Effect of dietary Centrosema pubescens leaf meal on growth and reproductive traits of Archachatina marginata snails

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ABSTRACT
This study was conducted to determine the effect on growth and reproductive performance of supplementing dried Centrosema pubescens leaf meal to Archachatina marginata snails. A total of 80 growing, medium-sized A. marginata snails were used. Twenty snails were randomly assigned to one of the four experimental diets as T1, T2, T3, and T4 containing 0%, 5%, 10%, and 15% dietary inclusion levels of dried C. pubescens leaves, respectively. Each diet constituted a treatment and each treatment was replicated four times with five snails per replicate. Results showed that snails under T4 had the highest (~0.05) final body weight, average weight gain, and daily weight gain (346.89, 47.60, and 0.85 g, respectively) with the best feed conversion to meat rate of 2.12. The weekly feed intake and average body weight gain revealed the superiority of dietary T4 in supporting the growth of snails more efficiently than other dietary treatments (T1, T2, and T3) used in this study. The number of eggs laid, percentage fertility, and hatchability were highest (~0.05) for snails fed dietary T4 with the least (~0.05) embryo mortality of 2.22%. Archachatina marginata snails fed 15% dietary inclusion of dried C. pubescens leaves (T4) were the best in growth and reproductive performance.

1. Introduction
The problem of protein malnutrition is real among human populations in most developing countries. The protein intake per individual per day in Nigeria represents about one-tenth the level of intake in the advanced countries (Esonu 2001). Micro livestock have the potential of a good source of animal protein in human diet (Merkramer 1992). The African giant land snail (Archachatina marginata), for instance, is one of the micro livestock that could serve as a ready cheap source of meat for the human populations where snails thrive widely (Ngoupayon 1992). Snails have been known as a valuable source of animal protein in many countries of the world (Akinnusi 1998; Jennifer 2009). Huet (2014) reported that there is a flourishing international trade of snails in Europe and North America, and that the annual requirement of snails in France is about 50,000 tonnes, over 60% of which is imported. The estimated annual consumption in Italy is 450 million snails of which 50% is imported and in Spain alone, more than 4000 tonnes are demanded every year (Lun Oscar 2013). However, in spite of the considerable foreign and local demands, commercial snail farms such as those in Europe, South-East Asia, and the United States hardly exist in Africa (Duah and Monney 2001). In Ghana, Nigeria, Cameroon, Côte d’Ivoire, and some other West African countries where snail meat is particularly popular, snails are gathered from the forest during the wet season (CADEV 2006).

The high iron content in snail meat is considered important in treating anaemia (Fagburo et al. 2006), and it has been recommended for the treatment of ulcers, asthma, high blood pressure, and other related ailments due to its relatively low cholesterol levels (Awasu 1980; Akinnusi 2002). Orthocalcium phosphate extracted from snails can cure kidney disease, tuberculosis, anaemia, diabetes, and asthma (Mead 1961; Ademolu et al. 2006). Snail meat is palatable, nutritious, and rich in essential amino acids such as lysine, leucine, isoleucine, and phenylalanine as well as high iron contents (Imevbore 1990; Stevenart 1996; Ebenebe 2000). In recent years, however, wild snail populations have declined considerably, primarily because of the impact of such human activities such as deforestation, pesticide use, slash-and-burn agriculture, spontaneous bush fires, and the collection of immature snails (Monney 1994). As snails are going into extinction, there is a need to conserve them in order to maintain our own life support system. This is in line with the worldwide campaign for biodiversity conservation (Barbara Corker 2003), and there are two main ways to conserve biodiversity. These are termed ex situ (i.e. out of the natural habitat) and in situ (within the natural habitat).

It is therefore important to encourage snail farming (heliculture) in order to conserve this important resource. Farmers are complaining of slow growth and high mortality (Omole et al. 2010). Hence, the growth, development, and reproduction of animals are highly dependent on the quality of its feed (Amaefule and Onwudike 2000; Ani and Okeke 2003; Esonu et al. 2003;
Oyeagu et al. 2015). Centrosema pubescens is a perennial, trail-
ing-climbing leguminous herb with high nutritional qualities. Centrosema pubescens is a good source of protein, calcium, and potassium for ruminant animals as forage (Nworgu and Faphunda 2002; Odunsi et al. 2002; Ige et al. 2006; Aka and Nnaji 2010; Etim et al. 2014), and it can be used as an alternative to soybean meal in feeding broiler chickens and broiler finishers as leaf meal in a quantity up to 20 g per day (Nworgu and Egbunike 2013). There is little or no information present as of now on the use of C. pubescens leaf meal to feed snails. Therefore, this study was designed to determine the effect of varying dietary levels of C. pubescens leaf meal on growth traits and reproductive performance of A. marginata snails.

2. Material and methods

2.1. Study site

The experiment was conducted at the Snailery Unit of the Department of Animal Science, University of Nigeria, Nsukka, Nigeria. Nsukka lies within latitude 06° 22' North and longitude 07° 24' East. It has an annual rainfall range of 1567.05 mm to 1846.98 mm. Natural day length is 12–13 hours, and mean minimum and maximum daily temperatures are 20.99°C and 30.33°C, respectively. Relative humidity ranges from 46.68% to 76.20% (Ugwu et al. 2011). Nsukka belongs to the humid tropical rainforest zone of south-eastern Nigeria. The entire study lasted for eight weeks.

2.2. Experimental diet

Centrosema pubescens leaves were harvested fresh and air dried to 40% dry matter. Four different levels of 40% dry matter (60% moisture content) C. pubescens leaves was supplemented in four experimental diets. The four experimental diets were represented with T1, T2, T3, and T4 containing 0%, 5%, 10%, and 15% dietary inclusion of air-dried C. pubescens leaves, respectively. The percentage compositions of the experimental diets are presented in Table 1.

2.3. Experimental snails and management

A total of 80 growing, medium-sized A. marginata snails of the same age with an average mean weight of 299.93 g were used for the study. Twenty snails were randomly assigned to one of the four experimental diets (T1, T2, T3, and T4) using a completely randomized design (CRD). Dietary treatment 1, which contains 0% inclusion of dried C. pubescens leaves, served as the control diet. Each diet constitutes a treatment and each treatment was replicated four times with five snails per replicate. Five snails were reared in wooden boxes measuring 60 cm × 64 cm × 40 cm placed inside the snailery unit. The boxes stood 30 cm off the ground. The sides of the boxes were constructed with nylon net to facilitate ventilation while the floor had holes for drainage. The boxes were filled with garden soil up to a height of 8 cm from the floor. The soil was thoroughly mixed before the snails were introduced into the boxes. All snails were kept under the same environmental conditions and managed similarly.

2.4. Egg collection and handling

The snails started laying eggs after three weeks of housing in the boxes. Eggs were collected twice daily (early morning and late evening) for five days before incubation. Eggs waiting to be incubated were held or stored at 16–17°C (~61–63°F) in a refrigerator with the main objective of stopping all embryonic development until the eggs can be set at normal incubation temperatures (37.5°C; 99.5°F) and a secondary objective of cool storage is to discourage bacterial growth (Fasenko 2007). At lower temperatures, the water loss of the eggs is reduced and the deterioration of albumen slowed down (Walsh et al. 1995; Reijrink 2010).

2.5. Growth performance parameters measured

The parameters measured are as follows:

Initial and final body weights (g): These were measured at the beginning and at the end of the experiment, respectively. Weight changes were measured on a weekly basis.

Average body weight gain (g) = Final body weight − Initial body weight.

Daily feed intake was measured as

\[
\text{Daily feed intake (g)} = \frac{\text{Total feed intake (g)} - \text{Feed refusal (g)}}{\text{Number of Snails in each replicate}}.
\]

Total feed intake was measured as (g) = Daily feed intake (g) × no of days within the period of study.

Feed conversion ratio = Feed intake (g)/Weight gain (g). (Jabeen et al. 2004)

2.6. Reproductive performance parameters measured

The total number of eggs laid by the snails was determined and recorded. All eggs that did not hatch after the 30th day were collected and opened to determine the ones with dead embryos and those that were not fertile ab initio. These were counted and recorded. From these, the following parameters are presented in Table 1.

| Ingredients              | T1  | T2  | T3  | T4  |
|--------------------------|-----|-----|-----|-----|
| Maize                    | 22  | 22  | 22  | 22  |
| Cassava flour            | 17  | 17  | 17  | 17  |
| Wheat offal              | 17  | 17  | 17  | 17  |
| Soybean meal             | 10.5| 8.5 | 8   | 6   |
| Groundnut cake           | 15  | 14  | 13  | 10  |
| Fish meal                | 10.5| 8.5 | 7   | 6   |
| Bone meal                | 3   | 3   | 3   | 3   |
| Oyster shell             | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin premix           | 0.5 | 0.5 | 0.5 | 0.5 |
| Centro                   | –   | 5   | 10  | 15  |
| Total                    | 100 | 100 | 100 | 100 |
New Multiple Range Test (Duncan 1955) as outlined by Obi respectively.

In the present study, snails fed dietary T4 had the highest TFI and DFI with a better FBW, AWG, DWG, and FCR as the intake of feed increased from weeks 1–8. However, at week 1, dietary T4 proved to be the best diet among other were calculated:

\[
\text{Fertility} \% = \frac{\text{No. of fertile eggs (w)}}{\text{Total no. of eggs incubated (x)}} \times 100,
\]

where \( w = \) No. of eggs that hatched + No. of dead-in-shell.

\[
\text{Embryo mortality} \% = \frac{\text{No. of dead-in-shell (y)}}{\text{Total no. of fertile eggs (z)}} \times 100,
\]

\[
\text{Hatchability} \% = \frac{\text{No. of eggs that hatched (z)}}{\text{Total no. of fertile eggs (w)}} \times 100.
\]

### 2.7. Proximate analysis

Samples of the four experimental diets and *C. pubescens* leaves were analysed for their proximate compositions according to AOAC (2006) methods. Tables 2 and 3 show the chemical composition of the experimental diets and *C. pubescens* leaves, respectively.

### 2.8. Statistical analysis

Data collected were subjected to analysis of variance for CRD as described by Steel and Torrie (1980) using Statistical Package for the Social Sciences (SPSS 2003), windows version 17.0. Significantly different means were separated using Duncan's New Multiple Range Test (Duncan 1955) as outlined by Obi (2002).

### 3. Results and discussion

Growth performance of *A. marginata* snails fed varying dietary levels of dried *C. pubescens* is presented in Table 4. The initial weight gain recorded no \( P > .05 \) effect while significant \( P < .05 \) differences existed for final body weight (FBW), average weight gain (AWG), daily weight gain (DWG), total feed intake (TFI), daily feed intake (DFI), and feed conversion ratio (FCR). Among all the treatments (\( T_1 = 0\% \) dried *C. pubescens* leaf inclusion, control; \( T_2 = 5\% \) dried *C. pubescens* inclusion; \( T_3 = 10\% \) dried *C. pubescens* inclusion; and \( T_4 = 15\% \) dried *C. pubescens* inclusion) used in the present study, *A. marginata* snails fed dietary *T*4 had the highest \( P < .05 \) FBW, AWG, DWG, TFI, and FCR. Feed conversion ratio was better for *A. marginata* snails fed dietary *T*4 (15% *C. pubescens* leaf inclusion) with FCR mean of 2.12 when compared with those fed other dietary treatments (3.14, 3.06, and 2.53 for *T*1, *T*2, and *T*3, respectively). The fact that *A. marginata* snails fed dietary *T*4 had the highest TFI and DFI with a better FBW, AWG, DWG, and FCR than their counterparts that received other treatments *T*1, *T*2, and *T*3 may be due to the higher digestibility of dietary *T*4. Martens et al. (2012) reported that voluntary intake of leafy forage legumes is generally higher than that of grasses due to their lower fibre content and higher digestibility. Omole et al. (2011) had reported a higher feed intake and weight gain of growing snails fed *C. pubescens* over pawpaw leaf and *Mucuna purensis*, meanwhile they attributed it to the higher digestibility of *C. pubescens*. Moreover, it has been established that the feed consumption increases with increased protein level (McDonald et al. 1987; Bright 1996). This may be the reason why *A. marginata* snails fed *T*1 (0% inclusion of dried *C. pubescens*) had the least TFI and DFI with a poor performance in other growth parameters as shown in Table 4. It has been reported that there is a positive correlation between the feed intake and the weight gain (Cobbinah 2008; Omole et al. 2008). Thus, the results on body weight and feed conversion ratio which were better for snails fed *T*4 than those fed the control diet indicate that snails being herbivores performed well on leaf-based diets. This also showed that the protein content in *C. pubescens* leaf meal up to the level of inclusion in dietary *T*4 was well utilized by the snails. This result is in consonance with the report of Ejidike (2001) who found that increased protein level of diets resulted in better efficiency of utilization and improved growth performance in snails. The results were however consistent with the finding of Akinnusi (2002) who noted that snails fed on pawpaw leaves took advantage of the higher crude protein content of the diet and were able to make appreciable weight gain. It seems evident that snails did better when higher levels of leaf proteins are included in the diet. Olomu (1995) had earlier reported that protein functions mainly in tissue growth of animals; this may also be the reason why *A. marginata* snails fed dietary *T*4 had an appreciable weight gain of 47.60 g compared to those fed *T*1, *T*2, and *T*3 with 28.00 g, 29.68 g, and 36.96 g, respectively. Figures 1 and 2 showed the results of weekly (0–8 weeks) average feed intake and average body weight gain of *A. marginata* snail. There was a progressive increase in body weight gain as the intake of feed increased from weeks 1–8. However, at week 1, dietary *T*4 proved to be the best diet among other

### Table 2. Chemical composition of the four dietary sources used for the study.

| Determined compositions | \( T_1 \) | \( T_2 \) | \( T_3 \) | \( T_4 \) | SEM |
|-------------------------|---------|---------|---------|---------|-----|
| Crude protein           | 22.81   | 23.18   | 23.90   | 25.09   |     |
| Crude fibre             | 2.95    | 3.22    | 3.49    | 3.90    |     |
| Moisture                | 8.00    | 8.05    | 8.25    | 8.40    |     |
| Ash                     | 10.80   | 10.95   | 11.25   | 12.00   |     |
| Oil                     | 1.85    | 2.30    | 2.30    | 2.65    |     |
| Nitrogen-free extract   | 53.59   | 52.30   | 50.81   | 47.96   |     |

### Table 3. Chemical composition of *Centrosema Pubescence* (CP) leaf.

| Parameters             | CP     |
|------------------------|--------|
| Ether extract          | 3.43   |
| Crude fibre            | 12.57  |
| Ash                    | 11.99  |
| Dry matter             | 22.82  |
| Nitrogen-free extract  | 45.94  |
| Crude protein          | 24.89  |

### Table 4. Effect of Centrosema leaf on the growth performance of *Archachatina marginata* snails.

| Parameters             | \( T_1 \) | \( T_2 \) | \( T_3 \) | \( T_4 \) | SEM |
|------------------------|---------|---------|---------|---------|-----|
| Initial weight (g)     | 298.34  | 300.15  | 297.95  | 299.29  | 2.82|
| Final weight (g)       | 326.34  | 329.83  | 334.91  | 346.89  | 4.26|
| Average weight gain (g)| 28.00   | 29.68   | 36.96   | 47.60   |    |
| Daily weight gain (g)  | 0.50    | 0.53    | 0.66    | 0.85    |    |
| Total feed intake (g)  | 88.15   | 90.62   | 93.55   | 100.88  | 1.95|
| Daily feed intake (g)  | 1.57    | 1.62    | 1.67    | 1.80    | 0.18|
| FCR                    | 3.14    | 3.06    | 2.53    | 2.12    | 0.43|

Note: Row means with different superscripts (a, b, c) differ significantly at \( P < .05 \).

FCR: feed conversion ratio; SEM: standard error of mean.
The observed increase in body weight gain recorded for *A. marginata* snails fed dietary T4 might be as a result of the fact that the sources of the nutrients utilized by the *A. marginata* snails are artificial diets that are supplemented with plant materials (natural food), thereby providing increased nutrients. This findings supported the report of Ejidike and Afolayan (2000) who supplemented natural food with artificially prepared diets for snails and it produced an improved growth in *A. marginata*. The result of this study is also in consonance with the reports of Ejidike (2007), who compared artificial diet, plant material (*Carica papaya*), and a combination of the two (artificial food + plant material). He found out that snails that fed diets containing artificial food + plant material performed better than those that received only artificial diet or natural food. It can also be observed that there may still be a potential positive result in body weight if the inclusion rate of *Centrosema* leaf meal increases further than the highest level used in this study (15%). Ejidike and Afolayan (2010) pointed out that availability of acceptable snail feed could contribute a lot in encouraging recent interest in snail farming, thereby adding to the efforts towards eradication of the current acute animal protein shortage. However, the use of artificial diets supplemented with natural food (plant material) in feeding snails will arouse the establishment of commercial farming of African giant land snails for constant supply to the market.

Table 5 shows the reproductive performance of *A. marginata* snails fed varying dietary levels of dried *C. pubescens*. The data show significant (*P* < .05) difference in all the reproductive performance traits except for the average weight of hatchlings that appear to be the same (*P* > .05) among the treatment means. *Archachatina marginata* snails that consumed dietary T4 (15% *C. pubescens* leaf inclusion) had greater number of total eggs laid, higher percentage hatchability and fertility of eggs, as well as least percentage embryo mortality compared to their counterparts that recieved other treatments used for the study. *Archachatina marginata* snails fed dietsry T1 (control diet with 0% *C. pubescens* leaf inclusion) had the least eggs laid, lower percentage hatchability and fertility of eggs, as well as higher embryo mortality percentage. This may be attributed to the low nutritional quality of the diet (control diet added up to 15% *C. pubescens* leaf inclusion). The poor nutritional quality of the diet (control diet with 0% *C. pubescens* leaf inclusion) that resulted in poor growth performance may also be the reason for their poor reproductive performance compared to their counterparts that recieved other treatments. It can be said that the nutritional quality in the T4 diet (15% *C. pubescens* leaf inclusion) was the best as it increased both the number of eggs laid, and hatchability as well as fertility of eggs with a lesser mortality of the embryo. Eniolorunda et al. (2007) reported that nutritional quality of diets is associated with diets of better ingredient combinations which were utilized more efficiently when fed to the animals. Ejidike et al. (2002) reported that both growth and reproductive performance of African giant land snails (*A. marginata*) were better when their diets are supplemented with their natural plant food materials than the snails that were placed on pure artificial diets. Thompson and Sheldon (2004) also reported that poor nutrition affects snail growth and causes a drop in the reproductive performance. According to Amaefule and Onwudike (2000), Ani and Okeke (2003), Esonu et al. (2003), and Oyeagu et al. (2015), the most important factor influencing the performance of animals under captivity, all other factors being constant, is the quality of diet offered to the animals.

### 4. Conclusion

It can be concluded from the results obtained in the present study that *C. pubescens* leaf meal inclusion up to 15% in snail diet increased average weight gain which was evident from...
the growth performance and it also improved the reproductive performance of *A. marginata* snails.

However, Figure 2 shows a potential body weight increase of *A. marginata* snails more than what was obtained (recorded) in this work if the inclusion rate of *Centrosera* leaf increases further than the rate used in this study (15%). Hence, further research is necessary to determine the inclusion rate of *C. pubescens* leaf that produces the peak weight gain of *A. marginata* snails.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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