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Chemical adjustments during aestivation by the giant African land snails, *Archachatina marginata* and *Achatina achatina*

J.M. Omoyakhi*[1], O.A. Osinowo[2], S.A. Onadeko[3] and M.O. Ozoje[4]

[1]Department of Animal Science, University of Benin, Benin City
[2]Department of Animal Physiology, University of Agriculture, Abeokuta
[3]Department of Forestry and Wildlife Management, University of Agriculture, Abeokuta
[4]Department of Animal Breeding and Genetics, University of Agriculture, Abeokuta

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ABSTRACT: The effects of duration (0, 2, 4 and 6 weeks) of dormancy of two species of giant African land snails on the chemical adjustment to aestivation were determined in groups of matured snails. The results indicate that the overall % CP content of *A. marginata* was 53.48 ± 1.82 % which was not significantly ($P > 0.05$) higher than that of *A. achatina* (49.50 ± 2.03 %). Duration of aestivation did not have any significant ($P > 0.05$) change in the CP in both species. Analysis indicates that both species maintained constancy of fat (lipid) over a measured period of first 4 weeks. Compared to control, the % EE was significantly lower ($P < 0.05$) in snails that aestivated for 6 weeks. While the % EE of *A. marginata* decreased by 39.9 % of the awake snails in 6 weeks, *A. achatina* only retained 52.6 % of the original EE of the awake snails after 6 weeks of dormancy. The ash and the NFE were not significantly affected ($P > 0.05$) by species and duration of aestivation except for crude fibre that declined ($P < 0.01$) with duration of aestivation.

Keywords**: C**hemical adjustments, Dehydration, Starvation, Giant African land snails; *Archachatina marginata*, *Achatina achatina*.

Introduction

Pulmonate land snails respond to unfavourable environmental conditions such as falling temperatures and dehydrating periods by entering a state of inactivity or dormancy (Bailey, 1981; Barnhart and Mcmahon, 1987; Whitwan and Storey, 1991; Fields, 1992 and Odiete, 1999). Aestivation is defined as dormancy that occurs in response to low water availability in the environment (Odiete, 1999), while hibernation is dormancy that occurs during the low winter temperatures (Schmidt-Nielson, 1996)
During dormancy, snails stop feeding, burrow in the ground or climb as high as possible on vegetation and withdraw deeply into their shell, greatly reducing many physiological functions. These behavioural responses to dormancy reduce the impact of stressful environmental conditions on snails and help to preserve the composition of the body fluids (Storey and Storey, 1990). Nevertheless during this period the snail, which is in the starve state, loses some water, with a consequent decrease in body weight.

*To whom correspondence should be addressed. E-mail: *omoyank@yahoo.com*

The information available on the aestivation of giant African land snails (*A. marginata* and *A. achatina*) has been on the behavioural adaptations to these inclement environments (Cobbinah, 1992; Odiete, 1999). The fact that changes in environmental factors and behaviour allow the animal to develop a suitable performance in complex situations represents a highly adaptive phenomenon. Thus, the objective of this study was to extend previous studies to establish the relative and comparative contribution of intrinsic control of aestivation and to discuss the contribution of some chemical alterations to the adaptation of dehydration and starvation of the two giant African land snails, *A. marginata* and *A. achatina.*

Materials and Methods

The experiment was carried out at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), University of Agriculture, Abeokuta. Abeokuta lies within the Rain Forest vegetation zone of Western Nigeria at latitude 7^{o} 13' 49.46"N, longitude 3^{o} 26' 11.98"E (Google Earth, 2006) and altitude 76 m above sea level. The climate is humid with a mean annual rainfall of 1,037 mm, an average temperature of 34.7^{o}C and an average relative humidity of 82 % throughout the year (60 % in January and 94 % in July to September).

Materials used in this experiment included a total of 40 apparently healthy snails (20 *A. marginata* and 20 *A. achatina*) of 150 g average liveweight, 40 well ventilated plastic basket cages of 40 cm by 25 cm by 20 cm with covers, 40 each of shallow feeders and drinkers, humus soil, sensitive electronic weighing scale, oil paint to mark for proper identification, dried pawpaw leaf meal, layer's mash and water

The experiment was conducted between the months of January and March. The experiment was laid out in a 2 X 4 factorial (spp X duration of aestivation) in a completely randomized design in 5 replicates.
The cages were prepared and filled with sun-dried humus soil up to a depth of 5 cm and moistened with 300 ml of water. Each cage was assigned a drinker and a feeder. The snails were weighed in grammes using a sensitive electronic balance. The snails randomly allocated to the treatments with one snail per basket. The snails were balanced for snail liveweight. Feed (layers mash + dried milled pawpaw leaves; 1:1; w/w; Table 1) and water were provided *ad libitum.* At the end of a 2-week adjustment period, the liveweight of the snails in all treatment groups were taken, feed and water were withdrawn.

The control group was sacrificed immediately at the end of the 2-week adjustment period. The treatment groups were sacrificed after 2, 4 and 6 weeks of aestivation respectively. Chemical analysis of the whole flesh was carried out according to AOAC (1994).

Data generated from the analyses were subjected to analyses of variance (ANOVA) in factorial arrangement (species X duration of aestivation) in a completely randomized design of 5 replicates using the Systat Analytical Computer Package, Version 5.0 (Systat Inc., 1992). Tukey's highest significant difference (HSD) was used to separate the means where significant differences existed.

Results

The results of the analyses of variance on the effects of duration of aestivation on the crude protein (CP), ash (ASH), ether extract (EE), crude fibre (CF), nitrogen free extract (NFE) and dry matter (DM) are presented. The least square means of % CP of *A. marginata* and *A. achatina* as affected by various length of aestivation are presented in Figure 1. The overall average % CP of *A. marginata* was 53.48 ± 1.82% which was not significantly ($P > 0.05$) higher than that of *A. achatina* (49.50 ± 2.03%). The % CP content of *A. marginata* and *A. achatina* that were actively feeding (control) were 50.35 ± 3.11% and 50.01 ± 3.11% which were not significantly different ($P > 0.05$). Duration of aestivation did not have any significant ($P > 0.05$) change in the CP in both species.

Table 1: Effects of duration of aestivation on the chemical compositions of *A. marginata* and *A. achatina*

Parameter	**Species**	**Length of Aestivation (Weeks)**			
		0	**2**	**4**	**6**
Dry matter (%)	*A. marginata*	92.61 ± 1.26	91.02 ± 1.26	91.54 ± 1.26	91.68 ± 1.99
	A. achatina	89.83 ± 1.26	91.29 ± 1.26	90.11 ± 1.62	92.27 ± 1.99
Ash (%)	*A. marginata*	8.06 ± 1.04	5.90 ± 1.04	5.46 ± 1.04	5.28 ± 1.64
	A. achatina	6.00 ± 1.04	4.10 ± 1.34	5.52 ± 1.34	5.07 ± 1.64
Crude fibre (%)	*A. marginata*	1.20 ± 0.10^{a}	0.78 ± 0.10^{b}	0.70 ± 0.10^{b}	0.66 ± 0.16^{b}
	A. achatina	1.03 ± 0.10^{a}	0.80 ± 0.13^{ab}	0.72 ± 0.13^{b}	0.72 ± 0.16^{b}
NFE (%)	*A. marginata*	18.94 ± 3.89	19.09 ± 3.89	18.33 ± 3.89	18.76 ± 6.15
	A. achatina	16.46 ± 3.89	23.53 ± 5.03	13.49 ± 5.03	17.18 ± 6.15

Values are least square means (± sem), n = 5.

[ab] Means with different superscripts within the same parametric row differ significantly ($P < 0.05$)

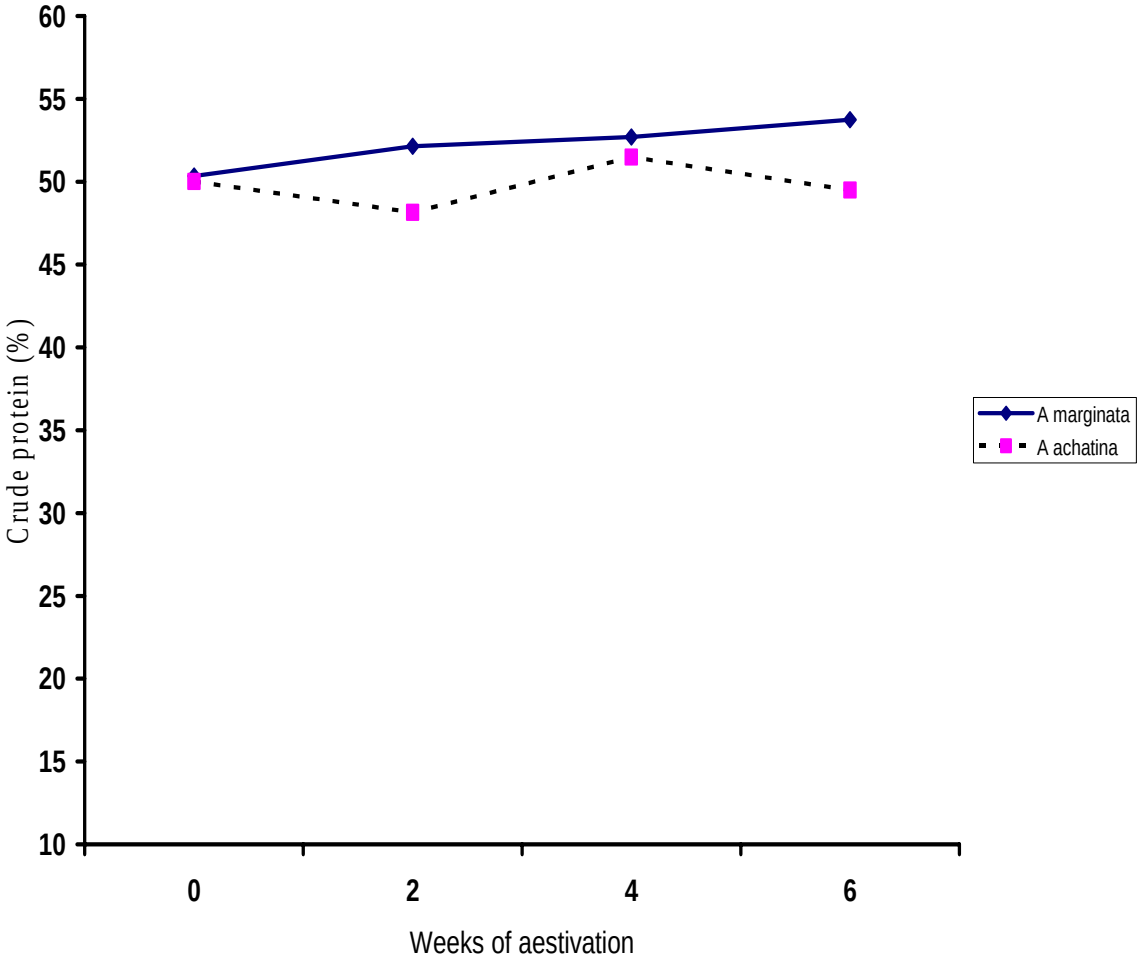

Figure 1. Effects of length of aestivation on the % crude protein content of *A. marginata* and *A. achatina*

Active feeding *A. marginata* and *A. achatina* had 8.06 ± 1.04% and 6.00 ± 1.04% ash content. There was no species difference ($P > 0.05$) and interaction was not statistically significant ($P > 0.05$). Increasing length of duration of aestivation caused a progressive decrease in the ash content of *A. marginata*. The ash content had reduced by 26.61% from active feeding (control) *A. marginata* by the 6th week of aestivation. These were not however significantly different ($P > 0.05$). The values of % ash content of *A. achatina* also showed a decreasing trend. They were also not significantly ($P > 0.05$) lower than the active feeding snails.

The % ether extract (EE) based on the duration of aestivation of *A. marginata* and *A. achatina* is presented in Figure 2 as least square means. Analysis indicated that both species maintained constancy of fat over the measured period of first 4 weeks. Compared to control snails, EE was significantly lower ($P < 0.05$) in snails aestivated for 6 weeks. While the % EE of *A. marginata* decreased by 39.9% of the awake snails in 6 weeks, *A. achatina* only retained 52.62% of the original EE of the awake snails after 6 weeks of dormancy. There was no significant difference ($P > 0.05$) between the species.

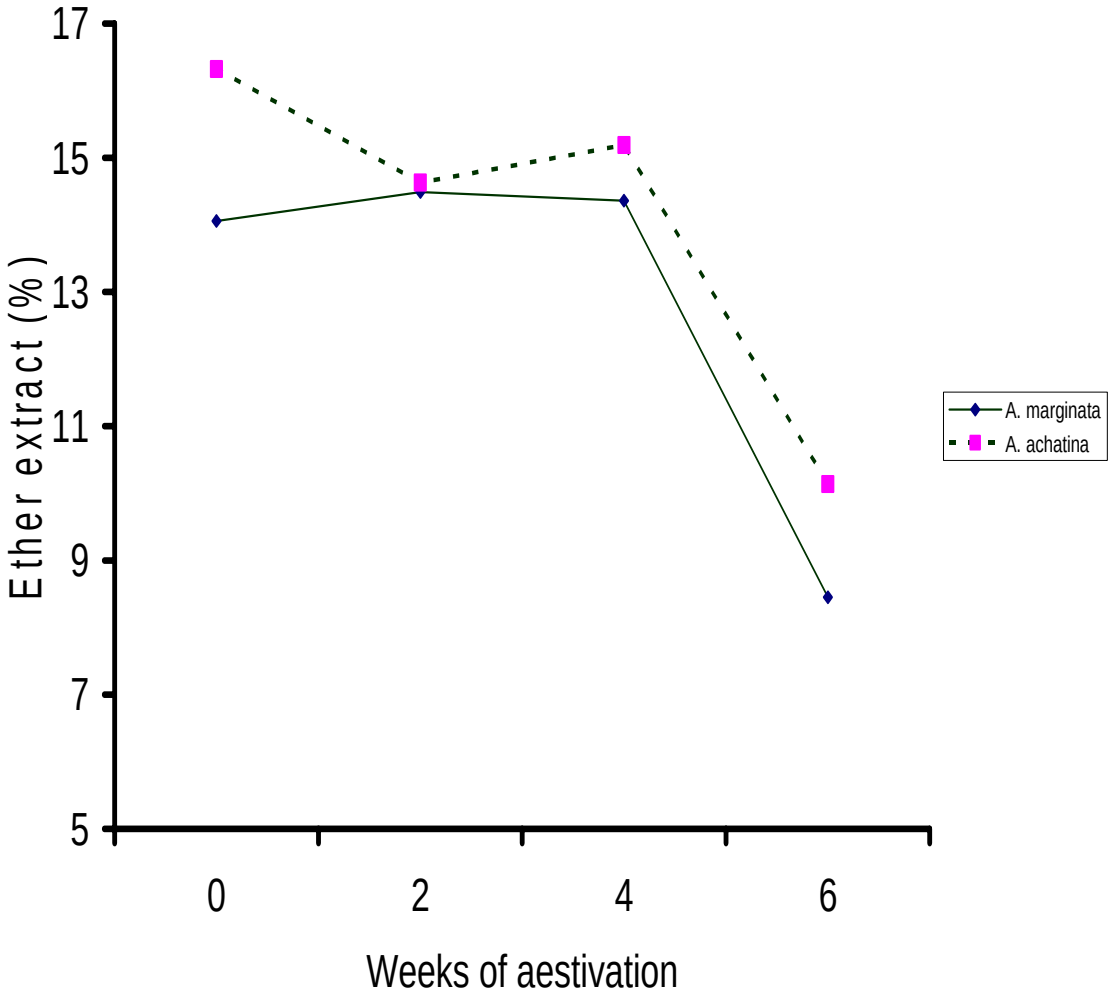

Figure 2. Effects of duration of aestivation on the ether extract (%) of the giant African land snails

CF was significantly depressed ($P < 0.05$) after 2 weeks of aestivation of *A. marginata*. Over 45% of the control CF was lost at the end of the 6th week of aestivation showing a highly significant ($P < 0.01$) lower % composition than the control. The CF content of the active feeding *A. marginata* was higher than that of *A. achatina* but were not significantly different ($P > 0.05$). Amongst the *A. achatina*, active feeding snails (control) showed only a significantly ($P > 0.05$) higher content than the component of the aestivating snails at the 4th week of aestivation. The values also indicated that *A. marginata* had higher rate of loss of CF than *A. achatina* as only about 16.5% of the CF was lost at the 6th week of aestivation as compared to over 45% loss recorded in *A. marginata*. There were however no significant differences ($P > 0.05$) amongst the aestivating snails.

The results showed that aestivation within the duration of 6 weeks did not have any significant ($P > 0.05$) change in the NFE of both *A. marginata* and *A. achatina*. The values did not also differ significantly ($P > 0.05$) between the species.

Discussion

The study presented here fundamentally shows that the crude protein of *A. marginata* is similar to that of *A. achatina*. Several conflicting results have been presented by several authors on the crude protein content of snail meat (edible portion). Hamzat *et al.* (2002) gave crude protein content of snail meat as 42.14 %. This was far below the earlier report of Imevbore and Ademosun (1988) whose range was between 82 –93 % and 72 – 88% recorded by Adegbite *et al.* (2006). In this study however, the crude protein of the actively feeding snails averaged 50.35 ± 3.29 % and 50.01 ± 3.29 % for *A. marginata* and *A. achatina* respectively. The low crude protein content recorded in this case may not be unconnected with the fact that the analysis was carried out on the whole flesh rather than the edible portion.

In both species, snails which entered extended periods of aestivation showed no significant variation in the protein composition of the flesh. Aestivation is known to be associated with depression of basal metabolic rate which helps the snails to survive for the extended periods on the stored fuel (Guppy and Withers, 1999). Protein synthesis is a major contributor to cell energy expenditure in a variety of organisms, tissues and cell types and typically accounts for 18 – 26 % of the total ATP turnover (Hawkins, 1991). Protein synthesis, if maintained at a similar rate during aestivation, would become an impossibly costly process in terms of this contribution to total energy consumption. It would therefore be expected that this major energy – consuming process is substantially down regulated which may be the reason why there was little change in the crude protein content.

The ash content of any material approximates the mineral component (Olomu, 1995). Church and Pond (1988) however cautioned that excessive or extreme values may be a result of contamination of sand. Since snails feed on soil, higher values at control may be due to the combine effect of the entire mineral content and sand present in the gut. The parallel decrease of the ash content may be an indication of utilization of the calcium and phosphorus which form the bulk of the minerals in snails for the formation and replacement of epiphragm and partly the voidance of the gut of sand during aestivation. Although these values are not significant absolute values may likely give incredible results.

The actively feeding *A. marginata* and *A. achatina* flesh contained as high as 14.05 ± 1.42 % and 16.32 ± 1.42 % of lipid respectively as against a trace value of 1.64 ± 0.16 % reported by Imevbore and Ademosun (1988) when they analyzed the edible portion of *A. marginata*. Mead (1961) also reported ether solutes (fat) of the foot and mantle of *A. fulica* to be about 0.75 % confirming the values quoted by the earlier authors. A test in this study in which the dry flesh deeply stained the filter papers debunks low fat content in the flesh. However, the possibility of fat storage in the visceral mass may not be completely ruled out. Moreover, there was a general tendency for fat content to remain constant as the snails entered into aestivation within the first 4 weeks. This was not however regular in *A. achatina* with depressed value at the second week of dormancy. This is likely due to inability of *A. achatina* to form epiphragm or completely enter into dormancy soon after the environment became unfavourable leaving the snails in a starve state and probably utilizing reserved energy.

The relatively stable lipid content recorded within the first 4 weeks of aestivation possibly resulted from the ability of the snails to dramatically reduce metabolic rates and enter a state of dormancy when environmental conditions are unfavourable. In such state, Gabriella and Hermes – Lima (2003) had observed that there was an overall decrease in the utilization of energy reserves and strong inhibition of anabolic routes. The losses observed in both species after the 4th week of aestivation agreed with the findings of Streit (1978) that during starvation in the fresh – water limpet *Ancylus fluviatilis*, lipid reserves are diminished first, followed by the carbohydrate fractions.

The percent crude fibre reported among the control groups may account for a significant proportion of the crude fibre component of the feed consumed during the active state. The significant decrease in the content that occurred between the actively feeding and aestivating snails within 2 weeks and subsequent reductions obviously shows that the GIT was being devoid of food materials during the period of dormancy. This assumption is similar to the observation of Odaibo (1997) that the alimentary canal may be empty of food during aestivation or starvation.

During the 6-week dormancy period, the nitrogen free extract which represents principally the carbohydrate fraction, showed no significant change. Several authors have observed that within a few days of aestivation, metabolic rate drops to 5 – 40 % of normal rates (Guppy and Withers, 1999; Bishop and Brand, 2000), phosphorylation of key regulatory enzymes occurs, as does binding of glycolytic enzymes to particulate matter (Storey and Storey, 1990; Brooks and Storey, 1997; Storey, 2002) and only enzymes

relevant to the maintenance of animal life would show increased activity (through biosynthesis) during aestivation (Gabriella and Hermes-Lima, 2003).

On the other hand, Cedeno-Leon (1984) demonstrated in *Pomacea urceus* that after the consumption of 50 % glycogen in the hepatopancreas and foot during the first month of aestivation, the level in the hepatopancreas tended to stabilize, although in the foot it decreased continuously up to almost 80 % after three months. Da Silva and Zancan (1994) however observed that the hepatopancreas, mantle and muscle glycogen levels and haemolymph glucose levels of *Megalobulimus oblongus* were significantly lower during winter months than during the other seasons. Inconsistent with the above findings, the result in this study revealed that the NFE of dormant snails remained stable through the 6 weeks of dormancy

During the dry harmattan period, snails are generally inactive (Cobbinah, 1990) and this appears to apply not only to snails in their natural habitat but also those kept in captivity. In the present study, the attempts made to disrupt this cyclic biological mechanism through application of water to arouse snails during dry harmattan period or withdrawal of feed and water during the cold wet season to induce aestivation produced reactions which confirm that aestivation and arousal are programmed phenomena in snails.

The general reduction in activity and feeding by snails in experiments moistened regularly over the period of low relative humidity however tend to suggest that this continued activity may be "forced" reaction of the snails in the face of prevailing climatic conditions which would normally have dictated otherwise. Similarly, the onset of appreciable rainfall and consequent raising of relative humidity in this study tended to arouse and increase activities of snails under aestivation studies. This support the view of Awesu (1980) that these factors play the most important part in restoring aestivating snails to normal activity. It would appear however, that only regular high moisture that has a lasting effect on the relative humidity of the air may be beneficial to snails. High environmental humidity, it was observed, induced snail movement, generally encouraged the snails to climb the sides of their baskets even when the culture medium remained dried. Hodasi (1982) observed that such rain did not help matters, but rather had an adverse effect. In the studies, high snail mortalities were recorded at such periods. This might be because aestivating snails were environmentally induced to spend energy in dissolving and reforming the epiphragm, increase movement and other muscular activities as well as resumption of normal respiratory and cardiac activities which were fuelled still by the reserved energy.

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