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# Impact of sundried mango seed kernel meal on the haemolymph of giant African snail *(Archachatina marginata)*

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**Abstract**

*Proximate analysis of mango seed kernel shows that it is high in carbohydrate and contains other essential minerals. Thus, this experiment was conducted to assess the impact of mango seed kernel meal on the haemolymph of giant African land snail (Archachatina marginata). One hundred and twenty (120) Archachatina marginata grower snails of similar sizes were randomly assigned to four treatments designated $T_1$, $T_2$, $T_3$ and $T_4$ and replicated three times in a completely randomized design (CRD). Each dietary treatment consisted of thirty (30) snails per treatment with ten (10) snails per replicate. 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 experimental diets were formulated to meet the nutrient requirements of snails included at 0% mango seed kernel meal ($T_1$), 5% mango seed kernel meal ($T_2$), 10% mango seed kernel meal ($T_3$) and 15% mango seed kernel meal ($T_4$), respectively. $T_1$ served as the control. The snails were fed once a day during the evening hours and adequately managed during this period. The feeding trial lasted for eight weeks. Proximate composition of the mango seed kernel meal and that of the experimental diets were conducted to determine the nutrient contents of the mango seed kernel meal and the experimental diets respectively. The proximate composition and the mineral content of the haemolymph of Archachatina marginata were also analyzed. The analysis of the proximate composition of mango seed kernel meal indicated that it is high in carbohydrate (58.6%), 13.72% moisture, 4.00% ash, 5.64% crude fiber, 15.94% fat, and 2.1% crude protein; which implies that it can be used as a carbohydrate source. The inclusion of mango seed kernel meal in the diets of Archachatina marginata significantly ($P<0.05$) increased the protein (2.80% in $T_1$ to 4.90% in $T_4$) and carbohydrate content (2.08% in $T_1$ to 5.6% in $T_2$); and reduced the moisture (93.39% in $T_1$ to 88.40% in $T_4$) and fat contents (0.80% in $T_1$ to 0.15% in $T_2$) of the haemolymph. The mineral analysis of snail haemolymph showed that it had varying effects on the mineral composition of the haemolymph with no deleterious effects up to 15% inclusion in the diets.*

**Keywords:** Snail, Mango, Haemolymph, ***Archachatina marginata***, Seed kernel

## Introduction

Animal protein plays a very important role in food and nutrient supply of human society. This is as a result of the relatively high content of essential amino acids of animal origin compared to proteins of plant origin, deficient in essential and limiting amino acids (Adebayo, 2003). Food and Agricultural Organization (FAO) recommended 35g of animal protein per person per day for normal growth and development (FAO, 2007, 2015). Beef, pork and mutton have been the major source of animal protein in Nigeria and Africa (Mallik and Dikko, 2009). However, these animal protein sources have continued to diminish in its capacity to meet the demands of the populace due to persistent drought, disease, primitive animal husbandry technique and low

productivity by local animal breeds. In the area of cattle production, the farmer-herdsmen conflicts have continued to affect animal production in Nigeria. High cost of feed and feed ingredients has been a threat to poultry industry in Nigeria (Oji, 2000; Adeyomo and Adeyomo, 2009; Ogogo *et al.,* 2011; Uwalaka and Ahaot, 2013). Therefore, there is need to harnessed other alternative animal protein sources that are equally nutritious and relatively cheap capable of bridging the gap between animal protein demand and supply. The challenge therefore falls on the micro livestock such as snail.

Giant African snail *(Archachatina marginata)* has voracious appetite (Agbogidi *et al.,* 2008; Agbogidi and Okonta, 2011 and Okonta, 2012). They are known to eat more than 500 different types of plant, fruits, vegetables, ornamental plants and many more. Their food comprises of fresh leaves/shoots (pawpaw, lettuce, cabbage, cassava, cocoyam, African spinach, waterleaf); ripe fresh fruits (pawpaw, banana, plantain, mango) and household/agricultural wastes (Omole *et al.,* 1999; 2007; Oayide and Okpeze, 2007; Funmilayo, 2008). Snail ability to utilize readily available feeding material to achieve appreciable weight under intensive management makes it suitable and cheaper alternative animal protein sources (Adedire, *et al*, 1999; Akinnusi, 1997, 1998, 2000; Akachuku, 2008; Okonta, 2012; Ademolu *et al*, 2013). There are need to assess other alternative feed resources for snail. One of such feed resource is mango seed kernel.

Mango (*Mangifera indica*) is an erect, branched, and medium to large sized tree with an alternately arranged evergreen leaves, with a wide crown and inflorescences having numerous flowers (Van Ee, 1997). The kernel is a good source of carbohydrate (58-80%), moderate protein (6-13%) and fat (6-16%) (Elegbede and Achoba, 1996; Diarra and Usman, 2008). It has the ability to supplement methionine and lysine **which**is limited in plant protein feed stuffs. The oil is also a good **source** of stearic acid and linoleic acid (Arogba, 1989).Therefore, this study was aimed to determine the proximate composition of mango seed kernel meal, evaluate the proximate composition and mineral composition of haemolymph of ***Archachatina marginata*** fed processed mango seed kernel meal.

## 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°08'31.9"E and latitude 6°15'10.1'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 (***Mangifera indica*****)** 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 $T_1$, $T_2$, $T_3$, $T_4$ *respectively.* $T_1$ served as the control. The gross composition and proximate compositions of experimental diets are presented in Table 1 and 2, respectively.

**Table 1: Gross Composition of the experimental diet (%)**

| Ingredients (%) | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| 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 pantot henate, 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 (%) | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| 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 |

***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 watered.

***Collection and treatment of soil substrate***

Humus soil was collected from the garden of Crop Science farm at the depth of 3cm and 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.

***Moisture content***

A petri-dish was washed and dried in the oven. 2g of mango seed kernel meal was weighed into a petri dish. The weight of the petri-dish and sample was noted before drying. The petri dish and sample were put in the oven and heated at 100°c for 1hour the result noted and heated another 1hour until a steady result is obtained and the weight noted. The drying procedure was continued until a constant weight was obtained.

% moisture content was calculated thus:

$\frac{W_1 - W_2}{\text{Weight of sample}} \times 100$

Where

$\mathbf{W_1}$ = weight of petri dish and sample before drying

$\mathbf{W_2}$ = weight of petri dish and sample after drying.

***Carbohydrate determination (differential method)***

100- (%Protein + %Moisture + %Ash + %Fat + % Fiber)

***Ash content***

The empty platinum crucible was washed, dried, weighed and noted. 2g of mango seed kernel meal was weighed into the platinum crucible and placed in a muffle furnace at 500°c for 3 hours. The sample was cooled in a desiccator after burning and weighed. The percentage ash content was calculated thus:

$$\textbf{\% Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

**Where:**

$\mathbf{W_1}$ = weight of empty platinum crucible,

$\mathbf{W_2}$ = weight of platinum crucible and sample before burning

$\mathbf{W_3}$ = weight of platinum and ash.

***Crude fibre content***

2g of mango seed kernel meal was defatted with petroleum ether. It was boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of $\mathbf{H_2SO_4}$ **per** 100ml of solution. The solution was filtered through linen or several layers of cheese cloth on a fluted funnel. It was wash with boiling water until the washings are no longer acidic. The residue was transferred to a beaker and boil for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible. It was dried in an electric oven and weighed. It was incinerate, cool and weigh again. The loss in weight after incineration x 100 became the percentage crude fiber. The crude fiber is calculated thus:

$$\textbf{\% Crude fibre} = \frac{\text{weight of fibre}}{\text{weight of sample}} \times 100$$

***Crude fat (Soxhlet fat extraction method)***

This method was carried out by continuously extracting a food with non-polar organic solvent such as petroleum ether for about 1 hour or more.250ml clean boiling flasks was dried in oven at 105 - 110°c for about 30 minutes. It was transfer into a desiccator and allowed to cool. Cooled boiling flasks were labeled and weighed. The boiling flasks were filled with about 300ml of petroleum ether with boiling point of about 40 - 60°c. The extraction thimble was plugged lightly with cotton wool. The soxhlet apparatus was assembled and allow to reflux for 6 hours. The thimble was carefully removed and the collected petroleum ether in the top container of the set - up and drain into a container for reuse. The flask was removed and dries as the flask was almost free of petroleum ether at 105°c - 110°c for 1 hour. The substance was transferred from the oven into a desiccator, cooled and finally weigh.

***Percentage crude proteins***

The method was the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample was reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution was made alkaline, and was distilled to release the ammonia. The ammonia was trapped in dilute acid and then titrated. 0.5g of mango seed kernel meal sample was weighed into a 30ml kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were shaken. Then 0.5g of the kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling about 100ml of distilled water was added to avoid caking and then 50ml was transferred to the kjedahl distillation apparatus. A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of bromocresol blue and 1 drop of methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution. The 5ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink color using 0.0IN hydrochloric acid. Percentage Nitrogen and protein was Calculated thus:

**% Nitrogen** = Titre value × 0.01 × 14×4

**% Protein** = % Nitrogen x 6

***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 $T_1$, $T_2$, $T_3$, $T_4$ *respectively.* Each treatment was replicated 3 times in a completely randomized experimental design. $T_1$ served as the control. After acclimatization, 120 snails were randomly assigned to twelve (12) bowels according to the level of mango seed meal inclusion $T_1$, $T_2$, $T_3$ and $T_4$. 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:

$Y_{ij} = U + T_i + e_{ij}$

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

$\mu$ = 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 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; 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** Proximate composition of mango seed kernel meal is presented in Table 3.

**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 |

The sun dried mango seed kernel meal used in the experiment contained 13.72% moisture, 4.00% ash, 5.64% crude fibre, 15.94% fat and oil, 2.1% crude protein and 58.6% carbohydrate, which implies that it can be used as a carbohydrate source. This agrees with the findings of Diarra *et al.* (2008) that mango seed kernel is a good source of carbohydrate (58-80%), moderate protein (6-13%), and fat (6-16%). Arogba (1989) reported that mango seed kernel meal has the ability to supplement methionine and lysine which is limited in plant protein feed stuffs. The ash content is similar to the values reported by Elegbede and Achoba (1996). The crude fibre was, however, lower than that reported by Faniyi (1998). These discrepancies might be due to differences in varieties of mango used, processing methods or the degree of ripeness before processing.

**Table 4: P roximate composition of *A. marginata* haemolymph fed graded levels of mango seed kernel meal**

| Treatment | Moisture Content (%) | Ash Content (%) | Fibre Content (%) | Fat Content (%) | Protein Content (%) | Carbohydrate (%) | Total |
|---|---|---|---|---|---|---|---|
| $T_1$ (0%) | $93.39\pm0.06^{a}$ | $0.49\pm0.0l^{b}$ | $0.44\pm0.06^{bc}$ | $0.80\pm0.02^{a}$ | $2.80\pm0.05^{b}$ | $2.08\pm0.06^{c}$ | 100.00 |
| $T_2$ (5%) | $87.41\pm0.06^{c}$ | $1.95\pm0.03^{a}$ | $0.69\pm0.01^{b}$ | $0.15\pm0.01^{c}$ | $4.20\pm0.02^{a}$ | $5.60\pm0.06^{a}$ | 100.00 |
| $T_3$ (10%) | $90.88\pm0.06^{bc}$ | $0.40\pm0.01^{b}$ | $0.28\pm0.01^{c}$ | $0.30\pm0.01^{b}$ | $4.38\pm0.06^{a}$ | $3.76\pm0.02^{b}$ | 100.00 |
| $T_4$ (15%) | $88.40\pm0.06^{c}$ | $0.38\pm0.01^{b}$ | $1.78\pm0.06^{a}$ | $0.35\pm0.01^{b}$ | $4.90\pm0.06^{a}$ | $4.19\pm0.06^{b}$ | 100.00 |

***Data represents mean ± Standard error of mean. [abcd]: Mean with different superscripts along columns aresignificantly different (P<0.05).**

The results of the proximate composition of snail haemolymph showed that there were significant differences($P<0.05$) on the moisture content, ash content, fiber, fat, protein and carbohydrate contents of the haemolymph of *Archachatina marginata* snails fed the experimental diets compared to the control.$T_1$ had the highest moisture content than those fed dried mango seed kernel meal. $T_2$ had the highest ash content of the snail haemolymph with statistically similar results for the control and the other treatments. The protein and the carbohydrate contents were significantly lower in the control diets, while the fat contents of the haemolymph increased in $T_1$ compared to those fed mango seed kernel meal. The fibre content was higher in $T_4$. From the foregoing results, it is observed that the inclusion of the mango seed kernel meal had varying effects on the proximate compositions of the haemolymph of *A. marginata*. Specifically, the inclusion of the mango seed kernel meal in the diets of *Archachatina marginata* increased the protein and carbohydrate content; and reduced the moisture and fat contents of the haemolymph. The mineral composition of *Archachatina marginata* haemolymph fed graded levels of mango seed kernel meal is presented in Table 5.

**Table 5: Mineral composition of *A. marginata* haemolymph fed graded levels of mango seed kernel meal**

| Parameters (ppm) | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| Sodium | 5.537±0.43$^{ab}$ | 4.653±0.38$^{b}$ | 6.536±0.21$^{a}$ | 4.893±0.17$^{b}$ |
| Potassium | 11.632±0.89$^{a}$ | 9.464±0.73$^{b}$ | 9.474±0.43$^{b}$ | 8.474±0.39$^{b}$ |
| Iron | 0.080±0.02$^{b}$ | 0.059±0.04$^{c}$ | 0.101±0.05$^{a}$ | 0.139±0.07$^{a}$ |
| Zinc | 1.221±0.01$^{a}$ | 0.498±0.11$^{c}$ | 0.872±0.20$^{b}$ | 0.767±0.19$^{b}$ |
| Copper | 0.083±0.04$^{b}$ | 0.055±0.01$^{bc}$ | 0.229±0.06$^{a}$ | 0.038±0.02$^{c}$ |
| Manganese | 0.000 | 0.000 | 0.000 | 0.000 |
| Magnesium | 1.220±0.60$^{c}$ | 1.339±0.40$^{bc}$ | 1.935±0.21$^{a}$ | 1.634±0.95$^{a}$ |
| Calcium | 12.574±0.80$^{b}$ | 6.717±0.38$^{c}$ | 14.648±0.46$^{a}$ | 11.352±0.43$^{b}$ |
| Phosphorus | 3.863±0.15$^{c}$ | 4.942±0.77$^{b}$ | 5.888±0.19$^{ab}$ | 6.292±0.86$^{a}$ |

***Data represents mean ± standard error of mean . abcd: Mean with different superscripts along rows are significantly different (P<0.05).**

From Table 5, all the mineral composition parameters analyzed were statistically ($P<0.05$) different compared to the control. The inclusion of mango seed kernel meal had varying effects on the mineral composition of the haemolymph *of Archachatina marginata*. The highest amount of sodium(6.536ppm) was observed on $T_3$, $T_1$ has the highest amount of potassium (11.632ppm), $T_4$ has the highest amount of iron (0.139ppm), $T_1$ has the highest amount of zinc (1.221ppm), $T_3$ has the highest amount of copper with (0.229ppm), none of the treatments contain manganese, $T_3$ has the highest amount of magnesium with (1.935ppm), $T_3$ has the highest calcium with (14.6476ppm) and while $T_4$ had the highest amount of phosphorus (6.292ppm).The effects of mango seed kernel meal on the mineral compositions of the haemolymph did not follow any specific pattern; thus, its representation with a chart.

The mineral content of *Archachatina marginata* haemolymph fed graded levels of mango seed kernel meal is presented in Chart 1.

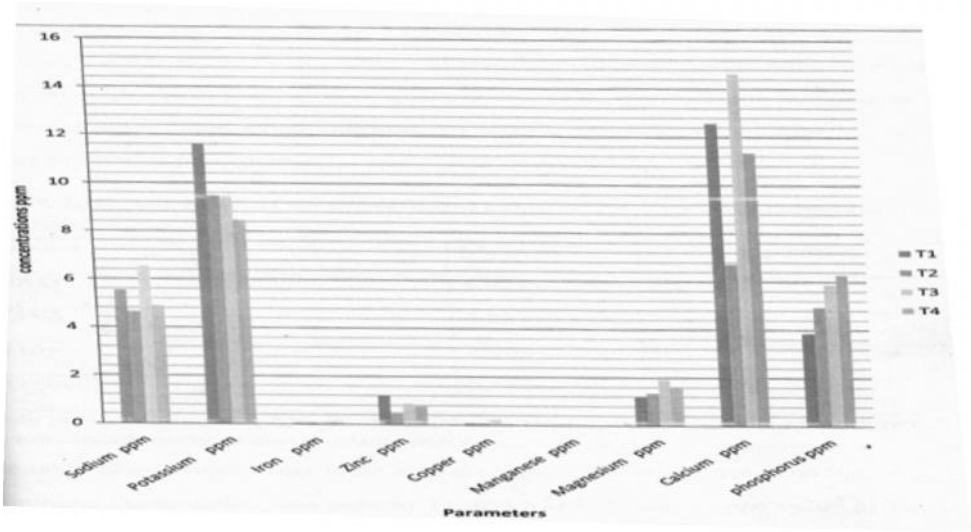


**Chart 1: Mineral content of *Archachatina marginata* haemolymph fed graded levels of mango seed kernel meal**

Chart 1 shows that $T_3$ has the highest amount sodium, $T_1$ has the highest amount of potassium, $T_4$ has the highest amount of iron, $T_1$ has the highest amount of zinc, $T_3$ has the highest amount of copper, none of the treatments contain manganese, $T_3$ as the highest amount of magnesium, $T_3$ has the highest calcium and $T_4$ has the highest amount of phosphorus. The chart also showed that inclusion of mango seed kernel meal had varying effects on the mineral composition of the haemolymph ***of Archachatina marginata*.**

**Conclusion and recommendation**

From the foregoing results, it could be concluded that the inclusion of the mango seed kernel meal had varying effects on the mineral compositions of the haemolymph of *A. marginata*. The inclusion of mango seed kernel meal in the diets increased the protein and carbohydrate content; and reduced the moisture and fat contents of the haemolymph of *Archachatin amarginata*. Furthermore, the proximate analysis of sun dried mango seed kernel meal showed that it contained 13.72% moisture, 4.00% ash, 5.64% crude fiber, 15.94% fat and oil, 2.1% crude protein and 58.6% carbohydrate, which implies that it can be used as a carbohydrate source. The utilization of mango seed kernel meal in feeding snails will help to reduce the wastage of mango seed in our environment thereby help to increase animal protein production in Nigeria.

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