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Growth response, organ morphometry, sperm production and reserve in rabbit bucks administered carrot fruit extracts

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Abstract

An experiment was conducted for 12 weeks to investigate the anti-oxidative effect of carrot fruit extracts administered to rabbit bucks on growth, organ characteristics, gonadal and extra-gonadal sperm reserves, and daily sperm production. A total of 35 rabbit bucks (four months old with average weight of 1.62±0.2kg) were randomly allotted into treatment groups each containing seven bucks per replicate. The bucks were subjected to the same environmental conditions and were assigned into five doses of carrot fruit extracts administration: Treatment 1 (0 mL of carrot fruit extracts (CE)/kg BW i.e. control), treatment 2 (0.5 mL CE/kg BW), treatment 3 (1.0 mL CE/kg BW), treatment 4 (1.5 ml CE/kg BW), treatment 5 (2 mL CE/kg BW). The extract was administered by drenching using corn oil as the carrier. The bucks were weighed weekly to determine weight gain and feed conversion ratio. At the end of the trial, the bucks were sacrificed, dissected, organs removed and weighed. Testes and epididymis were homogenised and filtered. The sperm cells in the testicular and epididymal homogenates were determined. Organ characteristics: eviscerated weight, adrenal gland, liver, lungs, kidney, pancreas, bile, testis, spleen, heart and GIT weight were measured. Data were subjected to analysis of variance at P = 0.05. The bucks administered with 1.0 mL CE had the highest relative eviscerated weight (48.74±6.73%) and also recorded the highest weight gain (0.21±0.15g/day) when compared to other treatments. There was non-significant difference for most of the organs assessed except for the GIT and spleen weight. The GIT weight was significantly (P<0.05) higher in T1 (15.41%), T3 (14.81%) and T4 (16.23%) than T2 (13.58) and T5 (13.09%) in rabbits administered 0.5mL and 2.0mL CE/kg BW, respectively. Relative spleen weight of rabbits administered 0.2mL CE/kg BW was similar to T1 but significantly (p<0.05) higher than T2, T3 and T4. Testicular and epididymal sperms reserves significantly (P<0.05) increased as the concentration of the carrot fruit extract administered to rabbits increased. Daily sperm production was significantly (P<0.05) higher in treated bucks than the control bucks. This study suggests that administering carrot fruit extracts up to 2mL/kg body weight increased growth, sperm cell production and testicular sperm reserve.

Keywords: Carrot fruit antioxidants, sperm reserve, daily sperm production, rabbits

Introduction

Rabbit meat production has been on the increase in Nigeria within the last two decades (Arijeniwa *et al.* 2000). The rabbit (*Oryctolagus cuniculus*) is one of the most productive animals among other domesticated livestock. The feeding habits offer no appreciable competition with humans because of the ability to subsist on forages as basal diet. In addition to this, rabbit has small body size, short generation interval with a relatively short gestation period of 30-32 days. The daily weight gain is high in proportion to the body weight which gives the rabbit a rapid growth rate and attains sexual maturity early. These factors result in the rabbit reaching the weight of a sexually mature animal 30% faster than other animals (Ajayi *et al.*, 2005) and also make them suitable as meat producing small livestock in developing countries (Arijeniwa *et al*., 2000). Although

rabbits can survive on forages, optimum performance can only be ensured in a mixed feeding regime involving forage and formulated feeds (Arijeniwa *et al*., 2000; Cross, 2013) without compromising their reproductive efficiency.

The ability of animals to reproduce efficiently is an integral component of animal agriculture. Reproductive failure due to oxidative stress which eventually causes cell death is one of the most significant factors that limit the productivity of animal production systems and result huge loss as profits annually (Etim *et al.,* 2014). Rabbit farmers are faced with challenges in finding practical, cost-effective ways to improve performance without compromising the production of safe and high quality meat products. However, the use of phyto-antioxidant source as feed additive or oral supplement is a promising alternative. The carrot (*Daucus carota*) is a root vegetable rich in antioxidants, it is usually orange in colour, though purple, black, red, white, and yellow cultivars exist. The most commonly eaten part of the plant is the taproot, although the greens are sometimes eaten as well. The cultivated carrot has been selectively bred for its greatly enlarged, more palatable; less woody-textured taproot (Furr and Clark, 1997) rich in Beta-carotene. Beta carotene is a plant pigment that belongs to a group of substances called carotenoids; it is one of only a few carotenoids that the human body converts into vitamin A (Paiva and Russell, 1999). In this role, beta-carotene maintains vision, regulates the growth of cells in the skin, keeps membranes lining the nose and respiratory tract healthy and also helps control the production of proteins. All carotenoids including beta-carotene, also possess antioxidant abilities. Besides canned pumpkin, carrots are the top source of alpha-carotene; which is another carotenoid the body coverts to vitamin A, they also contain lutein and zeaxanthin (Darvin *et al,* 2011). These two carotenoids work as antioxidants that protect the eyes from damaging blue light, reactive oxygen species and free radicals (Fiedor *et al.,* 2005; Cvetkovic *et al.,* 2013). Eating food like carrots that contain several carotenoids may improve overall health, growth and reproductive potential which have not been fully documented in rabbits. Therefore, this study was designed to assess the effect of carrot fruit extract on growth performance and sperm reserves in rabbits.

Materials and methods

Experimental material and treatment layout

A total of 35 males (bucks) mixed breed rabbits aged four months old were purchased and used for this study. The rabbits were housed individually in hutches under the same environmental conditions and acclimatized for a period of two weeks with a standard concentrate feed to meet nutritional requirements recommended by National Research Council (NRC, 1994). The rabbits were then grouped into five treatments with each treatment having seven replicates. The treatments consist of different concentration of carrot fruit extract using corn oil as vehicle, drenched into each animal at 48 hours interval for 12 weeks at varying doses according to their body weight. Fresh carrots were purchased from the open market and the carrot cloves were rinsed with distilled water and then peeled. The working solution of carrot was prepared by putting 11.4 kg of chopped carrot into a flat bottom flask and 100 mL of methanol was added, and it was covered for four hours, after which it was sieved with filter paper and transferred into a rotary evaporator for two hours at 40°C (Marcano *et al*., 1991) and 1 ml of the extract obtained contains 1 mg of the beta carotene. Beta carotene in the extract was quantified as described by Marcano *et al*. (1991). The experimental animals were randomly

allotted to five treatments in a completely randomized design with seven replicates. Carrot fruit extracts (CFE) was administered at five graded concentrations at 48 hours interval for a period of twelve weeks by oral drench and corn oil was used as a vehicle in the administration of the extract. The treatments were Treatment 1: 1 mL of water/Kg BW (Control); Treatment 2: 0.5 mL/Kg BW; Treatment 3: 1.0 mL/Kg BW; Treatment 4: 1.5 mL/Kg BW; Treatment 5: 2 mL/Kg BW

Organ assessment

At the end of the trial, five rabbits per treatment were sacrificed and dissected. The testes of each buch from the carcass were removed from the tunics and later separated into testis and epididymis, weighed and testicular volume determined as described by Ewuola and Egbunike (2010). Visceral organs like kidney, lung and the spleen) were also removed carefully and weighed while the gastro intestinal tract (GIT) length was measured with meter rule. The testes and epididymis were freed of fat and unwanted connective tissues. They were separated into right and left testes, right and left caput, corpus and caudal epididymis and weighed. The testes, caput, corpus and caudal epididymis were homogenized separately at 1g in 2mL normal saline solution (0.9% NaCl). The suspensions were mixed, filtered through a double layer cheese cloth and the filtrate transferred into test tubes. All samples were covered and stored for 24 hours at 40ºC. Counting was done haemocytometrically using an improved Neubauer haemocytometer. The daily sperm production was calculated using the formula outlined in Ewuola and Egbunike (2010).

$$DSP = \frac{\text{Testicular Sperm Count}}{\text{Time Divisor}}$$

Time divisor = length of one cycle of the seminiferous epithelium x Percentage of cycle represented by spermatids counted. A time divisor of 3.43 proposed by Amann (1970) was used.

Data analysis

Data obtained were subjected to descriptive statistics and one-way analysis of variance using Statistical Analysis System software version 9.3 (SAS, 2011). The means were separated using Duncan Multiple Range Test of the same software.

Results

Performance characteristics of rabbit bucks administered carrot fruit extracts

The growth performance of rabbit bucks administered carrot fruit extracts (CFE) is shown in Table 1. There were non-significant differences in the values obtained for final weight of the animal, while the weight gained per rabbit was significantly (P<0.05) higher in treated groups than the control.

Table 1: Growth Performance of rabbit bucks administered carrot fruit extract

Parameters (kg)	T1	T2	T3	T4	T5	SEM
Initial weight	1.64±0.36	1.61±0.27	1.63±0.29	1.60±0.25	1.60±0.26	0.05
Final Weight	1.68±0.22	1.72±0.24	1.84±0.34	1.70±0.18	1.77±0.32	0.04
Weight gain	$0.04±0.16^{b}$	$0.10±0.14^{ab}$	$0.21±0.15^{a}$	$0.10±0.10^{ab}$	$0.17±0.11^{ab}$	0.02

abc: Means with different superscripts in the same row are significantly (P<0.05) different

Organ assessment

The relative weight of the organs of rabbit bucks administered CFE is presented in Table 2. Among the organs assessed, only the values obtained for spleen and GIT were significantly (P<0.05) different across the treatments. Relative weight of spleen in bucks on (T5) was significantly (P<0.05) higher than bucks administered other treatments. The weight of GIT, for bucks on 2.0 mg (T5) was significantly (P<0.05) higher than those on 0.5 mg (T2) while

those on 1.5 mg (T4) were higher than 0.5 mg (T2). Comparing testis weight in animals of the five treatment groups showed that the weight in all groups was not significantly different from one another.

Gonadal and extra-gonadal assessment

Testicular volume, testicular and epididymal weight and sperm reserves in rabbit bucks administered CFE are shown in Table 3. There were non-significant differences in the values obtained for average weight of testis, testicular volume, sperm reserves in right testis and average weight of epididymis across the treatments. The concentration of sperm cells in left and right epididymis, left and total testes increased as the doses of the CFE administered increased. It was significantly ($P<0.05$) higher in treated bucks than the control, except for left epididymides which declined as the level of the CFE increased.

Table 2: *Relative organ weight of Rabbit Bucks administered carrot fruit extract

Parameters (%)	T1	T2	T3	T4	T5	SEM
Adrenal Gland	0.02	0.01	0.02	0.02	0.02	0.00
Liver	2.09	2.07	2.21	2.35	2.50	0.06
Heart	0.20	0.15	0.17	0.23	0.52	0.08
Spleen	0.01^{ab}	0.01^{b}	0.01^{b}	0.01^{b}	0.02^{a}	0.00
Testis	0.13	0.13	0.14	0.12	0.13	0.00
Bile	0.03	0.01	0.03	0.01	0.03	0.00
Pancreas	0.01	0.01	0.01	0.01	0.01	0.00
Kidney	0.43	0.51	0.50	0.55	0.51	0.02
GIT	15.41^{ab}	13.58^{b}	14.81^{b}	16.23^{b}	3.09^{a}	0.62
Lungs	0.50	0.42	0.53	0.51	0.51	0.03
Eviscerated Weight	48.38	46.79	48.74	48.24	48.41	1.01

abc: Means with different superscripts in the same row are significantly ($P<0.05$) different
*Relative to liveweight

Daily sperm production

The daily sperm production of rabbit bucks administered CFE is presented in Table 4. The result showed there was a significant ($P<0.05$) difference in 0 mg (T1) of rabbit bucks administered CFE and no significant differences in values obtained in 0.5mg (T2), 1.0mg (T3), 1.5mg (T4) and 2.0 (T5) of rabbit bucks administered carrot fruit extract.

Table 3 : Gonadal characteristics and extra -gonadal sperm reserves of rabbits administered carrot fruit extracts

Parameters	T1	T2	T3	T4	T5
Testicular Weight (g)	1.56±0.43	1.40±0.25	1.42±0.41	1.48±0.36	1.16±0.51
Volume of Testis (ml)	1.56±0.44	1.24±0.23	1.32±0.34	1.28±0.53	1.22±0.54
Average Weight of Epididymis (g)	0.74±0.18	0.64±0.17	0.66±0.29	0.58±0.19	0.66±0.28
Left Epididymal sperm reserves ($\times 10^6$/g)	1 25.00±1.39^{a}	24.40±2.67^{ab}	23.00±5.73^{ab}	19.70±1.44^{b}	19.25±1.55^{b}
Right Epididymal sperm reserves ($\times 10^6$/g)	19.80±4.91^{c}	20.20±7.82^{bc}	30.20±3.86^{ab}	35.00±4.41^{a}	36.40±9.74^{a}
Total Epididymal sperm reserves ($\times 10^6$/g)	44.80±4.2^{b}	44.60±6.99^{b}	53.20±6.39^{ab}	54.70±3.90^{a}	55.65±7.52^{a}
Left Testicular sperm reserves ($\times 10^6$/g)	6.20±2.42^{c}	7.20±3.96^{b}	9.60±1.79^{ab}	10.60±3.85^{a}	13.00±3.37^{a}
Right Testicular sperm reserves ($\times 10^6$/g)	11.60±1.15	12.60±1.14	12.40±2.88	13.60±2.30	12.80±2.59
Total Testicular sperm reserves ($\times 10^6$/g)	17.80±2.38^{b}	19.80±3.4^{b}	22.00±3.11^{ab}	24.20±4.10^{a}	25.80±3.97^{a}

abc: Means with different superscripts in the same row are significantly ($P<0.05$) different

Table 4: Daily sperm production estimated from Testicular sperm reserves of rabbit bucks administered carrot fruit extracts

Parameters	T1	T2	T3	T4	T5
Left Testicular sperm reserves ($\times 10^6$/g)	6.20±2.42^c	7.20±3.96^b	9.60±1.79^{ab}	10.60±3.85^a	13.00±3.37^a
Right Testicular sperm Reserves ($\times 10^6$/g)	111.60±14.15	12.60±1.14	12.40±12.88	3. 13.60±2.30	12.80±2.59
Total Testicular sperm reserves ($\times 10^6$/g)	117.80±11.93^c	19.80±1.86^b	9. 22.00±10.84^b	4. 24.2±1.81^a	25.8±1.79^a
Daily Sperm Cell Production (x10^6)	15.19±3.48^c	5.77±1.08^{bc}	6.41±3.12^b	2. 7.06±1.06^a	7.52±0.91^a

abc: Means with different superscripts in the same row are significantly ($P<0.05$) different

Discussion

The significant increase in the weight gain of rabbits administered the carrot fruit extracts (CFE) than the control, most especially those that received 1 mg CFE/BW is probably an indication of positive influence of the extracts in improving the growth performance of the rabbits. This conforms with the findings of Walaa *et al.* (2006) who observed the antioxidative effects of *Moringa oleifera* leaves extract at the dose of 200 and 400 mg/kg recording a positive weight gain in rabbits, which was attributed to antioxidant inherent in the plant material as in carrot used in this study. The observed non-significant influence of the extracts on the organ weights implies that administering the CFE would possibly pose no serious consequences on organ functions and developments. This agrees with the report of Karada *et al.* (2016) who reported that the effect of different dietary antioxidants on organs′ weight and performance of broiler chickens was not significant. Tavarez *et al.* (2011) also reported that dietary antioxidant inclusion had no effect on carcass weight, meat quality and lipid oxidation of broiler chickens.

The testis volume and weight of the gonadal and extra-gonadal organs of rabbit bucks administered the CFE revealed that there were non-significant differences in the values obtained for weight of testis, testicular volume, concentration of sperm cells in right testis and average weight of epididymis across the treatments. The knowledge of basic morphometric characteristics of the reproductive tract have been found to provide valuable information in the evaluation of breeding and fertility potential of the animals (Ogbuewu *et al*. 2011). Gage and Freckleton (2003) described the mammalian testes as infallible predictors of spermatozoa production. The authors further asserted that knowledge of the basic morphometric characteristics of the reproductive organs is mandatory for assessment and prediction not only of sperm production but also of the storage potential and fertilizing ability of the breeder male. The weight of the paired testes in the present study was not significantly influenced by dietary treatments. The result is similar to the report of Bitto *et al*. (2006) who observed non-significant effect of pawpaw peel meal up to 30% on testicular morphometry of male rabbits.

However, it was observed that the left testes had higher sperm reserve, compared to the right in this study. This is in line with the findings of Matthew (2015) who tested the effect of garlic (*Allium sativum*) on rabbit bucks. The sperm reserves in both epididymis and testes, and consequent daily sperm production which increased as the concentration of CFE administered increased might indicate that accumulation and increased concentration of the CFE progressively enhanced spermatogenesis and sperm production in the bucks. This could have invariably accounted for dose-

dependent sperm reserves in the rabbit bucks. This probably could be attributed to the presence of beta-carotene, and other carotenoid/antioxidants in carrot that has been implicated as fertility and reproductive booster (Fiedor *et al.,* 2005; Cvetkovic *et al.,* 2013).

Conclusion

The study showed that carrot fruit extracts exerted positive effects on growth, gonadal and extra-gonadal parameters of rabbits without advert effects on the visceral organs. Therefore, carrot fruit extracts can be used in reducing oxidative stress up to 2mL/ Kg body weight. It is recommended that beta-carotene supplement can be made available in feeding as a way of reducing or eliminating oxidative stress in animals, which consequently might lead to cell death causing a decline in reproductive ability.

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