

Effects of Varying Levels of Dietary Citric Acid on semen characteristics and reproductive tract morphometry of Rabbit Bucks

Abstract

Thirty (30) weaner rabbit bucks at 10 weeks old were used to investigate the effects of the varying levels of dietary citric acid on reproductive parameters of rabbit bucks. The rabbits were divided randomly into five dietary groups and assigned to five treatments tagged T1, T2, T3, T4 and T5 containing 0.00, 0.75, 1.50, 2.25 and 3.00% synthetic citric acid in the rabbit diets respectively. Each group had six rabbit bucks with one rabbit constituting a replicate. Semen samples were collected on the 111th day of the experiment and semen characteristics evaluated. Subsequently, 6 animals randomly selected per treatment were sacrificed and their reproductive tract morphometry evaluated. The results of the semen characteristics obtained revealed that there were no significant differences ($P>0.05$) in the values obtained across the dietary groups. The right testis weight, paired testis weight, right testis volume, left epididymis weight, paired epididymis weight, right epididymis length, right vas deferens weight, left vas deferens weight and paired vas deferens weight were significantly different ($P<0.05$) among the treatment groups. From the results obtained, it could be concluded that the inclusion of citric acid in the diets of rabbit bucks up to 3.00% did not cause detrimental effects on the testicular growth and semen characteristics of rabbit bucks.

Keywords: rabbit, semen, citric acid, testis, epididymis

Introduction

The inadequate supply of proteins from such livestock as cattle, sheep, goat, pig and poultry has led to a shift towards alternatives of these animals. The production of animal protein from small animals has been emphasized as an alternate option to mitigate the protein requirement problem for the nation. Considering this point, the rabbit would be an important micro-livestock as an alternative (Vietmeyer, 1985) and a more economical source of animal protein (Egbo *et al.*, 2001). Topmost in the attributes promoting rabbit production and making it attractive include high fecundity, short gestation length, ability to rebreed immediately after parturition and a short generation interval (Biobaku and Dosumo, 2003; Ani, 2006; Ogundele and Apata, 2006). Unfortunately, in spite of all these products and economic advantages over other livestock, the rabbit has not achieved its potential as a cheap source of animal protein in the tropics (Herbert and Adejumo, 1995).

Numerous attempts have been made to improve the productive performance of rabbit and other animals by using growth promoters (Ashour *et al.*, 2004; El-Kholy *et al.*, 2008a). A typical example of such growth promoters used in the livestock industry are the organic acids. Organic acids such as citric acid, lactic acid, fumaric acid and benzoic acid are exclusively used as livestock feed preservatives, acidifier, growth promoter and for improvement of the conversion efficiency in farm animals (Esteive *et al.*, 2001). Most of the literature on the use organic acids in livestock diets indicated a positive influence on weight

gain especially in poultry, pigs and rabbits (Islam *et al.*, 2008; Gorniewicz and Dziadek, 2002; Lesson *et al.*, 2005; Alikhosravi *et al.*, 2008).

There is a relationship between nutrition and productivity which has a significant effect on the production and reproductive performance of farm animals. Effects of nutrition on the reproduction of farm animals have been well reported. Togun and Egbunike (2006) reported that the efficiency of sperm production, libido and quality of sperm tend to be significantly altered by age, nutrition, environment, health status, drugs, and chemicals. Osinowo *et al.* (1981) also reported that a high correlation exists between age, body weight and sperm reserves in Bunaji bulls. Togun and Egbunike (2006) further reported that the testes size is a good indicator of the present and future sperm production in bulls. Omole (1982) also stated that sexual maturity is known to be delayed by a poor nutrition regiment during growth. Delayed sexual maturity also affects age at puberty and stimulation of hypothalamus indirectly to produce an interstitial cells stimulating hormone that acts in the testicular tissue (Cogan *et al.*, 2004). The dietary inclusion of growth promoters such as citric acids in enhancing increased body weight may have an improved effect on reproduction in farm animals. However, there is a dearth of information on the effects of dietary citric acids on the reproductive parameters of rabbit bucks. The objectives of this study were, therefore, to determine the effect of varying inclusion levels dietary citric acid on the semen characteristics and reproductive tract morphometry of rabbit bucks.

Materials and Methods

Location

The study was carried out at the Rabbitry unit of the Livestock Research Farm, Federal University of Agriculture, Makurdi, Nigeria. Makurdi is located at Latitude 7° 14' North and Longitude 8° 21' East and lies within the Southern guinea savannah region of Nigeria. The area is warm with a temperature range of 24 to 36°C and high temperature is experienced between late February and April. The rainfall is between 508 and 1016mm (TAC, 2009).

Experimental Diets

Five experimental diets were prepared using conventional feedstuffs. The diets were tagged T1, T2, T3, T4 and T5 which contained feed grade synthetic citric acid at 0.00%, 0.75%, 1.50%, 2.25% and 3.00% inclusion levels respectively. The anhydrous citric acid used for the experiment was procured from Jasper Biochemicals. The diet composition is presented in Table 1.

Experimental Animals and Design

A total of thirty crossbred weaner rabbit bucks (Newzealand white x Chinchilla) were used for the experiment, which had five treatments each replicated six times with one rabbit per replicate. Each rabbit was housed separately in a wire mesh cage with dimensions 40 x 60 x 40cm³. The rabbit bucks were obtained from the rabbitry unit of Dagwom Farm of the Veterinary Research Institute, Vom. On arrival, the rabbits were allowed to acclimatize to the environment for 14days. After the acclimatization period, the rabbits were weighed and randomly allocated to treatments. The rabbits were assigned to the five treatments using the Completely Randomized Design. The feeding trial lasted for sixteen weeks (16weeks).

Semen collection and evaluation

Semen was collected between 8:00 am and 9:30 am. This was to ensure that optimum quality of semen was obtained. Semen collection was done with the aid of an artificial vagina (AV) so constructed as used at the Rabbitry Unit of the National Animal Production Research Institute. The AV was basically made of polyvinyl chloride tube with an external and internal diameter of 27.5mm and 21.7mm respectively and a length of 37.9mm. Two rubber latex condom was used one as the liner and the other as the collecting tube. The tip of the liner was cut off in order to create room to overturn it into the other end of the AV. The one side of the AV was held in place by a rubber band folded 5 times for a tight grip. Glycerol was poured into the space between the inner part of the AV and pulled liner until it was three-quarters full. The held end of the liner was then turned over the end of the AV and held in place by a rubber band folded 5 times to ensure a firm grip. The collection tube was made from a rubber latex condom fix to one of the ends of the AV. Prior to semen collection; the locally constructed AV was warmed by allowing it stay for 15minutes in warm water at 40°C obtained by using a clinical thermometer. A matured cycling doe was used to tease the buck for proper semen collection. At the 13th week of the study, a 2 week training period was used to get the bucks to ejaculate with the aid of the AV.

The collected semen sample was analyzed for semen quality parameters such as semen volume, sperm concentration, live sperm percentage, sperm motility, semen colour and abnormal sperm percentage as reported by Ahemen *et al.* (2013).

Reproductive tract morphometry

Before slaughter, the scrotal length and scrotal circumference were taken from the three selected animals using a vernier calliper and measuring tape rule respectively, the measuring tape rule was passed around the broad part of the scrotum. The scrotal circumference measurement was done as would have been the case in a standing animal. The testicular characteristics of the animals were taken after slaughter.

For investigation of testicular morphometry, the weight of each testis was recorded after the epididymis has been trimmed off. The volume of each testis was also recorded, using Archimedes Principle of water displacement. The density of testis was calculated as testes weight (g) divided by testes volume (ml). The length, volume, weight and density of the epididymis were also taken. Only the length and weight of the vas deferens of the rabbit bucks were taken as reported by Ahemen (2013).

This research work was performed in accordance with ethical rules for animal welfare and research.

Statistical analysis

Data collected were subjected to Analysis of Variance (ANOVA) using Minitab Statistical Software (Minitab 2010). Significant difference among treatment was separated using Duncan's New Multiple Range Test as outlined by Steel and Torrie (1980).

Table 1 Ingredient and nutrient composition of experimental diets

Ingredients	T1 (0.00% CA)	T2 (0.75% CA)	T3 (1.50% CA)	T4 (2.25% CA)	T5 (3.00% CA)
Maize	32.3	32.1	31.9	31.6	31.6
Soybean meal	25.8	26.3	26.8	27.2	27.7
Rice offal (5%)	22.5	22.4	22.2	22.0	21.8
BDG	15.6	14.7	13.8	13.1	12.1
Citric acid	0.00	0.75	1.50	2.25	3.00
Bone ash	3.00	3.00	3.00	3.00	3.00
Premix*	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100
Calculated analysis					
ME (kcal/kg)	2580	2590	2590	2590	2600
CP (%)	18.0	18.0	18.00	18.0	18.0
CF (%)	12.1	11.9	11.8	11.6	11.4
EE (%)	3.90	3.85	3.80	3.75	3.70
Calcium (%)	1.15	1.14	1.14	1.14	1.14
Phosphorus	0.74	0.74	0.74	0.74	0.74

ME- Metabolizable energy; CP-crude protein; CF-crude fibre; EE-ether extract

* Per kilogram of vitamin-mineral premix contains: 4800000 I.U of vitamin A, 1200000 I.U of vitamin D₃, 12000 mg of vitamin E, 1000mg of vitamin K, 16000 mg of niacin, 4000 mg of cal-pan, 2000 mg of vitamin B₂, 8 mg of vitamin B₁₂, 800 mg of vitamin B₁, 1400 mg of vitamin B₆, 32 mg of biotin, 50000 mg of antioxidant, 100 mg of cobalt, 100 mg of selenium, 480 mg of iodine, 16000 mg of iron, 28000 mg of manganese, 3200 mg of copper, 24000 mg of zinc and 80000 mg of choline chloride.

Results and Discussion

The results of semen characteristics of rabbit bucks fed varying inclusion level of citric acid are presented in Table 2. The similar milky whitish colour observed in the semen of the rabbits across the dietary treatments is an indication that the acidic nature of citric acid up to the inclusion of

3.00% did not cause inflammation or malfunctioning of the reproductive tract or accessory sex organs and glands (Banerjee, 2010). The values obtained for semen volume, sperm concentration, live sperm, normal sperm and sperm motility were within the normal range for healthy rabbit bucks reported by Ajayi *et al.*

(2009) and similar to those reported by Ahemen *et al.* (2013). The highest values of semen volume, sperm concentration and live sperm obtained at 1.5% citric acid inclusion imply that citric acid at 1.5% inclusion of the diet supported spermatozoa production in the seminiferous tubule. Dietary treatments did not significantly influence the percentage of live sperm cells in this study range from 78.33% to 81.67%. Arthur *et al.* (1989) reported that good semen samples show an average of 25% dead sperms or a minimum of 75% live sperm. Therefore, the mean value of the percentage of live sperm cells obtained in

this study was within the range of good samples. The results of percentage live sperm obtained also showed that the sperm cells are available for fertilization (Ajala *et al.*, 2001). The percentage of abnormal sperm cells values in this study ranged from 12.00 to 17.33%. The values were within the range of 6.00 to 18.00% reported by Ajayi *et al.* (2009). The percentage of abnormal sperm cells in this study were below the upper limit of 20% recommended as the minimum for good reproductive potential and fertility in either normal mating or in artificial insemination (Oyeyemi and Okedirin 2007).

Table 2 Semen characteristics of rabbit bucks fed varying inclusion levels of citric acid

Parameters	T1 (0.00%)	T2 (0.75%)	T3 (1.50%)	T4 (2.25%)	T5 (3.00%)	SEM
Semen Colour	Milky White	Milky White	Milky White	Milky White	Milky White	----
Semen Volume (ml)	1.03	1.00	1.83	1.63	1.43	0.41
Sperm Conc. ($\times 10^7$ /ml)	75.0	63.3	91.7	65.0	58.3	15.5
Live Sperm (%)	80.0	81.7	81.7	81.7	78.3	2.36
Dead Sperm (%)	20.0	18.3	18.3	18.3	21.7	2.36
Normal Sperm (%)	88.0	86.0	85.3	88.0	82.7	3.08
Abnormal Sperm (%)	12.0	14.0	14.7	12.0	17.3	3.08
Sperm Motility (%)	70.0	75.0	71.7	71.7	68.3	3.08

Table 3 shows the results of reproductive tract morphometry (RTM) of rabbits bucks fed varying levels of citric acid. The paired testis weight, right testis length, left testis length and testis volume obtained in this study were similar to those reported by Ahemen *et al.* (2013). The inclusion of citric acid in the diets of the rabbit bucks seems to have caused a slight effect on the paired testis density of the rabbit bucks across the dietary treatments. It appears that the inclusion of citric acid in the diets did not cause detrimental effects to the development of spermatogenic potentials.

The significantly similar data obtained for most reproductive tract morphometry measured reveals that the inclusion of citric acid in the diets of the rabbit bucks across the dietary treatments did not have a harmful effect on the testis, scrotum, epididymis and vas deferens of rabbit bucks. Uneven variation in the RTM values obtained may therefore not be linked to the diets but perhaps to genetic differences. The RTM of rabbit bucks has been shown to vary within and between breeds (Ugwu *et al.*, 2009; Wilson *et al.*, 1977).

Table 3 Reproductive Tract Morphometry of rabbit bucks fed varying inclusion levels of citric acid

Parameters	T1 (0.00%)	T2 (0.75%)	T3 (1.50%)	T4 (2.25%)	T5 (3.00%)	SEM
MSL (cm)	3.71	3.88	3.49	3.57	4.04	0.23
MSC (cm)	2.44	2.49	2.79	2.44	2.62	0.19
MTC (cm)	1.79	2.06	1.91	1.73	2.12	0.25
RTW (g)	1.02 ^{ab}	0.99 ^{ab}	0.87 ^b	0.92 ^{ab}	1.54 ^a	0.21
LTW (g)	1.03	1.07	0.92	1.06	1.69	0.25
PTW (g)	2.05 ^{ab}	2.06 ^{ab}	1.79 ^b	1.98 ^{ab}	3.23 ^a	0.46
LTV (ml)	0.97	0.77	0.77	0.63	0.93	0.17
RTV (ml)	0.93 ^{ab}	0.67 ^{ab}	0.67 ^{ab}	0.50 ^b	1.00 ^a	0.14
PTV (ml)	1.90	1.43	1.43	1.13	1.93	0.30
PTD(g/ml)	1.10	1.43	1.24	1.85	1.68	0.30
RTL (cm)	1.67	1.94	1.68	2.03	2.21	0.26
LTL (cm)	2.08	2.06	1.79	1.77	2.32	0.19
REW (g)	0.78	0.93	0.92	0.71	0.87	0.08
LEW (g)	0.84 ^{ab}	0.78 ^{ab}	0.81 ^{ab}	0.72 ^b	0.95 ^a	0.06
PEW (g)	1.63 ^{ab}	1.70 ^{ab}	1.73 ^{ab}	1.43 ^b	1.82 ^a	0.10
REV (ml)	0.77	0.73	0.77	0.67	0.90	0.10
LEV (ml)	0.83	0.70	0.87	0.63	0.93	0.10
PEV (ml)	1.60	1.43	1.63	1.30	1.83	0.17
PED(g/ml)	1.04	1.24	1.11	1.10	1.00	0.15
REL (cm)	5.26 ^{ab}	4.48 ^b	5.69 ^a	5.26 ^{ab}	5.34 ^{ab}	0.33
LEL (cm)	5.14	4.90	6.64	6.68	4.97	0.66
RVdW (g)	0.08 ^{ab}	0.08 ^{ab}	0.08 ^{ab}	0.05 ^b	0.11 ^a	0.01
LVdW (g)	0.08 ^{ab}	0.09 ^{ab}	0.07 ^{ab}	0.06 ^b	0.10 ^a	0.01
PVdW (g)	0.17 ^{ab}	0.18 ^{ab}	0.15 ^b	0.12 ^b	0.21 ^a	0.02
RVdL (cm)	7.97	6.78	8.15	8.11	7.23	0.78
LVdL (cm)	9.39	7.96	8.62	8.02	7.91	0.94

PVdW-paired vas deferens weight; *RVdL*-right vas deferens length; *LVdL*-left vas deferens length

MSL-mean scrotal length; *MSC*-mean scrotal circumference; *MTC*-mean testis circumference; *RTW*-right testis weight; *LTW*-left testis weight; *PTW*-paired testis weight; *RTV*-right testis volume; *LTV*-left testis volume; *PTV*-paired testis volume; *RTL*-right testis length; *LTL*-left testis length; *PTD*-paired testis density; *REW*-right epididymis weight; *LEW*-left epididymis weight; *PEW*-paired epididymis weight; *REV*-right epididymis volume; *LEV*-left epididymis volume; *PEV*-paired epididymis volume; *REL*-right epididymis length; *LEL*-left epididymis length; *PED*-paired epididymis density; *RVdW*-right vas deferens weight; *LVdW*-left vas deferens weight;

Conclusion

It is concluded that inclusion of citric acid up to 3.00% in the diet of the rabbit bucks supported normal growth of the reproductive tract and semen quality of the rabbit bucks. Citric acid at 3.00% inclusion level in the diets of rabbit bucks is therefore recommended for use to Rabbit Breeders in farms and Research Stations.

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