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Original Research Article

Effect of zinc oxide on liveweight, reproductive organ dimensions and spermatozoa production of *Archachatina marginata* during dry season

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Abstract

Demand for Giant African Land Snail in Nigeria is so high such that it is very difficult to meet up with the supply as a protein source. However, during dry season, production at intensive level is often challenged with decline in growth and reproduction despite interventions to boost production. This study therefore evaluates the effect of zinc oxide on liveweight, reproductive organ dimensions and spermatozoa production of Giant African Land Snail (*Archachatina marginata)* during dry season. Thirty-two (32) snails with an average weight of 100–180 g were used for this experiment. The snails were allotted to four different feeding treatments which included concentrate diet only, concentrate +10 mg/kg of zinc oxide, concentrate +15 mg/kg of zinc oxide and concentrate +20 mg/kg of zinc oxide, respectively. Each treatment contained eight replicates. After nine weeks, five snails were selected from each treatment and dissected. Variables monitored were: weekly weight gain, shell circumference, shell length and shell diameter. Other reproductive data collected after dissections were: organ weight, reproductive tract weight, albumen weight, ovo-testis weight, gonado-somatic index (GSI) and spermatozoa concentration. The result showed that dietary zinc oxide significantly ($P < 0.001$) influenced feed intake, shell parameters, organ weight ovo-testis weight, albumen weight, gonado-somatic index, reproductive tract weight and spermatozoa concentration. Snails fed zinc oxide supplemented diet had better shell measurements, organ weight, ovo-testis weight, albumen weight, gonadosomatic index and reproductive tract weight. Considering spermatozoa concentration, 10 mg/kg of zinc oxide inclusion into concentrate diet gave the highest concentration. The results of the present study showed that dietary zinc oxide positively influenced feed intake, shell measurements, organ weight, ovo-testis and albumen weight. Similarly, gonado-somatic index and spermatozoa production were also positively influenced. Therefore, 10 mg/kg inclusion of zinc oxide is recommended for better reproductive tract development, and spermatozoa production at the desirable economic level during the dry season production of *Archachatina marginata*.

Keywords: aestivation; snail; feeding; growth; reproductive tract, spermatozoa; Nigeria

INTRODUCTION

The main target in snail production are reproduction, growth and intact shell architecture. These key features determine to larger extent the economic value of this animal. There are several factors often considered in production of land snails, notable among which are nutrition, management, reproduction and climatic variables. Prominent among these is nutrition. It plays a paramount role in animal growth and shell development. Feed type influences growth rate, reproductive organ function, spermatozoa and ova production. In the presence of favourable climatic variables (high humidity and moisture), reproductive performance of snails has been reported to be excellent (Michael, 2011). Good management of hatchling, breeder and grower snails are also important in sustaining their production. However, problems arise when there is a sudden increase in temperature especially during dry season that hamper the reproductive performance of land snail (Michael, 2011). Feed intake is also known to be seriously affected which translates into drop in live weight especially if the situation persisted for long period of time. It may also result in mortality after long period of aestivation. The use of dietary supplement such as zinc has also been reported in other livestock to improve reproductive activity (Smith and Akinbamizo, 2000). Bíreš et al. (1997)

elucidated the role of zinc in metalloenzymes involved in several enzymatic reactions influencing carbohydrate, protein, lipids and nucleic acid metabolism, and as such may promote growth and sperm livability in rams. Zinc also plays a prominent role in spermatogenesis (Kendall et al. 2000; Wong et al. 2002; Wroblewski et al. 2003). Recently in Nigeria, it was observed that sudden increase in temperature has often been experienced in different States of the Country more than what it used to be in the southern part of Nigeria that used to favour the production of Giant African Land Snails. Several aspects of snail biology and aestivation have recently been explored (e.g. Abussamad et al., 2010; Omoyakhi and Osinowo 2010). Under increased temperatures, production activity is often affected due to impaired general biology of the animal. It therefore becomes very important to incorporate substance(s) that have the capacity to eliminate some of these negative effects (i.e. reduction in activity of reproductive tracts, liveweight and wellbeing) during the dry season. We hypothesised that zinc would exert a positive effect on food intake, growth rate and reproductive indicators in the land snail. This study therefore aimed at determining the effect of zinc oxide on liveweight, reproductive organ dimension and spermatozoa production of *Archachatina marginata* during dry season.

MATERIALS AND METHODS

The research was carried out at the Snail Physiology Research Unit Abeokuta, Ogun State. The location lies within the rainforest belt of Western Nigeria (latitude 7°N, longitude 3°2′E and altitude 76 m.a.s.l.). The climate is humid with a mean annual rainfall of 1,037 mm, mean temperature of 28.5 °C and mean relative humidity of 82%.

A total of thirty two (32) *Archachatina marginata* snails weighing between 100 g and 180 g were purchased from local market in Nigeria. They were placed in plastic cages, washed with detergent and clean water before the commencement of experiments. The first two weeks of the experiment were used as acclimatization period. Snails were fed *ad libitum* and water was placed in their drinkers on a daily basis. The drinkers, feeders and the cages were cleaned daily. The feeding experiment lasted nine weeks. Table 1 shows the composition of experimental concentrate diet used as prepared in the laboratory.

Experimental design

The snails were randomly assigned to four treatments with eight replicates each. The experimental treatments consisted of supplementing the normal snail diet with 10, 15, 20 mg/kg of zinc oxide. The normal diet without zinc oxide served as the control. Zinc oxide used was purchased from Baofull, Guangxi, China.

Data collection

Liveweight and shell measurements

Liveweight gain was measured with the aid of sensitive scale (AR 2140). Snails were allowed to be at rest, i.e. after retraction into the shell before weighing to ensure accurate weight measurement. Liveweight measurement comprising both shell weight and the visceral mass of the animal was taken only once per week. Digital venire caliper and meter rule were used for shell measurements (shell length and diameter).

Determination of spermatozoa concentration

The thirty two snails were selected for sperm concentration determination. After dissection, little hermaphroditic ducts were removed and homogenized in 1 ml of normal saline. A dilution of 1:19 was made with the aid of formalin-bicarbonate solution after which 10 µl of the homogenate was loaded into improved haemocytometer. Sperm cells found within the four corner squares were counted. Cells counts were multiplied by a conversion factor (50,000) to obtain the sperm concentration.

Gonado-somatic index (GSI)

After dissection, separate weighing of visceral organs and ovo-testis were done. Thereafter, ovo-testis weight was expressed as proportion of visceral organ weight and multiplied by 100 according to Barber and Blake (1983). The formula is shown below:

$$GSI = \frac{\text{Ovo-testis weight}}{\text{Visceral mass}} \times 100$$

Reproductive Tract Dimensions

Weighing of visceral organ, ovo-testis, albumen gland and reproductive tract were carried out using microbalance (AR 2140) after dissection, see Figure 1 and 2.

Statistical analysis

Data collected from the experiment were subjected to a least square analysis of variance using SYSTAT Statistical Package (1999) in a completely randomized design (CRD). Significant treatment means were separated using Duncan multiple range test.

The statistical model used is:

$Y_{ij} = \mu + T_I + E_{ij}$

Where,

Y_{ij} = dependent variables

μ = population mean

T_I = effect of Zinc oxide (I = 1–4)

E_{ij} = random error

Table 1. Composition of experimental diet (g/100 g)

Ingredients	Quantity (g)
Maize	50
Wheat offal	27.5
Groundnut cake	12
Soya bean meal	4.25
Bone meal	3
Oyster shell	3
Salt	0.25
Total	100

Analysis of the diet: Energy: 10, 015 KJ/kg, Crude protein: 16.395%, Ether extract: 4.21%, Crude fibre: 4.42 %, Calcium: 2.37%, Phosphorus: 0.7%, Ash: 1.5%

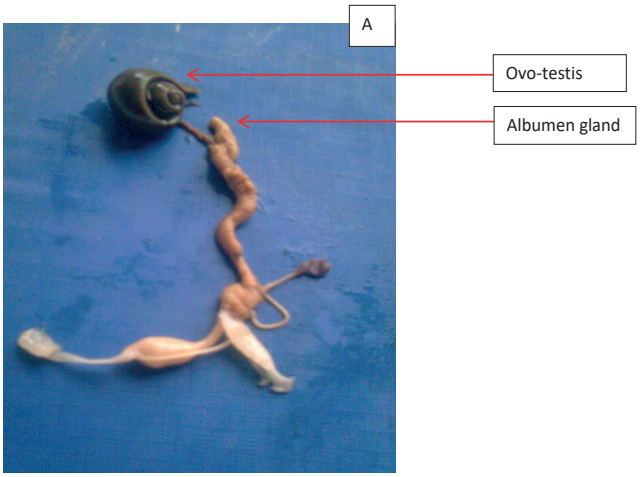

Figure 1. Reproductive tract of *A. marginata* showing ovo-testis and albumen gland

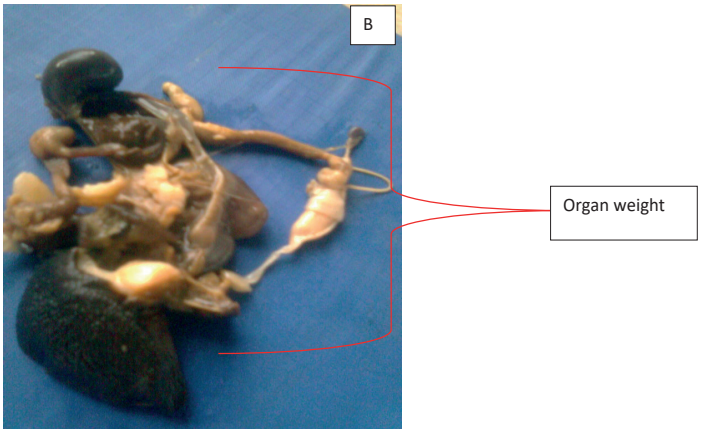

Figure 2. Dissection showing complete organ weight of *A. marginata* after shell removal

Table 2. Least-square means showing effect of dietary zinc oxide on weight gain of Giant African Land Snail (*Archachatina marginata*)

Treatment	Weight gain (g)	Total Feed intake (g)	FCR
Diet (Control)	14.03±2.11	215.60±5.16^{b}	15.30±1.57
Diet + Zinc (10 mg/kg)	15.18±2.11	200.32±5.16^{b}	13.78±1.57
Diet + Zinc (15 mg/kg)	17.13±2.11	207.73±5.16^{b}	13.15±1.57
Diet + Zinc (20 mg/kg)	19.47±2.11	239.14±5.16^{a}	13.36±1.57

Means in the same column with different superscripts differ significantly ($P < 0.001$)

RESULTS

The result of least-square means showed the effect of dietary zinc oxide on weight gain of Giant African Land Snail is shown in Table 2. Zinc oxide had no significant effect ($P > 0.05$) on weight gain and FCR. However, total feed intake was significantly affected ($P < 0.001$). Snails fed with 20 mg/kg of zinc oxide in concentrate had the highest feed intake compared to other levels (control, 10 and 15 mg/kg of concentrate).

Table 3 shows effect of dietary zinc oxide on shell measurements of Giant African Land snail. Inclusion of dietary zinc oxide at 10 mg, 15 mg and 20 mg/kg significantly influenced shell length (SHLNT) compared with those snails fed with control diet. Similarly, snails fed zinc oxide at 10 mg and 15 mg/kg had better shell circumference compared with control diet used in the present study. Response of shell diameter (SHLDIM) was differed significantly. Snails fed on diet supplemented with 10 mg and 15 mg/kg had an improved shell diameter compared with those that received other dietary treatments (control diet and 20 mg zinc oxide per kg diet) used in the present study.

Effect of dietary zinc oxide on organ weight, ovo-testis weight, albumen gland weight, gonado-somatic index and reproductive tract weight of *A. marginata* is shown in Figures 3–7. Results showed that dietary zinc oxide had significant influence on visceral organ weight (ORGWT), ovo-testis weight (OVOWT), albumen gland weight (ALBWT) as well as gonado-somatic index (GSI) and reproductive tract weight. In all these organs, dietary zinc oxide inclusion at 10, 15 and 20 mg/kg were better compared to the control (0 mg/kg) (Figures 3–7).

Effect of dietary zinc oxide on spermatozoa concentration of *A. marginata* is shown in Table 4. The result showed that 10 mg/kg diet produced the highest sperm concentration ($18.10 \pm 2.13 \times 10^6$), followed by 15 mg/kg ($12.83 \pm 2.13 \times 10^6$) and 20 mg/kg ($9.03 \pm 2.13 \times 10^6$), while the snails fed control diet recorded the least ($8.07 \pm 2.13 \times 10^6$).

DISCUSSION

Zinc supplementation levels used in this study (10, 15 and 20 mg/kg zinc oxid e) showed no significant influence on weight gain of land snail. This observation was contrary to what was reported in broilers and White Pekin ducks (Attia et al., 2013; Zhao et al., 2014). Although significant reduction in liveweight has been reported for different live weights of Land snail under aestivation (Abdussamad et al., 2010), compensatory increase in liveweight was also observed post-aestivation after arousal (Omoyakhi and Osinowo (2010). These changes occurred as a result of metabolic suspension and reinstatement of the system of the animal which are quite different from introduction of a substance like zinc oxide. Aestivation has also been reported to depress egg laying numbers in *A. marginata* (Omoyakhi et al., 2017).

The role performed by zinc oxide as reported may be linked to positive influence on metabolic activity which resulted in better protein and nucleic acid metabolism which are key determinants of growth (Eisler, 1993). Increased total feed intake recorded at the highest level of zinc oxide (20 mg/kg) supplementation in this study is in agreement with the report of Attia et al. (2013) and Zhao et al. (2014) who reported positive significant effect of zinc oxide on feed intake. Since feed intake is boosted in this study, it is possible that total amount of feed consumed is mobilized more towards the buildup of protective shell of the animal than flesh.

Role of zinc in calcium and phosphorus metabolism in mollusks is not yet fully elucidated; however, its function in growth and bone development has been documented (McClain et al., 1973; Batal et al., 2001). Zinc oxide at 10 mg and 15 mg/kg diet increased the shell circumference and diameter compared with the control diet. This is an indication that shell deposit was higher at these two treatment levels. Thus, zinc may influence calcium metabolism. Similarly, for shell length, all the three levels (10, 15 and 20 mg per kg diet) used in this study positively increased shell length. Increase in shell length may be as a result of tendencies of mollusks to tolerate and bioacummulate elements (Coughtrey and Martin 1976; Beeby and Richmond 2003; Viard et al. 2004 ; Wegwu and Wigwe 2006) like zinc which promote shell production via calcium and phosphorus mobilization. The tolerance and binding affinity of zinc to an element like calcium may also be aided by specific metallothioneins (Taylor et al. 1988; Dallinger et al. 1989) which eventually lead to their deposition in extracellular crystalline matrix of the shell (Howard et al. 1981; Beeby and Richmond 2003; Viard et al. 2004). Aside shell parameters, effect of zinc on reproductive tract was also evaluated considering visceral organ weight, albumen gland and ovo-testis.

Visceral organ weight is also a reflection of how several organs responsible for body function were influenced by dietary zinc oxide offered to the snail. In this study, all three zinc levels (10, 15, and 20 mg/kg) produced improved results compared to the control (0 mg/kg). This may indicate that zinc plays a crucial role in protein synthesis. Its role as a co-factor for more than 80 metalloenzymes necessary for protein synthesis has been reported (Park et al., 2015; Byun et al., 2017).

Albumen gland is known to be responsible for secretion of perivitelline fluid (Ademolu et al. 2013). This fluid provides nutrients for developing embryos (Mukail et al. 2004). Therefore, secretion levels of this fluid to a larger extent may determine survivability

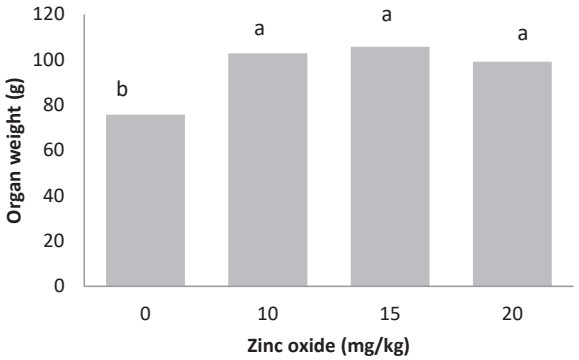

Figure 3. Effect of dietary zinc on organ weight of *Archachatina marginata*
a, b: different symbols denote mean values significantly different (P < 0.05)

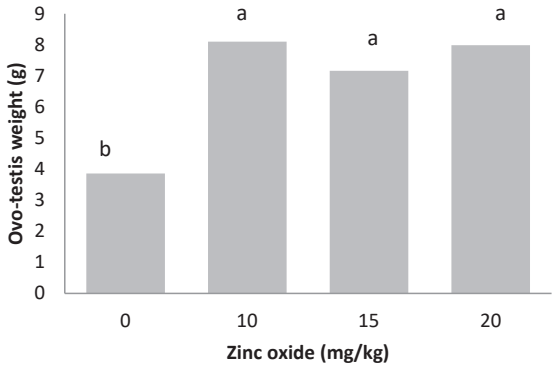

Figure 4. Effect of dietary zinc on ovo-testis weight of *Archachatina marginata*
a, b: different symbols denote mean values significantly different (P < 0.05)

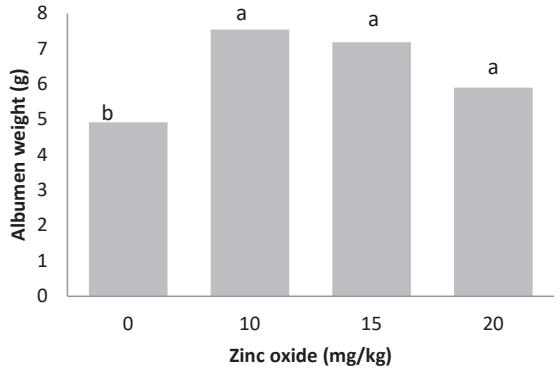

Figure 6. Effect of dietary zinc on albumen gland weight of *Archachatina marginata*
a, b: different symbols denote mean values significantly different (P < 0.05)

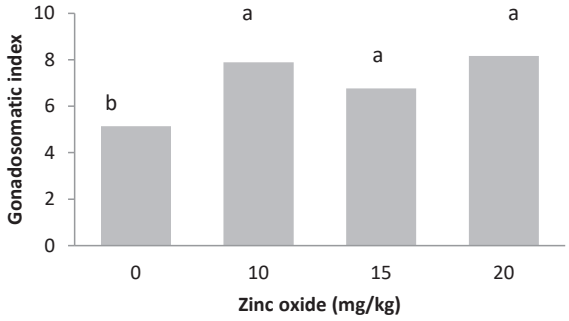

Figure 7. Effect of dietary zinc on gonado-somatic index of *Archachatina marginata*
a, b: different symbols denote mean values significantly different (P < 0.05)

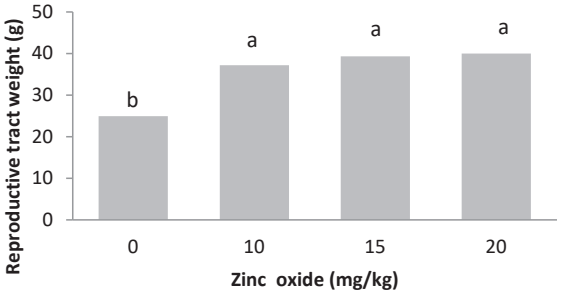

Figure 8. Effect of dietary zinc on reproductive tract weight of *Archachatina marginata*
a, b: different symbols denote mean values significantly different (P < 0.05)

of embryo during incubation or shortly before hatching. It was noticed that irrespective of the zinc supplementation levels used, albumen gland weight was positively influenced by all treatment levels except those that received the control diet. Zinc at these levels promoted increased activity of this organ. Ademolu et al. (2013) reported that albumen glands contain some elements which play prominent roles in body physiology of this animal (Na^+, K^+, Ca^{2+}, Cl^- and Po_4^{2-}). However, increase in weight of albumen gland of snails fed dietary zinc may result from an influx of some of these elements acting as co-factors for both metal and enzymes (Favier, 1992). Similarly, ovo-testis which is known to perform a crucial role in reproduction of giant African Land Snail was also positively influenced in this study.

In mollusks, the ovo-testis is known to be responsible for both oocytes and spermatozoa

Table 3. Least-square means showing effect of dietary zinc on shell measurements of Giant African Land Snail (*Archachatina marginata*)

Treatment	SHLCIR(cm)	SHLNT(cm)	SHLDIM(cm)
Diet (Control)	17.186 ±0.128[b]	9.623±0.092[b]	5.256±0.049[b]
Diet + Zinc (10 mg/kg)	17.655±0.128[a]	10.162±0.092[a]	5.618±0.049[a]
Diet + Zinc (15 mg/kg)	17.769±0.128[a]	10.146±0.092[a]	5.593±0.049[a]
Diet + Zinc (20 mg/kg)	17.393±0.128[ab]	10.153±0.092[a]	5.389±0.049[b]

[ab]Means in the same column with different superscripts differ significantly ($P < 0.01$; $P < 0.001$ and $P < 0.001$ for SHLCIR, SHLNT and SHLDIM, respectively)

Table 4. Least-square means showing effect of dietary zinc oxide on spermatozoa concentration of Giant African Land Snail (*Archachatina marginata*)

Treatment	Sperm concentration (× 10^6)	SEM (±) × 10^6
Diet (Control)	8.067[d]	2.13
Diet + Zinc (10 mg/kg)	18.100[a]	2.13
Diet + Zinc (15 mg/kg)	12.833[b]	2.13
Diet + Zinc (20 mg/kg)	9.033[c]	2.13

Mean in the same column with different superscripts differ significantly ($P < 0.05$)

production (Geoffroy, 2005). Zinc at 10 mg/kg which gave the best sperm concentration is a reflection of its support for reproduction. This result in snails confirms the role of zinc in sperm production and fertility as well as domestic animals including goats (Biswajit et al., 2013; Saleh et al., 1992). But the sharp decline that occurred in sperm concentration at 15 and 20 mg/kg of zinc indicates that higher amounts may serve as a testicular toxicant negatively affecting normal spermatogenesis. Negative effects of higher zinc concentrations on testicular tissue of mice have been reported (Talebi et al., 2013). Since in animals zinc is needed for normal regulation of the hypothalamus-pituitary-gonadal axis (Biswajit et al., 2013), its quantity must be at acceptable/tolerable amount. This study revealed that the ratio of ovo-testis to organ weight known as gonado-somatic index was also significantly affected.

Gonado-somatic index is a reflection of gonadal development (Rheman et al., 2002). The higher gonado-somatic index levels induced by increase zinc levels compared to control is the evidence that zinc promotes ovo-testis activity and gamete production.

The role of zinc in reproductive functions had been documented (Kaludin et al., 1983; Wong et al., 2001; Henkel et al., 1999; Carpino et al., 1998; Zhao and Xiong, 2005). Kumar et al. (2006) reported an increase in sperm concentration of bull fed zinc-supplemented diet. Zinc has anti-oxidative properties which play a vital role in sperm quality (Colagar et al., 2009). In this study, the highest sperm concentration was recorded at 10 mg/kg. This observation disagrees with the report of Sokol et al. (1990) and Mankad et al. (2006) that high concentration of zinc enhances sperm count. Some previous studies have also reported that a higher concentration of zinc is detectable in testis of which may have roles related sperm production (Hidiroglou and Knipfel, 1984; Miura and Miura, 2001). Although these assertions were made based on results from mammals, this study demonstrated that higher zinc concentrations also alter spermatogenesis in mollusks, specifically in giant African land snail.

CONCLUSION

Zinc oxide positively influenced feed intake, shell parameters, organ, ovo-testis and albumen weight. It is also obvious that gonado-somatic index and spermatozoa production were positively influenced. For spermatozoa production, zinc oxide at 10 mg/kg added to feed was the most effective inclusion level. Therefore, the additions of 10 mg, 15 mg and 20 mg of zinc oxide into feed are recommended for better reproductive tract development whereas 10 mg per kg of feed is recommended for spermatozoa production during the dry season production of *Archachatina marginata*.

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