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SEMEN INDICES, GROWTH RESPONSE AND SPERM RESERVE OF MALE RABBITS FED ZINC SUPPLEMENTED DIETS

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Abstract: This study was carried out to assess the effect of dietary levels of zinc on semen indices, growth parameters and testicular sperm reserve of heterogeneous stock of male rabbits. Twenty male rabbits aged 6 mo old were randomly allotted to four groups of five males each and fed diets containing 0, 50, 100 and 150 mg of zinc gluconate per kg diet, respectively, for eight weeks. Semen was collected from the males weekly using an artificial vagina and semen volume (mL), spermatozoa motility (%), sperm concentration ($\times 10^8$/mL) and live sperm cells (%) were assessed. Seminal plasma was separated from the semen by centrifugation, and its zinc concentration was determined using atomic absorption spectrometry. The weights of the males were taken weekly, and the daily feed intake was recorded. At the end of the feeding trial, the rabbits were stunned and slaughtered; the testes and epididymis were carefully removed and homogenised to determine sperm reserves using standard procedure. The result showed that semen indices were not significantly influenced by the dietary levels of zinc gluconate. Seminal zinc concentration was significantly higher in males fed 100 and 150 mg of zinc per kg compared to those in the control group. A higher body weight gain (454±50.3 g), testes weight (6.23±0.25 g) and epididymis weight (1.63±0.59 g) was recorded in males fed diet supplemented with 50 mg of Zinc gluconate per kg. No significant effect of dietary zinc supplementation was recorded in testicular sperm reserve. Epididymal sperm reserve was significantly higher in males fed the diets including 100 and 150 mg of zinc gluconate per kg. In conclusion, dietary levels of zinc gluconate did not improve semen quality and growth indices but increased seminal zinc concentration, which could result in improved prostate health in the heterogeneous stock of male rabbits in the tropics.

Key Words: epididymis, weight gain, testes weight, zinc gluconate, rabbit.

INTRODUCTION

In the tropics, rabbit populations are largely heterogeneous. The male rabbits are used for breeding purposes from about six months of age, when they are expected to produce semen consistently. Performance in this heterogeneous stock of rabbits is often below optimum, as their performance is influenced by several factors among which nutrition play a significant role. Diets low in micronutrients contribute to inefficient spermatogenesis, as these elements are essential for growth and development (Smith and Akinbamijo, 2000). Microelements are often provided to animals in diets as a component of the premix added and supplemented in the diet (Asaduzzaman, 2005) or in water as necessary. Certain microelements may be present in sufficient quantities in the feed. However, their deficiency symptoms may be observed in the animals due to their variable bioavailability or because the forms cannot be utilised in the body (Wang *et al.,* 2010). Mineral salts are often used in the form of oxides, carbonates, chlorides and sulphates, and the use of chelate forms is increasing (Wang *et al.,* 2010; Chrastinova *et al.,* 2015; Yan *et al.,* 2017). Zinc deficiency is the most widespread mineral deficit and can occur through at least five mechanisms - inadequate intake, increased requirements, mal-absorption and increased or impaired utilisation (Biswajit *et al.,* 2010). Zinc gluconate is a chelated form of dietary zinc and has been used extensively in human (Neve *et al.,* 1992; Wegmuller

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et al., 2014). Zinc is vital for growth and development, sexual maturation and reproduction, dark vision adaptation, olfactory and gustatory activity, insulin storage and release and for a variety of host immune defences (Biswajit *et al.,* 2013). The presence of zinc in the proper concentration in the animals' diet is of immense importance not only for their wellbeing, but also to optimise the overall performance of the animals and enhance their production potential (Shinde *et al.,* 2006). Improvement in the sperm production of domestic animals and fertility have been achieved following the supplementary feeding of zinc (Biswajit *et al.,* 2013). Intake of zinc chelate as glycine hydrate significantly increased the activities of superoxide dismutase and total antioxidant capacity in the kidney in rabbits 49 d old (Cobanova *et al.,* 2018). Zinc functions as a cofactor of numerous enzymes and is involved in cell division processes; higher levels are required for reproduction and fur production than for maintenance and meat production (Halls, 2010). The recommended zinc level for commercial rabbit diet ranges from 50 to 60 mg/kg diet to a maximum of 150 mg/kg diet (Mateos *et al.,* 2010). In order to select males on the basis of high fertility for breeding purposes, it is necessary to assess the growth and productivity of their reproductive organs, especially at the age they become sexually mature, and the probable role of supplemental levels of zinc may be necessary. Zinc supplementation in the diet of crossbred bulls improved qualitative and quantitative attributes of semen and serum testosterone levels (Kumar *et al.,* 2006). Injectable trace mineral supplementation in bulls did not affect growth, carcass characteristics or semen attributes of yearling bulls, although semen mineral concentrations may be related to semen quality (Blank *et al.,* 2016). Hence, this study aimed to evaluate semen characteristics, weight gain, testes weight, epididymis weight and sperm reserve in male rabbits fed dietary levels of zinc.

MATERIALS AND METHODS

Experimental design, animals and feeding

The study was conducted at the Rabbitry unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria. Twenty males aged 6 mo with an average weight of 1.88±0.16 kg were used for this study. The rabbits were randomly allotted into 4 groups with 5 males per group and housed individually in cages. The experimental diets containing 0, 50, 100 and 150 mg zinc gluconate per kg diet, designated Z_0, Z_{50}, Z_{100} and Z_{150}, respectively, were fed to the males for 8 wk. The experimental diet (Table 1) was presented to the animals in pelletised form and clean water was supplied daily. Feeds samples were analyzed for crude protein (CP), crude fiber (CF), ash and ether extract (EE) according to the methods of AOAC (2006). A sample of the ash was subjected to Atomic Absorption Spectrophotometry (AAnalyst 400 AAS) to determine the zinc concentration of the diets. Digestible energy was calculated.

Table 1: Ingredients and chemical composition of the experimental diet.

	Diets			
Ingredients (%)	Z_0	Z_{50}	Z_{100}	Z_{150}
Maize	10.6	10.6	10.6	10.6
Corn bran	18.5	18.5	18.5	18.5
Wheat bran	34.3	34.3	34.3	34.3
Rice bran	21.8	21.8	21.8	21.8
Soya bean meal	9.21	9.21	9.21	9.21
Palm kernel cake	3.90	3.90	3.90	3.90
Bone meal	1.06	1.06	1.06	1.06
Salt	0.33	0.33	0.33	0.33
Vitamin-mineral premix	0.30	0.30	0.30	0.30
Zinc gluconate (mg/kg feed)	0.00	50.0	100	150
Chemical Composition:				
Crude protein (%)	16.1	16.1	16.1	16.1
Crude fibre (%)	10.4	10.4	10.4	10.4
Ether extract (%)	5.43	5.43	5.43	5.43
Ash (%)	11.2	11.3	11.6	11.8
Zinc (mg/kg)	11.3	18.4	25.6	32.7
Digestible energy (kcal/kg)	2393	2393	2393	2393

Diets Z_0, Z_{50}, Z_{100} and Z_{150}, included 0, 50, 100 and 150 mg zinc gluconate per kg diet.

Zinc gluconate (Mason vitamins, Inc., Miami Lakes, United States) was procured, carefully ground in a sterile Petri dish and incorporated into the experimental diets. Two grams of ashed feed samples were dissolved separately in 5 mL of perchloric acid and nitric acid (3:1) and heated gently until it dissolved to give a clear solution. This was allowed to cool, transferred into a volumetric flask and marked up to 10 mL with deionised water. Zinc content of the solution and the blank were determined by atomic absorption spectrometry method.

Feed (120 g/rabbit) was supplied to the animals daily (in 2 servings to prevent wastage). Feed intake was estimated for each animal by deducting feed leftover from quantity supply every morning before supplying fresh feed.

Semen collection and evaluation

Semen was collected weekly from the males using an artificial vagina. An average of 102 ejaculates per experimental group (total=408) were assessed for semen volume (mL), spermatozoa motility (%), sperm concentration (per mL of semen) and live sperm cells (%). Semen volume was measured using a graduated collecting tube (excluding the gel mass). Spermatozoa motility was determined by placing a drop of undiluted fresh semen mixed with a drop of slightly warmed diluents (1% sodium citrate) on a sterile slide, covered with a cover slip and observed under the microscope at a magnification of 400× immediately after collection and rated within a range of 0-100%. Sperm concentration was evaluated by visual count under the microscope using the improved Neubauer haemocytometer method as described in Adeyemi *et al.* (2014). Live sperm cells were assessed by placing a drop of fresh semen and a drop of eosin nigrosin on a glass slide; gently mixed, smeared, left to dry and observed under the microscope at a magnification of 400×. The sperm cells that do not absorb the eosin stain represented the live cells, while the stained cells are the dead ones. The number of spermatozoa per ejaculate was calculated as the product of semen volume and spermatozoa concentration. Seminal plasma was separated from the sperm cell mass by centrifugation at 3000 rpm for 15 min. Seminal zinc concentration was determined by atomic absorption spectrophotometry. One millilitre of each seminal plasma sample was pipetted into different tubes. Nine mL of 0.1 N nitric acid (HNO_3) was added to make it up to 10 mL. The mixture was left overnight and then centrifuged to remove all precipitate. The supernatant and blank were analysed using atomic absorption spectrometer (AAnalyst 400 AAS).

Estimation of weights and sperm reserves

Live body weights of rabbit males were recorded at the beginning of the experiment and subsequently on a weekly basis to determine the weekly weight gain. Live body weights of the rabbit males were taken using a balance. The feeding trial lasted for 8 wk. The total live body weight gain was obtained as the difference between the final weight and initial weight.

At the end of the feeding trial, the rabbits were stunned and sacrificed. The testes (T) and epididymis (E) were carefully removed, weighed individually by placing in sterile Petri dish on a sensitive scale. The T and E were then homogenised separately for sperm cell count to determine sperm reserves. The T and E from each animal were placed separately in sterile beakers and macerated using sharp scissors in 1 mL of normal saline. The homogenates were diluted with normal saline at a ratio of 1 to 20 and filtered using sterile gauze to remove macerated tissue in the homogenate. Ten µL of diluted sample was taken for sperm count using the improved Neubauer haemocytometer method (Adeyemi *et al.,* 2014). Sperm cells in five chambers of the haemocytometer were counted diagonally for each testis and epididymis sample as observed under the microscope at a magnification of 400×. Sperm reserve was estimated as the total number of sperm cells present in the homogenates.

Statistical analysis

Data obtained from seminal, growth parameters and sperm reserve were subjected to general linear model procedure for complete randomised design of Statistical Analytical System (SAS, 2009). The treatments (zinc levels) were included as the main effect in the model. All means are presented as means±standard error. Statistical significance of means was assessed at $P<0.05$ using Duncan multiple range test and P-values below 0.05 were considered significant.

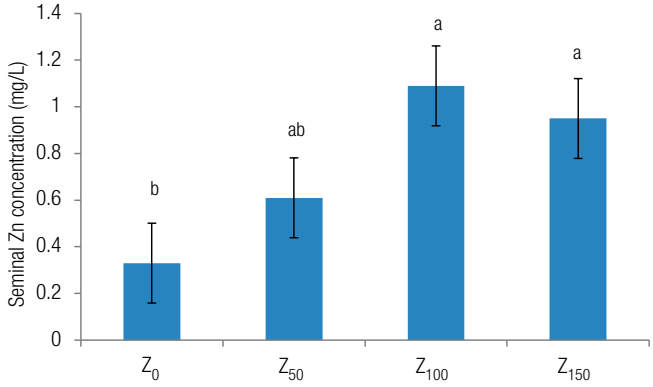

Figure 1: Seminal zinc concentration of male rabbits fed with Z_0, Z_{50}, Z_{100} and Z_{150}, (include 0, 50, 100 and 150 mg zinc gluconate per kg diet). Bars with different letters (a,b) differ at $P<0.05$.

RESULTS

Dietary zinc and semen indices

The zinc concentration of the diets was lower in the diet without zinc supplementation (11.3 mg/kg) compared to diets supplemented at 50, 100 and 150 mg zinc gluconate/kg (18.4, 25.6, 32.7 mg/kg, respectively). The seminal parameters of rabbit males fed different dietary levels of zinc are presented in Table 2. None of the seminal parameters assessed were significantly ($P>0.05$) influenced by the supplemental levels of zinc fed to the males. The result of seminal zinc concentration (mg/L) presented in Figure 1 showed a significantly ($P<0.05$) higher value in males fed Z_{100} and Z_{150} than those fed the control diet (Z_0). Zinc gluconate supplementation at 100 mg significantly ($P<0.05$) increased seminal zinc concentration by 69.7% compared to the Z_0 group. Animals fed Z_{50} had intermediate values.

Growth response

The body weight gain and feed intake of rabbit males fed zinc supplemented diets are shown in Table 3. Among the growth parameters assessed, only feed conversion ratio (FCR) was significantly ($P<0.05$) influenced by dietary zinc supplementation. The FCR was 27% lower in males fed Z_{100} diet compared to the control group (Z_0). Figure 2 shows the result of testes weight of males fed the different dietary levels of zinc. No effect of zinc supplementation on the testes weight was recorded.

Sperm reserve and epididymis weight

The sperm reserves in the left, right and paired testes were not significantly ($P>0.05$) increased or adversely affected by the dietary zinc supplementation. However, the treatment effect was recorded in the left, right and paired

Table 2: Semen parameters of rabbit males fed with the different experimental diets.

Semen indices	Z_0	Z_{50}	Z_{100}	Z_{150}	*P*-value
Semen volume (mL)	1.67±0.27	1.69±0.09	1.38±0.19	1.50±0.08	0.59
Spermatozoa motility (%)	89.9±2.97	84.7±5.04	80.3±3.61	87.2±3.78	0.40
Sperm concentration ($\times10^8$/mL)	4.07±1.94	3.78±1.30	5.05±0.84	4.08±0.48	0.53
Live sperm cells (%)	75.9±4.16	73.8±2.23	77.4±1.57	84.7±0.66	0.58
Number of spermatozoa /ejaculate ($\times10^8$)	6.80±2.21	6.39±1.65	6.97±0.69	6.12±0.27	0.98

Diets Z_0, Z_{50}, Z_{100} and Z_{150}, included 0, 50, 100 and 150 mg zinc gluconate per kg diet.

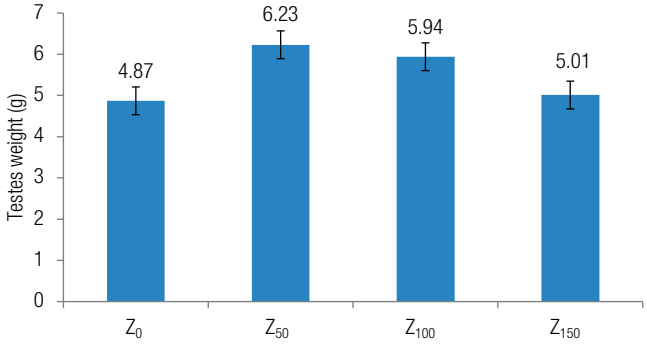

Figure 2: Testes weight of males fed with Z_0, Z_{50}, Z_{100} and Z_{150}, (include 0, 50, 100 and 150 mg zinc gluconate per kg diet). No significant differences were observed (P>0.05).

epididymis (Table 4). A higher non-significant weight of the paired epididymis was recorded in the males fed 50 mg dietary level of zinc.

DISCUSSION

Zinc is a trace micronutrient that is essential for various biological processes. Specific dietary levels are required by species of farm animals which are usually achieved with supplementation. Dietary supplementation of zinc as zinc gluconate at 100 and 150 mg per kilogram diet in this study resulted in increased (25.6 and 32.7 mg/kg respectively) levels of zinc in the diets administered. This implies that supplementing rabbit diet with zinc is essential to meet the nutritional requirement of mature rabbits as 35.94 mg/kg (Sun *et al.,* 2005) and a 50 to 60 mg/kg diet (Mateos *et al.,* 2010) is recommended.

Zinc supplementation has already proven beneficial in male sterility (Favier, 1992). Improvements in sperm production and fertility in male animals (bull, ram, etc.) have been achieved with dietary zinc supplementation (Biswajit *et al.,* 2013). In this study, dietary supplementation of a chelated form of zinc as zinc gluconate did not improve semen production significantly in male rabbits compared to those fed the control diet. Significantly increased semen volume, total live sperm concentration and sperm mobility due to dietary supplementation of zinc sulphate have been recorded in heat-stressed rabbit males (El-Masry *et al.,* 1994). Moce *et al.* (2000) also reported a significantly higher total sperm production in adult male rabbit administered 100 mg/kg dietary levels of zinc sulphate with no effect on semen quality parameters assessed during summer. Likewise, Oliveira *et al.* (2004) reported enhanced cellular mass volume of ejaculates in rabbits supplemented with 50, 100 and 150 ppm zinc oxide, revealing a possible increase in spermatozoa concentration in the breeder rabbits. Egwurugwu *et al.* (2013) also reported a significant effect of oral

Table 3: Growth parameters of rabbit males fed with the differents experimental diets.

Parameters (g)	Z_0	Z_{50}	Z_{100}	Z_{150}	P-value
Initial weight (g)	1896±174	1889±119	1867±119	1871±120	0.99
Final weight (g)	2231±184	2344±155	2315±140	2243±135	0.94
Total weight gain (g)	336±39.0	454±50.3	448±72.7	372±47.0	0.32
Average daily weight gain (g)	5.99±0.34	8.11±0.90	8.00±1.29	6.63±0.84	0.31
Average daily feed intake (g)	101±5.25	105±6.86	97.7±7.24	95.5±3.88	0.71
Feed conversion ratio	16.7±1.49^{b}	13.0±1.89^{ab}	12.2±2.63^{a}	14.4±2.93^{ab}	0.03

Diets Z_0, Z_{50}, Z_{100} and Z_{150}, included 0, 50, 100 and 150 mg zinc gluconate per kg diet.

^{a,b}Means in the same row with different superscript are significantly different (P<0.05).

Table 4: Sperm reserve and epididymis weight of male rabbits fed with the different experimental diets.

Parameters	Z_0	Z_{50}	Z_{100}	Z_{150}	*P*-value
Testicular sperm reserve ($\times 10^6$ sperm cells)					
Right	55.5±3.09	55.0±2.57	66.2±5.01	59.2±5.19	0.22
Left	52.5±1.89	54.7±1.92	54.2±5.44	65.3±2.09	0.13
Paired	108±4.92	109±4.49	120±10.11	124±5.22	0.35
Epididymal sperm reserve ($\times 10^6$ sperm cells)					
Right	153±14.75^{ab}	128±4.41^{a}	152±6.50^{ab}	165±12.42^{b}	0.04
Left	127±18.6^{a}	118±7.69^{a}	157±18.6^{b}	143±10.93^{b}	0.03
Paired	280±27.6^{ab}	246±11.4^{a}	309±25.8^{b}	308±24.9^{b}	0.03
Epididymis Weight (g)	1.25±0.35	1.63±0.59	1.35±0.33	1.440.36	0.46

Diets Z_0, Z_{50}, Z_{100} and Z_{150}, included 0, 50, 100 and 150 mg zinc gluconate per kg diet.

^{a,b}Means in the same row with different superscript are significantly different ($P<0.05$).

zinc administration on sex hormones and sperm quality in Wistar rats. The supplementation of 50 to 100 ppm zinc oxide has also improved different productive and reproductive parameters in goats (Kundu *et al.,* 2014).

Chrastinova *et al.* (2015) observed a weak influence of Glycinoplex-Zn in the performance of young rabbits. The addition of pure zinc supplement to the diets of local rabbit acts as an ameliorative tool for some productive traits of rabbits (Amen and Muhammad, 2016). Nonetheless, a significant decrease in follicle-stimulating hormone and interstitial cell-stimulating hormone was reported when dietary levels of zinc gluconate were fed to rabbits 2 mo old (Ogbu and Herbert, 2018). The result on semen indices in the current study, therefore, shows a possible underutilisation of zinc gluconate as a source of dietary zinc in the male rabbits at 6 mo of age.

Apart from red and white blood cells, zinc is also stored in the prostate gland (Bentley and Grubb, 1991). The increase in seminal plasma concentration of zinc as recorded in this study could indicate that dietary levels of zinc influenced the activity of the accessory sex gland (prostate gland). Seminal plasma trace elements were not influenced by zinc sulphate supplementation in males (Rahman *et al.,* 2014). Increasing the seminal zinc level may help to enhance prostate health, as dietary supplementation of zinc oxide significantly improved seminal vesicle volume in males (Emmanuel *et al.,* 2019). Hence, zinc gluconate improves zinc level in semen.

Adequate dietary levels of macro and micronutrients are essential for optimum performance of farm animals. This study showed that growth indices of rabbits fed supplemental levels of zinc were not enhanced. The daily live body weight gain of rabbits was not significantly higher in males fed the zinc diets compared to the control group in this study. Significantly higher live body weight gain has been reported in younger animals with dietary zinc supplementation. Ayyat and Marai (2000) reported significantly increased live body weight at 4 and 8 wk of age with supplementation of zinc oxide in diets of weaner rabbit. Garg *et al.* (2008) recorded an increase in average daily gain of lambs with 25 mg Zn/kg dry matter as Zn-methionine. Yan *et al.* (2017) reported improved growth performance in growing rabbits fed diet supplemented with 80 mg/kg Zn as Zn lactate. El-Moghazy *et al.,* (2019) also reported that zinc methionine at 100 mg/kg diet increased growth performance of rabbits at 9 and 13 wk of age. The result in this study, therefore, implies that zinc supplementation as zinc gluconate up to 150 mg/kg may not significantly enhance weight gain in a heterogeneous population of mature male rabbits, as the rate of gain at this age is minimal. It has been reported that zinc supplementation significantly reduces FCR in growing rabbits (Amen and Muhammad 2016, Yan *et al.,* 2017). In our study, the values obtained for FCR in males fed zinc supplemented diet were lower (12.2-14.4) than those of males fed Z_0 diet (16.7). These values are far higher than those of growing rabbits (4.03-4.49, Amen and Muhammad 2016; 3.69-3.82, Yan *et al.,* 2017). Findings from our study are at variance with those of Emmanuel *et al.* (2019) who reported 2.82 FCR in New Zealand white males 28 wk old fed 150 mg of zinc oxide. This confirms that the rate of weight gain in mature animals is extremely lower than in growing ones. This may also be influenced by breed differences.

Larger sized testes tend to possess more sperm producing ability than smaller ones contain more seminiferous tubules, Leydig cells and Sertoli cells (Britto *et al.,* 2004). Zinc overload has promoted growth and organ weight in male rats (Sun *et al.,* 2005). Zinc supplementation had no effect on the weight testes and epididymis of male rabbits

in this study. Yu *et al.,* (2014) and Amen and Muhammad (2016) reported no significant difference in the testes weight of rat and males, respectively, fed diets supplemented with zinc. In this study, males with the highest live body weight gain also had the largest testes size. The sperm reserves in the left, right and paired testes and epididymis were not significantly increased or adversely affected by zinc supplementation in male rabbits. Zinc supplementation as zinc oxide in males resulted in improved epididymal sperm traits compared to the control group (Emmanuel *et al.,* 2019). Leydig cell synthesis of testosterone is dependent on adequate dietary zinc (Biswajit *et al.,* 2013), which will boost the activities of the testes, thus higher storage in the epididymis. Higher testicular and epididymal sperm reserve were recorded with zinc supplementation at 100 and 150 mg zinc gluconate/ kg diet in this study; this shows the importance of adequate dietary zinc in the male reproductive tract/organs. The males (Z_{100} and Z_{150} group) with lower semen volume had higher seminal zinc concentration, as well as testicular ad epididymal sperm reserves. Hence, dietary zinc supplementation is essential for optimum semen production in the heterogeneous population of male rabbits in the tropics.

CONCLUSION

The study suggests that dietary levels of a chelated form of zinc (zinc gluconate) did not improve the semen quality of mature heterogeneous stock of rabbit males. However, supplemental levels of zinc may result in improved prostate health due to increased seminal zinc concentration in rabbit males. Other sources of zinc should be explored for the possible effect on young and adult male rabbits' reproduction.

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