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Effects of Varying Levels of Dietary Citric Acid on Semen Characteristics and Reproductive Tract Morphometry of Rabbit Bucks

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TA and PAA managed the analyses of the study. Authors TA and PAA managed the literature searches. All the three authors read and approved the final manuscript.

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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

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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: Citric acid; rabbit; semen; citric acid; testis; epididymis.

1. 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, rabbit would be an important micro livestock as an alternative [1] and a more economical source of animal protein [2]. 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 [3,4,5]. Unfortunately, in spite of all these production and economic advantages over other livestock, the rabbit has not achieved its potential as a cheap source of animal protein in the tropics [6].

Numerous attempts have been made to improve the productive performance of rabbit and other animals by using growth promoters [7,8]. A typical example of such growth promoters used in 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 [9]. Most of the literature on the use organic acids in livestock diets indicated positive influence on weight gain especially in poultry, pigs and rabbits [10,11].

There is a relationship between nutrition and productivity which has a great effect on the production and reproductive performance of farm animals. Effects of nutrition on reproduction of farm animals have been well reported. Togun and Oseni [12] reported that the efficiency of sperm production, libido and quality of sperm tend be significantly altered by age, nutrition, environment, health status, drugs, and chemicals. Osinowo [13] also reported that a high correlation exists between age, body weight and sperm reserves in Bunaji bulls. Togun and Oseni [12] further reported that the testes size is a good indicator of the present and future sperm production in bulls. Omole [14] 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 interstitial cells stimulating hormone that acts in the testicular tissue [15]. 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 varying inclusion levels dietary citric acid on the semen characteristics and reproductive tract morphometry of rabbit bucks.

2. MATERIALS AND METHODS

2.1 Location of Experiment

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 temperature range of 24 to 36˚C and high temperature is experienced between late February and April. The rainfall is between 508 and 1016 mm [16].

2.2 Experimental Diets

Five experimental diets were prepared using conventional feedstuffs. The diets was 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.

2.3 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 were housed separately in a wire mesh cage with dimensions 40 x 60 x 40 cm^3.

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 into treatments. The rabbits were assigned to the five treatments using the Completely Randomized Design. The feeding trial lasted sixteen weeks (16 weeks).

2.4 Semen Collection and Evaluation

Semen was collected between 8:00 am and 9:30 am. This was to ensure that optimum quality 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 external and internal diameter of 27.5 mm and 21.7 mm respectively and a length of 37.9 mm. 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-quarter 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. [17].

2.5 Reproductive Tract Morphometry

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

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 D_3, 12000 mg of vitamin E, 1000 mg of vitamin K, 16000 mg of niacin, 4000 mg of cal-pan, 2000 mg of vitamin B_2, 8 mg of vitamin B_{12}, 800 mg of vitamin B_1, 1400 mg of vitamin B_6, 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*

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 et al. [17].

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

2.6 Statistical Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using Minitab Statistical Software [18]. Significant difference among treatment was separated using Duncan's New Multiple Range Test as outlined by Steel and Torrie [19].

3. 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 [20]. 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. [21] and similar to those reported by Ahemen et al. [17]. The highest values of semen volume, sperm concentration and live sperm obtained at 1.5% citric acid inclusion implies that citric acid at 1.5% inclusion of the diet supported spermatozoa production in the seminiferous tubule. The percentage live sperm cells in this study range from 78.33% to 81.67% were not significantly influenced by dietary treatments. Ajala et al. [22] reported that good semen samples show an average of 25% dead sperms or a minimum of 75% live sperm. Therefore, the mean value of percentage 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 [23]. The percentage 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. [21]. 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 [24].

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. [17]. 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 [25].

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. (x 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. 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

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

4. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vietmeyer ND. Potentials of micro livestock in developing countries. Journal of Applied Rabbit Research. 1985;8:16-25.
2. Egbo ML, Doma UD, Lacdaks AB. Characterization of small scale rabbit production and management. Proc. Nigeria Society of Animal Production. 2001;26:160-161.
3. Biobaku WO, Dosumo EO. Effects of supplementing a diet based maize and rice bran with 3 different improved forages on feed intake, digestibility and growth in rabbits. Nigerian Society Animal Production. 2003;22:179-184.
4. Ani AO. Effect of toasted Bambara groundnut (*Voandzeia subterranean. L*.) waste on hematology, carcass and organ characteristics of growing rabbits. Proceedings of Nigeria Society Animal Production. Bayero University Kano, P.M.B.3011 Kano, Nigeria; 2006.
5. Ogundele OO, Apata DF. Influence of Theobromine from dietary cocoa bean shell on hematology and serum biochemical constituents of Young pigs. Proceedings of Annual Conference of the Nigerian Society of Animal Production. Bayero University, Kano, P.M.B.3011 Kano; 2006.
6. Herbert U, Adejumo DO. Construction and evaluation of Artificial Vagina for collecting rabbit semen. Journal of Delta Agriculture. 1995;2:99-108.
7. Ashour G, Ibrahim SA, Ismail AM, El-Kholy KH. Physiological reactions and biological performance of rabbits to summer heat stress. In Proceedings of 2nd Science Conference on Physiology Respiration to Environmental Conditions, El-Arish, Egypt. 2004;165-186.
8. El-Kholy KH, Seleem TS, El-Aasar TA, Hanaa Abdelharith A. Effect of dietary addition of Arak (*Salvadora persica*) on growth and reproductive performance in black baladi rabbit males. World Rabbit Sci. 2008;16:21-27.
9. Esteive-gracia EJ, Brufau A, Perez-vendrell Miquel A, Duven A. Bioefficacy of enzyme preparation containing B-glucanase and xylanase in broiler diet based on barley and wheat in combination with flavomycin. Poult. Sci. Sci. 2001;76:1728-1737.
10. Islam MZ, Khandakerl ZH, Chowdhury SD, Islam KM. Effect of citric acid and acetic acid on the performance of broilers. Journal of Bangladesh Agricultural University. 2008;6(2):315–320.
11. Gornowicz E, Dziadek K. The effects of acidifying preparation added to compound feeds on management condition of broiler chickens. Ann. Animal Science Supplement. 2002;1:93-96.
12. Togun VA, Oseni BS. Effect of low level inclusion of biscuit dust in broiler finisher diet apparently healthy red Sokoto goats. Proceedings of the 27th Annual Conference of Nigerian Society for Animal Production. 2005;50-53.
13. Osinowo EO, Molokwu EC, Osori DC. Growth and testicular Development in Bunaji Bulls. Journal of Animal Research. 1981;16:55-67.
14. Omole TA. The effects of dietary protein on growth and reproductive performance in rabbits. Journal of Applied Rabbit Research. 1982;52:83-88.
15. Cogan U, Yaron DE, Brek M, Zimmermen G. Effect of processing condition on nutritive value of isolated soybean protein. J. Agric. Food Chem. 2004;16:196-198.
16. TAC. Nigerian Air Force Tactical Air Command, Makurdi Metrological station, Makurdi, Benue state, Nigeria, Makurdi Weather Elements Records; 2009.
17. Ahemen T, Abu AH, Orakaanya TT. Sperm quality and testicular morphometry of rabbits fed dietary levels of water spinach (*Ipomoea aquatica)* leaf meal. Agriculture and Biology Journal of North America. 2013;4(3):352-357.
18. Minitab. Minitab statistical software version 16.1.1, Mintab Incoporation, U.S.A; 2010.
19. Steel RG, Torrie JH. Principles and procedures of statistics. An Approach. 2nd Ed. McGraw-Hill Book Company, Incorporation, New York; 1980.
20. Banerjee GC. A textbook of animal husbandry: Mechanisms of reproduction. 8th Edition. 2010;144-160.
21. Ajayi AF, Raji Y, Togun V. Caudal epididymal sperm characteristics nd testicular morphometric of rabbits fed graded levels of a blood-wild sunflower leaf meal (BWSLM) mixture diet. J. Compl. Integr. Med. V. 2001;6:1-26.

22. Ajala OO, Oyeyemi MO, Akusu MO, Eimunjeze HE. The effects of scrotal insulation on the testicles and spermatozoa characteristics of WAD goats. Journal of Veterinary Science. 2001;3(1):44-50.
23. Oyeyemi MO, Okediran BS. Testicular parameters and sperm morphology of chinchilla rabbits fed with different planes of soybean meal. International Journal of Morphology. 2007;25(1):139-144.
24. Ugwu SO, Onyimonyi AE, Foleng H. Testicular development and relationship between body weight, testis size and sperm output in tropical boars. African Journal of Biotechnology. 2009;8(6):1165-1169.

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