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Potentials of Watermelon (*Citrullus lanatus*: (Thunb.) Matsum, & Nakai) Extract as an Appetite Stimulant and Antioxidant in the Giant African Land Snail (*Archachatina marginata*: Swainson, 1821)

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ABSTRACT

The growth and development of *Archachatina marginata* are relatively slow but expected to improve given the most preferred feed materials, and appetite stimulants are provided at optimal periods. This study aims to assess *Citrullus lanatus* extract's potential as an appetite stimulant and an antioxidant in *A. marginata*. Growth parameters such as specific growth rate (SGR) were used as indices for measuring *A. marginata* growth. Morphometric parameters were taken, and biochemical markers such as total superoxide dismutase (SOD) activity, Catalase (CAT), was determined. A total of 30 juvenile *A. marginata* (124.18 ± 35 g) was procured for the study, carried out over four weeks, with a week of prior acclimatization. Five (5) experimental treatments, comprising six (6) *A. marginata* each, were gavaged with diets containing various concentrations of *C. lanatus* extracts. Results indicated that *C. lanatus* extract improved growth in snails with a significant regression of SGR ($F(2,12) = 0.06$, $p = 0.03$, $R^2 = 0.3495$), and SCI ($F(2,12) = 0.007$, $p = 0.048$, $R^2 = 0.4984$) recorded on extract concentration. Biochemical markers activity showed downward trends relative to increasing *C. lanatus* extract concentration. This indicates that *C. lanatus* extract reduced antioxidant activity and consequently oxidative stress in *A. marginata* and that it is possibly capable of augmenting feeding and subsequent growth in *A. marginata*. Hence, the incorporation of *C. lanatus* extract of concentrations ≥ 0.338 ml/g into snail feed to improve snail growth and productivity is therefore recommended.

Keywords: *Archachatina marginata*, *Citrullus lanatus*, Markers, Oxidative stress, Biochemical, Morphometrics.

Introduction

Archachatina marginata, commonly known as the 'Giant African Land Snail,' is a snail species that is most accepted and cherished in Nigeria. It is a species of air-breathing tropical land snails belonging to the Phylum: Mollusca, Family: Achatinidae, Class: Gastropoda, and Subclass: Pulmonata. It is one of the predominant snail species in West Africa and Central Africa, and other species include *Achatina achatina* and *Achatina fulica.*[2] Snails, especially land snails, are mostly edible and nutritious - hence they have an excellent farming prospect. The rearing and breeding of snails are known as heliciculture, heliculture, or snail farming.

According to Iyangbe and Orewa,[5] an estimated 31% of the nation's population met the minimum daily calorie intake recommended by the Food and Agriculture Organisation (FAO), while the more significant portion of the population was considered malnourished. Moreover, Nigeria's per capita daily intake, for instance, is estimated to be 45.4g, as against the 53.8g recommended by the FAO.[5] This incited the need to diversify the current farming system in order to complement the conventional animal sources of protein,[4] and an abundance supply of snail may result in affordability of animal protein in addition to other nutritional and health benefits it affords.[5]

Thus, there is an increasing tilt towards the rearing of micro livestock such as rabbits and snails, particularly within suburban and some urban communities, for subsistence and small-scale commercial purposes - this is the case mainly because of the limited resources, in terms of space, housing, feed, and care required to manage small-scale micro livestock.

Challenges associated with large-scale commercial heliculture in developing countries such as Nigeria include; inadequate funding, vulnerability to predators and pests, infections and diseases, availability of adequate soil and land, absence of proper veterinary services, the problem of poachers.[6] Heliciculture is also affected by cultural and religious beliefs against its consumption. The growth and development of the land snail are relatively slow and may take up to a year to reach marketable size - this is partly due to the feeding patterns, nocturnal nature, and narrow preferences for climatic conditions, humidity, and temperature.[7] The relatively slow growth rate and low market demand recorded in heliciculture compared with aquaculture and poultry in Nigeria is a fundamental challenge.[6]

A. marginata, a primarily herbivorous organism, also subsists on dead plants and animal material (coprophagy) and may eat up members of its species when weak or dead (cannibalism) under certain conditions.[8] As reported by Ogbu *et al*.[9] field and laboratory investigations have revealed that, although snails show a wide variety of food preference spectrum, they typically demonstrate selective feeding, showing preferences to certain feeds over others (orange, ripe cherries, and Curcubits – watermelon (*Citrullus lanatus)* and cucumber (*Cucumis sativus*). Similarly, Cobbinah *et al.*[7] reported that Giant African land

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snails are naturally predisposed to consuming appetizing foods (such as pawpaw) and feed mixtures containing feeding or appetite stimulants. Hence, growth and feeding performance in *Archachatina marginata* is expected to improve given the most preferred feed materials are provided at critical periods of developmental stages.

Citrullus lanatus possesses a characteristic pleasant aroma with a refreshing taste.[10] It is also reported to contain various antioxidants such as lycopene, beta-carotene, and ascorbic acid,[11] which are capable of inhibiting free radicals activities within the body. These free radicals are formed as crucial intermediates in a variety of biochemical reactions. However, when generated in excess or uncontrolled (a condition known as oxidative stress), they result in oxidative damage to proteins, lipids, and other macromolecules that form a relatively high component of the body tissues.[12] Various studies on *A. marginata* have focused on the effects of different feeds (fruit peels, mango, pawpaw), diet plans, environmental conditions on the growth performance and meat quality.[13-17] However, this study is essentially unique in that it aims at assessing the potential of *C. lanatus* extract as an appetite stimulant and an antioxidant in *A. marginata*.

Materials and Methods

Experimental Conditions and Design

Thirty juvenile *Archachatina marginata* (124.18 ± 35 g) were procured from an intensive free-range snail pen in Ota, Ogun State, Nigeria, for the study, which was carried out for 35 days (5 weeks). Prior to recruitment, the snails were inspected for research fitness, and this was conducted based on broken apexes, observable retractions, infections, and general weight.

Recruited snails were washed with distilled water, labeled, and held in a wooden cage demarcated into five compartments. Each compartment (20 x 30 x 30 cm) had a stocking density of: 6 snails/ 60 cm^2

6 snails * 124.18 g (mean weight) = 745.08

745.08 ÷ 60 cm^2 (surface area) = 12.42 g/cm^2

Stocking density = 12.42 g/cm^2

A 14-day acclimation was conducted during which experimental conditions were moderated. The regulated conditions include: a pH range of 6-8, a mean recorded temperature of 23 ± 1 ^oC, a low light intensity (shaded to simulate tropical rainforest), and an average relative humidity (RH) of 85%.[7]

A makeshift humidifier in the form of soaked polyurethane foam was employed in providing the humid environment. The cage was kept within a dark room, providing the required low light intensity, which is known to induce increased snail activity and, consequently, its feeding. Snails were labeled and randomly assigned to five diets as treatments; T1 (control), T2, T3, T4, and T5.

Snails within each treatment group were fed on feed formulated with varied watermelon extract concentrates, with the assigned concentration remaining unchanged throughout the experiment - feed mixtures were served using Petri dishes. Water supply was mediated via a wide, transparent plastic cover, and pest control was mediated via an oil lining around the cage bases.

Feed formulation and Concentrate Preparation

Fresh, ripe watermelon purchased from Covenant University farm, Ogun State, Nigeria, were carefully screened for infections and stored at room temperature. The rind (epicarp) was removed, and the flesh (mesocarp and endocarp) was cut into small pieces and processed within an electric blender (Magic Bullet Nutribullet) in the laboratory. The resultant *Citrullus lanatus* extract was sieved with a muslin cloth to remove residue. The filtrate was stored as an extract in -20^o C freezer to prevent fermentation.

Citrullus lanatus extract was mixed with a formulated feed mixture (Table 1) with varied liquid content gradients. The feed was constantly mixed as the extract was added. The addition of extract was discontinued at the point weighed feed became moist and almost wet. The volume of extract used was read, and feed concentrate was calculated using the volume of extract required against the weight of the feed thus:

$$\text{Extract concentration in feed (mL/g)} = \frac{Volume\ of\ extract\ added\ to\ feed}{Weight\ of\ feed}$$

The upper limit concentration was assigned T5, and concentration progressively decreased up to T2 with each initial treatment by a factor of 2, and the control diet T1, which constituted the snail feed mixture and water.

Feeding Regime

Five (5) experimental treatments (Table 2), which comprised six snail individuals each were gavaged with 0.338 ml deionized water/g of feed (T1- control), 0.0845 ml extract/g of feed (T2), 0.169 ml/g of feed (T3), 0.2535 ml/g of feed (T4), and 0.338 ml/g of feed (T5).

Snails were fed daily with treatment diets five times a week and fed on an untreated feed mixture on weekends. The feed intake for a specific day was calculated as thus:

Feed intake (g) = Initial feed weight – Final feed weight.

Values were recorded at 24-hour intervals with an electric weighing balance (Cole Palmer^{TM}) of 220 x 0.0001 g.

After documentation of the final feed weight, the Petri dishes were washed, and the cycle was repeated daily throughout the experimental regime.

Morphometrics

Changes in morphological parameters (morphometrics) were taken at the beginning of each week for four weeks. These include weight (W) measured in grams (g), shell length (SL), shell circumference/width (SC), aperture length (AL), aperture width (AW), and whorl length (WL) measured in centimeters (cm) each. Weights were measured with an analogue scale, while the other body parameters were measured with the aid of a fine piece of white thread, a pencil, and a 30 cm transparent ruler. The increments in each body parameter were calculated by subtracting the length recorded in the previous week from the given week's length. Three increment values were recorded for four weeks for each body parameter.

Tissue collection

Snails were retrieved from the cage. A two-step method for euthanasia employed by Gibertson and Wyatt (2016)[18] was employed: the snail was immersed into 5% ethanol to induce anesthesia, followed by immersion in a 70% ethanol solution to induce euthanasia (painless death). Afterward, they were stripped of their shell and the visceral mass. Snail tissues – muscle tissue of the foot - were minced with a sterile blade and collected in appropriately labeled EDTA bottles. Tissue samples were preserved at -10 ^oC before further analysis.

Assessment of biochemical indicators

Preserved snail tissue (2g wt w) was homogenized in 5 volumes of 25 mmol/L sucrose, containing 10mmol/L Tris-HCl, at a pH of 7.5 and temperature of 4 ^oC, using a homogenizer. The homogenates were sonicated for 30 seconds at 10 KHz on ice.

Total superoxide dismutase (SOD)

The total SOD activity in tissue homogenates was analysed following a modified procedure of Marklund and Marklund (1974)[19] based on the ability of SOD to inhibit the autoxidation of pyrogallol. One unit of SOD activity was defined as the amount of enzyme that can inhibit the auto-oxidation of 50% of the total pyrogallol in the reaction.

Catalase (CAT)

Catalase (CAT) was assayed colorimetrically at 620nm and expressed as moles of hydrogen peroxide (H_2O_2) consumed/min/mg protein as described by Quinlan *et al.* (1994).[20]

Reduced glutathione peroxidase (GSH-Px)

Reduced glutathione peroxidase (GSH-Px) was determined by the method of Ellman (1959)[21]. 10% TCA was added to the homogenate centrifuged. 1.0 ml of the supernatant was treated with 0.5 ml of

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Ellman's reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm.

Malondialdehyde (MDA)

Malondialdehyde (MDA) was considered as an index of lipid peroxidation. This was determined by the method of Buege and Aust (1978)[22]. MDA was calculated within the molar extinction coefficient for the MDA-TBA complex of 1.56 x 10^5 M^{-1} C^{-1}. MDA was calculated thus:

MDA = A x V_T / $\sum$ x V_S; where A =Absorbance, V_T = Total volume, $\sum$ = molar extinction.

To ensure quality control and quality assurance, all enzymes were measured in triplicates, and values were recorded in U/mg/prot.

Evaluation of Growth Performance

The effects of mixed feed on growth performance were determined by evaluating the weekly feed conversion ratio (FCR), specific growth rate (SGR), shell length increment (SLI), shell circumference increment (SCI), aperture length increment (ALI), aperture width increment (AWI), whorl length increment (WLI).

The feed conversion ratio (FCR) was ascertained thus:

$$FCR = \frac{Weight\ gained\ over\ the\ week}{Feed\ intake} \times 100^{23}$$

Specific Growth Rate was calculated thus:

$$SGR = \frac{W2 - W1}{t2 - t1} \times 100^{23}$$

Other values were calculated thus:

SLI, SCI, ALI, AWI, WLI = $l_2 - l_1$.

Where: W_2 is the weight of snail after a period of feed on a diet; W_1 is the weight before feeding regime; $t_2 - t_1$ is the duration in days; l_2 is the final length, and l_1 is the initial length of the given body parameter.

Statistical analysis

Data generated were presented as descriptive statistics subjected to Analysis of Variance (ANOVA) to test for significance. The outcome of ANOVA was further subjected to Duncan's Multiple Range test using SPSS v 25. Differences between the treatment means were considered significant at a 95% confidence level. Correlation and regression analysis was used to determine the relationships between feed intake and snail growth parameters. Scattered diagrams were plotted to determine the regression intercept and slope.

Results and Discussion

Feed Composition

The feed composition (%) is presented in Table 1, while the composition of experimental treatments presented as a volume of watermelon extract (ml) per weight of feed (g) is presented in Table 2.

Spatial and Temporal Variation of Snail Growth

Evaluation of Growth Parameters - Weight, SGR, SCI, SLI, ALI, AWI, and WLI

A steady weight and specific growth rate (SGR) increase over the experimental duration was observed from treatment 2 to treatment 5 and slightly inconsistent shell circumference increment (SCI) among the treatments (Figure. 1; Ai, Bi). The overall regression model of SGR and SCI on *C. lanatus* extract concentration was significant - suggesting that the SGR was impacted by the *C. lanatus* extract concentration gradient at a p-value of 0.05 (Figure. 1 Aii, Cii), but not significant on other growth parameters - SLI, ALI, AWI, and WLI as reflected by Figure. 1; Cii - Fii. However, the overall regression model of all growth parameters (SGR, SCI, SLI, ALI, AWI, and WLI) on feed intake was not significant, which implies that they were not impacted by the amount of feed consumed at a p-value of 0.05 (Figure. 1 Aiii - Fiii).

Also, as shown in Figure 1; B, the highest shell length (SL) growth was in the snails in treatments 5 (T5). The bar chart (Figure. 1; D, E) showed no observable trend in the aperture length in all treatments, with rapid aperture width increment recorded from T2 to T5. However, T4 had the highest increase in whorl length, aperture length, and width (Figure. 1; Di, Ei, and Fi).

Relationship between Extract Concentration and Feed Intake across the Treatments

The overall regression model of feed intake on watermelon extract concentration was not significant, (F (1,13) = 0.585, p = 0.585, R^2 = 0.0235). This connotes that the feed intake was uninfluenced by the watermelon extract concentration gradient at a p-value of 0.05 (Figure. 1G).

Overall, the results of morphometries (Figure. 1) of A. marginata estimated from weeks 1 to 4 showed a cumulative growth in the snails of all treatment groups (T2 to T5) when compared to the control treatment group (T1). The significant regression of SGR (F (2,12) = 0.06, p = 0.03, R^2 = 0.3495) on extract concentration suggests that the odorant receptors in the snails were stimulated by the watermelon extract. This was supported by the report of Ademolu *et al*.[13] that feeding in snails is preceded by the exploration of the diet with their tentacles and lips, indicating a highly dependent on olfactory and gustatory clues in exploring their environment for food. The insignificant regression (F (2,12) = 0.06, p = 0.57, R2= 0.0499) of SGR on feed intake in the snails further buttresses the fact that watermelon extract accounted for the increases recorded in the specific growth rates, similar to that of the SCIs. As reported by Ademolu *et al*.[13] and Okonkwo *et al*.[24] an increased shell growth in snails fed pawpaw leaves were observed in their separate studies; these corroborate this study result in that A. marginata growth, as observed by an increase in morphometric estimations (SGR, SCI, ALI) is directly proportional to the quantity and quality of their feeding.

Responses of SGRs and SCIs of the snails to the feeds formulated with *C. lanatus* extracts against unformulated feed (T1) imply that watermelon extract may serve as a more effectivegrowth enhancer in snails than conventional feed. Conversely, regression analyses on other growth indices suggest otherwise - SLI showed no significant regression on extract concentration and feed intake. Insignificant regression was also recorded in ALI, AWI, and WLI. Although it appears that *C. lanatus* extract conveys no overt effect on the growth of *A. marginata*, such observations could be attributed to the short duration of the experimental period, in conjunction with the relatively slow growth rate of snails.[7] Thus, the relatively short duration of the experimental period might be responsible for the insignificant increases in the growth indices, which might be too small to be detected upon physical observation of the snails. Moreover, increments in the weights, SGR, and shell circumferences could be detected possibly due to their relatively quicker increments as compared to the other growth parameters. This was in concomitant with the report by Medeiros *et al*.,[25] who observed that the growth is mediated through the addition of material from the mantle collar at the shell's opening, nutrient material is continuously added, and growth is therefore first observed at the anterior part of the shell, proceeding onward to the posterior.

ANOVA results revealed significant differences in specific growth rates (SGR), shell length increments (SLI), shell circumference increments (SCI), and aperture width increments (AWI) recorded in all five treatments. Upward trends were observed, as growth parameters steadily increased with a corresponding increase in *C. lanatus* extract concentration. Treatment 5 consistently had significantly higher growth and feed conversion levels than treatments 1 and 2, respectively. This could mean that slight changes in extract concentration, such as between T4 and T5, do not necessarily alter growth, and feeding but a greater increase in extract concentration would undoubtedly alter growth levels and feeding in *A. marginata* and possibly other closely related snail species. The results support the expectation that the volatile organic compounds in the watermelon extract would stimulate snail appetite via the inducement of olfactory cues. This makes *C. lanatus* a potentially viable growth booster, which could be incorporated into conventional snail feed. Increased growth performance was also observed in catfish (*Clarias gariepinus*), as Musa *et al*.[26] observed that catfish fed freshwater rotifers, *Artemia*

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nauplii showed slower specific growth rates than those fed with freshwater rotifers with fish meal and freshwater rotifers with maize bran. Other studies27,28 corroborate enhancement of growth through feeds formulation.

Assessment of Biochemical Markers

Superoxide Dismutase (SOD) Activity

SOD activity in the treatment group of A. marginata showed the trend: T1 (0.3008±0.06) > T2 (0.2423 ± 0.04) > T3 (0.1848 ± 0.05) > T4 (0.119 ± 0.08) > T5 (0.0455 ± 0.05) U/mg/prot (Table 3). SOD levels in all treatments were significantly different from one another (as indicated by different superscript on each value in Table 3).

Catalase (CAT) Activity

Significantly higher ($p < 0.05$) levels of catalase activity was recorded in treatments 1 (2.3625 ± 0.6) and 2 (2.6575 ± 1.06) U/mg/prot. compared to all other experimental treatments of *A. marginata*. CAT levels in T1 and T2 were significantly different from T3 to T5 (Table 3).

Glutathione Peroxidase (GPx) Activity

GPx activity in the treatment groups of A. marginata showed the trend: T1 (0.3165 ± 0.09) > T2 (0.3033 ± 0.08) > T3 (0.17600 ± 0.03) > T4 (0.0618 ± 0.04) > T5 (0.0438 ± 0.0) (U/mg/prot). GPx level in T1 and T2 was significantly different from that in T4 and T5 (Table 3).

Lipid Peroxidation (MDA) Activity

MDA activity in the treatment groups of A. marginata showed the trend: T1 (18.93 ± 4.21) < T2 (19.63 ± 2.44) < T3 (24.41 ± 3.77) < T4 (25.35 ± 2.11) < T5 (26.4 ± 1.09) nmol/ml. MDA levels in T4 and T5 were significantly different from T1 to T3 (Table 3).

Oxidative stress levels observed in T1 and T2 characterized by the antioxidant activities were significantly higher than the levels in treatments with greater concentrations of *C. lanatus* extract (particularly T4 and T5) - this suggests that the extract might have enhanced the natural antioxidant defense system in the snails fed greater extract concentrations. Therefore, 0.2535 mL/g and 0.338 mL/g in T4 and T5, respectively appeared to be the most promising among other treatments. This result, therefore, suggests that *C. lanatus* possesses a high antioxidant potential.

Activities of Catalase (CAT) and Superoxide dismutase (SOD) are the two major indicators of oxidative stress. SOD primarily neutralizes the oxygen radicals by quickening superoxide's dismutation to hydrogen peroxide, which ordinarily destroys biological structures and cell membranes. At the same time, CAT, on the other hand, further degrades the hydrogen peroxide produced by SOD in periods of prolonged stress.29 The observed rise in the CAT level from T5 to T1 may suggest prolonged stress in the snails with lower extract concentrations, particularly those in the control (with no extract). Similarly, the downward trend (from T1 to T5) observed in lipid peroxidation (MDA) activity could be attributed to increased *C. lanatus* extract concentrations, as MDA activity recorded reduced with increased extract concentration in the feed. This, therefore, buttresses the notion that *C. lanatus* extract is capable of preventing cell membrane disruption mediated through lipid peroxidation.

Table 1: Percentage Composition of Snail Feed

Ingredient	Percentage composition (%)
Maize	28.08
Soybean meal	17.55
Fish meal	3.51
Blood meal	3.51
Ground oyster shell	5.62
Bone mix	3.51
Palm kernel cake	14.04
Groundnut cake	7.02
Ground snail shell	14.04
Noodle mix	2.81
Methionine	0.07
Lysine	0.07
Animal pre-mix	0.17

Table 2: Composition of Experimental Treatments

Treatments	Concentration
1	0.338
2	0.0845
3	0.169
4	0.2535
5	0.338

Table 3: Mean antioxidant concentrations recorded in all treatments

Treatments	T1	T2	T3	T4	T5
SOD (U/mg/prot)	0.3008 ± 0.06^{a}	0.2423 ± 0.04^{ab}	0.1848 ± 0.05^{bc}	0.119 ± 0.08^{cd}	0.0455 ± 0.05^{d}
CAT (U/mg/prot)	2.3625 ± 0.6^{a}	2.6575 ± 1.06^{a}	1 ± 0.91^{b}	0.29 ± 0.12^{b}	0.1755 ± 0.11^{b}
GPx (U/mg/prot)	0.3165 ± 0.09^{a}	0.3033 ± 0.08^{a}	0.17600 ± 0.03^{b}	0.0618 ± 0.04^{c}	0.0438 ± 0.0^{c}
MDA (nmol/ml)	26.4 ± 1.09^{b}	25.35 ± 2.11^{b}	24.41 ± 3.77^{b}	19.63 ± 2.44^{a}	18.93 ± 4.21^{a}

Mean (± SD) values followed by different superscript letters are significantly ($P \leq 0.05$) different from each other, ascertained using the DMR test.

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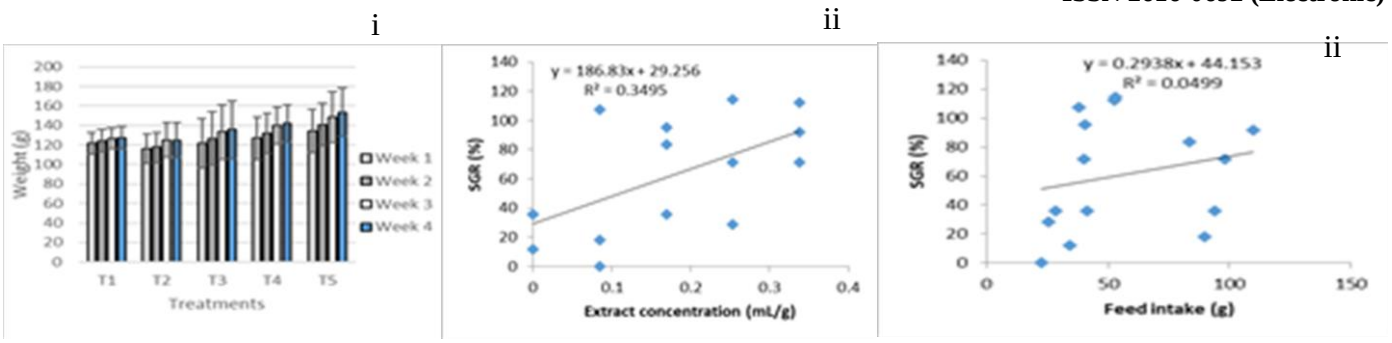
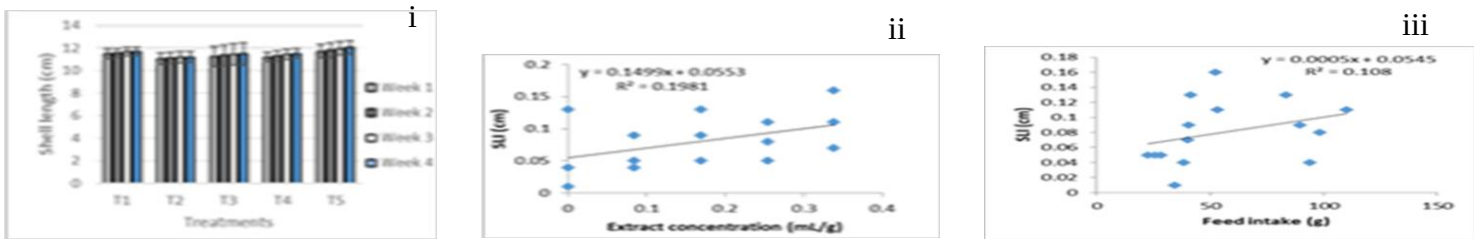
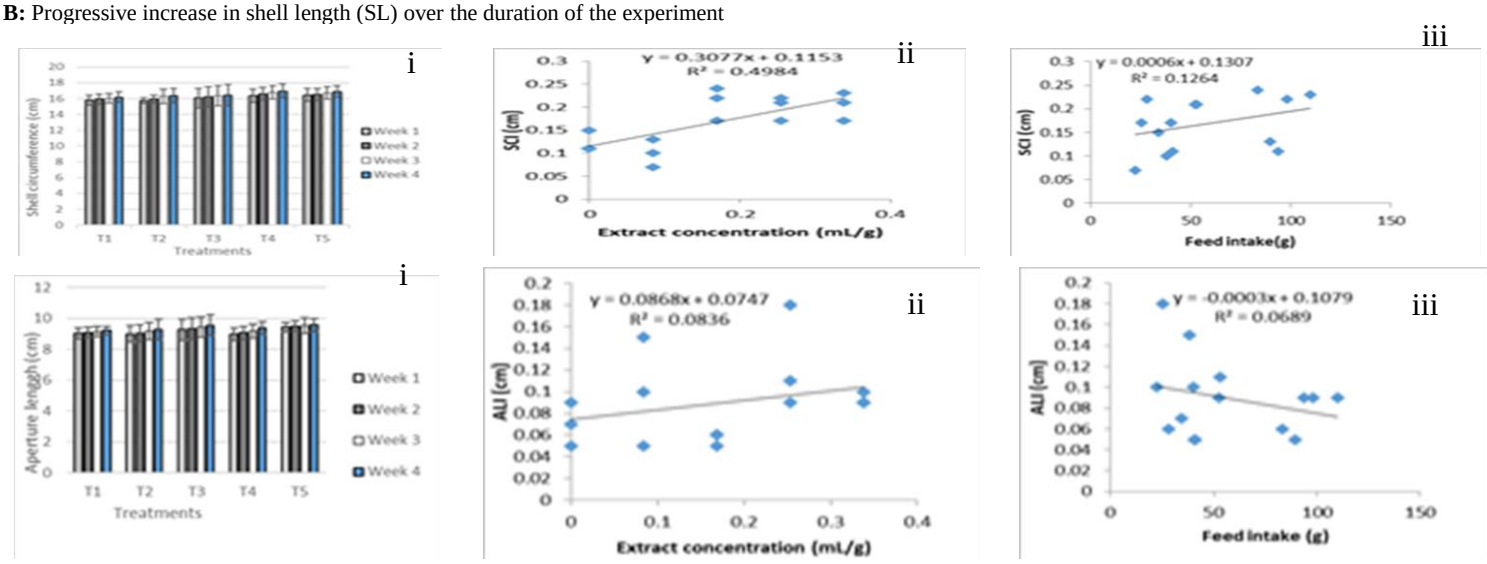


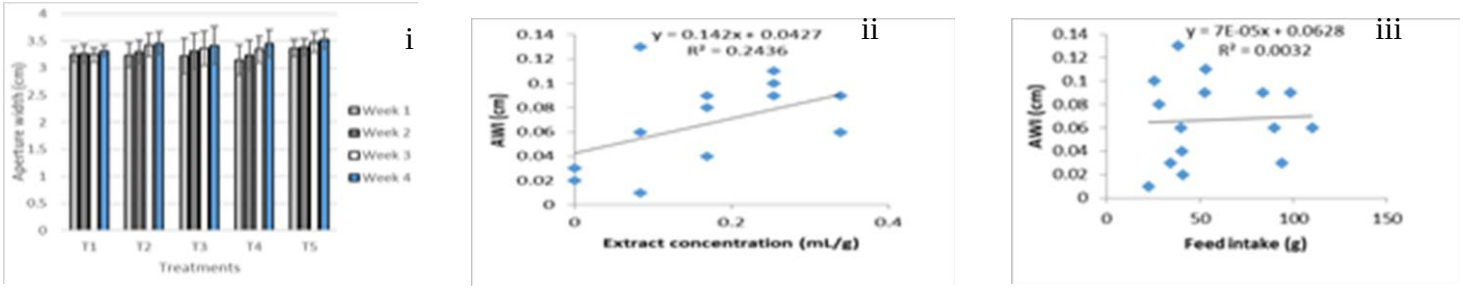
A: Increase in snail weights and specific growth rate (SGR) throughout the experiment.



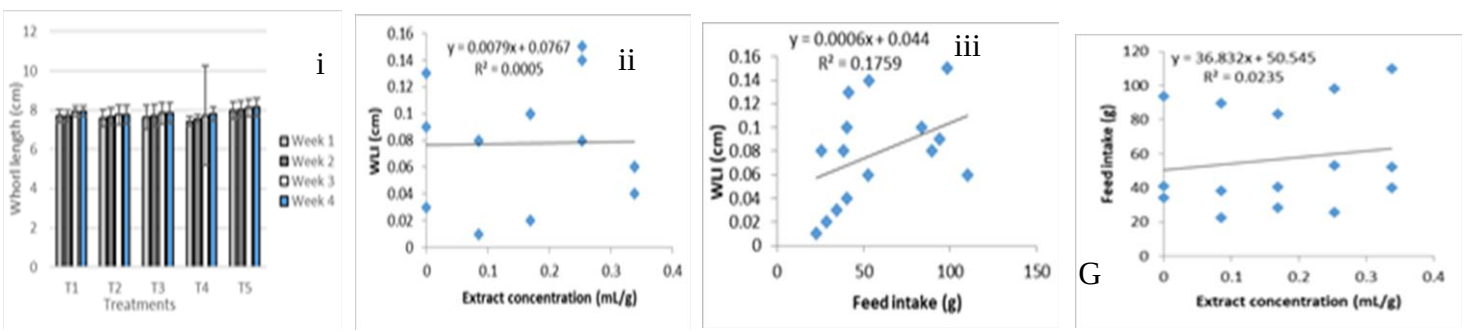
B: Progressive increase in shell length (SL) over the duration of the experiment



D: The progressive increase in aperture length (AL) for the experiment.



E: The progressive increase in aperture width (AW) throughout the experiment.



F: Progressive increase in whorl length (AW) over the duration of the experiment. **G:** Regression of Feed Intake on Extract Concentration.

Figure 1: Changes in Growth Indices of Treatment Concentrations

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Conclusion

This study established that *C. lanatus* extract is potentially capable of augmenting feeding, antioxidant production/activity, and subsequent growth in *A. marginata*. Further study on the appetite-stimulating potential of *C. lanatus* extract in *A. marginata* over an extended period is, however, recommended. This will fill the gap in knowledge created in the current study due to the short duration of this experimental regime. Incorporating concentrations of *C. lanatus* ≥ 0.338 ml/g in *A. marginata* feed to improve snail growth and productivity is recommended. The information provided by this study, if adopted by snail farmers in the preparation of feed mixtures, may improve snail production – given that an increased and improved heliculture practice is a sustainable approach to combating the problem of animal protein availability and dwindling snail availability and biodiversity in the wild.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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