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Haemolymph and mineral composition of East African land snail (*Achatina fulica*) fed sundried mango seed kernel meal

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Abstract

A hundred and twenty (120) growing Achatina fulica snail were used to assess the impact of sundried mango seed kernel meal (MSKM) on the haemolymph and mineral composition of Achatina fulica. The mango seed kernel meal used as test ingredient were collected, cracked and the kernel sundried for two weeks after which it was milled into a fine powder. Four dietary treatments were formulated to meet the nutrient requirements of snails. The mango seed kernel meal was included at 0%, 5%, 10%, and 15%, representing Diet 1, Diet 2, Diet 3 and Diet 4 respectively. Diet 1 served as the control. Snails of similar weights were randomly allocated to four dietary treatments and replicated three (3) times in a completely randomized design (CRD) experiment. Each dietary treatment consisted of thirty (30) snails per treatment with ten (10) snails per replicate. The snails were fed ad libitum. At the end of the 56 days feeding trial, four (4) snails sampled from each replicate were randomly selected for haemolymph and mineral analysis. Data collected on different parameters were analyzed using one-way analysis of variance (ANOVA). The results showed that there were significant differences ($p<0.05$) on the mineral and proximate composition of haemolymph among the treatments. The experimental diets significantly increased the sodium (3.54ppm to 5.64ppm), potassium (6.54ppm to 10.46ppm), magnesium (1.56ppm to 7.38ppm), calcium (14.79ppm to 19.18ppm) and phosphorus (4.86ppm to 7.44ppm) contents of the haemolymph. Also, mango seed kernel meal increased the crude fiber (0.10% - 1.02%), fat content (0.47% - 1.48%), crude protein (2.45% - 3.12%) and the carbohydrate contents (3.94% - 6.56%) of the haemolymph of Achatina fulica. The 15% mango seed kernel meal inclusion had relatively better results in terms of the crude fiber, fat content, crude protein and carbohydrate. The inclusion of varying levels of sundried mango seed kernel meal did not have detrimental effects on the mineral and proximate compositions of haemolymph of Achatina fulica snails. This study has shown that sundried mango seed kernel meal can be used to replace maize in the diets of Achatina fulica without affecting the haemolymph negatively. The use of mango seed kernel meal as feed ingredient for snails is recommended as an innovative strategy in ameliorating the health hazards of mango waste pollution in our environment.

Keywords: Snail, Mango, Haemolymph, Minerals, *Archatina fulica*, Seed kernel

Introduction

One of the major tasks facing underdeveloped and developing countries of the world today is that of providing sufficient quantity and quality of food for its rapidly growing population (Eneji *et al.*, 2008). Many developing countries in are currently faced with the alarming drop in per capita income and food production (Eneji *et al.*, 2008). The shortage of food supply is becoming more serious than never before. The meager amount of animal protein in the diet of many Nigerians cannot be overemphasized. FAO had recommended 35g of animal protein per person per day for normal growth and

development, but most Nigerians consume less than the recommended (Oayide and Okpeze, 2007; FAO, 2007; 2015). Hence, efforts should be made on developing the micro livestock to complement the animal protein supply from the macro livestock.

Micro-livestock such as snail has great potential as good sources of animal protein for daily consumption. Land snails mainly dwell in the humid forest areas from where they are gathered by villagers for consumption and other uses (Ademolu ***et al.,*** 2007). Akinnusi (2002) stated that snail meat is highly nutritious, high in digestible protein, low in fat and compete favorably with poultry eggs and flesh in amount of essential amino acids. Many species of Giant Land snails exist in African such as *Archachatina marginata* (West African Land Snail), *Achatina fulica* (East African Land Snail) and *Achatina achatina* (Ghana Tiger Snail).

Snails feed invariably on everything digestible; including feed resources of plant origin, agricultural waste products and domestic waste (Omole ***et al.,*** 2007, 2011; 2012; Funmilayo, 2008; Hamzat and Longe, 2014). But such vegetables and fruit peels/waste alone cannot meet the nutrient requirements of snails for growth and reproduction (Ejidike, 2000; Agbogidi *et al*, 2008; Ebenebe ***et al.,*** 2014). Mango seed kernels appear to be a useful supplement in this regard.

Mango (*Mangifera indica*) is a perennial crop of the family *Anacardiacea*. It is an erect, branched, and medium to large sized tree with an alternately arranged evergreen leaves, having numerous flowers. It is grown practically all over tropical and sub-tropical regions of the world. The fruits are oval or kidney shaped with smooth, leathery skin and the color ranges from light or dark green to clear yellow when ripe. The pulp of the fruit is consumed fresh as a dessert or processed into juices, jams and other products, while the seeds are discarded which often results in environmental pollution (Morton, 1987; Van Ee, 1997; Gebremedhn and Alemu, 2015; Asma, 2017). The littering of roads, streets, our environments and refuse dump sites with mango seeds during mango seasons has constituted issues of concern to environmental health workers. Fresh mango seeds increases the multiplication of flies and other insects in our environments. The use of mango seed kernel as feed ingredient for snails can be an innovative strategy in ameliorating this health hazard in our environmental. Therefore, this work was designed to assess the effects of sundried mango seed kernel on the haemolymph and mineral compositions of East African Land snails.

Materials and methods

Experimental location

The experiment was carried out at the snail and edible insect section of the Teaching and Research farm of the Department of Animal Science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State. It lies within the rainforest region of the South Eastern Nigeria, having an annual rainfall of 1500mm and mean ambient temperature of about 34°c within longitude of 7°08'31"E and latitude 6°15'10'N. The location experiences seven months of heavy tropical rains (April-October), followed by five months of dryness (November-March).

Collection and preparation of mango seeds

Dried mango seeds were collected from different locations around Awka, Anambra State metropolis. Thereafter, the seeds were broken with a hammer, the kernels removed and sundried for 2 weeks to reduce the anti-nutritional factors, and to make the kernels easier to grind. The dried mango seed kernels were milled using a hammer mill to produce mango seed kernel meal. The mango seed kernel meal was stored in an airtight container, in a cool dry environment until when needed. The mango seed kernel

meal was used in formulating the experimental diets. *Four experimental diets were formulated to meet the nutrient requirements of snails included at 0%* mango seed kernel meal, 5% mango seed kernel meal, 10% mango seed kernel meal and 15% mango seed kernel meal; representing Diet 1, Diet 2, *Diet 3 and Diet 4respectively.* Diet 1 served as the control. The gross composition and proximate compositions of experimental diets are presented in Table 1 and Table 2 respectively, while the proximate composition of mango seed kernel meal is shown in Table 3.

Table 1: Gross Composition of the experimental diet

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4
Maize	43.25	36.25	31.25	26.25
Mango seed kernel meal	0.00	5.00	10.00	15.00
Soybean cake	30.00	30.00	30.00	30.00
Fish meal	5.00	5.00	5.00	5.00
wheat offal	10.00	10.00	10.00	10.00
Palm kernel cake	3.00	5.00	5.00	5.00
Bone meal	5.00	5.00	5.00	5.00
Limestone	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vit/min. Premix*	0.25	0.25	0.25	0.25
Total	100	100	100	100

***Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50. 00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium panthenate, 10mg; biotin,0.08mg;vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25 mg; selenium, 0.10mg; antioxidant, 200mg.**

Table 2: Proximate composition of the dietary treatments

Feed composition (%)	Diet 1	Diet 2	Diet 3	Diet 4
Crude protein	23.01	23.05	22.93	22.80
Crude fibre	4.08	4.50	4.73	4.95
Ether extract	3.54	3.95	4.32	4.70
Calcium	3.37	3.38	3.39	3.40
Phosphorus	1.16	1.16	1.16	1.15
Lysine	1.49	1.49	1.47	1.46
Methionine	0.65	0.64	0.63	0.62
Energy (ME Kcal/kg)	2693.05	2706.25	2744.75	2783.25

Table 3: Proximate composition of sundried mango seed kernel meal

Parameter (%)	Percentage composition
Moisture content	13.72
Ash content	4.00
Crude fiber	5.64
Fat and oil	15.94
Crude protein	2.10
Carbohydrate content	58.60
Total	100.00

Experimental animals and management

Housing and management of snails

A platform made from bamboo sticks constructed inside the snail house were used to hold the bowls which served as improvised housing unit according to treatments and replicates. The housing was roofed with zinc to prevent intrusion of

rainfall as the experiments were carried out during the rainy season. Hundred and fifty (150) snails of similar sizes, twelve (12) plastic bowls and mosquito netting materials were purchased from Ochanja market, Onitsha, Anambra state. The bowls were used as alternative housing unit for the snails. The bowl was covered with mosquito netting material to avoid intrusion of insects and mites and to prevent the snails from escape. The snails were acclimatized for one week after which hundred and twenty (120) snails of similar weights were used for the experiment. Care was taken during transportation and handling of snails to avoid cracking or breaking of the shells. The snails were fed *ad-libitum* once a day during the evening hours between 4.00-5.00pm local time. The feed was served with a feed saucer which was thoroughly cleaned daily and the soil subsequently moistened.

Collection and treatment of soil substrate

Humus soil was collected from the garden of Crop Science farm at the depth of 3cmand was sterilized by heating to kill soil micro-organisms and insects which might pose a threat to the performance and development of the snails. The top soil was removed weekly to prevent the soil from sticking together which is caused mostly by their slime, while the remaining soil is being tilled and moistened with water.

Identification of snails

The snails were marked with a permanent marker from 1-120 for easy identification and were randomly placed inside the bowl. The bowls were also marked to identify the treatments and replicates.

Proximate analysis

The proximate analysis of the mango seed kernel was carried out using the method of A.O.A.C (2000) and the following parameters were determined: moisture content, carbohydrate content, crude fiber, crude protein, ash and fat content.

Experimental design

The study was a completely randomized design (CRD) with 4 treatments *consisting of 0%* mango seed kernel meal, 5% mango seed kernel meal, 10% mango seed kernel meal and 15% mango seed kernel meal; representing Diet 1, Diet 2, *Diet 3 and Diet 4 respectively.* Each treatment was replicated 3 times in a completely randomized experimental design. Diet 1 served as the control. After acclimatization, 120 snails were randomly assigned to twelve (12) bowels according to the level of mango seed meal inclusion Diet 1, Diet 2, *Diet 3 and Diet 4.* Ten (10) snails were placed into each bowel with dimensions 15cm depth and a diameter of 56cm in a circular pattern filled with sterilized humus soil. The experimental model is as follows:

$\mathbf{Y}_{ij} = U + T_i + e_{ij}$

Where Y_{ij}= individual observation on the snail characteristics.

μ = overall mean

T_i = treatment effect

e_{ij} = random error assumed to be independently, identically and normally distributed with zero means and constant variances.

Data collection

At the end of the experiment, four (4) snails were randomly selected from each replicate and used for laboratory analysis. The snails were washed to remove soil particles. The haemolymph were extracted as recommended by Ademolu, ***et al.*** (2004 and 2006), Babalola and Akinsoyinu, (2011). The apex of the shell was opened and the snail haemolymph drained into a clean conical flask. The haemolymph samples were taken to the laboratory for both proximate and mineral analysis.

Data analysis

Data collected on different parameters were subjected to ANOVA in accordance with the methods of Steel and Torrie

(1980). Significant means were separated using Post Hoc Test according to Duncan's Multiple Range Test (Duncan, 1955).

Results and discussion

The results of the mineral analysis of haemolymph of ***Achatina fulica*** fed varying levels of sundried mango seed kernel meal is presented in Table 4.

Table 4: Mineral analysis of the haemolymph of *Achatina fulica* fed varying levels of sundried mango seed kernel meal

Minerals (ppm)	Diet 1 (0%)	Diet 2 (5%)	Diet 2 (10%)	Diet 2 (15%)
Sodium	3.54±0.23^{c}	4.78±0.77^{b}	5.18±2.45^{ab}	5.64±1.40^{a}
Potassium	6.54±0.83^{b}	9.27±0.11^{a}	10.46±1.82^{a}	9.65±0.25^{a}
Iron	0.12±0.76	0.10±0.65	0.16±0.04	0.13±0.81
Zinc	0.54±0.42	0.46±0.24	0.65±0.09	0.54±1.65
Copper	0.03±0.01	0.06±0.03	0.07±0.01	0.04±0.04
Manganese	0.03±0.04	0.05±0.07	0.04±0.01	0.03±0.01
Magnesium	1.56±0.34^{c}	4.77±0.58^{b}	3.78±1.05^{b}	7.38±0.74^{a}
Calcium	14.79±0.21^{c}	18.92±0.94^{a}	17.10±2.89^{ab}	19.18±1.55^{a}
Selenium	4.29±0.18	6.87±0.90	7.36±1.18	6.58±1.40
Phosphorus	4.86±0.60^{b}	6.25±0.71^{a}	6.29±2.10^{a}	7.44±1.94^{a}

***Data represents mean ± standard deviation.$^{abc:}$ Mean with different superscripts along rows aresignificantly different (P<0.05)**

The results in Table 4 shows that no significant differences (P>0.05) were observed on iron, zinc copper, manganese and selenium of the haemolymph of *Achatina fulica* snails fed varying levels of sundried mango seed kernel meal compared to the control. These mineral compositions are statistically similar with the control. This indicates that the inclusion of sundried mango seed kernel meal did not have any negative impact on these minerals. Furthermore, significant increases (P<0.05) were observed on sodium, potassium, magnesium, calcium and phosphorus of *Achatina fulica* snails fed varying levels of sundried mango seed kernel meal compared to the control. The experimental diets significantly increased the sodium, potassium, magnesium, calcium and phosphorus contents of the haemolymph of the snails compared to the control. The increased on these minerals might be attributed to the sodium, potassium, magnesium, calcium and phosphorus content of mango seed kernel. Several researchers who studied the chemical and phytochemical composition of mango seed kernel have documented that it has appreciable amount of these minerals (Dangoggo *et al*, 2001; Fowomola, 2010; Ogunsina *et al*, 2012 and Orijajogun *et al.*, 2014).

The result of proximate composition of the haemolymph of *Achatina fulica* fed varying levels of sundried mango seed kernel meal is presented in Table 5.

Table 5: Proximate composition of the haemolymph of *Achatina fulica* fed varying levels of sundried mango seed kernel meal

Parameter (%)	Moisture content	Ash content	Crude Fibre	Fat content	Crude Protein	Carbohydrate content
Diet 1	91.93±0.84^{a}	1.11±0.02^{a}	0.10±0.00^{c}	0.47±0.01^{c}	2.45±0.06^{c}	3.94±0.06^{c}
Diet 2	90.74±0.22^{a}	0.99±0.02^{a}	0.17±0.00^{bc}	0.64±0.01^{c}	2.94±0.02^{b}	4.52±0.06^{bc}
Diet 3	89.20±0.04^{a}	1.00±0.03^{a}	0.31±0.01^{b}	0.99±0.01^{b}	2.80±0.58^{b}	5.70±0.06^{b}
Diet 4	86.46±0.12^{b}	1.36±0.02^{a}	1.02±0.01^{a}	1.48±0.04^{a}	3.12±0.12^{a}	6.56±0.01^{a}

***Data represents mean ± Standard deviation. $^{abcd:}$ Mean with different superscripts along columns are significantly different (P<0.05).**

The results of proximate analysis of the haemolymph showed that there were significant differences on the moisture content, crude fiber, fat, crude protein and carbohydrate contents of *Achatina fulica* snails fed dietary inclusions of sundried mango seed kernel meal compared to the control. The moisture contents ranges between 91.93% in Diet 1 and 86.46% in Diet 4, while the ash content increased from 0.99% in Diet 2 to 1.36% in Diet 4. The fiber contents ranges between 0.10% in Diet 1 and 1.02% in Diet 4, while the fat content was between 0.47% in Diet 1 and 1.48% in Diet 4. The crude protein ranges between 2.45% in Diet 1 and 3.12% in Diet 4, while the carbohydrate content of the haemolymph increased from 3.94% in Diet 1 to 6.56% in Diet 4. The inclusion of mango seed kernel meal in the diets of *Achatina fulica* reduced the moisture contents of the haemolymph. Also, the increase on the inclusion levels of the sundried mango seed kernel meal increased the crude fiber, fat content, crude protein and the carbohydrate contents of the haemolymph of *Achatina fulica.* And this increase becomes more obvious as the levels of inclusions of mango seed kernel meal increased. Therefore, the significant increases on these parameters of the haemolymph is attributable to the appreciable amount of crude fiber, fat content, crude protein and the carbohydrate contents of mango seed kernel as seen in Table 3.

From the foregoing results, it could be inferred that sundried mango seed kernel meal can be used to replace maize in the diets of *Achatina fulica* with respect to the mineral and the proximate compositions of *Achatina fulica haemolymph.*

Conclusion and recommendations

The study on the haemolymph and mineral analysis of *Achatina fulica* fed varying levels of sundried mango seed kernel meal have shown that sundried mango seed meal did not have detrimental effects on the mineral and proximate compositions of haemolymph of *Achatina fulica* snails. This study has also shown that sundried mango seed kernel meal can be used to replace maize in the diets of *Achatina fulica without* affecting the haemolymph negatively. Therefore, the use of mango seed kernel meal as feed ingredient for snails is recommended as an innovative strategy in ameliorating the health hazards of mango waste pollution in our environmental.

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