Figure 1: West African Dwarf (WAD) goat with cutaneous papillomatosis

Effect of common salt (Nacl) on haemocyte count and haemolymp biochemical parameters of Giant African Land snail (*A. marginata*) during dry season

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Abstract

A study was conducted on effect of common salt (NaCl) on haemocyte count and haemolymph biochemical parameters of Giant African Land snail (*Archachatina marginata)*. Forty (40) snails with weight range of 150g to 180g were used for this study. Four (4) treatment with each consisting of ten (10) replicate include: T1 (Control: concentrate + 0g of salt (NaCl)), T2 (concentrate + 0.05g of salt (NaCl)), T3 (concentrate + 0.15g of salt (NaCl) and T4 (concentrate + 0.25 of salt (NaCl)). The experiment lasted 13 weeks. Haemolymph were collected at the end of 13 weeks. The parameters measured include: haemocyte count, total protein, albumin, globulin, alanine transaminase (ALT) and aspartate transaminase (AST). The haemocyte count was determined using Nubeurheamocytomer, while haemolymph biochemical parameters were determined using spectrophotometric methods. Mortality record was taken as the experiment progresses. Result showed that serum biochemical parameters were not significantly ($P>0.05$) affected by various level of salt used. However, common salt (NaCl) significantly affected ($P<0.01$) haemocyte count and mortality record of GALS. Snails fed 0.25g NaCl had the highest haemocyte count, followed by those fed 0.15g while snails fed 0g and 0.05g recorded the least. It was also discovered that 0

g Nacl inclusion into the diet of snail (A. marginata) recorded the highest mortality (60%) followed by 0.05 g Nacl inclusion level which gave 30 % mortality. However, inclusion level of 0.15 g and 0.25g Nacl into snail feed recorded no mortality.It is obvious from this study that NaCl had significant effect on haemocyte count and mortality record of A. marginata without negatively affecting the haemolyme biochemical parameter of GAL. It is therefore recommended that both 0.15 g and 0.25g/kg be included into diet of *A. marginata* .
Keywords: Sodium chloride, Haemolymph biochemical parameters, Haemocyte,

Introduction

Dry season is known to be a period in which all reproductive activities partially or totally seized depending on the degree of dryness. Feed consumption during this period may be affected depending on the housing condition. It is therefore important to consider feed fortification during this period to avoid mortality. One of such approach is to include appropriate vitamins or salts into the diet of this animal to stabilize production during this season. The use of salt (Nacl) in snail feed has being a subject of debate for decades due to the belief that snails do not consume salt. Machiels and Henken (1987) asserted that salt promote weight gain, Wu *et al*.(2017) reported it positive influence on oesteoclast differentiation. It has also been reported to modulate homeostatic accessory function of renal mononuclear phagocytes (Schatz *et al*., 2017). Cytokines chemokines and tumor necrosis factor release had also been reported to be influenced by Nacl (Long et al., 2002; Kostvk *et al*., 2006). In order to maintain the wellbeing of this animal during the dry season, this study therefore aimed at investigation the effect of common salt (Nacl) on haemocyte count and haemolymph biochemical parameters of *A. marginata* during the dry season.

Material and Method

Experimental Site:

This experiment was carried out at the snail Unit of College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun-State. The location lies within the rainforest belt of Western Nigeria (latitude 7^0 N, longitude 3^0 2' E and altitude 76m.a.s.l.). The climate is humid with a mean annual rainfall of 1,037mm, mean temperature of 34.7^0 and mean relative humidity of 82% .

Materials

A total of forty snails (40) *Archachatina marginata* weighing between 150g to 180g were used for this experiment. The snails were kept in plastic cages with dimension of 30 cm by 40 cm by 24 cm, with small plastic feeding and drinking troughs in each of the cages.

Snails and their management

The plastic cages were clean prior to the commencement of the experiment; two weeks was set aside as a period of acclimatization. The snails were fed *ad libitum* throughout the period of the experiment. Drinking water was also provided daily *ad-libitum* in drinking troughs. Feed and water troughs were washed daily while the cages were also clean daily. The experiment lasted for thirteen (13) weeks.

Experimental design

The snails were randomly assigned to four treatments with ten (10) replicates each. The experimental treatments consisted of inclusion of 0, 0.05, 0.15 and 0.25 g/kg of the concentrate diet. Summarized below is the experimental set up:

Treatment 1: 0 gram salt inclusion
Treatment 2: 0.05 gram salt inclusion

Treatment 3: 0.15 gram salt inclusion
Treatment 4: 0.25 gram salt inclusion
Concentrate was given to the animal on a daily basis. Table 1 shows the composition of concentrate supplement (g) for treatments.
Table 1: Composition of concentrate supplement (g) for treatments

INGREDIENT	Composition			
	T_1	T_2	T_3	T_4
Maize	50.00g	50.00g	50.00g	50.00g
Wheat Offal	27.75g	27.70g	27.65g	27.50g
Groundnut cake	12.25g	12.25g	12.25g	12.25g
Soya bean meal	4.00g	4.00g	4.00g	4.00g
Bone meal	3.00g	3.00g	3.00g	3.00g
Oyster shell	3.00g	3.00g	3.00g	3.00g
Salt	0.00g	0.05g	0.15g	0.25g
Total	100g	100g	100g	100g

Haemolymph collection and total haemocyte count
Haemolymph from ten (10) snails per treatment were collected via the anterior part of the head with the aid of needle at the end of thirteen (13) weeks. A dilution of 1:19 was made with the aid of 5% eosin solution which was loaded into improved haemocytometer. Haemocyte found in the four squares were counted. Thereafter, numbers of cells counted were multiplied by a conversion factor (50,000) to obtain the total haemocyte count.

Haemolymph biochemical parameters
Haemolymph total protein, albumin, AST and ALT were determined via the usage of spectrophotometric method with the use of appropriate kits. Globulin values were calculated by subtracting total protein values from albumin values.
Mortality Record
The total numbers of snail that die under each treatment were recorded as the weeks of experiment increases. Thereafter, percentage mortality was calculated.
RESULTS AND DISCUSSION

Table 2 shows the least square means showing effect of salt (NaCl) on heamocyte count. Result showed that inclusion of 0.25g of salt in concentrate feed had the highest mean followed by 0.15g 0.05g while 0g had least haemocyte count. From the result it can be deduced that salt inclusion into the diet of snail has an immunomodulatory effect which is in agreement with the findings of Machiels and Henken (1987), Wu *et al*.(2017) and Schatz *et al*. (2017) since haemocytes are known to be the specialized immune cells in mollusc. Effect of Salt (NaCl) on haemolymph

biochemical parameters of Giant African Land snail (A. marginata) is shown in Table 3. All the biochemical parameters determined were not significantly influenced by various levels of salt used in this study. This observation is an indication that various levels of the salt used do not negatively affect the system of the animal. This means that normal biochemical part-ways were not affected; it only mobilized the haemocytes to be ready to eliminate any opportunistic infection that may alter the system of the animal negatively during this period.

Table 4 showed that snails fed 0g of Nacl recorded highest mortality (60%), followed by those fed 0.05g (30%) while those that were fed 0.15g and 0.25g of common salt were not affected by the salt inclusion i.e had no mortality. The reason for this observation could be as a result simulative role played by Nacl in the release of immune cells (Machiels and Henken, 1987; 2017; Schatz *et al*., 2017, Wu *et al*.,)

Conclusion

It was made clear from this study that Nacl significantly influenced haemocyte count and mortality pattern of *A. marginata* fed different levels without necessarily obstructing the haemolymph biochemical parameters. It is therefore recommended that both 0.15 g and 0.25g/kg be included into diet of *A. marginata* .

Table 2: Least square means showing the effect of treatment on haemocyte count of GALS.

Treatment	Least square mean (x 10^6) ±S.E.M
0g salt inclusion	10.723 ± 1.215^{c}
0.05g salt inclusion	11.623 ± 0.918^{b}
0.15g salt inclusion	12.187 ± 0.768^{b}
0.25g salt inclusion	15.502 ± 0.768^{a}

Means on the same column with different superscript differs significantly (P<0.01)

Table 3: Effect of Salt (NaCl) on haemolymph biochemical parameters of Giant African Land snail (GALs) (*A. marginata*)

TREATMENT	TP	ALB	GLOB	AST	ALT
T1(0g)	8.84±1.77	1.36±0.37	7.47±1.58	21.75±8.37	81.25±12.20
T2(0.05g)	6.43±1.77	0.72±0.37	5.71±1.58	28.25±8.37	76.50±12.20
T3 (0.15)	9.86±1.77	1.33±0.37	8.54±1.58	23.50±8.37	97.75±12.20
T4(0.25)	8.08±1.77	0.89±0.37	7.19±1.58	34.50±8.37	85.00±12.20

Table 4: effect of common salt (Nacl) on mortality record of GALs (*a.marginata).*

TREATMENT	% MORTALITY
0g NaCl + Concentrate	60
0.05g NaCl + Concentrate	30

0.15g NaCl + Concentrate	0
0.25g NaCl + Concentrate	0

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