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EFFECTS OF AESTIVATION DURATION ON TESTOSTERONE, HAEMOLYMPH BIOCHEMICAL PARAMETERS AND REPRODUCTIVE TRACT DIMENSIONS AND WEIGHT OF GIANT AFRICAN LAND SNAIL (*Archachatina marginata*) DURING DRY SEASON

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ABSTRACT

Aestivation is a process of metabolic inactivity under which energy reserve are manipulated for survival. Reproductive apparatus and haemolymph biochemical agents also undergo phase manipulation as the duration continues. To determine the physiological influence on key hormone of reproduction and reproductive apparatus, seventy five *A. marginata* snails were used for this study. The snails were divided into five treatments with fifteen replicate each. Treatment include: Zero (0) week, Three (3) weeks, Six (6) weeks, nine (9) weeks and six (6) weeks post-aestivation. Parameters measured were: Testosterone concentration, haemolymph biochemical parameters (Total protein, albumin, globulin, Aspartate transaminase (AST) and Alanine transaminase (ALT), dimensions (length) and weight of the organs and tissues of the reproductive tracts (Ovo-testis, penis, vaginal, oviduct, little hermaphrodite duct, common hermaphrodite duct, vas deferens and albumen gland) gonado-somatic index and percentage mortality. Result showed that level of testosterone at three and six weeks of aestivation significantly reduced compared to the control. Also, at nine weeks of aestivation, the reduction was significantly greater than what was recorded at both three and six weeks of aestivation. But the testosterone levels were reversed at nine weeks post aestivation. Total protein and globulin were significantly influenced with both reaching a peak value at 9 weeks of aestivation while ALT, AST and albumin were not significantly affected. So also, of all the reproductive tract parts measured, organ weight, ovo-testis weight, penis weight and length were significantly influenced ($P<0.001$; $P<0.001$; $P<0.05$; $P<0.001$). Similarly, vaginal weight, oviduct weight, little hermaphrodite duct weight and length were also significantly affected together with vas deferens length and albumen gland length while aestivation duration had no significant influence on reproductive tract weight, ovo-testis length, vaginal length, oviduct length, little hermaphrodite duct length, vas deferens weight and albumen gland weight. Similarly, gonado-somatic index was also not significantly affected by aestivation duration. It was also obvious from this study that the highest mortality was recorded at 6 weeks of aestivation, followed by 9 weeks of aestivation while 3weeks and 6 weeks post-aestivation had the least mortality with the control intact.

In conclusion, it is clear from this study that aestivation duration significantly influenced testosterone concentration, haemolymph biochemical parameters and some selected reproductive apparatus of A. marginata.

Keyword: Aestivation, testosterone concentration, A. marginata, Haemolymph, biochemical parameters

INTRODUCTION

Commercial production of Giant African Land Snails is gaining support gradually in this era in Nigeria. This move is a proactive step to close the wide gap between the demand and supply of this animal as bulk of the supply is from the wild. Seasonal influence on productivity remains a main challenge as reproduction decline during the dry season. But production and breeding activity is known to be at peak during the raining season. Previous studies have shown that climatic variables during this period favor reproduction. But the opposite is the case during the dry season. Depending on the environmental variables of immediate environment of the animal, the effects are felt at different degrees and thus decline the rate of reproduction. Under natural condition when the environment becomes unfriendly the animal goes into a state called aestivation. This is a period of dormancy characterized by lack of food and water availability (Storey and Storey, 2012). It is a period in which animal suppress metabolic activity and translate to hypometabolic state (Storey and Storey, 1990; Guppy, 2004; Withers and Cooper, 2010). Under this condition, animal conserve energy, retain body fluid, stabilize organs, cells and macromolecules (Storey and Storey, 2012).

Physiological adaptation put in place to reduce moisture loss during this condition is the secretion of mucous epiphragm by snails to seal the shell aperture (Carvalho *et al*., 2010; Storey and Storey, 2012). As a result of both moisture loss and suppressed metabolic activities, haemolymph metabolites which constitute biochemical parameters are also affected. Transaminase and aspartate enzymes were reported to be depleted during aestivation in Giant African Land Snail and thus leading to cell injury due to thermal and oxidative stress (Christian *et al*., 2014). Reduction in tissue content of the reproductive organs was also reported during this period (Omoyakhi and Okhale, 2015). Liveweght reduction of 52.4 % and 35.6 % of initial weight for *A. marginata* was reported by *Abdusamad et al*. (2010). Considering various effects of aestivation, there is need for proper understanding of this phenomenon in other to annex the positive influence of these effects and at the same time averts the negative sides in commercial production. For example, Snails to be transported for export purpose needed to be in aestivated form as such, appropriate duration of time must be taken into consideration not to hamper the health and reproductive status of this animal. Sound understanding of aestivation will also made it possible to know different points at which quick intervention must be made to nullify negative effects on both health and reproductive status of the animal. Effect of this phenomenon on reproductive hormone and apparatus could also supply useful information on how to manipulate environmental variables to suit reproductive activity of this animal. Such information may also be used for prediction of land snail population in the wild, especially during this era of climate change. This study therefore aimed at evaluating the effects of aestivation on testosterone, haemo-

lymph biochemical parameters and reproductive apparatus of Giant African land Snail (*Archachatina marginata*) during dry season

MATERIALS AND METHODS

Experimental Area

The research was carried out at the Snail Physiology Research Unit of the College of Animal Science and Livestock Production, University of Agriculture, Abeokuta, Ogun State. The location lies within the rainforest vegetation zone of Western Nigeria, latitude 7 ^{o}N, longitude 3 ^{o} 2' E and Altitude 76 meter above sea level (m.a.s.l). The climate is humid with a mean annual rainfall of 1,037 mm, mean temperature of 34.7 ^{o}C and mean relative humidity of 82 % (Abdussamad, 2010).

Materials

A total of seventy five (75) *Archachatina marginata* weighing between 150 g to 180 g were used for this experiment. The snails were housed in plastic cages with each having a dimension of 30 cm by 40 cm by 24 cm, with small plastic feeding and drinking troughs in each cage to take care of the animal.

Snails and their management

The plastic cages, water trough and feeders were clean prior to the commencement of the experiment, three weeks was set aside as a period of acclimatization. Poultry layers mash was fed *ad libitum* to all the snails during this period. After three weeks, feed and water were withdrawn.

Epiphragm Formation

Whitish calcareous substance appeared and sealed up the aperture of the shell within 7-9 days of withdrawal of feed and water. Care was also taken not to shake or move the basket to prevent the removal of already formed epiphragm.

Experimental Procedure

The snails were randomly assigned into five (5) different treatments with 15 replicates for each treatment. Below is the experimental layout:

Treatment 1: Control (15)
Treatment 2: 3 weeks (15)
Treatment 3: 6 weeks (15)
Treatment 4: 9 weeks (15)
Treatment 5: 6 weeks post-aestivation (15)

Reproductive tract dimension

Snails were weighed and dissected at week zero, 3 weeks, 6 weeks, 9 weeks and 6 weeks post aestivation. Reproductive tract organs which include: Albumen, Ovo-testis, oviduct, common duct, vas deferens, little hermaphrodite duct and penis were weighed, their length were also taken.

Gonado-somatic index

At designated weeks after dissection, visceral organ and ovo-testis were weighed separately. 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:

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

Haemolymph Collection

Haemolymph was collected from the mantle (oxygenated) cavity region, after the removal of first two or three whorls. Haemolymph from each group were measured using measuring cylinder and temporarily stored in universal bottle before the commencement of other analysis.

Haemolymph Biochemical Parameter assay

The haemolymph total protein, albumin, aspartate transaminase (AST) and alanine transaminase (ALT) were assayed using spectrophotometer with appropriate kit (Randox). The globulin value was calculated by subtracting the albumin values from the total protein value of the haemolymph.

Testosterone assay

Haemolymph testosterone levels were assessed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit.

Statistical Analysis

The data generated from this study were subjected to Least squares analysis of variance using SYSTAT statistical computer package (SYSTAT, 1992) in a Completely Randomized Design (CRD). Significant treatment means were separated using Duncan multiple range test (Gomez and Gomez, 1985). The statistical model used was:

$$Y_{ij} = m + T_i + \varepsilon_{ij}$$

Where

Y_{ij} = Dependent variable
m = Population mean
T_i = Effect of aestivation status (i=1 - 5)
ε_{ij} = Random residual error.

RESULTS

Figure1 showed effect of aestivation duration on haemolymph testosterone level of Giant African Land snail (*Archachatina marginata*). The testosterone level of the control group was significantly higher than those group of snails under three weeks (3wks), six weeks (6wks) and nine weeks (9wks) of aestivation but not significantly different (P>0.05) from those group of snails under six weeks (6 weeks) post-aestivation. Although, group of snails at three weeks (3wks) and six weeks (6wks) of aestivation were not significantly different from each other but both groups were significantly higher than those group of nine weeks (9wks) of aestivation. The result of analysis of variance showing effect of aestivation duration on haemolymph biochemical parameters are shown in Table 1. Aestivation duration had significant (P<0.01) effect on total protein and globulin. Albumin, ALT and AST were not significantly (P>0.05) affected by aestivation status. The result of effect of duration of aestivation on haemolymph biochemical parameters of Giant African Land snail (A. marginata) is presented in Table 2. The highest value for total protein was obtained at nine weeks (9wks) of aestivation (27.67±2.19) followed by six weeks (6wks) post aestivation (3.93±0.47) however, total protein values remained unchanged significantly (P>0.05) considering the control; those snails under three weeks (3 weeks) and six weeks (6wks) aestivation, while those under six weeks (6wks) aestivation and those at six weeks (6 wks) post-aestivation were not significantly different (P>0.05) from each

other. For globulin, the highest value was recorded at nine weeks (9wks) aestivation (5.70±0.52), followed by those group of snails under control and six weeks (6wks) post-aestivation (2.12±0.45 vs 2.06±0.52). Those snails under six weeks (6wks) recorded the least value after those under three weeks (3wks) aestivation (1.61±0.64 vs 1.17±0.52).

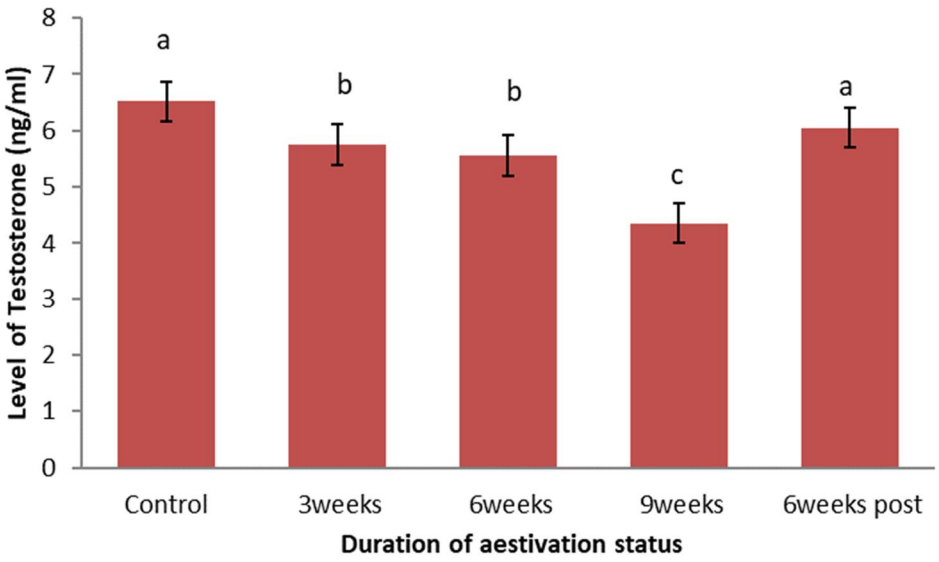

Figure 1: Effect of Duration of aestivation and post-aestivation on testosterone level of GALS

Table 1: Analysis of variance showing effect of aestivation duration on haemolymph biochemical parameters of GALS (A. marginata)

SOURCE	DF	Mean square				
		TP	ALB	GLOB	ALT	AST
TRT	4	9.996*	0.733^{NS}	9.662*	20.475^{NS}	7.713^{NS}
ERROR	10	0.661	0.359	0.817	14.383	4.808

*=P<0.05; NS= Not significant
TP - Total Protein
ALB - Albumin
GLOB - Globulin
AST - Aspartate Transaminase
ALT- Alanine TransaminaseAwqs

Table 2: Effect of duration of aestivation on haemolymph Biochemical parameters of GALS (*A. marginata*)

Treatment	Biochemical parameters				
	ALT	TP	ALB	GLOB	AST
Control	23.00±1.90	3.12±0.41^{c}	1.00±0.30	2.12±0.45^{b}	12.25±1.10
3 weeks	29.50±2.68	3.12±0.58^{c}	1.52±0.42	1.61±0.64^{c}	17.00±1.55
6 weeks	23.67±2.19	3.42±0.47^{bc}	2.25±0.35	1.17±0.52^{d}	13.67±1.27
9 weeks	27.67±2.19	7.41±0.47^{a}	1.72±0.35	5.70±0.52^{a}	14.00±1.27
6 weeks post	26.00±2.19	3.93±0.47^{b}	1.87±0.35	2.06±0.52^{b}	14.33±1.27

Means in the same column, for a given parameter with different superscripts differ significantly (P< 0.05)
TP - Total Protein
ALB - Albumin
GLOB - Globulin
AST - Aspartate Transaminase
ALT- Alanine Transaminase

Effect of aestivation duration on reproductive tracts dimensions of Giant African Land snail (A. marginata) is shown in Table 3. Aestivation duration had significant effect (P<0.001) on organ weight. The control snails had significantly higher organ weight compared to those on aestivation duration of 3wks, 9wks and those on 6wks post-aestivation. For ovo-testis weight, aestivation duration does not significantly influence this organ up to 6wks of aestivation compared to the control group. For penis length, similar trend was noticed, except that only those snails on 6wks post-aestivation were significantly different from the control group. Considering the penis length, at 3wks aestivation duration, the penis increased in length compared to the control group that was significantly lowers (4.50±0.30 vs 3.28±0.29), while at 6wks and 9wks of aestivation duration, the two were not significantly different from each other (P>0.05), but at 6wks post-aestivation, the penis length has attained similar dimension compared to that of the control group. For vaginal weight, similar weight was maintained throughout except the difference seen at 9wks of aestivation compared to that which was observed at the control group (0.54±0.05 vs 0.28±0.06). Considering the oviduct weight, oviduct weight decreased immediately after 3wks aestivation compared to the control group but the decreased noticed was not significantly different as aestivation duration continued i.e at 6wks, 9wks and 6wks post- aestivation except the weight at 6wks aestivation that was not significantly different from the weight of oviduct of the control group. For little hermaphrodite duct weight, effect of aestivation duration was

seen to have significant effect at 9wks of aestivation (0.04±0.03) while the control and other weeks were not significantly different from each other. Considering common hermaphrodite duct weight, at 3wks, 9wks and 6wks post-aestivation, the weight of these organs were not significantly different from each other but were lower to the control with the exception of those group of snails under 6wks aestivation which was not significantly different from the control. So also, the common hermaphrodite length, 3wks, 6wks and 9wks aestivation were not significantly different in length except for those snails under 6wks post-aestivation which were significantly shorter in length compared to the control group. Another organ is vas deferens length, at 3wks aestivation, the length increased compared to the control (10.95±0.80 vs 6.35±0.75) while at 6wks and 9wks, the length were not significantly different from each other. But at 6wks post-aestivation, the length was statistically similar to those in the control group (4.37±0.85 vs 6.35±0.75). For albumen length, aestivation duration (i.e 3wks, 6wks and 9wks) had significant effect on the length but the length of those snails under 6wks-post aestivation was significantly lower to those in the control group. However, aestivation duration does not significantly (P>0.05) influence reproductive tract weight, ovo-testis length, vaginal length, oviduct length, little hermaphrodite duct, vas deferens weight and albumen weight.

Table 4 showed effect of aestivation duration on gonado-somatic index. It was observed that aestivation had no significant effect (P>0.05) on gonado-somatic index in this study. Figure 2 showed effect of aestivation on percentage mortality of *A. marginata* . The control group had no mortality while 3wks and 6wks post-aestivation duration both had 10 % mortality. However, snails under 6wks aestivation duration had the highest mortality (65%) followed by 9wks aestivation duration (40 %).

DISCUSSION

Circulating testosterone that went down at 3wks, 6wks with high level of decrease at 9wks of aestivation is an indication that duration of aestivation had effect on testosterone production. Grizard *et al*.(1997) reported that under-nutrition or starvation is associated with different disorder in male pituitary gonadal axis in rat. It was also reported that starvation depending on level of severity decreased circulating concentration of testosterone and gonadotropin in different species of animal (Howland and Skinner, 1973; Campbell *et al*., Root and Russ, 1972; 1977; Klibanski *et al*., 1981; Bergendahl *et al*., 1989). The concentration of testosterone recorded by those groups of snails under 6wks post-aestivation which was not different from the control group is also an indication that effect of starvation after period of 9wks in this species is reversible.

Considering the elevated values recorded for both total protein and globulin at 9wks of aestivation duration compared to other durations is an indication that high level of dehydration had set in at this level (Beetham *et al*., 2005). The adaptive physiological mechanism during aestivation is very critical to the survival of this animal. The key mechanism focuses on water retention and sufficient energy reserve (Christian *et al*., 2014; Giokas *et al*., 2005).

Table 3: Effect of aestivation duration on reproductive tracts weight and dimension of Giant African Land snail (*A. marginata*)

Reproductive organs	Aestivation duration					P-Value
	Control	3wks	6wks	9wks	6wks post-aestivation	
Organ weight (g)	108.34±3.45^{a}	84.80±3.66^{b}	88.88±6.00^{ab}	70.36±4.64^{bc}	65.89±3.92^{c}	0.001
Reproductive tract weight (g)	15.30±1.17	12.94±1.24	14.50±2.03	11.76±1.57	10.35±1.33	0.089
Ovo-testis weight (g)	8.08±0.40^{a}	6.46±0.42^{ab}	5.99±0.69^{ab}	5.52±0.53^{b}	4.80±0.45^{b}	0.001
Ovo-testis length(cm)	2.42±0.13	2.60±0.14	2.57±0.23	2.37±0.18	2.39±0.15	0.771
Penis weight (g)	0.81±0.06^{a}	0.65±0.06^{ab}	0.77±0.10^{ab}	0.72±0.08^{ab}	0.52±0.65^{b}	0.026
Penis length (cm)	3.28±0.29^{bc}	4.50±0.30^{a}	3.65±0.50^{abc}	4.59±0.38^{ab}	2.16±0.32^{c}	0.001
Vaginal weight (g)	0.54±0.05^{a}	0.37±0.05^{ab}	0.32±0.08^{ab}	0.28±0.06^{b}	0.34±0.05^{ab}	0.017
Vaginal length (cm)	2.02±0.24	1.20±0.25	1.13±0.41	1.72±0.31	1.23±0.27	0.098
Oviduct weight (g)	0.46±0.03^{a}	0.20±0.32^{b}	0.34±0.05^{ab}	0.27±0.04^{b}	0.30±0.03^{b}	0.001
Oviduct length (cm)	1.25±0.11	1.15±0.11	1.15±0.19	1.17±0.14	0.89±0.12	0.294
Little hermaphrodite duct weight (g)	0.13±0.02^{ab}	0.08±0.02^{abc}	0.17±0.03^{a}	0.04±0.03^{c}	0.05±0.02^{bc}	0.005
Little hermaphrodite duct length (cm)	1.93±0.34	2.50±0.36	2.17±0.58	1.28±0.45	0.94±0.38	0.053
Common hermaphrodite duct weight (g)	2.45±0.18^{a}	1.18±0.19^{b}	1.74±0.32^{ab}	1.26±0.24^{b}	1.18±0.21^{b}	0.001
Common hermaphrodite duct length (cm)	5.58±0.28^{a}	4.45±0.20^{ab}	5.73±0.48^{a}	4.45±0.73^{ab}	3.19±0.32b	0.000
Vas deferens weight (g)	0.15±0.07	0.25±0.08	0.08±0.13	0.15±0.10	0.13±0.08	0.762
Vas deferens length (cm)	6.35±0.75^{bc}	10.95±0.80^{a}	10.31±1.31^{ab}	8.18±1.01^{abc}	4.37±0.85^{c}	0.001
Albumen gland weight (g)	3.87±0.62	2.67±0.65	3.92±1.08	2.41±0.84	2.05±0.71	0.295
Albumen gland length (cm)	4.72±0.42^{a}	3.75±0.44^{ab}	4.64±0.73^{ab}	4.51±0.56^{ab}	2.61±0.47^{b}	0.024

Means within the same row with different superscripts differs significantly (P<0.05)

Table 4: Effect of aestivation duration on gonado-somatic index of GALS (*A. marginata*)

Aestivation duration	GSI	SEM (±)
Control	7.41	0.37
3 weeks aestivation	7.63	0.39
6 weeks aestivation	6.81	0.63
9 weeks aestivation	7.77	0.49
6 weeks Post-aestivation	7.24	0.42

P>0.05

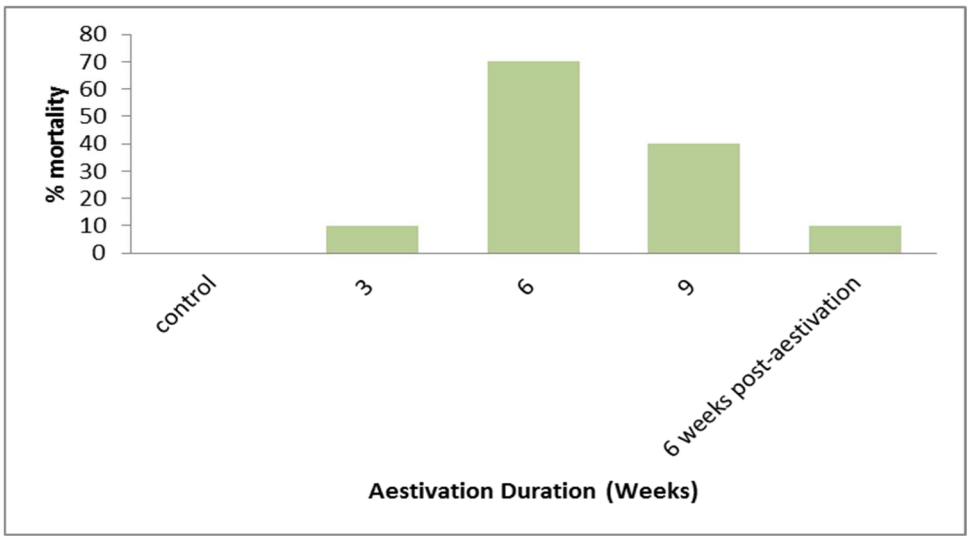

Figure 2: Effect of aestivation duration on percentage mortality of *A. marginata*

The prevailing environmental temperature also plays a vital role in the total amount of water that is loose despite the thickness of the epiphragm (Prior, 1985; Arad and Avivi, 1998). It is not unlikely that during aestivation, oxidative stress may occure, but in this study, values recorded for both AST and ALT were not significantly different among all the aestivation duration used. This observation is an indication that cell injury has not occurred considering the aestivation duration used in this study. Both AST and ALT are known to be enzymes present in hepatocytes and other organs in Land snails (Mahmoud, 2006). Elevated values are indication of tissue damage and hepatic injury (Farkas *et al*., 2004). Lower values than normal are also an indication of problem to these internal organs.

Significant reduction seen in organ weight may be as a result of depletion of energy reserve and body fluid losses which are common occurrence during aestivation (Omoyakhi and Okhale, 2015; *Abdusamad et*

al., 2010). This trend is expected since the reserve is been depleted for physiological maintenance of the animal. For Ovo-testis, this structure is known to be responsible for both spermatozoa and ova production (Abiona, 2005). Sharp decrease in weight observed at 9wks of aestivation duration compared to the control is an indication that aestivation/starvation can alter reproductive activity of this organ. Tamburi and Martin (2011) and Vega *et al*. (2006) had reported the negative effect role of aestivation on ovo-testis function. This negative role may be linked to other reproductive apparatus since their activity are controlled by hormones which are secreted by this organ (Ovo-testis). Prominent among such organs are penis and vaginal. With critical look, penis weight seems not to be affected significantly but its values also witness some form of decrease which fully manifested during post-aestivation state. The reason for this observation may be as a result of tissue mediated moisture loss which makes it not to be so rapid for it not to become obvious. The trend for vaginal was almost similar, but the moisture lost was distinct at 9wks of aestivation. The reason for this observation may be as a result of the tubular nature of the vaginal which may make fluid lost to be easily noticeable at this aestivation duration. The trend for oviduct weight lost was noticeable also at 3wks and 9wks of aestivation duration. This observation may be due to the fact that this organ is fluid rich which may be traced to the role it performed during egg envelope process as it passes through it (Buckland-Nicks and Chia, 1990; Mann *et al*., 1994). Differences seen among other organs i.e little hermaphrodite duct, common hermaphrodite duct, vas deferens and albumen gland are all traceable to moisture lost among the different aestivation duration used in this study. For gonado-somatic index which was not significantly affected is an indication that the decrease in weight recorded by ovo-testis is not so high compared to the organ weight of the animal thus not establishing any noticeable damage in relation to spermatogenic activity (Simanainen *et al*., 2008).

The highest percentage for mortality recorded at 6wks aestivation is an indication that physiological adaptation to moisture loos and energy reserve reached peak which may be dependent on prevailing environmental temperature, but thereafter, physiological adjustment for survival took place at 9wks and the mortality came down. This observation may as a result of switch over in energy sources for survival.

CONCLUSION

This study has shown that aestivation duration significantly influenced haemolymph testosterone, biochemical parameters and some selected reproductive apparatus of *A. marginata*. It is therefore important to note the peak points where this species of snail may be affected during dry season in order not to delay reproduction during aestivation.

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