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Modification of some biochemical activities in response to transition of giant african land snails, *Archachatina marginata* and *Achatina achatina* from aestivation to an active state

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Abstract

The role of biochemical activities in the mechanisms that might control the transition of the giant African land snails, A. marginata and A. achatina, from aestivation to arousal were investigated in the study. Twenty snails each of the species were divided into four groups each. The experiment was conducted in a completely randomized design laid out in a species x week factorial arrangement. All groups were aestivated for 6 weeks. The control group was slaughtered at the end of the aestivation period, whereas the other groups were moistened and fed. These were slaughtered 2, 4 and 6 weeks after arousal. The results showed that no significant species differences ($P > 0.05$) were observed in chemical composition during post-aestivation. The crude protein significantly ($P < 0.05$) increases over the first 4 weeks and then recovered slowly towards the control values. The increase and recovery of crude protein reflect the rate of upgrading of protein synthesis and an increase in the tissue nutrient levels stimulated by arousal and active feeding. Rehydration and feeding triggered increase in the crude fibre content of both snails at the second week which were though, not significantly ($P > 0.05$) higher than the aestivated snails (control). This however, resulted in significant ($P < 0.05$) decrease of ether extract with increase length of post-aestivation period in both species. It is reasonable to conclude that the changes in protein synthesis and lipid utilization may account for a significant proportion of the mechanism that favours the transition.

Keywords: Composition biochemical transition aestivation rehydration active

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INTRODUCTION

Seasonally arid region of the earth support fauna that are only active (reproducing, eating) during the rainy season but the then retreat into a dormant state, called aestivation, to endure long period of prolonged dryness.numerous behavioural, biochemical and physiological adaptations support long term aestivation [1- 3] adaptations that aid water retention within the body or increase dehydration tolerance are very crucial [4]. Also important is a profound metabolic rate depression typically to 30 % or lower of the corresponding resting rate of awake individuals, that allows aestivating animals to greatly extend the time that a fixed reserved of metabolic fuels can support survival [2]. For example, many species of polmunate land snails (such as *Otala lacteal*, *Helix pomatia* and *Helix aspersa*) can aestivate for many months or even years, yet aroused within minutes when environmental humidity soars [5,6].

Under dry conditions, land snails withdraws into their shells, closing the entrance with a calcified mucous membrane called a epiphrgm to minimize evaporative water loss [4]. Oxygen consumption is reduced to 10-30 % of awakeresting level [5] and overall metabolic rate, measured by heat production, is similarly depressed.

Our previous study showed that dormancy is maintained by both *A. marginata* and *A. achatina*, apparently fueled by the utilization of stored lipid reserve, downregulation of protein synthesis and adjustments of some other chemical components [7].

In view of the above considerations, the objective of the present study was to extend the investigation of chemical adjustment through the transition fron the dormant state to the active feeding state and its contribution to the compensatory growth of 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 [8] 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 150-200 g liveweight, 40 well ventilated plastic basket cages of 40 cm by 25 cm by 20 cm with cover, 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 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 were 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) 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.

At the end of the aestivation period, the control group (6 weeks aestivation) was slaughtered. The soils of others were moistened, feed and water were provided to awake and activate out of aestivation. Weekly weights were recorded while snails in the respective treatments were slaughtered 2, 4 and 6 weeks post-aestivation. Chemical analysis [9] was carried out on the flesh. Data generated from the analyses were subjected to analyses of variance (ANOVA) in factorial arrangement (species X duration of post-aestivation) in a completely randomized design of 5 replicates using the Systat Analytical Computer Package, Version 5.0 [10]. Tukey's highest significant difference (HSD) was used to separate the means where significant differences existed.

RESULTS

The summary of the analyses of variance of the effects of length of post-aestivation on the chemical composition of *A. marginata* and *A. achatina* is presented in Table 1. The table shows the least square means of the dry matter basis (%) on which the chemical compositions were determined.

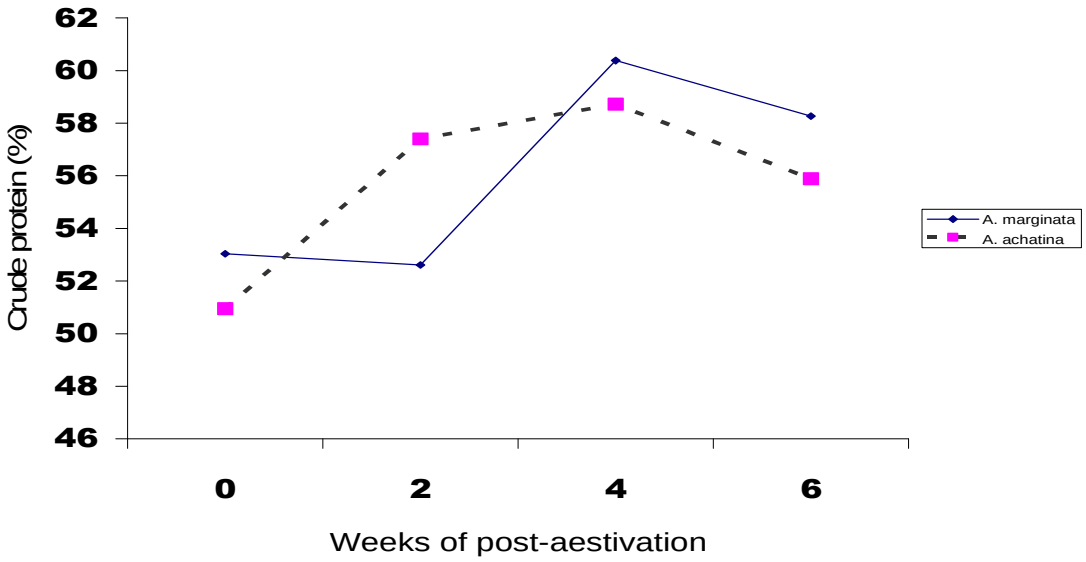

Figure 1. Effects of duration of post-aestivation on the crude protein of the giant African land snails

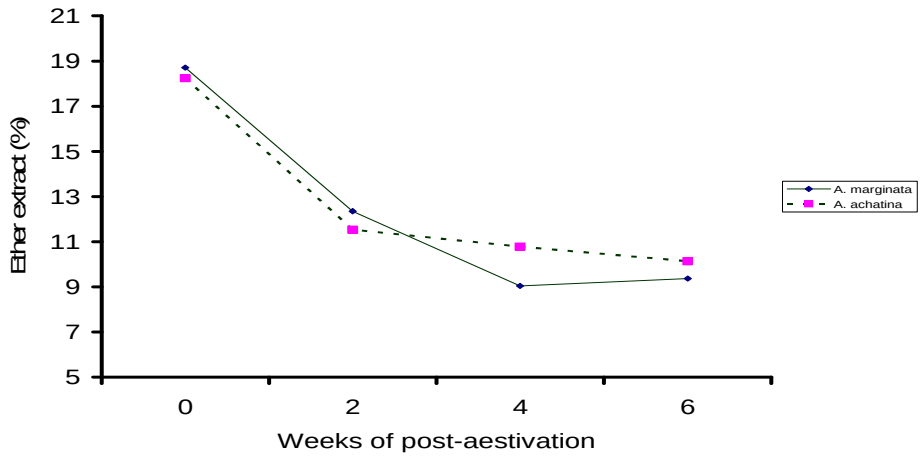

Figure 2. Effects of duration of post-aestivation on the ether extract of the giant African land snails

The effects of length of post-aestivation on the CP (%) of *A. marginata* and *A. achatina* are presented as least square means in Figure 1. CP significantly ($P < 0.05$) increased by 11.2 and 8.6% of the CP composition of the aestivated snails by the 4^{th} and 6^{th} weeks of post-aestivation in *A. marginata* while *A. achatina* had the CP increased significantly ($P < 0.05$) by 12.6, 15.3 and 9.7% at 2, 4 and 6 weeks of arousal. There was no significant difference ($P > 0.05$) between the overall averages CP of the species.

The ash content of *A. marginata* and *A. achatina* as influenced by duration of arousal from dormancy are also summarized in Table 1 as least square means. The overall average of *A. marginata* (95%) and *A. achatina* (92%) over 6 weeks of post-aestivation were not significantly ($P > 0.05$) different. Rehydration and feeding triggered an elevation of ash content in both species. The ash contents of the actively feeding snails were significantly larger ($P < 0.05$) than their respective aestivated values. After 2 weeks of significant increase in ash content, both species showed a tendency towards decrease as they were continuously fed. However, these changes were not significant ($P > 0.05$) except at 6th week of post-aestivation in *A. marginata* which significantly depressed ($P < 0.05$) below other post-dormancy values.

The least square means of the effects of length of post-aestivation on the ether extract (%) of *A. marginata* and *A. achatina* are presented in Figure 2. After 6 weeks of dormancy, *A. marginata* and *A. achatina* had 18.71 ± 1.79% and 18.24 ± 1.79% respectively which were not statistically different ($P < 0.05$). Rehydration and feeding resulted in significant ($P > 0.05$) decrease of EE with the duration of post-aestivation in both species.

Table 1. Effects of length of post-aestivation on the chemical composition of *A. marginata* and *A. achatina*

Parameter	**Species**	**Length of post-aestivation (Weeks)**			
		0	**2**	**4**	**6**
Dry matter (%)	*A. marginata*	95.62 ± 0.80	91.94 ± 0.90	92.68 ± 1.04	91.82 ± 0.80
	A. achatina	91.56 ± 0.80	92.01 ± 1.04	91.06 ± 0.90	91.12 ± 0.90
Ash (%)	*A. marginata*	5.93 ± 1.04^{c}	12.35 ± 1.16^{a}	10.88 ± 1.34^{a}	8.81 ± 1.04^{b}
	A. achatina	6.00 ± 1.34^{c}	11.05 ± 1.34^{a}	9.51 ± 1.16^{ab}	10.34 ± 1.16^{a}
Crude fibre (%)	*A. marginata*	3.45 ± 0.39^{a}	5.56 ± 0.43^{a}	3.33 ± 0.50^{b}	3.29 ± 0.39^{b}
	A. achatina	3.62 ± 0.39^{b}	4.61 ± 0.50^{ab}	3.30 ± 0.43^{b}	3.77 ± 0.43^{b}
NFE (%)	*A. marginata*	10.93 ± 1.66	9.63 ± 1.86	7.42 ± 2.14	12.10 ± 1.66
	A. achatina	12.74 ± 1.66	7.41 ± 2.14	8.75 ± 1.86	7.15 ± 1.86

Values are least square means (± sem), n = 5
^{abc} Means with different superscripts within the same parametric row differ significantly

Rehydration and feeding triggered increase in the crude fibre (CF) content of both snails at the second week which were however, not significantly ($P > 0.05$) higher than the aestivated snails (control). Subsequent feeding did not significantly affect the CF composition of both *A. marginata* and *A. achatina*.

The effects of length of post-aestivation on the nitrogen free extract of *A. marginata* and *A. achatina* are also presented as least square means in the table. Rehydration and feeding did not significantly affect the NFE content in both species. NFE depressed inconsistently with increasing length of post-aestivation which did not show any significant difference ($P > 0.05$) among treatments and between species.

DISCUSSION AND CONCLUSION

The increase in % protein in both species post-dormancy may have suggested an upregulation of protein synthesis. This may also be due to an increase in the tissue nutrient level triggered by active feeding. Hawkins [11] identified protein synthesis as a major contributor to cell energy expenditure in tissues and cells and typically accounts for 18 – 26 % of the total ATP turnover. It may therefore become economical to embark on excessive protein synthesis soon at arousal. However, the fact that the protein level shot up and above the pre-dormancy level presented in this experiment indicates a possibility of physiological and biochemical benefits of aestivation on a long term. Further studies therefore, on protein synthesis before, during and after metabolic depression will need to focus on regulatory mechanism and perhaps investigating changes in the specific organs and tissues in each of the translational machinery.

Omoyakhi, *et al*. [7] in an earlier submission indicated that the ash content of the whole snail may likely be divided into two fractions: the basic mineral component and the sand fraction contributed by the feeding on soil. The sharp rise in the ash content of the snails at the second week of active feeding indicates that both may have a contributory effect.

According to Odaibo [12], *Achatinidae* are herbivorous and feed on a wide variety of plants. The snails have remarkable ability for converting dead and decaying plant into highly nutritious flesh. Therefore, crude fibre naturally forms an indispensable component of the snail feed. The increase or rise in the content of crude fibre in this study post-dormancy therefore suggested the feeding activities of the snails.

The lipid content significantly dropped from the control to the aroused groups in both *A. marginata* and *A. achatina*. This is contrary to the earlier observation in which the percentage ether extract decreased significantly at the 6^{th} week of dormancy [7]. Supporting the report in this experiment however, Wieser [13] and Churchill & Storey [14] noted that the main end products that accumulate in the tissues and haemolymph of land snails during dormancy are D-lactate and succinate. During prolonged dormancy, pyruvate is directed into fatty acid biosynthesis, resulting in an increased in fatty acid levels used for lipid synthesis. The sudden dropped at the second week of arousal in this experiment therefore, may not be unconnected to the resumption of normal breathing. Herreid [5] and Hermes-Lima & Storey [15] observed that at arousal, oxygen concentration rises and stabilizes in the tissues and oxygen consumption increases rapidly to peak at levels at least 2-fold higher than the pre-dormant state values and

about 6-fold higher than consumption in the dormant state in *O. lacteal*. It is obvious therefore, that these physiological and biochemical changes would result in excessive demand of energy substrates. In all the actively feeding groups, the lipid contents were similar showing no significant changes. This was probably as a result of the stabilization in the utilization of energy and replenishment from feed.

As noted in the previous experiment, there was also no significant change in the nitrogen free extract which represents the bulk of the carbohydrate when hydrated and fed. There was no appreciable or consistent effect to the utilization or recovery of this energy source in both cases. Earlier observations in *O. lacteal*, for example, showed that there are coordinated changes in the phosphorylation state of both glycolytic (phosphofructokinase, pyruvate kinase) and tricarboxylic acid cycle (pyruvate dehydrogenase) enzymes which act to suppress net carbohydrate oxidation during dormancy [16-18]. These biochemical adjustments may partly be responsible for the stability of NFE recorded during the study.

It is reasonable to conclude therefore from our studies that protein biosynthesis appears to be arrested during aestivation but triggers soon after arousal. This change in protein synthesis may account for a significant proportion of the mechanism that favours the adaptation. Moreover, the reserved energy utilization during aestivation seems to come from the lipid component.

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