*. (1999) that within a species of animals, there is a good correlation between spermatozoa production, testicular sizes and the age of the animal.

Epididymal characteristics

Inconsistent trend was recorded for paired epididymal weight and mean epididymal weight. Bucks fed CSC diet supplemented with ginger had the highest epididymal weight. This implies that although gossypol depressed epididymal weight, ginger supplementation ameliorated the depression. Bucks fed diet supplemented with carrot had the lowest epididymal weight; this might be because carotene (a precursor of vitamin A) present in carrot was used by other body organs thereby making it insufficiently available for mitigating the effect of gossypol contained in the CSC. The observation that mean epididymal length was highest in bucks fed the control diet and lowest in those fed CSC-based diet without supplementation suggests that gossypol in the CSC significantly depressed epididymal length. Although supplementation with ginger and carrot tended to counter the effect, ginger showed a greater ameliorative potential than carrot. Since epididymis is essentially for storage of spermatozoa, it was possible that the effect of gossypol in the CSC on the testis was carried over to the epididymis. This result corroborates the recent report of Amao *et al*. (2012)

Conclusion

It could be concluded from this study that feeding CSC-based diets to rabbit bucks may not have apparent adverse effect on testicular characteristics. However, CSC depressed epididymal characteristics significantly. Supplementing CSC-based diets with carrot or ginger showed some degree of promise in correcting the depressive effect of the diet on the epididymal characteristics; with ginger tending to be more effective than carrot.

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Table 1. Gross Composition of experimental diets

Ingredients (%)	T1 (Control)	T2 (100%CSC)	T3 (CSC+Carrot)	T4 (CSC+Ginger)
Maize	43.57	42.88	42.88	42.88
Soya bean meal	21.43	___	___	___
Cotton seed meal	___	22.12	22.12	22.12
Rice husk	30.00	30.00	29.50	29.50
Fish meal	2.00	2.00	2.00	2.00
Bone meal	0.25	0.25	0.25	0.25
Oyster shell	2.00	2.00	2.00	2.00
Vita/min premix*	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Carrot	___	___	0.50	___
Ginger	___	___	___	0.50
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein (%)	16.00	16.00	16.01	16.00
Crude fibre (%)	11.28	12.64	12.51	12.50
Metabolizable Energy(ME)(Kcal/kg)	2552.00	2523.00	2517.00	2517.00

* Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg

Table 2. Proximate composition of the experimental diets

	Treatment			
Parameter (%)	**T1 (Control)**	**T2(100%CSC)**	**T3(CSC+carrot)**	**T4(CSC+ginger)**
Crude protein	16.38	15.85	15.93	16.30
Crude fiber	12.55	13.48	13.68	13.75
Ether extract	3.79	3.82	2.05	3.54
Ash	11.54	11.64	9.88	11.64
Moisture	9.85	9.92	7.58	9.68
NFE	42.89	46.41	57.18	46.89

Table 3. Effect of CSC based diets supplemented with carrot or ginger on testicular characteristics of rabbit buck.

Parameters	T1 Control (SBM)	T2 (CSC)	T3 (CSC+ Carrot)	T4 (CSC+ Ginger)	SEM
Left Testis Weight (g)	1.03^{a}	0.53^{b}	0.60^{b}	0.50^{b}	0.10
Right Testis Weight (g)	0.97	0.60	0.53	0.55	0.09
Paired Testis Weight (g)	2.00	1.13	1.13	1.05	0.19
Relative Paired Testis Weight (%)	0.12	0.09	0.09	0.09	0.01
Mean Testis Weight (g)	1.00	0.57	0.57	0.52	0.10
Relative Mean Testis Weight (%)	0.06	0.05	0.05	0.04	0.01
Left Testis Length (cm)	2.17	1.77	1.77	1.70	0.09
Right Testis Length (cm)	2.10	1.80	1.80	1.75	0.09
Mean Testis Length (cm)	2.13	1.78	1.78	1.72	0.09
Left Testis Volume (cm^3)	1.10	0.75	0.63	0.75	0.09
Right Testis Volume (cm^3)	0.97	0.75	0.63	0.80	0.08
Paired Testis Volume (cm^3)	2.07	1.50	1.27	1.55	0.17
Mean Testis Volume (cm^3)	1.03	0.75	0.63	0.78	0.08
Left Testis Width (cm)	1.13^{a}	0.83^{b}	0.80^{b}	0.75^{b}	0.06
Right Testis Width (cm)	1.07	0.80	0.80	0.85	0.05
Mean Testis Width (cm)	1.10^{a}	0.82^{b}	0.80^{b}	0.80^{b}	0.05

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

Table 4. Effect of CSC based diets supplemented with carrot or ginger on epididymal characteristics of rabbit bucks on epididymal characteristics of rabbit bucks

Parameters	T1 Control (SBM)	T2 (CSC)	T3 (CSC+ Carrot)	T4 (CSC+ Ginger)	SEM
Left Epididymis Weight (g)	1.10	0.90	0.73	1.27	0.11
Right Epididymis Weight (g)	1.47^{a}	1.00^{ab}	0.67^{b}	1.30^{ab}	0.12
Paired Epididymis Weight (g)	2.57^{a}	1.90^{ab}	1.40^{b}	2.57^{a}	0.22
Paired Epididymis Weight (%)	0.16^{ab}	0.16^{ab}	0.12^{b}	0.21^{a}	0.01
Mean Epididymis Weight (g)	1.28	0.95	0.70	1.28	0.11
Mean Epididymis Weight (%)	0.08^{ab}	0.08^{ab}	0.06^{b}	0.11^{a}	0.01
Left Epididymis Length (cm)	13.07	7.73	11.67	11.40	0.99
Right Epididymis Length (cm)	14.37^{a}	8.90^{b}	9.90^{b}	11.50^{ab}	0.77
Mean Epididymis Length (cm)	13.37^{a}	8.32^{b}	10.79^{b}	11.45^{ab}	0.88

a,b: Means along the same row with different superscripts differ significantly ($p<0.05$)