Journal of Agriculture and Rural Development in the Tropics and Subtropics
Vol. 125 No. 1 (2024) 9–19

https://doi.org/10.17170/kobra-202402239636 ISSN: 2363-6033 (online); 1612-9830 (print) – website: www.jarts.info

Effect of different diets containing varying inclusion levels of *Moringa oleifera* leaf meal on growth, mineral composition and meat quality of the edible land snails *Archachatina marginata* and *Achatina fulica*

Maduabuchi Inwele Amobi [a], Amara Chibuzo Anazodo [b], Bede Izuchukwu Ezewudo [b,c,*], Valentine Obinna Okpoko [b,d]

[a] *Department of Biological Sciences, Federal University of Kashere, Nigeria*
[b] *Department of Zoology, Nnamdi Azikiwe University, Nigeria*
[c] *Department of Zoology and Environmental Biology, University of Nigeria, Nigeria*
[d] *Department of Biology and Forensic Science, Admiralty University of Nigeria, Nigeria*

Abstract

This study examined the effect of different dietary inclusion levels of *Moringa oleifera* leaf meal on growth, haemolymph mineral composition and meat quality of *Archachatina marginata* and *Achatina fulica*. A total of one hundred and forty four (144) juvenile snails of *A. marginata* = 72 and *A. fulica* = 72, were used for the study. Eighteen (18) snails of each species were subjected to four dietary treatments (0 %, 10 %, 15 % and 20 %) of *M. oleifera* leaf meal in three replicates of six (6) snails per replicate over a period of 16 weeks. Growth was measured using growth indices while haemolymph mineral composition and meat quality were determined spectrometrically and by proximate method. *A. marginata* and *A. fulica* fed diets containing 20 % *M. oleifera* leaf meal showed the best growth performance compared to the other treatments with increase in mean weight gain of 132.14 % and 62.95 %, mean shell length gain of 51.87 % and 59.47 %, mean shell circumference gain of 22.81 % and 47.53 % and mean shell thickness gain of 2.18 % and 83.43 %, respectively, as compared to the control diet. The haemolymph mineral composition results of the two species showed that magnesium, calcium, potassium, iron, phosphorus and chlorine were highest in snails fed diet with 20 % *M. oleifera* leaf meal. However, copper and sodium were recorded highest in *A. marginata* fed with control diet. The results of the proximate meat analysis showed that the highest crude protein contents were recorded in *A. marginata* and *A. fulica* fed with 20 % *M. oleifera* leaf meal, with an increase in crude protein content of 80.42 % and 114.28 %, compared to the control diet. Based on the results of this study, the inclusion of *Moringa oleifera* leaf meal in the snail diet up to 20 % is recommended for optimum productivity of the two snail species.

Keywords: dietary treatments, growth indices, haemolymph, proximate, non-conventional livestock, heliciculture

1 Introduction

Globally, there is a rise in human population on daily basis. This daily increase in human population has culminated to the scarcity and high cost of food especially proteins from animal sources. In order to alleviate this serious situation, animal nutritionists are searching for unconventional animal proteins that are cheap, nutritious and capable of replacing the conventional animal proteins such as beef, pork, poultry etc. (Edet *et al.*, 2017). Snail, a bilaterally segmented and soft bodied invertebrate bearing a calcareous shell exoskeleton is adjudged to be a good alternative to the conventional animal proteins (Edet *et al.*, 2017), due to its richness in protein, essential minerals like phosphorous, calcium, iron, zinc and low cholesterol content (Amobi *et al.*, 2019).

Worldwide consumption of snails is estimated at 400,000–450,000 tonnes per year, most of which come from the wild (Aromolaran *et al.*, 2019). In Africa, the demand for snail meat has increased due to health concerns as more people

* Corresponding author: ib.ezewudo@unizik.edu.ng; Phone: +2347035167101

Published online: 25 February 2024 – Received: 7 Juni 2023 – Accepted: 5 February 2024

have started avoiding red meat (Aduloju *et al*., 2019). In Nigeria, snail meat, commonly known as "Congo meat" is widely consumed in many homes and it can be consumed in fried, cooked and roasted forms (Nnodim & Ekpo, 2019; Oguntoye *et al*., 2020). The Giant West African snail (*Archachatina marginata*) and Giant African snail (*Achatina fulica*) are among the breeds of snails readily found in Nigeria alongside Giant tiger land snail (*Achatina achatina*) and are the most cultured in snail farms in Nigeria (Babalola & Akinsoyinu, 2009; Okon *et al*., 2017). Their high acceptability by snail farmers could be due to their massive size, excellent adaptation to available feed, and moderate to high egg-laying capacity (Raut & Barker, 2002; Omole & Kehinde, 2005).

However, the operation of snail farms on a commercial scale in Nigeria has not been fully realised. Majority of the snails sold in most Nigeria markets are sourced from the wild while the few existing farms are saddled with multidimensional problems such as lack of finance, technical know-how and the right feeds that can promote early maturation, improve meat quality and overall well-being of the snails (Nnodim & Ekpo, 2019). Studies have shown that snails can feed on various available feeds, preferably plant leaves (Orsa *et al*., 2018; Amobi & Ezewudo, 2019; Amobi *et al*., 2019) such as Moringa oleifera leaves (Ani *et al*., 2014; Edet *et al*., 2017).

Moringa (*Moringa oleifera* Lim.) is a tropical plant used for different purposes as virtually all parts of the tree can be utilised as food, medicinal, industrial and agricultural purposes including animal diets (Khalafalla *et al*., 2010; Mahfuz & Piao, 2019). The *M. oleifera* leaves are rich in vitamins, proteins and essential minerals such as calcium, phosphorous, iron, zinc, making it the most nutrient-rich part of the plant (Leone *et al*., 2015). The leaves of *M. oleifera* are also rich in antioxidant compounds like carotenoids, ascorbic acids, phenolic compounds and flavonoids, hence can be utilised as a natural antioxidant (Sahay *et al*., 2017).

Assessment of the nutritional benefits of feed supplied to an animal can be properly studied by evaluating the effect of the feed dietary components on the blood (Babalola & Akinsoyinu, 2011). The blood of pulmonate snails, known as haemolymph provides the medium for the transport of minerals and waste removals from the body of snails (Abdussamad *et al*., 2010). Its constituents include water, inorganic ions such as Na^+, Cl^-, K^+, Mg^{2+} and Ca^{2+} and other compounds like protein, carbohydrates and lipids (Abdussamad *et al*., 2010), making it fit for both clinical and nutritional assessment studies. The use of *M. oleifera* leaves in snail diets to improve production yield and wellbeing has received little attention, despite offering both nutritional and medicinal benefits. Based on this finding, the present study focused on the effect of different amounts of *M. oleifera* leaf meal in the diet on growth, haemolymph mineral compositions, and meat quality of two edible land snails, *A. marginata* and *A. fulica*.

2 Materials and methods

2.1 Study site

The present research was conducted at the snail farm of the Department of Animal Science, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The station where the study was conducted is located at 06°14'42.4" N and 07°07'05.4" E. It has an average temperature which fluctuates between 22 °C and 34 °C with an annual rainfall of 1450–1524 mm in two seasons – the rainy and dry season (Ozoemene *et al*., 2022). subsectionMoringa leaves preparation and feed formulation Fresh *M. oleifera* leaves were collected from Amansea in Awka Metropolis, the Capital City of Anambra State, Nigeria, while other feed ingredients were procured from local sellers at Afor Nnobi Market, Anambra State, Nigeria. The freshly collected *M. oleifera* leaves were dried in shade under ambient temperature until they became crispy. The drying lasted for two (2) weeks after which it was milled using local milling machine with 1 mm mesh size sieve and then incorporated into the feed at 0 %, 10 %, 15 % and 20 % inclusion levels. The experimental diets were formulated at equicalorie and isonitrogenous levels by adopting Pearson Square method (Table 1).

Table 1: *Gross composition of the experimental diets on a dry matter basis.*

	Moringa leaf meal inclusion level			
Ingredients	*0 %*	*10 %*	*15 %*	20 %
Yellow maize	40	40	40	40
Groundnut cake	15	10	12	10
Brewers grain	10	10	7.5	7
Palm kernel cake	12	10	7.5	5
Moringa leaf meal	0	10	15	20
Blood meal	15	12	10	10
Oyster shell	1.5	1.5	1.5	1.5
Bone meal	1.5	1.5	1.5	1.5
Vitamin premix[†]	5.0	5.0	5.0	5.0

[†]1 kg of premix contains: Vitamin A (5,000,000 I.U), Vitamin D3 (1,000,000 I.U), Vitamin E (16,000 mg), Vitamin K3 (800 mg), Vitamin B1 (1,200 mg), Vitamin B2 (22,000 mg), Niacin (22,000 mg), Calcium pantothenate (4,600 mg), Manganese (948,000 mg), Iron (40,000 mg), Zinc (32,000 mg), Copper (3,400 mg), Iodine (600 mg), Cobalt (120 mg), Selenium (48 mg), Anti-oxidant (48,000 mg).

2.2 Experimental snails, design and set-up

A total of 150 juvenile snails (*Achatina marginata*, n = 75 and *Achatina fulica*, n = 75) were purchased from the Ministry of Agriculture, Awka, Anambra State, Nigeria. The snails were acclimatised for two weeks and fed with moistened pawpaw (*Carica papaya*) leaves which served as sources of food and water. For the feeding trial, one hundred and forty four snails (144) belonging to the two species were used for the study: seventy two (72) *Archachatina marginata* (28.90 ± 0.116 g) and seventy two (72) *Achatina fulica* (16.03 ± 0.200 g) species. The experiment adopted a 4 × 3 completely randomised design (CRD) with four dietary treatments having 0 % (control), 10 %, 15 % and 20 % inclusion levels of *M. oleifera* leaf meal. The study strictly adhered to the guidelines approved by the Nnamdi Azikiwe University, Awka, Ethical Committee on the Use of Live Animals for Experimental Studies. Eighteen snails (18) were assigned to each of the dietary treatments and replicated thrice so that, each replicate contained six (6) snails. Each replicate was housed in a mini-paddock measuring 120 cm × 60 cm × 30 cm, in accordance with the housing structure and stocking density recommended by Cobbinah *et al*. (2008). The mini-paddocks were sheltered from rain and sunlight in a covered enclosure. There were trees such as plantains, around the farm to cushion wind effects and regulate sudden temperature changes.

The snails in all the treatments were further subjected to the same management conditions: they were fed daily, and their beddings were made with humus soil to a depth of 20 cm. Additionally, all snail beddings were repeatedly provided with 50 g of ground egg shell to ensure adequate calcium supply and sprinkled daily with equal amount of water (0.75 litre) to increase humidity and prevent aestivation due to hot weather condition common in tropical regions (Agbogidi *et al*., 2008).

2.3 Determination of growth performance

Data were collected on the growth performance of the snails by measuring the following parameters – body weight was taken with the aid of a sensitive weighing balance (model: BL7501), and shell length, which was taken along the axis of the snail using vernier callipers (Okonta, 2012). Also, the widest part of the snail was measured with vernier callipers to ascertain the shell circumference while shell thickness was taken with the aid of a micrometre screw gauge (Amobi *et al*., 2019). These data were taken on weekly basis over a period of 16 weeks.

2.4 Haemolymph collection and mineral composition analysis

Prior to the commencement of the feeding trial, three juvenile snails from each species were randomly selected for haemolymph mineral composition assay. Also, at the end of the experimental period, three snails from each species were randomly selected from each replicate (n = 9) of the four (4) dietary treatments for haemolymph mineral composition analysis. The haemolymph collection followed the procedure of Cooper (1994). The haemolymph collected was analysed in the Springboard Research Laboratories, Awka, Anambra State, for mineral compositions using Atomic Absorption Spectrophotometry (Varian AA240, Agilent Technologies Inc., Santa Clara, California, USA). Calcium was analysed by flame photometry using Jenway digital flame photometer (Model: PFP7).

2.5 Determination of meat quality

Three juvenile snails from each species were randomly selected and sacrificed before the start of the experiment for proximate composition of the snail meat and served as the initial result. These same snails were used for haemolymph mineral composition assay. Also, at the end of the 16weeks feeding trial, three snails from each species were randomly selected from each replicate (n = 9) of the four (4) dietary treatments. The selected snails were thoroughly washed and the shell broken with the aid of a stone. The snail meats were collected and properly washed with alum and water until the slime was washed off. The meat was analysed in laboratory for proximate composition by adopting AOAC (2005) methodology.

2.6 Statistical analysis

The data generated were subjected to descriptive statistics, student's t-test, analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 2001) and significant means was separated using Turkey's test at 0.05 % significance.

3 Results

3.1 Proximate composition of the formulated diets

Proximate composition of the experimental diets showed that highest dry matter was observed in diet with 20 % inclusion level of *M. oleifera* leaf meal whereas highest crude protein content was noted in diet with 0 % inclusion level of *M. oleifera* leaf meal (Table 2). In addition, lowest values in crude ash were recorded in control diet (0 % *M. oleifera* leaf meal) and diet with 10 % inclusion level of *M. oleifera* leaf meal.

Table 2: *Proximate composition of experimental diets (% dry weight).*

	Inclusion level of Moringa oleifera *leaf meal*			
Parameters	*0 %*	*10 %*	*15 %*	20 %
Dry matter	57.17	59.20	60.10	60.25
Crude protein	22.35	19.73	20.50	20.63
Ash	6.50	6.50	6.70	6.80
Crude fat	7.00	7.29	7.30	7.32

3.2 Growth performance

Before the start of the feeding trial, the initial wet weight of *A. marginata* was 28.90 ± 8.50 g, 28.86 ± 9.60 g, 28.90 ± 9.40 g and 29.26 ± 10.00 g, for the four diets 0 %, 10 %, 15 % and 20 % inclusion levels, respectively, while at the completion of the trial, it was observed as 71.12 ± 12.40 g, 85.10 ± 15.00 g, 91.90 ± 20.50 g and 110.67 ± 25.70 g, respectively (Fig. 1). Overall wet body weight gain of *A. marginata* fed diets with varying inclusion levels (0 %, 10 %, 15 % and 20 %) of *M. oleifera* leaf meal was 42.22 ± 15.00 g, 56.24 ± 18.60 g, 63.00 ± 19.00 g and 81.41 ± 20.60 g (Fig. 1). The data of mean weight gain of *A. marginata* when subjected to One way ANOVA showed a significant difference ($p < 0.05$) in the mean weight gains of snails fed diets with varying inclusion levels of *M. oleifera* leaf meal. There was corresponding increase in mean shell length of *A. marginata* as inclusion levels of *M. oleifera* increased (Table 3). The lowest value for shell length was recorded in snails fed with 0 % inclusion level of *M. oleifera* leaf meal.

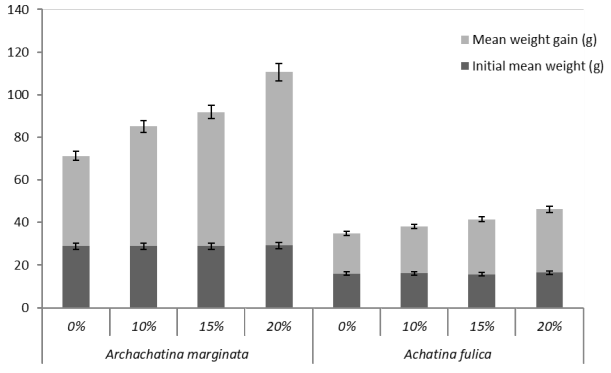

Fig. 1: *Growth performance of snails (*Archachatina marginata *and* Achatina fulica*) based on different inclusion levels of* Moringa oleifera *leaf meal. Error bars denote standard deviation.*

There was non-significant difference ($p > 0.05$) in shell length of snails (*A. marginata*) fed diets with 15 % and 20 % inclusion levels of *M. oleifera* leaf meal whereas when compared with other diets, significant differences ($p < 0.05$) were observed. There was increase in mean shell thickness gains of *A. marginata* fed diets with 0 %, 10 %, 15 % and 20 % inclusion levels of *M. oleifera* leaf meal, however, when subjected to One way ANOVA, non-significant differences ($p > 0.05$) were observed in *A. marginata* fed with varying inclusion levels of *M. oleifera* leaf meal. Still, the least performed group in terms of the mean shell thickness was recorded in those control diet with slight variation (Table 3).

At the end of the feeding trial, the weight gain of *A. fulica* fed diets with varying inclusion levels (0 %, 10 %, 15 % and 20 %) of *M. oleifera* leaf meal was recorded as 34.96 ± 16.60 g, 38.10 ± 18.70 g, 41.63 ± 19.00 g and 46.26 ± 19.50 g, respectively. Overall mean body weight gain of *A. fulica* fed with varying inclusion levels (0 %, 10 %, 15 % and 20 %) of *M. oleifera* leaf meal was 18.93 ± 10.50 g, 21.94 ± 15.00 g, 25.93 ± 17.50 g and 29.80 ± 18.00 g, respectively (Fig. 1). Highest value for mean weight gain was recorded in *A. fulica* fed diet with 20 % inclusion level of *M. oleifera* with percentage difference of 62.95 % compared to the treatment with lowest value recorded (snails fed control diet). The effect of inclusion levels of *M. oleifera* leaf meal in the diet of *A. fulica* showed that *A. fulica* fed with 20 % inclusion level of *M. oleifera* had the highest mean shell length gain (Table 3). For mean shell circumference, there was non-significant difference ($p > 0.05$) in *A. fulica* fed diet with 10 % and 15 % inclusion levels of *M. oleifera* leaf meal while significant difference (P<0.05) was observed in those fed diet with 0 % and 20 % inclusion levels of *M. oleifera* leaf meal. From the results of mean shell circumference, *A. fulica* fed diet with 0 % inclusion level of *M. oleifera* leaf meal recorded the lowest value (Table 3).

3.3 Haemolymph mineral compositions

Upon completion of the feeding trial, the two snail species were further analysed for haemolymph mineral compositions and the values obtained are recorded in Tables 4 and 5, respectively. There was a progressive increase in all the minerals (magnesium, calcium, copper, potassium, iron, phosphorous, chlorine and sodium) analysed in *A. marginata* and *A. fulica* fed with the experimental diets. Results obtained showed that the values recorded for haemolymph mineral compositions (magnesium, calcium, potassium, iron, phosphorus and chlorine) of *A. marginata* and *A. fulica* were higher in both species fed diet with 20 % inclusion level of *M. oleifera* leaf meal (Tables 4 and 5). However, copper and sodium had the highest values in *A. marginata* fed control diet, while in *A. fulica*, only copper was found to have

Table 3: *Linear body performance of snails (*Archachatina marginata *and* Achatina fulica*) fed with different inclusion levels of* Moringa oleifera *leaf meals after the feeding trial.*

Mean shell gain	*Snail species*	*Inclusion level of* Moringa oleifera *leaf meal*			
		0 %	*10 %*	*15 %*	20 %
length (cm)	*AM*	3.76 ± 1.50^{c}	4.46 ± 2.00^{b}	5.03 ± 2.50^{a}	5.56 ± 3.00^{a}
	AF	2.66 ± 1.15^{c}	3.13 ± 1.20^{b}	3.77 ± 1.50^{b}	4.43 ± 2.50^{a}
circumference (cm)	*AM*	3.57 ± 1.15^{c}	5.27 ± 2.00^{b}	5.83 ± 2.60^{b}	6.16 ± 3.50^{a}
	AF	2.24 ± 1.00^{c}	3.06 ± 1.20^{b}	3.60 ± 2.00^{b}	4.83 ± 2.50^{a}
thickness gain (mm)	*AM*	0.12 ± 0.10^{a}	0.13 ± 0.10^{a}	0.14 ± 0.30^{a}	0.15 ± 0.50^{a}
	AF	0.09 ± 0.05^{b}	0.11 ± 0.50^{b}	0.12 ± 0.10^{b}	1.12 ± 0.10^{a}

AM: *Archachatina marginata*; AF: *Achatina fulica*. Rows with different superscripts (a,b,c) are significantly different at 5 %. Values are mean ± SE.

Table 4: *Summary of mineral composition of haemolymph (ppm) of snails (*Archachatina marginata*) after the feeding trial.*

Minerals	*Initial value n = 3*	*Final values of mineral composition of haemolymph (ppm) of snails fed with graded levels of* M. oleifera *leaf meal (n = 9 per treatment)*			
		0 %	*10 %*	*15 %*	20 %
Magnesium (Mg)	9.88 ± 5.00	20.23 ± 7.10	20.24 ± 8.00	20.25 ± 8.10	20.25 ± 7.90
Calcium (Ca)	24.15 ± 9.00	42.63 ± 12.10^{d}	43.08 ± 13.00^{c}	44.62 ± 14.20^{b}	46.46 ± 15.00^{a}
Copper (Cu)	0.28 ± 0.30	0.48 ± 0.50	0.46 ± 0.20	0.46 ± 0.20	0.44 ± 0.15
Potassium (K)	66.10 ± 20.00	102.20 ± 35.10^{c}	152.20 ± 50.00^{c}	261.30 ± 70.10^{a}	275.10 ± 75.50^{a}
Iron (Fe)	1.06 ± 0.25	2.74 ± 0.80^{b}	2.82 ± 0.90^{b}	2.91 ± 0.90^{a}	3.05 ± 0.85^{a}
Phosphorous (P)	4.86 ± 0.90	10.22 ± 2.00^{c}	11.31 ± 2.25^{b}	11.78 ± 2.80^{b}	12.37 ± 3.00^{a}
Chlorine (Cl)	2.67 ± 1.00	8.43 ± 2.25^{c}	8.82 ± 2.90^{c}	9.37 ± 3.00^{b}	10.23 ± 3.90^{a}
Sodium (Na)	2.24 ± 0.50	11.48 ± 2.00^{a}	10.97 ± 1.00^{b}	10.65 ± 0.90^{b}	9.44 ± 0.75^{c}

Rows with different superscripts (a,b,c,d) are significantly different at 5 %. Values are mean ± SE.

the highest value in snails fed control diet. Additionally, copper and sodium were observed to have the lowest values in *A. marginata*, while only copper had the least value in *A. fulica* fed diet with 20 % inclusion level of *M. oleifera*

Table 5: *Summary of mineral composition of haemolymph (ppm) of snails (*Achatina fulica*) after the feeding trial.*

Minerals	*Initial value n = 3*	*Final values of mineral composition of haemolymph (ppm) of snails fed with graded levels of* M. oleifera *leaf meal (n = 9 per treatment)*			
		0 %	*10 %*	*15 %*	20 %
Magnesium (Mg)	7.56 ± 2.50	16.32 ± 5.00	16.56 ± 6.00	16.60 ± 6.10	16.82 ± 6.20
Calcium (Ca)	18.34 ± 5.00	33.19 ± 10.00^{d}	38.09 ± 10.50^{c}	40.44 ± 12.00^{b}	48.28 ± 15.00^{a}
Copper (Cu)	0.21 ± 0.05	0.57 ± 0.50	0.55 ± 0.40	0.54 ± 0.35	0.53 ± 0.30
Potassium (K)	29.66 ± 9.00	59.10 ± 12.20^{d}	75.20 ± 15.00^{c}	81.30 ± 28.50^{b}	89.10 ± 30.10^{a}
Iron (Fe)	0.17 ± 0.10	2.64 ± 0.50^{b}	2.85 ± 0.60^{a}	2.86 ± 0.60^{a}	3.00 ± 0.80^{a}
Phosphorous (P)	4.38 ± 1.00	8.56 ± 1.20^{c}	9.89 ± 2.00^{b}	10.03 ± 2.00^{b}	10.79 ± 2.50^{a}
Chlorine (Cl)	2.86 ± 0.80	7.26 ± 1.00^{b}	8.11 ± 1.50^{a}	8.29 ± 1.70^{a}	8.54 ± 1.60^{a}
Sodium (Na)	20.12 ± 5.00	29.21 ± 5.90^{c}	38.81 ± 9.00^{b}	38.79 ± 10.00^{b}	42.77 ± 12.10^{a}

Rows with different superscripts (a,b,c,d) are significantly different at 5 %. Values are mean ± SE.

Table 6: *Proximate composition of snail meat (*Archachatina marginata*) (%) after the feeding trial.*

Minerals	*Initial value n = 3*	*Final values of mineral composition of haemolymph (ppm) of snails fed with graded levels of* M. oleifera *leaf meal (n = 9 per treatment)*			
		0 %	*10 %*	*15 %*	20 %
Crude protein	9.70 ± 2.00	14.4 ± 5.00^{c}	20.10 ± 7.20^{b}	20.90 ± 8.00^{b}	22.20 ± 8.50^{a}
Ash	2.40 ± 1.00	5.0 ± 1.20^{c}	5.80 ± 1.30^{c}	6.20 ± 1.50^{b}	7.50 ± 1.70^{a}
Moisture content	25.8 ± 5.00	74.60 ± 10.00d	77.20 ± 10.50^{c}	80.00 ± 12.50^{b}	84.60 ± 15.00^{a}

Rows with different superscripts (a,b,c,d) are significantly different at 5 %. Values are mean ± SE.

Table 7: *Proximate composition of snail meat (*Archachatina marginata*) (%) after the feeding trial.*

Minerals	*Initial value n = 3*	*Final values of mineral composition of haemolymph (ppm) of snails fed with graded levels of* M. oleifera *leaf meal (n = 9 per treatment)*			
		0 %	*10 %*	*15 %*	20 %
Crude protein	6.30 ± 1.00	14.20 ± 5.00^{c}	19.50 ± 8.20^{b}	20.30 ± 9.00^{a}	21.40 ± 9.00^{a}
Ash	1.80 ± 0.20	2.70 ± 0.50^{b}	3.00 ± 0.70^{a}	3.20 ± 0.70^{a}	3.60 ± 0.80^{a}
Moisture content	21.00 ± 5.00	65.00 ± 10.00^{d}	69.60 ± 15.00^{c}	70.10 ± 15.90^{b}	76.80 ± 16.00^{a}

Rows with different superscripts (a,b,c,d) are significantly different at 5 %. Values are mean ± SE.

leaf meal (Table 4 and 5). Results in all the haemolymph mineral parameters were statistically significant ($p < 0.05$) while the values for magnesium and copper were statistically non-significant ($p > 0.05$).

3.4 Meat quality analysis

The meat of *A. marginata* and *A. fulica* were analysed at the beginning and upon completion of the feeding trial using proximate analysis to ascertain the effect of different dietary levels of *M. oleifera* on the nutritional quality of the snails' meat. Proximate composition of the snail meat exhibited wide variations in the parameters studied viz. crude protein, ash and moisture content as the inclusion level of *M. oleifera* leaf meal increased (Tables 6 and 7). In *A. marginata*, the meat crude protein content ranged from 14.4 % to 22.20 % while in *A. fulica*, it ranged from 14.20 to 21.40 %.

Highest body meat protein contents were observed in *A. marginata* and *A. fulica* fed diet with 20 % *M. oleifera* leaf meal while the least values were observed in those fed diet with 0 % *M. oleifera* leaf meal (Tables 6 and 7). Lowest crude ash was recorded in snails (*A. marginata* and *A. fulica*) fed with control diet. Still, results of crude ash in *A. marginata* when statistically analysed showed significant differences (P<0.05) in diets with inclusion levels of *M. oleifera* leaf meal, while in *A. fulica*, non-significant difference was recorded (P>0.05). Whole body moisture contents of both snail species were significantly different (P<0.05). Highest value for body moisture levels were recorded in *A. marginata* and *A. fulica* fed diet with 20 % inclusion level of *M. oleifera* leaf meal.

4 Discussion

4.1 Proximate composition of formulated diets

The inclusion of *M. oleifera* leaf in animal diets including snails have been considered as a potential source of protein in formulated animal feed (Ani *et al*., 2014, Ayodele *et al*., 2014). The crude protein content for the control diet (22.35 %) was slightly higher than the diets with *M. oleifera* leaf meal (19.73–20.63 %). The moderate concentration of crude protein in *M. oleifera* leaf meal formulated diets compared to that of the control further confirmed that *M. oleifera* can provide the required protein needed for the replacement of worn-out tissues and enhancing enzymatic processes needed for body building. The crude protein contents of the diets with varying inclusion levels of *M. oleifera* leaf meal is slightly lower than the finding (23.78–24.06 %) of Ani *et al*. (2014). The differences in crude protein contents could be a result of variations in methods of drying of the leaves, the age of the plants, the cultivar, the fertility of the soil on which the plants were grown, or the climatic and environmental conditions prevalent in the area where the plants were grown (Moyo *et al*., 2011; Amobi *et al*., 2019). Also, the increase in protein content in the diets with varying inclusion levels of *M. oleifera* leaf meal with increased inclusion of

M. oleifera leaf meal shows that *M. oleifera* leaf meals are rich in nitrogenous compounds.

The addition of *M. oleifera* leaf meal improved the ash contents of the diets which in the control diet had a value of 6.50 % but in diets with *M. oleifera* leaf meal, it ranged between 6.50-6.80 %. Since ash contents measure the mineral content present in diets (Usunobun & Egharebva, 2014), the increase in ash content in diets with *M. oleifera* leaf meal shows that *M. oleifera* leaves are rich in minerals and this is in line with the report of Moyo *et al*. (2011).

Furthermore, it was observed that the inclusion of *M. oleifera* leaf meal in the formulated diets improved the crude fat contents of the diets (7.29–7.32 %) compared to the control diet (7.00 %). This suggests that *M. oleifera* leaf meal contains relatively moderate to high concentrations of fatty acids (Moyo *et al*., 2011) and the variation could be linked to the age of the plant, soil and environmental conditions (Sanchez-Machado *et al*., 2010). As reported by Moyo *et al*. (2011), *M. oleifera* leaf was found to be richer in polyunsaturated fatty acids than saturated fatty acids. A higher content of polyunsaturated fatty acids (PUFAs) such as α-linoleic, linoleic and g-linoleic acids and lower content of saturated fatty acids (SFAs) is required (Hoffman & Wilklund, 2006). Its inclusion in animal diets may help to improve meat quality as well as the general well-being of animals (Colin *et al*., 2012).

4.2 Growth performance

In both species of snails (*A. marginata* and *A. fulica*), there was a progressive increase in general growth performance with an increased inclusion level of *M. oleifera* leaf meal, indicating the acceptability of *M. oleifera* leaf meal by the snails within these levels of inclusion. This finding is in agreement with the observation of Ani *et al*. (2014) who reported a better growth performance of snails (*A. marginata*) fed graded levels of *M. oleifera* leaf meal at 0 % to 20 % inclusion levels, with 20 % performing best when compared to other treatments.

The study by Aboagye *et al*. (2015) has shown that *M. oleifera* leaf meal is of anthelmintic value when incorporated in the diets of A. achatina. Edible land snails are susceptible to infection with parasitic nematodes (Angiostrongylus catonensis), which can retard growth and general body development (Aboagye *et al*., 2014). The higher body weight gain in snails fed *M. oleifera* leaf meal when compared to snails fed the control diet may be due to the fact that these snails were able to resist parasitic infection due to the anthelmintic value of *M. oleifera* leaf meal, resulting in an increase in their body weight. Additionally, in terms of mean weight gain per treatment, both snail species fed the 20 % inclusion level of *M. oleifera* leaf meal performed best when compared to the other treatments. This could be due to moderate protein content and high crude fats of this diet which support tissue growth and replacement of worn-out tissues as well as improving the health status of the snails. The observed significant increase in both the shell length and circumference of the snails fed with different dietary treatments of *M. oleifera* leaf meal is not out of place. As expected, the increase in body weight leads to a corresponding increase in size, probably due to the expansion of the shell (Amobi & Ezewudo, 2019; Amobi *et al*., 2019). Also, the best performance in terms of shell length and circumference in snails fed 20 % *M. oleifera* leaf meal may be due to the presence of more minerals in the diets, as reflected in the ash content of the diet compared to the other diets, that the snails were able to ingest and utilise. The significant increase in shell thickness of both species fed different dietary supplements of *M. oleifera* leaf meal could be a result of the addition of calcium (ground eggshells) in their beddings (Amobi *et al*., 2019).

Furthermore, snails fed with 20 % *M. oleifera* leaf meal had the best shell thickness when compared to snails fed with other diets. This may suggest that snails fed with 20 % *M. oleifera* were able to optimally assimilate the essential minerals inherent in their diet for shell development. According to Leone *et al*. (2015), Moringa leaves are rich in essential elements such as calcium and phosphorous and since diet with 20 % *M. oleifera* leaf meal is the diet with most Moringa leaf meal, this could be the reason for the best shell thickness recorded in this study. As reported by Funmilayo (2008), calcium and phosphorous are necessary for proper shell development.

4.3 Haemolymph mineral compositions

The haemolymph in pulmonate snails serves as the medium for the transport of nutrients including minerals to the cells (Abdussamad *et al*., 2010). Noticeable increase in mineral compositions were observed in all the snails fed the formulated diets, however, better performance were recorded in snails fed diets with varying inclusion levels of *M. oleifera* leaf meal than the control diet. This could be linked to the ability of the experimental animals to absorb and utilise the minerals abounding in their diets. Also, the high mineral compositions in the haemolymph of snails fed diets with varying inclusion levels of *M. oleifera* treated diets could be explained by the findings of Anwar *et al*. (2007), Anjorin *et al*. (2010) and Moyo *et al*. (2011). These authors reported that *M. oleifera* leaf meal is richly endowed with both macro- and micro-elements such as calcium, phosphorous, iron, magnesium, copper, potassium, manganese, zinc, sul-

phur, sodium and selenium, which are highly beneficial to the general well-being of animals and humans. Potassium was observed to be higher compared to other minerals found in both species of snails. Potassium in synergy with sodium plays a crucial role in the regulation of acid-base balance as well as in maintaining internal water balance in humans (Otten *et al*., 2006). This could be the reason for prescribing snail meat to hypertensive patients taking diuretic drugs against high blood pressure, which usually lead to potassium depletion due to excessive excretion of potassium in the urine (Youn & McDonough, 2009). Potassium is found to play a key role in muscular contraction and relaxation as well as in co-ordination of nerve cells (Moczydlowski, 2009). This is the reason why pregnant women are being advised by their doctors to consume snail meat and haemolymph during pregnancy, as this helps in the contraction and relaxation of cervical muscles during child birth (Adeyeye, 1996; Moczydlowski, 2009). Calcium and phosphorous which are also found at appreciable amounts in snails fed diets with varying inclusion levels of *M. oleifera* leaf meal suggests that intake of such snail meat can fight osteoporosis and assist in building strong teeth and bones. In addition, calcium also supports blood clotting and in synergy with magnesium, aids in nerves coordination (Engmann *et al*., 2013; Olagbende-Dada, 2015). This explains the external use of snail haemolymph to shorten the clotting time of open wounds that require immediate reduction of blood loss, such as in accidents and child delivery (Agbogidi *et al*., 2008; Olagbende-Dada, 2015). Imbalance in calcium and magnesium levels can incite the actions of nerve cells which may lead to convulsion in children or neurological disorders in adults (Cheng *et al*., 2005; Jahnen-Dechent & Ketteler, 2012). Incorporating snail haemolymph in the diets of children and adults could go a long way in suppressing convulsion in children and reducing neurological disorders in adults. Iron was discovered to increase in snails fed diets with varying inclusion levels of *M. oleifera* leaf meal compared to the control. This is likely because iron was discovered to be abundant in Moringa leaves but was found to be deficient in most plant leaves (Moyo *et al*., 2011).

The concentrations of sodium and chlorine were observed to be increased in all the treatments at the completion of the feeding trial. This is in line with the findings of Bamidele *et al*. (2018) and Ademolu *et al*. (2011) who recorded higher concentrations of chlorine and sodium in the haemolymph of *A. marginata* and *A. marginata* ecotypes- normal skin and albino snail, respectively. The function of sodium in nervous coordination is significant. Hence, increased level of sodium observed in the haemolymph of both species of snails across treatments could support the contraction and relaxation of their muscular foot (Bamidele *et al*., 2018).

4.4 Meat yield composition

Mixed feed is an excellent means of improving the meat quality in animals, including snails (Edet *et al*., 2017; Cheng *et al*., 2019). The inclusion of *M. oleifera* leaf meal in the formulated diets improved the meat quality of *A. marginata* and *A. fulica*. The reason for the improved performance of the snails is attributed to the nutrient-rich nature of the leaves of the plants, which are rich in proteins, fats, minerals and vitamins and have anti-microbial properties (Leone *et al*., 2015). Snails fed a diet containing 20 % *M. oleifera* leaf meal performed best in both species, probably due to the moderate crude protein and high ash content of the diet. This agrees with the finding of Ademolu *et al*. (2004) who reported that diets with crude protein contents in the range of 19.12 to 20.00 % enhanced the crude protein content in the flesh of African giant land snail (*A. marginata*). Additionally, snails fed diets with *M. oleifera* leaf meal showed higher ash contents than the control diet, underlying that *M. oleifera* leaves are not only rich in protein but also contain abundant minerals (essential minerals) necessary for the growth of the snails (Abiona *et al*., 2018).

5 Conclusion

This study has shown that the Moringa oleifera leaves can be efficiently utilised as feed ingredients in snail diets. The incorporation of *M. oleifera* leaf meal at 20 % in snail diets can boost growth performance, serves as a good source of minerals for grow-out snails as well as improve the nutritional make-up of snail meat. Future research should investigate the roles of different dietary levels of *M. oleifera* leaf meal on snails' reproductive performance and egg hatchability rate.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors wish to acknowledge in a special way the roles of Mr. B. U. Ononye for assisting in interpretation of the data analysis and Dr. T. C. Mogbo for identifying the two snail species.

References

Abdussamad, A. M., Osinowo, O. A., Smith, O. F., & Onadeko, S. A. (2010). Some haemolymph biophysical parameters in the giant African land snail *Archachatina marginata* during a six-week aestivation period. *Global Veterinaria*, 4, 400–408.

Abiona, J. A., Abioja, M. O., Fabinu, O. O., Ehimiyein, A. O., Ladokun, A. O., Olapeju, Y. A., Sodipe, O. G. , Oke, O. E.,& Onagbesan, O. M. (2018). Effect of *Moringa oleifera* on live weight and reproductive tract dimension of giant African land snail (*Archachatina marginata*). *Archiva Zootechenica*, 21(2), 81–90.

Aboagye, I. F., Mensah, D., & Boadu, F. (2015). Anthelmintic effect of *Moringa oleifera* Lam. In wild caught *Achatina achatina* Linnaeus, 1758 from the Sefwi Wiawso district, Ghana. *West African Journal of Applied Ecology*, 23, 27–33.

Aboagye, I. F., Nsiah-Yeboah, F., & Aboagye-Antwi, F. (2014). Assessment of roundworm infection in the organs of wild-caught *Achatina achatina* from two districts in the eastern and central regions of Ghana. *International Journal of Natural and Applied Science*, 10, 34–39.

Ademolu, K. O., Idowu, A. B., Mafiana, C. F., & Osinowo, O. A. (2004). Performance, proximate and mineral analyses of African giant land snail (*Archachatina marginata*) fed different nitrogen sources. *African Journal of Biotechnology*, 3, 412–417.

Ademolu, K. O., Jayeola, O. A., Dedeke, G. A., & Idowu, A. B. (2011). Comparative analysis of the growth performance and haemolymph biochemical properties of normal and albino Giant land snail- *Archachatina marginata*. *Ethiopian Journal of Environmental Studies and Management*, 4, 101–106. http://dx.doi.org/10.4314/ejesm.v4i2.11.

Adeyeye, E. I. (1996). Waste yield, proximate and mineral composition of three different types of land snail found in Nigeria. *International Journal of Food Sciences and Nutrition*, 47, 111–116.

Aduloju, A. R., Oyelami, B. A., & Alarape, A. B. (2019). Socio-economic analyses of snail consumption pattern in Ibarapa central local government, Oyo state. *Journal of Forestry Research and Management*, 16, 27–33.

Agbogidi, O. M., Okonta, B. C., & Ezeanya, E.L. (2008) Effects of two edible fruits on the growth performance of African giant land snail (*Archachatina marginata* Swainson). *Journal of Animal Protection Research*, 9, 546–577.

Amobi, M. I., & Ezewudo, B. I. (2019). Utilisation of common leafy vegetables in the diets of giant West African snail *Archachatina marginata* (Swainson, 1821) (Stylommatophora: Achatinidae). *Brazilian Journal of Biological Sciences*, 6, 181–187. https://doi.org/10.21472/bjbs.061217.

Amobi, M. I., Ezewudo, B. I., Okpoko, V. O., Ugokwe, C. U., & Okereke, H. N. (2019). Effects of three leafy vegetables on the growth performance of giant African snail *Achatina (Lissachatina) fulica*. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 120, 15–20. https://doi.org/10.17170/kobra-20190219195.

Ani, A. O., Ogbu. C. C., & Okonkwo, A. M. (2014). Growth response of African giant land snails (*Archachatina marginata*) to varying dietary levels of *Moringa oleifera* leaf meal. *Advances in Life Science and Technology*, 27, 21–27.

Anjorin, T. S., Ikokoh, P., & Okolo, S. (2010). Mineral composition of Moringa oleifera leaves, pods and seeds from two regions in Abuja, Nigeria. *International Journal of Agriculture and Biology*, 12, 431–434.

Anwar, F., Sajid, L., Muhammad, A., & Anwarul, H. G. (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research*, 21, 17–25.

AOAC (2005). *Association of official analytical chemists, official methods of analysis.* 15th edition Washington D.C. pp. 71-79.

Aromolaran, A. K., Alarima, C. I., Awotunde, J. M., & Olatunde, O. A. (2019). Willingness of snail consumers to choose snail production as livelihoods in Ibadan southwest local government area of Oyo state, Nigeria. *Journal of Tropical Agriculture, Food, Environment and Extension*, 18, 29–34.

Ayodele, A. E., Adeola, J. R., & Mayowa, A. T. (2014). Reproductive performance of rabbit does fed graded levels of *Moringa oleifera* leaf meal based diet. *International Journal of Sciences*, 3, 50–53.

Babalola, O. O., & Akinsoyinu, A. O. (2009). Proximate composition and mineral profile of snail meat from different breeds of land snail in Nigeria. *Pakistan Journal of Nutrition*, 8, 1842–1844.

Babalola, O. O., & Akinsoyinu, A. O. (2011). Proximate, mineral and biochemical evaluation of the haemolymph of growing snails fed fresh lettuce waste, whole lettuce, pawpaw leaves and cabbage waste. *Asian Journal of Agricultural Sciences*, 3, 1–4.

Bamidele, J. A., Ademolu, K. O., Idowu, A. B., Aladesida, A. A., & Oladele, A. O. (2018). Biochemical and nutritional composition of Giant African land snail (*Archachatina marginata*) from southwest Nigeria. *Pertanika Journal of Tropical Agricultural Science*, 41, 129–138.

Cheng, S., Lyytikainen, A., Kröger, H., Lamberg-Allardt, Alen, C., Koistinen, A., Wang, Q. J., Suuriniemi, M., Suominen, H., Mahonen, A., Nicholson, P. H., Ivaska, K. K., Korpela, R., Ohlsson, C., Väänänen, K. H., & Tylavsky, F. (2005). Effects of calcium, dairy product, and vitamin D supplementation on bone mass accrual and body composition in 10-12-y-old girls: a 2-y randomized trial. *The American Journal of Clinical Nutrition*, 82, 1115–1126. https://doi.org.10.1093/ajcn/82.5.1115.

Cheng, Y., Chen, Y., Li, J., Qu, H., Zhao, Y., Wen, C., & Zhou, Y. (2019). Dietary β-Sitosterol improves growth performance, meat quality, antioxidant status, and mitochondrial biogenesis of breast muscle in broilers. *Animals*, 9, 71. https://doi.org.10.3390/ani9030071.

Cobbinah, J. R., Vink, A., & Onwuka, B. (2008). *Snail Farming, Processing and Marketing*. Agromisa Foundation, Wayeningen. pp 78.

Colin, M., Xi, C., & Prigent, A. Y. (2012). The enrichment of rabbit feeds with long and short chains omega 3 fatty acids: an opportunity for the producer and the consumer. *Cuniculture Magazine*, 39, 33–43.

Cooper, J. E. (1994). Bleeding of pulmonate snail. *Laboratory Animals*, 28, 277–278. https://doi.org.10.1258/002367794780681606.

Edet, D. I., Ekwugha, E. U., Uluocha, O. B., & Nnanyereozo, B. I. (2017). Weight response and proximate analysis of African giant land snail, *Achatina achatina* (Linnaeus, 1758) fed with mixture of Moringa leaf powder and brewery waste. *International Journal of Agriculture and Rural Development*, 20, 2851–2855.

Engmann, F. N., Afoakwah, N. A., Darko, P. O., & Sefah, W. (2013). Proximate and mineral composition of snail (*Achatina achatina*) meat; any nutritional justification for acclaimed health benefits? *Journal of Basic and Applied Scientific Research*, 3, 8–15.

Funmilayo, S. M. (2008). Preliminary investigation of the growth performance of Giant Land Snail (*Archachatina marginata*) fed with selected household wastes. *African Journal of Agricultural Research*, 3, 647–649.

Hoffman, L. C., & Wiklund, E. (2006). Game and vension-meat for the modern consumers. *Meat Science*, 74, 197–208. https://doi.org/10.1016/j.meatsci.2006.04.005.

Jahnen-Dechent, W., & Kettler, M. (2012). Magnesium basics. *Clinical Kidney Journal*, 5, i3-i14. https://doi.org.10.1093/ndtplus/sfr163.

Khalafalla, M. M., Abdellatef, E., Dafalla, H. M., Nassrallah, A. A., Aboul-Enein, K. M., Lightfoot, D. A., El-Deeb, F. E., & El-Shemy, H. A. (2010). Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma. *African Journal of Biotechnology*, 9, 8467–8471. https://doi.org.10.5897/AJB10.996.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. *International Journal of Molecular Sciences*, 16, 12791–12835. https://doi.org.10.3390/ijms160612791.

Mahfuz, S., & Piao, X. S. (2019). Application of Moringa (*Moringa oleifera*) as natural feed supplement in poultry diets. *Animals*, 9, 431. https://doi.org.10.3390/ani9070431.

Moczydlowski, E. G. (2009). Electrophysiology of the Cell Membrane. In: Boron, W. F., & Boulpaep, E. L. (eds.), *Medical Physiology*, 2nd Ed. Saunders/Elsevier, Philadelphia.

Moyo, B., Masika, P. J., Hugo, A., & Muchenje, V. (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*, 10, 12925–12933. https://doi.org.10.5897/AJB10.1599.

Nnodim, A. U., & Ekpo, S. U. (2019). Factors constraining commercial farming of snail among farmers in rural areas of Rivers state. *International Journal of Rural Development, Environment and Health Research*, 3, 151–157.

Oguntoye, T. O., Fatoki, O. A., Adetola, O. O., Arowolo, O. V., & Tokede, A. M. (2020). Household's consumption pattern of snail (*Archachatina* species) in Oluyole local government area of Oyo state, Nigeria. *Journal of Applied Sciences and Environmental Management*, 24, 1267–1271.

Okon, B., Ibom, L. A., Sam, I. M., & Ibiang, N. O. (2017). Comparison of growth and heterosis of body parameters of two varieties of snail [*Archachatina marginata* (Swinson, 1821)] and their crosses reared under tropical conditions. *Journal of Animal Science and Veterinary Medicine*, 2, 1–7.

Okonta, B. O. (2012). Performance of giant African land snail *Archachatina marginata* (Swainson) fed with selected diets. *Global Journal of Bio-Science & Biotechnology*, 1, 182–185.

Olagbende-Dada, S. O. (2015). Effects of the bluish fluid (hemolymph) from the African giant snail (*Achatina marginata*) on the blood coagulation time and erythropoietic volume. *African Journal of Pharmacy and Pharmacology*, 9, 295–298.

Omole, A. J., & Kehinde, A. S. (2005). *Backyard snail farming at a glance: back to agricultural series (1).* Technovisor Agricultural Publications, Ibadan, Nigeria.

Orsa, T. J., Danjuma, J., & Tyowua, B. T. (2018). Growth performance and carcass characteristics of African giant land snail (*Archachatina marginata*) fed different vegetable based diets. *Advances in Life Science and Technology*, 66, 1–7.

Otten, J. J., Hellwig, J. P., & Meyers, L. D. (2006). *Dietary reference intakes: the essential guide to nutrient requirements.* National Academies Press, Washington D.C. pp. 370-379.

Ozoemene, M. I., Nwosu, A. C., Obikwelu, M. C., & Igboanugo, R. I. (2022). Examination of trend and patterns of rainfall and temperature in Awka, Anambra State. *Journal of Geography and Environment*, 5, 17–34.

Raut, S. K., & Barker, G. M. (2002). *Achatina fulica* Bowdich and other Achatinidae as Pests in Tropical Agriculture. In: Barker, G. M. (ed.), *Molluscs as Crop Pests*. CABI Publishing, Hamilton, New Zealand. pp. 55–114.

Sahay, S., Yadav, U., & Srinivasamurthy, S. (2017). Potential of *Moringa oleifera* as a functional food ingredient: a review. *International Journal of Food Science and Nutrition*, 2, 31–37.

Sanchez-Machado, D. I., Nunez-Gastelum, J. A., Reyes-Moreno, C., Ramirez-Wong, B., & Lopenz-Cervantes, J. (2010). Nutritional quality of edible parts of *Moringa oleifera*. *Food Analytical Methods*, 3, 175–180. https://doi.org/10.1007/s12161-009-9106-z.

Usunobun, U., & Egharebva, E. (2014). Phytochemical analysis, proximate and mineral composition and in vitro antioxidant activities in *Telfaria occidentalis* aqueous leaf extract. *Journal of Basic and Applied Sciences*, 1, 74–87.

Youn, J. H., & McDonough, A. A. (2009). Recent advances in understanding integrative control of potassium homeostasis. *Annual Review of Physiology*, 71, 381–401. https://doi.org.10.1146/annurev.physiol.010908.163241.