GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN AFRICAN GIANT LAND SNAIL (*Archachatina marginata* Swainson, 1821) FED DIETS SUPPLEMENTED WITH ENZYMES, PREBIOTIC AND PROBIOTICS

By

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CERTIFICATION

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DEDICATION

Absolutely dedicated to the Glory of God the Father, God the Son, and God the Holy Spirit.

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ABSTRACT

African Giant Land Snails (AGLS) (*Archachatina marginata*) are a good source of protein with low-fat content but with slow growth rate which may be due to genetic or nutritional factors. Conventional high fibre feed resources fed to AGLS have a marginal nutrient profile. Growth enhancers such as enzymes, prebiotics, probiotics, and organic acids have been used to optimise productivity in other animals but reports on their use in AGLS nutrition is scanty. Therefore, effects of diets supplemented with enzyme, prebiotics and probiotics on performance of AGLS were investigated.

Grower AGLS (n=240), weighing 92.03±1.55 g, procured from Ikire market, Osun State and certified at the Department of zoology, University of Ibadan. They were allotted to diets containing 0.00 (T_1, Control), 0.10 (T_2), 0.15(T_3) and 0.20 g/kg (T_4) *β-D-mannanase* in five replicates for 98 days. Grower AGLS (n=350), weighing 100.08±1.73 g were allott ed to another seven compounded diets containing 0.00 (M_1, Control), 0.1 mg/kg oxytetrac ycline (M_2), 0.50(M_3), 1.00(M_4), 1.50(M_5), 2.00(M_6) and 2.50 g/kg(M_7) mannanoligosacch aride plus organic acids blend in five replicates for 98 days. Another batch of grower AGLS (n=400) weighing 94.12±1.77 g were allotted to eight different diets containing 0.00 probiotics (D_1, Control), 0.1 mg oxytetracycline/ kg (D_2), $1x10^8$ (D_3), $2x10^8$ (D_4) and $3x10^8$ CFU *Lactobacillus plantarum* (Lacp)/ kg (D_5), $1x10^8$ (D_6), $2x10^8$ (D_7), and $3x10^8$ *Saccharomyces cerevisiae* (Sac)/ kg (D_8) in five replicates for 98 days. Using standard procedures, Feed Intake (FI, g/snail/day), Body Weight Gain (BWG, g/snail), Feed Conversion Ratio (FCR), Live Weight (LW, g/snail), Dressing Percentage (DP), Protein Digestibility-PD (%) and Fibre Digestibility-FD were assessed. Haemolymph (5 mL) was sampled to determine Total Protein (TP) and Packed Cell Volume (PCV) using standard procedures. Data were subjected to descriptive statistics, polynomial regression and ANOVA at $\alpha_{0.05}$.

The FI of AGLS was similar for all treatments, however, BWG and PD were significantly higher in T_4 (295.0±6.6 and 69.4±2.0) than T_1 (276.1±10.3 and 65.1±0.6) and T_2 (280.0±5.8 and 66.4±1.1), but similar to T_3 (290.0±10.3 and 68.9±1.5), respectively with *β-D-mannanase* supplementation. Increased *β-D-mannanase* inclusion in the diet of AGLS significantly enhanced FD (R^2=0.9), DP (R^2=0.8) and LW (R^2=0.7) of AGLS. Grower AGLS on M_7 had the highest BWG and LW of 178.6±1.07, and 181.7±2.1 respectively. The LW of Grower AGLS on M_7 (181.7±2.1) was similar to M_6 (181.7±2.1) but higher than M_5 (167.4±1.71), M_4 (166.1±1.52), M_3 (164.5±2.1), M_2 (171.8±1.11) and 161.8±1.55 (M_1). The AGLS fed M_7 had higher serum TP (8.8±0.52 g/L) and PCV (54.1±2.98%) compared to other treatments. The FI significantly increased linearly (R^2=1) with dietary inclusion of Lacp and Sac. The BWG of AGLS for D_6 (142.5±13.6) and D_7 (143.9±12.3) were similar. Optimum BWG of AGLS (R^2 = 1.0) on Lacp diets was obtained at 2.0×10^8 CFU (D_4, 142.9±18.4).

Sole dietary supplement of African giant land snail with any of *β-D-mannanase* at 0.5 g/kg, mannanoligosaccharides plus organic acid blend (2.5 g/kg), *Lactobacillus plantarum* (2.0×10^8 CFU/ kg) and *Saccharomyces cerevisiae* (3.0×10^8 CFU/ kg) enhanced growth performance and nutrient digestibility.

Keywords: African giant land snails, *β-D-mannanase*, Fibre digestibility, Feed additives
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LIST OF ABBREVIATIONS

AB:	Antibiotics
ADF:	Acid Detergent Fibres
ADL:	Acid Detergent Lignin
AGLS:	African Giant Land Snail.
AL:	Apertural Length
ALP:	Alkaline Phosphatase
ALT:	Alanine aminotransferase
ANOVA:	Analysis of Variance.
AOAC:	Association of official Analytical chemists, Washington DC, USA.
AST:	Aspartate Aminotransferase
BDG:	Body Weight Gain.
CC:	Carcass Characteristics.
CFU:	Colony forming Unit, Measure for concentrations of living microorganisms.
CRD:	Completely randomized design
DFMs:	Direct-fed microbial
Digesta:	Content of the gastrointestinal tract
DP:	Dressing percentage
FAO:	Food and Agriculture Organisation.
FC:	Feed Cost
FCR:	Feed conversion ratio.
FDE:	Fibre degrading enzyme

FI:	Feed intake
HDL:	High-density lipoprotein cholesterol
HSLP:	Haemolymph Serum lipid profile
HTV:	Haematological variables
LAB:	Lactic acid bacteria
LDL:	Low-density lipoprotein cholesterol
Lacp:	Lactobacillus plantarum
MOS:	Mannanooligosaccharide
MPCTH:	Morphology and cell types of Haemocytes.
MRS:	De Man Rogosa Sharpe Agar
ND:	Nutrient digestibility
NDF:	Neutral detergent fibres
NDS:	Neutral detergent solubles
SBP:	Serum biochemical parameters
FD:	Fibre Digestibility
Sac:	Saccharomyces cerevisiae
SEM:	Standard error of means
SL:	Shell length
ST:	Shell thickness
SW:	Shell width
TBC:	Total bacteria count
TC:	Total cholesterol
TFC:	Total fungi count

PCV:	Packed Cell Volume
LW:	Live Weight
VLDL:	Very low density lipoprotein cholesterol
WHO:	World Health Organisation.
WBC:	White Blood Cell
CP:	Crude Protein
CF:	Crude Fibre
EE:	Esther Extract
DM:	Dry Matter
EU:	European Union

CHAPTER ONE

INTRODUCTION

1.1 Background

No less than three scores animal species provide for human's daily needs of food, clothing, housing, and energy (Peters, 1987) with only the conventional species of cattle, sheep, goats, pigs, fish and poultry playing prime role in the livestock industry. Food seems to be the most important need of man with food sufficiency posing a major challenge to developing countries. Food serves as a supply of that function in; provision of energy for essential metabolic processes, repairs of worn-out tissues, growth and increase in body weight, production of offsprings, production of milk and other animal products.

Feed can be classified into carbohydrates, proteins, fats and oils, minerals, vitamins and water. For proper maintenance of the livestock, every kind of feedstuff supplied should contain all the classes of food listed above. However, the importance of proteins as a nutrient for life sustenance cannot be over emphasized. The major source of proteins are animals and plants but animal proteins are of greater quality than plant proteins. This is due to the balanced amino acid profile of animal protein. With continuous increase in the world's population, particularly in the developing countries such as Nigeria, satisfying the requirements of animal protein in human diets for adequate supply of all essential amino acids that the body cannot synthesise is a great challenge. In many developing counties, there is still deficiency of dietary animal protein. The situation is quite different in many developed countries that have well developed animal production industries. According to a CBN (2004) report, animal protein consumption is inadequate in Nigeria. While the daily protein requirement for adults is 65 g (CBN, 2004) per caput per day, the average protein intake per adult in Nigeria is 45 g per caput per day, of which 8 g per caput per day is supplied by animal protein. This reflects a deficient of FAO (2006) recommendation. Peacock, (1996) indicated that adequate nutrition is a prerequisite for sound health, good

reproduction, improved milk yield, fast growth rate and a successful animal production system.

Microlivestock are considered a class of cheap, quality sources of animal protein, consisting of species such as rabbits, rodents, cane-rats (grass cutters), reptiles, turtles, edible frogs, quails, snails, crabs, insects, etc (Peters, 1987). These form a rich pool of genetic, resources with high potentials to supply proteins and augment supply from the conventional livestock in the developing countries.

Snails (Gastropoda) are a group of microlivestock which are found all over the world and in diverse habitats. They are invertebrates with soft bodies that are covered with hard calcareous coiled shells. The shells are spiral right from the beginning. They are naturally endowed with these calcareous, hard (especially at adult stage) protective devices, often referred to as their armours, which they retract into when they sense any danger from the environment, particularly the presence of predators, or hostile environment. However, some snails such as slugs do not possess shells and some with reduced shells. Every snail has one shell. Snails have natural ability to adapt to different kinds of climates and are therefore found in a diverse range of environments, including dishes, land, deserts, sea, and fresh water. The three most popular snails found abundantly in West Africa are the Giant land snails; *Achatina achatina*, *Archachatina marginata* and *Achatina fulica*. African Giant Land Snails (AGLS) are known to have originated from the eastern part of Africa and later spread through some decades to different parts of the world.

In Nigeria, the two species, *Archachatina marginata* and *Achatina achatina*, commonly reared are pulmonates, air breathing gastropods that thrive mostly in moist and cool environment, especially in the rain forest area of Nigeria (Kayode *et al.*, 2014). Snails are usually very scarce and expensive to procure during dry seasons in Nigeria due to the scarcity of water and natural fresh feeds; therefore compounding balanced rations with a view to providing steady supply of snails at all seasons is worthwhile.

The greatest challenge in snail production is reduced growth performance. This makes it urgentl y necessary to explore all available options of enhancing snail growth and production to provide an additional source of protein to augment those derived from the

conventional livestock, as well as making snails available as vital ingredient in traditional medicine for many ailments, and for cosmetic industry.

The snails in the wild feed on a variety of feed in their habitat. What they feed on depend on the species, size, age, habitat and individual nutritional requirement. Snails can be herbivorous, carnivorous, omnivorous, and detritivorous (feeding on decaying plants and animals). Some conventional feeds are plants, fruits, vegetables and algae. They also feed on soil in search for calcium that is used for shell developments. Varieties of feed additives are available in the market and are used to improve rate of weight gain and feed conversion efficiency or prevent certain diseases. Until recently, Antibiotic Growth Promoters (AGP) had been commonly used in livestock nutrition all over the world to improve productivity and profits. For instance, antibiotics such as penicillin, and oxytetracyclines had been administered for many years to promote performance of livestock such as birds, swine, goat, sheep and cows and reports revealed appreciable benefits to the animal production by improving the health, the body weight gain and the feed conversion. However, the use of antibiotics as growth promoters have been banned from animal production in many parts of the world. The reason being that their use had led to the production of multiple resistant microorganisms which can cause health problems to the animals, as well as the consumers of the animals and their products (Rolfe, 2000). Strict restraint on the consumption of antibiotics in feeds/diets of animals has been recommended in order to reduce the risk of antimicrobial resistance to medically used antibiotics.

Since the ban on antibiotics (because the use of antibiotics have been abused such that residues of antibiotics find their way into human's food chain, in addition to the risk presented to resistant bacteria, which can give rise to problems in animals and humans health. (EUC, 2005)) as growth promoters in animal production, scientists have been challenged to explore alternative growth enhancers such as essential oils, prebiotics, probiotics, organic acids, antioxidants, metals, enzymes, and spices, among others. Addition of enzymes to animal feed is an option to promote animal growth and other performance indicators. They function in the degradation and easy digestion of plant materials. In-feed enzymes can also reduce the available, substrates for microbial

proliferation in the ileum and ceacum, while stimulating the more beneficial organisms, as a result of oligosaccharides and/or sugars they are able to release.

Several studies have demonstrated beneficial effects of specific enzyme complexes in such diets on growth and efficiency of feeds (Gradient *et al.,* 1992). The effectiveness of some In-feed enzymes has been tested as substitutes growth promoters and the results were very encouraging especially in monogastric animals. *β-D-mannanase,* a fermentation product of *Bacillus lentus,* has been implicated to improve growth performance of monogastric animals such as pigs, and poultry by degrading galactomannans present in the feed (Pettey *et al.,* 2000). Soyabeans contain hemicellulose and require *β-D-mannanase* to break down its major components (Jackson, 2007). Another possible option of promoting the performance indicators in animal production is the use of probiotics and/or prebiotics. The use of probiotics and/or prebiotics came as well as their safety in humans, animals, and the environment had been guaranteed (FEFENA, 2005). In animal nutrition, probiotics, are defined as viable live culture of microorganisms administered as feed additives (e.g bacteria, yeast and fungi) which improves the balance of microbial population in the gastrointestinal tract and act against the pathogen microorganisms. According to Fuller (1992), probiotics are:

> *"Live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance'*

According to the currently adopted definition by FAO/WHO (2001), probiotics are:

> *"Live microorganisms which when administered in adequate amounts confer a benefit on the host"*

As feed additive, probiotics have a good impact on the monogastrics' performance. These live organisms after colonizing the animals intestinal tract and their metabolites can act as immunomodulatory agent by activating specific and non-specific host immune responses in monogastric animals, which in turn aid in prevention and control of diverse infectious disease (Fuller, 1992).

Probiotics have not been labelled to have exhibited any residual effect in animal products and could be used alternatively to antibiotics that had posed serious health consequences to animals and humans that consume such products (Abe *et al.,* 1995). Researchers are

currently replacing antibiotics with prebiotics (or probiotics) in their work as therapeutic and growth promoters (Martins *et al.*, 2010).

Probiotics are live microorganisms whose activity in the digestive tract has been confirmed to be beneficial in animal nutrition. Probiotics used as feed supplements could be lactic acid bacteria, Bacillus spores or yeasts. As indicated above, the greatest challenge in snail production is reduced growth performance. This research work therefore aims to assess the beneficial effects of these additives. Enzymes (β-D-mannanase), prebiotics, (mannanoligosaccharide + organic acids) preparation and probiotics; Lactobacillus plantarum and saccharomyces cerevisiae in snail production.

1.2 Statements of the Problem

Satisfying the requirements of animal protein in human diets for adequate supply of all essential amino acids that the body cannot synthesis is a great challenge in Africa, especially in Nigeria Peacock (1996), indicated that adequate nutrition is indispensable for sound health and good reproduction system.

Microlivestock form a rich pool of genetic resource with high potentials to supply protein and augment supply from the convensional livestock in Africa. Snails belong to microlivestock and its meat is rich in protein, essential minerals and vitamins but low fat content.

However, the greatest challenge in snail production is reduced growth performance. This makes it urgently necessary to explore all available options of enhancing snail growth and production to provide an additional source of protein to augment those derived from the conventional livestock.

This work therefore explored the possibility of enzymes, prebiotics, and probiotics supplementation in the diets of snails to accelerate their growth.

1.3 Research Questions/Hypothesis

Experiment 1

Ho: That no significant effect would be observed by feeding different sole-fruits on growth performance and nutrient digestibility in African giant land snail growers.

Hi: That effect of the different sole-fruits on growth performance and nutrients digestibility in African giant land snail growers would be different.

Experiment 2

Ho: That no differences would be observed in growth and nutrient digestibility in African giant land snail hatchlings fed enzyme supplemented diets.

Hi: That the effect of enzyme addition on growth and nutrient digestibility in African giant land snail hatchlings would be different.

Experimental 3

Ho: That no differences would be observed in growth response and nutrient digestibility in African giant land snail growing African Giant Land Snails in response to enzyme supplemented diets.

Hi: That the effect of enzyme addition on growth response and nutrient digestibility in African giant land snail growers would be different.

Experiemental 4

Ho: That the effect of prebiotic-organic acid preparation on the performance of snails would be similar.

Hi: That significant differences would be observed in performance of snails fed diets supplemented with prebiotic-organic acid preparation.

Experiment 5

Ho: That the effect of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (Yeast) on the performance of snails would be similar.

Hi: That significant differences would be observed in the performance of snails fed diets supplemented with *Lactobacillus plantarum* and *Saccharomyces cerevisiae*.

1.4 Justification of the Study

- The greatest challenge in snail production is slow growth rate, and nutritional manipulation may be used to address this challenge.
- Despite its generally recognized advantages, information on snails' nutritional requirements and response of their performance to nutritional intervention are not well documented in Nigeria.
- Since snails in natural environment are known to harbor diverse pathogenic organisms in their gastrointestinal tracts due to contamination and their mode of feeding (Nwuzo *et al.*, 2016), intensive rearing of the animals in captivity could produce healthy and high quality snail meat for human consumption.

1.5 Significance of the Study

The investigation will reveal some factors that are responsible for the reduced growth performance of the snails. The chemical and fibre analyses as well as qualitative and quantitative phytochemical analyses of the feedstuffs fed to the snails will expose possible anti-nutritional factors present in their common feedstuffs.

Since snails do not have endogenous enzyme that can effectively digest fibre-rich diet and cellulose, addition of infeed enzyme such as β-D-mannanase may enhance the snails growth performance.

The study will also reveal the efficacy of some microorganisms such as Saccharomyces cerevisiae and Lactobacillus plantarum added to snail diets to enhance nutrition and accelerate the growth of the snail and health status.

1.6 Scope of the Study

Five studies were carried out to achieve the purpose of this research work, which is to provide means of accelerating the growth performance of snails.

The first step is to investigate the possible factors that may be responsible for the reduced growth performance. This involved proximate analysis of the conventiona feedstuffs, fibre analysis, qualitative and quantitative phytochemical analysis of the feedstuffs and preliminary feeding trial.

After this, two studies were conducted using hatchling snails and growers fed formulated diets supplemented with *β-D-mannanase* at varying inclusion levels. Another study was conducted using formulated diets supplemented with prebiotic-organic acid blend.

The last experiment was carried out using formulated diets supplemented with *Saccharomyces cevevisiae* and *Lactobacillus plantarum.*all the studies channeled towards enhancing the growth performance and health status of the snails.

1.7 Aim of the Study

This research work aims to assess the beneficial effects of these additives; Enzymes (*β – D – mannanase),* pribiotics (mannanoligosaccharide + organic acids) preparation and probiotics; *Lactobacillus planetarium* and *Saccharomyces* cerevisiae in promoting the growth of snails.

1.7.1 General Objective

This research aims to assess the beneficial effects of diets supplemented with enzymes, prebiotics and probiotics on performance in Africa Giant Land Snail (AGLS).

1.7.2 Specific Objectives

The specific objectives are to:

1) improve the grower African giant land snail (*Archachatina marginata*)'s performance and nutrient digestibility through sole-fruit feeding.

2) examine the feed digestibility and growth of African giant land snail (*Archachatina marginata*) hatchlings and growers in response to an enzyme that breaks down fibers in the feed.

3) determine the effect of a prebiotic blend supplementation on the growth performance and nutrient digestibility of feed in grower African giant land snails (*Archachatina marginata*).

4) evaluate the effects of adding *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (Yeast) to a grower African giant land snail's diet on its growth performance and digestibility of nutrients.

CHAPTER TWO

LITERATURE REVIEW

2.1 Concept of Snail

Snails are as old as man and have existed in familiar relationship with man. The relationship is epitomized in the coining of the proverbial statement; "*as slow as snail".* Though snail is characteristically slow in movement, it was still able to survive the first flood that wiped off the first world. Afterwards, snail was one of the animals that entered the Noah's Ark (Gen 7: 2-3; 7-10) before God caused the flood to destroy all the rest of the living things from the surface of the earth. Snails are invertebrates, terrestrial gastropods with soft-bodies that are covered with hard calcareous, coiled or spiral shells. The shell which is often referred to as armored shield acts as the snail's protective device against predators and also help to conserve moisture within. The shell is formed by the mantle and it varies in form and ornamentation.

Snails are nocturnal, monogastric and hermaphroditic animals belonging to the phylum mollusca (soft-bodied animals) which consist between 50,000 to 80,000 species and is the second largest phylum in the animal kingdom (Mead, 1961). Snails have natural endowment to survive in different environments such as deserts, sea, freshwater, on the trees and land. Some species of snails are very versatile that they can remain alive for years of desert heat in a state of estivation. Some gastropods live in the thermal spring where temperature rises to 40°C (104°F) while others can easily survive winter frozen into the ice cover. Some molluscs live in an indefinitely long time darkness of caves.

The molluscs are coelomate invertebrates, that is, animals lacking a backbone and possessing an internal cavity or coelom that is usually reduced in most molluscs to small pericardial, renal and gonadial cavities (Rosenberg, 2014).

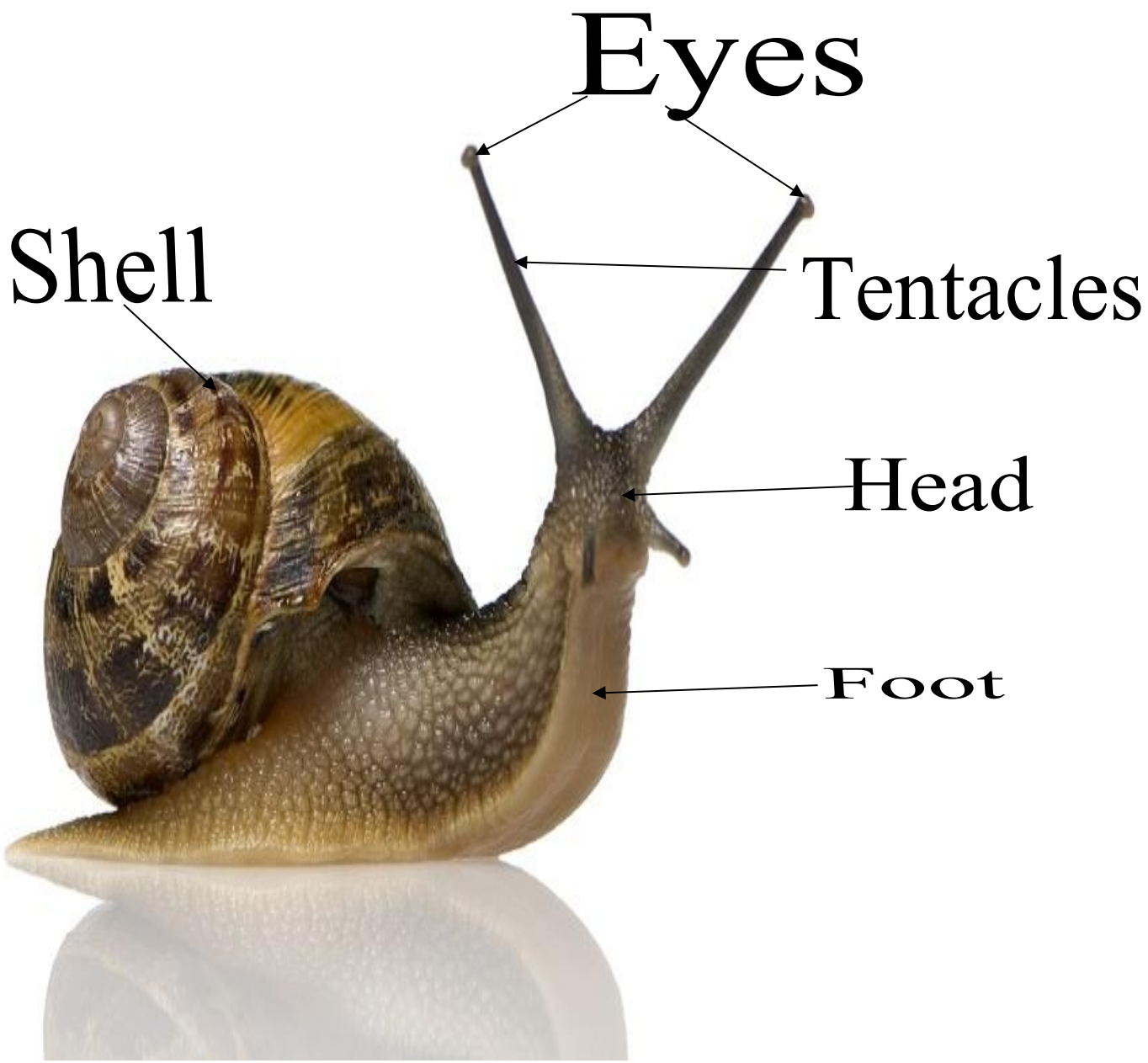


Figure 2.1: A Typical Snail, *Cornu aspersum* (formerly *Helix aspersa*)

Source: Hickman *et al.*, (2009)

Some members of the phylum do not possess shells. These are predominantly aquatic but some are terrestrial. Members of the group are found throughout the world. The phylum includes the mussels, chitons, clams, squids, octopus and cattle-fish.

2.1.1 Brief Description of Snail's Biology

The snail`s morphology typically consists of two parts; the body and the shell. The body is divided into three parts – a well developed head, a ventral muscular foot and a dorsal visceral mass covered by a mantle. The mantle secrets a calcareous shell which grows out peripherally to enclose a mantle cavity where the clenidia (or gills) lie, and into which the anus and the kidney ducts open. In snails, the shell account for about a third of the entire body and it acts as a protective device (or casing). The entire body mass is retractable into the shell when at rest or when in danger or threatened by an enemy such as predator. This is made possible by contraction of the collumellar muscle and emerges by forcing haemolymph into sinuses in the foot (Ademolu et al, 2016).

The head is not actually well demarcated and it carries two pairs of tentacles. The longer pair of the tentacles possess the eyes in the knobbed end. When the snail is on motion. The long muscular foot occupies almost the entire ventral surface and like the head is not clearly demarcated from the rest of the body. The digestive, reproductive, and respiratory organs are contained within the visceral mass, which is encased in the shell above the muscular foot. The mouth, which is located beneath the head and does not have any teeth but does have a spiky tongue known as the radula. The snails use these to rasp their food. According to Punchon (1997), the circulatory system of mollusks consists of a central contratile heart which is linked to the various parts of the body by a series of distributing vessels, arterial and a series of collecting vessels.The normal red colour of blood is absent in the mollusks because of its content of the respiratory pigment known as haemocyanin which is responsible for the bluish colour of the blood (Haemolymph).

Snails are hermaphrodites, that is, each individual posseses both male and female sexual organs, the gonads. Therefore, each individual is a producer of eggs. However, they do not produce by self fertilization in most species. The reason is that the male part cannot reach the female part. Cross fertilization is still necessary for the production of fertile/viable eggs (Mead, 1961). However, the African giant landsnail, *Achatina*

achatina reproduces by self-fertilization (Mead, 1961), but the most common form of reproduction is simultaneous reciprocal exchange of sperm.

Perga (1961) who had repeatedly observed self-fertilization in *Achatina fulica* was persuaded that a strain type of snail that is being introduced to a country is enough to make a colony, as fertilization of egg batches occur intermintently over a period of time. Seasonal rainfall and variations in relative humidity are significant factors that influence breeding of the African giant land snails. He further reported that *Archachatina marginata* in captivity bred throughout the year although a decline in performance was noticed from October to November. Mating usually takes place in the evening or sometimes in the morning or afternoon when the sky is over cast and the weather is humid. According to Omole (2003), the sexual maturity age for *Archachatina marginata* is 10 months. Awesu (1980), reported 18months for maturity of *Archachatina marginata* while Akinnusi (1998) observed that it would take 9-10 months for *Archachatina marginata* to lay eggs. Bright (1996) reported that sexual maturity in snails depend on proper management practices and feeding regime.

Mating occurs after several hours of courtship, as reported by Amusan (1999). A healthy and sexually matured snail prepares for egg laying by digging out holes in the soil, with the aid of the radula. Snails, as suggested by Cobbinah (1993), need loamy soil at 2-5cm deep for easy egg laying. The dirt soil that turns hard after a little lack of water, may diminish the conceptive rate in light of the fact that the snails can't cover their eggs and the hatchlings might have the trouble in rising up out of the dirt subsequent to bring forth (Amusan *et al.,* 1998). The incubation period is described as the time taken by the eggs to hatch from the first day of laying. The incubation period of *Archachatina marginata*, 29-35 days (Akinnusi, 1998 Bright 1999). Lazaidon and Barley (1991), reported 3-4 weeks as incubation period for the Helix species. Aribisala (1999) reported an average of 28 days, (range, 24-32 days) as incubation period for *Archachatina marginata*. The hatchability of the egg is a function of the temperature of the soil, soil humidity and the nature of the soil (Lacarilao 1990). Akinnusi (1998) reported an average incubation period of 30.25 days (range 29-32 days). The percentage hatchability as observed by Aribisala (1999) for *Archachatina marginata* was 42.5%.

As indicated by Bright (1999), the quantity of eggs laid (grip size) for *Archatina achatina* went from 37 to 305 eggs for each grasp, contingent upon the species' egg size

and grip size. Omole (2001) claims that *Archachatina marginata* has a significantly smaller clutch size than *Archatina achatina*. Results obtained from observation made by Aribisala (1999) indicated on the average a matured *Archachatina marginata* snail lays 7 eggs per clutch and the average weight of a newly weighed healthy egg was between 1.52 and 4.24 g. Furthermore, he opined that any egg that weighed below 1.5 g was not viable for reproduction. He also reported that the average length of a newly laid egg ranged between 1.47 and 1.70 cm while that of the width ranged between 1.10 and 1.14 cm. In addition to these, the colour of a newly laid *Archachatina marginata* was yellow with oval-almond shape. The colour of the egg gradually whitened with time, especially when the eggs were exposed to dryness and immediately began to crack because of the thin egg-shell thickness. Eggs were usually on top of one another in the hole. Plummer (1975) in his observations gave the average clutch size of the giant land snail as 8.6, the average width 1.57 cm. Aribisala (1999) also observed that *Archachatina marginata* can lay eggs throughout the year round if put in a very favorable condition. Akinnusi (1998) reported an average live weight of 2.3 g at day old for *Archachatina marginata* while Bright (1996) reported mean live weight of 4.95 at day old for the same species. Amusan *et al.*, (1998) cited by Omole (2003) as reporting a positive correlation between the size of the egg and the hatchling produced. Depending on the nature of the soil, young snails may remain below it for several days after hatching before emerging. Snails are nocturnal animals, so they are most active at night, at dusk, or in moist, humid conditions with a high relative humidity. It is better to feed snails between 5-7:30 pm daily. *Archachatina marginata* are much less prolific than *Archatina achatina* and/or *Archatina fulica*, laying larger eggs in clutches of about 7-10, looking like small birds' eggs (Leigh and Long, 2000). The snail shell thickness is determined by the availability of calcium in their diets and/or in the soil substratum.

2.2 Taxonomy of the Molluscs:

Molluscs are grouped into the following classes:

- Amphineura (polyplacophora)
- Monoplacophora (Neopilina)
- Scaphopoda (Tusk shells)
- Bivalis
- Cephalopoda

- Gastropods

Edible land snails belong to the class Gastropoda (Molluscs with well-marked head, a creeping “belly foot” and a radula, generally with a single shell). The molluscs were further divided into three subclasses. The subclass pulmonates which the African giant land snails belong to, distinguishable by a large visceral mass, covered by a short calcareous shell and highly concentrated nervous system. Pulmonates do not possess clenidia (or gills) which is present in other classes of molluscs, the mantle cavity is modified into a lung so that the land snails could breath air while the hard calcareous shell is retained as protective device against desiccation and osmotic changes (Purchon, 1997). Two orders of the pulmonates were acknowledged by Purchon, (1997) they are:

- Basommanatophora
- Stylommatophora

The commonly accepted scheme of classifying the African giant land snail, outlined by Purchon (1997) is as follows:

Phylum:	Mollusca
Class:	Gastropoda (shell bearing snails)
Subclass:	Pulmonata
Order:	Stylommatophora
Family:	Achatidae
General:	Archatina archachatina
Species:	Over 40 of either genera

Examples of species includes

a) *Archachatina marginata*: the big black snail
   (Yoruba name: Igbin apinnu, egbun)
b) *Achatina achatina: (*Linne)
   (Yoruba name: Ilako, Isan, Aginiso)
c) *Achatina fulica:*
   Common name: The garden snail or the follish snail (It is sometimes also called the African giant snail)

d) *Limicolaria spp:*

(Yoruba name: Esan, Ipere)

Source: Omole, *et al.* (2007)

Plate 2.1: Three Species of Snail:

A = *Achatina folica*,

B = *Achatina achatina*,

C = *Archachatina marginata*

2.2.1 *Archachatina Marginata*

In Nigeria, it is the most widely raised snail species. In the West African rain forest belt, it is prevalent. It belongs to the group of snails that are typically referred to as African giant land snails. When compared to other breeds, its shell has no distinct coloration and is wider at the posterior end (apex). Typically, the foot is dark brown. In the Congo basin and the southern part of Nigeria, Archachatina marginata is more prevalent. As revealed in Plate 2.1, its shell is brown, with dark brown stripes, and obviously pink tips to the end of the shell. The apex is usually blunt and the inside of the lip of the shell is brightly pink, whereas that of *Achatina fulica's* is white. It lays between 5-20 eggs per clutch. A freshly laid egg is yellow in colour, and an average daily egg weight of 2.07± 0.02 g. (Akanni *et al.,* 2014).

2.2.2 *Achatina Achatina*

The Republic of Benin, Liberia, and other West African nations are the primary markets for this breed. It is prevalent in Rivers and Akwa-Ibom states in large numbers in Nigeria. Because of its high mortality rate, the population of the South-Western region of Nigeria is relatively lower (Omole, 2003). The ovate shell has regular conical spines that are narrow at the posterior end and are conical in shape (Plate 2.1). It lays between 150-500 eggs per clutch and the egg is very small i.e. between 0.30-0.70 g each (Omole *et al.,* 2003). Mature adult can weigh up to 500 g.

2.2.3 *Achatina Fulica*

This is a tropical snail, but can survive also in cold conditions, such as snow. It is smller in size compared to both *Archachatina marginata* and *Achatina achatina*. It resembles *Archachatina marginata* at an early stage. The colour of this species is predominantly brown with weak, darker banded markings across the spirals (Plate 2.1). The fleshy part can be grayish or whitish in colour. The colour of the snail sometimes can be affected by the nature of the soil in its habitat. *Achatina fulica* is highly adaptable to a wide range of environments, modifying its life cycle to suit local conditions. Mature adult weighs between 20 – 35 g and it lays 10-15 eggs per clutch, the eggs weight is between 0.6-1.4 g and it is of lower economic yield when compared to *Archachatina marginata.*

2.2.4 *Limicolaria spp*: Garden snail is another name for this species. It is common in farms, where it becomes a major pest of leafy vegetables, plantains, maize, cocoyam,

and yams, among other crops. It can also be seen and picked at the backyards during rainy season. It can lay up to 25-50 eggs per clutch. The eggs are usually very tiny and weigh less than 1 gram. *Limicolaria spp* are rarely reared perhaps because of their small sizes except for experimental purpose. *L\imicolaria spp* are picked up in farms and bushes and prepared for consumption. They are commonly sold in local measures in Nigerian markets and they serve as a source of protein for rural dwellers.

2.3 Snail Feed and Feeding

Snails will feed on live and dead plant and animal materials. They can also feed on fresh vegetables and fruits available in their environment. *Archachatina marginata* can feed on broad types of leaves and fruits particularly if they are succulent. They can also feed on compounded feed from the feed mill. They can feed on both dry and wet feed materials. Some snails are carnivores that feed on other species or their own but most species are herbivores. (Ajayi *et al*, 1998; Akinwumi, 1998; Cobbinah 1990), some species are omnivores. Snails have preference for feed that are rich in calcium and their population is larger on the soils rich in calcium. This is because they need calcium for the shell formation and thickness since the shell is a protective device. Pesticides and herbicides are highly deadly to snails (Olayinka, 2014). Hence the use of agro-chemicals around the areas where snails are being reared should be avoided. In addition, the soil from the farms where pesticides, herbicides, insecticides have been used should not be used for snail pen bedding materials. Such soil, if used can increase mortality rate and can even kill the entire animals in the snailery. Since a distinction between their senses of smell and taste is seldom possible, they are usually considered together.

Table 2.1 Leaves of some plants/crops that can be fed to snails

English	***Scientific name***
Pawpaw	*Carica papaya*
Sweet potato	*Ipomea batatas*
Plantain	*Musa paradasiaca*
Green Vegetable	*Amaranthus spp*
Waterleaf	*Talimum triangulare*
Centro	*Centrosema pubescence*
Groundnut	*Arachis hypogea*
Stylo	*Stylosanthes gracilis*
Lab-lab	*Lab-lab purarium*
Lettuce	*Lactuca sativa*
Carbage	*Brassica oleracae*
Sunflower	*Titonia*

Source: Omole (2019)

Table 2.2: Some tubers, Stems and roots that can be fed to snails

English	*Scientific name*
Sweet cassava	*Manihot Utilisima*
Yam	*Discorea spp*
Sweet potato	*Ipomea ibatatas*
Carrot	*Daucus carota*
Cocoyam	*Colacasia esculenta*

Source: Omole (2019)

Table 2.3: Some fruits that can be fed to snails

English	***Scientific name***
Pawpaw	*Carica papaya*
Mango	*Mangifera indica*
Sweet orange	*Citrus sinensis*
Pineapple	*Ananae sativa*
Cucumber	*Cucumis sativus*
Carrot	*Daucus carota*
Water melon	*Citrullus lanatus*
Banana	M*usa paradisiaca*

Source: Omole (2019)

2.3.1 Compounded Ration

Compounded feeds are usually formulated in the right proportions that enriches the animal in terms of supplying needed amount of nutrients. They must contain protein, energy, minerals, adequate amount of fibre, vitamins, calcium and phosphorus. They should be used as supplementary feeds. They are rich in all nutrients but are expensive. According to Bright (1996), adding broiler starter mash, which contained 23% crude protein, to snails' feed led to an increase in growth rate. Similar results were observed by Radrizzni (1992) when 22.60 percent crude protein was added to the carrot and vegetable portions of Helix promatia, H. locorum, and H. aspersa. In developing nations like Nigeria, there is currently a lack of information regarding the use of compounded diets to feed snails. This is due to the fact that the snails do not yet have standardized nutrient requirements (Amusan and Omidiji, 1999). Figured out feed for *H. aspersa* was analyzed to have diminished the development period from incubating to gather by 10 months.

As per Bright (1996), adding grill starter squash, which contained 23% rough protein, to snails' feed prompted an expansion in development rate. When 22.60 percent crude protein was added to the carrot and vegetable portions of *Helix promatia, H. locorum*, and *H. aspersa*, Radrizzni (1992) observed comparable outcomes. There is currently a lack of information regarding the use of compounded diets to feed snails in developing nations like Nigeria.

This is due to the fact that the snails do not yet have standardized nutrient requirements (Amusan and Omidiji, 1999). The growth period from hatching to harvest for H. aspersa was found to have been slashed by ten months using formulated feed.

2.3.2 Edible snails of European Origin

Helix aspersa common name: petis-gris (the small grey snail)

Helix promatia common name: the Burgundy snail

Helix lucorum common name: escargot turn (small snail of Turkey)

Helix cibeta common name: the snail of Adana

Helix anctostoma

Helix melanstroma

Helix melanonixia

Helix theissian

Helix nucula

Helix aprta common name: the burrowing snail

Iberu alonensis *common name:* Spanish cabaret

Arianta arbustorium

Cepaea nemoralis common name: The wood snail

Cepaea hortensis common name: the banded snail

Otala puntala common name: Vaqueta

Otala factea

Perforatella incarnate

Sphincterochila candidisma common name: cagol mangeta.

Source: Hickman *et al* (2009)

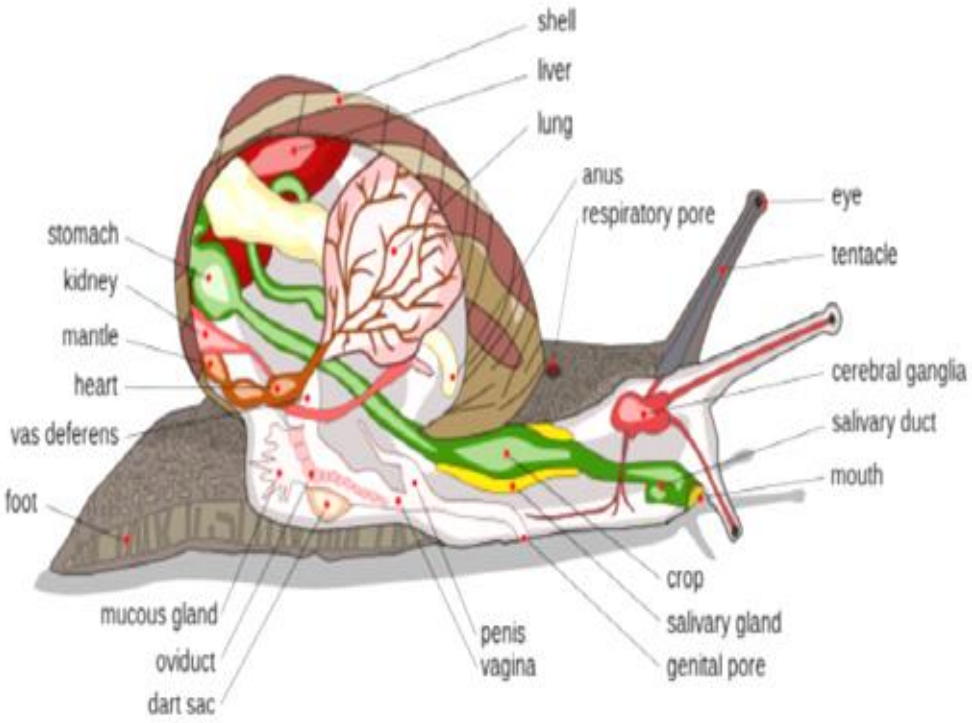


Figure 2.3: A well-labeled Anatomy of Snail Showing the Digestive System

Source: *Hickmanet al.*, (2009).

2.4 Nutrition in snail

Animal nutrition has to do with various biological and chemical transduction that allows nutrients from feed resources to be effectively utilized for both physical and maintenance activities. Nutrition involves the ingestion, digestion, absorption and assimilation of various nutrients, their transportation to all body cells as well as the removal of unutilized elements and waste products of metabolism. The first requirement for a nutritious, complete, and well-balanced snail diet is that it contain the right balance of protein and energy. Snails have a straightforward yet effective digestive system. Their radula, or rasping tongue, ingests food or feed. The buccal mass of the snail is where the digestive system begins. Its mouth is the buccal mass, where food is taken in. The radula, which grinds and cuts the food into pieces after it passes through the mouth, is the next step. While the radula grinds the food, the snail holds it in place with its jaws. After the snail has taken in the food, rasping it into tiny bits with its radula, the product disappears in the snail's gullet (oesophagus) to be digested. Contrary to the siliva, the digestive fluids from hepatopancreas can digest all classes of food items. From the oesophagus the food goes into the crop, where salivary glands are found. There are bacteria in the crop which help the snail to digest cellulose. The food later enters the sac-shaped stomach which is just an extension of the crop. Digestion of the food also takes place in the stomach (Appleton *et al.,* 2009). As the food moves into the intestine, nutrients are absorbed into the large intestine, while undigested items evacuate the system through the anus (Mackenstedt and Markel, 2001) which opens at the head region.

2.4.1 Enzymes in the gut of the snails: The achievement in animal husbandry is a function of the efficiency of the animals in converting and utilizing the feed materials administered to them. This is highly made possible and effective by the availability and activities of the enzymes in the digestive system. Snails feed on various food items such as dead plants and animals that are available in their environments. They are naturally endowed with the ability to break down these materials and hydrolyze them effectively because of the presence of various enzymes along their gut regions. Some enzymes that had been found in the snail gut regions include proteinase, lipase, cellulase, glucosidase, and α-glucosidase (Ademolu *et al.,* 2016). Ademolu *et al.,* (2016) investigating on the feeding pattern and gut enzymes of AGLS (*Archachatina marginata*) reported that the activity of the enzymes were significantly affected at the growth phases, with the adult phase having the largest enzymes activity level for all the enzymes, followed by the

grower phase (sub-adult) while the hatchling phase has the lowest activity. In term of enzyme population, the stomach region had the highest, while the oesophagus had the lowest according to the report of the same research group. In addition, the presence of lipase, α-glucosidase, amylase, cellulase and proteinase in the snail gut revealed the omnivorous nature of the snail as suggested by Ademosun and Imevbore (1998).

Ademolu et al. (2013) noted that during aestivation in African giant land snail (Archachatina marginata), all of the digestive enzymes—amylase, -glucosidase, cellulase, lipase, and proteinase—were found to be present in the oesophagus, crop, stomach, and intestinal tract. They also said that aestivated snails had significantly lower enzyme activities in all parts of their stomachs than snails fed a regular diet. This might invariably lead to reduction in the nutrient uptake and in poor performance. The undigested food is passed through the anus that is located above the head.

2.4.2 The digestive system of snail

The buccal mass, which includes the mouth, pharynx, retractor muscles of the pharynx, and salivary glands with salivary ducts, is the most well-developed internal structure of any snail species. The oesophagus and its crop; gastrointestinal tract; Rectum, anus, intestine, and digestive gland

2.4.2.1 Buccal mass: The buccal is subdivided into mouth and pharynx and both function in digestion of feeds. The pharynx are generally small, but may be large in carnivorous species. The salivary duct extends to the mouth and the oesophagus contains the digestive enzymes that function in the hydrolysis of feeds. The carnivorous species of snails are characterized by special features such as the development of proboscis that contains the radula and oral cavity.

2.4.2.2 Radula: The radula is that organ in snail that is specialized for obtaining food items and feeding. It is a rasping tongue used for cutting food into smaller pieces. The structure is common in all molluscs. The membrane of the radula possess many continuous teeth attached to it. These pseudo teeth are used for rasping and breaking down food particles. Some molluscs can have about 250,000 of these tiny teeth. According to the scientists, the radula, apart from helping in cutting food particles into pieces, presumably assist in bringing a continuous flow of food into the body (Dimitriadis, 2001).

2.4.2.3 Oesophagus and oesophagal crop: Oesophagus can be described as a sort of bridge between the mouth and the stomach. The oesophagus can lie around the stomach due to torturing, and it opens at its posterior ends, far from the mouth. On the other hand, species that have undergone de-torsion may have an oesophagus that opens into the stomach's anterior part instead of the usual way. Because of the presence of some enzymes, digestion can take place to some extent.

2.4.2.4 Stomach: The stomach is a straightforward sac-shaped structure that extends from the crop in most gastropod species. The literature indicates that the stomach is the primary digestion site in the snail gut because it contains the greatest number of enzymes. The hind part of the oesophagus of some herbivorous snails is enlarged to form a crop. The crop section of the stomach would have the most cellulase activity. However, it was thought that the stomach of some aquatic herbivores had evolved into a gizzard that assisted in the digestion of the food. The gizzard may be filled with abrasive sand grains or have a tough cuticle. According to Van Iten *et al.* (2002), the "style sac," or part of the stomach closest to the oesophagus, is always lined with cilia in all gastropods. The food particles are pulled forward from the mouth by these cilia. According to Dimitriadis (2001), two glands were found to have entered the stomach and were producing enzymes that aid in the digestion of food through the gastropod digestive tract.

2.4.2.5 The Hepatopancreas (Digestive glands): The hepatopancreas, an organ with glandular and enzymatic functions, provides the functions of the liver (hepato-) and pancreas in mammals. It produces enzymes, participates in food digestion, absorbs nutrients, and stores them. It has been reported to be the largest organ in stylommatopharan gastropods (Dimitriadis, 2001).

2.4.2.6 Intestine: Food materials are transported through the intestinal tract and nutrient are taken in when the digestive glands break the food substances. The intestine also helps to re-absorb water from the food, producing faecal pellets.

2.4.2.7 Anus: The anus of the snail was reported to have opened above the head in virtually all the gastropods/molluscs (Dimitriadis, 2001).

2.4.2.8 Time to feed the snails

Snails are nocturnal animals. They are more active in the night, at dusk or when the atmospheric condition is cool. Snails usually move out to search for food when the relative humidity is high (60% and above) and when the temperature of the environment of the snailery is between 23°C-28°C (Omole, 2018). He also indicated that the best time to feed snails is when the sun has set, around 5-7:30 pm. If the feed is supplied in the morning, it might have lost its freshness before evening when they come out to feed.

2.4.2.9 How to feed snails: Feed should be supplied in the feeding trough made of clay or put on the slab provided in the pen. If it is leaf materials, they can be put at different locations in the pen. Fruits and tubers cut into 2-4 pieces with the surface or fleshy parts faceup down.

2.5 Excretory system of Gastropods

The role of the excretory system of gastropods is to remove nitrogenous waste and maintain the internal water balance of these animals. The waste product of excretion is either ammonia (NH_3) or uric acid. The nephridium does not play the role of maintaining water balance in terrestrial species alone but also in fresh water snails.

2.5.1 Grouping of animals on basis of types of nitrogenous compounds excreted

2.5.1.1 **Ammonotelic**: Ammonia (NH3) is the most common form of nitrogen excreted by members of this group. This mostly applies to aquatic animals, such as teleosts, tadpoles, certain unicellular animals, polycheate annelids, aplysisa, molluscs, crocodiles, and crustaceans.

2.5.1.2 **Ureotelic**: In this group of animals, nitrogen is mostly released as urea. Mammals, reptiles, and elasmobranchs, for example Ammonotelic and ureotelic metabolism are present in fish.

2.5.1.3 **Uricotelic**: Uric acid is the most common form of nitrogen excretion in this group of animals. Insects, lizards, snakes, and some gastropods are all susceptible to this (Frederick, 1968).

2.6 Circulatory system of gastropods

Haemolymph, an extra-cellular circulating fluid, is the transport fluid in their open circulatory system. It flows directly through the sinuses and bathes the tissues. Snails lack haematins and have blue cells instead of red blood cells. The haemolymph is the fluid that circulates in the circulatory systems of arthropods, crustaceans (like crabs and shrimps), insects (like stone flies), and mollusks (like snails). It is comparable to the water, fats, proteins, sugar, and hormone-rich cells and fluids found in vertebrate blood and interstellar fluid.

As reported by Wyatt (1998) haemolymph is the fluid that completely fills interior cavity or (haemocoel) of the animal body and bathes the surrounding cells. The snail's heart is present in the anterior part of the visceral mass. In most species of snails, the heart is made up of two chambers: an auricle and a ventricle. The auricle receives haemolymph from either the gill (aquatic snail) or lung (terrestrial snail) and a ventricle pumps it into the aorta (Wikipedia, the free encyclopedia). The flesh and haemolymph of *Achachatina marginata* is rich in inorganic ions such as Mg^{2+}, Ca^{2+}, K^{+}, Cu^{2+}, Na^{+}, Zn^{2+}, Fe^{2+}, and Cl^{-} (Ademolu *et al.*,2004).

Haemolymph is also made up of water and organic compounds like proteins, carbohydrates, and lipids. As a result, it's a useful medium for studying human and animal nutrition and clinical research. Because blood/haemolymph components are frequently used in nutritional assessments and animal surveys due to the fact that dietary constituents also have quantifiable effects over blood constituents. All chemical exchange within the snail tissues is facilitated by the presence of the haemolymph according to Dimitriadis (2005). Its circulation is enforced by the rhythmic contractions of the heart and foot muscles (Sembrat, 1981).

2.6.1 Haemocyanin: The respiratory pigment known as haemocyanin, which is a Copper-based protein, is found in the haemolymph of a number of invertebrate species that are members of the Mollusca and *Anthropoda phylas* and are responsible for oxygen transport and/or storage. Metal proteins called haemocyanins are found in vertebrate haemoglobin, but they contain copper rather than iron. Haemocyanin is the substance that gives the haemolymph its light blue color and ensures that oxygen reaches every part of the snail. Deoxy-haemocyanin and oxy-haemocyanin are the two functional forms of the protein, and their relative abundance varies with oxygen concentration. The

dinuclear copper (I) site is present in the deoxy-haemocyanin form. Each copper ion is attached to three histidine residues in both forms (Beltramin *et al.,* 1995). According to Dolashka *et al.* (1996) the oxygenation results in a color shift between the blue Cu2+ oxygenated form and the Cu2-deoxygenated form.

$$\underset{\text{(colourless)}}{2Cu^{2-}} + 2O_2 \leftrightarrow \underset{\text{(blue)}}{2Cu^{2+}} + O_2 \qquad 2.1$$

A chemical equation of the reversible combination of copper with oxygen

As a molecule that transports oxygen, haemocyanin comes in second place to haemoglobin in terms of frequency of use. According to Waxman (1975), haemocyanin has a larger size (400-20,000 KDa) than haemoglobin (65 KDa). They are not bound to haemocytes like haemoglobin is. On the other hand, because haemoglobin is so small in vertebrate red blood cells, it could easily clog and damage organs that filter blood, like the kidney, if it were not attached to cells but instead suspended directly in the haemolymph. Haemocyanin's slow performance as an oxygen transporter is compensated for by its free-floating nature (Strobel, 2012). Haemocyanin, in contrast to hemoglobin, which only has four subunits, is composed of numerous subunit proteins that each have active sites. Each subunit is arranged in bundles or chains with masses greater than 1500 KDa and has a mass of approximately 75 KDa. According to Barimah (2013), haemocyanin's copper atoms are bound in prosthetic groups coordinated by histidine residues, in contrast to haemoglobin, which carries its iron atoms in porphyrin rings (heme groups).

2.6.2 Snail haemocytes

Heamocyte is a cell that is found in the haemolymph of various invertebrates particularly arthropods and the molluscs. They are often referred to as amoebocytes or phagocytes of invertebrates. e.g Snails. Haemocytes are the major defense cells in molluscs immunity, whether terrestrial or acquatic. The internal defense systems of snails are in two folds – cellular and humoral factors. The haemolymph's cellular component is made up of haemocytes or amoebocytes, which can also be found in connective and vascular tissues.

The presence of these cells was implicated to be central to maintenance of haemostasis within an animal and is extremely important for defense against infection.

Haninton *et al.* (2010) stated that the most significant function that haemocytes perform in mollusks is:

• Detection of viruses and the initiation of defense responses

• Phagocytosis

• Encapsulation

• The production of cytotoxic molecules that aid in pathogen elimination (Humphries *et al*., 2008). Other significant processes, such as wound healing (Franchini *et al.*, 2000), as well as the formation and repair of the shell, the digestion of nutrients, the transport of nutrients, and excretion.

Haemotopoisis is the process that makes sure there are enough blood cells in the tissues and in the blood. It is very important for an animal's defense against infection and is crucial to maintaining homeostasis (Ottaviani *et al.,* 1992). All animals with open circulatory systems go through this process. Around the 1980s, observational studies served as the foundation for the investigation into this process. There is still very little information available regarding this procedure. It was reported that the three mollusc families known as Gastropoda, Cephalopoda, and Bivalia served as the foundation for this research. Additionally, haemocytes are involved in the molluscan stress response, which involves invertebrate-shared endocrine molecules (Ottaviani *et al.,* 1992).

2.6.3 Types of haemocytes

The mollusca's haemocytes are not fully understood. Several investigations had been conducted, with diverse species- and mollusc-specific results in conflict. In this setting, the majority of studies had focused on Bivalves. Gary *et al.* (2007) claim that they could only identify a single type of circulating haemocyte in the blood of two marine gastropods, *Megathura crenulata* and *Aplysia califonica*, after investigating the haemocyte type in those animals. Abiona *et al.* (2013) conducted a study found that four distinct types of haemocytes were present in the haemolymph of both *Archachatina marginata* and *Achatina achatina* species of African giant land snails, indicating their presence and types. In addition, the haemocytes indentified in *Archachatina marginata* had significantly higher dimension than those found in *Achatina achatina*. It was also observed that the haemocytes were morphologically different in terms of shapes, nucleus position and cytoplasm types.

2.7 Behavioural nature of snail

African giant land snails have no physiological means of regulating the intake or loss of moisture other than sealing themselves in their shells, making them naturally intolerant of extreme cold or heat as well as dry conditions. During the hamattan season in Nigeria, this response to severe weather is easily observed. In order to survive, land snails have developed adaptive physiological responses to deal with these conditions. These physiological responses are referred to as aestivation and hibernation. During aestivation or hibernation, there is no opportunity for nourishment. It is a period of dormancy and growth is retarded and this will affect the productivity invariably and consequentially a loss of money to the farmer.

2.7.1 Aestivation: This is a state of inactivity and metabolic depression, a psychological response to unfavorable weather conditions, when the weather becomes too hot or dry, and the moisture content in the environment is very low, the snails resume a torpid state by sealing the opening/aparture of the shell with a whitish, fragile muco-calcareous film or layer known as epiphragm. This response is typical of all land snail species whenever conditions are not favorable, they go into dormancy. In this condition, they are able to survive many months without food or water. Snails will also aestivate if dry spells occur during the wet seasons. The duration of inactivity often depends on how long the unfavorable conditions prevail. During aestivation, there is always weight loss and retarded growth. This phenomenon is common during dry season in Nigeria when the relative humidity is usually very low, especially during hamattan periods between December and February yearly (Akinnusi, 1997).

2.7.2 Hibernation: Snails can also survive unfavorable periods by hiding themselves in crevices, underground, burying their faces into the soil, clinging to fence posts, trunks and objects or hiding under the dry leaves especially in cocoa farms, until the weather improves. As recorded by Akinnusi (1997), the snail being reared under intensive system can behave thesame way if deprived of water and advised that it is necessary to keep their surroundings moist always.

Mead (1961) concluded that hibernation is a physiological phenomenon that requires elaborate metabolic preparations, whereas aestivation, which is the belief of many investigators that reduced moisture is the major factor responsible and that a low water

content could also be the result of the snails' naturally occurring hydration cycle or environmental dryness.

2.7.3 Epiphragm: Epiphragm is a calcareous substance (a kind of whitish film, when dried) that seals the aperture of the snail shell, usually at the commencement of aestivation. This is a physiological reaction of snails during dry spells or shortage of moisture or water or at low relative humidity. Aestivation in snails occurs to prevent loss of moisture. During this period, the animal withdraws into its shell, mobility, nourishment and reproductive behavior are reduced or stopped and growth suspended.

Compared to Achatina achatina and Achatina fulica, archachatina marginata was observed to form epiphragms more quickly and to replace them (if removed) more frequently. The use of a discontinuous breathing pattern, in which the pneumostome opens intermittently to facilitate a rapid exchange of carbon dioxide (CO_2) and oxygen (O_2), further reduces the loss of water (moisture). It is important to note that the epiphragm does some of the work of the operculum. However, it is only a temporary secretion in comparison to an operculum, which is a permanent structural component of the animal that possesses it (the operculates).

2.7.4 Operculum: Only operculates possess this organ; It is a calcareous or horny plate that seals the opening when the snail retreats into the shell. It serves as a security measure. Operculates are solid land snails with a round aperture and often spiral-shaped shells.

2.7.5 Homing: Snails live during the night. When it is dark outside or at night, they are more active. In the dark, they leave in search of food. The use of mucus makes it possible to hominate. The ability to follow another snail or its own mucus track is known as trail following. The mucus contains both the olfactory and trail-following pheromones, which are chemical substances secreted externally that inform and elicit specific responses in members of the same species. The pheromone's trail remains even if one of the olfactory pheromones is destroyed, such as by water.

2.8 Importance of water in snail production

Snails are always at their best when their environment is dark and damp hence, fresh clean water must be supplied at all times for them. This can be done by putting enough water to moisture the environment of the snails very early in day and late at night.

Chlorine-free water is preferable and the harder the water the better for the snails because it enhance shell development. Chlorinated water (tap water), or water with insecticides, herbicides or fungicides should be avoided. Water is a mandatory requirement for maintenance, production and to keep the body moist at all times. Water and feed consumption depend on the environmental conditions such as temperature, relative humidity and the microbial balance in the gut.

2.9 Mineral in snail nutrition

Minerals are very important for livestock's maintenance and production, even though animals only need a small amount for nutrition. However, some inherent factors typically determine the amount that animals require.

The element sodium (Na) is primarily provided to animals as sodium chloride (NaCl). The majority of feeds and forages lack Na, but plants that thrive in alkaline soil do (Menecty *et al.*, 1976). Omole (2003) says that these electrolytes in body fluids also play a role at the cellular level in water metabolism, nutrient absorption, and nerve impulse transmission. Additionally, NaCl controls the excretion of waste products and the absorption of nutrients into the cells. According to McDonald *et al*. (1987), an inadequate supply of sodium decreases the utilization of digested protein and energy.

Chloride (Cl^-), one of the most important anions in the extracellular fluid and also present in the gastric secretions, is an essential component in food digestion and absorption. Chlorine is required to activate intestinal amylase, whereas hydrochloric acid is necessary for protein digestion. According to NRC (1980), terrestrial animals have higher salt requirements under hot, semi-arid conditions in which they sweat out a lot of water and salt (NRC, 1980). The salt requirement for livestock generally ranges from 0 to 0.50% of the ration. However, the requirements differ based on animal class, feed type, animal activity, and production stage (Underwood, 1981). According to Omole (2003), weight gain and feed conversion ratio of hatchling and sub-adult snails were enhanced with ration containing between 0.10 – 0.20% level of sodium chloride (NaCl). In addition, Omole (2003) indated in his reports that feed intake, dry matter digestibility, feed conversion ratio, number of eggs laid and size of the hatchlings were improved in the ration containing 0.2% NaCl in breeding snails. In livestock, sodium deficiency can lead to reduction in growth, protein and energy utilization, lowering of egg production in birds and reproductive disturbances (Menecty *et al.*, 1976).

According to Bright (1996), feeding snail a compounded ration of broiler starter and layer mash increased the snail's growth and reproductive indicators. These apportions contained between 0.2 - 0.3% NaCl (NRC, 1985). In livestock, however, toxicity is characterized by an increase in water consumption, weight loss, nervousness, and anorexia—and, of course, death in snails—when excessive amounts of sodium chloride are administered in conjunction with inadequate water intake (NRC, 1974).

Animals require a large quantity of macro minerals, which are essential minerals. Calcium, phosphorus, magnesium, sodium, potassium, sulfate, and chlorine are all substances that should be present in snail feed. Snails, like all animals, require micro-minerals (also known as trace elements) like manganese, iron, copper, iodine, fluorine, vanadium, cobalt, chromium, tin, silicone, and others. However, if consumed in large quantities, some of the trace elements may be toxic. By and large, lacks of these minerals could prompt decreased hunger and creation, hindrance of development and intermittent demise (Babayemi *et al*., 1999; Matnard, *et al*., 1983).

2.10 Importance of Calcium (Ca) and phosphorus (P) in Snail Nutrition

The importance of calcium in snail nutrition cannot be over emphasized. Calcium plays a prime role in the diet for snail. Its importance in snail shell and egg shell formation is well known in research circle. Calcium ions (Ca^{2+}) are also required by all green plants which are beneficial to snails. Snails derive their calcium from the feed they consume and from the soil by ingesting soil particles or from scrapping rocks. Any calcium dissolved in water can also be absorbed through their skin or from the drinking water. It has also been implicated that growth of snails is enhanced by the presence of Ca^{++} both in the feed supplied and in the soil substratum (Bradford *et al*., 1994). Calcium also has an impact on the heart and a portion of the body's enzyme system. The skeleton and bodily fluids contain the majority of calcium (McDonald et al., 1987). Skeletal calcium is always calcium phosphate with some calcium carbonate. However, the main compound in the shell is calcium carbonate (Arthur, 1975). In addition to the absorption of water, it has also been thought that essential amino acids and soluble calcium ions (Ca2+) could be absorbed through the body wall.

Calcium deficiency can result in soft egg shells, low blood calcium levels, and demineralization of bone (fracture) in animals. Animals consume less protein and fat when their diets contain too much calcium (Barison *et al.*, 1994). Vitamin D3 controls

calcium absorption, which typically occurs in the upper intestine. Phospholipids, phosphoprotein, and nucleic acids are all sources of phosphorus in the body. Phosphorus plays a significant part in the metabolism of energy. According to NRC 1980, the body usually has very little phosphorus in comparison to calcium. Bone and shell formation requires both calcium and phosphorus. Appetite loss is linked to low phosphorus intake in the diet. As a result, the animals will begin to chew strange things like bones, rags, and wood (Arthur, 1975). These conditions have also been linked to infertility problems and abnormal young animal growth (McDonald et al., 1987). The ratio of calcium to phosphorus in an animal's diet should be 25:1. According to Omole (2003), this is also necessary for snail shell and egg shell development. The recommended ratio for laying birds should be 3.5:0.5 due to the fact that laying hens require a greater ratio of calcium to phosphorus during the laying period and that the egg shell is primarily composed of calcium (McDonald et al., 1998). Snail diet deficiencies in calcium and phosphorus have also been linked to poor shell formation, sluggish growth, and shell leakage (Dauda, 1995). Chalk, dicalcium phosphate, limestone, oyster shell, and other substances have been included in the diets of snails by a number of researchers by directly incorporating it into the soil or incorporating it into their diets.

2.11 Soil Influence

A good soil fit for snail rearing should also provide snails with some nutrients and minerals such as calcium and phosphorus. The absence of these essential nutrients required by snails in the soil negatively affect the life and life cycle of the snails (Cobbinah *et al.*, 2008).

Cobbinah *et al.*, (2008) reiterated further that soil composition, water content and texture are important factors to consider in the site selection because snails take in calcium ion from the soil for shell development, egg-laying and aestivation during dry weather to conserve their body moisture. African giant land snails tend to prefer fairly loose top soil surface, and fine loamy soil, the mucus collects soil particles which have a dragging effect on the snail’s movement.

Soil with high organic matter supports the growth and development of snails. Snails majorly derive calcium for shell structural formation from the soil. It is therefore essential that the calcium and water content of the soil is high and that the soil is loose. It does not really appear that snail show any definite preference for any kind of soil,

their pattern of selection might be determined by the availability of nutrients and minerals present in the soil. They always feed on soil particles probably because of the nutrient and water content. However, Moyin-Jesu and Ajao (2008), reported from their investigation that loamy soil had the best value of pH, organic matter, nitrogen, potassium, calcium, sodium, magnesium and the least bulk density, permeability and temperature while sand and silt soil had the least soil nutrients. However, it is expedient to change the soil periodically to prevent pollution of the soil by mucus and faeces of the snails. It is also necessary to till the soil especially when you observe that the snails have reached the point of laying. According to Omole, (2003) if a soil supports good growth of cocoyam, tomatoes and leafy vegetables, it should be suitable for snail farming.

2.12 Diseases, parasites, pests and predators of snails.

The enemies of edible mollusks are found throughout the range of groups of animal kingdom, from protozoans to vertebrates (Mead 1977 and 1979). The most important pests of snails are phyton, termites, ants, especially soldier ants. Pest attack can lead to the deaths of the snails. A predatory ant, *Phildelogetan affinis* swallowing a batch of snail eggs that were first hatched was observed in Indonesia by Green (1996). Pagga (1969) also reported that these ants sometimes attack newly hatched *Achatina fulica* snails in Malasia. According to Elmslie (1985), the common rat, *Rallus rallus*, is an important pest of the *Archachatina* species. In West Africa, particularly in Ghana, the worst natural enemy of the local Achatinid (*Achatina achatina)* seemed to be the red and black driving ants. These ants normally give the snails painful stings making them to show thigmatactic behavior by adhering to branches of plants. Snails in this condition usually withdraw their heads into the shells and remain in the state for weeks without feeding or moving which leads to loss of weight (Cobbinah, 1993).

In another study by Awesu (1980), the thigmactic behavior was also observed in *Archachatina marginata*. She also noted that snails responded to insect attack by adhering to branches of shade plants like banana and remained in this state with the withdrawn heads until a favourable condition resulted. Many deaths have been caused by predators commonly reffered to as shell breakers which attack land snails. The worst among them being mammals such as rodents (rats, mice and shrews), birds and some

reptiles (e.g. snakes), and beetles. Other known predators includes frogs, toads, crabs, bees, flies and human beings (FAO, 1986).

Studies have also revealed injured snails with broken shells as well as dying ones offer more attention to soldier ants in comparing to healthier ones. Much is yet known about the parasites of the giant land snails. This is important since various kind of snails are already known to serve as intermediate hosts for a number of parasites. *Achatina fulica* has so far been implicated in this regard. It was identified by Trotti and Tofehenton (1979) as a carrier of *Angistanylus cantonesis* a nematode that causes meningitis in man. Also Rabditordes nematodes and their eggs (Bongianni *et al.,* 1982*).* According to Elmslie (1984), animals that feed or prey on giant land snails, particularly the young ones (hatchlings) include mammals, reptiles, amphibians, some avians, beetles, crabs, mites and some fishes. Snails such as *Achatina fulica* and *Arcachatina marginata* will really and even avidly feed on the flesh of injured dying, dead and decaying individuals of its own species, though the eggs, and delicated young ones (hatchlings) are safe in the presence of larger individuals (Green, 1996). Imevbore (1990) observed that common rat preyed on the hatchlings in particular as revealed by their attraction to traps with such snails as baits.

He further noted that stomach examination of captured rats revealed in one out of three trapped rats and partly chewed snails flesh in another.

Table 2.4: Ectoparasites associated with *Archachatina marginata*

Ectoparasite	**Common name**	**Parts of snail located/found**
Ricadoella limacoum	White mite	Mantle, cavity, head and foot
Proistoma stachi	Spring tail	Shell, head, foot and lip,
Selenia uricolar	Drilid beetle	Shell, head, food and collar
Prenolepis imparia	Honey ant	Head, sole of foot, inner collar and lining

Source: Imevbore (1990).

2.13 Endoparasite associated with *Archachatina marginata*

Rhabditis spp. a pale white coulour, with an almost transparent body, cylindrical and non-segmented and varied in length from about 1.00 mm to 3.00 m

2.14 Digestibility

Everything refereed to as matter has potential energy. One of the most important tools for determining a feed's or feed component's nutritive value is its digestibility. Only the unavoidable losses that occur during digestion, absorption, and metabolism are taken into account when determining the feed's actual value to the animal.

The most precise way to describe a nutrient's digestibility in food or feed is the proportion of the intake that is absorbed—that is, the amount of feed or nutrient that is digested and used by the animal as it moves through the intestines—less the amount of feces that are eliminated. It is assumed that the proportion of the feces that is excreted is not absorbed.

McDonald *et al.* (1991) defined digestibility as the portion of a food that is assumed to have been absorbed by the animal because it is not excreted in the feces. The digestibility coefficient and dry matter percentage are common ways to measure digestibility. It contributes to the biological value in some way. For instance, the digestibility of the hay's dry matter would be as follows if a cow consumed 10 kg of hay per day that contained 70% dry matter and excreted 6 kg of feces that contained 60% dry matter:

$$\text{Dry matter intake } \frac{70}{100} x10 = 7kg \qquad 2.2$$

$$\text{Dry matter output (faeces) } \frac{60}{100} x6 = 3.6kg \qquad 2.3$$

Therefore, dry matter (DM) digestibility:

$$= \frac{7-3.6}{7} x \frac{100}{1} = 48.6\% \qquad 2.4$$

Digestibility can be expressed thus:

$$\%\text{Digestibility} = \frac{intake - output}{intake\ DM} \qquad 2.5$$

Coefficient can be calculated in the same way for each consistuent of the DM.

The coefficient so obtained is referred to as apparent value because not all the dry matter or nutrient in the faeces would originate from food consumed. Part of the nutrient would have arisen from bile secretions, enzymes, wears and tears along the alimentary canal or GIT. These components are referred to as metabolic nutrients or DMin faeces (metabolic faecal nutrients). That is to say that metabolic nutrients are that nutrient in faeces which is not a dietary origin but have arisen from the secretions of bile, microbes, enzymes and wears and tears along the GIT. These values may be small but when corrected in the faeces, the digestibility is called true instead of apparent coefficient. Unless otherwise stated digestibility coefficients are universally acceptable. The value if undesignated as true is known as digestibility value/coefficient which is one of the indices of biological value.

Digestibility coefficient of protein is defined as the nitrogen in feed less nitrogen in faeces divided by nitrogen in the feed, expressed as a percentage.

Protein digestibility

$$= \frac{N.feed - N.faeces}{N.feed} \times 100 \qquad 2.6$$

Where N = Nitrogen

The total protein digestibility (TPD) is expressed as

$$\text{TPD} = \frac{N.feed - FN - MFN}{N.feed} \times 100 \qquad 2.7$$

N = Nitrogen

FN = Faecal Nitrogen

MFN = Metabolic faecal Nitrogen

TPD = Total protein digestibility

2.14.1 Measurement of digestibility

Proximate analysis is performed on the diet or feed under investigation. After that, it is given to the animal in known quantities, weighed, and the feces output is measured. However, the fact that feces and urine are voided together from the same orifice makes

it more or less difficult to determine digestibility for snails, just as it is for poultry. As a result, snail droppings contain both feces and urine. Therefore, the faecal and urinary components ought to be separated in order to determine the digestibility coefficient in snails. At this time, there is no evidence that this kind of work has ever been done on snails. To ensure a uniform composition, the test food and feed should be thoroughly mixed prior to use. Before beginning collection of feces, it is given to the animal for a week (for rats, guinea pigs, poultry, and monogastrics) or two weeks (for ruminants) to familiarize the animal with the diet and remove any leftover foods or feeds from the GIT. After this initial phase, feed/food intake and faecal output are recorded daily at approximately the same time before feeding.

2.14.2 Factors influencing digestibility

Certain factors have been identified to influence digestibility of any type to feed consumed by animals. Some of these factors are:

2.14.3.2 Feed factors

2.14.2.2 Feed composition: The chemical makeup of a feed has a direct impact on how easily it can be digested. There is little variation in digestibility between samples for feeds whose composition is relatively constant. Cell content (made of protein) are almost completely digestible but the digestibility of cell walls (made of lignin and cellulose) depend on the extent to which they are liquefied (i.e on the lignin content of acid detergent fibre (ADF). Fibre is capable of absorbing amino acids and peptides, withholding them from absorption one to the hydrophobic binding of amino acids to it. Studies have suggested that fibres may absorb trypsin and chymotrypsin and thereby decreasing the activities of the proteolytic enzymes. Fibres may form gels which obstructs the access of enzymes to the protein. An increase in crude fibre content of many foods by 1% unit causes a reduction in the digestibility of the total organic nutrients of 0.7 to 1.0 unit for ruminant and twice this value for pigs (McDonalds *et al.*, 1991).

2.14.2.3 Ration Composition: These are the composition of other foods incorporated into the feed for the animal. The digestibility of feed is therefore influenced not only by its own composition but also the composition of other feeds consumed with it (Soest, 1982). A sample of GNC may differ in digestibility according to whether it was eaten with cassava flour or maize offals, or hay or silage.

2.14.2.4 Preparation of feeds: Chopping, chaffing, crushing or grinding, and cooking are all common treatments that are applied to feeds. Cement grain should be ground for pigs and crushed for cattle to maximize digestibility; any other way they might go through the guts unblemished. Ground roughages go through the rumen quicker than long or hacked materials and their sinewy parts might be less totally matured. The grinding of roughages therefore reduces digestibility of their crude fibre by as much as 20% units, and of the dry matter as whole by 5-15% units.

Roughages such as cereal straws or maize stovers may be pre-treated chemically to separate the two components. Straw is soaked with 2.3% solution of NaOH for two days and washed to remove residual alkali. Solution of wood ash (lyle) can be used to achieve the same objective after soaking for five days. Both processes are capable of increasing the dry matter digestibility of straw from 40% to 60% or more. Cooking of foods does little to improve their digestibility except in the case of maize and potato fed to pigs and poultry. Pre-cooked soyabeans presents higher digestibility coefficients for monogastrics due to anti-nutritional factors that are heat labile.

2.14.4 Animal factors

2.14.3.1 Interspecies differences: The incidence of interspecies difference in digestibility estimation with feeding trials has been recognized. The ruminant is more efficient in the digestion of high fibre, low protein forages while non-ruminant such as pig is more efficient in digestion of high protein low fibre feedstuffs (McDonald *et al.,* 1968). The rate of digestion of food protein by broilers may be different from that of sheep. It must however be noted that interspecies differences are small and of no practical importance for ruminants, though Nowt *et al.,* (1965) observed notable differences in digestive abilities of cattle and sheep. So animal of the same quality and quantity of feed may not digest the feed to the same extent.

2.14.4 Level and rate of feeding

The rate at which digesta travels through the animal's digestive tract increases when the animal consumes more feed. As a result, the feed is subjected to digestive enzymes for shorter periods, which may make it harder to digest. The quantity of feed required by the animal for what is known as body maintenance—the quantity that prevents the animal from losing weight but does not permit growth—is frequently expressed in

multiples of the level of feed. Unity is the definition of this level. According to McDonald *et al.*, (1991), the level in poultry may reach 2.0 to 3.0 times maintenance in non-ruminants.

2.14.5 Anti-nutritional factors

Anti-nutritional factors, many of which mediate their effects via changes in the digestive and absorptive processes (thereby increases the endogenous amino acid loss) are often responsible for depressing nutrient digestibility in animal diets. Examples of anti-nutritional factors include the trypsin inhibitors, tannins, lectins, and saponins. Legumes contain a wide range of these toxic factors and are known to provoke deleterious reaction in animals unless the legumes are properly processed. The effect of these anti-nutritional factors may be partially prevented or corrected through processing (e.g. the development of varieties of field beans with a low tannin content).

2.15 Feed Additives in Animal Nutrition

Feed additives are non-nutrient substances that are added to animal feeds to improve the efficiency of feed utilization, feed acceptance or improve health status and metabolism of animal to certain extent. According to Zahid, (2000), feed additives can be grouped into two broad classes viz:

2.15.1 Additives that influence the animal feed.

The antifungal agents are included in this. The growth of fungi like Aflatoxins and Fusarium in stored feeds like cereals, grains, or legumes is typically prevented by antifungal agents. Drying stored grains to a moisture level of 12 percent or less prevents mold growth. When grains have a moisture content of more than 13 percent or 14 percent, a relative humidity (RH) of 80-85%, and a temperature of 55 degrees Fahrenheit or higher, mold inhibitors should be used. Additionally, 1% calcium propionate can be applied to stored grains. According to Saheed (2020), propionic acid provides protection for 90 days. Another type of additives that influence the feeds are the antioxidants. Antioxidants inhibits auto-oxidation of fat (rancidity).

Unsaturated fatty acid + oxygen = toxic products with bad odour and this can destroy the fat-soluble vitamins (i.e Vit. E & Vit C).

1. Additives that influence animal feed intake, growth, feed efficiency, metabolism, health and performance.

These include:

2.15.2 Feed flavor- increase feed palatability and intake

- Buffers
- Methane inhibitors
- Ionophores
- Prebiotics
- Probiotics
- Probiotic-yeast
- Organic acids (Acidifiers)
- Antibiotics

At the beginning, a greater variety of antibiotic were being used as growth promoters in livestock diets. The mode of action of antibiotic is to reduce the hazards caused by pathogenic micro-organisms in the gut and consequently lowering the occurrence of enteric diseases. However, the use of antibiotics had been abused, such that residue of the antibiotic found their way into human's food chain in addition to the risk presented by resistant bacterial which can give rise to problems in animals and human health (EUC 2005).

2.15.3 In-feed Enzymes: The metabolic reactions (chemical reactions) that take place in animals' bodies are sped up by enzymes, which are chemical substances. Enzymes aid in the utilization and conversion of various feed ingredients, thereby enhancing animal performance and growth rate (Huyghebaret *et al.,* 2013). Animal scientists have recently been able to find some in-feed enzymes that can help animals grow when they are added to their diets in the right amounts since the use of antibiotics as growth promoters (AGPs) was banned.

Huyghebaret *et al.* (2013) claim that these proteins help the creatures proficiently hydrolyze and separate plant materials. Some enzymes, like xylases, beta-glucanasses, and beta-D-mannanase, are already present in chicken feed. Huyghebaret *et al*. (2015) noted that the mechanism behind in-feed enzymes' effectiveness as growth promoters is still poorly understood. It may require altering the gut microbiota, repairing or

preventing damage caused by undigested plant residues rubbing against the intestinal surface layer and degrading macromolecules (Elijah *et al*., 2013). The results were encouraging when chicken were fed enzymes in their food to speed up growth and get more nutrients (Huyghebaret *et al.,* 2009). In point of fact, this group discovered that adding enzymes to feed increased the feed conversion ratio by 2% to 5%. Numerous studies, including systematic reviews and meta-analyses, have shown that these enzymes decrease the incidence of intestinal lesions and necrotic enteritis, which is primarily caused by intestinal lesions (Robert *et al*., 2006). However, the low pH of the gut, which may inactivate infeed enzymes, rendered in-feed enzyme growth promoters in pigs ineffective. Only those, like phytases, that are stable at low pH would be able to boost swine growth. Additionally, it had been suggested that ruminant animals are ineligible for in-feed enzymes due to the presence of rumen, which prevents enzymes from reaching the intestine (Robert *et al*., 2006).

As products of fermentation from fungi and bacteria, in-feed enzymes are likely to only have a beneficial effect on the animals. They enhance the hydrolysis of feed components like glucans, proteins, and phytases, which are difficult for animals to digest, and they are frequently added to manogastric animal feeds. According to Hughes and Heritage (2002), in-feed enzymes have few drawbacks and are very effective at increasing feed consumption and feed conversion efficiency.

2.15.4 Application of -*β-mannanase* (Hemicell®)

It was discovered that the cell wall structure of leguminous seeds like soyabeans and other legumes contains a variety of non-starch polysaccharides (NSP). Some seeds and grains used as monogastric feed contain these components, which are called hemicelluloses. Snails, like all monogastric animals, lack the enzyme that breaks down galactomannans. Galactomannans can be broken down by the fermentation product of Bacillus lentus, the enzyme beta-D-mannanase.

It is now acceptable to add enzymes to animals' diets to increase the feed's nutritional value, such as in monogastric animals (Gradient *et al.,* 1992). *β*-D-*mannanase* improves the growth performance of all monogastric animals tested, as stated by Chesson (1987). Hahn *et al.* (1995) found that pigs fed diets containing *β*-D-*mannanase* had consistently higher feed efficiency than pigs fed diets similar to those without *β*-D-*mannanase*. In addition, Pettey *et al.* (1999) reported that during the late nursery phase, pigs fed diets

supplemented with β- D-*mannanase* converted feed to gain more effectively than pigs fed the control diet. β-D- *mannanase* is Hemicell®'s active ingredient. Additionally, it contains -galactosidase, xylanases, cellulases, and amylase. β-D- *mannanase* boosts growth performance in two ways, namely

2.15.5 Reaction Mechanisms of *β-D-mannanase*

The hydrolysis of the -1-4-glycosidic linkage in -Mannans is how -D-mannanase works. According to Mc Cleary (1986), the enzyme randomly cleaves the 1, 4-D-Mannan main chains of galactoglucomannan, galactomannan, and mannan. Another mode of action of -D-mannanase is to reduce -Mannan levels in the gastrointestinal tract, which may decrease the stimulation of innate immunity (Jackson et al., 2001). 2004). Excitement of the inborn resistance occurs because of the retention of β-Mannan from the gastrointestinal substance and in this way improves the expansion monocytes and microphages as well as resultant cytokine creation. Additionally, it is well established that -Mannans (-galactomannans), which can be found in common feed ingredients like soya bean meal and other leguminous feeds, are antinutritive fibers (Hsiao, et al., 2006).

β-D-mannanase when added to takes care of corrupts β-mannans to save energy for execution while most catalyst items are energy-relaeasing compounds. They "open up" parts of the feed that the animals can't get to on their own. D-mannanase is a source of energy. Through the degradation of -Mannans in feed (Whistier et al., 1957), β-D-mannanase lessens metabolisable energy (ME) misfortune brought about by the FIIR from the β-mannans permitting more energy to be accessible for development and execution.

Dietary supplementation with -Mannanase helps to reduce the viscosity of the intestina digesta and has negative effects when accompanied by an increase in the viscosity of the intestine's contents (Almirall et al., 1995). According to Danicke et al., the -Mannans found naturally in guar meal (say, 60-80 g/kg) bind with large water molecules to increase the viscosity of animal digestive fluids. 2000). In addition, Karimi and Zhandi (2014) reported that the feeding of -gluconase and -Mannanase enzymes altered the gut's morphology. Gutierrez *et al*. (2008) stated that these diets may increase resistance to pathogenic bacteria when supplemented with enzymes.

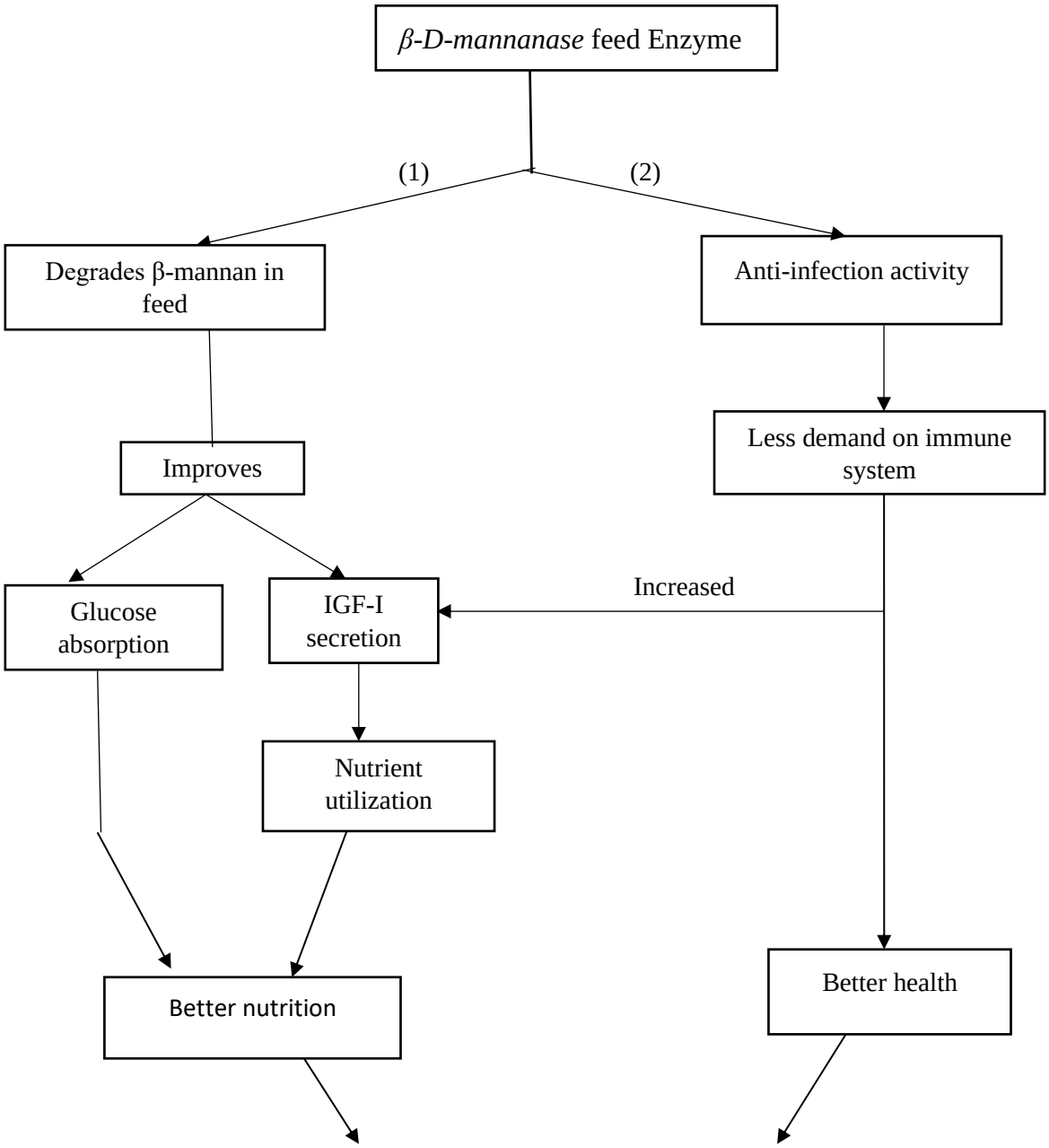


Figure 2.4: Reaction Mechanism of *β-D-mannanase*

Source: Southern Poultry Research, (USA) and Chem Corp (1989).

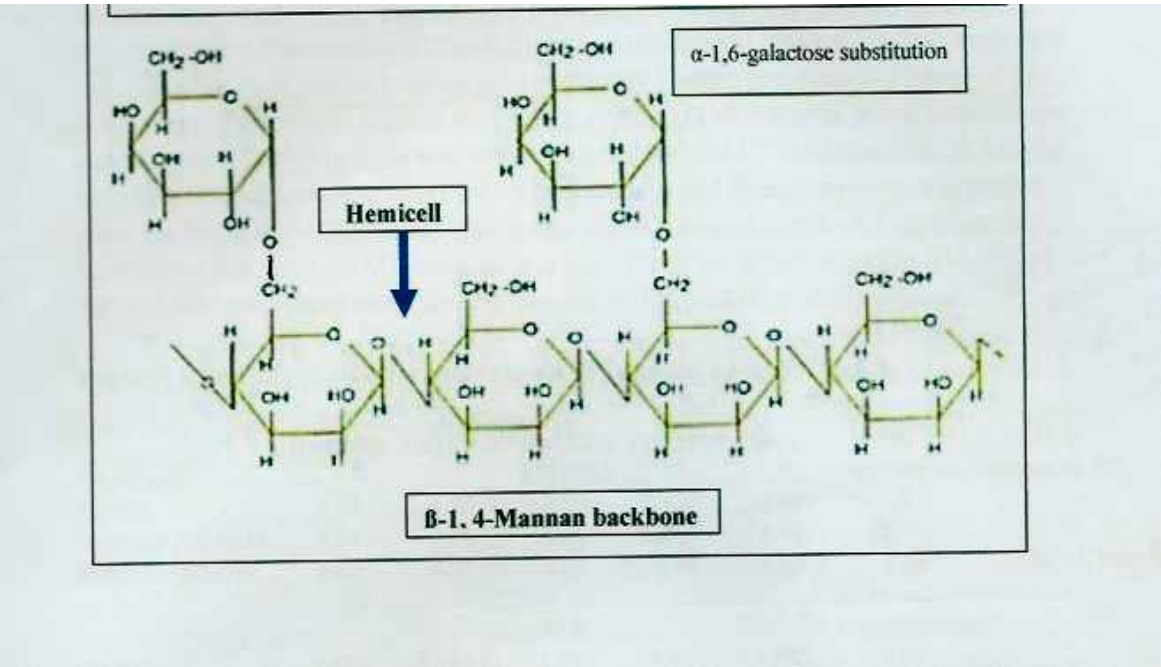


Figure 2.5*:* Structure of *Soy* β-1, 4-*galacto-mannan* and its *hydrolysis by ß-D-mannanase*

Source: Jackson (2003)

The following steps can be used to summarize these actions:

• It affects the viscosity of the intestina digesta; • It prevents the growth of pathogenic bacteria in the gastrointestinal tract and allows for the production of some sugars like D-Mannose as a source of energy.

• Effect on immune response and nutrient release

The live weight consistency is a significant component influencing the benefit of domesticated animals handling plants. The reason for this is that improved final products will result from increased live body weight uniformity in animal output.Anderson *et al*, (2001) after a number of trials in order to access the effects of diets supplemented with *β-D-mannanase* on live body weight uniformity in monogastric animals concluded that they had an encouraging result. Mannans are very common in nature and available in legumes and beans and their respective enzyme-mannanase has been used to improve monogastric nutrition. Soyabean meal contain some amounts of β-mannans. β-mannans are known to be anti- nutritional in monogastric animals and as such require the action of conjugate enzymes.

2.16 Probiotics in Animal Nutrition

Probiotics are viable, live cultures of microorganisms that are thought to be beneficial to the host organisms in animal nutrition. They were also described as viable live organisms (like bacteria, yeast, and fungi) added as feed additives that help maintain a healthy balance of microbial populations in the gastrointestinal tract and fight pathogens. "Live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance," Fuller (1992) said of probiotics. The word "probiotic" comes from the Greek words "pro" and "bios," which mean "for the life" (Gibson and Fuller 2000). Probiotics are "Live microorganisms which when administered in adequate amounts confer a benefit on the host," as defined by FAO/WHO. Probiotics have been reported in the literature as the most scientifically advanced microbial feed additives in the world. It prevents the growth of undesirable microorganisms in the gastrointestinal tract while simultaneously encouraging the growth of beneficial bacteria. Probiotics have accelerated resistance to pathogenic bacteria colonization and enhanced host mucosa immunity, both of which contribute to reduced pathogenic load and improved animal health (Choct 2009; William et al, 2001), and a diminished gamble of food-borne microbes in food varieties.

The most widely recognized sorts of microorganisms utilized as probiotics in creature takes care of include: Bifidobacteria, bacilli, and some yeasts (Saccharomyces cerevisiae) that produce lactic acid Probiotics are one of the additives used in feed regulation to keep the microbial communities in monogastric and ruminant digestive tracts stable. Direct-fed microbes (DFMs) and digestive bio-regulators are other names for them. By producing enzymes in the gut that are effective in converting certain components of the diet into more digestible nutrients for the host animal, probiotics help improve nutrient utilization and control or prevent intestinal infections (Stanley Gilliland, 2014). Stanley (2014) also suggested that certain probiotic bacteria could be fed to animals or their products to alter certain body compositions, like the lipid composition. "The specific function of probiotics might be different depending on the host animal and more importantly on the characteristics of the probiotic," he added in his report.

- According to Parker (1974), the term "probiotic" refers to substances or microorganisms that help maintain a healthy intestinal microbial balance. Afterward, Crawford (1979) characterized probiotic as
- "A culture of explicit living microorganisms (essentially Lactobacillus species) which inserts in the creature to which it is taken care of and guarantee the powerful foundation of the gastrointestinal populaces of the helpful organic entities. According to the definition, "the culture must contain a specific minimum number of bacteria, be maintained in a dry and stable form for storage purposes, be temperature dependent, and produce an optimal response with a specific dose range." However, several researchers had proposed a number of hypotheses, some of which are listed below:
- The mode of action of probiotics is the production of lactic acid and some fatty acids with fewer carbon atoms, which help lower the pH of the intestinal tract. A lower pH of the intestinal tract encourages the growth and survival of beneficial bacteria and reduces the replication of pathogens along the gut regions. • By colonizing and adhering to intestinal cells, probiotic organisms eliminate harmful pathogens through competitive antagonism (Yirga, 2015)
- Lowered production of toxic compounds such as toxic amines, sulphides and ammonia have been observed with probiotics use (Nino Binns, 2013).

- Probiotics form hydrogen peroxide (H_2O_2), a bactericide which attack pathogenic organisms in the gut (McDonald *et al.,* 2010).
- Digestive enzymes and B-group vitamins that functions in enhancing digestion of ingested feeds are being produced by probiotics (Saarel *et al.,* 2000).

• Probiotics increase the activity of the host antibiotics, activation of macrophages, proliferation of T cells, and production of interferon (Fuller, 1992; Jin et al. ,1997).

• In addition to their ability to mask enterotoxins, probiotics also produce anti-enterotoxins (FEFENA, 2005). When probiotics attach to intestinal receptors, enterotoxins are excluded.

• The host animal benefits from probiotic supplementation by increasing appetite (Naharson et al., 1992). In layers and turkey, probiotics increase egg size, weight, and production (Thayer et al., 1978; Naharson *et al*.; Jin et al., 1998). Increase the host's feed conversion ratio (Raymane, 2000; Cavit, 2003) Lower animal mortality rates and beneficial effects on host health (Samanta, 2000; Biswa, 1995; Soomro and others, 2002) . Probiotics have a significant impact on poultry performance as a feed additive (Stavric and Kornegay, 1995). Probiotics subsequent to appending to the gastrointestinal mucosa and metabolites can go about as resistant modulatory specialist by actuating explicit and vague host safe reactions in chicks, which thusly prompts the counteraction and control of different irresistible sicknesses (Fuller, 1992; Koenen, et al., 2004; Rowghani *et al*., 2007). One major advantage of probiotics over antibiotics, according to Abe *et al.* (1995), is that they do not persist in animal products, as opposed to antibiotics, which can have serious side effects like drug resistance and harmful changes in the bacterial population in the proximal part of the gut. At the end of the day, probiotics comprises no wellbeing risk for the creature since they are not moved from the digestive system into the body of the creature. According to FEFENA (2005), they have no effect on the animal's metabolic processes. According to Huang *et al.* (2004), this success may be in part due to the animal's health, the probiotic's preparation method, the strain of selected microorganisms, and the dosage.

Dissimilar to anti-microbials, probiotics needn't bother with any withdrawal times before the butcher. The issue with probiotics is the absence of by and large satisfactory proof concerning their component of activity and of consequences for have creatures

even man, however they are extremely helpful in supporting development rate, weight gain and feed transformation proportion.

Probiotics are safe for humans: People can come into contact with probiotics used in animal nutrition in two ways: a) as workers in compound feed production facilities.

(b) During animal feeding, when a farmer comes into contact, but neither of these situations poses a threat to probiotic users. There are sufficient studies to suggest that registered probiotic products do not harm human health when applied directly to the skin, mouth, or nose. Standard trials have also shown that probiotic use for an extended period of time or on a regular basis does not pose a health risk (FEFANA, 2005). Additionally, probiotics are only introduced through feed, so they do not enter the food chain.

2.16.1 Safety for animals: All of the probiotics that have been approved for animal nutrition by FEFANA (2005) have safe records. There are still no signs of dysbiosis in the gut, even in the event of overdoses of more than a thousand times the recommended levels in feed. As a result, probiotics pose no threat to the animal's health, have no effect on metabolic processes, and have no adverse effects.

2.16.2 Safety for the environment: The importance of the harmlessness of the probiotics to the environment is a factor to consider before registration. Probiotics usually exhibit their effect in the gut. As the probiotics get to the exit of the gastrointestinal tract in the digesta, they die off, in addition to all other intestinal microorganisms (Simon, 2005).

2.17 Prebiotics in animal nutrition:

Aside from probiotics, prebiotics are additionally utilized as regular feed added substances. Feeds containing prebiotics encourage the growth and activity of some beneficial microorganisms like fungi and bacteria. Most of the time, dietary prebiotics are non-digestible fiber compounds that pass through the upper part of the gut undetected and serve as a substrate for the beneficial bacteria that live in the large intestine (the colon) and stimulate their growth or activity. They are short-chain carbohydrates that improve the gastrointestinal microbiota's composition or metabolism. In their reports, Marcel Robertfoid (1995) defined prebiotics as "Non-digestible food ingredients that try to improve the health of the host animals by selectively stimulating

the growth and/or activity of one or a limited number of bacterial species that are already residents of the colon."

According to Gibson et al.'s additional definition of prebiotic, 2011) was described as "a selectively fermented ingredient that confers beneficial host health by causing specific changes in the composition and/or activity of the gut microbiota."

Prebiotics and probiotics serve as a conceptual bridge between food and drugs because they are a functional component of food. It is known that prebiotics have a function that complements but is distinct from that of probiotics. The prebiotic concept, on the other hand, is based on the selective stimulation of the host's own beneficial microbiota, with the prebiotic serving as the substrate that is (selectively) fermented, to stimulate the growth and activity of the particular microorganism of interest and thereby resulting in the desired health effect. Probiotics, as is already known, are exogenous microorganisms that are ingested to promote a specific health effect. In order for a prebiotic to reach its site of action without being distorted in its configuration, it must resist all effects of gastric acidity and digestive enzymes because the large intestine is their primary site of action. Prebiotics are thought to be beneficial because they stimulate the growth and/or metabolic activities of the bacteria that ferment them in the colon. Bifidobacteria and lactobacilli are currently the primary targets of prebiotics' actions that are known to be general.

According to research, prebiotics boost the immune system, which in turn improves animal health. It aids in gut health maintenance and thus prevents the spread of pathogens throughout the food chain when added to livestock feed. There are two distinct categories of fermented ingredients that are frequently utilized in animal nutrition: probiotics and prebiotics. Synbiotics are the combination of prebiotics and probiotics.

2.17.1 Prebiotic Selection criteria

Based on the reports from ILSI Europe (2013), the following factors are considered for selecting prebiotics:

- For prebiotics to reach the colon intact, they must be able to resist the effects of digestive enzymes and acid in the stomach.

- The fermentation must have a positive impact on the health of the host by altering metabolic processes and enhancing immune system function.
- It must be able to selectively promote the probiotic bacteria's growth.
- However, it should not be overlooked that prebiotics must directly influence health, such as through the immune system or the binding of microorganisms to receptors.

Disaccharides, oligosaccharides, and polysaccharides are the three main types of prebiotics. Prebiotics used in animal nutrition frequently include:

- Fructo-oligosaccharides (FOS)
- Gluco-oligosaccharides (GOS)
- Isomalto-oligosaccharides (IMOS)
- Xylo-oligosaccharides (XOS)
- Lactulose
- Mannon-oligosaccharides (MOS)
- Polysaccharides Inulin (Fructans)
- Cereal fibre

Note that overdose of prebiotics may lead to flatulence and diarrhea.

2.18 Probiotics and Prebiotics Combination (Synbiotics)

The synergistic combination of probiotics and prebiotics is commonly referred to as a synbiotic. The combination of fructooligosaccharides (FOS) with a bifidobacteria strain or lactitol with lactobacilli is an example of a product for which the prebiotic compounds selectively favor particular probiotic organisms. Bifidobacteria, for instance, transform FOS into propionic acid, a short-chain fatty acid (SCFA) that the liver uses to lower serum triglycerides and cholesterol. Gibson and Robertfoid (1995) presented the idea of "synbiotics" suggesting a mix of probiotics and prebiotics that usefully impacts the host by working on the endurance and adherence of live microbial dietary enhancement in the guts, by specifically prompting the development as well as enacting the digestion of one or a set number of wellbeing advancing microorganisms, consequently further developing host government assistance. This reduces the likelihood of intestinal diseases and certain microbial disorders. It is important to note that using synbiotics, prebiotics, and probiotics is safe and does not harm the natural environment.

2.18.1 Probiotic use of Yeast (*Saccharomyces cerevisiae)* in Livestock feed

According to Suarez *et al*. (2018), the interest of animal scientists in probiotics as feed additives actually began well before 1950. This was established by the literature. However, as of late after the restriction on anti-microbials as development advertisers, endeavours of the creature researchers looking for the substitutes (non-anti-toxin choices) prompted the rise of different probiotics being utilized as development advertisers in animals takes care of. It is now known that including probiotics in livestock feed increases feed digestibility, improves production parameters, reduces diarrheal diseases, and lowers domestic animal mortality. Yeast strains have been used for several years in the production of food, such as bakery yeast, and in the production of ethanol from any starch source, such as cereals, tubers, and so on, and palm wine through the process of fermentation, which turns glucose into ethanol.

In addition, according to Suarez *et al.,* (2018), Hippocrates around 370 BC (2018) recognized yeast's diuretic properties and considered it a drug. A clergyman used yeast to treat leprosy and prevent contamination in the Middle Ages. It was also used to treat scarlet fever and rubella. Doleza *et al.* (2011), which Suarez *et al.* cited, in 2018) asserted that the yeast *Saccharomyces Cerevisiae* was utilized as a supplement for ruminant feed; which improved the ecology of the rumen because there was less oxygen, which made anaerobiosis and cellulolytic bacteria more likely (Garcia *et al*., 2012). Additionally, fresh palm wine-derived yeast had traditionally been used to treat measles in Nigerian communities.

2.18.2 Selection criteria for yeast strains with prebiotic properties:

Perez (2007) opined that yeast strains are selected based on their properties, as those to function as prebiotics are selected based on their tolerance to extreme acidity, bile salt resistance, and adhesion of intestinal cells. However, their antisecretory effect against pathogenic substances as well as immunostimulants cannot be ignored.

In the report of Rodriguez *et al.,* (2000) and Baptista (2002), live yeast cells can be used as detoxifying agents. Severe organs damage caused by dietary toxins are removed by *Saccharomyces cerevisiae* and as such been beneficial in reducing stress in clinical animals as it provides vitamins, enzymes and proteins. Yeasts are reported to affect fermentation by production of volatile fatty acid; Reduction of methane (CH_4) gas; reduction in ammonia concentration; enhancement of pH stability and enhance lactate

to propionate conversion. The bioregulatory action of yeast when used in animal feed involves microbial antagonism suppression of pathogens; inducing the immune system of the animal and attachment and displacement of the pathogens as it accelerates the activity of specific enzyme bacteria (Perez, 2007). However, these mechanisms are not yet completely clarified. It has been demonstrated that adding yeast (Saccharomyces cerevisiae) to ruminant feed has an effect (Lezcano et al., 2005), as animals' consumption of feed results in weight gain, increased productivity, and improved milk quality. According to Hill et al., it is important to know that dead yeast still have significant amounts of polysaccharides and proteins in their cell walls. These polysaccharides and proteins are still able to help the immune system and the absorption of nutrients (2006).

2.19 Lactic Acid Bacteria (LAB)

Lactic acid is a good growth promoter that can be produced naturally as a byproduct of fermentation and is found in living things. It is an excellent alternative to antibiotics. By fermentation, certain kinds of sugars are transformed into lactic acid by lactic acid bacteria. As probiotic feed additives, a variety of well-known species and some suitable strains were selected. The genera Lactobacilli, Pediococci, Bifidobacteria, and Enterococci are some common lactic bacteria used as probiotics. Enterococcus faecium (recently known as *Streptococcus faecium*) is the main species utilized in creature sustenance. Probiotics' production of lactic acid is primarily characterized by their metabolic activity in the intestine, which results in the release of antimicrobial substances and the formation of a biofilm to safeguard the intestinal mucous membrane.

Different species of microorganisms used for probiotic preparations include:

- *Lactobacillus bulgaricus*
- *Lactobacillus acidophyllus*
- *Lactobacillus casei*
- *Lactobacillus plantarum*
- *Lactobacillus helveticus*
- *Lactobacillus thermophilus*
- *Enterococcus faecium*
- *Enterobactris faecalis*

- *Bifidobacteria species*
- *Saccharomyces Cerevisiae*
- *Toulopsis sphaevica*

But of all these, Lactobacillus and Streptococci are the most popularly used to produce probiotics. This work emphasized the effects of *Lactobacillus plantarum,* and *Saccharomyces cerevisiae* and other growth promoters on the growth rate, body weight gain, feed intake, FCR on snails (*Archachatina marginata*).

2.20 Organic Acids (Acidifier)

Organic acids (carbonxylic acids) are compounds that contain the carboxyl group (-COOH) attached to either an alkyl group or aryl group (ArCOOH). Organic acids especially the aliphatic ones have manifested favorable impacts when administered as feed additives. They are very effective in altering the physiology of bacteria, affecting metabolic disorders that prevent proliferation and cause death. They aid in lowering stomach pH and thereby preventing digestive disorder. When animal feeds are supplemented with organic acids, they help to combat susceptible microorganisms, such as pathogenic bacteria and some fungi which would otherwise cause feed spoilage and thereby reduce the nutritive value by their metabolic processes. Organic acids commonly used in animal nutrition include citric or acetic acid, fumaric acid, propionic acid, lactic acid, ascorbic acid etc. These organic acids possess aliphatic structures and serve as a sources of energy for the cells. Aromatic acid possess different metabolic and absorption characteristics. Organic acids are good alternatives for growth promotion and disease prevention. Supplementation of organic acids at the adequate or appropraite doses in animal feeds can increase the body weight gain, improve feed conversion ratio and minimize colonization of pathogenic organisms in the gut of livestock (Kirgessner, 1958). Supplementation of 1-2% fumaric or citric acid would increase 4-7% average feed intake and feed efficiency by 5-10%. They are said to be beneficial especially in milk replacer by improving milk clot formation in the abomasum.

Organic acids' positive effects on cattle performance and prevention of certain digestive diseases like acidosis had also been demonstrated in some studies (Martins et al., 1999). The following are additional suggestions that had been made:

• A decrease in the feed's antibacterial and antifungal properties, pH value, and buffering capacity.

• A decrease in the feed's pH level.

• A decrease in pH caused by the stomach's release of H+, which makes protein digestion easier and activates pepsinogen to make pepsin.

• Restraint of gram-negative native microflora in the gastrointestinal plot (GIT).

• Increase the amount of energy used in the intermediate metabolism.

The efficiency of an organic acid to inhibit the growth of the pathogens is a function of its pKa value which indicates the pH value at which the acid is available 50% in its dissociation and undissociation form respectively. It is only in its undissociated form the organic acid has its antimicrobial power as they can penetrate through the walls of bacterial and fungi to alter microbial metabolism. This implies that the anti-microbial efficacy of organic acids is higher in acidic conditions, in the stomach, and reduce at neutral pH in the intestine (Roth and Ettle, 2005).

2.21 Buffers

The pH must not deviate significantly from a predetermined value in virtually all biological processes and many other chemical processes. The close to consistency of pH in a framework to which corrosive or base is added is expected to buffering activity of a corrosive base balance. Cushions are substances that assistance to oppose pH change. Additionally, buffers are used in animal nutrition. Sodium bicarbonate (NaHCO3), magnesium oxide (MgO), and calcium carbonate (CaCO3) are examples of weak acids and bases that are resistant to changes in pH. Feed acidosis is reduced or prevented by buffer.

2.22 Methane Inhibitors

The production of methane (CH4) lowers feed efficiency. Inhibiting production also reduces methane, which is linked to global warming, and improves efficiency.

2.23 Ionophores

These form a class of antibiotic additive for cattle. These are produced by strains of streptomyces fungi and include monesin and Iasalocid. They improve feed efficiency and milk production.

2.24 Antioxidants

Antioxidants are substances that contribute to the shelf life of feeds, flours, premixes and animal fats. They allow them to be kept for longer period because the oxidant protect them from deterioration effected by oxidation, keeping their sensory characteristics intact, thereby preventing them from rancidity and decolouration. These substances prevent an oxidative reaction or hydrolysis. Autoxidation or oxidative rancidity alters organoleptic qualities and lowers the nutritional value of fats. Sometimes, it produces toxic compounds due to its exposure to oxygen. The process involves addition of oxygen to the α-carbon of the double bond forming a hydroperoxide or peroxides. This reaction is irreversible but can be slowed down or delayed. Oxidation of fatty acids involves the processes of initiation, propagation and termination. Initiation is the creation of active free radicals by external energy. Propagation entails free radical forming peroxide radicals that attack fatty acids. These peroxides later split into highly volatile by-products that cause bad odour. Termination occur when reactive compounds interact with each other, and subsequently decreasing the amount of peroxide radicals. The process of hydrolysis is enhanced in the presence of moisture, calalytic agents or lipases, releasing glycerol as an end product. Methylketones and their esters can be form by their hydrolytic reactions. Antioxidants exist naturally but are easily lost during the processing or storage of products, so it is often advisable to add exogeneous antioxidants. Examples of natural antioxidants are Tocopherol, Ascorbic acid and its derivatives, Rosemary extract, Greentea extract while the examples of synthetic antioxidants are BHA, BHT, TBHQ, Propylgallate, Ethoxyquin.

2.25 Mycotoxins and Toxin binders

Certain fungi produce poisonous chemicals called mycotoxins. They are toxic fungal secondary metabolites that, when consumed by animals, including humans, can result in intoxication. Toxigenic fungi are fungi that produce mycotoxins. Grains like maize, cereals, soyabeans, sorghum, millet, peanuts, and silage frequently contain toxic fungi. During transportation, mycotoxins are also found in feed crops, hay, and grain. It can also be found in foods for humans or animals that have been exposed to conditions that encourage the growth of toxic fungi in the past. Mycotoxins thrive in conditions that are humid, warm, and aerobic. Inadequate storage in conditions that encourage the toxin-producing fungi's growth can also result in the growth of mycotoxins in feeds.

There are approximately 300,000 harmful mycotoxins, but only a few have been characterized as of yet. According to the Food and Agriculture Organization, approximately 25% of the world's crops contain mycotoxins, and no region of the world is immune. These toxins can also be found in some processed foods and feed that is made from feed that has been contaminated. Fungi belonging to the genera Aspergillus, Penicillium, and Fasarium produce the majority of mycotoxins. Animals and humans both contract mycotoxicosis when they consume feedstuffs and foods contaminated with mycotoxins. When animals or humans consume contaminated feeds or food in large quantities, mycotoxicosis can cause death or chronic illness due to damage to the kidney and liver.

The following issues can be found in livestock, which result in significant financial losses:

- Feed refusal and poor feed conversion.
- Increased restlessness, agalactia and lameness
- Depressed growth rate
- Weak shell eggs
- Skeletal disorders
- Impaired immune response
- Damage cardiovascular, endocrine, reproductive and nervous system.

This means that the presence of mycotoxins in the feed can be very detrimental to both animals and humans. Mycotoxins can enter into a human being through secondary food chain when infested livestock and their products are consumed.

2.26 Zeolite

Zeolites are solid, crystalline structures made of oxygen, silicon, and aluminum. They have cavities and channels inside that can hold water cations or other small molecules. They are also known as molecular mass on occasion. Aluminosilicate microporous minerals are frequently utilized as commercial adsorbents and catalysts. The open, cage-like framework structures that are contained within zeolites are one peculiar feature. Zeolite crystals are formed in this manner by water molecules and positively charged atoms with too few electrons (also known as cations) in alkali or alkaline–earth metal ions. Cation exchange is the process by which positively charged ions originally trapped within zeolites can be exchanged for other positively charged ions. They can easily gain or lose water molecules (reversible dehydration) as well. Known as molecular sieving, zeolites have regular, fixed-sized openings that allow smaller molecules to pass right through, but trap larger ones.

2.26.1 Uses of Zeolites

They are used on petrochemical cracking, water softening purification, in the separation and removal of gases and in agriculture. Zeolites have the ability to act as catalysts for chemical reaction which take place within the internal cavity.

2.26.2 Megawin

Megawin is a composite mixture of toxin binder, prebiotic and organic acids.it is non-nutritive used in mongastric feeds. Inclusion of Megawin to animal feed prevents and arrests the Mycotoxins in the feeds, improves immunity and has a positive effect on digestion and metabolism throught the following steps:

- The zeolites in Megawin selectively binds mycotoxins such as aspergillus/aflatoxins (B1, B2, G1, G2 and M1), Fasarium, vomitoxin and zearalenone in the feed without affecting the vitamins and minerals. The resultant mycotoxin-zeolites complez leaves no residues in the animal products (e.g. meat or eggs).

- Megawin provides Mannanoligosaccharides (MOS) – a-non-digestible protein carbonhydrate which supports the availability of nutrients used by the beneficial bacteria to provide antagonistic effect against harmful bacteria.
- Abundance of Megawin promotes the probiotic functions and supports e.g a healthy gut flora and maintenance and management of gut health.
- In addition, Megawin also contains a blend of organic acids that reduces the pH in guts, and low pH favours the multiplication of beneficial flora/bacteria and at the same time suppresses the growth of harmful bacteria/pathogens.

2.27 Economic importance of Snail

The knowledge about the nutritional value, medicinal, cosmetic, ornamental values of snails and mullusc shell waste for civil and construction engineering applications is still growing all over the world and more researches are going on.

Snail meat as source of nutrients: Since the beginning of time, humans have eaten snail meat (Cobbinah *et al*., 2008). According to a number of studies, snail meat has a high protein content (16.72-20.26 percent), high levels of iron (45-50 mg/kg), calcium, and phosphorus, and relatively low levels of sodium (2.32 g/100 g), fat, and cholesterol (0.05-0.09%). Cobbinah (1997) says that *A. marginata* meat has 45-50mg/kg iron, 0.05-0.8% fat, and 12-60% protein. Additionally, most of the essential amino acids for humans can be found in snail meat (Cobbinah *et al*., 2008). According to Ajayi's report from 1978, snails contain lipids and saturated fatty acids, have important health implications, and may be beneficial to elderly people who do not consume fatty foods or hypertensive patients. Magnesium, zinc, manganese, copper, and no phosphorus are found in snail meat. (Ajayi *et al.,* 1978). The snail shell can be used in formulation of poultry feeds particularly feeds for layers which requires high percentage of calcium. In European countries such as France, prepared land snails are taken as delicacy or appetizers and are very expensive and sought out meal. Escargot is the name given to a dish of cooked land snails, usually served as an appetizer in France and French restaurant. In America and Australia, where it is usually called abalone, it is consumed as main meal.

Here in Nigeria, Ghana and South Africa, the common African giant land snail is a traditional meat. It is popularly called "*Congo meat"*. It is traditionally consumed all over the southern parts of West Africa.

2.28 Medicinal value of snails: In West Africa, especially in Nigeria and Ghana, the use of snails for medicinal preparations cannot be overemphasized. In the south western part of Nigeria for instance, various medicinal preparations containing snail and snail products are being used in local areas for restoring fertility, virility as well as relieving pains during child delivery. It is also used to prevent blood loss in pregnancy. A substance known as mucin which can be isolated from *A. marginata* had also been implicated for lowering blood glucose (Akinnusi, 1984). According to Barman's (2013) report, haemolymph was used as an aphrodisiac, a treatment for tuberculosis, whooping cough, stroke, and foot rot. Due to its high iron content, haemolymph had also been thought to help treat anemia (Okafor, 2001; Cobbinah *et al.,* 2008). Because snail contains very little fat and cholesterol, it can also be used to treat hypertension, high blood pressure, and other health issues related to fat (Wosu, 2003; Adegbola, 1998, and Imovbore and Ademosun, 1988).

According to Akinnusi (2004), the glandular substances found in the edible giant land snails agglutinated some bacteria, making them potentially useful against whooping cough and other diseases. Snail shell powder (properly dried and ground) has been used by humans to speed up the healing process after burns. According to Osemeobo (1992), he reported that the fluid from snails can be used to halt blood loss from wounds, bring health to severed fingers, in removal of male foreskin, and suppress small pox. In folk medicine, the bluish fluid from the snail (haemolymph) is thought to be good for infant development when added to their meals (Ademosun and Omidiji, 1999).

Snails have been used for medicinal purposes for a long time, especially since Hippocrates confirmed that crushed snails could alleviate pain and inflammation on the skin. The discovery that the Chilean snail's slime could speed up the healing of skin lesions without leaving scars led to the production of "Elicina," a fairness product made from snails. It was said that Missha, a cosmetics company based in the United States, introduced a fairness cream under the brand name "Aquacell renew Snail cream" in 2010. 70% of them were snail slime. Additionally, the company was linked to claims that this cream reduced wrinkles, acne, pigmentation, and acne scars. Another exciting discovery is that snail slime may also be useful in orthopedics due to its unusual calcite crystals. This is because a researcher from Herriot-Watt University stated that calcite can be used to make bone cement using inorganic crystals in an organic matrix (Aitke, 2012). The treatment of kidney sickness, tuberculosis, iron deficiency, diabetes, asthma,

urticarial messes, circulatory issues, improvement of stoppage and hemorrhoids, avoidance of flu, and rebuilding of virility and essentialness were completely ascribed to this compound. Additionally, according to Jimenez (2012), this chemical was thought to maintain beauty by reversing the skin's aging process and removing burn marks, wrinkles, and scars. Ogunsanmi and co. 2003) noted that the haemolymph of Archachatina marginata has a ratio of albumin to globulin that is comparable to that of sheep and pangolin. It was found to be present in the snails' examined haemolymph enzymes, including aspartate transaminase (AST), alanine aminotransferase (ALT), and bicarbonate (HCO3-), as well as sodium (Na+), potassium (K+), calcium (Ca2+), magnesium (Mg2+), zinc (Zn2+), and other ions. According to Abere and Lameed (2008), a specific form of snail-derived calcium phosphate has been linked to the treatment of kidney diseases, tuberculosis, diabetes, asthma, heart conditions, and circulatory disorders. This was discovered in the study.

Utilizations of snail slime Snails typically leave behind slime trails. Special glands in the muscular foot of snails produce slime. This secretion influences the snails' ability to adhere to substrata, or surfaces. Depending on the species and the age, the slime is made up of high-molecular-weight glycoproteins and approximately 91.0-98.0% water (Denny, 1984).

Another function of the slime is to assist the snails in moving and lubricating themselves. The variety of slime a snail can produce is determined by the kind of stimulus it is exposed to. The sludge is gooey (tacky) when excitement is typical, however assuming that the snail is over and over or brutally upset, it delivers clear, frothing discharges (Danny, 1984). A wide range of gastropods, including marine gastropods, land snails, and slugs, produce mucus. Snail slime also helps to stimulate the production of collagen and elastin, protects the skin from free radicals, rehydrates it, and repairs damaged tissue. Dry skin, wrinkles and extended imprints, hurts and rosacea, age spots, consumes, scars, razor knocks, and, surprisingly, level moles can be in every way treated with snail sludge. In ancient Greece, crushed snails and sour milk are said to have been prescribed by the well-known Greek physician Hippocrates to treat inflammation. However, it wasn't until Chilean farmers who handled snails for the French market noticed that their skin appeared to be smoother that snail creams were first used.

Be that as it may, due to natural impurities, the snail ooze should be refined preceding use. E. coli and other harmful bacteria, as well as rat lungworm, can be carried by the slime. Lungworms are dangerous because they quickly travel to the brain after being ingested, where they can cause meningitis, which damages the brain, causes swelling, and damages tissue. Snail ooze, otherwise called snail filtrate in beauty care products, is stacked with supplements like hyaluronic corrosive, glycoprotein, and proteoglycan, which have been all demonstrated to be great for the skin and are usually utilized in magnificence items. These materials protect the snail's skin from UV rays, infection, dryness, and damage. Water makes up 91% to 98% of snail slime. The slime is typically filtered multiple times to increase its concentration and ensure its purity. Some snail slime products, it has been claimed, contain approximately 97% snail secretion filtrate.

However, when looking for a reasonably good product, the consistency and quality of the snail mucus should be taken into consideration. Snail secretion filtrate is found in the majority of Korean beauty products, including serums, facial masks, moisturizers, and fading creams. The majority of products don't stick as well as one might think. The majority of them are the same in texture, smell, and appearance. Thailand has a lot of snail health clubs, and major beauty-conscious people in Asian countries like Japan and Korea are interested in them. Residing snails are put on the client's face during the spa treatment and permitted to normally move.

2.29 Snail meat contamination

Snail living in natural environment or in the wild has been known to harbour pathogenic enterobacteriaceae. About forty genera had been identified in this family. Example of members of this family are, Salmonella, Proteus, Servatia, Enterobacter, Cirobacter, Pseudomonas, and Klsebsiella (Fagbuaro and Nwuzo, 2006). Some of the members had been discovered, however to live in the gut without causing any health problems in individuals while others manifest some signs of infection such as vomiting, diarrhea, and related symptoms. It has also been indicated that most diseases in human are caused by enteric pathogens which are rampart in animal rearing environment. Moreover, report about the occurrence of pathogenic strains of E. coli from snails' sources and outbreak of illnesses due to them were also increasing. A lot of cases of food poisoning are caused by infection with enteric bacteria. Microbial food poisoning is a term that describes an illness caused by taking or drinking food contaminated with bacteria, viruses, parasites,

toxins or chemicals. Snails are usually proned to contamination because of their nature of feeding. They are multi-feeders. They feed on filthy substances in the environment, decaying and rotten plant and animal materials. There are therefore possibilities of regular interaction of microbes with snails. It is therefore very necessary that the snails especially from the wild or backyard be boiled properly before consumption.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Facility

Five experiments were conducted at the Mini-Zoo unit, Department Wildlife and Ecotourism Management: 7.4490993N, 3.896667^{o}E, 7^{o}26^{1}56.76''N, 3^{o}53^{1} 48.0^{11}E. altitude 207.00m/679.13ft above the see level. Coordinates of University of Ibadan 7.4433^{o}N, 3.9003^{o}E, 7^{o}23^{1} 28.19''N, 3^{o} 54^{1}59.99^{o}E. The average temperature ranges from 25 to 29 degrees Celsius, and the average annual rainfall is about 1250 millimeters.

The snails were reared under intensive system of production, using cages with dimensions 50 x 45 x 45 cm. The cages were covered on all sides with mesh and wood. There were five hutches per cage. The floors of the hutches were fitted with wire mesh and lined with woven polypropylene layers. Loamy soil was used as bedding materials inside the hutches (8-10 cm thick).

3.2 Routine Health Management

The housing were swept/dusted and thoroughly washed with water, disinfected and allowed to dry for two days before the arrival of the snails. The experimental houses/pens and the environment were kept clean throughout the duration of the experiment. The soil were turned regularly to avoid accumulation of pathogens, germs, ants and other organisms, and leftover feeds were removed daily.

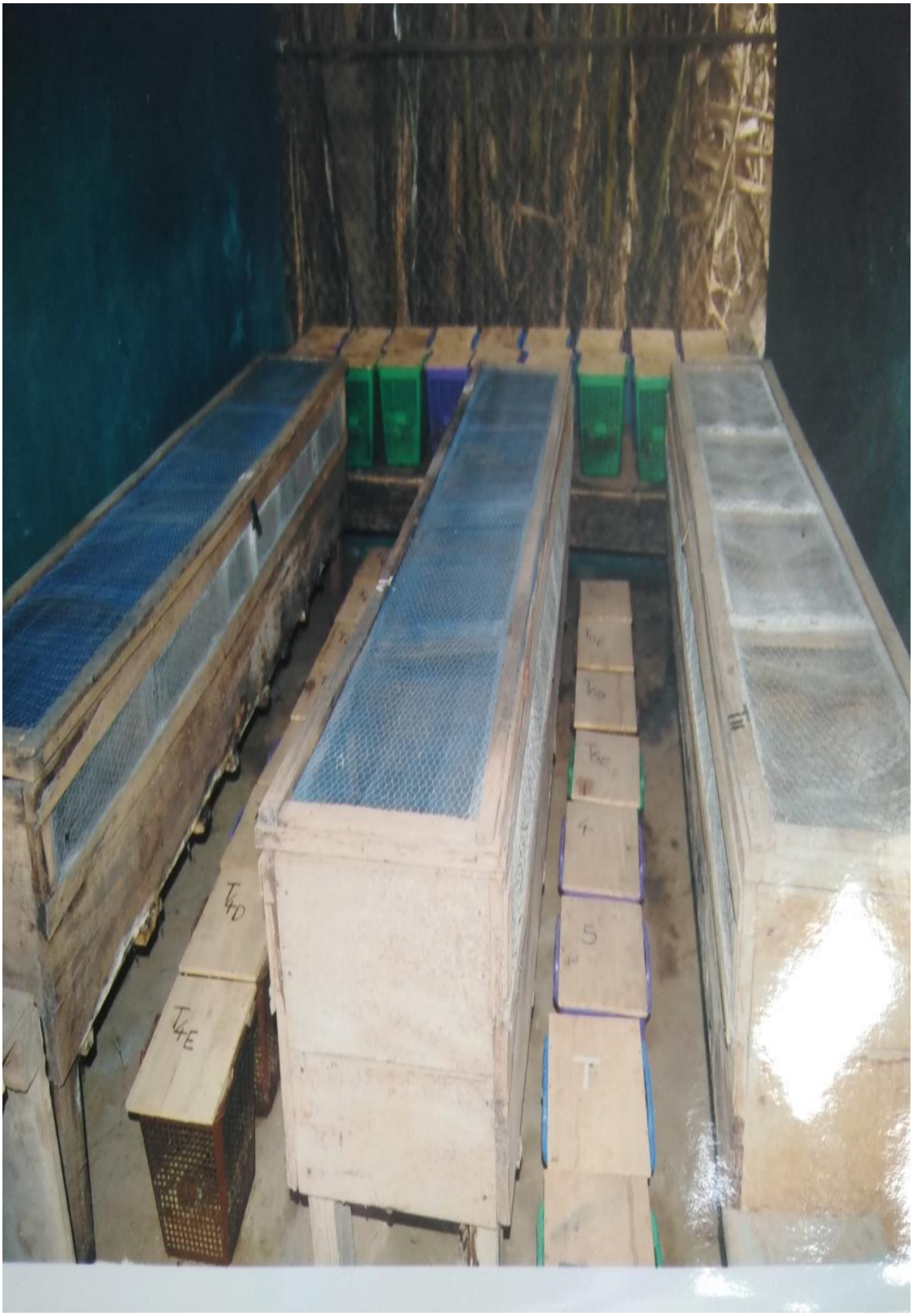


Plate 3.1: Experimental Cages used for the Snail

Experiment 1: Effects of sole-fruit feeding on growth performance and nutrient Digestibility in Grower African Giant Land Snail (*Archachatina marginata*).

3.3 Experimental Animals Allotment: Snails (*Archachatina marginata*) were procured from Apomu-Ikire market, Osun state. Snails were rinsed in chlorine-free water on arrival, weighed (93.04 $\pm$ 0.63 g) and indiscriminately assigned to five dietary treatments. Each treatment consisted of fifty snails, each replicate of ten snails, in a completely randomized design. The snails were allowed to acclimatize for two weeks and fed fresh pawpaw leaves only.

3.3.1 Experimental Diets: Sole-feeding of green pawpaw leaves (*Cavica papaya*) (7 months old), unripe pawpaw fruits 7-10 months old, pineapple fruits (*Ananae sativa*) (2-3 months), sweet orange (*Citrus sinensis*) (7-10 months), and coconut milt (*Cocos mucifera*) (9-11 months) were deployed in this experiment and all feedstuffs and water supplied ad libitum for seventy days. The feedstuffs were all supplied "as fed" and values consumed later converted to dry matter basis.

3.4 Sampling and Data Collection

3.4.1 Measurement of Growth Performances

Individual body weight and feed intake (consumption) per hutch were recorded weekly. Body weight gain was calculate as the difference between initial and final body weights and feed conversion ratio (FCR) was estimated as the ratio of feed intake (consumption) to body weight gain over the study period.

The lengths, widths, and the apertural length of the snails' shell were recorded weekly using Venier Calipers. The dimensions of the shell measured were: shell length (SL), Shell Weight (SW), and Apertural Length (AL) as described by Ebenebebe et al: (2014) and shell thickness measured using a micrometer screw gauge. The SW/SL, a description of the degree of globosity of the shell and AL/SL% (description of the spire (i.e winding) and the shape of the aperture) were estimated (Ndifon, 1979). Increase in shell length and shell width were estimated by deducting initial values from latter values.

Carcass Assessment: In the last part of 70 days, two snails were indiscriminately picked per duplicate, cleansed with paper towels and weighed prior to killing. The snails were killed by immersion in boiled water for a while and then carcass pulled out of the shells. The empty shells were weighed, the entire carcass in each case was weighed and recorded before separating it into its component parts. The components of the carcass were then measured and recorded. The ratios of these weights to the live weight were expressed as percentages. The foot samples were further used for the proximate analysis according to methods of AOAC (2000).

The dressing percentage was determined by the following relationships:

$$\text{Dressing percentage} = \frac{Carcass\ wt}{live\ wt} \times \frac{100}{1} \qquad 3.1$$

Carcass wt = The wt of the animal after harvesting and removoal of internal organs (and/or hides).

Live wt = The wt of the animal before harvesting

$$\text{Meat: Shell (M/S)} = \frac{Total\ flesh\ (g)}{Total\ shell\ (g)} \qquad 3.2$$

Nutrient Digestibility: At the end of 70 days, the digestibility of the experimental feedstuffs were determined using 15 snails, (three snails for each treatment, including the control over a 21 days period. The snails were housed in individual enclosure that allowed a complete/separation and collection of excreta. The snails were allowed four days adjustment period before collections were carried out. The feed consumed and the excreta voided out during the period were weighed and recorded. The freshly voided excreta from each treatment group was weighed and oven-dried and dry matter determined. The samples were then stored in air-tight containers ready for chemical analysis.

3.5 Chemical Analyses

All proximate analyses were performed at The Biochemical Laboratory, Department of Animal Science, University of Ibadan, using standard procedures.

The proximate composition determination: Dry matter, Crude protein, Crude fibre, Ether extract and Nitrogen – free extract of meat, excreta, and feedstuff.

In a forced air oven, Dry matter (DM) was measured by drying to a constant weight at 105^{o}C, and Ether extract (EE) was measured using the soxhlet method and petroleum ether as the solvent (AOAC, 2000). After detergent digestion, solubilization of non-fiber constituents, and correction for ash (AOAC, 2000), crude fiber (CF) was measured gravimetrically.

The nitrogen-free extract (NFE) was calculated as the balance after the sum of percentages of ether extract, crude protein, ash, and crude fiber were deducted from 100 percent on a DM basis. Crude Protein (CP) was estimated using the kjeldahl method (AOAC, 2008).

3.5.1 Metabolisable Energy (ME) Determination: the ME was obtained by the prediction equation according to [(Pauzenga, 1985) ME (kcal/kg = (37 x % crude protein + 818 x % ether extract + 35.5 x % nitrogen-free extract)]

Fibre Fractions: Neutral detergent fibre (NDF), Neutral detergent solubles (NDS), Acid detergent Fibre (ADF) and acid detergent lignin (ADL) were determined by the methods of (Soest, 1963).

Determination of NDF and NDS involved solubilisation in a neutral detergent solution consisting of sodium borate ($Na_2B_4O_7$) + disodium EDTA ($C_{10}H_{14}N_2Na_2O_8$) + sodium lauryl-sulphate ($C_{12}H_{25}N_aO_4S$) + disodium phosphate (Na_2HPO_4) + 2-ethoxyethanol.

Determination of ADF and ADL involved solubilisation in an acid detergent solution consisting of (CTAB) cetyltrimethyl ammoniumbromide ($C_{19}H_{42}BrN$) and sulphuric acid (H_2SO_4).

Proximate composition of the snail (*Archachatina marginata*) meat was determined according to the method of AOAC, (2000).

Phytochemical Analysis of the Feedstuffs: All sample materials were collected fresh, washed with clean water to remove the dust. The samples were oven-dried at 105^{o}C to constant weight, pounded into powder and stored in polythene bag for extraction.

3.5.2 Ethanol Extraction: 2.0g of each dried sample powder was weighed into an extraction bottle and 20 ml of 70% ethanol added, allowing the sample to be submerged. The mixture was then agitated using a mechanical shaker for 20 minutes. The agitated mixture was immediately filtered by using a funnel. The secondary metabolites, tannin saponins, flavonoids, photosteroid, alkaloids, phenols, steroids, cardiac glycosides and anthraquinone were determined qualitatively. These were identified using characteristic colour manifestations by the method described by Harborne (1998) and Ayoola *et al*, (2008). Each sample extract was tested individually with specific chemical reagent according to the standard procedures. Each test was qualitatively expressed as negative (-), positive (+), and the intensity of the characteristic colour was expressed as (++), or (+++), (Ayuk, *et al*, 2015).

Expansion of 2 milliliters of Ferric chloride ($FeCl_2$) answer for 1 milliliter of the example separate yielded a dull blue or greenish dark tone, which demonstrated the presence of tannins. Before adding 2 ml of distilled water, saponin was measured by vigorously shaking 2 ml of the sample extract in a test tube for 15 minutes. The formation of a transparent layer of foam indicated the presence of saponin. In order to confirm the presence of flavonoids in the selected plant product extract, 0.5 grams of each sample extract were added to 10 milliliters of distilled water in a test tube, 5 milliliters of diluted ammonia solution (NH_4OH) were added to a portion of the aqueous filtrate of each sample, and 1 milliliter of conc H_2SO_4 was added. The addition of one milliliter each of chloroform, concentrated tetraoxosulphate (vi) acid, and sample extract caused the yellow color, which suggested the presence of flavonoids or phytosteroids. The presence of phytosteroid was demonstrated by the presence of an earthy colored ring.

For phenol detection, 2 ml of distilled water was added to 1 ml of the sample extract after a few drops of 10% Ferric chloride were added. When a blue or green color appeared, phenols were present. For the purpose of alkaloid detection, two milliliters of concentrated hydrochloric acid (HCL) were also added to two milliliters of the sample extract. After that, a few drops of Mayer's reagent were added to the mixture to enhance it. The presence of a white or green precipitate indicated the presence of an alkaloid.

Glycoside determination in the Heart: Shook 0.5 ml of the sample extract and added 2 ml of glacial acetic acid and a few drops of 5% ferric chloride. After layering the mixture with 1 ml of concentrated tetraoxosulphate (vi) acid (Conc H_2SO_4), a brown ring formed at the interface, indicating the presence of cardiac glycosides.

Determination of Anthraquinone: Few drops of 2% hydrochloric acid (HCL) were added to 1 ml of the sample extract. The precipitate's red appearance indicated the presence of anthraquinone. For the purpose of detecting terpenoids, 10 milliliters of methanol (CH_3OH) were also added to approximately 0.9 grams of sample extract in a test tube. The mixture was thoroughly shaken and then filtered before being put into a test tube with two milliliters each of chloroform and tetraoxosulphate (vi) acid (H_2SO_4). Terpenoids are available in the example, as proven by the development of a rosy earthy colored tone.

Flavonoids: Quantitative phytochemical analysis 10 milliliters of 80% methanol was used to extract 1 gram of the test sample, which was left to stand for two hours. The mixture was then filtered into a petri dish that was weighted. The contents of the petri dish were transferred to an oven at 40 degrees Celsius to dry, and the petri dish was weighed until it dried to a constant weight.

Alkaloids: 20 ml of 10% acetic acid in ethanol was added to 1 g of the sample extract (W). After that, the mixture was shaken, and it was left to stand for four hours. After that, it went through a filter, and about a quarter of the filtrate evaporated, added a small amount of concentrated ammonia. After that, a weighed (W1) filter paper was used to filter the precipitate that was produced. After the filter paper had dried in the oven at 60 degrees Celsius, it was weighed to ensure a constant weight (W2).

$$\% \text{ Alkaloids} = \frac{W_1 + W_2}{W} x \frac{100}{1} \qquad 3.3$$

3.5.3 Saponins: 5 ml of 20% ethanol was added to 1 g of the sample extract, and it was placed in a 55 °C water bath for four hours. After that, it was filtered and the residue was washed twice with 20% ethanol. In the oven, the extract was reduced to about 5 milliliters. A separating funnel was used to add 5 milliliters of petroleum ether to the concentrated extract. After the petroleum ether layer was separated, 3 milliliters of butanol were added,

and 5 milliliters of 5% sodium chloride were used to clean it. After that, the butanol was put into a weighed petridish. After that, it was put into an oven to dry out, and the rest was weighed.

3.5.4 Tanins: 25 milliliters of the 80:20 acetone solvent mixtures were used to extract 1 gram of the sample: 10% Glacial Acetic Acid, with a 5 oonm absorbance permitted. Also, the absorbance of the reagent black was measured. Then, use 10, 20, 30, 40, or 50 mg/100 mg of tannic acid to create a standard graph. Take into account any dilution factors when determining the concentration of Tanin.

3.5.5 Phenolics, also known as Phenolic Acids: 20 ml of 80:20 acetone was used to extract 2 grams of the sample: 0.5% formic acid for the filter and two minutes. The extract was then combined with 1.5 milliliters of sodium carbonate (20 percent) and 0.5 milliliters of Folin-ciocalteau reagent. After mixing for 15 seconds, the color was left to develop for 30 minutes at 40^{o}C. Determine the GAE/ g Gallic Acid equivalent for A765.

3.5.6 Steroids: 5 grams of the sample were mixed with 100 milliliters of water, 0.1 millimeters of ammonium hydroxide (NH_4OH), 2 milliliters of petroleum ether, 3 milliliters of acetic anhydride, and conc. Added H_2SO_4. At 420 nm, the absorbance was measured.

3.5.7 Glycosides for the Heart: 1 gram of the sample was extracted using 40 milliliters of water and baked for 15 minutes at 100 degrees Celsius. 5 ml of glacial acetic acid, one drop of $FeCl_3$, and 1 ml of conc H_2SO_4 were added to 1 ml of the extract. Then, at 410 nm, the absorbance of the resulting solution was measured.

3.5.8 Terpenoids: Weighing 1 gram of the sample, it was placed in 10 milliliters of petroleum ether for 15 minutes. The absorbance was then measured at 420 nm using the filter.

3.6 Mortality: Mortalities were recorded as they occurred and the percentage mortality determined for each treatment.

3.7 Climate Data: The data on minimum and maximum temperature, rainfall and relative humidity within the study area (University of Ibadan) was collected from the Department of Geography.

3.8 Calculations and Statistical Analysis: All the information gathered were processed through analysis variance (ANOVA) using the General Linear Model of SAS (2003). Averages were divided with the use of Duncan's multiple range test at $p<0.05$.

Experiment 2: Growth and Nutrient Digestibility of Feed in African Giant Land Snail Hatchlings (*Archachatina Marginata*) in Response to Enzyme Supplemented Diets.

3.9 Experimental Animals' Allotment and Design:

300 hatchlings of Archachatina marginata (16 weeks old) were procured from Apomu-Ikire market in Osun State. The snails were cleansed with chlorine-free water on arrival, weighed and housed in cages for a period of seven days for acclimatisation and fed pawpaw (*Carica papaya*) leaves only. The snails were thereafter randomly assigned by weight (25.60 ± 0.39g) into four treatment groups of 75 snails, each treatment replicated five times with 15 snails per replicate, in a completely randomized design. The study lasted for 98 days.

A fibre degrading preparation from Bacillus lentus was used for the study. Its active ingredient is *β-D-mannanase*. It also contains other enzymes; amylase, xylase (or xylanase), cellulose and *α*-galactosidase.

3.9.1 Experimental Diet: To the formulated diets were added the fibre degrading enzyme at the inclusion rate of 0.00g (control), 0.10 g, 0.15 g and 0.20 g/kg diet for treatment H_1, H_2, H_3 and H_4 respectively.

Throughout the experiment, the environment was kept moist and the snails were given diets and water at will (*ad libitum*).

Table 3.1: Gross Composition of Experimental Basal Diets Fed to Snail Hatchlings.

Ingredients	%
Maize	9.00
Brewers Dry Grain	29.00
Wheat Offals	10.00
Soyabean Meal	33.00
Palm Kernel Cake	10.00
Fish meal	3.00
Oyster Shell	4.50
Dicalcium phosphate	1.00
Salt	0.10
Premix	0.25
L.Lysine	0.10
DL Methionine	0.10
Total	**100.05**
Calculated Composition	
Metabolishable Energy (Kcal/Kg)	2367.37
Crude protein (%)	26.11
Cacium (%)	4.45
Phosphorus (%)	0.64

Premix composition: Vitamin A; 10,000 IU, Vitamin $D_{3;}$ 200,000IU, Vitamin $K_{3;}$ 20,000mg, Vitamin B;30,000mg, Vitamin $B_{2;}$ 50,000mg, Niacin 4,500mg, Calcium pantothenale 10,000mg, Vit B_6 40,000mg, Vit B_{12} 20mg, Chlorine chloride 300,000mg, Biotin 100mg, Manganese 50mg, Iron 300,000mg, Zinc 120,000mg, Copper 80,000mg, Iodine 15,000mg, Cobalt 300mg, Selenium 130mg, Antioxidant 120,000mg. Metabolisable Energy (Kcal/kg) was obtained from prediction equation ([Pauzenga, 1985). ME (Kcal/kg = 37x% crude protein + 818 x % Ether extract +35.5 x % Nitrogen – free extract)

3.10 Measurement of Growth Performance

The mean daily feed intake (FI), mean weekly body weight gain (BWG), feed conversion ratio (FCR), and shell dimensions (weekly increases in shell length and width as described in experiment 1) were used to calculate performance indices. By subtracting the weight of the diets that were left over from the weight of the diets that were provided, we were able to determine the average daily amount of feed that the snails consumed. The difference between the mean weights of two consecutive weeks was used to determine the experimental snails' mean weekly body weight gain for each treatment. The ratio of feed intake to body weight gain was used to measure the feed conversion ratio.

3.11 Carcass Characteristic Evaluation

After terminating the feeding trial on day 98, three snails from each treatment were indiscriminately picked for carcass assessment. The snails were cleansed thoroughly with paper towel and weighed. The snails were then killed by immersion in boiled water treatment by treatment. The weight of each of the component parts of the carcass, were measured and recorded. The ratio of each weight to live weight was expressed as percentage.

3.12 Nutrient Digestibility

On day 98, three snails were indiscriminately picked from each treatment for metabolic trails in clean hutches. The snails were fed *ad libitum* with the respective diets. Excreta collection started after four days of acclimatisation and was carried out for ten days in order to get enough excreta samples. Concentrated H_2SO_4 was added in drops into the containers to trap the possible NH_3 gas escaping. Samples of dialy collectiions were pooled together for each treatment, oven-dried at 60^{o}C for two days at constants weights, then stored in an air-light container in readiness for the sub-sequent analyses according to the methods of AOAC (2000).

$$\% \text{ Digestibility} = \frac{Intake - Output}{Intake\ DM} \text{ x } \frac{100}{1} \text{ i.e CP } = \frac{N.Feed - N\ Excreta}{No\ feed} \text{ x } \frac{100}{1} \qquad 3.4$$

Where n = nutrient in feed/excreta

3.13 Mortality: Mortalities were recorded as they occurred and the percentage mortality determined for each treatment.

3.14 Proximate investigation of the experimental foods and faecal samples from the snails were carried out according to AOAC (2000) at the Biochemical Laboratory, Department of Animal Science, University of Ibadan.

3.15 Calculations and Statistical Analysis

All the Information gathered were processed through descriptive statistics polynomial regression and ANOVA at $\alpha_{0.05}$

Experiment 3: Growth and Nutrient Digestibility of feed in Grower, African Giant Land Snail *(Archachatina marginata)* in response to Enzyme Supplemented Diets.

3.16 Experimental Animals Allotment and Design.

A total of 240 grower snails *(Archachatina Marginata)* were procured from Apomu-Ikire market, Osun State. The snails were cleansed with chlorine-free water, weighed (92.03 ± 0.62 g) and indiscriminately assigned to four dietary treatment groups of 60 snails for each treatment each replicated five times and twelve snails per replicated in a completely randomized design. The animals were allowed to acclamatise for a week while being fed fresh pawpaw leaves only.

Four diets were formulated and to them were added the fibre degrading enzyme (β-D-mannanase/ kg diet for treatment T_1, T_2, T_3 and T_4 respectively. The snails were permitted *ad libitum* right to diets and water and the environment kept moist throughout the experimental period. The investigation lasted for 98 days.)

Experimental diet: To the formulated diets were added the fibre-degrading enzyme (β-D-mannanase) at the inclusion levels of 0.00g (central), 0.10g, 0.15g, and 0.20g/jg diet for treatments T_1, T_2, T_3 and T_4, respectively.

Table 3.2: Gross composition of experimental basal diets fed to grower snails

Ingredients	%
Maize	13.70
Soyabean Meal	27.50
Brewers dry grain	25.50
Fish meal	2.50
Wheat offal	10.00
Palm Kernel Cake	12.00
Oyster Shell	4.00
Dicalcium phosphate	4.05
Salt	0.20
Premix	0.25
L.Lysine	0.20
DL Methionine	0.10
Total	**100.00**
Calculated Composition	
Metabolisable Energy (Kcal/kg)	2413
Crude Protein (%)	24.00
Calcium (%)	4.90
Phosphorus	2.75

Premix composition: Vitamin A: 10,000 IU, Vitamin $D_{3:}$ 200,000IU, Vitamin K_3 20,000 mg, Vitamin B: 30,000 mg, Vitamin $B_{2:}$ 50,000 mg, Niacin 4,500 mg, Calcium pantothenale 10,000 mg, Vit B_6 40,000 mg, Vit B_{12} 20 mg, Chlorine chloride 300,000mg, Biotin 100 mg, Manganese 50 mg, Iron 300,000 mg, Zinc 120,000 mg, Copper 80,000 mg, Iodine 15,000 mg, Cobalt 300 mg, Selenium 130 mg, Antioxidant 120,000 mg. Metabolisable Energy (Kcal/kg) was obtained from prediction equation (pauzenga (1985) ME (Kcal / kg = 37 x % crude protein + 818 x % Ether extract + 35.5 x % Nitrogen-free extract)]

3.17 Measurement of Growth Performance

By measuring the mean daily feed intake (FI), mean weakly body weight gain (BWG), feed conversion ratio (FCR), and shell dimensions (weekly increases in shell length and width as described in experiment 1), performance indices were established. The snail's average daily diet consumption was defined by deducting the weight of the remaining diets from the weight of the diets provided. The difference between the mean weights of two consecutive weeks was used to determine the experimental snails' mean weekly body weight gain for each treatment. The ratio of feed intake to body weight gain was used to measure the feed conversion ratio.

3.18 Evaluation of the Characteristics of the Carcass:

On day 98, the feeding trial came to an end, and three snails from each treatment were chosen at random to be examined for their carcasses. After being thoroughly cleaned with a paper towel, the snails were weighed. After that, the snails were killed through treatment by immersion in boiling water. Each of the carcass's individual parts' weights were measured and recorded. Each weight was expressed as a percentage of the live weight.

3.19 Nutrient Digestibility

On day 98, three snails were indiscriminately picked from each treatment for metabolic trials in clean hutches. The snail were fed *adlibitum* with the respective diets. Excreta collection started after four days of acclimatization and was carried out for ten days in order to get enough excreta samples. Concentrated H_2SO_4 was added in drops into the containers to trap the possible escaping. Samples of daily collections were pooled together for each treatment, oven-dried at 60^0C for two days at constant weights, then stored in an air-tight container in readiness for the subsequent analyses according to the methods of AOAC (2000).

$$\% \text{ Digestibility} = \frac{Intake - Output}{Intake\ DM} \text{ x} \frac{100}{1} \qquad 3.5$$

$$\% \text{ protein digestibility} = \frac{n.feed - n.excreta}{n.feed} \text{ x } \frac{100}{1} \qquad 3.6$$

Where n = nutrient in feed/excretes

3.20 Mortality

Mortality were recorded as they occurred and the percentage mortality determined for each treatment

3.21 Analysis of the Experimental Diets and excreta samples from the snails were carried out according to AOAC (2000) at the biochemical laboratory, department of Animal Science, University of Ibadan

3.22 Mineral Compositions of the Experimental Diets. Wet digestion was done in a nitric acid (HNO_3) and perchloric acid ($HCLO_4$) mixture and Ca, P, Na, K, Mg, Mn, Fe, Cu etc determined in digest using atomic absorption spectrophotometry (Perkin elemer model 403) as described by AOAC (2000)

3.23 Calculations and Statistical Analysis

Data collected were analysed through descriptic statistics, polynomial regressions and ANOVA at $\propto_{0.05.}$

Experiment 4: Growth Performance and Nutrient Digestibility of Feed in Grower African Giant Land Snail (*Archachatina marginata)* in Response to Dietary Supplementation of Prebiotic-organic Acid Preparation.

3.24 Experimental Animals Allotment and Design

A total of 350 grower snails (*Archachatina marginata*) were used for this study. The snails were cleansed with chlorine-free water weighed (100.08 ± 1.73g) and indiscriminately assigned to seven dietary treatment groups of 50 snails for each treatment and each treatment replicated five times with ten snails per replicate in a completely randomized design. The animals were allowed to acclimatize for seven days while being fed fresh unriped pawpaw leaves only. Seven diets were formulated and to them were added mannanoligosaccharide organic acid blend at the inclusion rate of 0.00 g (control), 0.50 g, 1.00 g, 1.50 g, 2.00 g and 2.50 g/kg feed for treatments M_1, M_3, M_4, M_5, M_6 and M_7 respectively. To treatment M_2 was added 0.1mg oxytetracycline soluble powder to serve as the positive control. Oxytetracycline was added as an additive just like the prebiotic. The

snails were permitted *ad libitum* right to the diets and water. The investigation continued for 98 days.

The feed additive used in this study was a composite mixture of toxin binder, prebiotic and organic acids. It is non-nutritive additive used in monogastric diets. The inclusion of the mixture to animal diets prevents and arrests the mycotoxins in the diets, improves immunity and has a positive effect on digestion and metabolism. For instance mannanoligosaccharide supports the availability of nutrients used by the beneficial bacteria to provide antagonistic effect against harmful bacteria and enhances the probiotic functions and supports healthy gut flora and maintenance and management of gut health. In addition, it contains a blend of organic acids that reduces the pH in guts and low pH favours the multiplication of beneficial flora/bacteria and at the same time suppresses the growth of harmful bacteria/pathogens.

3.25 Measurement of Growth Performance

Performance parameters were determined by measuring the mean daily feed intake (FI), mean weakly body weight gain (BWG), feed conversion ratio (FCR), and shell dimensions (shell length, shell width, apartural increases obtained weekly as described in experiment 1. The mean increments in shell thickness were measured bi-weekly by taking the difference between the mean thickness of shell for two successive weeks, using a micrometer screw gauge (0.10 mm: 0 = 25 mm).

The mean amount of diets consumed by the snails on daily basis was calculated by subtracting the weight of the left over diets from the weight of the diets supplied. The mean weekly body weight gain of the experimental snails for each treatment was obtained by taking the difference between the mean weights of two successive weeks. Feed conversion ratio was measured by relating the feed intake to the body weight gain i.e

$$\text{FCR} = \frac{\text{Feed intake/snail (g)}}{\text{Body weight gain / snail (g)}} \qquad 3.7$$

Table 3.3: Gross Composition of Experimental Basal diet fed to Grower Snail.

Ingredients	%
Maize	38.00
Soyabean Meal	35.00
Fish meal	3.00
Wheat offal	12.95
Oyster Shell	9.00
Dicalcium phosphate	1.50
Salt	0.10
Premix	0.25
L.Lysine	0.10
DL Methionine	0.10
Total	**100.00**
Calculated Chemical Composition	
Metabolisablel Energy (Kcal/Kg)	2479.10
Crude protein (%)	23.10
Crude Fibre	4.40
Lysine	1.40
Methionine	0.50
Calcium	4.10
Phosphorus (%)	0.70

Composition of the premix: Vit A 10,000 IU, Vit D_3 200,000IU, Vit K_3 20,000 mg, Vit B 30,000 mg, Vit $B_2$50,000 mg, Niacin 4,500 mg, Calcium pantothenale 10,000 mg, Vit B_6 40,000 mg, Vit B_{12} 20 mg, Chlorine chloride 300,000 mg, Biotin 100mg, Manganese 50mg, Iron 300,000 mg, Zinc 120,000 mg, Copper 80,000 mg, Iodine 15,000 mg, Cobalt 300mg, Selenium 130 mg, Antioxidant 120,000mg. Metabolisable Energy (Kcal/kg) was obtained from the prediction equation [(pauzenda, (1985) ME (Kcal/kg=37 x % crude protein + 818 x % ether extract + 35.5 x % nitrogen-free extract)] 35.2 measurement of growth performance.

3.26 Carcass Characteristics Evaluation

At the end of the feeding trial on 98 days, three snails from each treatment were indiscriminately picked for carcass assessment. The snails were cleansed thoroughly with paper towel and weighed. The snails were killed by immersion in boiled water. The weight of each component part of the carcass was measured. The ratio of each weight to the live weight was expressed as percentage.

3.27 Nutrient Digestiability

On day 98, three snails were indiscriminately picked from each treatment for metabolic trials in clean hutches. The snails were fed *ad libilum* with the respective diets. Excreta collection started after four days of acclimation and was carried out for ten days in order to get enough excreta samples. Concentrated H_2SO_4 was added in drops into the containers to trap the possible ammonia gas (NH_3) escaping. Samples of daily collections were pooled together for each treatment, oven-dried at 60^0C for two days at constant weights, then stored in an air-tight container in readiness for the subsequent analyses according to the method of AOAC (2000).

$$\% \text{ digestibility} = \frac{\text{Intake} - \text{Output}}{\text{Intake DM}} \text{ x} \frac{100}{1} \qquad 3.8$$

$$\% \text{ protein digestibility} = \frac{\text{n.feed} - \text{n.excret}}{\text{n.feed}} \text{ x } \frac{100}{1} \qquad 3.9$$

where n = nutrient in feed / excrets

3.28 Serum Biochemistry and Haematological Indices

3.28.1 Collection of Haemolymph Samples:

After the 98 days duration of the experiment two snails were indiscriminately picked from each treatment. They were cleansed with clean water in order to remove the slime, excreta and soil particles. The shell was turned in such a way that the shell aperture was upward. The shell was perforated a little to make a small hole below the first and the largest whorl at the front just before the boundary between the first and the second whorl.

The syringe with needle size 26 (0.45 mm x 13 mm) was inserted to draw the haemolymph. With this method, you can collect as much as 5 ml at a time from each snail especially if the snail is big. The haemolymph sample for haematology were collected into 5ml sterile vacutainer tubes containing CMF while that of the serum biochemistry were without EDTA to allow blood / haemolymph cotting and serum decantation in readiness for the analyses. The samples collected were kept in the fridge pending the various analyses. The volume of the haemolymph collected was a function of the size or / and age of the snail. The bigger the size, the more the volume of haemolymph obtained. Moreover, this method used was not fatal to the snail since it would easily rejuvenate to seal up the small perforation by itself.

The only disadvantage is that during this period of rejuvenation, the snail would divert part of its nutrients and energy to repair the broken portion of the shell and may slightly slow down its growth.

3.28.2 Haemolymph Determination

Haemolymph determination was carried out as described by Schalm *et al*. (1975) through the cyanometh haemoglobin method. Blood/haemolmph profiles are relevant parameters of the physiological state of animals (Khan and Zafara, 2005). The competence to expound the state of blood profiles in normal and diseased condition is a fundamental objective of haemotological and biochemical studies. The blood / haemotolymph picture varies with certain factors such as stress, infections, and toxicity (Khan and Zafar, 2005). The intake of many dictary materials has been reported by Church *et al.*, (1984) to have significant effects on blood / haemolymph gives the closest measure for long term nutritional status of animals.

3.28.3 Packed Cell Volume (PCV)

The number of cells per unit volume of blood and the size of the erythrocyte, also known as a red blood cell, determine the packed cell volume. Haemocyanin, a protein made of copper, is present in the snail's blood, which is not red but rather light blue. According to Schalm *et al.* (1975) method, the conventional method of filling the tube with haemolymph determines the PCV (1975). Anemia occurs when the PCV falls below the maximum of the

normal hemoglobin range, while a fall below the minimum range indicates hemoglobin deficiency.

3.28.4 White Blood Cells

White blood cells (WBC) or leukocytes are transported from their site of origin to their various destinations through the blood/haemolymph medium in the body of the animals. They are usually produced in the bone marrow. The cells provides the body with defences against foreign bodies such as viral, bacterial and parasitic infections. Increased level of circulating WBC indicates the presence of a disease condition while low level of WBC in the blood / haemolymph is an indication of the end of a disease condition. White blood cells were determined as described by Schalm et al., (1975)

3.28.5 Haemocytes Count (HC)

This was determined by the haemocytometer method as described by Coles (1968)

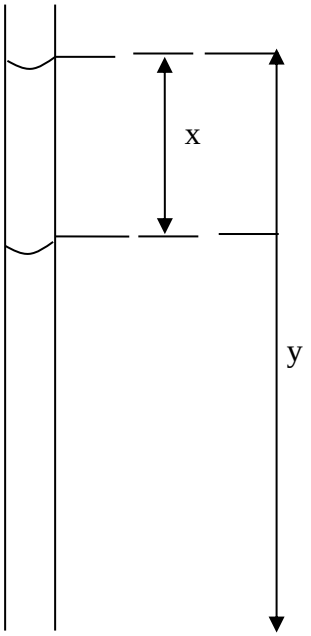
3.28.6 Erythrocytes Sedimentation Rate (ESR)

An erythrocytes sedimentation rate is a type of blood test that measures how fastly erythrocytes (red blood cells) or haemocyanins settle at the bottom of a test tube that contains a haemolymph sample. Under normal conditions, red blood cells settle relatively slowly.

A faster-than-normal rate may indicate inflammation of the body. Inflammation is part of immune response system. It can be a reaction to an infection or injury. Inflammation may also be a sign of a chronic disease, an immune disorder or other medical problems. ESR reveals the health status of the animal. High ESR value indicates that the internal organs of the animals are diseased especially the liver, kidney, heart, or rheumatoid arthritis etc.

3.29 Method of Determining ESR

The haemolymph was filled into the capillary tube to the brim and positioned vertically for one hour, after which a fall in the fluid surface was observed



$$\frac{x}{y} \times \frac{50}{1} \text{ (minutes)} \qquad 3.10$$

The unit is always in minutes. 50 cm^3 is used because of the error in handling.

3.30 Serum Biochemical Parameters

Total protein (g/l)

Total protein was measured using Biuret method. This method for estimating the total serum protein concentration is one of the simplest chemical quantitative analytical techniques. This principle is based on the formation of copper-protein complex which in turn react with Buiret reagent i.e.

Cu^{2+} + Protein $\rightarrow$ Cu-protein complex 3.11

In alkaline medium, cupric ion formed a violet coloured complex with protein nitrogen. Absorbance measured at 520-560 nm is proportional to the total protein concentrational in a sample.

3.30.1 Albumin (g/l)

Albumin was measured by colorimeter using the sigma diagnostics albumin reagent (Sigma Diagnostic, U.K.), containing Bromocresol green (BCG) (Peter *et al*, 1982) Albumin concentration is proportional to absorbance increase at 628 nm due to the albumin bromocresol green complex formation.

3.28.2 Globulin (g)

The globulin concentration was obtained by subtracting albumin value from the total protein i.e. Total protein-Albumin= Globulin.

3.30.3 Albumin/Globulin ratio

This was obtained by diving the albumin value by the calculated globumin value.

3.30.4 Creatinine (mg/dl)

Blood creatinine or urine creatinine is a waste product made by the muscles as part of normal daily activity. Under normal conditions, the kidneys filter creatinine from the blood and send it out of the body through the urine. If there is a problem with the kidneys, creatinine can accumulate in the blood. An increased level of creatine in the blood is an

indication of poor kidney function. Serum creatinine was also evaluated using photoelectric colorimeter as described by Toro and Ackermanin (1995).

Aspartate Aminotransferase (AST)

Although Aspartate transaminate (AST) is not a liver specific enzyme but it is used to measure the level of liver necrosis if no disease exists in other tissues in which the enzyme is found in high concentration. AST was determined by monitoring the concentration of oxaloacetale hydrazine formed with 2, 4 – dinitrophenylhydrazine, using Randox kit and photoelectric colorimeter (Gallenkam and Sons Ltd; English).

Alkaline Phosphatase (ALP) in Serum and Plasma.

ALP was determined using colorimetric method. This is an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur klinische chemie. Alkaline phosphatase catalyses the hydrolysis of various phosphate esters in an alkaline medium. It may be up in rickets and hyperparathyroidism. Its level may also be increased in congestive heart failure as a result of injury to the liver Mitruka and Rawnsley (1977).

3.30.5 Glucose

Glucose is one of the most essential nutrients in the body of every animal. It is a primary source of energy. A regular supply of glucose is essential as a source of energy, especially for the nervous system and erythrocyte. It is also required in adipose tissues as a source of glycerideglycerol, and it is the only fuel, that supplies energy to skeletal muscle under anaerobic conditions and the precursor of milk sugar (lactose) in mammary gland.

Glucose was assessed by enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts under catalysis of peroxidase (POD), with phenol and 4-aminophenazone to form a red-violet quinoneimine due as indicator

$$\text{Glucose} + O_2 + H_2O \rightarrow \text{glyconic acid} + H_2O_2 \qquad 3.12$$

$2H_2O_2$ + 4-aminiophenazone + phenol $\rightarrow$ quinoneimine + $4H_2O$. Randox kit was also applied here. 3.13

Haemolymph Serum Lipid Profile:

3.30.6 Total Serum Cholesterol (TCHOL)

Cholesterol measurements are used in the, diagnosis and treaments of lipid lipoprotein metabolism disorders. Lipid play an important role in the body; they serve as hormones or hormone precursors, enhance digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allowo nerve conduction and prevent heat loss.

Total serum cholesterol was determined according to Trinder (2009), using dialab kit

Cholesterol (mg/dl) = $\frac{absorbance\ of\ test\ sample}{absorbance\ of\ calibrator}$ x $concentration\ of\ calibrator$

3.30.7 Serum Triglycerides (TG)

Triglycerides are lipids, a type of fat in the body. It provides the body of animal with energy, but their main function is to store energy for future use. Fat cell hold the triglyceride molecules until the body needs energy such as between meals or during fasting. Triglyceride was analysed enzymatically (Gott Fried and Roseberg, 2010).

3.30.8 High Density Lipoprotein (HDL)

This was determined using dialab HDL direct Kit.

3.30.9 Very Low Density Lipoprotein (VLDL)

This was calculated by dividing the Triglycerides by 5.

i.e VLDL = Triglyceride ÷ 5 3.15

3.30.10 Low Density Liproprotein (LDL):

This was also calculated mathematically using Friedeward Formula

LDL = $\frac{Total\ Serum\ Cholesterol}{Total\ HDL\ Cholesterol}$

= $\frac{TC}{HDL}$ 3.16

Morphology and cell types of haemocytes of African giant land snail (*Archachatina marginata*) haemolymph. Samples were taken from the original haemolyph collected for the previous analyses.

3.31 Haemocyte Dyeing:

Archachatina marginata haemolymph from the seven treatments were placed each on 22 x 22 mm slides and dried at room temperature, fixed with methanol and dyed with May-Guwald Giemsa. Various types of haemocytes were morphologically differentiated in addition to their dyeing affinity of the nucleus and cytoplasm of the cells available in the haemolymph obtained from *Archachatina marginata*. Observations were made by immersion into a Leitz photomicroscope.

3.32 Mortality: The number of snails that died within the experimental period was recorded.

Mineral Composition of the snail shell powder.

These were determined as described in experiment one, using atomic absorption spectrophostometer.

3.33 Proximate Analysis:

Proximate investigation of the experimental foods and faecal matters samples from the snails were determined according to AOAC (2000) at the Department of Animal Science, University of Ibadan.

3.34 Statistical Analysis:

Information gathered was processed through Analysis of Variance (ANOVA) of the completely randomized design using the SAS (2003) package. The statistical differences were compared using Duncan's multiple range test at $P<0.05$

Experiment 5: Effects of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* supplementation on growth performance nutrients digestibility and carcass yield in grower African giant land snails (*Archachatina marginata*)

3.35 Experimental Animals Allotment and Design

Another batch of 400 grower African giant land snails (*Archachatina marginata*) were used for this experiment. The snails were cleansed with chlorine-free water, weighed (92.12 ± 1.77 g) and indiscriminately assigned to eight dietary treatment groups of 50 snails for each treatment, replicated five times with ten snails per replicate in a completely randomized design. The animals were allowed to acclimatise for seven days while being fed fresh unripe pawpaw leaves only.

Eight diets were formulated and to them were added Lactobacillus plantarum and saccharomyces cerevisiae at the inclusion rate of 0.00 probiotics (D_1, control), 0.1mg oxytetracycline/kg (D_2), $1x10^8$(D_3), 2 x 10^8(D_4), 3x108 CFU Lactobacillus plantarum/ kg (D5), 1 x 108 (D_6) 2 x 108 (D_7) and $3x10^8$ CFU Saccharomyces cerevisiae/ kg (D_8). Oxytetracycline was added as an additive just like the probiotics but serves as a positive control. The snails were permitted *ad libitum* right to the diets and water. The investigation continued for 98 days.

3.35.1 Experimental Materials:

Two different probiotics, Lactobacillus plantarum and Saccharomyces cerevisiae (Yeast) were prepared for the study.

3.35.2 Preparation of *Lactobacillus Plantarum*

The Lactobacillus plantarum culture was collected from the culture centre of the Department of Microbiology, University of Ibadan. The Lactobacillus plantarum was maintained on Agar slant and glycerol broth. The Lactobacillus plantarum was subcultured from the slant to get the 24 hrs old culture on the De Man Rogosa Sharpe (MRS) Agar. It was then transferred into MRS broth after 24 hours. The broth was centrifuged at 15,000 revolutions per minute (rpm) for fifteen minutes to obtain the cells. The cells were washed

twice in normal saline. The washed cells were diluted in sterile water and the turbidity was adjusted to get the cells of $\times 10^8$ CFU/kg.

One litre was prepared for each treatment. Each preparation was mixed with the formulated diet before the commencement of the feeding trial at once.

3.35.3 Preparation of *Saccharomyces Cerevisiae* (Yeast)

The yeast culture was collected from the culture center of the Department of Microbiology, University of Ibadan. The yeast was maintained on Agar slant and glycerol broth. The yeast was then sub-cultured from the slant to get 24 hours old cultured on malt extract agar. It was then transferred to malt extract broth. The broth was then centrifuged at 15,000 revolution per minute (rpm) for 15 minutes to obtain the cells. The cells were washed twice in normal saline. The washed cells were diluted in sterile water and were adjusted to get cells of $\times 10^8$ CFU / kg. One litre was prepared for each treatment. Each preparation was mixed with the formulated diet before the commencement of the feeding trial at once.

3.36 Measurement of Growth Performance:

As described in experiment 1, performance indices were determined by measuring the mean weekly body weight gain (BWG), feed conversion ratio (FCR), and shell dimensions (length, width, apertural, and increase) obtained weekly. Using a micrometer screw gauge (0.01 mm;) the difference between the mean thickness of the shell for two consecutive weeks was used to measure the biweekly mean increments in shell thickness. 0-25 mm).

By dividing the weight of the diets that were left over from the weight of the diets that were provided, we were able to determine the average daily amount of diets that the snails consumed. The difference between the mean weights of two consecutive weeks was used to calculate the experimental snails' mean weekly body weight gain for each treatment. The feed intake was linked to body weight gain to determine the feed conversion ratio, which was as follows:

$$\text{FCR} = \frac{Feed\ intake/snail(g)}{Body\ weight\ gain/snail\ (g)} \qquad 3.17$$

3.37 Carcass Characteristic Evaluation

At the end of 98 days feeding trial, three snails from each treatment were indiscriminately picked for carcass assessment. The snails were cleansed thoroughly with paper towel and weighed. The snails were killed by immersion in boiled water. The weight of the entire carcass were weighed and the weight of each component part of the carcass was measure. The ratio of each weight to the live weight was expressed as percentage.

3.38 Nutrient Digestibility

On day 98, three snails were indiscriminately picked from each treatment for metabolic trials in clean hutches. The snails were fed *ad libitum* with the respective diets. Excreta collection started after four days of acclimatisation and was carried out for ten days in order to get enough excreta samples. Concentrated H_2SO_4 was added in drops into the containers to trap the possible ammonia gas (NH_3) escaping. Samples of daily collections were pooled together for each treatments oven-dried at 60^{o}C for two days at constant weights; then stored in an air-tight container in readiness for analyses according to the method of AOAC (2000).

$$\% \text{ Digestibility} = \frac{intake - Output}{intake\ DM} \text{ x} \frac{100}{1} \qquad 3.18$$

$$\% \text{ Protein digestibility} = \frac{\text{n.feed} - \text{n.excreta}}{n.feed} \text{ x} \frac{100}{1} \qquad 3.19$$

Where n = nutrient in feed/excreta

Table 3.4: Gross Composition of the Experimental Basal diets fed to Grower Snails

Ingredients	%
Maize	38.00
Soyabean Meal (45% CP)	35.00
Fish meal (72%CP)	3.00
Wheat offal	12.95
Oyster Shell	9.00
Dicalcium phosphate	1.50
Salt	0.10
Premix (growers)	0.25
L.Lysine	0.10
DL - Methionine	0.10
Total	**100.00**
Calculated Chemical Composition	
Metabolisable Energy (Kcal/kg)	2479.10
Crude Protein (%)	23.10
Crude Fibre (%)	4.40
Calcium (%)	4.10
Phosphorus (%)	0.70
Lysine	1.40
DL Methionine	0.50

Premix composition: Vitamin A: 10,000 IU, Vitamin $D_{3:}$ 200,000IU, Vitamin $K_{3:}$ 20,000 mg, Vitamin B: 30,000 mg, Vitamin $B_{2:}$ 50,000 mg, Niacin 4,500 mg, Calcium pantothenale 10,000 mg, Vit B_6 40,000 mg, Vit B_{12} 20 mg, Chlorine chloride 300,000 mg, Biotin 100 mg, Manganese 50 mg, Iron 300,000 mg, Zinc 120,000 mg, Copper 80,000 mg, Iodine 15,000 mg, Cobalt 300 mg, Selenium 130 mg, Antioxidant 120,000mg. Metabolisable Energy (Kcal/kg) was obtained from prediction equation [(Pauzenga, (1985) ME (Kcal/kg = 37x% crude protein + 818 x % Ether extract + 35.5 x % Nitrogen-free extract)]

3.38.1 Dissection Procedures

Three live snails were indiscriminately picked from each treatment group. The snails were washed with clean water to remove the soil or dust and outer shells thoroughly cleansed with ethanol for surface sterilization according to Oyeleke *et al,* (2013). The shells were broken to remove the flesh. Each snail flesh was then dissected to isolate the gut and other organs, using surgical instruments. The entire alimentary canal (gut) was carefully separated from the common haemophroditic duct and other internal organs were totally removed using flame forceps and scissors. The length of the gut was measured using a calibrated tape rule and recorded.

3.39 Morphometrics of the Alimentary Canal:

The detached gut whose length had been measured was then cut into various sections; oesophagus, stomach, large intestine and small intestine and the rectum. The length of each section was measured (excluding the rectum) and recorded using the calibrated tape rule. The weight of the stomach was taken with the aid of a sensitive weighing balance (Digital scale capacity; 500x0.01 g). The length and weight of the hepatopancrease after separation were also taken using the same measuring devices. The gut contents of each section were finally emptied into separate labeled sterile plastic bottles (20 ml) for gut microbes estimation.

3.39.1 Microbial Analysis of the GIT of Snails (*Archachatina Marginata*)

Sample collection: The GIT contents/digesta were collected from the oesophagus, stomach, large intestine, and small intestine. The snails were dissected and the contents/digesta of each section collected into sterile bottles and transported to the Department of Microbiology, University of Ibadan for analysis.

Culture Media Preparation: Different culture media were used for the isolatioin of culturable bacteria from the snails that were dissected and the contents/digesta of each section collected into sterile bottles and transported to the Department of Microbiology, University of Ibadan for analysis.

Culture Media preparation: Different culture media were used for the isolation of culturable bacteria from the snails. They were Plate Count Agar (PCA), Mac Conkey Agar (MA), Eosin Methylene Blue Agar (EMBA) Samonella/Shegella Agar (SSA), Mannitol salt' Rogosa Sharpe (MRS) Agar, Potatoe Dextrose Agar (PDA).

Each media was prepared by weighing appropriate quantity as specified by the manufacturer and dissolved in appropriate quantity of distilled water and sterilized by autoclaving at 121^{o}C for 15 minutes except SSA which was only brought to boil for 10 minutes. They were allowed to cool to 45^{o}C before pouring it into petri dishes.

3.39.2 Isolation Procedures

The isolation was done using standard procedures. The aliquots of different dilutions of the gut samples were made and labelled properly. Each was serially diluted using 10 fold serial dilution. Dilutions 10^{-2}, 10^{-4}, and 10^{-6} were selected pout plate method of isolation was used by putting 1ml from selected dilution factors (10^{-2}, 10^{-4}, and 10^{-6}) into already labeled petri dishes and the 15-20 ml of sterilized molten Agar was put into petri dishes swirled to mix properly. The plates were then allowed to be solidified and incubated at 37^{o}C for 18-48 hours for bacteria and 2-5 days for fungi. The colonies formed on each plate were counted and recorded properly.

3.39.3 Identification of Bacteria

Each of the organisms was sub-cultured to obtain pure culture, and identified based on their colonial morphology, gram reactions, sugar fermentation and biochemical tests.

3.40 Proximate Analysis

Proximate investigation of the experimental foods and faecal matters samples from the snails were determined according to AOAC, (2000) at the Department of Animal Science, University of Ibadan.

3.41 Mortality: Mortalities were recorded as they occurred and the percentage mortality determined for each treatment.

3.42 Calculations and Statistical Analysis

Data collected were processed through descriptive statistics, polynomial regression and ANOVA at $\alpha_{0.05}$.

CHAPTER FOUR

RESULTS

4.1 Chemical Composition of the Experimental Feedstuffs offered to Snails in Experiment One.

The outcomes are revealed in Table 4.1. The outcomes revealed that the protein matters in the pawpaw leaves (control) was the highest (31.26%), followed by coconut milt (11.28%) and unripe pawpaw fruit (10.28%), while pineapple fruit had the least protein content (5.69%) among all the feedstuffs.

The crude fibre values were very high across the feedstuffs; PPL (9.31%), PPF (12.01%), SOF (16.50%), PAF (10.50%) and CCM (16.35%). The values for ether extracts of the feedstuffs were appreciably low except that of the coconut milt. (63.64%). Generally, all the feedstuffs, with the exception of coconut milt had high NFE values, which is a measure of their carbohydrate contents and since the protein and NFE contents of the feedstuffs were appreciably high, they can be tried as feedstuffs for snails. Gross energy in CCM (5808.93) was significantly ($P<0.05$) greater relative to PPL (3000.19), PPF (2937.33), SOF (2756.88) and PAF (2930.34).

Table 4.1: Chemical composition of the feedstuffs fed to growing African giant land snail (*Archachatina marginata*)

Parameter	PPL	PPF	SOF	PAF	CCM
Dry Matter (%)	24.72	9.50	18.00	13.50	47.00
Moisture (%)	75.28	90.50	82.00	86.50	53.00
Crude Protein (%)	31.26	10.28	6.28	5.69	11.38
Crude Fibre (%)	9.31	12.01	16.50	10.50	16.35
Ether Extract (%)	0.86	1.01	0.77	0.77	63.64
Ash (%)	8.62	7.00	7.10	8.20	3.50
Nitrogen Free Extract (%)	49.95	69.70	69.61	74.84	5.13
Gross energy (kcal/kg)	3000.19	2937.33	2756.88	2930.34	5808.93

PPL - Pawpaw Leaf, PPF - Pawpaw fruits, SOF - Sweet Orange Fruit, PAF - Pineapple Fruit, CCM - Coconut Milt

4.1.2 Fibre Analysis of different feedstuffs offered to growing snails (*Archachatina marginata*)

The outcomes of the chemical breakdown of the fibre fractions of the feedstuffs are revealed in Table 4.2. There were significant ($P<0.05$) variations among all the parameters determined for the fibre fractions; Neutral detergent solubles (NDS), Neutral detergent fibres (NDF), Acid detergent fibres (ADF), Acid detergent lignin (ADL), Cellulose and hemicellulose contents.

The Neutral detergent soluble represents the soluble fraction of the cell contents. The values were significantly ($P<0.05$) different from one another, ranged from 5.62% (CCM) to 9.61% (PAF). The highest value (9.61%) was obtained from PAF. The NDF values significantly differed ($P<0.05$); PPL (21.05%), PPF (28.29%), SOF (24.18%), PAF (23.79%) and CCM (17.41%) respectively.

Acid detergent fibre (ADF). The ADF highlights the quality of the cell wall (Ologhobo, 2018). The acid detergent fibre values were significantly high ($P<0.05$). Their values ranged from 8.59% (CCM) to 19.93% (PPF), the highest value 19.93% obtained from PPF. High ADF could lead to decrease in feed intake and reduction of feed digestibility.

Acid detergent lignin. Lignin provides protective function, rigidity and structure to seeds, stems and vascular tissues of leaves and it is commonly referred to as a non-carbohydrates component of the plant cell and can be considered non-digestible (Ologhobo, 1980). The values for ADL differed ($P<0.05$) significantly among the different feedstuffs and were generally low.

The values of hemicellulose significantly differed among the feedstuffs with highest and least values recorded for sweet orange fruit (9.12%) and coconut milt (8.82%) respectively. The cellulose contents among the feedstuffs also differed significantly ($P<0.05$) the least value (6.39%) observed for sweet orange fruit while the highest value (14.78%) was observed for pawpaw fruit.

Table 4.2: Fibre Analysis of different feedstuffs fed to grower African giant land snail (*Archachatina marginata*)

Parameters (%)	PPL	PPF	SOF	PAF	CCM	SEM	P value
Crude Fibre	9.31^{e}	12.01^{c}	16.50^{a}	10.50^{d}	16.35^{b}	0.01	<0.0001
Neutral Detergent Soluble	9.32^{b}	8.69^{c}	6.39^{d}	9.61^{a}	5.62^{e}	0.01	<0.0001
Neutral Detergent Fibre	21.05^{d}	28.29^{a}	24.18^{b}	23.79^{c}	17.41^{e}	0.01	<0.0001
Acid Detergent Fibre	12.04^{d}	19.93^{a}	15.06^{b}	14.68^{c}	8.59^{e}	0.01	<0.0001
Acid Detergent Lignin	2.76^{d}	5.14^{a}	4.26^{b}	3.87^{c}	2.03^{e}	0.01	<0.0001
Hemicellulose	9.01^{b}	8.36^{d}	9.12^{a}	9.11^{a}	8.82^{c}	0.003	<0.0001
Cellulose	9.28^{c}	14.78^{a}	6.39^{e}	10.81^{b}	6.56^{d}	0.01	<0.0001

^{abcde} Means of treatments along a row with diverse superscripts varied considerably (P<0.05).

PPL- Pawpaw Leaves, PPF-Pawpaw Fruit, SOF-Sweet Orange Fruit, PAF-Pineapple Fruit, CCM- Coconut Milt, SEM+Standard Error of means, P value -Probability

4.1.3 Mineral composition of the experimental feedstuffs fed to grower African giant land snail.

Table 4.3 shows the mineral composition of experimental diets fed to AGLS (*Archachatina marginata).* Total phosphorus has highest value in PPL (1.18%) compared to other feedstuffs, while PAF (0.27%) had the lowest total phosphorus. The PPL had the highest Calcium value of 2.62%, while CCM had the lowest value of 0.24%. However, PPF and SOF had similar values of 0.67% and 0.65%, respectively. Magnesium has highest value ($P<0.05$) in PPF (0.45%) and lowest in CCM (0.01) and PPL (0.07%). The SOF and PAF had similar magnesium values of 0.18 and 0.16%. Higher ($P<0.05$) potassium value of 8.70% was observed in PPL while PAF and CCM had the lowest values of 1.40% and 1.45%, respectively. Sulphur was significantly higher in PPF (2.21%) compared to PPL (2.18), SOF (0.42), PAF (0.36), and CCM (0.36). The PPL (0.27) and PPF (0.30) had significantly ($P<0.05$) higher sodium concentration compared to SOF (0.14%), PAF (0.12%) and CCM (0.13%). Chloride value has highest value $p<0.05$) in CCM (11.52%) and PPF (10.80). The SOF had the highest concentration of Lead (Pb) (102.00 mg/kg). The value of cobalt was found to be highest ($P<0.05$) in SOF (3.40 mg/kg) and lowest in PPL (0.07 mg/kg). The PPF had significantly higher chromium concentration of 12.80 mg/kg compared to other feedstuffs. However, the least chromium concentration was observed in SOF (4.40 mg/kg). Zinc was abundant in PPL as indicated by value of 44.60 mg/kg, while it is lowest in SOF (13.20 mg/kg) and PAF (9.10 mg/kg).

Table 4.3: Mineral composition of the experimental feedstuffs fed to grower African giant land snail (*Archachatina marginata*)

Parameters	PPL	PPF	SOF	PAF	CCM	SEM
Total phosphorous (%)	1.18[a]	0.69[b]	0.67[b]	0.27[c]	0.46[b]	0.71
Calcium (%)	2.62[a]	0.67[d]	0.65[d]	0.34[e]	0.24[f]	0.19
Magnesium (%)	0.07[c]	0.45[a]	0.18[b]	0.16[b]	0.01[c]	0.06
Potassium (%)	8.70[a]	4.60[b]	2.55[c]	1.40[d]	1.45[d]	0.53
Sulphur (%)	2.18[b]	2.21[a]	0.42[c]	0.36[c]	0.36[c]	0.26
Sodium (%)	0.27[a]	0.30[a]	0.14[b]	0.12[b]	0.13[b]	0.02
Chloride (%)	6.48[b]	10.80[a]	6.12[b]	6.12[b]	11.52[a]	0.50
Lead (mg/kg)	3.10[b]	1.01[c]	102.00[a]	2.70[b]	1.50[c]	0.82
Iron (mg/kg)	209.00[a]	105.00[b]	63.00[c]	61.00[c]	77.00[c]	12.54
Cobalt (mg/kg)	0.70[d]	1.10[c]	3.40[a]	1.60[b]	1.70[b]	0.16
Chromium (mg/kg)	9.40[b]	12.80[a]	4.40[e]	5.80[d]	7.90[c]	1.05
Zinc (mg/kg)	44.60[a]	28.00[b]	13.20[c]	9.10[c]	30.20[b]	3.03

[abc] Means in the same row followed by the different letters are significantly different $(P < 0.05)$. PPL - Pawpaw Leaf, PPF - Pawpaw fruits, SOF - Sweet Orange Fruit, PAF - Pineapple Fruit, CCM - Coconut Milt.

4.1.4 Qualitative phytochemical analysis of experimental feedstufss fed to grower African giant land snail (*Archachatina marginata*)

Phytochemicals form a large class of the plants secondary metabolites thought to be responsible for much of the disease protection achieved by diets rich in fruits, vegetables, beans, cereals, and plant-base beverages such as tea and wine. The outcomes of the qualitative phytochemical analyses are presented in Table 4.4. The outcomes revealed that tannins, phenols, glycosides, photosteroids, and flavonoids were present in trace amounts as indicated by single plus (+). Sapnins and alkaloids were predominantly present across all the feedstuffs tested (++ and +++). Their presence would most likely confer possible medicinal and dietary values on health status of the grower African giant land snail (AGLS). Antraquinone was observed in SOF in high concentration (++++) but not found in other feedstuffs.

Table 4.4: Qualitative phytochemical analysis of the experimental feedstuffs

Parameters	PPL	PPF	SOF	PAF	CCM
Tannins	+	+	++	+	+
Saponins	++	++	+	++	+++
Phenol	+	+	+	+	+
Steroid	-	-	+	+	-
Glycosides	+	+	+	+	+
Photosteroids	+	+	-	+	+
Antraquinone	-	-	++++	-	-
Alkaloids	++	+	+	++	+++
Flavonoids	+	+	+	+	+

+	-	indicates presence of phytochemicals in trace amounts	**PPL**	- Pawpaw Leaves
-	-	indicates absence of phytochemicals	**PPF**	- Pawpaw Fruits
++	-	indicates presence of phytochemicals in small amounts	**SOF**	- Sweet Orange Fruits
+++	-	indicates presence of phytochemicals in moderate concentration	**PAF**	- Pineapple Fruits
++++	-	indicates a very high concentration of phytochemicals	**CCM**	- Coconut Milt

4.1.5 Quantitative phytochemical analysis of experimental feedstuffs fed to grower African giant land snail (*Archachatina marginata*)

The result of the quantitative phytochemical analyses are presented in Table 4.5. Both saponins and alkaloids were largely abundant ($P<0.05$) across all feedstuffs assessed. The highest value (11.89 mg / 100 g) was recorded for saponins in coconut milt (CCM) while the least value was found in treatment four (PAF) 2.11 mg / 100 g comparing the fruits, but in the control treatment it was 2.18 mg / 100 g.

The values of alkaloids differed significantly ($P<0.05$) across the feedstuffs. The highest value of alkaloids was obtained in SOF (16.89 mg/ 100 g), followed by PPL (9.70 mg/100g), PAF (8.71 mg / 100 g), CCM (8.15 mg / 100 g) and the least in PPF (0.40 mg /100 g) respectively.

Tannins were present in all the feedstuffs but in minute amounts; PPL (0.03 mg/100g), PPF (0.01 mg /100 g), SOF (0.08 mg/ 100 g), PAF (0.01 mg /100 g) and CCM (0.02 mg /100 g). Phenols were also observed across the treatments. The values ranged from 0.03 mg/100g to 0.43 mg/100g. The values of steroids were not significant ($P>0.05$), they were similar across the treatments; PPL (0.07 mg/ 100 g), PPF (0.09 mg /100 g), SOF (0.07 mg/ 100 g), and CCM (0.01 mg/ 100 g). The values for the glycosides were significantly low ($P<0.05$), the highest value (0.32 mg/ 100 g) was obtained in PPL while the least value (0.13 mg / 100 g) was observed in PAF. The amounts of photosteroids across the treatments were very small. The values ranged from 0.01 mg /100 g in SOF, PAF and CCM to 0.05 mg /100 g in PPF. The values for flavonoids were not significant and were also very low. They ranged from 0.01 mg /100 g (PPL) to 0.09 mg /100 g in PPF, SOF, and PAF respectively.

Table 4.5: Quantitative phytochemical analysis of the experimental feedstuffs fed grower African giant land snail (*Archachatina marginata*)

Parameter (mg /100 g)	PPL	PPF	SOF	PAF	CCM	SEM
Tannin	0.03[a]	0.01[f]	0.08[g]	0.01[h]	0.02[c]	0.02
Saponin	2.18e	4.90b	2.38d	2.11e	11.89a	0.83
Phenol	0.43a	0.12d	0.11d	0.06d	0.19c	0.03
Steroid	0.07	0.09	0.07	0.07	0.01	0.01
Glycosides	0.32c	0.16e	0.14f	0.13g	0.17d	0.02
Photo-steroids	0.03	0.05	0.01	0.01	0.01	0.01
Alkaloids	9.70c	0.40h	16.89a	8.71d	8.15e	1.34
Flavonoid	0.01	0.09	0.09	0.09	0.06	0.01

PPL - Pawpaw Leaves, PPF - Pawpaw Fruits, SOF - Sweet Orange Fruits, PAF - Pineapple Fruits, CCM - Coconut Milt, SEM - Standard Error of Mean

[abcde] Means of treatments along a row with dissimilar superscript varied considerably ($P<0.05$). P value- probability, SEM- Standard error of means.

4.1.6 Growth performance characteristics of snails fed sole-fruits

The performance characteristics of grower African giant land snails (*Archachatina marginata*) fed experimental feedstuffs are revealed in Table 4.6. There were major difference ($P<0.05$) in the average weekly feed ingestion for the snails fed the different feedstuffs. The values ranged from 0.24 g/snail (CCM) to 0.25 g/snail (SOF). Although the values for feed ingestion differed ($P<0.05$) they were generally low. The highest feed ingestion was obtained in PPL (8.56 g/ snail) while coconut milt (CCM) had the lowest value for feed intake (0.24 g/snail). The low feed intake (FI) of these snails on CCM could be attributed to its high fat content (63.44%).

The body weight gain (BWG) is a measure of the growth performance of the snails in response to the feed intake for the prescribed period. There were significant difference ($P<0.05$) obtained for the weight changes of the snails fed the different feedstuffs. Among the tropical fruits used, the highest value for weekly body weight gain was observed in treatment four (PAF), which was 1.23 g/snail while the least value (0.03 g/snail) was recorded for treatment five (CCM).

Feed Conversion Ratio (FCR) relates the feed intake to the body weight gain. Analysis of variance showed significant variations across dietary treatments; PPL (3.60), PPF (1.49), SOF (2.16), PAF (1.11), and CCM (8.00) respectively. Treatment four (PAF) had the best value (1.11) of FCR.

Both the shell length increment and the shell width increment are growth performance indicators in snails and they differed significantly ($P<0.05$) among the different treatments. The values were different from one another across the treatments. Highest increase in shell length was recorded for snail on the control diet (0.32 cm) PPL and PPF (0.28 cm) respectively.

The least increase in shell length was recorded in snails on the PAF (0.07 cm) and CCM (0.03 cm) diets. On the other hand, highest increase in shell width was recorded in snails on the PPF (0.13 cm) and SOF (0.13 cm) diets which were similar to snails on the PAF (0.09 cm) diets. However, the least increase in shell width was found in CCM (0.04 cm).

Table 4.6: Performance characteristics of grower African giant land snail (*Archachatina marginata*) fed experimental feedstuffs.

Parameter	PPL	PPF	SOF	PAF	CCM	SEM	P value
Feed Intake (g/d/snail)	8.56^{a}	1.27^{d}	2.05^{b}	1.36^{c}	0.24^{e}	0.002	<0.0001
Weight gain (g/wk/snail)	2.38^{a}	0.85^{d}	0.95^{c}	1.23^{b}	0.03^{e}	0.03	<0.0001
Feed conversion ratio	3.60^{b}	1.49^{d}	2.16^{c}	1.11^{e}	8.00^{a}	0.03	<0.0001
Shell length (cm)	0.32^{a}	0.28^{a}	0.14^{b}	0.07^{c}	0.03^{c}	0.01	<0.0001
Shell width (cm)	0.03^{bc}	0.13^{a}	0.13^{a}	0.09^{ab}	0.004^{c}	0.02	0.004

PPL - Pawpaw Leaves, PPF - Pawpaw Fruits, SOF - Sweet Orange Fruit, PAF - Pineapple Fruit, CCM - Coconut Milt

^{abc}… means in the same row followed by the different letter are significantly different ($P < 0.05$)

4.1.7 Nutrient digestibility of different feedstuffs fed to growing African giant land snail (*Archachatina marginata*)

Apparent digestibility of the nutrients is the proportion of the feed consumed which is not excreted in the faeces/excreta which is assumed to be absorbed by the animal. The digestbilities of the feedstuffs are presented in Table 4.7.

Dry matter digestibility values in snails on PPL, PPF, SOF, PAF and CCM were 37.52%, 72.57%, 79.52%, 74.85% and 90.39%, respectively, with the latter (CCM) having the highest ($P<0.05$) DM digestibility. Crude protein digestibilities were observed to vary significantly ($P<0.05$), the highest value found in snails on PAF (63.41%) diet. Crude protein digestibility in SOF and PAF were similar and values were 62.8% and 63.41% respectively. However, the lowest CP digestibilitiy was observed in PPF (38.39). Crude fibre digestbilities differed significantly ($P<0.05$) across the treatments with SOF having the highest value of 26.16% while PPL (14.76%) had the lowest CF digestibility. Snails on SOF (79.78%) had significantly higher crude fat digestibility compared to PAF (56.36), PPL (49.29%), PPF (50.00%) and CCM (undetermined). Nitrogen free extract digestibility values in snails on PPF (84.66%), SOF (84.23%) and PAF (85.04%) were similar ($P>0.05$) and significantly greater relative to PPL (40.48%) and CCM (undertermined).

Table 4.7 Nutrient digestibility of different feedstuffs (DM) fed growing African giant land snails (*Archchatina marginata*)

Parameter	PPL	PPF	SOF	PAF	CCM	SEM
Dry matter digestibility (%)	37.52^{e}	72.57^{d}	79.52^{b}	74.85^{c}	90.39^{a}	4.73
Crude protein digestibility (%)	42.80^{b}	38.39^{c}	62.80^{a}	63.41	ND	6.45
Crude fibre digestibility (%)	14.76^{d}	17.04^{b}	26.16^{a}	16.65^{c}	ND	10.05
Ether extract digestibility (%)	49.29^{d}	50.00^{c}	79.78^{a}	56.36^{b}	ND	6.95
Nitrogen free extract digestibility (%)	40.48^{d}	84.66^{b}	84.23^{c}	85.04^{a}	ND	4.55

ND – Not determined.

^{abc}----- are the averages with the varied superscripts along the similar row which are expressively dissimilar

PPL - Pawpaw Leaves, PPF - Pawpaw fruits, SOF - Sweet orange, PAF-Pineapple fruit, CCM-Coconut milt

SEM-Standard Error of Means

4.1.8 Carcass evaluation of grower African giant land snail (*Archachatina marginata*) fed different feedstuffs.

This analysis gives insight into the meat yield of grower African giant land snail fed different feedstuffs. The outcomes of the carcass analyses were presented in Table 4.8. The carcass yield of snails differed significantly across the treatment groups. The highest live weight was obtained in treatment PPL (133.82 g) (control), followed by PAF (131.02 g), PPF (130.82 g), SOF (129.40 g) and the least value was 114.96 g (CCM).

There was a significant ($P<0.05$) difference in the foot (main edible portion) weights expressed in percentages of the live weights. The highest foot weight was observed in the treatment one, PPL (24.00%). This was followed by PAF (22.38%), then PPF (21.85%), SOF (21.41%) and the least was on CCM (11.53%). The shell weight is also a growth parameter. The values were significantly different across the treatments ($P<0.05$), with the highest value obtained in the control treatment, PPL (23.33%), followed by SOF (21.96%), PAF (21.69%) then PPF (21.16%) and the lowest value in CCM (10.75%). Concerning the dressing percentage, the values were generally low across the treatment groups; 43.80% (PPL), 41.96% (PPF), 41.56% (SOF), 42.43% (PAF) and 30.80 (CCM) respectively. The values for the dressing percentage were significantly ($P<0.05$) different across the feedstuffs. The maximum value was achieved in PPL (43.80%) while the minimum value was noted for CCM (30.80%). Meat to shell ratio was significantly ($P<0.05$) higher in snails on CCM diet (3.32) compared to other feedstuffs. Snails on PPL have the highest value of shell to live weight ratio of 23.33, but did not differ significantly ($P<0.05$) from SOF (21.96) and PAF (21.69), while snails on CCM had the lowest ratio of 10.44.

Table 4.8: Carcass analysis of grower African giant land snail (*Archachatina marginata*) fed different feedstuffs

Parameter	PPL	PPF	SOF	PAF	CCM	SEM
Live weight (g)	133.82^{a}	130.13^{C}	129.40^{d}	131.02^{b}	114.96^{e}	1.77
Foot weight (%)	24.00^{a}	21.85^{c}	21.41^{d}	22.38^{b}	11.53^{e}	1.19
Shell weight (%)	23.33^{a}	21.16^{d}	21.96^{b}	21.69^{c}	10.75^{e}	1.22
Dressing percentage (%)	43.80^{a}	41.96^{b}	41.56^{b}	42.43^{b}	30.80^{c}	1.20
Meat/shell ratio	2.51^{c}	2.58^{b}	2.45^{d}	2.56^{bc}	3.32^{a}	0.09
Shell/live weight	23.33^{a}	21.16^{b}	21.96^{a}	21.69^{a}	10.44^{c}	1.25

^{abc} are the averageswith the diverse superscripts along the similar row which are considerably dissimilar

SEM-Standard Error of Mean

PPL - Pawpaw Leaves, PPF - Pawpaw fruits, SOF - Sweet orange, PAF-Pineapple fruit, CCM-Coconut milt

4.1.9 Proximate composition of the grower African giant land snail meat (*Archachatina marginata*)

The proximate composition of the meat of grower African giant land snails is presented in Table 4.9. The outcomes revealed the nutritive value of the snail meat compared to the meat of other conventional livestock. The crude protein levels were significantly different ($P<0.05$) and the values ranged from 16.72±0.01% to 20.10±0.10%. The crude fat values were relatively low. The values ranged from 0.68±0.01% to 0.98±0.01%. The low crude fat content contributes to the nutritive quality of the snail meat. The lower the fat content of the meat, the better the quality for the consumers. Ash content of snail meat was similar across the treatments. PPL (11.89±0.01%), PPF (11.86±0.01%), SOF (11.89±0.01%), PAF (12.01±0.01%), and CCM (11.91±0.01%) respectively. However, the NFE values observed in snail meat on CCM (58.69±0.01%) has highest value, while the lowest value was observed in those on PPF (42.60±0.01%).

Table 4.9: Analysis of the proximate constituents of grower African giant land snail meat (*A. marginata*) fed different feedstuffs.

Parameters (%)	PPL	PPF	SOF	PAF	CCM	SEM	P value
Dry matter	23.50±0.01^{d}	24.70±0.01^{b}	23.89±0.01^{c}	22.80±0.01^{e}	24.80±0.01^{a}	0.006	<0.0001
Crude protein	19.20±0.01^{d}	19.80±0.01^{c}	20.26±0.01^{a}	16.72±0.01^{e}	20.10±0.10^{b}	0.03	<0.0001
Ether extract	0.98±0.01^{a}	0.96±0.01^{b}	0.98±0.01^{a}	0.89±0.01^{c}	0.68±0.01^{d}	0.006	<0.0001
Ash	11.89±0.01	11.86±0.01	11.89±0.01	12.01±0.01	11.91±0.01	0.006	<0.0001
NFE	44.47±0.01^{d}	42.60±0.01^{e}	47.91±0.01^{b}	47.50±0.01^{c}	58.69±0.01^{a}	0.006	<0.0001

PPL - Pawpaw Leaves, PPF - Pawpaw Fruits, SOF - Sweet Orange Fruits, PAF-Pineapple Fruits, CCM-Coconut Milt

^{abcde} Means of treatments along a row with dissimilar superscript varied considerably (P<0.05). P value- probability,

SEM- Standard error of means.

4.2 Growth performance characteristics of African giant lnd snail hatchling in response to fibre degrading enzyme

The outcomes of performance behaviours of African giant land snail hatchlings fed diets supplemented with varying inclusion levels of fibre degrading enzyme, (*β-D-mannanase)* are revealed in Table 4.10.

No significant (P>0.05) effect of *β-D-mannanase* dosage was noted on feed ingestion of AGLS hatchlings. A similar trend was detected in last weight, overall weight gain and FCR of AGLS hatchlings fed diets supplemented with *β-D-mannanase*.

Increasing dosage of *β-D-mannanase* considerably (P<0.05) improved final weight (FW) and total weight gain (TWG) in a linear fashion, while improving FCR from 4.63 in H1 to 4.13 in H_4. However, increasing *β-D-mannanase* dosage from 0.15 g to 0.20 g/kg diet had no significant (P>0.05) effect on FW, TWG and FCR for AGLS hatchlings. Moreover, the shell dimensions; shell length and shell width were not (P>0.05) affected by *β-D-mannanase* dosage. Figure 4.1 shows the correlation among varying inclusion levels of *β-D-mannanase* and feed conversion ratio of the hatchling snails. It was noted that a negative linear relationship exists between *β*-D-mannanase and FCR of AGLS hatchlings. The R^2 value (0.91) indicated that about 91.0% of the observed differences in FCR of AGLS hatchlings were as a result of *β-D-mannanase*. A unit increase in *β-D-mannanase* dosage will result in a 1.88 decrease in FCR of AGLS hatchlings. The intercept is set at 4.64 when the *β-D-mannanase* value is at zero.

The correlation among varying inclusion levels of *β-D-mannanase* and the Body Weight Gain of AGLS hatchlings is revealed in Figure 4.2. A positive linear relationships exists between *β*-D-*mannanase* and the body Weight Gain of AGLS hatchlings. Increasing level of *β-D-mannanase* resulted in a corresponding increase in weight gain of AGLS hatchlings. The R^2 value (0.92) indicated that about 92.0% of the observed changes in the BWG of hatchling snails were as a result of *β-D-mannanase*. A unit increase in *β*-D-*mannanase* will result in a 128.29 g weight gain.

The outcomes of the cost analysis reveal that there was a significant (P<0.05) difference in the cost/g feed of the snails fed experimental diets with increasing enzyme inclusion.

However, the cost/body weight gain decreased as the levels of *β-D-mannanase* inclusion in the diets increased, demonstrating an inverse relationship. The minimum cost/body weight increase was obtained in the diet comprising 0.15 g *β-D-mannanase*/kg feed (H3). Based on the cost/body weight gain record therefore, it would be expedient to advise snail famers to go for diets with inclusion of 0.15 g *β-D-mannanase*/kg feed to accelerate the growth of snails at this phase of growth.

Table 4.10: Growth performance of African giant land snail hatchlings (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β-D-mannanase*

PARAMETERS	H1 BD + 0.00g/kg feed	H2 BD + 0.01g/kg feed	H3 BD + 0.15g/kg feed	H4 BD + 0.20g/kg feed	SEM
Total feed intake (g/snail)	343.22	344.70	345.30	347.30	7.20
Initial weight (g)	26.01	25.90	25.03	25.46	3.90
Final weight (g)	100.14	103.71^{b}	108.84^{a}	109.58^{a}	5.60
Total weight gain (g/snail)	74.13^{c}	77.81^{b}	83.81^{a}	84.12^{a}	4.50
Feed conversion ratio	4.63^{a}	4.43^{b}	4.12^{c}	4.13^{c}	0.02
Total shell length (mm)	19.13	19.21	19.28	19.31	2.30
Total shell width (mm)	16.29	16.31	16.33	16.38	2.10
Cost/g feed (₦)	0.05^{b}	0.05^{ab}	0.06^{a}	0.07^{a}	1.50
Cost/g body weight gain (₦/g)	82.13^{a}	80.27^{a}	78.12^{d}	79.48^{c}	3.90

a,b,c are the averages with varied superscripts along the similar row which are considerably different ($p<0.05$)

BD - Basal diet; SEM-Standard error of means.

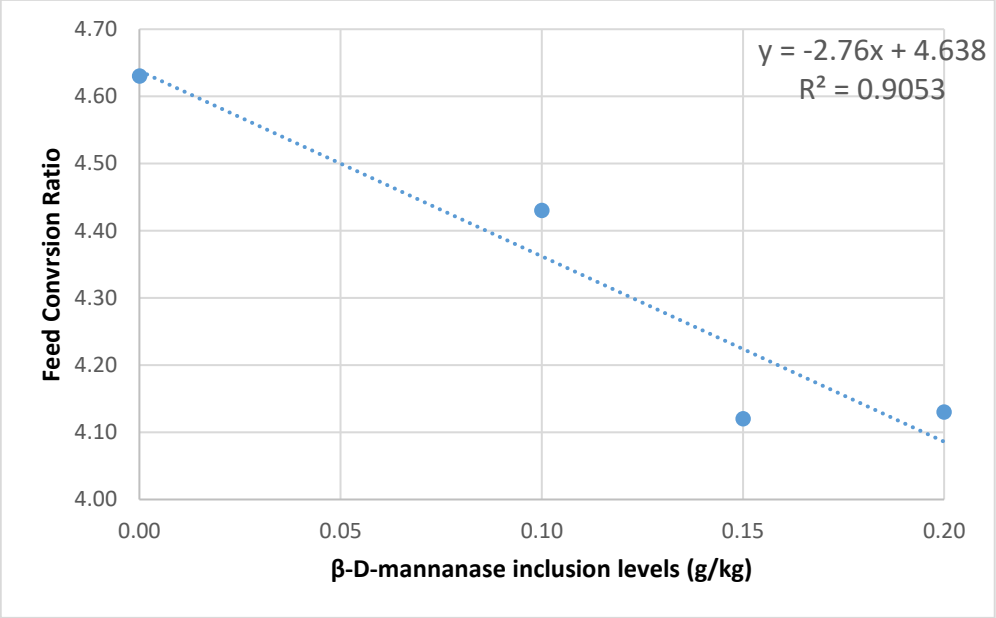


Figure 4.1: Correlation between varying inclusion levels of *β-D-mannanase* and feed conversion ratio of hatchling snails

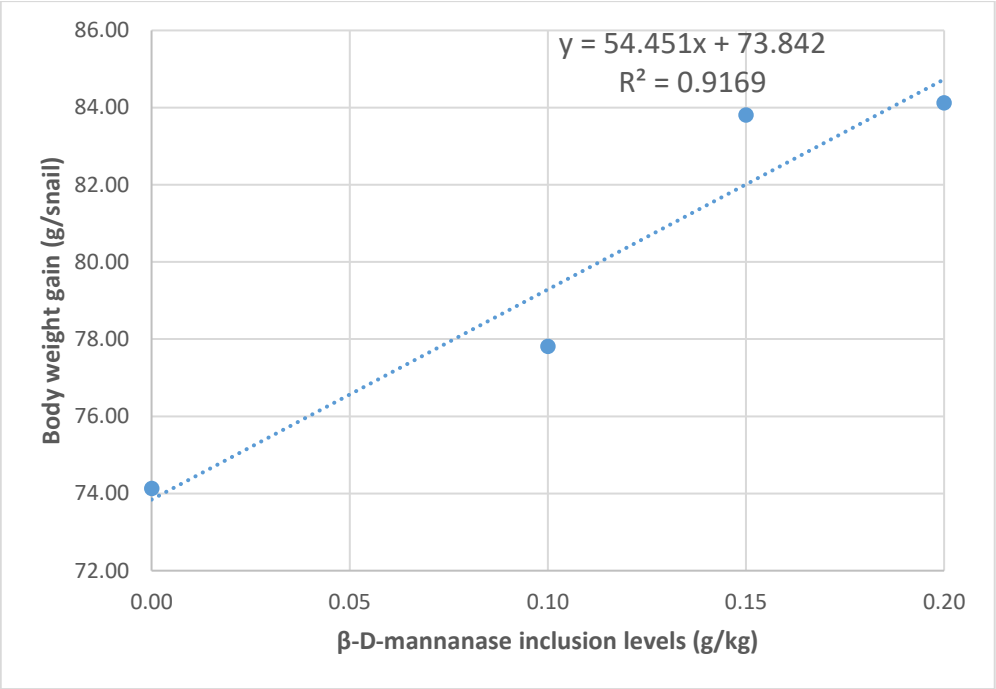


Figure 4.2: Correlation among varying inclusion levels of *β-D-mannanase* and body weight gain of hatchling snails

4.2.1 Nutrient digestibility of African giant land snail hatchlings fed diets supplemented with varying inclusion levels of *β-D-mannanase*.

The outcomes of the digestibility of AGLS diets supplemented with varying inclusion levels of *β-D-mannanase* are revealed in Table 4.11 Dry matter, crude protein and crude fibre digestibility ($P<0.05$) increased with increased *β-D-mannanase* dosage in the diets of AGLS hatchlings Supplementation of the AGLS hatchlings diets with *β-D-mannanase* at either 0.15 or 0.20 g/kg diet resulted in better DM, CP and CF digestibility compared to the other treatments. For each parameter evaluated, treatment, H4 had the highest value, which corresponded to 0.20 g *β-D-mannanase*/kg feed. Figure 4.3 shows the correlation among crude protein digestibility in hatchlings AGLS and varying inclusion levels of *β-D-mannanase*. It was observed that the R^2 value was 0.915, indicating that about 91% of the observed changes in protein digestibility were as a result of varying inclusion levels of *β-D-mannanase*. A unit increase in *β-D-mannanase* level will result in 78.49% protein digestibility. The intercept is set at 69.84. However, an optimum level of inclusion was obtain at 1.2 g/kg inclusion of *β-D-mannanase*.

The correlation between fibre digestibility and levels of *β-D-mannanase* in diets of hatchling AGLS is revealed in Figure 4.4. The R^2 value noted was 0.88 and indicated that about 88.0% of the observed changes in fibre digestibility was a result of the varying inclusion levels of *β-D-mannanase* in feed. A unit increase in *β-D-mannanase* dosage will result in 75.87% fibre digestibility. The intercept is set at 62.79. The optimum inclusion level of *β-D-mannanase* was observed at 1.20 g/kg.

Table 4.11: Nutrient digestibility of African giant land snail hatchlings (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β-D-mannanase*

PARAMETERS (%)	H1	H2	H3	H4	SEM
Dry matter	70.05[b]	72.15[b]	76.01[a]	76.41	2.6
Crude protein	60.63[b]	64.13[b]	65.80[a]	66.90[a]	3.1
Crude fibre	60.33[c]	63.10[b]	67.01[a]	67.20[a]	2.8
Crude fat	62.10	62.64	63.03	63.40	2.3

[abc] are averages with varied superscripts along the similar row are which considerably different.

H1-0.00 g/kg; H2 - 0.10 g/kg; H3=0.15 g/kg; H4 – 0.2.0 g β-D-mannanase/kg feed. SEM - Standard error of mean

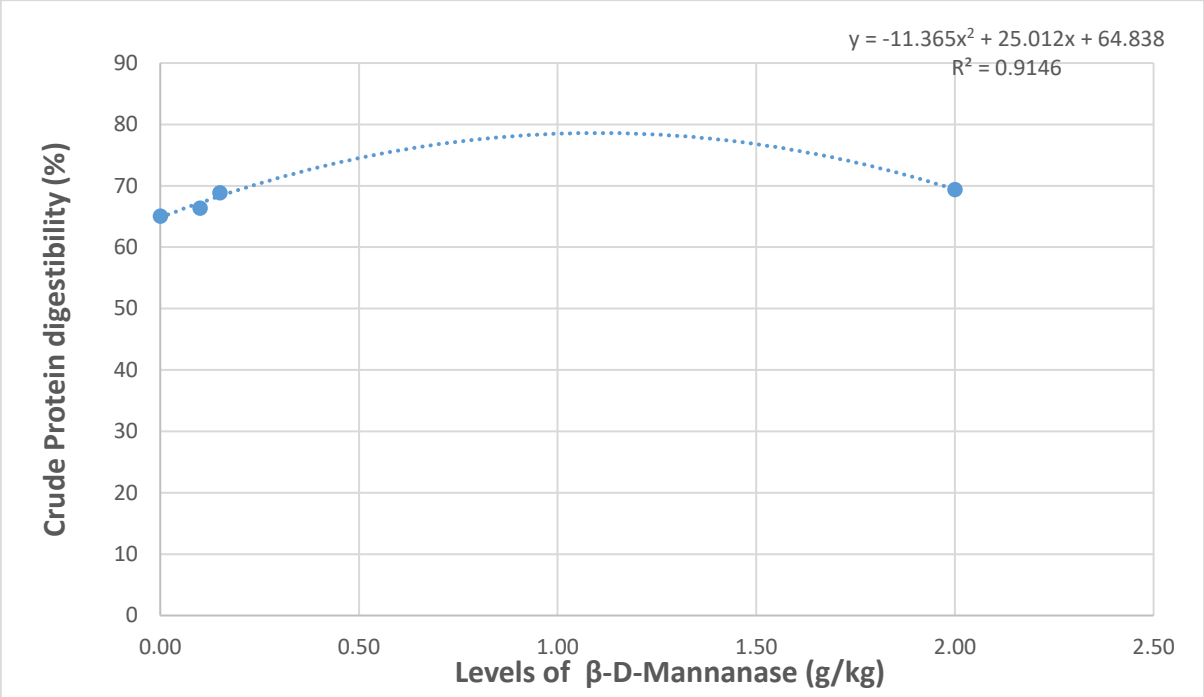


Figure 4.3: The correlation between crude protein digestibility in hatchling AGLS and varying inclusion levels of *β-D-mannanase*

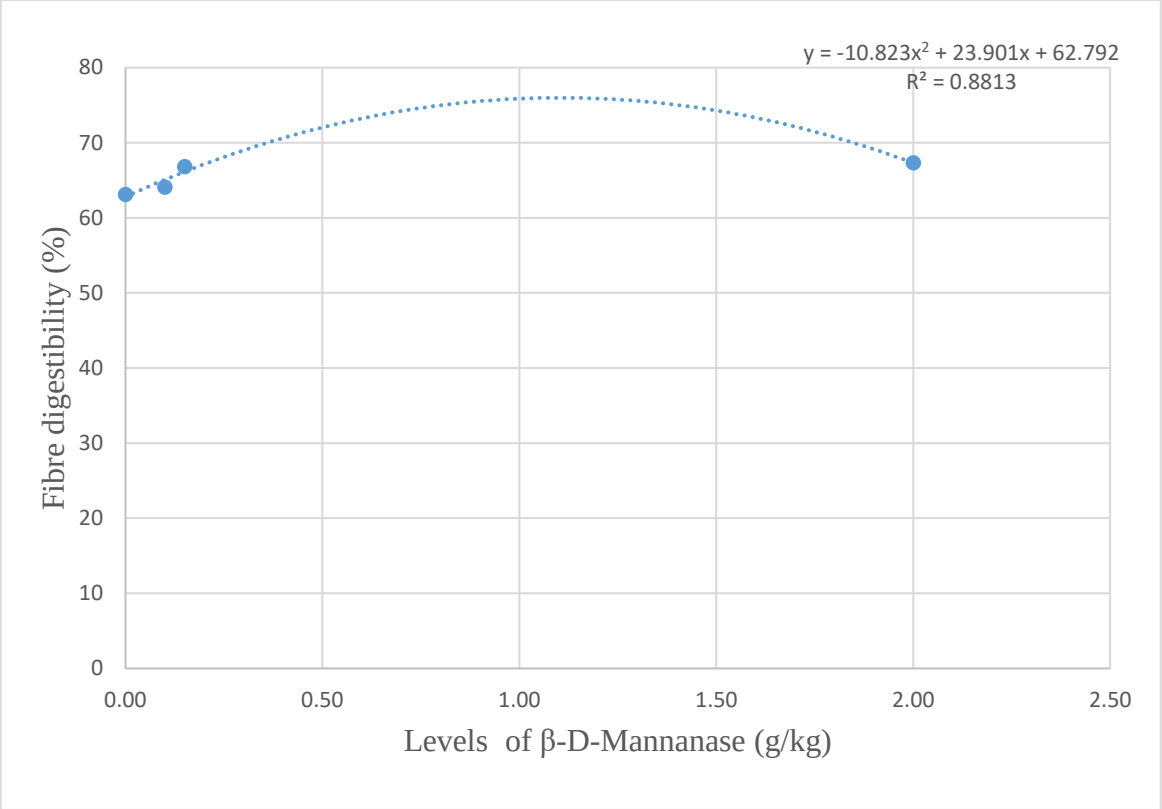


Figure 4.4: The correlation between fibre digestibility and levels of *β-D-mannanase* in diets of hatchling AGLS

4.2.2 Carcass evaluation of African giant land snail hatchlings fed diets supplemented with varying inclusion levels of *β*-D-mannanase.

The outcomes of carcass yield of AGLS hatchlings fed diets supplemented with varying inclusion levels of *β-D-mannanase* are presented on Table 4.12. The carcass yield differed significantly ($P<0.05$) across the treatment groups. Increasing levels of *β-D-mannanase* resulted in a corresponding increase in live weight of the AGLS hatchlings. A similar trend was observed on the foot (most edible portion) weight. The foot weight increased as the level of *β-D-mannanase* dosage increased. The highest value for foot weight (53.84 g) was recorded for treatment H_4 with 0.20 g/kg feed *β-D-mannanase*. The dressing percentage was also significantly affected ($P<0.05$) by the increasing dosage of *β-D-mannanase*. The dressing percentage is increased as the level of enzyme inclusion increased.

Figure 4.5 shows the correlation among varying inclusion levels of *β-D-mannanase* and live weight of AGLS hatchlings fed diets supplemented with *β-D-mannanase*. A positive linear correlation exists between the *β-D-mannanase* and the live weight of the hatchling snails. The intercept is set at 99.43 when the *β*-D-mannanase dosage is zero. Increasing levels of *β*-D-mannanase resulted in a corresponding increase in live weight of AGLS hatchlings. The R^2 value (0.91) indicated that about 91.0% of the observed changes in live weight of AGLS hatchlings were as a result of *β-D-mannanase*. A unit increase in *β-D-mannanase* will result in 148.10g live weight of AGLS hatchlings.

Table 4.12: Carcass characteristics of African giant land snail hatchlings fed diets supplemented with varying inclusion levels of β-D-mannanase

Parameters	T1	T2	T3	T4	SEM	P value
Live weight (g)	99.84^{d}	102.70^{c}	108.30^{b}	108.79^{a}	0.01	<0.0001
Foot weight (%)	50.15^{d}	51.33^{c}	53.45^{b}	53.84^{a}	0.01	<0.0001
Shell weight (%)	20.05^{b}	20.51^{a}	19.95^{c}	19.93^{c}	0.01	<0.0001
Offal weight (%)	26.22^{c}	24.88^{d}	27.30^{b}	27.56^{a}	0.01	<0.0001
Shell: live weight	0.20^{b}	1.20^{a}	0.18^{b}	0.18^{b}	0.01	0.0003
Offal: live weight	0.26^{a}	0.24^{c}	0.25^{b}	0.25^{b}	0.09	0.0003
Visceral weight (%)	29.80^{a}	28.16^{b}	26.59^{c}	26.23^{d}	0.01	<0.0001
Dressing percentage	50.23^{a}	50.16^{b}	49.35^{d}	49.46^{c}	0.01	<0.0001

^{abcd} Averagesof treatments along a row with dissimilar superscript varied considerably (P<0.05).

T1 - 0.00 g/kg, T2 - 0.10 g/kg, T3 - 0.15 g/kg, T4 - 0.20 g/kg β-D-mannanase SEM - Standard error of means, P value - probability.

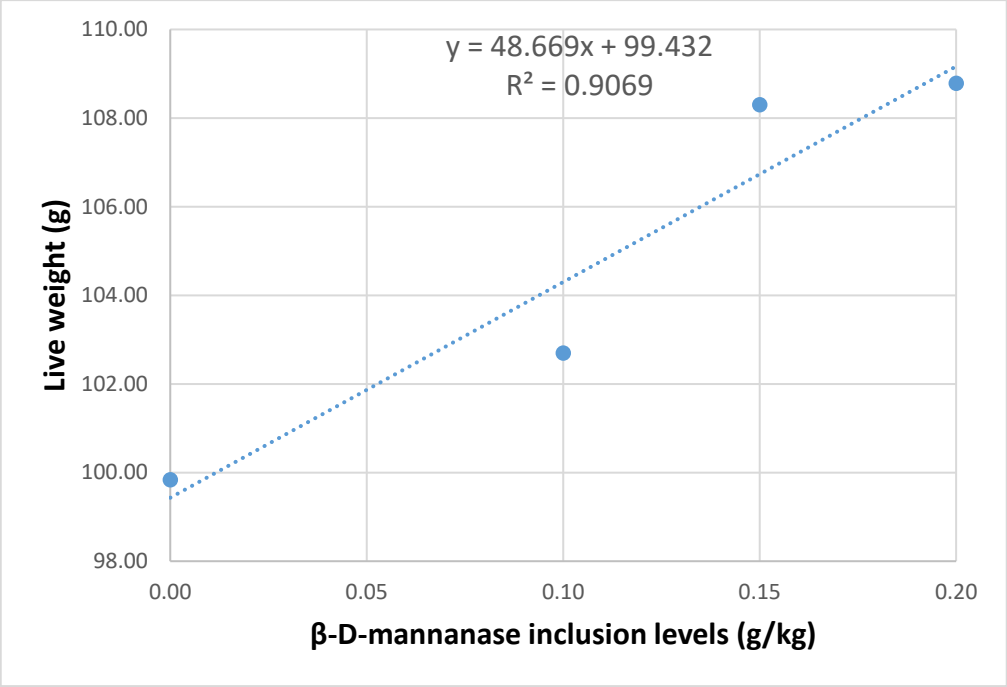


Figure 4.5: Correlation between varying inclusion levels of *β-D-mannanase* and live weight of hatchling snails

4.3: Growth performance characteristics of grower African giant land in response to in-feed fibre degralina enzyme *β-D-mannanase*

The outcomes of the performance indices of grower African giant land snail fed diets supplemented with fibre degrading enzyme, *β-D-mannanase* are presented in Table 4.13.

There was no significant (P>0.05) effect of *β-D-mannanase* dosage observed on feed intake of grower African giant land snails. The mean total feed intake were relatively similar for all the treatment groups. However, the BWG and FCR were significantly (P<0.05) affected by the increasing dosage of *β-D-mannanase*. The correlation among varying inclusion levels of β-D-mannanase and body weight gain of grower African giant land snails is revealed in Figure 4.6. A positive linear correlation exists between *β-D-mannanase* and the BWG of the grower AGLS. The intercept is set at 274.34 when the *β-D-mannanase* dosage is zero. Increasing levels of β-D-mannanase resulted in a corresponding increase in BWG of grower AGLS. The R^2 value (0.90) indicated that about 90.0% of the observed changes in BWG of grower AGLS were as a result of β-D-mannanase. A unit increase in *β-D-mannanase* will result in a 371.63 g body weight gain of grower AGLS. Figure 4.7 shows the correlation among varying inclusion levels of *β-D-mannanase* and FCR of grower AGLS. A negative linear correlation exists between the β-D-mannanase and the FCR of grower AGLS. Increasing levels of *β-D-mannanase* resulted in decrease in FCR of grower AGLS. The R^2 value (0.86) indicated that 86.0% of the observed differences in FCR of grower AGLS were as a result of varying levels of *β-D-mannanase*. The intercept is set at 3.83 when the *β-D-mannanase* dosage is zero. A unit increase in *β-D-mannanase* will result in a 0.53 decrease in FCR of grower AGLS.

The shell dimensions; shell length and shell width were affected (P<0.05) by the increasing dosage of β-D-mannanase. The cost/g body weight gain (₦/g) also increased (P<0.05) with the increasing dosage of *β-D-mannanase* in the diets of grower AGLS.

Table 4.13: Performance characteristics of grower African land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β-D-mannanase*

Parameter	T1	T2	T3	T4	SEM
Total feed intake (g/snail)	913.09	912.09	910.03	914.03	6.90
Initial weight (g)	91.03	93.02	91.07	93.01	5.12
Final weight (g)	368.02^{b}	371.07^{b}	375.08^{a}	380.04^{a}	4.20
Total weight gain (g/snail)	276.09^{b}	280.03^{b}	290.01^{a}	295.01^{a}	4.39
Feed conversion ratio	3.94^{c}	3.20^{ab}	3.13^{ab}	3.09^{a}	0.05
Shell length increment (mm)	12.51^{b}	12.51^{b}	12.53^{a}	12.53^{a}	0.4
Shell width increment (mm)	9.66^{d}	9.67^{c}	9.68^{b}	9.69^{a}	0.28
Cost/g Feed (₦)	0.05	0.05	0.05	0.05	2.90
Cost/g body weight gain (₦/g)	144.88^{c}	148.23^{a}	143.67^{d}	147.73^{b}	2.46

^{abc} Averages with varying superscripts along similar rows with diverse superscripts are expressively dissimilar from each other (P>0.05).

T1- 0.00 g/kg; T2 - 0.10 g/kg; T3 - 0.15 g/kg; T4 - 0.20 g *β-D-mannanase*/kg feed SEM - Standard Error of Mean

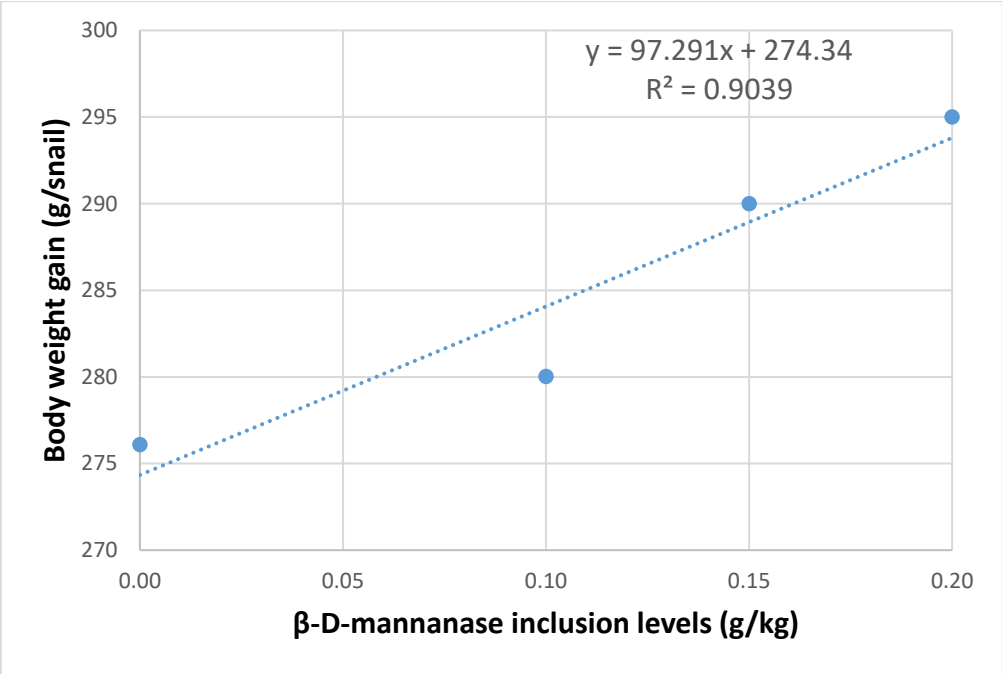


Figure 4.6: Correlation among varying inclusion levels of *β-D-mannanase* and weight gain of grower AGLS

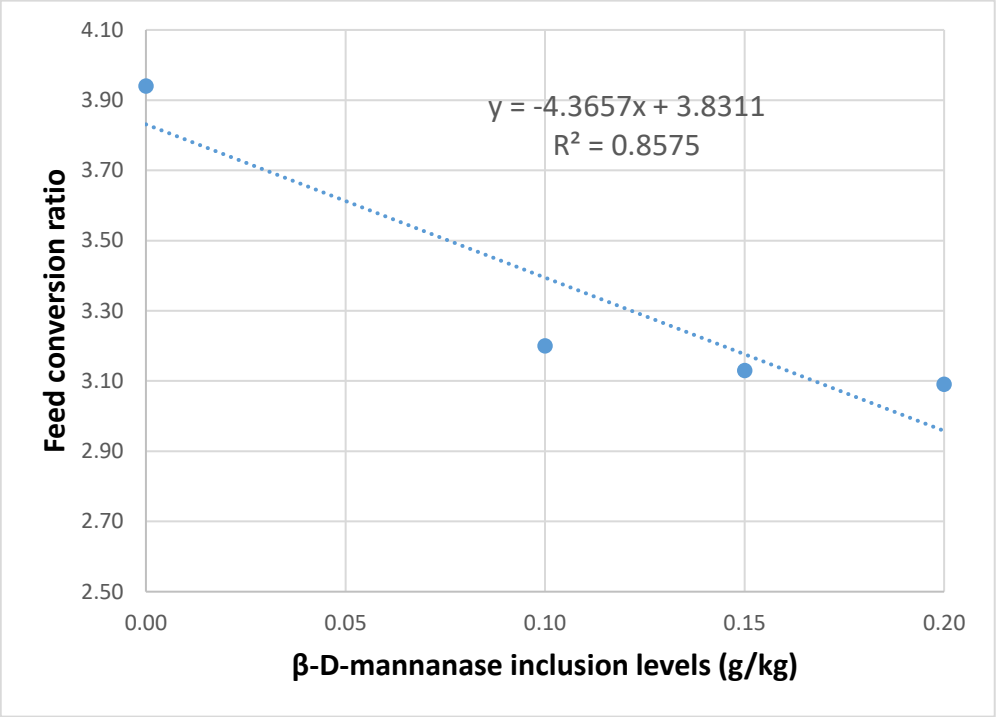


Figure 4.7: Correlation among varying inclusion levels of *β-D-mannanase* and Feed conversion ratio of grower AGLS

4.3.1 Nutrient Digestibility of grower African giant Land Snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β*-D-*mannanase*.

The nutrient digestibility of grower AGLS (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β*-D-*mannanase* is revealed in Table 4.14. Dry matter, crude protein, crude fibre and crude fat digestibility ($P<0.05$) increased with increased dosage in the diets of AGLS growers. For each parameter evaluated, treatment T_4 had the highest result. Figure 4.8 shows the correlation among varying inclusion levels of *β*-D-*mannanase* and fibre digestibility of grower AGLS. It was observed that a positive linear correlation existed between *β*-D-*mannanase* dosage and fibre digestibility of AGLS growers. The R^2 value (0.889) indicated that about 88.9% of the observed differences in fibre digestibility of grower AGLS were as a result of *β*-D-*mannanase* dosage will result in 85.35% fibre digestibility. The intercept is set at 62.78 when *β*-D-*mannanase* value is at zero. Crude fat digestibility in T_3 and T_4 were similar and significantly higher than in T_1 and T_2.

Table 4.14: Nutrient digestibility of grower African giant land snails (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β-D-mannanase*

Nutrient Digestibility (%)	T1	T2	T3	T4	SEM
Dry matter	80.20^{b}	80.90^{a}	83.40^{a}	84.10^{a}	2.9
Crude protein	65.10^{b}	66.40^{b}	68.90^{a}	69.40^{a}	2.3
Crude fibre	63.10^{d}	65.10^{c}	66.80^{b}	68.30^{a}	2.8
Ether extract	64.50^{b}	66.30^{b}	68.10^{a}	68.50^{a}	2.5

^{abc} Averages along same rows with varying superscripts are considerably dissimilar from each other ($p<0.05$). T1-0.00 g/kg; T2-0.10 g/kg; T3-0.15 g/kg; T4-0.20g *β*-D-*mannanase*/ kg feed. SEM-Standard error of mean.

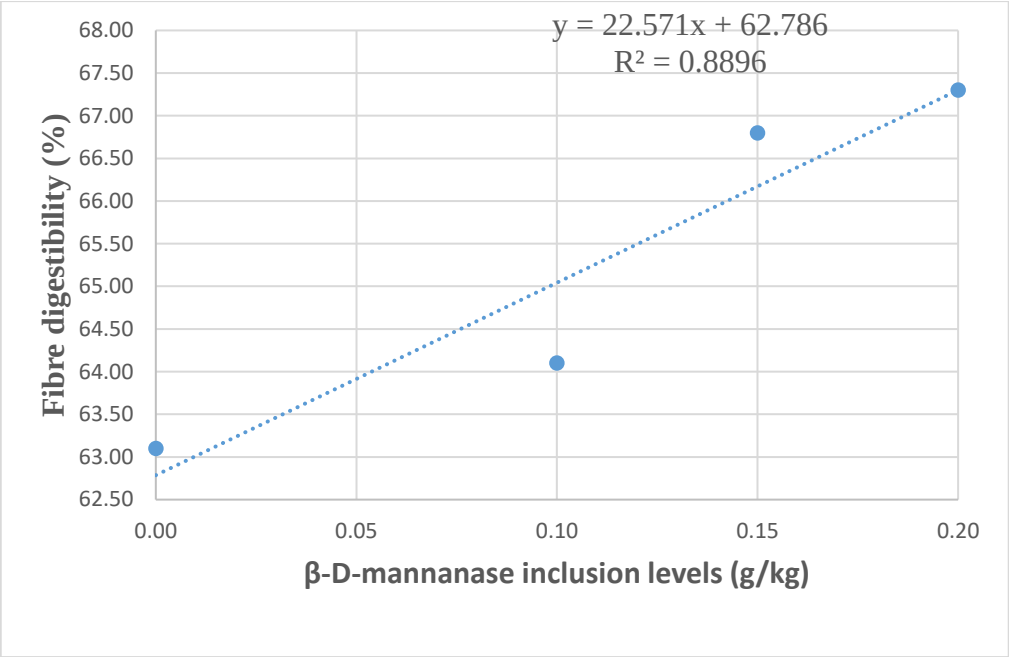


Figure 4.8: Correlation among varying inclusion levels of *β*-D-mannanase and fibre digestibility of grower AGLS.

4.3.2 Carcass Characteristics of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β*-D-mannanase.

The outcomes of the carcass characteristics of grower African giant land snail (*Archachatina marginata*) fed diets with *β-D-mannanase* supplementation are presented in Table 4.15. The values of the live weight across the treatments were affected (P<0.05) by the increasing supplementation of *β-D-mannanase*. A similar trend was observed on the foot weight. The foot weight increased (P<0.05) at the increasing levels of *β-D-mannanase*. The dressing percentage was also affected by the increasing dosage of *β-D-mannanase* (P<0.05) shell to live weight ratio ranged from 0.06 to 0.07. The correlation among varing inclusion levels of *β-D-mannanase* and live weight of grower AGLS is revealed in Figure 4.9. A positive linear correlation exists between *β-D-mannanase* and the live weight of grower AGLS. The intercept is set at 3.66.29 when *β*-D-mannanase dosage is zero. Increasing levels of *β-D-mannanase* resulted in increase in live weight of grower AGLS. The R^2 value (0.72) indicated that about 72.0% of the observed differences in live weight of grower AGLS were as a result of varying levels of *β-D-mannanase.* A unit increase in *β-D-mannanase* will result in 416.12 g live weight of grower AGLS. Figure 4.10 shows the correlation among varying inclusion levels of *β-D-mannanase* and dressing percentage of grower AGLS. It was observed that a positive linear correlation existed between *β-D-mannanase* and the dressing percentage of grower AGLS. The R^2 value (0.79) indicated that about 79.0% of the observed differences in dressing percentage of grower AGLS were as a result of *β-D-mannanase*. A unit increase in *β-D-mannanase* will result in 47.22% dressing percentage. The intercept is set at 41.17 when *β-D-mannanase* dosage is at zero.

Table 4.15: Carcass characteristics of Grower African giant land snails (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of *β-D-mannanase*

Parameters	T1	T2	T3	T4	SEM	P value
Live weight (g)	367.50^{c}	370.50^{b}	370.50^{b}	379.10^{a}	0.03	<0.0001
Foot weight (%)	41.31^{d}	41.40^{c}	42.64^{b}	42.80^{a}	0.01	<0.0001
Shell weight (%)	23.70^{d}	23.81^{c}	24.04^{a}	23.92^{a}	0.01	<0.0001
Offal weight (%)	22.04^{d}	22.40^{b}	22.54^{a}	22.11^{c}	0.01	<0.0001
Shell: live weight	0.07^{a}	0.06^{b}	0.07^{a}	0.06^{b}	0.004	<0.0001
Offal: live weight	0.06	0.06	0.06	0.06	0.01	0.10
Visceral weight (%)	82.31^{c}	82.39^{b}	82.50^{a}	82.51^{a}	0.01	<0.0001
Dressing percentage	41.30^{d}	41.40^{c}	42.30^{b}	42.40^{a}	0.01	<0.0001

$^{a, b, c, d}$: Averages of treatments along a row with dissimilar superscript varied considerably (P<0.05).

T1-0.00 g/kg, T2-0.10 g/kg, T3-0.15 g/kg, T4-0.20 g/kg SEM-Standard error of means, P value=probability.

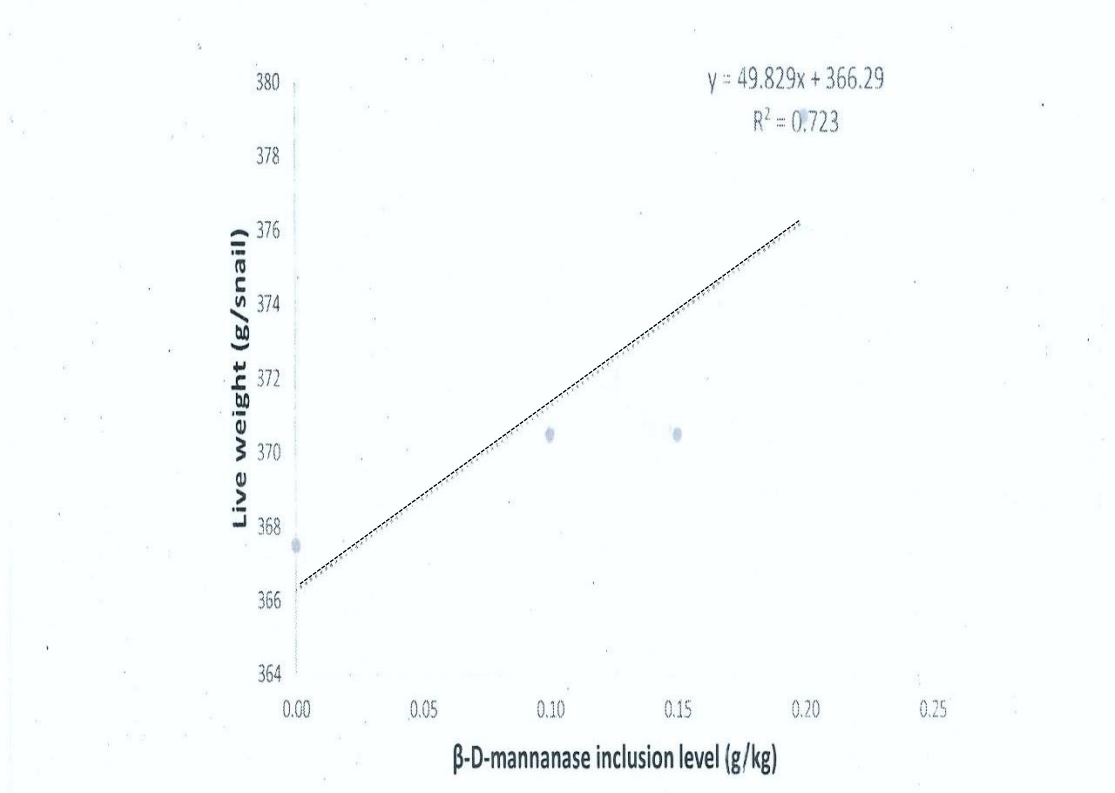


Figure 4.9: Correlation among varying inclusion levels of *β-D-mannanase* and live weight of grower snails

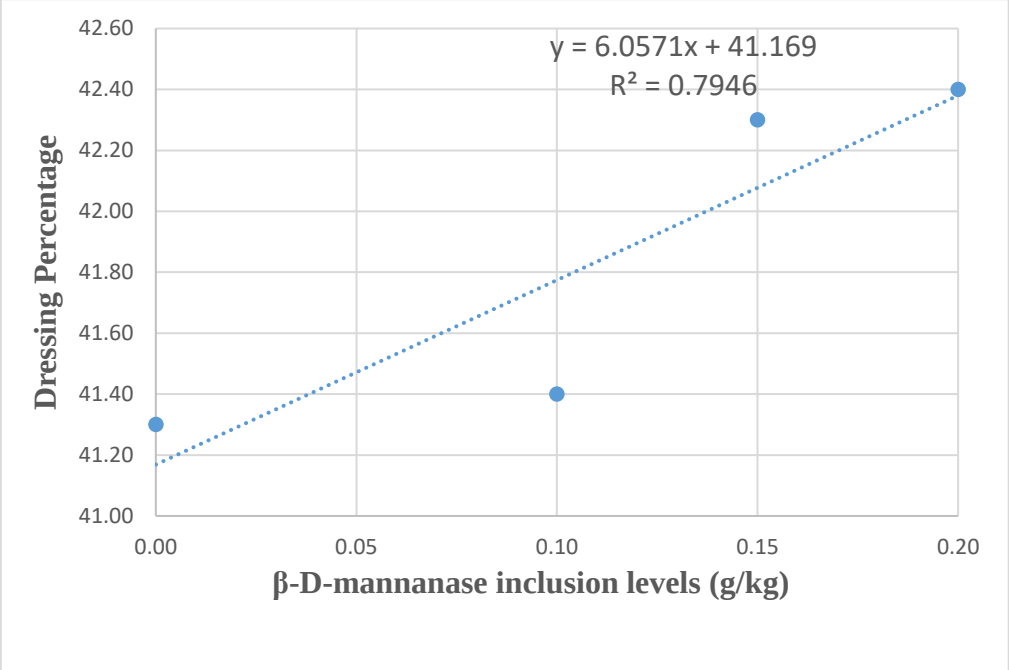


Figure 4.10: Correlation between varying inclusion levels of *β-D-mannanase* and dressing percentage of grower AGLS.

4.4.1 Growth performance characteristics of grower African giant land snails fed - diets supplemented with varying inclusion levels of prebiotic preparation.

Table 4.16 shows the outcomes of the performance indicators of grower African giant land snail fed diets supplemented with varying levels of prebiotic preparation. There were no significant differences ($P>0.05$) in the feed intake across the treatments aside from the first two treatments (i.e negative and positive controls). The values ranged from 27.65 g (M3) to 31.00 g (M5) compared to the control treatments. M1 (13.49 g) and M2 (23.72 g). However, the body weight gain was significantly affected ($P<0.05$) by the increasing dosage of prebiotic. The body weight gain in snails on M7 (189.61) has highest value and the lowest weight gain was observed on M1 (157.99 g). The FCR values of snails on different inclusion levels of prebiotic blend did not vary significantly and values ranged from 0.09 to 0.17. The shell length, shell width, apertural length and shell thickness were all, significantly affected ($P<0.05$) by the increasing dosage of prebiotics. As the amount of inclusion of prebiotics increased across the treatments. The values of these parameters increased. There was no mortality recorded throughout the experimental period.

Figure 4.11 shows the correlation between varying inclusion levels of prebiotic blend and body weight gain of grower AGLS. A positive linear correlation exists between the prebiotics and the body weight gain of grower AGLS. The intercept is set at 169.02 g when the prebiotic dosage is zero. Increasing levels of prebiotics resulted in increase in BWG of grower AGLS. The R^2 value (0.517) indicated that about 51.7% of the observed differences in BWG of grower AGLS were as a result of varying levels of prebiotics. A unit increase in prebiotics outcomes in 177.50 g BWG of grower AGLS.

The correlation between varying inclusion levels of prebiotics and FCR of grower AGLS is revealed in Figure 4.12. A polynomial correlation existed between the prebiotics and FCR of grower AGLS. The optimum level was set at 1.50 g/kg. The R^2 value (0.72) indicated that about 72.0% of the observed differences in FCR of grower AGLS were as a result of varying inclusion levels of prebiotics. A unit increase in prebiotics results in FCR value of 0.5.

Table 4.16: Performance characteristics of African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of Prebiotics

Parameters	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**		
	NC	PC	0.5	1.0	1.5	2.0	2.5	SEM	P value
Feed Intake (g/wk/snail)	13.49	23.72	27.65	29.51	31.00	28.98	29.58	17.34	0.51
Body Weight gain (g/snail)	157.99^{f}	183.69^{c}	166.80^{c}	178.57^{d}	183.87^{c}	186.27^{b}	189.61^{a}	0.79	<0.0001
FCR	0.09	0.13	0.17	0.17	0.17	0.16	0.16	0.10	0.54
Shell thickness (mm)	0.95^{c}	1.05^{b}	0.96^{c}	0.98^{c}	1.06^{b}	1.14^{a}	1.15^{a}	0.01	<0.0001
Apartural length(cm)	6.43^{d}	6.48^{c}	6.51^{c}	6.53^{c}	6.54^{b}	6.56^{b}	6.58^{a}	1.98	0.03
Shell width (cm)	5.62^{d}	5.72^{c}	5.73^{c}	5.81^{b}	5.82^{b}	5.85^{a}	5.89^{a}	0.06	0.09
Shell length (cm)	10.34	10.01	10.33	10.45	10.46	10.48	10.49	0.75	0.43

[a, b, c, d, e, f] Averagesof treatment along a row with dissimilar superscript varied considerably (P<0.05). NC- Negative control, PC-Positive control, SEM-Standard error of means, P value – probability, FCR – Feed conversion ratio. M1 = 0.00g/kg, M2 = 0.10 mg/kg Oxytetracycline, M3 - 0.50 g/kg, M4 - 1.00 g/kg, M5 - 1.5 g/kg, M6 - 2.0 g/kg, M7 - 2.50 g Prebiotic/ kg feed.

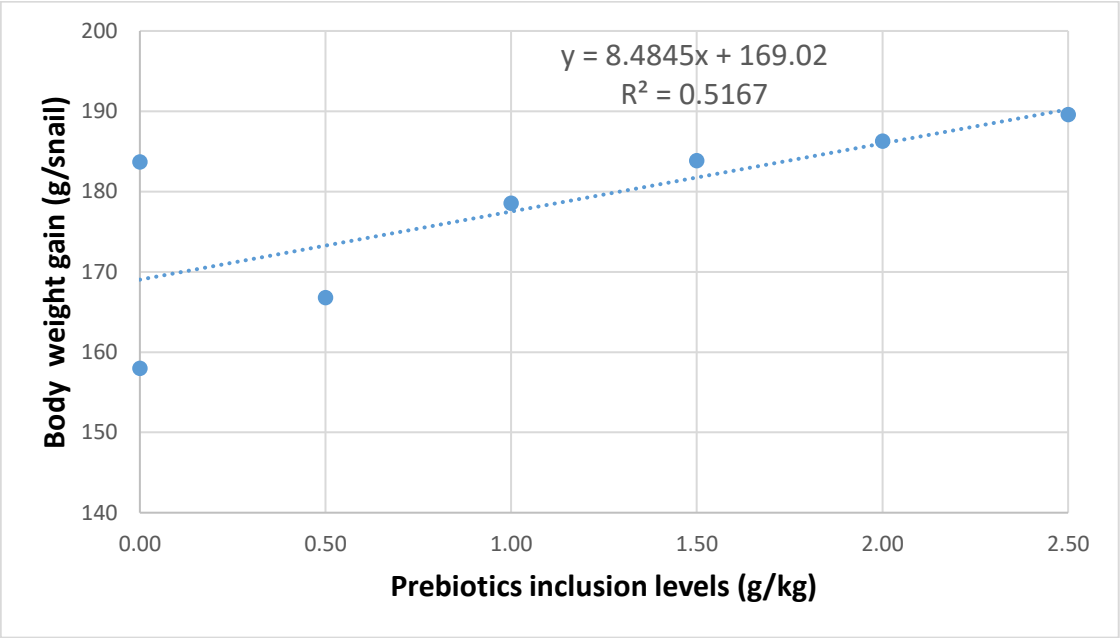


Figure 4.11: Correlation between varying inclusion levels of prebiotics and Body weight gain of grower AGLS

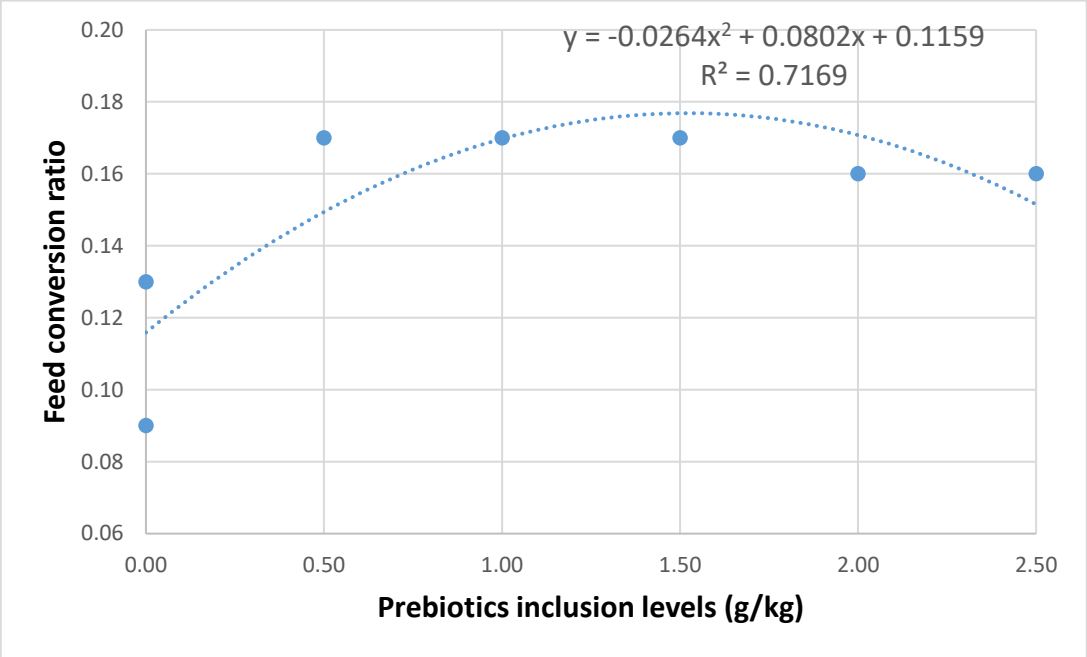


Figure 4.12: Correlation between varying inclusion levels of Prebiotics and Feed conversion ratio of grower AGLS

4.4.2 Nutrient digestibility of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics

The nutrient digestibility of grower AGLS (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics is revealed in Table 4.17. There were fluctuations in the dry matter digestibilities but the highest was observed in treatment M_7 with the highest amount of prebiotics (2.5 g/kg). Crude protein digestibility was affected ($P<0.05$) by the treatments. Crude protein digestibility values were generally high with the highest value obtained in treatment M_7 (83.82%). Crude fibre digestibilities were also affected ($P<0.005$) by the increasing levels of prebiotics in the diets. The values were however on the averages, the highest value noted was 53.79% compared to the negative treatment (45.99%). Digestibility of crude fat decreased as the amount of prebiotics added increased. The values ranged from 66.49% to 71.37%.

Table 4.17: Nutrient digestibility of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics

	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**		
Nutrients (%)	NC	PC	0.50	1.00	1.50	2.00	2.50	SEM	P value
Dry matter	52.35^{g}	57.23^{a}	53.65^{d}	56.69^{c}	53.07^{e}	52.89^{f}	58.61^{a}	0.01	<0.0001
Crude Protein	68.15^{g}	78.68^{e}	75.16^{f}	79.69^{g}	79.95^{c}	82.25^{b}	83.82^{a}	0.01	<0.0001
Crude Fibre	45.99^{f}	48.56^{d}	47.66^{e}	51.64^{b}	52.90^{c}	52.49^{g}	53.79^{a}	0.01	<0.0001
Ether Extract	67.00^{cd}	68.25^{b}	71.37^{a}	67.50^{c}	67.41^{c}	67.32^{c}	66.49^{d}	0.22	<0.0001

$^{a, b, c, d, e, f}$ Averagesof treatment along a row with different superscript differed significantly (P<0.05). NC- Negative control, PC-Positive control, SEM-Standard error of means, P value – probability

T1 - 0.00 g/kg, T2 - 0.10 mg/kg Oxytetracycline, T3 - 0.50 g/kg, T4 - 1.00 g/kg, T5 - 1.5 g/kg, T6 - 2.0 g/kg, T7 - 2.5 g/kg Megawin

4.4.3 Carcass characteristics of grower African giant snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics

The outcomes of the carcass characteristics of grower AGLS fed with diets supplemented with changing inclusion levels of prebiotics are presented in Table 4.18. The carcass traits were influenced ($P<0.05$) by the supplementation of the grower AGLS diets with varying inclusion levels of prebiotics. The live weight were influenced ($P<0.05$) by the increasing dosage of prebiotics. The highest live weight of 181.7 g were observed in treatments M_6 and M_7. Foot is the major edible portion of the snail. The foot weight was also influenced ($P<0.05$) by the increasing dosage of the prebiotics with the maximum values observed in treatments M_6 (24.64%) and M_7 (24.98%). The dressing percentage was affected by the increasing dosage of prebiotics, the highest values observed in M_6 (53.59%) and M_7 (54.10%). Shell to live weight ratio increased significantly in treatment M_5 (28.68), but did not differ from M_6 (18.32), M_7 (21.10) and M_1 (20.92). Similar trend was observed with offal to live weight ratio.

Figure 4.13: Shows the correlation among variable inclusion levels of prebiotics and live weight of grower AGLS. A positive linear correlation exists between prebiotics and live weight of grower AGLS. The intercept is set at 163.81 when the prebiotics value is zero increasing dosage of prebiotics resulted in increase in LW of grower AGLS. The R^2 value (0.60) indicated that about 60.0% of the observed differences in LW of the grower AGLS were as a result of varying levels of prebiotics inclusion. A unit increase in prebiotics results in 170.26 g live weight.

Table 4.18: Carcass characteristics of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of Prebiotics

Parameters	M1	M2	M3	M4	M5	M6	M7		
	NC	PC	0.50	1.00	1.50	2.00	2.50	SEM	P Value
Live weight (g)	161.78^{d}	171.77^{b}	164.52^{c}	166.13^{bc}	167.37^{bc}	181.73^{a}	181.73^{a}	11.43	0.24
Shell weight (%)	20.80^{cd}	25.48^{b}	22.39^{c}	25.47^{b}	26.18^{b}	27.05^{a}	27.68^{a}	3.18	0.29
Whole flesh (%)	52.62^{d}	69.58^{a}	60.54^{cd}	61.21^{d}	62.15^{d}	64.94^{c}	66.68^{b}	5.09	0.19
Foot weight (%)	16.28^{d}	20.44^{b}	20.47^{b}	21.42^{a}	22.68^{c}	24.64^{e}	24.98^{cd}	1.86	0.37
Visceral hump (%)	34.76^{e}	42.23^{b}	34.72^{e}	47.53^{a}	39.48^{dc}	38.92^{dc}	40.42^{bc}	2.93	0.45
Edible visceral (%)	22.32^{d}	32.69^{a}	25.15^{c}	34.50^{a}	32.85^{ab}	25.38^{c}	27.15^{b}	3.05	0.19
Offal weight (%)	11.67	11.69	12.86	12.16	12.30	11.62	13.18	1.28	0.59
Dressing (%)	38.85^{d}	51.93^{c}	27.73^{e}	36.08^{d}	52.84^{b}	53.59^{a}	54.10^{a}	3.82	0.15
Shell: live weight	20.92^{ab}	14.94^{c}	13.61^{d}	15.33^{c}	28.68^{a}	18.32^{ab}	21.10^{ab}	4.26	0.26
Offal: Live weight	11.71^{ab}	11.64^{ab}	7.82^{c}	7.32^{c}	12.14^{ab}	11.55^{ab}	13.25^{a}	0.85	0.19

$^{a, b, c, d, e, f}$ Averagesof treatment along a row with dissimilar superscript varied considerably ($P<0.05$). NC- Negative control, PC-Positive control, SEM-Standard error of means, P value – probability

M1 - 0.00 g/kg, M2- 0.10 mg/kg Oxytetracycline, M3- 0.50 g/kg, M4- 1.00 g/kg, M5 -1.5 g/kg, M6 - 2.0 g/kg, M7- 2.50 g prebiotics/kg feed

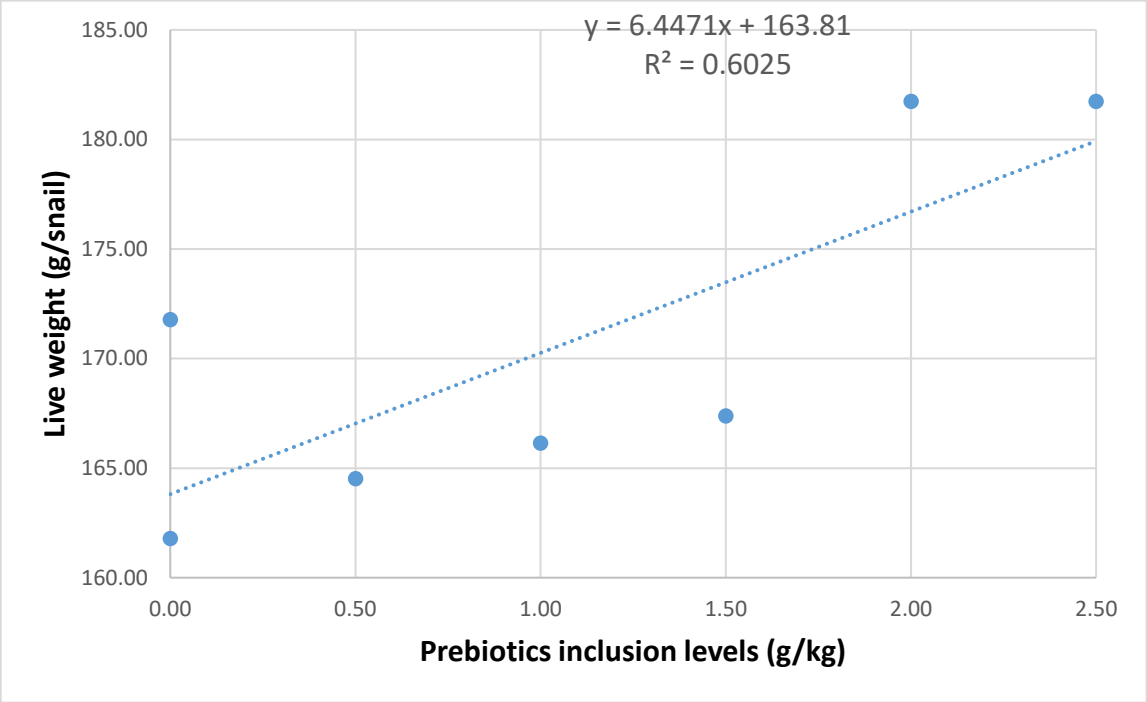


Figure 4.13: Correlation between varying inclusion levels of Prebiotics and live weight of grower snails

4.4.4 Serum biochemical indices of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying Levels of Probiotics

Table 4.19 displays the serum biochemical indices of grower AGLS fed diets augmented by varying amounts of prebiotics. The prebiotics were included in the diet, and their presence had a significant impact on the total protein in the serum ($P<0.05$). When matched to the other treatments, the total protein value in M7 (8.78 g/L) was greater ($P<0.05$), whereas the minimum value was found in M2 (4.56 g/L). The albumin value also increased ($P<0.05$) as prebiotic dosage in AGLS diets increased. The albumin-to-globulin ratio and globulin were also trending similarly. However, as the prebiotic dosage in AGLS diets increased, creatinine values decreased ($P>0.05$). M3 had the maximum value (68.00 mg/dL), while M2 had the minimum (42.00m g/dL). ALP, AST, and ALT generally decreased as prebiotic dosage increased across the treatments. Prebiotic levels increased with serum glucose, which also followed the same pattern. Treatment M2 had the lowest value (66.00 percent), while M7 had the highest value (90.00 percent).

Table 4.19: Serum biochemical indices of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics

Parameter	M1	M2	M3	M4	M5	M6	M7	SEM
Total Protein (g/L)	5.72^{e}	4.56^{f}	6.50^{d}	6.72^{d}	6.88^{c}	7.20^{b}	8.78^{a}	0.28
Albumin (g/L)	2.10^{e}	1.24^{f}	2.30^{d}	2.62^{c}	2.80^{b}	3.10^{a}	3.12^{d}	0.14
Globulin (g/L)	3.62^{c}	3.32^{d}	4.20^{a}	4.10^{b}	4.08^{b}	4.10^{e}	5.66^{b}	0.17
Alb-Glo ratio	0.58^{c}	0.37^{e}	0.55^{d}	0.64^{b}	0.69^{a}	0.76^{a}	0.55^{d}	0.33
Creatinine (mg/dl)	56.00^{c}	42.00^{e}	68.00^{a}	60.00^{b}	58.00^{c}	56.00^{c}	51.00^{d}	1.63
ALP (U/L)	42.00^{f}	40.00^{f}	74.00^{a}	72.00^{b}	65.00^{c}	54.00^{e}	50.00^{e}	3.08
AST (U/L)	56.00^{f}	54.00^{g}	86.00^{a}	84.00^{b}	75.00^{c}	60.00^{e}	57.00^{e}	3.01
ALT (U/L)	45.00^{f}	42.00^{g}	80.00^{a}	78.00^{b}	68.00^{c}	57.00^{d}	54.00^{e}	3.37
Glucose (%)	70.00^{d}	66.00^{e}	67.00^{e}	76.00^{c}	84.00^{b}	85.00^{b}	90.00^{a}	2.34

a, b, c - Mean with diverse superscripts along the similar row are significantly ($P<0.05$) different

SEM - Standard error of mean. ALP-Alkaline phosphatase; AST- Aspartate aminotransferase; ALT- Alanine amino transferase; M1 - 0.00 g/kg, M2 - 0.10 mg/kg Oxytetracycline, M3 - 0.50 g/kg, M4 - 1.00g/kg, M5 - 1.5 g/kg, M6 - 2.0 g/kg, M7 - 2.50 g prebiotics/kg feed.

4.4.5 Haematological response of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying levels of prebiotics.

The outcomes were presented in Table 4.20. The PCV values increased with the increasing dosage of prebiotics. The values ranged from 36.05% (M_2) to 54.05% (M_7). However, WBC of grower AGLS decreased with increasing dosage of prebiotics, the lowest ($6.00x10^7/\mu L$) was observed in treatment M_7. The ESR was significantly ($P<0.05$) higher in M_1 (8.00 mm) than other treatments while the least value was observed in M_7 (2.00 mm). Haemocyte type I were more predominant in population than the haemocyte type II. The values increased with the increasing inclusion of prebiotics. Haemocyte type I has highest value M_7 (66.00) while Haemocyte type II has highest value in M_2 (46.00) and the lowest in M_7 (33.00). The population of haemocyte type II was about half of that of haemocyte type I. Haemocyte type II cells do not take part in immune activity in snails.

Table 4.20: Haematological response of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics.

Parameter	M1	M2	M3	M4	M5	M6	M7	SEM
PCV (%)	38.11^{c}	36.05^{e}	38.14^{d}	40.04^{d}	47.90^{c}	51.11^{b}	54.05^{a}	1.66
WBC $X10^{7}$/UL	11.20^{a}	4.20^{e}	10.20^{b}	9.60^{b}	7.80^{c}	7.20^{cd}	6.00^{d}	0.46
ESR (mm)	8.00^{a}	2.11^{f}	6.19^{b}	4.00^{c}	3.30^{d}	3.24^{e}	2.00^{g}	0.58
Heamocyte 1	61.00^{d}	54.00^{e}	62.00^{d}	64.00^{c}	64.50^{c}	65.00^{b}	66.00^{a}	0.84
Haemocyte 2	39.00^{b}	46.00^{a}	38.00^{c}	36.00^{d}	37.00^{d}	35.00^{e}	33.00^{e}	0.88

^{abcde} Averagesof treatments along a row with varying superscripts vary significantly at $P<0.05$

PCV- Packed Cell Volume; WBC- White Blood Cells; ESR- Erythrocytes Sedimentation Rate

SEM- Standard Error of Mean

4.4.6 Haemolymph serum lipid profile of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics.

The outcomes of haemalymph serum lipid profile of grower AGLS fed diets supplemented with varying levels of prebiotics were presented in Table 4.21. The values of cholesterol and triglycerides significantly ($P<0.05$) decreased with increasing dosage of prebiotics. Highest cholesterol value (78.00 mg/dL) was obtained in treatment M_3 while the lowest value (73.00 mg/dL) was observed in treatments M_6 and M_7. The highest value for triglycerides (70.00 mg/dL) was observed in treatment M_3 while the least value (50.00 mg/dL) was obtained in treatments M_6 and M_7. Increasing dosage of prebiotics across the treatments resulted in a corresponding increase in the values of HDL ($P<0.05$). However, increasing dosage of prebiotics resulted in decreased values of LDL, VLDL and the risk ratio. LDL: HDL followed the same trend; values decreased with increasing dosage of prebiotics. The values ranged from 0.39 (M_1) to 0.05 (M_7). The risk ratio decreased with increasing dosage of the prebiotic blend.

Table 4.21: Haemolymph serum lipid profile of grower African giant land snail (*Archachatina marginata*) fed diet supplemented with varying inclusion levels of prebiotics.

	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	SEM	P value
Parameters (mg/dl)	NC	PC	0.50	1.00	1.50	2.00	2.50	-	-
Cholesterol	62.00^{e}	56.00^{f}	78.00^{a}	76.00^{b}	74.00^{c}	73.00^{e}	73.00^{d}	0.58	<0.0001
Triglycerides	60.00^{c}	48.00^{f}	70.00^{a}	68.00^{b}	55.00^{d}	50.00^{e}	50.00^{e}	0.58	<0.0001
HDL	36.00^{c}	40.00^{d}	48.00^{c}	50.00^{b}	54.00^{b}	57.00^{a}	60.00^{a}	0.70	<0.0001
LDL	14.00^{b}	5.00^{f}	16.00^{a}	12.00^{c}	9.00^{d}	6.00^{e}	3.00^{e}	0.43	<0.0001
VLDL	12.00^{c}	11.00^{cd}	14.00^{a}	13.60^{b}	11.00^{cd}	10.00^{d}	10.00^{d}	0.44	<0.0001
LDL:HDL	0.39^{a}	0.13^{c}	0.33^{a}	0.24^{b}	0.17^{b}	0.11^{c}	0.05^{d}	0.01	<0.0001
Risk ratio (TC:HDL)	1.72^{a}	1.40^{d}	1.63^{b}	1.52^{c}	1.37^{d}	1.28^{e}	1.22^{e}	0.03	<0.0001

[a, b, c, d, e, f:] Averagesof treatment along a row with dissimilar superscript varied considerably (P<0.05). NC- Negative control, PC- Positive control, SEM-Standard error of means, control, SEM-Standard error of means, P value – probability, HDL- High density lipoprotein; LDL-Low density lipoprotein; VLDL- Very low density lipoprotein; TC- Total cholesterol, M1 - 0.00 g/kg, M2 - 0.10 mg/kg Oxytetracycline, M3 - 0.50 g/kg, M4 - 1.00 g/kg, M5 - 1.5 g/kg, M6 - 2.0 g/kg, M7 - 2.50 g Prebiotics/kg feed

4.4.7 Haemolymph mineral composition of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics.

The outcomes of haemolymph mineral composition of the grower African giant land snail (*Archachatina marginata*) fed diets with varying inclusion levels of prebiotics were revealed in Table 4.22. Sodium (Na), Potassium (K), Calcium (Ca), Copper (Cu), Zinc (Zn), chloride (Cl^-) and phosphorus (P) values were significantly higher ($P<0.05$) in haemolymph of AGLS on M_3 compared to other treatments. However, Iron (Fe) has highest value in treatment M_6 (6.67 mg/kg) and lowest in treatment M_1 (5.60 mg/kg). Manganese has highest value in treatment M_1 (5.82 mg/kg) Haemolyph values were particular high in sodium (Na) (48.00-70.00%), Potassium (K) (38-66.00%), chloride (Cl^-) (60-96%), Calcium (Ca) (7.63-8.72%), and phosphorus (P) (2.20-4.80%).

Table 4.22: Haemolymph mineral composition of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with varying inclusion levels of prebiotics

	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	SEM	P value
Parameters	NC	PC	0.50	1.00	1.50	2.00	2.50		
Sodium (%)	65.00^{b}	64.00^{b}	70.00^{a}	62.00^{c}	56.00^{d}	48.00^{f}	52.00^{e}	0.58	<0.0001
Potassium (%)	56.00^{b}	55.00^{bc}	66.00^{a}	54.00^{c}	45.00^{d}	38.00^{e}	44.00^{d}	0.58	<0.0001
Iron (mg/kg)	5.60^{e}	6.20^{c}	6.12^{c}	5.80^{d}	5.69^{de}	6.67^{a}	6.40^{b}	0.05	<0.0001
Calcium (%)	7.63^{f}	7.82^{e}	8.72^{a}	6.92^{g}	7.99^{d}	8.59^{b}	8.23^{c}	0.01	<0.0001
Manganese (mg/kg)	5.82^{a}	5.06^{f}	5.58^{b}	5.45^{e}	5.57^{bc}	5.57^{bc}	5.51^{d}	0.01	<0.0001
Copper (mg/kg)	1.58^{c}	1.54^{d}	1.79^{a}	1.48^{e}	1.42^{f}	1.53^{d}	1.66^{b}	0.01	<0.0001
Zinc (mg/kg)	0.66^{c}	0.68^{b}	0.73^{a}	0.65^{cd}	0.60^{f}	0.64^{d}	0.62^{e}	0.01	<0.0001
Chloride (%)	62.00^{f}	60.00^{g}	96.00^{a}	85.00^{b}	72.00^{c}	64.00^{e}	70.00^{d}	0.58	<0.0001
Phosphorus (%)	2.20^{f}	3.20^{e}	4.80^{a}	4.50^{b}	3.80^{c}	2.20^{f}	3.40^{d}	0.06	<0.0001

$^{a, b, c, d, e, f, g}$ Averages of treatment along a row with dissimilar superscript varied considerably (P<0.05). NC- Negative control, PC- Positive control, SEM-Standard error of means, P value – probability, T1 - 0.00g/kg, T2 - 0.10 mg/kg Oxytetracycline, T3-0.50 g/kg, T4 - 1.00 g/kg, T5 - 1.5 g/kg, T6 - 2.0 g/kg, T7 - 2.50 g prebiotics/kg feed.

4.4.8 Mineral Composition of grower African giant land snail (*Archachatina marginata*) shell powder as affected by the dietary supplementation with Prebiotics.

Table 4.23 shows the outcomes of the mineral composition of AGLS shell powder as affected by the diets supplementation with prebiotics. Generally, all the parameters evaluated were significantly ($P<0.05$) influenced by the treatments. The effect of the additive (prebiotic was more of stabilizing the amount of Sodium (Na) in each treatment). There was no appreciable increase ($P>0.05$) as the levels of prebiotics inclusion increased. The value of Sodium was stabilized at 0.02% across the treatments, the trends with Potassium (k) were similar to that of Sodium (Na). The values for the Iron (Fe) increased correspondingly with increased levels of prebiotics. The negative treatment was 477.00 mg/kg while the positive treatment was 515.00 mg/kg. The values for the main treatments ranged from 319.00 mg/kg to 673 mg/kg. The values of Calcium (Ca) also increased as the levels of prebiotics inclusion increased ($P<0.05$). The values across the treatments ranged from 74.60% to 96.03% while those of the control treatments were 73.70% (M_1) and 73.90% (M_2). The values of Manganese were not different ($P>0.05$) across the treatments. The values ranged from 24.80 mg/kg (M_1) to 25.43 mg/kg (M_4). The values of Copper (Cu) were also affected by the addition of prebiotics to the diet. The values ranged from 1.60 mg/kg (M_1) to 5.40 mg/kg (M_4). Copper is very important in snail because it is a component of haemocyanin, the oxygen carrier pigment in snail haemolymph.

Table 4.23: Mineral composition of grower African giant land snail (*Archachatina marginata*) shell powder as affected by dietary supplementation with Probiotics

	M1	M2	M3	M4	M5	M6	M7	SEM	P value
Parameters	NC	PC	0.50	1.00	1.50	2.00	2.50	-	-
Sodium (%)	0.02^{b}	0.04a	0.02^{b}	0.02^{b}	0.02^{b}	0.03^{b}	0.02^{b}	0.002	0.002
Potassium (x 10^{-2} %)	4.30^{bc}	4.80a	4.40^{b}	4.20^{c}	3.70^{d}	4.70^{a}	3.80^{d}	0.001	<0.0001
Iron (mg/kg)	477.00^{f}	515.00d	673.00^{a}	511.00^{e}	526.00^{c}	579.00^{b}	319.00^{g}	0.58	<0.0001
Calcium (%)	73.70^{e}	73.90e	74.60^{e}	82.30^{d}	91.00^{b}	96.03^{a}	89.67^{c}	0.33	<0.0001
Manganese (mg/kg)	24.80	25.00	25.20	25.43	24.98	25.10	24.97	0.22	0.57
Magnesium (x10^{-2} %)	1.40^{d}	1.50d	1.40^{d}	1.70^{c}	1.20^{e}	2.20^{a}	1.90^{b}	0.001	<0.0001
Copper (mg/kg)	1.60^{f}	4.00cd	5.20^{ab}	5.40^{a}	3.80^{d}	4.60^{bc}	2.40^{e}	0.22	<0.0001
Zinc (mg/kg)	13.30^{g}	18.40^{f}	22.90^{c}	20.10^{e}	21.20^{d}	31.50^{b}	45.00^{a}	0.22	<0.0001
Chloride (%)	0.9^{a}	0.50^{c}	0.31^{d}	0.72^{b}	0.72^{b}	0.90^{a}	0.73^{b}	0.04	<0.0001
Phosphorus (x10^{-2} %)	7.80^{a}	7.20^{b}	6.60^{d}	6.80^{c}	6.80^{c}	7.10^{b}	6.60^{d}	0.001	<0.0001
Sulphate (%)	0.08^{e}	0.11^{c}	0.1^{c}	0.11^{c}	0.14^{c}	0.13^{b}	0.10^{d}	0.002	<0.0001

a, b, c, d, e, f, g Averagesof treatment along a row with dissimilar superscript varied considerably (P<0.05). NC- Negative control, PC-Positive control, SEM-Standard error of means, P value – probability.

M1 - 0.00 g/kg, M2 - 0.10 mg/kg Oxytetracycline, M3 - 0.50 g/kg, M4 - 1.00 g/kg, M5 - 1.5 g/kg, M6 - 2.0 g/kg, M7 - 2.50 g/prebiotics/Kg feed

4.5.1 Performance characteristics of grower snails fed diets supplemented with *Lactobacillus plantarum* and *Saccharomyces cerevisiae*

The main effect of the probiotics and the inclusion levels on performance of African giant land snail (*Archachatina marginata*) were revealed in Table 4.24. Feed intake (g/wk/snail) of grower African giant land snail (AGLS) for Lactobacillus plantarum (Lacp) supplemented diets (11.47) and Saccharomyces cerevisiae (Sac) supplemented diets (11.49) did not differ (P>0.05). However, shell length observed in snails on Lacp diets (9.85 cm) was significantly (P<0.05) lower compared to Sac-supplemented diets (10.07cm). Shell width of snails on Sac-supplemented diet (5.90 cm) was higher (P<0.05) compared to Lacp-supplemented diet (5.78 cm). In addition, apartural length increase of grower AGLS shell for Lacp-supplemented diet (6.50 cm) and Sac-supplemented diet (6.56 cm) did not differ (P>0.05) and the shell thickness increase observed in snails on Lacp-supplemented diet (1.20 mm) was lower (P<0.05) than the shell thickness increase (1.26 mm) observed in Sac-supplemented diets. Moreover, the Body Weight Gain (BWG) (g/wk/snail) observed in Sac-supplemented diet (225.18) was higher (P<0.05) compared to Lacp-supplemented diet (141.37). However, in each case the values of those treated with the *Saccharomyces cerevisiae* were much more higher than those supplemented with the *Lactobaccilus plantarum*.

The feed intake observed in snails on 3% probiotics (12.97) was higher (P<0.05) compared to 2.0% (11.62) and 1.0% (9.85). However, the lowest (P<0.05) feed intake was observed in snails on 1.0% (9.85) probiotics. The shell length (cm) values in snails on 2.0% (9.99) and 3.0% (10.12) probiotics were similar, and higher (P<0.05) than 1.0% probiotics (9.78). Shell width in snails on 1.0% (5.82) and 2.0% (5.83) were lower (P0.05) compared to 3.0% (5.93) probiotics.

Regarding apartural length (cm) in snails on 1.0% (6.48) and 2.0% (6.48) were similar and were lower (P<0.05) compared to 3.0% (6.63) probiotics. On shell thickness (mm), measured fortnightly in snails on 1.0% (1.21) and 2.0% (1.21) were lower (P<0.05) compared to 3.0% (1.27) probiotics. Also, the Body Weight Gain (g/wk/snail) in snails on 1.0% (140.21) and 2.0% (143.40) were significantly (P<0.05) lower compared to 3.0% (266.21). Probiotics enhanced appetite in African giant land snail at levels of 1.0% to 3.0%.

Fig. 4.14 shows the correlation between varying inclusion levels of Lactobacillus plantarum and feed intake of grower AGLS fed diets supplemented with varying inclusion levels of probiotics. It was observed that a positive linear correlation existed between the independent variable (*Labtobacillus plantarum*) and the dependent variable (feed intake) of grower AGLS. The R^2 value (0.999) indicated that about 99.9% of the observed differences in feed intake of grower AGLS were as a result of Lactobacillus plantarum. A unit increase in Lactobacillus plantarum dosage will result in 10.27 g feed intake. The intercept is set at 9.07 when the Lactobacillus plantarum value is at zero.

The correlation between varying inclusion levels of Saccharomyces cerevisiae and feed intake of grower AGLS is presented in Figure 4.15. It was observed that a positive linear correlation existed between the independent variable (Saccharomyces cerevisiae) and the feed intake of the grower AGLS. The R^2 value (0.99) indicated that about 99.0% of the observed differences in feed intake of grower AGLS were as a result of Saccharomyces cerevisiae inclusion. A unit increase in Saccharomyces cerevisiae dosage will result in 9.57g feed intake. The intercept is set at 7.65 when the Saccharomyces cerevisiae dosage is at zero.

Figure 4.16 shows the correlation between varying inclusion levels of Lactobacillus platarum and FCR of grower AGLS. A positive linear correlation existed between the independent variable (Lactobacillus plantarum) and the dependent variable FCR of grower AGLS. The intercept is set at 6.67 when the independent variable is zero. Increasing levels of Lactobacillus plantarum resulted in increase in FCR of grower AGLS. The R^2 value (0.99) indicated that about 99.0% of the observed differences in FCR of grower AGLS were as a result of varying levels of Lactobacillus plantarum. A unit increase in Lactobacillus plantarum resulted in FCR value of 7.37.

The correlation between varying inclusion levels of Saccharomyces cerevisiae and FCR of grower AGLS is presented in Figure 4.17. A negative linear correlation exists between the independent variable (Saccharomyces cerevisiae) and the dependent variable (FCR) of grower AGLS. The intercept is set at 6.67 when the independent variable (Saccharomyces cerevisiae) dosage is zero. Increasing levels of Saccharomyces cerevisiae dosage resulted

in decrease in FCR of grower AGLS. The R^2 value (0.86) indicated that about 86.0% of the observed differences in FCR of grower AGLS were as a result of varying levels of Saccharomyces cerevisiae dosage. A unit increase in Saccharomyces cerevisiae dosage results in a 1.65 decrease in FCR.

The correlation between varying inclusion levels of *Lactobacillus plantarum* and the body weight gain of grower AGLS is presented in Table 4.18. It was observed that a polynomial correlation existed between the independent variable (Lactobacillus plantarum) and the dependent variable (Body Weight Gain) of grower AGLS. The R^2 value (1.00) indicated that about 100.0% of the observed differences in the body weight gain of grower AGLS were as a result of Lactobacillus plantarum. A unit increase in Lactobacillus plantarum dosage will result in 138.0 g BWG. The intercept is set at 126.9 when the independent variable (Lactobacillus plantarum) value is at zero.

Figure 4.19 shows the correlation between varying inclusion levels of Saccharomyces cerevisiae and body weight gain of grower AGLS. It was observed that a linear correlation existed between the independent variable (Saccharomyces cerevisiae) and the dependent variable (body weight gain) of grower AGLS. The R^2 value (0.77) indicated that about 77.0% of the observed differences in the body weight gain of grower AGLS were as a result of Saccharomyces cerevisiae. A unit increase in Saccharomyces cerevisiae dosage will result in 135.18 g BWG. The intercept is set at 111.83 when the independent variable (Saccharomyces cerevisiae) value is set at zero.

Table 4.24: Main effect of the probiotics and the inclusion levels on performance of grower African giant land snail (*Archachatina marginata*) fed probiotics supplemented diets

Parameters	Probiotics			Inclusion level (g/kg)			P values	SEM
	Lactobacillus	Yeast	1.00	2.00	3.00	Probiotics	Level	
Feed intake (g/wk/snail)	11.47	11.49	9.85^{c}	11.62^{b}	12.97^{a}	0.93	<0.0001	0.34
Shell length (cm)	9.85^{b}	10.07^{a}	9.78^{b}	9.99^{a}	10.12^{a}	0.004	0.002	0.08
Shell width (cm)	5.78^{b}	5.90^{a}	5.82^{b}	5.83^{b}	5.93^{a}	0.0001	0.03	0.04
Apartural length (cm)	6.50	6.56	6.48^{b}	6.48^{b}	6.63^{a}	0.16	0.003	0.04
Shell thickness (mm)	1.20^{b}	1.26^{a}	1.21^{b}	1.21^{b}	1.27^{a}	0.0011	0.01	0.02
Body weight gain (g/snail)	141.37^{d}	225.18^{b}	140.21^{d}	143.40^{c}	266.21^{a}	0.31	0.36	25.34

^{abc} Averagesof treatments along a row with dissimilar superscript varied considerably (P<0.05). SEM- Standard error of means.

P value - Probability

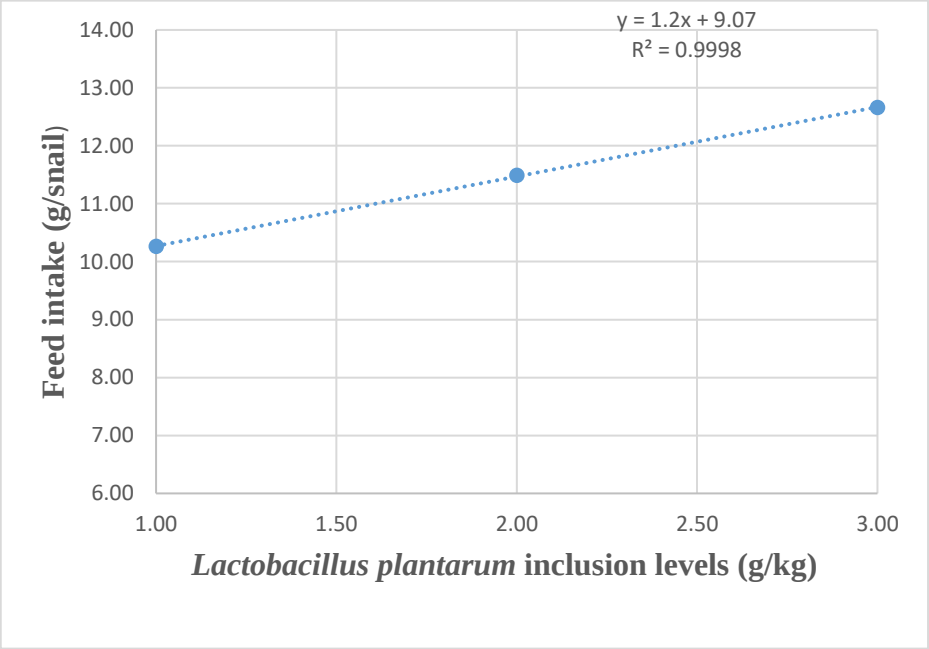


Figure 4.14: Correlation between varying inclusion levels of Lactobacillus plantarum and feed intake of grower AGLS.

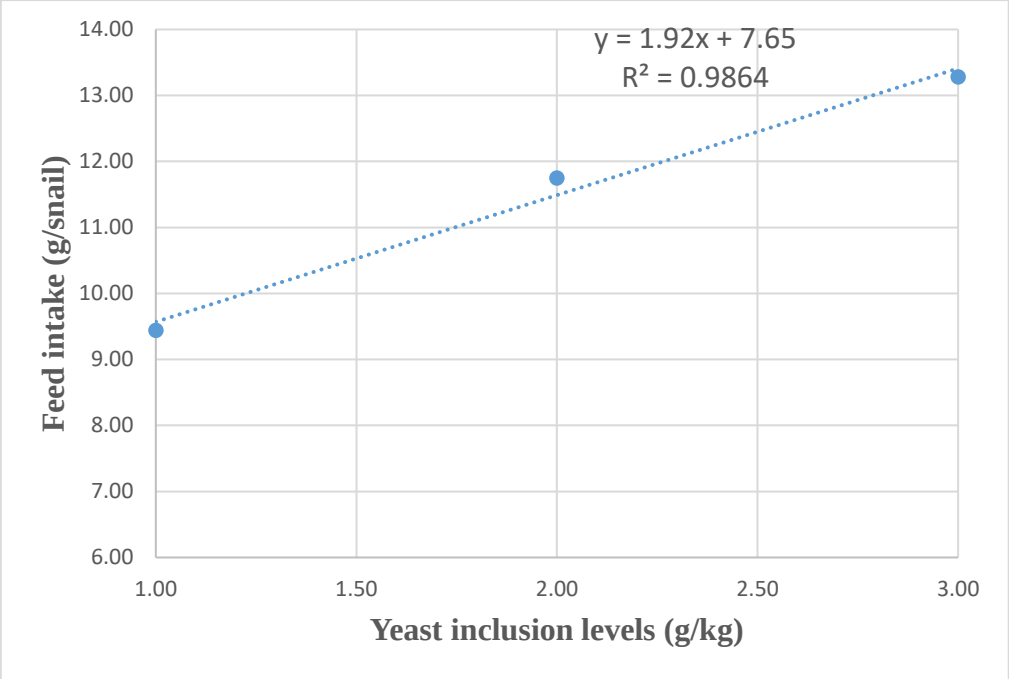


Figure 4.15: Correlation between varying inclusion levels of Saccharomyces cerevisiae and feed intake of grower AGLS.

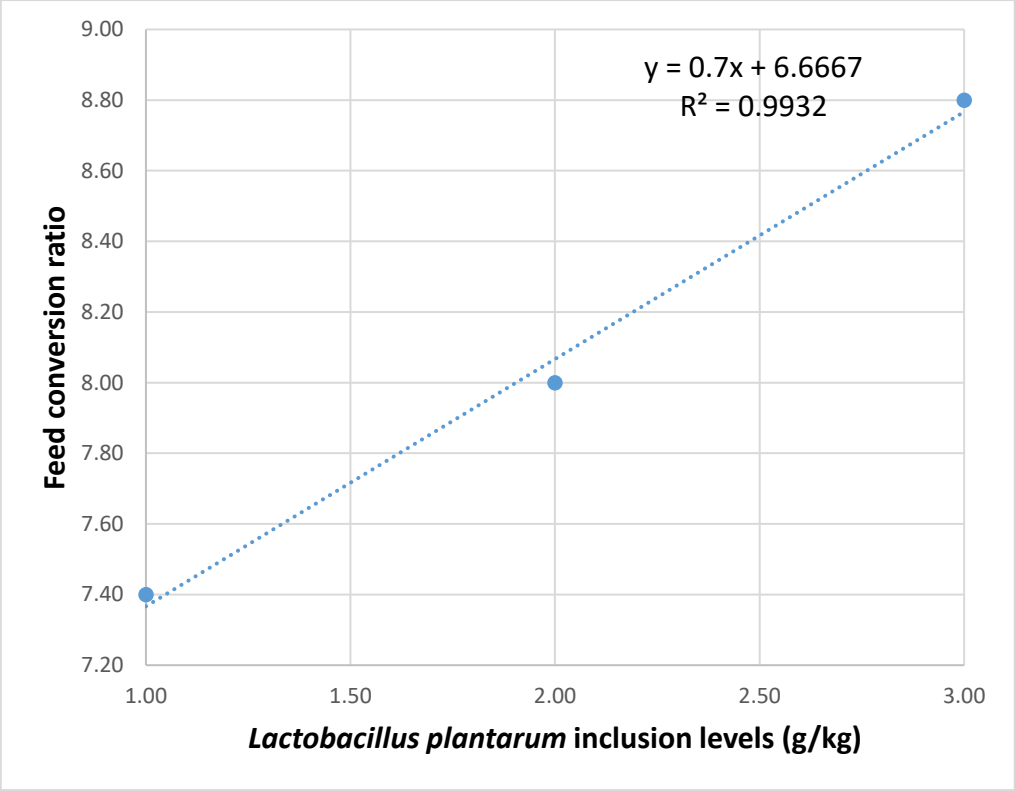


Figure 4.16: Correlation between dietary inclusion levels of *Lactobacillus plantarum* and feed conversion ratio of grower AGLS

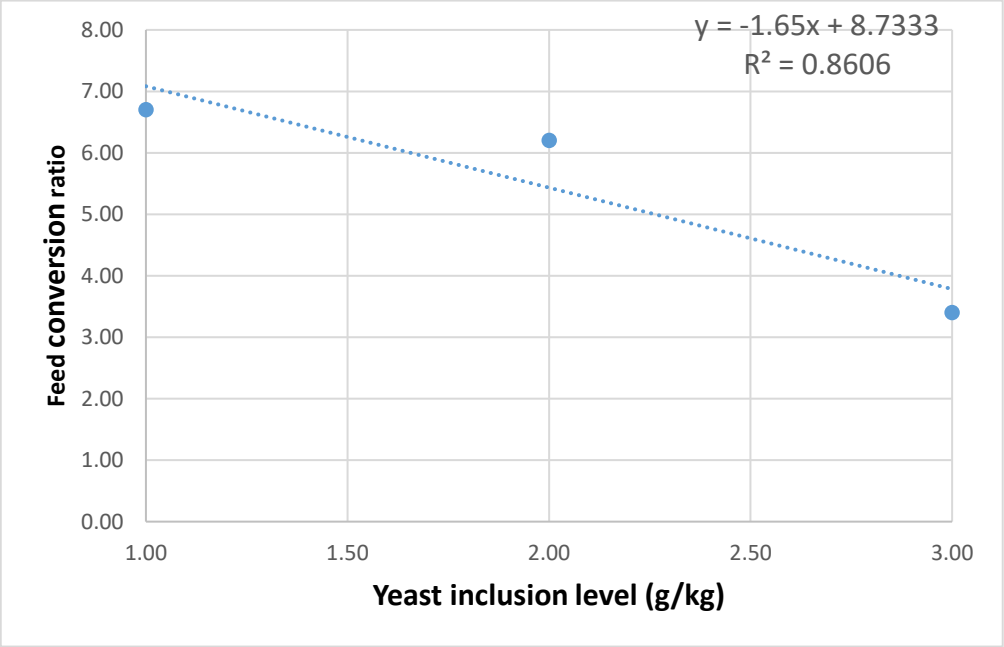


Figure 4.17: Correlation between dietary inclusion levels of yeast and feed conversion ratio of grower snails

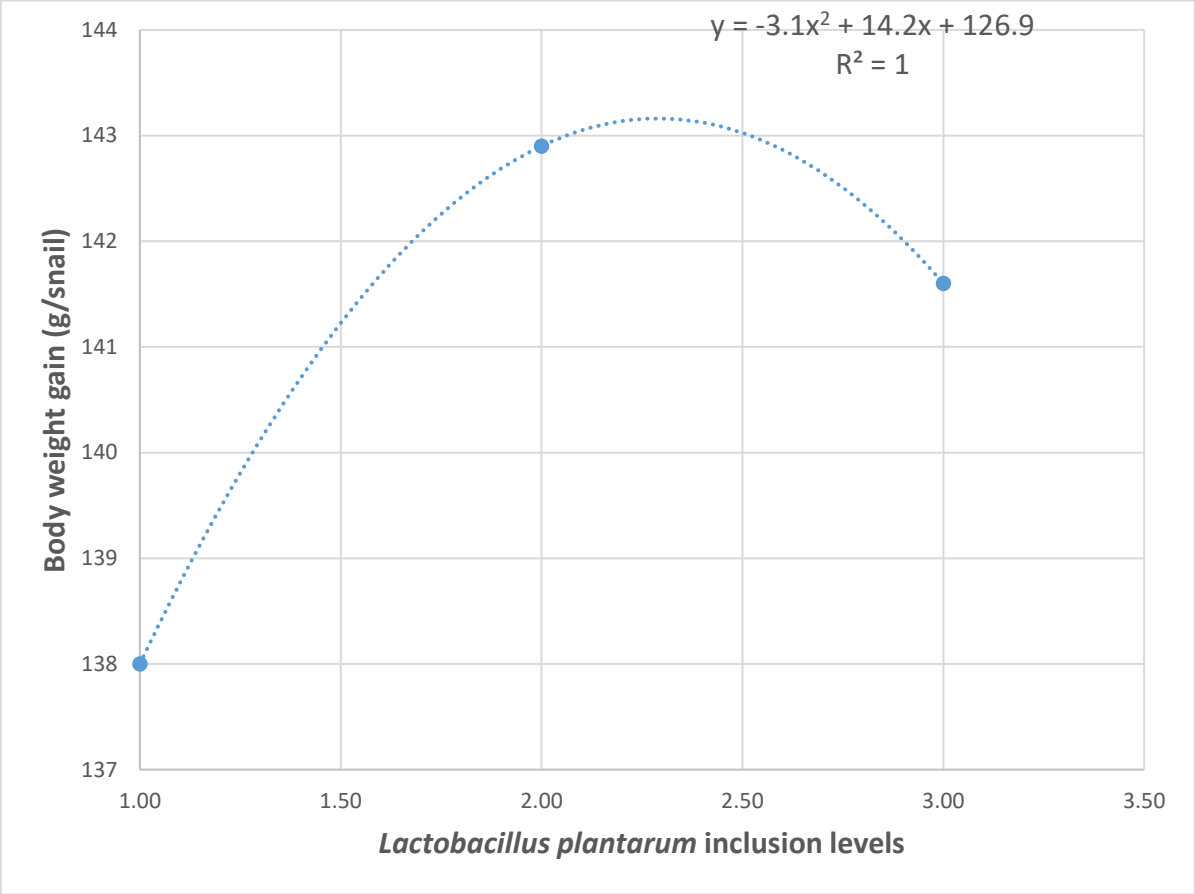


Figure 4.18: Correlation between body weight gain of grower AGLS and inclusion levels of Lactobacillus plantarum.

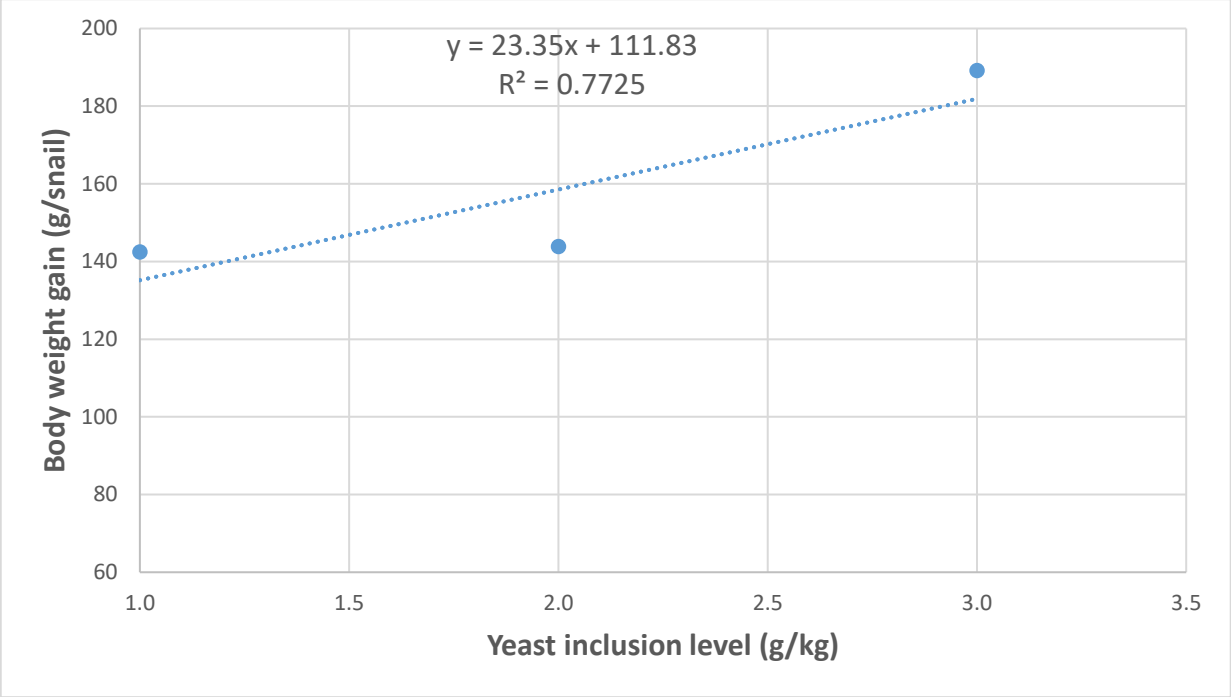


Figure 4.19: Correlation between body weight gain of grower AGLS and inclusion levels of *Saccharomyces cerevisae*.

4.5.2: Interaction Effect of probiotics and inclusion levels on performance of grower African giant land snail (*Archachatina marginata*) fed probiotics supplemented diets

Interaction effect of probiotics and inclusion levels on performance of African giant land snail was presented in Table 4.25. It was observed that the feed intake in D8 (13.28) was significantly (P<0.05) higher compared to D1 (6.72), D2 (9.97), D3 (10.26), D4 (11.49), D6 (9.44) and D7 (11.75), but did not differ (P>0.05) from D5 (12.66). The lowest (P<0.05) feed intake was observed in snails on D1 (6.72). Feed conversion ratio in D8 (3.40) was lower (P<0.05) compared to other dietary treatments. Treatment D5(8.80) had the highest (P<0.05) FCR value. Shell length of snails on D8 (10.22), was highest (P<0.05) compared to other dietary treatments, although not differ significantly (P<0.05) from D_4 (10.02), D5 (10.03) and D_6 (10.05). For the shell width of snails, D8 (6.03) was the highest value compared to other dietary treaments but not different (P<0.05) from D_6 (5.96). As for D_4 (5.85), D_5 (5.83) and D_7 (5.82), they were however, significantly (P<0.05) different from D_1 (5.69), D_2 (5.75) D3(5.67).

Regarding apartural length, treatment D_8 (6.66) had the highest (P<0.05) value compared to other dietary treatments but slightly different from treatment D_5 (6.61). Treatments D_6 (6.54) was not significantly (P>0.05) different from treatment D_7 (6.58). The least in value (6.35) was observed in treatment D_1. Generally, apartural length increased with increased dietary supplementation of probiotics. Considering the shell thickness (mm), the highest value (1.28) was obtained at treatment T_8 and was different (P<0.05) compared to other dietary treatments. Treatments D_5 (1.25), D_6 (1.24) and D_7 (1.26) did not differ (P>0.05) but differed (P<0.05) from treatments D_1 (1.19), D_3 (1.19), and D_4 (1.19), and D_4 (1.19) while the least value (1.15) was found in treatment D_2.

The outcome of the interaction effect of probiotics and inclusion levels on performance of AGLS showed that the inclusion of varying levels of probiotics significantly influenced the body weight gain (g/wk/snail). As the levels of the inclusion of probiotics increased, the body weight gain increased. The highest body weight ain (189.20) was observed in Treatment D_1 and significantly (P<0.05) h igher than other treatments. The least value (134.20) was recoreded against treatment D_1. The value of treatments D_2 (142.70), D_4 (142.90) and D_6 (142.50) did not differ (P>0.05) significantly.

Table 4.25: Interaction effect of Probiotics and Inclusion level on performance of grower African giant land snail (*Archachatina marginata*) fed probiotics supplemented diets

Treatment	Probiotics	Inclusion level	Feed Intake (g/wk/snail)	FCR ($x10^{-2}$)	Shell Length (cm)	Shell Width (cm)	Apartural Length (cm)	Shell thickness (cm)	Body weight gain (g/snail)
D1(NC)	0	0	6.72^{e}	5.00^{e}	9.80^{b}	5.69^{c}	6.35^{d}	1.19^{c}	134.20^{e}
D2(PC)	0	0	9.97^{d}	7.00^{cd}	9.85^{b}	5.75^{bc}	6.45^{c}	1.15^{d}	142.70^{c}
D3	Lab	1.0	10.26^{d}	7.40^{c}	9.50^{c}	5.67^{c}	6.42^{cd}	1.19^{c}	138.00^{d}
D4	Lab	2.0	11.49^{c}	8.00^{b}	10.02^{ab}	5.85^{b}	6.49^{bc}	1.19^{c}	142.90^{c}
D5	Lab	3.0	12.66^{ab}	8.80^{a}	10.03^{ab}	5.83^{b}	6.61^{ab}	1.25^{ab}	142.30^{c}
D6	Yeast	1.0	9.44^{d}	6.70^{d}	10.05^{ab}	5.96^{a}	6.54^{b}	1.24^{b}	142.50^{c}
D7	Yeast	2.0	11.75^{bc}	6.20^{cd}	9.95^{b}	5.82^{b}	6.58^{bc}	1.26^{ab}	143.90^{b}
D8	Yeast	3.0	13.28^{a}	3.40^{f}	10.22^{a}	6.03^{a}	6.66^{a}	1.28^{a}	189.20^{a}
SEM			0.34	0.35	0.08	0.04	0.04	0.02	85.34
P value			<0.0001	0.0001	<0.00001	0.45	0.45	0.004	0.41

^{a,b,c,d,e,f} Means of treatments along a column with dissimilar superscript varied considerablyly ($P<0.05$). FI – Feed intake, NC-Negative control, PC-positive control, FCR-Feed Conversion Ratio, SEM-Standard Error of Means.

4.5.3: Main effects of probiotics and inclusion levels on nutrient digestibility in grower African giant land snail (*Archachatina marginata*) fed diets supplemented with probiotics.

The main effects of probiotics and inclusion levels on nutrient digestibility in grower African giant land snail (*Archachatina marginata*) were presented in Table 4.26. Dry matter (%) digestibility of African giant land snail (AGLS) on Lactobacillus plantarum (Lacp)-supplemented diets (73.37) and *Saccharomyces cerevisiae* (Sac) supplemented diets (73.39) did not differ (P>0.05). The value for Sac was similar to that of Lacbacillus plantarum but for protein digestibility, the value (63.27) for Lactobacillus plantarum was higher (P<0.05) than that of the *Saccharomyces cerevisiae* (61.03), indicating that the digestibility of protein by Lactobacillus plantarum was better. The digestibility of fibre in both probiotics was very high. However, crude fibre digestibility (%) in African giant land snail on Lactobacillus plantarum-supplemented diets (80.49) was lower (P<0.05) than that of the Saccharomyces cerevisiae-supplemented diets (81.72). the ether extract digestibility in both probiotics were above average. The digestibility (%) in Lactobacillus plantarum-supplemented diets (65.59) was higher (P<0.05) than that of the *Saccharomyces cerevisiae*-supplemented diets (65.07).

For the main effect of inclusion levels of probiotics type, a 1% increase in inclusion level of the probiotics improved dry matter, crude protein, crude fibre and ether extract digestibility of grower AGLS. The dry matter digestibility obtained on 3.0% probiotics (73.88) was higher (P<0.05) compared to 2.0% (73.19) and 1.0% (73.08). However, the lowest dry matter digestibility (73.08) was observed in snails on 1.0% probiotics. Crude protein (%) digestibility value for 2.0% (62.96) was higher (P<0.05) than 1.0% (62.49) and 3.0% (61.01) probiotics. Crude fibre digestibility (%), for 3.0% probiotics (81.42) was significantly (P<0.05) higher than that of 2.0% (81.17) and 1.0% (80.73) which had the least value.

Generally, probiotics supplementation enhanced the digestibility of fibre at levels 1.0% to 3.0% probiotics. Ether extract digestibility (%) value for 3.0% (66.35) probiotics significantly (P<0.05) differed from 2.0% (66.16) and 1.0% (65.49).

Table 4.26: Main effects of probiotics and inclusion levels on nutrient digestibility in grower African giant land snail (*Archachatina marginata)* Fed probiotics supplemented diets.

Parameters (%)	Probiotics			Inclusion level (g/kg)		P values		Pooled SEM
	Lactobaccillus	Yeast	1.00	2.00	3.00	Probiotics	Level	
Dry matter	73.37[b]	73.39[a]	73.08[c]	73.19[b]	73.88[a]	0.001	<0.0001	0.01
Crude protein	63.27	61.03	62.46	62.46	61.01	<0.0001	<0.0001	0.01
Crude fibre	80.49[b]	81.72[a]	80.73[c]	81.17[b]	81.42[a]	<0.0001	<0.0001	0.01
Ether extract	65.59[c]	65.60	65.49	66.16[b]	66.35[a]	<0.0001	<0.0001	0.006

[a, b, c] Averagesof treatments along a row with dissimilar superscript varied considerably (P<0.05). SEM- Standard error of means.

P value - Probability

4.5.4: The Interaction Effect of Probiotics and Inclusion Level on Nutrient Digestibility in Grower African Giant Land Snail (*Archachatina Marginata*) fed diets Supplemented with Probiotics

The interaction effect of probiotics and inclusion on nutrient digestibility in African giant land snail was presented in Table 4.27. It was observed that dry matter digestibility in D_8 (73.93) was significantly ($P<0.05$) greater relative to D_1 (72.88), D_2 (73.17), D_3 (73.06), D_4 (73.23), D_5 (73.83), D_6 (73.10) and D_7 (73.15). The lowest ($P>0.05$) nutrient digestibility was observed in D_1 (72.88) (negative control). Between treatments D_6 (73.10) to D_8 (73.93) digestibility of dry matter increased as the level of *Saccharomyces cerevisiae* - supplementation in the diets increased. This implied that *Saccharomyces cerevisiae*-supplementation in the diets improved digestibility of its dry matter. The same trend was observed between D_3 (73.06) and D_5 (73.83) i.e digestibility of the dry matter increased with increasing supplementation levels of either *Lactobacillus plantarum* or *Saccharomyces cerevisiae*. However, this did not hold for the digestibility of protein. The highest value (66.25) was observed in D_1 (control) while the least value (60.36) was obtained in D_8. Increased diet-supplementation of probiotics decreased the protein digestibility by the snails. It was observed that crude fibre digestibility in D_8 (82.53) was higher ($P<0.05$) compared to D_1 (80.26), D_2 (80.70), D_3 (80.70), D_4 (80.46), D_5 (80.31), D_6 (80.76) and D_7 (81.88). The least value (80.26) was obtained in D_1. It could be stated that dietary supplementation of probiotics had positive influence ($P<0.05$) on the fibre digestibility. The ether extract followed the same trend as protein digestibility. Digestibility decreased as the levels of diet-supplementation with probiotics increased.

Table 4.27: Interaction effect of probiotics and inclusion levels on nutrient digestibility in grower African Giant Land Snail (*Archachatina marginata*) fed Probiotics Supplemented Diets

			Digestibility			
Treatment	**Probiotics**	**Inclusion level**	**Dry matter (%)**	**Crude Protein (%)**	**Crude Fibre (%)**	**Ether extract (%)**
D1(NC)	0	0	72.88^{h}	66.25^{a}	80.26^{c}	65.22^{a}
D2(PC)	0	0	73.17^{d}	66.49^{b}	80.70^{e}	65.25^{d}
D3	Lab	1.0	73.06^{g}	65.14^{c}	80.70^{e}	63.12^{b}
D4	Lab	2.0	73.23^{c}	63.01^{d}	80.46^{f}	64.10^{e}
D5	Lab	3.0	73.83^{b}	61.65^{f}	80.31^{g}	65.57^{c}
D6	Yeast	1.0	73.10^{f}	59.84^{h}	80.76^{d}	63.87^{f}
D7	Yeast	2.0	73.15^{e}	62.90^{e}	81.88^{b}	63.21^{g}
D8	Yeast	3.0	73.93^{a}	60.36^{g}	82.53^{a}	63.13^{h}
SEM			0.006	0.01	0.01	0.006
P value			<0.0001	<0.0001	<0.0001	<0.0001

$^{a, b, c, d, e, f, g}$ Averages of treatments along a column with dissimilar superscript varied sconsiderably (P<0.05). NC- Negative control, PC- positive control, SEM- Standard error of means.

4.5.5: Main effects of probiotics and inclusion level on Carcass characteristics of Grower African giant land snail (*Archachatina marginata*) fed probiotics-supplemented diets

The outcomes of the main effects of probiotics and inclusion level on carcass characteristics of grower African giant land snail (*Archachatina marginata*) were presented in Table 4.28. Live weight gain (g) of African giant land snail on Yeast-supplemented diet (119.78) significantly (P<0.05) differed from the *Lactobacillus plantarum*-supplemented diet (112.72). This indicated that Yeast was more effective as a growth enhancer than *Lactobacillus plantarum.* This study also revealed that probiotics supplementation in the diet of snail positively (P<0.05) influenced its performance (i.e carcass yield). Shell weight (%) observed in snail on Yeast-supplemented diet (24.18) was observed to be significantly (P<0.05) higher than that of the shell weight (%) (23.77) on *Lactobacillus plantarum*.

The foot weight (edible part) of African giant land snail on *Lactobacillus plantarum*-supplemented diet (20.74) was observed to be lower (P<0.05) than that of the foot weight of the snail on the Yeast-supplemented diet (27.49). The edible visceral portion (%) of African giant land snail fed *Lactobacillus plantarum*-supplemented diet (25.17) was significantly higher than that of the Yeast-supplemented diet (24.99). The dressing percentage (%) of grower African giant land snail on *Lactobacillus plantarum*-supplemented diet (45.79) was lower (P<0.05) than the dressing percentage of the snail on Yeast-supplemented diet (53.76), implying that yeast is more effective than Lactobacillus plantarum.

The main effect of inclusion of probiotic type on carcass yield of African giant land snail also revealed that live weight gain in snail on 3.0% probiotics (117.53) was higher (P<0.05) compared to 2.0% (115.62) and 1.0% (115.60). However, the values for live weight on 1.0% (115.60) and 2.0% (115.62) were not different statistically (P>0.05) from each other. Whole flesh (%) of snail on 3.0% (77.92) significantly (P<0.05) differed from that of snail on 2.0% (69.04), which was also different (P<0.05) from the snail on 1.0% (65.49) probiotics. For the foot weight, it was observed that snails on 3.0% probiotics (28.06) was higher (P<0.05) than those on 2.0% probiotics (21.44) and 1.0% probiotic (22.85). However, the lowest foot weight was observed in snails on 2.0% probiotics (21.44). For the edible visceral, the snails

on 2.0% probiotics (26.96) was higher ($P<0.05$) than either the snails on 1.0% probiotics (21.52) or the snails on 1.0% probiotics (26.77). The least value was observed in snails on 1.0% probiotic (21.52). The results of the dressing percentage revealed that snail on 3.0% probiotics (53.84) was higher ($P<0.05$) than that on 2.0% probiotics (50.37). The least value (45.12) was observed on snail on 1.0% probiotics. This meant that a 1.0% to 3.0% increase in probiotic inclusion levels would increase the carcass yield of the grower AGLS.

Table 4.28: Main Effects of Probiotics and Inclusion level on Carcass Characteristics of African Giant Land Snail (*Archachatina marginata*) Fed Probiotics Supplemented Diets.

	Probiotics		Inclusion Level (%)			P Values		
Parameters	**Lactobacillus**	**Yeast**	**1.0**	**2.0**	**3.0**	**Probiotics**	**Level**	**SEM**
Live weight (g)	112.72	119.78	115.60	115.62	117.53	0.06	0.86	3.59
Shell weight (%)	23.77	24.18	21.21	22.53	21.13	0.09	0.86	2.57
Whole Flesh(%)	67.8	73.76	65.49^{b}	69.04^{ab}	77.92^{a}	0.12	0.04	3.98
Foot weight (%)	20.74^{b}	27.49^{a}	22.85^{b}	24.44^{b}	28.06^{a}	0.003	0.03	2.33
Visceral hump (%)	45.44	46.53	41.47^{b}	47.59^{ab}	48.90^{a}	0.65	0.05	2.53
Edible visceral (%)	25.17	24.99	21.52	26.96	26.77	0.93	0.08	2.18
Offals weight (%)	20.55^{b}	24.13^{a}	22.01^{b}	19.42^{b}	25.59^{a}	0.02	0.01	1.62
Offal: live weight	18.39	21.02	19.07	17.89	22.15	0.14	0.14	1.85
Shell: live weight	21.24^{a}	16.24^{b}	18.37	19.87	17.99	0.04	0.77	2.39
Dressing percentage (%)	45.79^{b}	53.76^{a}	45.12^{b}	50.37^{ab}	53.84^{a}	0.01	0.03	2.77

$^{a, b, c, d, e, f}$ Averagesof treatments along a column with dissimilar superscript varied considerably ($P<0.05$). FI- Feed intake, NC- Negative control, PC- positive control, FCR-Feed Conversion Ratio, SEM- Standard error of means.

4.5.6: Interaction effect of probiotics and inclusion level on carcass characteristics of grower African giant land snail (*Archachatina marginata*) Fed probiotics supplemented diets.

Table 4.29 shows the effects of probiotic levels and interaction on grower African giant land snail (*Archachatina marginata*) carcass characteristics. It was observed that the live weight (g) in D_8 (125.07) was higher ($P<0.05$) compared to D_1 (105.27), D_2 (116.43), D_3 (112.97), D_4 (116.17), D_5 (119.03), D_6 (118.23), and D_7 (116.03). The lowest value was observed to be D_1 (165.27). The shell, weight (%) of the snail in D_8 (19.82) was higher ($P<0.05$) compared to D_7 (17.55) which in turn was lower ($P<0.05$) compared to D_6 (21.06), D_5 (24.71), D_4 (25.24), D_3 (21.36), D_2 (27.99) and D_2 (22.25 Treatment D8 (86.18%) was significantly (P0.05) higher than D7 (71.23), D6 (63.89), D5 (69.66), D4 (67.84), T3 (67.10), D2 (72.30), and D1 (68.18%) in the interaction effect of probiotics and inclusion levels on the whole flesh (percent). D6 had the lowest value, 63.89, while D8 had the highest value, 86.18.

D8 was found to have a significantly ($P<0.05$) higher foot weight percentage than D7 (26.31), D6 (25.51), D5 (25.46), D4 (17.58), D3 (20.19), D2 (17.81), and D1 (17.14). Treatment D1 had the lowest foot weight percentage. The snail's foot weight was influenced by the probiotics (p 0.05), according to the findings.

The visceral hump (percent) interaction effects of probiotics and inclusion levels. The visceral hump in D8 (52.98) was found to be significantly (P0.05) higher than that in D7 (46.55), D6 (40.07), D5 (44.82), D4 (43.63), D3 (42.87), and D1 (49.74), but it was not significantly higher than that in D2 (53.34) or D1 49.74. Treatment D6 produced the lowest value (40.07). The presence of the dietary probiotics incorporation didn't actually influence the instinctive protuberance.

Additionally, the dressing percentage in D8 (58.78 percent) was higher (P0.05) than in D7 (53.39 percent), D6 (48.90 percent), D5 (48.90 percent), D4 (47.14 percent), D3 (41.34%), D2 (45.23%), and D1 (50.74 percent). The most reduced esteem was found in D3 (41.34). The proportion of African giant land snails (*Archachatina marginata*) with dressings

significantly increased (P0.05) as a result of the interaction effect of probiotics and inclusion levels.

Figure 4.20 shows the correlation between varying inclusion levels of Lactobacillus plantarum and live weight of grower AGLS. A positive linear correlation exists between the independent variable (Lactobacillus plantarum) and the dependent variable (LW) of grower AGLS. Increasing levels of Lactobacillus plantarum resulted in increase in LW of grower AGLS. The R^2 value (0.99) indicated that about 99.9% of the observed changes in LW of grower AGLS were as a result of varying levels of Lactobacillus plantarum. A unit increase in Lactobacillus plantarum dosage results in a 3.03 g increase in LW of AGLS.

The correlation between varying inclusion levels of *Saccharomyces cerevisiae* and live weight of grower AGLS is presented in Figure 4.21. A polynomial correlation exists between the independent variable (*Saccharomyces cerevisiae*) and the dependent variable (LW) of the grower snails. The intercept is set at 131.67 when the independent variable is zero. The R^2 value (1.00) indicated that the observed differences in LW of grower AGLS were as a result of varying levels of *Saccharomyces cerevisiae.* A unit increase in *Saccharomyces cerevisiae* dosage results in a 13.44 g decrease in live weight, but subsequently increase above 2.00 g/kg.

Table 4.29: Interaction Effect of Probiotics and Inclusion level on Carcass Characteristics of Grower African Giant Land Snail (*Archachatina Marginata*) fed probiotics Supplemented Diets.

Treatments	Probiotics	Level	Live weight (g)	Shell weight (%)	Whole flesh (%)	Foot weight (g)	Visceral hump (%)	Edible visceral (%)	Offal weight (%)	Offal: LW	Shell: LW	Dressing percentage (%)
D1 (NC)	0	0	105.27^{c}	22.35^{ab}	68.18^{b}	17.14^{c}	49.74^{ab}	32.36^{a}	19.34^{c}	18.65^{b}	21.25^{ab}	50.74^{ab}
D2 (PC)	0	0	116.43^{b}	27.99^{a}	72.30^{b}	17.81^{c}	53.34^{a}	28.37^{b}	24.96^{ab}	21.79^{ab}	24.48^{a}	45.23^{bc}
D3	Lab	1.0	112.97^{bc}	21.36^{ab}	67.10^{b}	20.19^{b}	42.87^{cd}	21.48^{c}	21.39^{bc}	19.00^{ab}	19.19^{ab}	41.34^{c}
D4	Lab	2.0	116.17^{c}	25.24^{ab}	67.84^{b}	17.58^{c}	43.63^{b}	30.64^{a}	17.99^{c}	16.97^{b}	23.78^{ab}	47.14^{bc}
D5	Lab	3.0	119.03^{ab}	24.71^{ab}	69.66^{b}	25.46^{ab}	44.82^{cd}	23.39^{bc}	22.27^{bc}	19.19^{ab}	20.77^{b}	48.90^{b}
D6	Yeast	1.0	118.23^{ab}	21.06^{ab}	63.89^{b}	25.51^{ab}	40.07^{d}	21.56^{c}	22.63^{b}	19.14^{ab}	17.56^{bc}	48.90^{b}
D7	Yeast	2.0	116.03^{b}	17.55^{b}	71.23^{b}	26.31^{ab}	46.55^{c}	23.27^{bc}	20.85^{bc}	18.83^{b}	15.95^{bc}	53.59^{ab}
D8	Yeast	3.0	125.07^{a}	19.82^{ab}	86.18^{a}	30.67^{a}	52.98^{ab}	30.14^{ab}	28.90^{a}	25.10^{a}	15.22^{c}	58.78^{a}
SEM			3.59	2.57	3.98	2.33	2.53	2.18	1.62	1.85	2.39	2.77
P-value			0.02	0.18	0.04	0.003	0.02	0.01	0.01	0.15	0.11	0.02

abcd Averagesof treatments along a column with varying superscripts varied considerably ($P<0.05$).

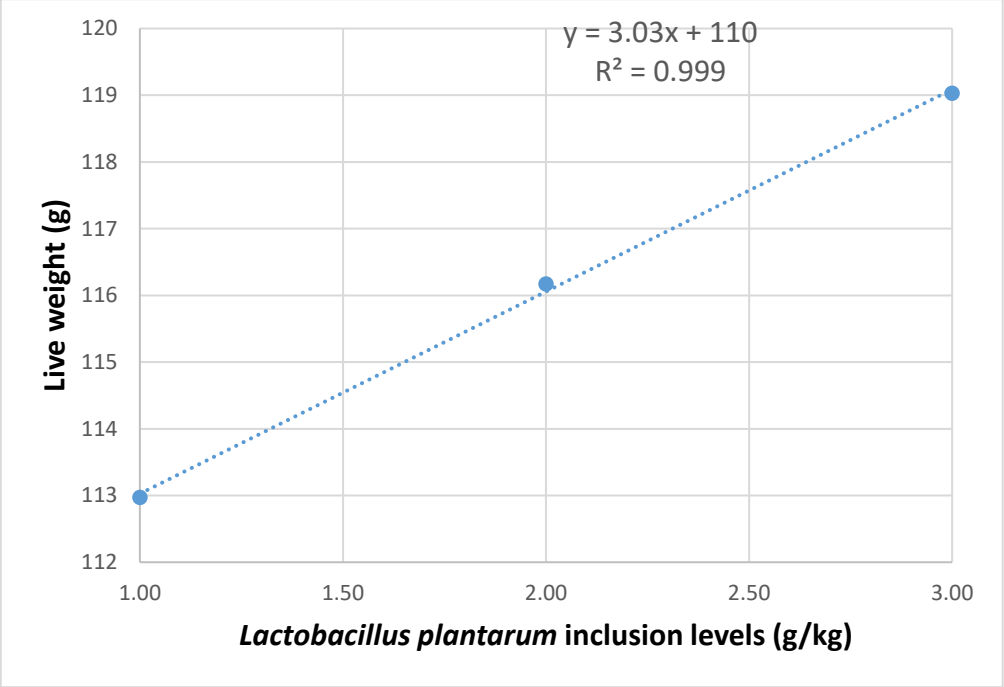


Figure 4.20: Correlation between Dietary Inclusion levels of *Lactobacillus plantarum* and live weight of grower African giant land snails

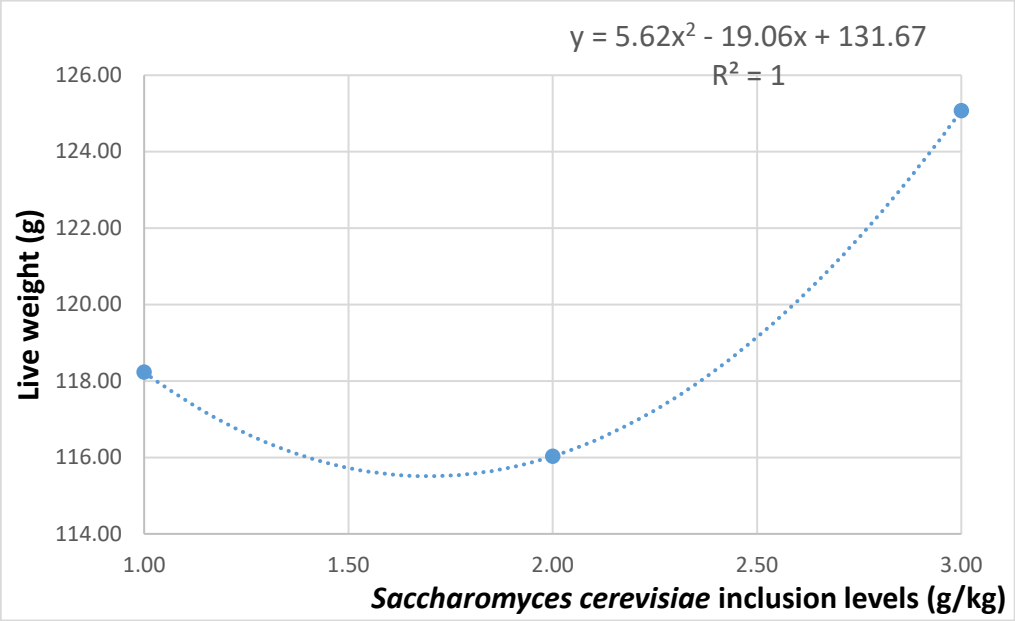


Figure 4.21: Correlation between dietary inclusion levels of *Saccharomyces cerevisiae* and live weight of grower snails

4.5.7 Interaction effect of probiotics and inclusion levels on gut sections of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with probiotics

The outcomes of the interaction effect of probiotics and inclusion levels on the gut sections of grower African giant land snail (*Archachatina marginata*) is presented in Table 4.30. It was observed that the gut length (cm) in D_8 (12.49) was significantly longer ($P<0.05$) compared to D_7 (11.79), D_6 (11.70), D_5 (11.52), D_4 (11.50), D_3 (11.11), D_2 (10.13) and D_1 (8.80). For the length of Oesophagus, it was observed the oesophagus's length (cm) in D_8 (4.07) was longer ($P<0.05$) compared to D_7 (3.85), D_6 (3.45) D_5 (3.70), D_4 (4.21), D_3 (3.48), and D_1 (2.80). However, D_8 (4.07) and D_2 (4.07) did not differ significantly ($P>0.05$). D_2 (4.07) was for the positive control (with antibiotic). The least value (2.80) was obtained in D_1, the negative control.

For the length of stomach (cm), it was observed that D_8 (1.94) was longer ($P<0.05$), D_4 (1.49), D_3 (1.47), D_2 (1.40) and D_1 (1.25). The least value (1.25) was observed in control treatment. The result took the same trend as that of the gut length. As the probiotics and inclusion levels increased, the length of the stomach and the gut length increased, indicating a positive influence of the probiotics added to the diets. The length of the intestine (cm) in D_8 (5.61) was longer ($P<0.05$) compared to D_7 (5.07), D_6 (5.24), D_5 (5.50), D_4 (5.25), D_3 (5.31), D_2 (4.80) and D_1 (3.50).

The interaction effect of probiotics and inclusion level on the length of hepatopancreas (cm) is revealed in Table 4.30. The length of the hepatopancreas in D_8 (5.58) was significantly ($P<0.05$) longer compared to D_7 (5.50), D_6 (4.88), D_5 (5.20), D_4 (5.00), D_3 (4.95), D_2 (5.00) and D_1 (4.20). However, D_4 (5.00), D_3 (4.88) and D_2 (5.00) did not differ ($P<0.05$) significantly. It could be seen from the results that probiotics and inclusion level positively influenced the size of the hepatopancreas. The least value was obtained from treatment D_1 (4.20). As the probiotics and the inclusion level increased, so the length of hepatopancreas increased ($P<0.05$), for the interaction effect of probiotics and inclusion level on the weight of hepatopancreas in African giant land snail growers, it was observed that the weight of hepatopancreas (g) in D_8 (5.75) was higher ($P<0.05$) compared to D_7 (4.70), D_6 (4.50), D_5

(5.70), D_4 (4.87), D_3 (5.20), D_2 (5.60), and D_1 (4.00). The lowest value was obtained in the control treatment D_1 (4.00).

The results revealed that the weight of the hepatopancreas increased as the probiotics and levels of inclusion in the diets increased. This was a positive influence on the snails.

Table 4.30: Interaction effect of probiotics and inclusion level on gut sections of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with probiotics

[a, b, c, d, e, f, g] Averagesof treatments along a column with dissimilar superscript varied considerably (P<0.05). NC- Negative

Treatment	Probiotics	Inclusion level	Gut Length (cm)	Length of Oesophagus (cm)	Lenth of Stomach (cm)	Length of Intestine (cm)	Length of hepatopancreas (cm)	Weight of hepatopancrease (g)
D1(NC)	0	0	8.80[g]	2.80[d]	1.25[g]	3.50[e]	4.20[d]	4.00[g]
D2(PC)	0	0	10.13[f]	4.07[a]	1.40[f]	4.80[d]	5.00[c]	5.60[b]
D3	Lab	1.0	11.11[e]	3.48[e]	1.47[e]	5.31[b]	4.95[c]	5.20[c]
D4	Lab	2.0	11.50[d]	3.51[c]	1.49[e]	5.25[b]	5.00[c]	4.87[d]
D5	Lab	3.0	11.52[d]	3.70[b]	1.80[d]	5.50[a]	5.20[b]	5.70[ab]
D6	Yeast	1.0	11.70[c]	3.45[e]	1.84[c]	5.24[b]	4.88[c]	4.50[f]
D7	Yeast	2.0	11.79[b]	3.85[b]	1.90[b]	5.07[c]	5.50[a]	4.70[e]
D8	Yeast	3.0	12.49[a]	4.07[a]	1.94[a]	5.61[a]	5.58[a]	5.75[a]
SEM			0.03	0.02	0.009	0.04	0.05	0.04
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

control, PC- positive control, SEM- Standard error of means.

4.5.8 The main effects of probiotics types and the inclusion levels on microbial load in the intestinal segments of African giant land snail.

The main effects of probiotics types and the inclusion levels on microbial load in the intestinal segments of African giant land snail were revealed in Table (4.31a) and (4.31b).

Oesophagus section:

Total bacteria count (TBC) observed in oesophagus section of AGLS on *Lactobacillus plantarum*-supplemented diet (3.31) was significantly (P<0.05) greater relative to Yeast-supplemented diet (2.50). However, enterobacterioceae count was lower (P<0.05) with *Lactobacillus plantarum*-supplementation (1.96) compared to Yeast-supplemented diet (2.63). Staphylococcus load observed in AGLS on *Lactobacillus plantarum*-supplemented diet (1.47) was lower (P<0.05) compared to Yeast-supplemented diet (1.83).

Salmonella/Shigella load observed in African giant land snail (AGLS) on *Lactobacillus plantarum*-supplemented diet (1.67) did not differ (P>0.05) compared to Yeast-supplemented diet (1.70). The population of salmonella/shigella was the same at the oesophagus section of the gut. *Lactobacillus spp* load observed in African giant land snail oeasophagus on *Lactobacillus plantarum*-supplemented diet (1.77) was higher (P<0.05) than that of the Yeast-supplemented diet (1.37). Total fungi count (TFC) observed in oesophagus section of AGLS on *Lactobacillus plantarum*-supplemented diet (1.40) was lower (P<0.05) than Yeast-supplemented diet (1.70).

Main effects of inclusion levels of probiotic type, as revealed in (Table 4.31a)1, showed that total bacteria count in oesophagus section on 3.0% probiotics (2.25) was lower (P<0.05) compared to 2.0% (2.47) and 1.0% (3.00). Enterobacteriae count on 1.0% of probiotics was higher (P<0.05) (2.55) than 2.0% (2.20) and 3.0% (2.13). Staphylococcus load on 2.0% probiotics was higher (1.75) compared to 1.0% (1.60) and 3.0% (1.60). The staphylococcus load on 1.0% (1.60) was similar to 3.0% (1.60). Salmonella/shigella count on 1.0% (2.00) was different (P<0.05) from 2.0% (1.55) and 3.0% probiotic levels (1.50). For *Lactobacillus,* 1.0% probiotics (2.10) significantly differed compared to 2.0% (0.85) and 3.0% (1.75) probiotics. A 1.0% - 3.0% increase in inclusion level of probiotics depressed the microbial load in oesophagus section of the GIT of African giant land snail.

Total Fungi Count (TFC) in oesophagus section of AGLS on 1.0% probiotics (3.55) differed (P<0.05) from 2.0% (1.10) and 3.0% (0.00). This implied that a 3.0% inclusion of probiotics reduced the fungi load to zero in the oesophagus section of African giant land snail (*Archachtina marginata*).

Stomach section:

Total bacteria count (TBC) observed in the stomach section of African giant land snail (AGLS) on *Lactobacillus plantarum*-supplemented diet (4.27) was significantly (P<0.05) greater relative to Yeast (3.81). Similarly, enterobacteriaceae count on *Lactobacillus plantarum*-supplemented diet (2.77) was higher (P<0.05) compared to Yeast-supplemented diet (2.50). However, Staphylococcus count was lower (P<0.05) with *Lactobacillus plantarum*-supplemented diet (1.61) compared to Yeast-supplemented diet (2.26). This showed that Lactobacillus plantarum was more effective in reducing staphylococcus in the stomach.

Salmonella/shigella load observed in African giant land snail on *Lactobacillus plantarum*-supplemented diet (2.70) was higher (P<0.05) compared to the Yeast-supplemented diet (2.20). For *Lactobacillus spp* load observed in the stomach of African giant land snail on *Lactobacillus plantarum*-supplemented diet (3.40) was higher (P<0.05) compared to Yeast-supplemented diet (1.47). This implied that *Lactobacillus plantarum* was more effective in adhering to the stomach mucosa.

Total Fungi count (TFC) in the stomach of African giant land snail on *Lactobacillus plantarum*-supplemented diet (4.04) was significantly lower (P<0.05) compared to Yeast-supplemented diet (4.32). This result also supported the fact that *Lactobacillus plantarum* was more effective in the stomach than yeast. Total bacteria count in the stomach of snail on 1.0% probiotics (5.15) was significantly (p<0.05) greater relative to 2.0% (3.27) and 3.0% (3.70). The least value (3.27) was observed at 2.0% probiotics. Enterobacteriaceae values in snail's stomach at 1.0% (2.80) and 2.0% (2.70) probiotics were similar (P>0.05) higher than 3.0% probiotics (2.40). Staphylococcus in snail's stomach on 2.0% (1.78) and 3.0% (1.88) were similar but significantly (P<0.05) lower compared to that on 1.0% (2.13) probiotics. Salmonella/shigella in snail's stomach on 1.0% (3.60) was higher (P<0.05) compared to 2.0% (2.75) and 3.0% (1.00).

Lactobacillus load increased as the percentage of probiotics in the diets increased. *Lactobacillus* in the snail's stomach on 2.0% (2.70) was higher (P<0.05) compared to 1.0% (2.15) and 3.0% (2.45). The total fungi count in the stomach section on 1.0% probiotic (5.55) was significantly (P<0.05) greater relative to 2.0% (3.50) and 3.0% (3.50) which were similar. A 2.0% to 3.0% probiotics inclusion levels decreased the total fungi load in snail's stomach.

Small intestine:

The total bacteria count (TBC) observed in the small intestine of African giant land snail (*Archachatina marginata*) on *Lactobacillus plantarum*-supplemented diet (2.20) was lower (P<0.05) compared to Yeast-supplemented diet (3.83). Similarly, Enterobacteriaceae on *Lactobacillus plantarum*-supplemented diet (2.07) was lower (P<0.05) than that of the yeast-supplemented diet (2.29). Same trend was observed on staphylococcus on *Lactobacillus*-supplemented diets (1.60) significantly reduced compared to that on the Yeast-supplemented diet (1.87). However, Salmonella/shigella in the small intestine of *Archachatina marginata* on *Lactobacillus plantarum*-supplemented diet (2.33) differed (P<0.05) from the Yeast-supplemented diet (1.50). *Lactobacillus* species on *Lactobacillus plantarum*-supplemented diet (2.73) was significantly (P<0.05) higher than that on Yeast-supplemented diet (1.33). for the total fungi count on *Lactobacillus plantarum*-supplemented diet (4.68) was observed to be significantly higher than that on Yeast-supplemented diet (2.00).

The main inclusion levels of probiotic type were presented in Table 4.31b. Total bacteria count (TBC) in the small intestine of snail on 1.0% probiotic inclusion (3.50) was significantly higher (P<0.05) compared to 2.0% (2.65) and 3.0% (2.60) which was the least value. Enterobacteriaceae in small intestine of snail on 1.0% (2.78) probiotic inclusion was higher (P<0.05) than that on 2.0% (2.30) and 3.0% (2.05). However, the highest value was observed on 1.0% (2.78). Enterobacteriaceae count decreased with the increase in percentage of probiotics in the diets. Staphylococcus on 1.0% probiotics inclusion (2.15) was significantly higher (P<0.05) than 2.0% probiotics (1.70) which in turn was higher (P<0.05) compared to 3.0% probiotics (1.05). This implied that the Staphylococcus load in the small intestine decreased as the percentage of the probiotics increased.

Salmonella/Shigella on 1.0% (2.50) probiotics inclusion level was significantly higher ($P<0.05$) compared to 2.0% (1.05) and 3.0% (1.00). The least value was recorded for 3.0% (1.00). Increased probiotics inclusion levels led to a decrease in the salmonella/shigella load in the small intestine.

For the *Lactobacillus* species on 1.0% probiotic inclusion, the value noted (1.75) was lower ($P<0.05$) than that of 2.0% (2.05), which in turn was lower ($P<0.05$) compared to 3.0% (2.30). The observation here was that, as the probiotic inclusion levels increased the *Lactobacillus* load increased. This is beneficial for the snail and this could result into better health status and improved snail performance. The total fungi count on 1.0% probiotic inclusion level observed was 5.02, which was higher ($P<0.05$) compared to 2.0% (2.00) and 3.0% (2.00) probiotic inclusion levels. It could be concluded that 1.0% to 3.0% probiotics inclusion level led to the reduced total fungi count along the gastrointestinal tract of the snail.

Large intestine: the total bacteria count (TBC) observed at the large intestine of the African giant land snail (*Archachatina marginata*) on *Lactobacillus plantarum* (Lacp)-supplemented diet (4.36) and Yeast-supplemented diet (3.93) differed significantly ($P<0.05$). The value was higher in *Lactobacillus plantarum*-supplemented diet (4.36). Enterobacteriaceae on *Lactobacillus*-supplemented diet (2.23) was significantly lower compared to the Yeast-supplemented diet (2.90). Staphylococcus in the large intestine on *Lactobacillus*-supplemented diet (1.17) was significantly ($P<0.05$) lower compared to the Yeast-supplemented diet (1.80) and for Salmonella/Shigella on *Lactobacillus*-supplemented diet (2.01) significantly ($P<0.05$) lower than that of the Yeast-supplemented diet (2.37). *Lactobacillus* species on the *Lactobacillus plantarum*-supplemented diet (3.11) was however, significantly ($P<0.05$) higher than that of the Yeast-supplemented diet (1.93). Considering the total fungi count (TFC) in the large intestine of the snail on *Lactobacillus*-supplemented diet (5.68), it was higher ($P<0.05$) compared to Yeast-supplemented diet (4.01).

Main effects of inclusion levels of probiotic type for the large intestine was on the same Table 4.31b. Total bacteria count observed in snail on 1.0% probiotics (5.20) was higher ($P<0.05$) than 2.0% (2.83) and 3.0% (2.40) probiotics but the least value (2.40) was obtained

at 3.0% probiotics inclusion level. Enterobacteriaceae value in snails on 1.0% (3.00) was significantly ($P<0.05$) higher than 2.0% probiotics level (2.60), which in turn was substantially higher ($P<0.05$) than that of the 3.0% (2.10) inclusion level. This implied that as the percentage inclusion levels of probiotic increased, enterobacteriaceae load decreased. Staphylococcus value in snails on 1.0% (1.65) was significantly higher ($P<0.05$) compared to 2.0% (1.40) which did not significantly differ ($P>0.05$) from 3.0% (1.40) inclusion level of probiotics. Same trend was observed as in enterobacteriaceae.

Salmonella/shigella value in snails on 1.0% (3.00) was higher significantly ($P>0.05$) than 2.0% (0.12) inclusion level of probiotics as well as 3.0% (0.05) probiotics inclusion ($P<0.05$). *Lactobacillus* species value in the snail's large intestine on 1.0% (2.17) was lower ($P<0.05$) than 2.0% (2.80) and 3.0% (2.60). For the total fungi count (TFC) in the large intestine of the snail on 1.0% (5.50) was higher ($P<0.05$) compared to 2.0% (5.02) and 3.0% (4.02). There is a gradual decrease in TFC as the percentage of probiotic inclusions increased.

Table 4.31a: Main effects of probiotics and inclusion levels on microbial load in intestinal segments of African giant land snail (*Archachatina marginata*)

Sections	Microbes (x 10^4CFU/g)	Probiotics		Inclusion level (g/kg)			P values		SEM
		Lactobacillus	Yeast	1.00	2.00	3.00	Probiotics	Level	
Oesophagus	TBC	3.31^{a}	2.50^{b}	3.00^{a}	2.47^{b}	2.25^{c}	<0.0001	<0.0001	0.06
	Enterobacteriaceae	1.96^{b}	2.63^{a}	2.55^{a}	2.20^{b}	2.13^{b}	<0.0001	<0.0001	0.05
	Staphylococcus	1.47^{b}	1.83^{a}	1.60^{b}	1.75^{a}	1.60^{b}	<0.0001	0.03	0.11
	Salmonella/shigella	1.67	1.70	2.00^{a}	1.55^{b}	1.50^{b}	0.53	<0.0001	0.05
	Lactobaccillus	1.77^{a}	1.37^{b}	2.10^{a}	0.85^{c}	1.75^{b}	<0.0001	<0.0001	0.05
	TFC	1.40^{b}	1.70^{a}	3.55^{a}	1.10^{b}	0.00^{c}	<0.0001	<0.0001	0.04
Stomach	TBC	4.27	3.81	5.15^{a}	3.27	3.70^{b}	0.42	0.04	0.57
	Enterobacteriacea	2.77^{a}	2.50^{b}	2.80^{a}	2.70^{a}	2.40^{b}	0.001	0.0003	0.07
	Staphylococcus	1.61^{b}	2.26^{a}	2.13^{a}	1.78^{b}	1.88^{b}	<0.0001	0.0004	0.11
	Salmonella/shigella	2.70^{a}	2.20^{b}	3.60^{a}	2.75^{b}	1.00^{c}	<0.0001	<0.0001	0.23
	Lactobaccillus	3.40^{a}	1.47^{b}	2.15^{c}	2.70^{a}	2.45^{b}	<0.0001	<0.0001	0.06
	TFC	4.04^{b}	4.32^{a}	5.55^{a}	3.50^{b}	3.50^{b}	0.0004	<0.0001	0.25

Table 4.31b: Main effects of probiotics and inclusion levels on microbial load in intestinal segments of African giant land snail (*Archachatina marginata*)

Sections	Microbes (x 10^4CFU/g)	Probiotics		Inclusion level (g/kg)			P values		SEM
		Lactobacillus	Yeast	1.00	2.00	3.00	Probiotics	Level	
Small intestine	TBC	2.20^{b}	3.83^{a}	3.50^{a}	2.65^{b}	2.60^{c}	<0.0001	<0.0001	0.06
	Enterobacteriacea	2.07^{b}	2.29^{a}	2.78^{a}	2.30^{b}	2.05^{c}	0.001	<0.0001	0.06
	Staphylococcus	1.60^{b}	1.87^{a}	2.15^{a}	1.70^{b}	1.05^{c}	<0.0001	<0.0001	0.06
	Salmonella/ shigella	2.33^{a}	1.50^{b}	2.50^{a}	1.05^{c}	1.00^{c}	<0.0001	<0.0001	0.44
	Lactobaccillus	2.73^{a}	1.33^{b}	1.75^{c}	2.05^{b}	2.30^{a}	<0.0001	<0.0001	0.30
	TFC	4.68^{a}	2.00^{b}	5.02^{a}	2.00^{c}	2.00^{b}	<0.0001	<0.0001	0.32
Large intestine	TBC	4.36^{a}	3.93^{b}	5.20^{a}	2.83^{c}	2.40^{b}	<0.0001	<0.0001	0.08
	Enterobacteriacea	2.23^{b}	2.90^{a}	3.00^{a}	2.60^{b}	2.10^{c}	<0.0001	<0.0001	0.06
	Staphylococcus	1.17^{b}	1.80^{a}	1.65^{a}	1.40^{b}	1.40^{b}	<0.0001	0.0012	0.09
	Salmonella/shigella	2.01^{b}	2.37^{a}	3.00^{a}	0.12^{b}	0.05^{a}	<0.0001	<0.0001	0.06
	Lactobaccillus	3.11^{a}	1.93^{b}	2.17^{c}	2.80^{a}	2.60^{b}	<0.0001	<0.0001	0.14
	TFC	5.68^{a}	4.01^{b}	5.50^{a}	5.02^{b}	4.02^{c}	<0.0001	<0.0001	0.47

$^{a, b, c, d, e, f, g}$ Averagesof treatments along a row with dissimilar superscript varied considerably (P<0.05). TBC-Total bacteria count, TFC-Total fungi count, SEM - Standard error of means, P-value - Probability.

4.5.9: The interaction effect of probiotics and inclusion levels of microbial load in the oesophagus of grower AGLS fed diets supplemented with probiotics.

The results of the interaction effect of probiotics and inclusion levels of microbial load in the oesophagus of grower African giant land snails is revealed in Table 4.32. It was observed that the total bacteria count ($x10^6$ cfu/g) in D_1 (4.20) was higher ($P<0.05$) compared to D_2 (1.60), D_3 (3.10), D_4 (2.43), D_6 (2.90), D_7 (2.50), and D_8 (2.10), but significantly lower compared to D_5 (4.40). The lowest value was found in D_2 (1.60), the positive control (with antibiotic). The total bactaria count (TBC) decreased with increased levels of *Saccharomyces cerevisiae*-supplemented diets, while there were fluctuations in the values of TBC on the Lactobacillus plantarum-supplemented diets. The variation in the values in this could be due to the microbial environment within the oesophagus flora.

For enterobacteriacea, the interaction effect of probiotics and inclusion levels revealed D_8 (1.90) to be significantly ($P<0.05$) lower compared to D_7 (3.00) and D_6 (3.00) (which were similar), D_5 (1.37), D_3 (2.10) and D_1 (2.87). The lowest value in D_2 (1.20) must have been attributed to the presence of antibiotic.

For staphylococus ($x10^4$ CFU/g), it was observed that staphylococcus in D_8 (1.50) was lower ($P<0.00$) compared to D_7 (2.00), D_6 (2.00), which were together similar in values but was higher ($P<0.05$) compared to D_5 (1.50), D_4 (1.50). Treatment D_8 (1.50) was also significantly higher ($P<0.05$) compared to D_3 (1.20), D_2 (1.43) and D_1 (1.10) interaction effect of probiotics and inclusion levels on salmonella/shigella ($x\ 10^4$CFU/g), it was observed that Salmonella/shigella were absent in D_2 (0.00), D_4 (0.00), D_5 (0.00), D_7 (0.00) and D_8 (0.00). The values in D_1 (2.00) and D_3 were similar ($P>0.05$) but together were higher than D_6 (1.10). The results reflected the effectiveness of probiotics in reducing the Salmonella/shigella load in the oesophagus of the grower AGLS, thereby enhancing the health status of the snails and in turn enhanced their growth.

The interaction effect of probiotics and inclusion levels on Lactobacillus species ($x\ 10^4$ cfu/g). It was observed that D_1 (1.30), D_2 (1.30) and D_6 (1.30) did not significantly differ but were significantly ($P<0.05$) lower compared to D_5 (2.40), D_7 (1.70). They were however higher ($P<0.05$) significantly than D_8 (1.10).

For the interaction effect of probiotics and inclusion levels of probiotics on the total fungi load in the oesophagus of grower AGLS, it was observed that there were zero fungi count on D_8 (0.00), D_5 (0.00), and D_2 (0.00), D_1 (2.00) was significantly ($P<0.05$) higher than D_3 (1.10), D_4 (1.10), D_6 (1.50) and D_7 (1.10). However, the value of fungi count in D_3 (1.10), D_4 (1.10) and D_7 (1.10) were similar ($P>0.05$).

Table 4.32: Interaction effect of varying inclusion levels of probiotics microbial load in the oesophagus of African giant land snails

Treatment	Probiotics	Inclusion level	TBC (x 10^6 CFU/g)	Enterobacteriacea (x 10^4 CFU/g)	Staphylococcus (x 10^4 CFU/g)	Salmonella/ shigella (x 10^4 CFU/g)	Lactobaccillus (x 10^4 CFU/g)	TFC (x 10^6 CFU/g)
D1 (NC)	0	0	4.20^{a}	2.87^{a}	1.10^{d}	2.00	1.30^{d}	2.00
D2 (PC)	0	0	1.60^{g}	1.20^{e}	1.43^{cd}	0.00^{d}	1.30^{d}	0.00^{d}
D3	Lab	1.0	3.10^{b}	2.10^{b}	1.20^{cd}	2.00	2.90^{a}	1.10^{b}
D4	Lab	2.0	2.43^{e}	1.40^{d}	1.50^{b}	0.00^{e}	3.00^{a}	1.10^{c}
D5	Lab	3.0	2.40^{e}	1.37^{c}	1.50^{b}	0.00^{b}	2.40^{b}	0.00^{d}
D6	Yeast	1.0	2.90^{c}	2.80	1.50^{a}	1.10^{a}	1.30^{d}	1.50
D7	Yeast	2.0	2.50^{d}	3.00^{a}	2.00^{a}	0.00^{f}	1.70^{c}	1.10^{c}
D8	Yeast	3.0	2.10^{f}	1.90^{b}	1.50^{b}	0.00^{e}	1.10^{e}	0.00^{d}
SEM			0.06	0.05	0.11	0.05	0.05	0.4
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

a, b, c, d, e, f, g, h Averagesof treatments along a column with dissimilar superscript varied considerably (P<0.05). TBC-Total bacteria count, TFC-Total fungi count. NC- Negative control, PC-positive control

4.5.10: The interaction effect of probiotics and inclusion levels of microbial load in the stomach of grower AGLS fed diets supplemented with probiotics

The results of the interaction effect of probiotics and the inclusion levels on microbial load in the stomach of AGLS (*Archchatina marginata*) are presented in Table 4.33. It was observed that the total bacteria count (x 10^6 cfu/g) in D_8 (2.80) was significantly lower ($P<0.05$) compared to D_7 (3.23), D_6 (5.40), D_5 (4.60), D_4 (3.30), D_3 (4.90) and D_1 (6.30). However, the lowest value was obtained in D_2 (2.10). The total bacteria count decreased with increased probiotic inclusion levels.

For the Enterobacteriaceae, it was observed that D_8 (1.70) was significantly ($P<0.05$) lower compared to D_7 (3.00), D_6 (2.80), D_5 (3.10), (D_4 (2.40), D_3 (2.80), D_2 (2.90) and D_1 (4.40), the highest value was obtained in D_1 (4.40). this was in no doubt due to the effectiveness of the probiotics in displacing the pathogens in the gastro intestinal tract of the snails. It was observed that staphylococcus load in D_8 (1.97) was lower ($P<0.05$) compared to D_7 (2.17), D_6 (2.63), D_2 (2.67), and D_1 (2.90). However, D_8 (1.97) was significantly ($P<0.05$) higher than D_5 (1.80), D_4 (1.40) and D_3 (1.63).

Salmonella/shigella in D_8 (1.00) was significantly ($P<0.05$) lower compared to D_7 (1.50), D_6 (5.10), D_5 (2.00), D_4 (4.00), D_3 (2.10), D_2 (2.50), and D_1 (7.00). The lowest value in all the treatments was in D_8 (0.00), while the highest value was obtained in D_1 (7.00) (control treatment).

For the Lactobacillus species, it was observed that Lactobacillus plantarum load in D_1 (1.80) was significantly lower ($P<0.05$) compared to D_2 (2.80), D_3 (3.10), D_4 (3.60), D_5 (3.50) but higher than D_6 (1.70), but was however similar to D_7 (1.80).

For the total fungi count (TFC), the interaction and inclusion levels of probtiotics on TFC showed that in D_8 (3.97) was similar to D_7 (4.00) but lower ($P<0.05$) compared to D_6 (7.00), D_4 (5.00), D_3 (4.10), D_1 (4.10) but significantly higher ($P<0.05$) compared to D_2 (3.37).

Concerning the total fungi count, the effects of inclusion of probiotics on the fungi showed that the total fungi count in D_8 (2.00) was lower ($P<0.05$) compared to D_7 (4.03), D_6 (6.00), D_5 (6.03), D_4 (6.00), D_3 (5.00), D_2 (3.33) but did not significantly differ ($P>0.05$) from D_1 (2.10). The total fungi cunt in the stomach was higher than any other section of the gastrointestinal tract of AGLS.

Table 4.33: Interaction effect of probiotics and varying inclusion levels on microbial load in the stomach of African giant land snails (*Archachatina marginata*) fed diets supplemented with probiotics

Treatment	Probiotics	Inclusion level	TBC (x 10^6 CFU/g)	Enterobacteriacea (x 10^4 CFU/g)	Staphylococcus (x 10^4 CFU/g)	Salmonella/ shigella (x 10^4 CFU/g)	Lactobaccillus (x 10^4 CFU/g)	TFC (x 10^6 CFU/g)
D1(NC)	0	0	6.30^{a}	4.40^{a}	2.90^{a}	7.00^{a}	1.80^{d}	4.10^{c}
D2(PC)	0	0	2.10^{d}	2.90^{bc}	2.67^{a}	2.50^{d}	2.80^{c}	3.37^{cd}
D3	Lab	1.0	4.90^{ab}	2.80^{c}	1.63^{cd}	4.10^{c}	3.10^{b}	4.10^{c}
D4	Lab	2.0	3.30^{c}	2.40^{d}	1.40^{d}	4.00^{c}	3.60^{a}	5.00^{b}
D5	Lab	3.0	4.60^{b}	3.10^{b}	1.80^{c}	2.00^{e}	3.50^{a}	3.03^{d}
D6	Yeast	1.0	5.40^{a}	2.80^{c}	2.63^{a}	5.10^{b}	1.70^{e}	7.00^{a}
D7	Yeast	2.0	3.23^{cd}	3.00^{bc}	2.17^{b}	1.50^{e}	1.80^{d}	4.00^{e}
D8	Yeast	3.0	2.80^{cd}	1.70^{e}	1.97^{bc}	0.00^{f}	1.91^{d}	3.97^{c}
SEM			0.57	0.07	0.11	0.23	0.06	0.25
P value			0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a, b, c, d, e, f}$Averagesof treatments along a column with dissimilar superscript varied sconsiderably (P<0.05). TBC-Total bacteria count, TFC-Total fungi count. NC- Negative control, PC-positive control.

4.5.11: The interaction effects of the probiotics and varying inclusion levels on microbial load in the small intestine of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with probiotics

The interaction effects of the probiotics and varying inclusion levels on microbial load in the small intestine of grower African giant land snail are presented in Table 4.34. It was observed that TBC in D_8 (3.00) was significantly ($P<0.05$) lower compared to D_7 (3.20), D_6 (5.30), but was higher ($P<0.05$) compared to D_5 (2.80), D_4 (2.10), D_3 (1.70), D_2 (1.23). However, D_1 (6.10) (control treatment) was the highest value. The results showed that the TBC decreased with increased levels of each probiotic. This implied that the probiotics significantly influenced microbial load to depress them in the GIT of AGLS. It was observed entcrobactericeae load in iD_8 (2.10) was lower ($p<0.05$) compared to D_7 (2.60), D_6 (2.17), D_5 (2.80), D_2= (2.60), and D_1 (3.10). However, D_8 (2.10) did not differ significantly ($P<0.05$) from D_4 (2.00). Generally, increased inclusion levels of probtiotics resulted in decreased microbial (enterobactericeae) load. This is beneficial to the host animals (snails).

For the interaction effects of probiotics inclusion levels on staphylococcus load, it was observed that D_8 (1.10) was significantly ($P<0.05$) lower compared to D_7 (2.10), D_6 (2.40), D_5 (1.60), D_4 (1.30), D_3 (1.90), D_2 (1.97) and D_1 (1.90). However, D_1 (1.90), D_2 (1.9), and D_3 (1.90) did not differ significantly ($P<0.05$). Interaction effect of probiotics and varying inclusion levels had effective beneficial influence on the hosts.

For the interaction effect of probiotics inclusion levels on salmonella/shigella load, ite was observed that treatment D_1 (4.00) was higher ($P<0.05$) compared to D_2 (3.07), D_3 (3.00), D_4 (1.00), D_5 (1.00), D_6 (2.00), D_7 (1.10) and D_8 (1.10) and D_8 (1.10). With increased probiotics levels of inclusion, there was a steady decrease in Salmonella/shigella load. The probiotics hence demonstrated their effectiveness at the snall intestine section of the gut as health enhancing agents and altimately good growth boosters. The interaction effects of probiotics and varying inclusion levels on microbial (Lactobacillus spp) load: it was observed that Lactobacillus in D_1 (1.10) was significantly lower ($P<0.05$) compared to D_2 (1.93), D_3 (2.40), D_4 (3.00), D_5 (2.80), and D_8 (1.80). However, D_1 (1.10) was not different ($P>0.05$) from D_6 (1.10), and D_7 (1.10). The results showed the evidence of the presence of

Lactobacillus spp in the small intestine of AGLS gastrointestinal tract and tended to increase with increased levels of probiotics dosage.

Regarding the interaction effects of probiotics and varying inclusion levels on total fungi count, it was observed that total fungi count (TFC) in D_1 (4.00), D_4 (4.00) D_5 (4.00) and D_6 (4.00) did not differ ($P>0.05$), but were significantly lower compared to D_2 (4.33) and D_3 (6.03). D_1 (4.00) was much greater than D_7 (0.00) and D_8 (0.00). The total fungi count (TFC) was higher ($P<0.05$) than that of the TBC in the small intestine of AGLS.

Table 4.34: Interaction effect of probiotics and varying inclusion levels on microbial load in the small intestine of grower African giant land snails (*Archachatina marginata*) fed diets supplemented with probiotics

Treatment	Probiotics	Inclusion level	TBC (x 10^6 CFU/g)	Enterobacteriacea (x 10^4 CFU/g)	Staphylococcus(x 10^4 CFU/g)	Salmonella/ shigella (x 10^4 CFU/g)	Lactobaccillus (x 10^4 CFU/g)	TFC (x 10^6 CFU/g)
D1(NC)	0	0	6.10^{a}	3.10^{a}	1.90^{b}	4.00^{a}	1.10^{d}	4.00^{b}
D2(PC)	0	0	1.23^{h}	2.60^{c}	1.97^{b}	3.07^{ab}	1.93^{c}	4.33^{b}
D3	Lab	1.0	1.70^{g}	2.40^{d}	1.90^{b}	3.00^{ab}	2.40^{b}	6.03^{a}
D4	Lab	2.0	2.10^{f}	2.00^{e}	1.30^{d}	1.00^{c}	3.00^{a}	4.00^{b}
D5	Lab	3.0	2.80^{e}	2.80^{b}	1.60^{c}	1.00^{c}	2.80^{ab}	4.00^{b}
D6	Yeast	1.0	5.30^{b}	2.17^{d}	2.40^{a}	2.00^{b}	1.10^{d}	4.00^{b}
D7	Yeast	2.0	3.20^{c}	2.60^{c}	2.10^{b}	1.10^{c}	1.10^{d}	0.00^{d}
D8	Yeast	3.0	3.00^{d}	2.10^{e}	1.10^{e}	1.10^{c}	1.80^{c}	0.00^{c}
SEM			0.06	0.06	0.06	0.44	0.30	0.32
P value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a, b, c, d, e, f, g, h}$ Averagesof treatments along a column with dissimilar superscript varied considerably (P<0.05). TBC-Total bacteria count; TFC-Total fungi count, NC- Negative control, PC-positive control.

4.5.12: The interaction effects of probiotic and varying inclusion levels on microbial load in the large intestine of grower African giant land snail (*Archachatina marginata*) fed diets supplemented with probiotics

The interaction effects of probiotics and varying inclusion levels on microbial load in the large intestine of African giant land snail are presented in Table 4.35. It was observed that the total bacterial count in D_8 (2.50) was lower compared to D_7 (3.70), treatments D_6 (6.30) and D_5 (6.30) were the same value. Enterobacteriaceae: It was observed that similar trend occurred as happened with TBC enterobacteriaceae load in D_8 (2.00) was significantly ($P<0.05$) lower compared to D_7 (3.30), D_6 (3.40), D_5 (2.20), D_3 (2.60) and D_1 (2.67). The lowest value was found in D_2 (1.30). In addition, increased probiotics inclusion levels resulted in a decreased microbial load (i.e enterobactericeae load) in the large intestine.

The interaction effects of probiotics and inclusion levels on the staphylococcus showed that staphylococcus in treatment D_8 (1.60) was significantly ($P<0.05$) lower compared to D_7 (1.70), D_6 (2.10) but the same was higher than D_5 (1.20), D_4 (1.10), D_3 (1.20). However, D_1 (2.50) was significantly ($P<0.05$) greater relative to D_2 (2.03).

Generally, along the treatments, increased probiotic levels of inclusion led to decreased microbial load. This meant that increased levels of probiotics inclusion depressed the staphylococcus load in the large intestine and a corresponding increase in positive bacteria colonization in the colon, and invariably enhance the growth performance of AGLS.

For salmonella/shigella, the results of the interaction effect of probiotics and inclusion levels showed that salmonella/shigella in D_8 (1.10) was significantly ($P<0.05$) lower compared to D_6 (6.00) and D_5 (0.00) had no salmonella/shigella. The lowest values were observed from D_7 (0.00) and D_3 (0.00). Increased inclusion of probiotics resulted in a decrease salmonella/shigella load in the large intestine.

For the interaction effect of probiotics and inclusion on Lactobacillus load in the large intestine of AGLS, it was observed that Lactobacillus in D_8 (2.10) was not significantly ($P>0.05$) different from D_1 (2.10), but was lower ($P<0.05$) compared to D_7 (2.20), D_5 (3.10), D_4 (3.40), D_3 (2.83) and D_2 (2.57). The inclusion of probiotics into the diets of AGLS positively ($P<0.05$) influenced the Lactobacillus load in the large intestine.

Table 4.35: Interaction effect of probiotics and varying inclusion levels on microbial load in the large intestine of grower African giant land snails (*Archachatina marginata*) fed diets supplemented with probiotics

Treatment	Probiotics	Inclusion level	TBC (x 10^6 CFU/g)	Enterobacteriacea (x 10^4 CFU/g)	Staphylococcus (x 10^4 CFU/g)	Salmonella/ shigella (x 10^4 CFU/g)	Lactobaccillus (x 10^4 CFU/g)	TFC (x 10^6 CFU/g)
D1 (NC)	0	0	3.70^{c}	2.67^{b}	2.50^{a}	2.10^{c}	2.10^{e}	2.10^{d}
D2 (PC)	0	0	1.70^{f}	1.30^{e}	2.03^{b}	1.70^{d}	2.57^{c}	3.33^{c}
D3	Lab	1.0	4.10^{b}	2.60^{b}	1.20^{d}	0.00^{f}	2.83^{bc}	5.00^{b}
D4	Lab	2.0	2.67^{e}	1.90^{d}	1.10^{d}	0.03^{e}	3.40^{a}	6.00^{a}
D5	Lab	3.0	2.30^{a}	1.20^{c}	1.10^{d}	0.00^{b}	3.10^{ab}	5.03^{b}
D6	Yeast	1.0	6.30^{a}	3.40^{a}	2.10^{b}	6.00^{a}	1.50^{f}	6.00^{a}
D7	Yeast	2.0	3.00^{d}	3.30^{a}	1.70^{c}	0.00^{f}	2.20^{de}	4.03^{bc}
D8	Yeast	3.0	2.50^{e}	2.00^{d}	1.60^{c}	0.10^{e}	2.10^{e}	2.00^{d}
SEM			0.08	0.06	0.09	0.06	0.14	0.47
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a, b, c, d, e, f, g, h}$ Averagesof treatments along a column with dissimilart superscript varied considerably (P<0.05). TBC-Total bacteria count, TFC-Total fungi count. NC- Negative control, PC-positive control

4.5.13: The effects of probiotics on the frequency of occurrence of selected bacteria species in grower African giant land snails (*Archachatina marginata*)

The effects of probiotics on the frequency of occurrence of selected bacteria species in grower African giant land snail is presented in Figure 4.22. It was observed that the highest frequency of occurrence of Bacillus subtillis was in snails on D_3 (19.50), compared to D_2 (17.50) and D_1 (13.00). Treatment D_1 (13.00) had the lowest frequency of occurrence of Bacillus subtilis.

Streptococcus species was lowest in AGLS on D_2 (3.00) compared to D_1 (9.00) and D_3 (10.00) Snails on D_2 (24.00) had the highest frequency of occurrence of staphylococcus aureus. However, snails on D_1 (4.50) revealed the occurrence of Escherichia coli but not present in D_2 and D_3. Occurrence of salmonella species has highest value in snails on D_3 (11.00, compated to D_2 (9.00) and D_1 (8.00). the lowest frequency of occurrence of salmonella species was found in D_1 (8.00). The highest frequency of occurrence of shigella species was observed in snails on D_2 (15.00) compared to other treatments. The lowest frequency of occurrence was observed in D_1 (6.00) compared to D_2 (6.50) and D_3 (15.00).

4.5.14: The effect of probiotics on the frequency of occurrence of selected fungi species in grower African giant land snail (*Archachatina marginata*)

The effect of probiotics on the frequency of occurrence of selected fungi species in grower African giant land snail is revealed in Figure 4.23. Although, there were slight difference in the frequencies of occurrence of Aspergillus Niger, it was observed that in snails on D_2 (14.00) has highest frequency of occurrence of Aspergillus Niger compared to D_1 (13.00) and D_3 (10.00). However, the highest frequency of occurrence of fungi species was in Aspergillus flavus with D_2 exhibiting the highest frequency of 40.00, but the snails on D_3 (10.00) had the lowest frequency of occurrence.

For Fasarium species, highest frequency was observed in snails on D_3 (28.00) compared to D_2 (21.50) and D_1 (17.00). For Aspergillus species, it was indicated that snails on D_2 had no observable occurrence of Aspergillus species, which we present in those of D_1 (9.50) and D_3 (4.0) but at lower frequencies compared to other fungi species. Similar frequencies of occurrence of penicillum species was observed in snails on dietary treatment D_2 (20.00)

and D_3 (20.00) but just a little difference from those on D_1 (21.00). The frequency of occurrence of Rhizopus species has highest value in snails on D_3 (19.00) compared to D_1 (9.50) and D_2 (7.50).

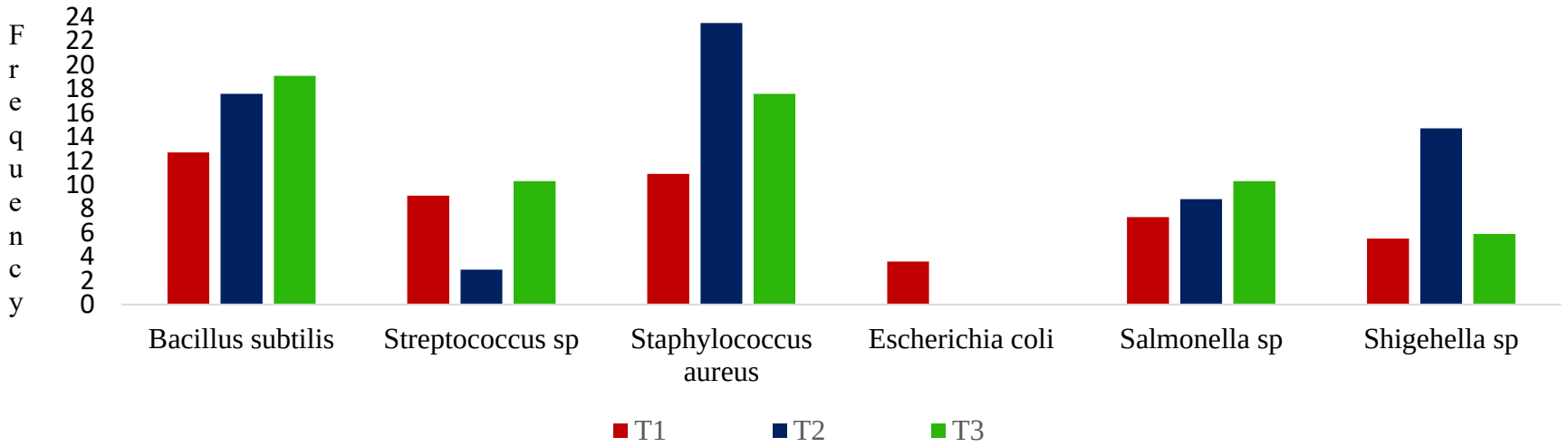


Figure 4.22: Effect of probiotic type on the frequency of occurrence of selected bacteria species in grower African giant land snails

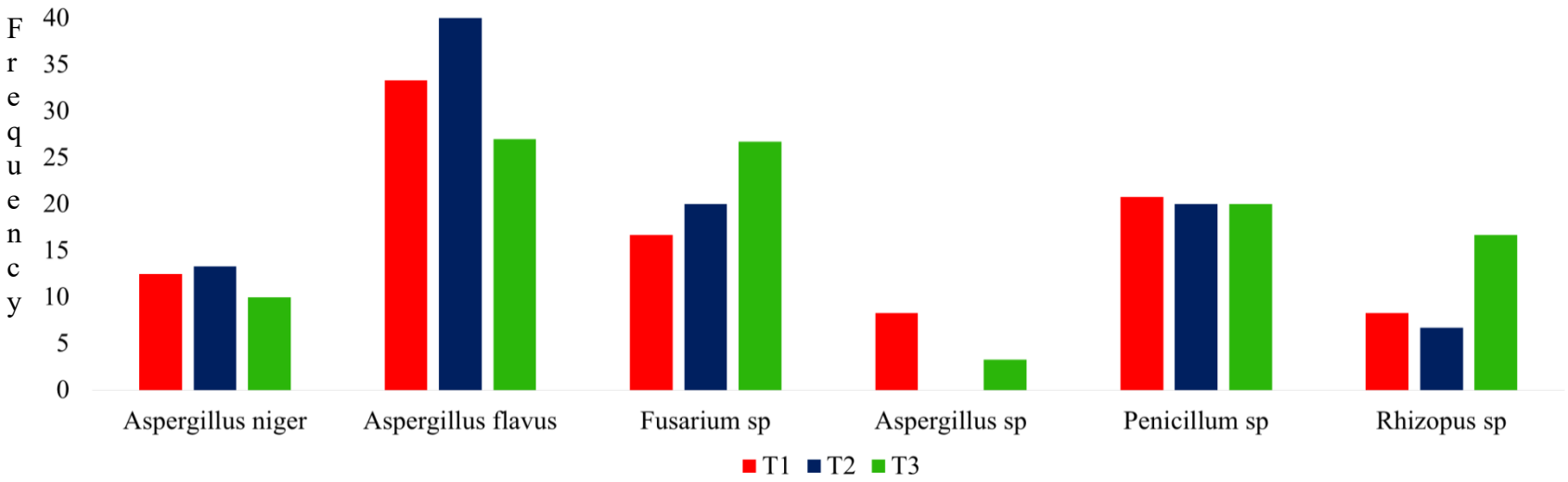


Figure 4.23: Effect of probiotics type on the frequency of occurrence of selected fungi species in grower African giant land snail.

CHAPTER FIVE

DISCUSSION

5.1 Chemical composition of the experimental feedstuffs offered to AGLS in experiment one.

The results showed that the protein content of the pawpaw leaves (control) has highest value, followed by coconut milt and unriped pawpaw fruit while pineapple fruit had the least protein content. The value of protein content of the pawpaw leaves being higher (31.26%) than that of the pawpaw fruit was similar to the report of Owosibo *et al.,* (2014) who indicated the protein content of pawpaw leaves as 22.3% and that of the fruits as 8.41%. Emelue and Donodawa (2017) in their report recorded the crude protein value for pawpaw leaves as 14.00%. Moreover, the results from this work for the pawpaw leaves was not too different from the earlier reports by Oyemuga (1963) who reported the protein content of pawpaw leaves to be 32.60%. Crude fibre in the feedstuffs in this work ranged from 10.50% (PAF) to 16.5% (SOF), this were rather high. High fibre could affect both the feed intake and digestibility of the feed. Fibre is a prime factor to consider in animal nutrition, especially monogastrics. Fibre consists of cellulose, hemicellulose, insoluble protein, lignin, gum, mucilage and bound nitrogen. Crude fibre also maintain micro-ecological balance of the gut, enhancing digestion. However, low level of fibre in the diet can result in diarrhea in animals (Fraga, 1990). Dietary Fibre (DF) was regarded as antinutritional factor because of its negative effects on feed intake (FI), and Nutrient Digestibility (ND) (Rajesh Jha *et al*, 2021). High fibre levels of the feed also tend to reduce the movement of indigested feed in the gut (increase the indigested feed in the digesta). Reduction in feed intake eventually results in reduced growth performance and animal production. Fraga (1990) in his report affirmed that high fibre level above tolerant level has adverse effect on absorption of calcium and phosphorus. As of today, there is scarce or no information regarding fibre nutrient requirement of snail, as available for other conventional livestock species. The observed low-fat contents of the feedstuffs might be of advantage to the snail

because it would result in low fat content of the snail meat. The lower the fat content the more preferable to the consumers.

Regarding the chemical analysis of the fibre fractions of the feedstuffs; Neutral detergent soluble (NDS), Neutral detergent Fibres (ADF), Acid detergent lignin (ADL), cellulose, and hemicellulose contents. Neutral detergent soluble (NDS): This is the soluble fraction of the cell contents. The values varied from pineapple fruit (PAF) having the highest value (9.61%) while coconut milk (CCM) had the least value (5.62%).

Neutral detergent fibre (NDF): NDF is a useful measure of the feeding value and commonly used to evaluate feed and formulate rations. Generally, low NDF values are preferable because NDF increases as forages mature (for ruminants). NDF is also used to calculate how much food an animal can retain. In the study, the NDF values differed across the treatments with the lowest and best value in treatment four (PAF) (23.79%). High NDF leads to a decrease in feed intake and reduced digestibility of feed in the animal. It was affirmed that plants with low NDF concentration are most of the time of great digestibility (Bakshi and Wadhawa, 2004).

Acid detergent fibre (ADF): ADF are important measurements used in forage food consumed by animals. ADF value can influence the feed intake as well as the digestibility of feed/diet. ADF value of a feedstuff is inversely proportional to the feed intake and nutrient digestibility in livestock (Babayemi *et al*, 2010). In table 4.2, the values of ADF differed significantly in the feedstuffs under study. The values were fairly high and this might have contributed to the poor digestibility of the feedstuffs (Babayemi *et al*, 2010): Both NDS and NDF precisely predict the proportion of digestible fractions found in the feedstuffs. (Soest *et al*, 1990) – (Van Soest) Acid Detergent Lignin (ADL). Lignin provides protective function, rigidity and structure to seeds, stems and vascular tissues of leaves and can be considered non-digestible component of the plant cell (Van Soest *et al*, 1982).

The values for ADL differed significantly among the different feedstuffs and were generally low. The ADF and ADL represent the most indigestible proportion of the feedstuffs. NDF-Land ADF will provide an estimate of hemicellulose content (Van Soest *et al*, 1982).

Hemicellulose: It is a complex polymer of xylose, arabinose, mannose, gluconic acid, galactose and some amount of other simple sugars like hexoses and pentoses. The values of hemicelluloses across the fruits used in this work were statistically the same.

Cellulose: Cellulose is the carbohydrate in plant. It is a polymer of glucose molecules in ± (1→ 4) linkages. It is a skeletal carbohydrate in plants. Cellulose enzyme is not secreted by mammals. The cellulose content across the foodstuffs differed significantly with the highest and least values observed in pawpaw fruit (14.78%) and sweet orange fruit (6.39%) respectively. Digestibility of cellulose varies with the amount of lignin, silica, and cutin (Van Soest, 1982).

5.1.1 Mineral composition of the experimental feedstuffs offered to grower African giant land snail (*Archachatina marginata*).

Minerals are synthetic components that can't be deteriorated nor incorporated by common compound responses. They are available in both plant and creatures to carry out unambiguous roles. The minerals that are known to play crucial roles and must be present in the diet or feed are called essential minerals. The dietary fundamental minerals are those that have shown by exploration to play fundamental metabolic parts in the body. The results of the mineral analysis of the feedstuffs fed to grower AGLS revealed that all the target elements assessed were present in different amounts. The mineral elements examined were Sulphur, Phosphorus, Calcium, Magnesium, Potassium, Sodium, Chlorides, Lead, Iron, Cobalt, Chromium and Zinc, which are among the dietary essential minerals. PPL had the highest values of Calcium (2.62%), Phosphorus (1.18%), Potassium (8.70%), Iron (109.00 mg/kg) and Zinc (44.60 mg/kg). Calcium (Ca^{2+}), and Phosphorus in particular are very important dietary essential mineral elements in shell formation in snails. They give rigidity, strength and shape to the structures of snails. Calcium is also an essential element in egg production and growth. Snail shell powder is about 95% calcium carbonate (Amusan, 2014).

The values of Calcium in the feedstuffs were all below the Calcium requirement for grower AGLS which is 4.5% as reported by Omole, (2003). However, PPL, PPF and SOS met the requirement for Phosphorus (0.64% P) according to Omole (2003), while PAF and CCM could not.

As at today, there is still scarcity of minerals requirement tables for different phases of snail growth. Low Calcium level (below the minimum requirement) could reduce the growth of snails.

Iron (Fe) was also present in large amounts in PPL and PPF compared to other feedstuffs. Iron deficiency is not common in snails, yet snail does not require iron as a respiratory pigment in snail.

Sodium is usually supplied to animals (snail) mainly in the form of sodium chloride (Nacl). The values of sodium across the feedstuffs, ranged from 0.12% to 0.30%. This range is adequate for snail growth. Omole (2003) reported a range of 0.1 to 0.2% for both hatchling and growers. Two major functions of Na^+ in animals are; regulation of osmotic pressure and maintenance of acid-base balance. Chloride (Cl^-) ion is also well distributed across the feedstuffs, ranged from 6.12% to 11.52%. Chlorine is als-o supplied to the snail in form of Nacl and the function of chlorine are regulation of osmotic pressure and acid-base equilibrium. All eleven basic dietary essential elements evaluated were present across the feedstuffs.

5.1.2 Qualitative phytochemical analysis of experimental feedstuffs offered to grower African giant land snail (*Archachatina marginata*).

Phytochemicals form a large class of the plants secondary metabolites thought to be responsible for much of the disease protection achieved by diets rich in fruits, vegetables, beans, cereals, and plant-base beverages such as tea and wine.

They are bioactive, non-nutritive compounds observed in plants that work with nutrients and dietary fibres to prevent or protect the body against disease (Tony *et al,* 2016). Phytochemicals can also affect the availability of nutrients required by the body and interfare with chemical (metabolic) process in such a way that growth and developing of the body is negatively affected (Singh *et al,* 1982).

The results revealed that tannins, phenol, glycosides, photo steroids, and flavonoids were present across the feedstuffs tested. Presence of antinutritional factors such as tannins and saponins might contribute to poor performance of snails.

5.1.3 Quantitative phytochemical analysis of experimental feedstuffs offered to grower African giant land snail (*Archachatina marginata*).

The quantitative phytochemical analysis of feedstuffs revealed that both saponins and alkaloids were largely present across all feedstuffs assessed, the rest (tannins, phenols, steroids, glycosides, photosteroids and flavonoids) were in trace amounts. Low level of tannins (toxicant) could contribute significantly to the health status of the snails. Saponin is an antinutritional factor, its presence could contribute to poor performance of the animal (snail). However, saponins had been implicated in many biological activities with potential health benefits such as hypochoterolemic, anti-coagulant, anti-carcinogenic, anti-inflamatory, hepato-protective, hypoglycemic, neuroprotective, immunomodulatory, anti-oxidant activity, inhibition of dental cavies and platelet aggregation (Rao and Gurfinked, 2000). Saponins are believed to possess deleterious properties such as cytotoxicity and permeabilisation of intestine (Price *et al*, 1987).

Moreover, Saponins are also known to inhibil Na^+ efflux by the blockage of the exist of Na^+ from the cells. This results in higher Na^+ concentration in the cells, thereby activating a Na^+ - Ca^{2+} antiporter in cardiac muscle. The increase in Ca^{2+} influx through this antiporter, strengthens the concentration of the heart muscle.

5.1.4 Growth performance characteristics of grower African giant land snail (*Archachatina marginata*) fed experimental feedstuffs.

Although the values for feed intake differed, these values were generally low. The low FI of the snails could be attributed to high fibre contents of the feedstuffs. High fibre feeds tend to reduce feed intake and increase digesta viscosity while reducing digestibility (Babayemi *et al*, 2010). It was opined by Abdelsamie *et al*, (1983) that high dietary fibre adversely affects growth rate and feed conversion ratio. Gidenne (2015) also reported that the essence of dietary fibre fractions in animal feeding is attributed to its impact on the rate of passage, mucosal functionality and its function as substrate for gut microbiota that relates to performance and digestive health, High fibre contents in the feed make it less preferred by non-ruminant animal such as pigs, poultry as well as snails. Since snails are known and preferred for having low fat and cholesterol, snails might have been having very low preference for fat – rich feeds, hence the rejection of coconut milt. It might be natural for snails to avoid excess fat which might make the snail to maintain the quality of its meat.

The body weight gain is a measure of the growth performance of the snails in response to the feed intake for the prescribed period. There were general depression in the body weight gain across the feedstuffs, which could be attributed to poor feed intake. Similar trend was recorded for FCR. FCR relates the feed intake to the body weight gain. The results from this work showed poor feed conversion ratio which was a direct reflection of the effect of the high fibre contents of the feedstuffs. These observations indicate that a minimum dietary fibre supply is important to prevent digestive challenges in the growing snail for improved growth performance. In addition, there is an urgent needs to determine the optimum fibre levels for every phase of snail growth because high dietary fibre has been reported to affect FI, BWG, digestibility and nutrient utilization in many animals. Treatment four (PAF) had the best FCR (1.11). Both the shell length and shell width were affected by the high fibre in the feedstuffs.

5.1.5 Nutrient digestibility in the experimental snail (dm) (*Archachatina marginata*) feds different feedstuffs.

The DM and NFE digestibility were high but CP, CF and EE digestibility were low. Low digestibility of CP, CF and EE must have been influenced by the high fibre content of the feedstuffs.

5.1.6 Carcass analysis of grower African giant land Snail (*Archachatina marginata*) fed experimental feedstuffs.

This analysis gives an insight into the snail meat yield. The carcass yield of snails differed significantly across the treatments but were generally low. The live weights were unexpectedly low. The foot weights (which represents the edible portion of the snails) were very low across the treatment groups. This must have been attributed to inefficient digestibility of feedstuffs, caused by the high fibre contents. The shell weight followed the same trend. The shell development also largely depends on the availability of calcium in both feed and soil. Since the calcium content of the feedstuffs were found to be low, it would have been one of the contributing factors. The presence of snails anywhere is usually an indication of available lime supply. Calcium is also a major ingredient for the formation of the epiphragm. The dressing percentage was on the average. The best dressing percentage (42.43%) was found in the PAF.

5.1.7 Proximate constituents of grower African giant land snail meat (*Archachatina marginata*) fed experimental feedstuffs.

The crude protein contents of the grower AGLS meat were relatively high, the values ranged from 16.72±0.01% to 20.26±0.01%. This can favourably be compared to the crude proteins of the meat of other common livestock. For example; Grass cutter (17.80 – 18.30 g /100 g CP); Goat (20.00 g /100 g CP); Pig (19.40 g /100 g CP); Chicken (21.00 g /100 g CP); Rabbit (14.00 – 15.00 g /100 g CP) as reported by Adu *et al* (2010), Popoola *et al* (2014) reported the protein content of grower AGLS meat that ranged from 19.21% to 20.26%. Ebemebe *et al*, (2014) working on fresh water snail (*Bulinus truncatus*) observed the protein content of its meat to be 21.35% and its fat content to be 1.88%. Malik *et al* (2011) reported the crude protein content of 20.35% for AGLS, 19.60% for beef, 20.10% for, rabbit and 18.30% for chicken meat.

The crude fat values for this current work were 0.68±0.1% to 0.98±0.01%. This low fat content and high protein content contribute to the nutritive quality of snail meat since the value of any meat of animal depends highly on its nutritional composition. It is this low fat content of the snail meat that makes it pharmacologically important for the treatment of hypertension according to Shobola (1986). The mineral contents of the snail meat are of fairly uniform values but low while the NFE (carbohydrate contents) were on the average.

5.2 Growth performance characteristics of AGLs hatchlings fed enzyme supplemented diets. No significant effect of *β-D-mannanase* was observed on feed intake of AGLS hatchlings. A similar trend were observed in final body weight (FBW), Total body weight gain (TBWG) and Feed conversion ratios (FCR) of AGLS hatchlings fed diets supplemented with *β-D-mannanase.*. Increasing dosage of *β-D-mannanase* significantly increased final and total body weight gain in a linear fashion, while improving FCR from 4.63 (H1) to 4.13 (H4). This result is in agreement with the report of Pettey *et al*, 2000 who evaluated the effects of *β-D-mannanase* addition to the diets of weanling pigs on growth performance. It was found that pigs fed diets supplemented with *β-D-mannanase* improved feed: gain ratio compared with pigs fed diets without *β-D-mannanase.* Observation from the present study suggests that *β-D-mannanase* improved growth performance and

ameliorate the efficiency of converting diets to weight gain in AGLS hatchlings. Another reports by Hahn *et al*, (1995) opined that pigs fed corn-SBM diets with *β-D-mannanase* grew faster and were more efficient than pigs fed simple corn-SBM diets implying that *β-D-mannanase* has proved to improve feed conversion ratio. Similarly, Chegeni *et al, (*2011) reported that dietary *β-D-mannanase* supplementation improved performance of broilers fed corn-soya bean meal during growing as well as whole period of the experiment but feed intake of the birds was unaffected by the enzyme.

However, the shell dimension: shell length and shell width were unaffected by the addition of *B-D-mannanse* to the diets. Since the development of shells in snails depends mainly on the level of calcium, this result could be attributed to the deficiency of calcium in the diets. On the cost analysis, there was a significant increase in the cost/g feed of the snails fed experimental diets with increasing enzyme inclusion. The implication is that it would lead to increase in the cost of production and ultimate rise in the market price of the snails. However, the cost/weight gain decreased as the level of *β-D-mannanase* dosage in the diets increased, demonstrating an inverse correlation. The lowest cost/weight gain was obtained in the diet containing 0.15 g *β-D-mannanase*/kg feed. Based on the cost/weight gain records. Therefore, it would be expedicant to advise the snail farmers to go for diets with inclusion level of 0.15 g *β-D-mannanase* feed to accelerate the growth of snails at this phase of growth.

5.2.1 Digestibility of AGLS hatchling diets supplemented with *β-D-mannanase*: Dry matter, crude protein and crude fibre digestibility increased as the levels of fibre degrading enzyme (*β-D-mannanase*) in the diets increased in a linear fashion. This demonstrates the efficacy of *β-D-mannanse* in breaking down the *glactomannan*, mannans and other fibres in the diets. These findings also agreed with reports from Mc Naughtem *et al* (1998) and Odetallah *et al*, (2002), that both indicated that the addition of *β-D-mannanase* to corn-soya bean diets enhanced performance of birds fed soya bean-based diets. However, there was no significant effect of *β-D-mannanase* on the digestibility of ether extract. This might be responsible for the usual observed low-fat content of the snail meat.

5.2.2 Carcass Characteristics of AGLS hatchlings fed diets supplemented with *β-D-mannanase*

The carcass charateristics of AGLS hatchlings fed diets supplemented with *β-D-mannanase* were positively influenced. The live weight and foot weight (The main edible portion of the snail) increased with increasing dosage of *β-D-mannanase*. The dressing percentage followed the same trend but the values of treatments H3 (50.16) and H4 (50.23) were statistically the same.

These observation could be attributed to the positive effect of the inclusion of *β-D-mannanase*. The positive performance exhibited by the AGLS hatchlings showed that the fibre degrading enzyme (*β-D-mannanase*) supplementation was very effective at the hatchling phase and could therefore be used to enhance the growth of the snail hatchlings. In conclusion, addition of fibre degrading enzymes to monogastric diets enhanced the digestibility of feeds, leading to better performance in animals and eventually better productivity and health status. Moreover, *β-D-mannanase* is effective in improving feed/weight gain performance of snails due to the degradation of *galactomannans, mannanas* and other fibres in corn-soya bean meal-based diets. Moreover, dietary *β-D-mannanase* supplementation improved performance of AGLS hatchlings fed corn-SBM-based diets.

5.3 Growth Performance Characteristics of grower AGLS fed enzyme supplemented diets. No significant effect of *β-D-mannanase* dosage was observed on feed intake of grower African giant land snails. However, the final weight (FW), total Body weight gain, (TBWG) and FCR improved with increasing dosage of the fibre degrading enzyme (*β-D-mannanase*). This could be attributed to the supplementation of exogenous enzyme, (*β-D-mannanase*) that had helped in breaking down *β-D-mannans,* galactomanans and other fibres present in the diets, preventing or depressing the formation of viscous digesta and thereby allowing the snails to utilize nutrients in the diets effectively. Yang *et al, (*2009), reported that addition of exogenous enzyme into a diet leads to reduction in the viscosity of intestinal digesta and subsequent increase in nutrient uptake and improved animal performance. The findings of this study are consistent with those of Leigh *et al.* (2000), who conducted research on grower AGLS fed guar meal diets supplemented with -

D-*mannanase*. They found that the inclusion of -D-*mannanase* reduced the viscosity of the intestinal digesta, which in turn increased feed efficiency and growth performance. According to Karimi and Zhandi (2014)'s research on poultry, adding -D-*mannanase* to a diet high in B-mannans may increase the concentration of beneficial intestinal bacteria, improve the digestibility of mannans, boost immunity, slow the growth of harmful intestinal bacteria, and improve the digestion and absorption of nutrients in the intestines. This study's findings were comparable to those of Pettey et al.'s (2000) study, which looked into how adding -D-*mannanase* to corn-soya meal diets affected growth, carcass traits, and apparent nutrient digestibility in growing-finishing pigs. Expansion of β-D-*mannanase* expanded the body weight gain and remains lean tissue contrasted with pigs took care of the control diet. This result also agrees with Hahn et al.'s findings from 1995, which stated that pigs fed diets with -D-*mannanase* exhibited consistent trends toward improved feed efficiency in comparison to pigs fed a diet similar to this one but without -D-*mannanase*. In one more preliminary examination led by Pettey et al, (1999), it was comparably seen that pigs took care of diets with β-D-*mannanase* were 4% more proficient in switching feed over completely to body weight gain than pigs not took care of β-D-*mannanase*.

This results have demonstrated the effectiveness of supplementation of *β-D-mannanase* to corn-soyabean-based snail diets in converting diets to weight gain.

The shell dimensions: shell length and shell width were not significantly affected by *β-D-mannanase* dosage. The possible reason is that the shell development in snail depends on availability of calcium and phosphorus in the environment.

Regarding cost analysis; the values of cost/g feed (₦) and cost/g body weight gain (₦/g) both increased at the increasing levels of *β-D-mannanase* in the diet increases. It is therefore not economical to include *β-D-mannanase* in the diets of the grower AGLS, and if it should be added, it has to be at a moderate level of 0.15 g *β-D-mannanase* per kg feed.

5.3.1 Digestibility of grower AGLS diets supplemented with *β-D-mannanase.*

Digestibility of DM, CP, CF and EE were significantly affected by increasing dosage of *β-D-mannanase*. This revealed the impact of the enzyme, *β-D-mannanase* in hydrolyzing the *mannans* and other fibres in the diets, permitting digestive enzymes access to substrates such as protein and starch and consequently improving the digestibility of nutrients (Fuente *et al*. 1995; Nian *et al*. 2011). Addition of *β-D-mannanase* to diets also led to the reduction

of viscosity of the digesta, increased nutrient uptake and improved animal performance (Yang *et al*. 2009) *β-D-mannanase* had therefore demonstrated its potential efficacy in enhancing performance of snails.

5.3.2 Carcass Characteristics of grower AGLS fed diets supplemented with *β-D-mannanase* with reference to the mean live weight (92.03 ± 0.98 g) at the beginning of the experiment compared to the live weight ranging from 367.50 g to 379.10 g within an experimental period of 98 days, one can conclude that *β-D-mannanase* had demonstrated its effectiveness and potential for promoting the growth of snails. The Live Weight (LW), and Foot Weight (FW) increased with the increasing dosage of *β-D-mannanase* the dressing percentage followed the same trend. The values increased with the increasing dosage of *β-D-mannanase*. This showed that *β-D-mannanase* used as the additive possessed potential beneficial effect on growth performance, health and nutrient utilization. It can therefore be concluded that addition of fibre degrading enzyme, *β-D-mannanase* to monogastric diets could enhance digestibility of feeds/diets, resulting in better animal performance.

5.4 Growth performance characteristics of grower AGLS in response to dietary supplementation of organic acid-prebiotic preparation. The feed intake (FI), feed conversion ratio (FCR), shell length, width, apartural length, and shell thickness, were not significantly affected by the dietary supplementation of prebiotic mixture. There was however, an indication that the FCR would be improved if the dosage of the prebiotic blend in the diets could be increased further. There was however an enhancement in the body weight gain of grower AGLS fed diets supplemented with prebiotic blend. The body weight gain increased with increasing dosage of the prebiotic mixture. The improvement in the body weight could be attributed to the synergistic effects of the components of the prebiotic mixture.

The positive control treatment (containing oxytetracyline) was affected favourably by the addition of antibiotic. The mode of action of the antibiotic may simply be explained by an inhibiting effect on certain intestinal bacteria that produce some toxins and competed with the hosts for available nutrient. It has already been known that administration of antibiotic to animal's feed from birth to slaughter may improve meat industry profits but it puts the

consumer's health at risk. This is because of the development of resistant strains and residual effects, knowing that man is the final consumer of eggs, meat and other animal products. The zeolite in the composite mixture, activated in the presence of moisture, ensures absorption of mycotoxins irreversibly (Boonchuvit, *et al*, 1975). The resultant mycotoxins-Zeolite complex leaves no residues in the animals products. The zeolite in the mixture selectively binds mycotoxin in the diets without binding vitamins and minerals. Thus, zeolotedetoxified the diets, effecting protection against mycotoxicoces (Boonchuvit, *et al*, 1975).

Mannanoligosaccharides (MOS), a non-digestible protein carbohydrate also supported the availability of nutrients used by the beneficial bacterial in the gut, accelerated the rapid growth of beneficial bacterial to provide improved defence against harmful bacteria. The MOS, in the composite mixture thus promotes the probiotic functions and supported a healthy immune system, natural defence against pathogens, a healthy gut flora, and maintenance and enhancement of gut health (Boochuvit *et al*, 1975). The propanoic acid in the mixture also help in inhibiting the development of mould in the diets, thereby preserving the quality of the diets for a long period. Moreover, it was indicated that organic acid helped to lower the pH of the gut, creating a condition that prevents the growth of the pathogenic organisms and at the same time accelerates the proliferation of the beneficial bacteria (Boonchuvit, *et al* 1975).

It should however be recalled that this prebiotic preparation inclusion to the diet resulted in non-significantly lower feed intake yet improved the body weight gain of grower AGLS. This was similar to the report of Bailey *et al* (1998) who used a combination of formaldehyde and zeolites in the diets of broilers. Non-significant feed intake was observed but improved body weight gain was recorded. The unexpected non-significant effect of the feed intake in this particular study might be explained by variations in gastro intestinal tract flora. Also, Miazazo *et al* (2000); Mutus *et al* (2006) and Rowghani (2007) similarly reported that probiotic alone had no significant effect on feed consumption but this was inconsistent to the findings of Yeo and Kim, (1997) who indicated that probiotic enhanced growth indicators in broiler chicks diets, significantly improved the daily body weight gain, feed intake and feed efficiency. Therefore, it would be noted that the possible reasons could be variables effects of biological addictives and/or the unexpected lowering effects or non-

significant effect on the feed intake in this particular study might be confounded by variations in gastro intestinal tract flora and environmental conditions (Mahdivi *et al*, 2005). The FCR was also slightly improved across the treatments. There was an indication that the FCR would be improved more if the amount of the prebiotic mixture in the diet could be increased further. The non-significant effect of the supplementation on the shell dimensions was in agreement with the report of Omole, (2003), who reported that the development of the snail shell did not depend on the constitution of the diets but on the amount of calcium in the environment.

5.4.1 Nutrient digestibility in grower AGLS diets supplemented with prebiotic preparation:

All the parameters measured were positively affected by the treatments. Even though there were flunctuations in the DM digestibility, it still increased with increasing dosage of the prebiotic blend. This could be attributed to the senergistic effects of the components of the prebiotic blends. Digestibility of E.E increased initially with increasing dosage of prebiotic blend and later after treatment three (M.3), started decreasing with increasing amount of prebiotic blend. This was a favourable observation because it would lead to reduction in the absorption and utilization of fatty acid and glycerol and subsequent lowering of the fat-content of the snail meat and invariably maintain its meat quality.

5.4.2 Carcass characteristics of grower AGLS fed diets supplemented with organic acid-prebiotic preparation: It should be noted that the animal growth traits and carcass criteria have great influence on the value of live animal as well as the retail meat value. The Live weights (LW) were affected by the treatments, compared to the negative control. The Live Weights increased with increasing dosage of the prebiotic blend. This could be attributed to effect of the prebiotic blend inclusion in the diets of the snails. The dressing percentage also improved with increasing amount of prebiotic blend in the diets. The higher the foot weight, the more the meat yield.

It is not only the foot portion of the snail that is edible. Some portions of the visceral hump are edible organs, including the muscles. These parts are referred to as edible visceral. These portions were also affected by the treatments. The dressing percentage also improved with increasing amounts of prebiotic blend. The observations could be attributed to the

senergistic effect of the components of prebiotic blend inclusion in the diets of grower AGLS.

5.4.3 Serum biochemical parameters for grower AGLS fed diets supplemented with prebiotic blend.

5.4.3.1 Serum total protein: Analysis of blood serum proteins is an important tool for monitoring health status of livestock. The serum total protein of grower AGLS were significantly influenced by increasing dietary supplementation of prebiotic blend. The serum total proteins were generally higher than that of both negative control (5.72/L) and the positive control (4.56 g/L). This could be attributed to the supplementation of the diets with prebiotic mixture. Moreover, the values observed for the total serum protein among the treatments were all above the mean serum total protein reported as 5.35± 0.55 g/L by Brockelman (1978). The values in this work was comparable to that of rabbit which was reported by Ogunbode *et al* (2016) in which the highest value was 7.33 g/dL while the least value was 4.45/dL. The normal range for total proteins according to Mitruka and Rawnsky (1977) was 5.4 – 7.5 g/dL for rabbit. Since the test diets gave a range of 5.72 – 8.78 g/dL, this suggested that the diets supplied to the snails had beneficial effects while elevated protein values translated to higher quality of feeds. Therefore, it could be stated that supplementation of the snail diets with prebiotic mixture possessed potential beneficial effects on the health and nutrient utilization.

5.4.3.2 Serum albumin: is a reliable predictor of good health status of a particular animal. A low albumin concentration is a sign of poor health (Gbore and Akele, 2010). The values obtained in this study varied from 2.30 to 3.12 g/L within the dietary treatment groups excluding the two control treatments whose values were both lower (i.e. 2.10 g/L and 1.24 g/L respectively) than the main treatments. In literature, two conflicting range values had been given for *Archachatina marginata* particularly Brockelman (1978) reported 3.82 ± 0.58 g/dL. While Maxwell (2017) reported 3.5 – 5.5 g/dL. It is therefore possible for the range to vary slightly in different laboratories and the physiological condition of the animals is a factor to be considered. It should be noted also that the blood/haemolymph constitution may vary with certain conditions such as stress, infections and toxicity etc. (Khan and Zafar, 2005). The ingestion of various dietary materials had also been reported by church *et al*, (1984) to have quantifiable results on blood/haemolymph constituents.

5.4.3.3 Globulin (g/L): The results showed that the supplementation of the basal diets with varying inclusion levels of prebiotic blend significantly affected the globulin concentration of the haemolymph of the grower AGLS. The values within the treatment groups ranged from 3.10 g/L to 5.66 g/L, the others, M1 (3.62 g/L) and M2 (3.32 g/L). Normal mean value for *Archachatina marginata* from Brockelman (1978) was 2.71 $\pm$ 0.34 g/dL. The values obtained from this study were above the range given by Brockelman (1978). Akinmutimi (2004) and Ogunbode *et al* (2016), reported that low levels of globulin reduced the ability of the animal to counteract diseases (i.e. break down immunity). The higher values of globulin in this work could be attributed to the supplementation of prebiotic blend.

5.4.3.4 Albumin – globumin ratio: The values were significantly influenced by the supplementation of the diets with prebiotic blend. The values ranged from 0.55 to 0.85. The values for the negative and positive control treatment were 0.58 (M1) and 0.37 (M2) respectively. High albumin/globulin ratio usually indicate that the animal is not suffering from the presence of any toxin (antigen) or pathogenic infection, which could have been the situation for any low albumen/globulin ratio. For this particular study the albumin/globulin ratio was significantly high compared to the control treatments. This must have been due to dietary supplementation of varying level of prebiotic blend. It also suggested a positive influence of the said additive and improved health status of the snails, i.e. the snails had no health challenge.

5.4.3.5 Creatinine and blood urea nitrogen (BUN): The amount of creatinne and blood urea nitrogen (BUN) in the blood/heamolymph is often used as renal function test (Duncan *et al*, 1994). In this study, it was not possible to carry out the BUN analysis but the observation on the blood creatinine indicated that this parameter was influenced. The value for the positive control was the least in all the treatments. The values for the main treatments were slightly higher than that of the negative control. The value ranged from 56.00 to 68.00 mg/dL. The value of creatinine for healthy snail (*Archachatina marginata*) given by Brockelman (1978) was 0.83$\pm$ 0.01 μmol/L. The values from this study were not too far from that of Brockelman. However, there is presently no universally accepted standards for all biochemical parameters for snails.

5.4.3.6 Alkaline phosphates (ALP): The values of the ALP were significantly influenced by the inclusion of varying levels of prebiotic blend. The values first rose to 80.00 (U/L)

and then decreased with increasing dosage of prebiotic blend. The values were all higher than any of either negative (42.00 U/L) or positive (40.00 U/L) control treatment. These result seemed to be consistent with the results in literature in other animals. The decrease in ALP observed were suggested to be indications of the safety the feed can provide for the animals (Leigh *et al*, 2010).

Moreover, the results in this study was similar to the reports of Kayode *et al* (2014) whose value of ALP were from 4.3×10^4 to 6.4×10^4 μmol/L for *Archachatina marginata*. According to Kayode *et al* (2014), the high level of ALP in the heamolymph could be due to the fact that ALP is a poly functional enzyme which hydrolyses a broad group of phosphomonoester substrates and acts as an early indicator of cell differentiation in the ostegenic lineage in bivalve mollusk. ALP in serum and haemocytes are important than any other enzymes in immune defense.

5.4.3.7 Aspartate aminotransferase (AST): The values were generally high, ranged from 60.00 to 92.00U/L, while the control treatments were 56.00U/L (M_1) and 54.00U/L (M_2) respectively. The trend was similar to that of the ALP. They gradually decreased with increasingly dosage of the prebiotic blend AST and ALT are known as cytosolic marker enzymes and are used as indicators for hepatic damage. The significant increase in the activities of these enzymes might be due to increased permeability of membrane or leakage of cytosol into the serum. The value noted in this work were unexpectedly high. However, as at now there are scarcity of information about serum biochemical indicators in snails and the liver functions. More comprehensive studies are still required on biochemical parameters on the haemolymph of African giant land snails.

5.4.3.8 Alamine aminotransferase (ALT): In current study, ALT values were also influenced by the supplementation of prebiotic mixture. The result followed the same trend with the AST. As the amount of prebiotic mixture increased the values of ALT increased and shortly after decreasing slightly with further increasing dosage of the prebiotic blend. This seemed to be an inconsistent result compared to what happened in vertebrate animals. ALT is a liver specific enzyme, its analysis is usually a sensitive and reliable test applied to detect the presence of hepatic necrosis both mild and chronic. ALT transfers the amino group of alamine to ketoglutamic acid, to form glutamic acid. Liver tissue in vertebrate is always rich in both ALT and AST but it contains more ALT than AST. Even though both

ALT and AST are elevated in sera of animals with acute hepatic disease, ALT, which is usually only slightly elevated by cardiac necrosis, is therefore a more specific indicator of liver damage (Rodwell, 1979).

5.4.3.9 Glucose: Glucose is usually a quick source of energy to the body tissues. It is one of the most important blood constituents. A continual supply of glucose is necessary as a source of energy, especially for the nervous system and erythrocytes. Generally animals glucose is also needed in adiopose tissue as a source of glyceride-glycerol and it was suggested to be playing an intermediate role in citric cycle in many tissues. The values of glucose in the haemolymph of snails in this study were increased with increasing dosage of prebiotic mixture. The values ranged from 66.00 to 96.00% while those of control treatments were 70.00% (M_1) and 66.00% (M_2). The values of glucose in this study were enough to supply the energy required for all metabolic activities of the snails.

5.4.4 Haematological Parameters

Haematological parameters are an index and refelction of the effect of dietary treatments on the animals. The results of current study indicated that the diets supplied were valueabl e for the snails. The Packed cell volume (PCV) is a function of erythrocytes (red blood cells) size and number of cells per volume of blood/haemolymph. So a fall in PCV below the minimum normal range is an indication of the existence of anemia, while haemoconcentration results when PCV exceeds the maximum of blood normal range. In this study the PCV values for the test ingredients besides the controls, ranged from 38.04 to 54.05%. This range showed that the snails were in good health status. Badawi and Al-Hadith, (2004) reported a range of 28.00-45.00% with an average of 34.90% for sheep while Mitruka and Rawsley (1977) gave a range of 29.90-33.60% with an average of 31.76% for male sheep. Ogunbode *et al*., (2016) evaluated the haemolymph of weaner rabbit fed graded levels of fermented-boiled ackee apple seed meal observed the highest PCV value of 30.14% while the least value was 25.00%. Therefore, the range value obtained in this work is favourable and portrayed a good health status of the grower AGLS.

5.4.4.1 White Blood Cells (WBC): White blood cells and neutrophils are responsible for defense and phagocytic activities of the body against invading foreign bodies (antigens).

The values of the WBC among the test ingredients ranged from $7.20x10^7$/UL to $11.20x10^7$/UL. The results in this study was comparable with the work of Ogunbode *et al.*, (2016) who assessed the WBC of weaner rabbits fed fermented seed meal. The highest value they obtained was $8.00x10^3$/UL while the least value noted was $5.2x10^3$/UL. The results of the WBC in this study showed that the WBC have the potentials to fight any invading toxins or pathogenic organisms and hence the animal could maintain good health condition. Since the function of the WBC is to provide the body of the animal with defenses against foreign bodies. The number of the WBC was affected by the levels of inclusion of the prebiotic blend. This would have also contributed to the increase in the body weight gain.

5.4.4.2 Erythrocyte sedimentation rate: Erythrocyte sedimentation rate (ESR) gives you the health status of an animal. If the rate is high, it is an indication that the internal organs are diseased. It is however better for ESR value to be normal. In current study, the ESR values were influenced by increasing amount of prebiotic blend in the diets. The values were generally low and decreased with increasing dosage of the prebiotic blend. This implied that the animals were healthy. The least value was observed in treatment M_7 (2.00 mm) compared to the negative control M_1 (8.00 mm). This could be attributed to the effectiveness of the prebiotic blend. It could also justify the reason why there was no mortality throughout the experimental period.

5.4.4.3 Haemolymph, haemocytes and functions

Heamolymph is the circulating fluid in the snails. It is the blood analogous that is found in snails. Haemolymph contains amoebocytes, the cells which are usually referred to as the haemocytes. The haemocytes are thought to have an important role in immune system in snail. The internal defense system in snails actually consists of both cellular and humoral components. The haemocytes are known to encapsulate the foreign bodies and kill or releasing cytotoxic compounds against the pathogens (or antigen or toxins). The first mode of action is referred to as phagocytosis (Farahnak *et al.,* 2004). The dyeing of haemolymph of *Archachatina marginata* with Wright Giemsa stain revealed two cell types.

5.4.4.4 Haemocyte type 1: Small in size, sub-spherical in shape, consists of small nucleus and little cytoplasm and uniformly basophilic. They were more predominant than the haemocyte type 2. (i.e much larger in population). The values increased significantly with increasing dosage of the prebiotic blend. Haemocyte type 1 had been reported to be the major principle for the snails internal defense system. That might probably be the reason for its predominant population far beyond that of haemocyte type 2. In this study, the numbers of haemocyte type 1 in each treatment was about twice the corresponding numbers of haemocytes type 2 in the same treatment.

5.4.4.5 Haemocyte type 2: It was larger in size, spherical in shape and nucleated and it was also uniformly basophilic. Its population was about half that of haemocyte type 1. Haemocyte type 2 cells do not take part in immune activities in snails. Farahnak *et al.*, (2004) working on Biomphalaria gibrata discovered four types of haemocytes and identified them as granocytes, plasmatocytes, oermocytoids and prohemocytes among which granulocytes were the main immune haemocytes that fight the invading foreign substances. In their findings, Abiona *et al.,* (2003) reported four circulating haemocyte types present in both *Archachatina marginata* and *Achatina achatina* snails. They indicated that *Archachatina marginata* had higher numbers of each type of haemocyte than *Achatina achatina.* In addition, they also stated that those four haemocyte types were morphologically different from one another in both species of African giant land snails. Sodipe *et al* (2014) in their study on Achatina achatina and *Archachatina marginata* reported that the haemocytes concentration of haemolymph of Achatina achatina was $8.16x10^6 \pm 1.465x10^6$ per mL. their report was silent about the haemocyte type.

5.4.5 Haemolymph serum lipid profile of grower AGLS fed diets supplemented with prebiotic blend.

5.4.5.1 Serum cholesterol: The results of the serum cholesterol were influenced by the inclusion of the prebiotic blend, at varying levels. However, there was a sharp rise in the amount of cholesterol i.e 85.00m g/dL in treatment M_3 with 0.50 g/kg prebiotic blend but decreased with increasing dosage of prebiotic blend in the diets given the least cholesterol level of 62.00 m g/dL at 2.00 g/kg prebiotic blend level. This suggested that the cholesterol level of the basal diet could be maintained with 2.00 g/kg prebiotic inclusion level. High

serum cholesterol level may have negative effect on the animal. There could be possibility of lowering the cholesterol level by further increasing the dosage of prebiotic blend. Lower level of cholesterol would increase the quality of the snail meat.

5.4.5.2 Triglycerides: Triglycerides are a type of fat in animal body. They are esters formed from glycerol and three molecules of fatty acids. They are the constituents of natural fats and oils. Their values across the treatments were significantly affected by the supplementation of prebiotic blend. The trend was similar to that of the serum cholesterol. There was a sharp rise at M_3 (75.00 mg/dL) with 0.5 g/kg prebiotic blend but decreased with increasing dosage of prebiotic blend resulting in the least value of triglycerides of 50.00 mg/dL observed at 2.00 g/kg prebiotic. Decreased triglycerides were beneficial to the snails as well as the consumers of snails. High triglycerides combined with high LDL cholesterol speeds up the build-up of plague in the arteries.

5.4.5.3 High-density liproprotein cholesterol (HDL): The results showed increased values of HDL with increasing dosage of prebiotic blend. The values for the negative and positive control treatments were 36.00 mg/dL and 40.00 mg/dL respectively while the values for the treatment groups ranged from 37.00 to 48.00 mg/dL. HDL lipoprotein is usually referred to as good cholesterol, therefore increase in HDL lipoprotein cholesterol is beneficial to the snails. On the contrary, decrease in HDL cholesterol is a disadvantage.

5.4.5.4 Low-density lipoprotein cholesterol (LDL): It is often referred to as bad cholesterol. The results revealed a gradual reduction across the treatments with increasing dosage of the prebiotic blend. This demonstrated a crucial impact of the prebiotic supplementation in the diets supplied to the snails. The values decreased from 22.00 mg/dL (M_1) to 13.60 mg/dL(M_7) at the inclusion level of 2.50 g/kg prebiotic blend. The values observed for LDL in this study portrayed a beneficial effect of the supplementation of prebiotic blend in the diets of snails. The observed increase in the level of HDL cholesterol with increasing levels of prebiotic inclusion in the diets and the corresponding decreased values of the LDL cholesterol would lead to an improved health status of the animals. The implication of this result was that the haemolyph cholesterol concentration would be reduced and invariably increased the quality of the snail meat. Having high LDL cholesterol

and low HDL cholesterol may contribute to the formation of fatty plaques in the arteries of animals which can lead to heart disease.

5.4.5.5 Very low density lipoprotein (VLDL): The values decreased generally across the treatments with increasing dosage of prebiotic blend. This parameter followed the same trend as the LDL cholesterol. VLDL has been considered one of the bad form of cholesterol along with LDL cholesterol and triglycerides. This is because high levels cholesterols in animal can clog the arteries and lead to heart attack. However, the body still needs both LDL and VLDL. VLDL is usually synthesized in the body. It plays a role in the renal excretion of uric acid.

5.4.5.6 Risk ratio (TC:HDL): is also referred to as cholesterol ratio. It is importantn because it helps to know the animal's risk of heart disease. The values were influenced by the treatment groups. The trend in the results tended to suggest that the more the increase in the dosage of the prebiotic blend, the better the result would be. When any animal has high TC it may be that the LDL is going up. A good cholesterol ratio shows that the body is working properly- the body is healthy. High risk ratio increases the risk of heart disease.

5.4.6 Haemolymph Mineral Composition of Grower AGLS (Archachatina marginata) fed diets supplemented with varying levels of prebiotic blend.

Parameters evaluated were influenced by dietary treatments. The haemolymphs were rich in minerals such as sodium (Na), potassium (K), Iron (Fe), Calcium (Ca), Manganese (Mn), Zinc (Zn), Copper (Cu), Chloride (Cl^-) and Phosphorus (P). The haemolymph mineral compositions were particularly high in sodium (48.00-70.00%), Potassium (38-66.00%) and Chloride (60-96.00%) Calcium (7.63-8.72%) and Phosphorus (2.20-4.80%). This was consistent with the report of Ademolu *et al.*, (2007) that the flesh and haemolymph of A. marginata was rich in minerals such as Ca, Mg, Na, Zn, Fe and Cl^-. Ademola et al. (2004) had also reported that in some parts of southwest Nigeria, the snail haemolymph was known to be fried and eaten like a chicken egg. It was also made into a drink that pregnant women could drink. This could be because the hemolymph contains a lot of minerals, especially calcium and iron. Additionally, the haemolymph of large-scale snail processing could be

coagulated, dried, and ground into a powder for use as a protein and mineral supplement in livestock feed.

5.4.7 Mineral composition of grower African giant land snail (*Archachatina marginata*) shell powder as affected by the dietary supplementation of prebiotic blend.

The effects of additive was more of stabilising the amount of sodium in each treatment group. The values of sodium was stabilized at 0.02% across the treatments despite the increasing dosage of the prebiotic blend. Same trend was observed with Potassium. The values for the iron increased correspondingly with increasing levels of prebiotic blend, but dropped at treatment M_7 (319 mg/kg). The values of Calcium also increased with increasing dosage of prebiotic blend. The values of Calcium also increased with increasing dosage of prebiotic blend. The values amongst the treatments ranged from 74.60% to 96.03% while those of the negative and positive control were 73.70% (M_1) and 73.90% (M_2). With these levels of Calcium in shell powder, it could reliably be used as a replacement for oyster shell or Dicalcium phosphate which are commonly used in commercial livestock feeds and are more expensive because they are imported into the country. The use of calcium carbonate from snail powder will be useful or good as a cheap source of Calcium for poultry and other livestock. The values for Phosphorus across the treatments were also high and stabilised significantly across the treatments. The values of Calcium and Phosphuorus in snail shell powder would make it a good alternative source of Calcium and Phosphorus to the conventional sources. Moreover, it could be suggested that if the snail shells are properly washed, dried and ground into powder, it could moderately be added to the infant formula for the supply of calcium ions for bone and teeth formation. The values of Manganese were not significant across the treatment groups. The values ranged from 24.50 mg/kg (M_1) to 25.43 mg/kg. The values of Magnesium, Zinc and Copper were slightly affected by increasing dosage of the prebiotic blend. Copper is very important in snail because it is a component of haemocynin, the oxygen carrier pigment in snail haemolymph. The values for copper ranged from 2.40 mg/kg to 5.40 mg/kg. The least value was observed in treatment M_1 (1.60 mg/kg). The shell powder was rich in Copper element. The shell powder was also rich in chloride (Cl^-) and sulphate ions (SO_4^{2-}).

The importance of Calcium in livestock industry cannot be over emphasized. Calcium in the form of calcium carbonate is a major component of snail shell. The calcium carbonate

in the form of a mineral known as “Calcite” that is 100% Calcium carbonate is useful for the snail for shell and egg development (Adeniyi, 2014). The use of calcium carbonate powder from snail shell in Nigeria will decrease the amount of fund spent on the importation of the conventional Calcium sources for livestock and snail production. Snail shells are found virtually everywhere wasting away in dumping grounds in villages and even in towns.

5.6 Performance Characteristics of glower AGLS in response to diets supplemented with probiotics (*Lactobacillus plantarum* and *saccharomyces cerevisaie*)

This study involved investigations on the performance of *Archachatina marginata* as affected by the inclusion of Lactobacillus plantarum and Saccharomyces cerevisiae. The main effect of the probiotics and the inclusion levels on performance of grower African giant land snail (*Archachatina marginata)* revealed that, feed intake (g/wk/snail) of grower African giant land snail (AGLS) for Lactobacillus plantarum (LP) supplemented diet (11.47) and Saccharomyces cerevisiae (Yeast) supplemented diets did not differ (11.49). However, the shell length, and shell width of LP – supplemented diets were significantly lower than that of the Yeast-supplemented diet. Moreover, the body weight gain (g/wk/snail) observed in Yeast-supplemented diet (225.18) was greater relative to the LP-supplemented diet (141.37). These results showed that probiotics significantly affected the feed intake as well as the body weight gain. However, in each case the values of those treated with the yeast (i.e Saccharomyces cerevisiae) were much higher than those treated/supplemented with Lactobacillus plantarum, indicating that Yeast must be more effective than Lactobacillus plantarum and that the Yeast might have increase the appetite of the snails.

Considering the main effects of inclusion levels of probiotic type, it could be asserted that a 1% increase in inclusion level of probiotics enhanced feed intake, shell configuration and body weight gain of AGLS. The FI observed in snails on 3% probiotic (12.97) was greater relative to 2.0% (11.62) and 1.0% (9.85). However, the lowest FI was observed in snails on 1.0% (9.85) probiotics. The shell length (cm) values in snail on 2.0% (9.99) and 3.0% (10.12) probiotics were similar, and higher than 1.0% probiotics (9.78). Shell width in snails on 1.0% (5.82) and 2% (5.83) were similar but lower compared to 3.0% (5.93) probiotics. Regarding apartural length (cm) in snail on 1.0% (6.48) were the same and were lower compared to 3.0% (6.63) probiotics. The shell thickness followed the same trend as the

apartural length. Also the body weight gain (g/wk/snail) in snails on 1.0% (140.21) and 2.0% (143.43) were significantly lower compared to 3.0% (266.21). Probiotics enhanced appetite in AGLS at level of 1.0% to 3.0%.

An increase in Lactobacillus plantarum supplementation has a negative impact on feed efficiency for African giant land snails. In AGLS, however, the addition of yeast had a positive impact on feed efficiency. Delgado *et al.* (2015) reported that the use of Saccharomyces cerevisiae as a nutritional additive possessed a probiotic effect that helped to reduce the incidence of diarrhea and that when produced, its duration was shortened. The findings of this study were consistent with these findings. According to Rodriquez *et al.* (2000), Saccharomyces cerevisiae moved along the digestive tracts alive and active without adhering to the gut walls because yeast were not typically hosts of digestive microbial biota in monogastric animals, like snails. Bio-regulators that are able to colonize through a variety of mechanisms rely on strains that are unable to adhere to intestinal epithelia for their effectiveness. Baptista *et al.* (2005) stated that they referred to live yeast cells as agents to detoxify mycotoxins and other bacterial toxins, such as those secreted by *C. difficile* and its receptors in the large intestine mucus and vibric cholera, from diets that might contain these toxins (Baptista *et al*., 2005). Saccharomyces cerevisiae's ability to suppress animal stress and make available vitamins, enzymes, and protein led It had likewise been accounted for in different logical examinations by Elias *et al*. (2009), Castillo (2010), and Caridad *et al.* (2018) that the presence of live yeast in the stomach related arrangement of creatures would cause an event called serious prohibition, in which certain microbes equipped for causing illness stick to the outer layer of the yeast in this way eliminating a lot of destructive microorganisms and aiding the creature safeguard all the more successfully.

The interaction effect of probiotics and inclusion level on AGLS performance revealed that the live body weight gain was significantly influenced by the inclusion of varying levels of probiotics. Body weight gain increased as the amount of probiotics included increased. Treatment D8 had the highest body weight gain (189.20 pounds), significantly more than the other treatments. For treatment D1, the lowest value (134.20) was recorded. Snails' performance could be improved by supplementing their diets with Lactobacillus plantarum or Saccharomyces cerevisiae in varying amounts, but Saccharomyces cerevisiae would work better.

Above all other known additives, the primary effects of probiotics were increased host mucosal immunity and improved resistance to pathogenic bacterial colonization, resulting in a lower pathogenic load and improved snail health. Interestingly, it has been reported in the literature that dead yeasts still contain significant quantities of proteins and polysaccharides in their cell walls that are still able to influence nutrient absorption and the immune system positively. In addition, yeasts contain minerals (Mn, Co, Zn) and vitamins (A, B12, and D3) that enhance the activity of beneficial microorganisms and can produce njtritive metabolites in the digestive system, which improves animal performance (Hill *et al.,* 2006).

5.6.1 Nutrient digestibility in grower AGLS diets supplemented with probiotics (Lactobacillus plantarum and Saccharomyces cerevisiae):

Main effects of probiotics and inclusion levels on nutrient digestibility in AGLS revealed that the DM (%) digestibility of grower AGLS on Lactobacillus plantarum (LP)-supplemented diets (53.37) and Saccharomyces cerevisiae (Yeast)- supplemented diet (53.39) were slightly different. For CP digestibility, the value (63.27) for Lactobacillus plantarum was higher than that of the Yeast (61.03), indicating that the digestibility of protein by Lactobacillus plantarum was better. The digestibility of CF in both probiotic were very high. However, CF digestibility of AGLS on Lactobacillus plantarum supplemented diets (80.49) was lower than that of Yeast-supplemented diet (81.72). The EE digestibility in both probiotics were above average. A 1.0% increase in inclusion levels of probiotics improved DM, CP, CF, and EE digestibility of feed in AGLS.
Generally dietary supplementation of probiotics enhanced nutrient digestibility in AGLS at levels of 1.0% to 3.0%.

5.6.2 Carcass characteristics of grower AGLS diets supplemented with probiotics (Lactobacillus plantarum and saccharomyces cerevisiae)

Live weight gain (g) of AGLS on yeast-supplemented diets (119.78) significantly differed from the Lactobacillus plantarum supplemented diet (112.72). This indicated that Yeast was more effective as a growth enhancer than Lactobacillus plantarum-. The foot weight (majoredible part) of AGLS on Lactoballus plantarum-supplemented diet (20.72) was

observed to be lower than that of the foot weight of the snail on Yeast-supplemented diet (27.49). The dressing percentage (%) of AGLS on Lactobacillus plantatum-supplemented diet (45.79) was significantly lower than the dressing percentage of snail on Yeast-supplemented diet (53.76). The main effect of inclusion of probiotic type on carcass yield of AGLS showed that the live weight gain in snail on 3.0% probiotics (117.53) was greater relative to 2.6% (115.62) and 20% (115.60). The results of the dressing percentage also revealed that snail on 3.0% probiotics (53.84) was higher than that of 2.0% probiotics (50.37) and that on 1.0% probiotics (45.12). This indicated that a 3.0% yeast inclusion on diets of AGLS would enhance carcass dressing percentage.

Regarding the gut sections: The gut length as well as every gut section increased with increasing dosage of the probiotics. However, Saccharomyces cerevisiae supplementation in the diets of AGLS was more efficient in increasing the length of the gut as well as the sections than Lactobacillus-supplemented diet. The overall result was that the weight or length of each section of the gut increased with increasing dosage of the probiotics and a better result in each case with Saccharomyces cerevisiae supplementation.

5.6.3 Microbial load in the intestinal segments of AGLS fed diets supplemented with probiotics (Lactobacillus plantarum and Saccharomyces cerevisiae)

From the current study, total bacterial count (TB) observed in oesophagus section of grower AGLS on Lactobacillus plantarum-supplemented diet was greater relative to yeast-supplemented diet. However, enterobacterioceae count was lower Lactobacillus plantarum-supplementation compared to Yeast-supplemented diet. The total bacteria count decreased with increased probiotics inclusion levels. This revealed one of the beneficial functions of probiotics by adhesion to intestinal epithelia cells, triggered the mucus secretion, and prevented the adhesion of pathogens as probiotics blocked intestinal receptors, thereby excluding the pathogens. This concept is referred to as “competitive exclusion”.

Salmonella/Shigella load observed in grower AGLS on Lactobacillus plantarum-supplemented diets did not differ from Yeast-supplemented diet. This result showed the effectiveness of probiotics in reducing the Salmonella/Shigella load in the oesophagus of the snails, thereby enhancing the health status of the snails and in turn promote their growth.

Total fungi count observed in oesophagus section of grower AGLS on Lactobacillussupplemented diet was lower than Yeast-supplemented diet. This implied that a 3.0% inclusion of probiotics reduced the fungi load to zero in the oesophagus section of the gut of AGLS Lactobacillus plantarum was more effective in reducing staphylococcus in the stomach. The staphylococcus load in the small intestine decreased with increasing dosage of probiotics. The observation here was that, as the probiotic inclusion level increased the Lactobacillus load increased. This is beneficial for the snails and this could result into better health status and improved snail performance.

Generally, as the dosage of probiotics increased the total fungi count (FTC) was observed to decrease. This occurred in the all snails. In a similar trend, with increased dosage of probiotics the total bacterial count decreased. This would invariably lead to improved health status of the snails and enhanced performance. These observed results must have been due to the fact that probiotics organisms displaced harmful pathogens through competitive antagonisms by colonization and adhesion to gastro-intestinal cells (concept referred to as competitive exclusion) among other actions. The staphylococcus load tended to decrease as the levels of probiotics inclusion increased but there were some fluctuations that could be due to some changes within the micro-environment of the stomach.

Generally, this result portrayed the positive influence of the probiotics on the health of the animals. Increased inclusion levels of probiotics supplementation load in the large intestine was to the benefit of the host organisms. However, it could be seen that yeast was more effective in reducing the intestinal microbial load.

These results were in agreement with the work of Agbonlahor *et al*, (2010) who recorded the presence of Eshrichia coli (15.7%) proteus species (10.4%), Psedomonae aeruginosa (4.2%), Shigella species (0.30%) and Yersisa (0.6%) in snails. Similarly, Nwuzo *et al*, (2016) reported that they isolated 61 bacterial belonging to 6 bacterial species. These included E. coli, Psedomonas spp, Shigella spp, Enterobacter spp, Salmomella spp and Klebsiella spp, all found in snails.

It was also observed in this study that there were more bacterial species than fungi species. This section of the study clearly showed that snails could harbore highly pathogenic bacteria of potential public health hazard especially where the consumption of snail is high and most of these snails are collected from the bush close to residential houses without modern toilet

system or from the wild. Barimah (2013) in his work, isolated 32 species of bacteria including Salmonella spp, Klebsiella spp, Shigella spp, Yersnia spp, Clostridium tetani, Giardia spp, Balantidium coli and Trophosites and cysts. He also isolated some fungi such as Candida spp, Cryptococcus neoformans, Aspergillus spp, and Rhizopus spp.

The aim of this section of the study was to create an awareness about the possible health hazards the users of snail and snail products could be exposed to, especially when consumed without proper processing or raw meat or other products such as haemolymph for treatment of ailments or use live Snail (slime) for beauty therapy, snails meat for the cosmetic or beauty therapy should be raised from day-old in an intensive system.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

A total of five major studies were carried out.

1. Effect of Sole-Fruit feeding on growth performance and nutrient digestibility of feed by grower African giant land snail (*Archachatina marginata*).
2. Growth and nutrient digestibility of feed in African giant land snail hatchlings (*Archachatina marginata*) in response to in-feed fibre degrading enzyme.
3. Growth and nutrient digestibility of feed in grower African giant land snail (*Archachatina marginata*) in response to in-feed fibre degrading enzyme.
4. Growth performance and nutrient digestibility of feed in grower African giant land snail (*Archachatina marginata*) in response to dietary supplementation of organic acid-prebiotic preparation.
5. Growth performance and nutrient digestibility of feed in grower African giant land snail (*Archachatina marginata*) in response to diets supplemented with probiotics (*Lactobacillus plantarum and Saccharomyces cerevisiae*).

The results of chemical composition of the experimental feedstuffs showed that pawpaw leaf had the highest crude protein content (31.26%), followed by coconut milt (11.38%), unripe pawpaw fruit (10.28%) while pineapple fruit had the least value (5.69%). The fibre contents of the fruits differed from one another and were generally high. The high fibre contents of the feedstuffs must have accounted for the poor feed intake and consequent reduction in growth performance. The ether extract for coconut milt was too high (63.64%) for snails, hence they avoid it but preferred other fruit with very low fat-contents.

The results of the chemical analysis of the fibre fractions of feedstuffs revealed significant variation among all the parameters determined. The values of NDS were different with

treatment PAF having the highest value (9.61%). The NDF values also different with the lowest value noted in treatment PAF (23.79%). The best NDF value was observed in treatment PAF (23.79%). High NDF led to a decrease in feed intake and reduced digestibility in the animals. The values of ADF were significantly different and on the high side. This could contribute to the decreased feed intake and poor digestibility of feedstuffs. The results of the qualitative phytochemical analysis revealed that tannins, phenols, glycosides, photosteriods and flavonoids were only present in trace amounts while the quantitative analysis showed that the phytochemicals that were predominantly present across the feedstuffs were saponins and alkaloids.

The performance characteristics of grower AGLS fed experimental feedstuffs showed that the feed intakes were generally low, and this could be due to the high fibre contents of the feedstuffs. The body weight gain was significantly affected by the poor FI. Treatment PAF had the best body weight gain the best FCR value and the most economical feed, and as well the best record of nutrient digestibility and carcass yield.

The performance of the AGLS hatchlings fed basal diets supplemented with increasing dosage of *β-D-mannanase* revealed that there was no significant difference in FI despite different levels of inclusion of the enzyme. However, the body weight gain increased with increasing dosage of the fibre-degrading enzyme implying a positive influence. The best FCR value was obtained in treatment T_3 (0.15 g *β-D-mannanase* /kg feed) and both shell length and shell width increment increased with increasing dosage of *β-D-mannanase*. This must have been attributed to the influence of the enzyme (*β-D-mannanase*) inclusion, demonstrating the efficacy of *β-D-mannanase* in degrading *galactomannans* and *mannans* in the diets, converting the diets to flesh. All carcass parameters including live body weight increased with increasing dosage of *β-D-mannanase.* The results of the cost analysis indicated that the feed cost increased as the level of enzyme in the diets increased. However, the cost/g body weight gain (₦/g) decreased with increasing dosage of *β-D-mannanase.* Based on the feed cost per weight gain records, it would be economically preferable to add 0.15 g *β-D-mannanase* /kg feed to the diet of the AGLS hatchlings.

There was no significant difference in the mean FI despite the varying levels of *β-D-mannanase* inclusion in the diets of grower AGLS. The values ranged from 910.03 (T_3) to 914.03(T_4). The body weight gain of the grower AGLS across the treatments were significantly affected by the increasing dosage of B-D- *mannanase.* The FCR was at its best in the diet containing 0.20 g *β-D-mannanase* /kg diet (3.09) in treatment T_4. The digestibility of feed in the snail increased with increasing dosage of *β-D-mannanase* with the highest value at treatment T_4. The foot weight increased with the increasing levels of the enzymes in the diets and the dressing percentage was also affected, although the values were low. The values of cost/g weight gain also increased with increasing dosage of enzyme in the diets. Since the inclusion of *β-D-mannanase* in the diets of grower AGLS increased the total feed cost, and the cost per weight gain, it would be economical not to add *β-D-mannanase* to the diet of grower AGLS. However, if it must be added, it should not exceed 0.15 g *β-D-mannanase* /kg feed.

Performance characteristics of grower AGLS fed diets supplemented with organic acid-prebiotic preparation showed that there was no significant difference in FI across the treatments but there was a significant increase in the body weight gain with increasing dosage of prebiotic blend in the diets. This might be attributed to the synergetic effects of the components of the prebiotic blend. The FCR and the shell dimensions were positively influence by the increasing level of prebiotic blend. The nutrients digestibility, carcass traits and the dressing percentage were significantly affected by prebiotic blend supplementation. The serum total protein, serum albumin and serum globulin in the *haemdymph* were all positively affected by the supplementation of the diets with prebiotic blend.

The values of the ALP, AST and ALT were significantly affected by the supplementation of diets with increasing dosage of prebiotic blend. However, their values were unexpectedly high. These results were not consistent compared to what happened in the vertebrate animal, yet there was no indication that the animals had any health challenge. The values of glucose in the *haemolymph* of grower AGLS is this study were increased with increasing dosage of prebiotic blend. It ranged from 66.00% to 96%.

All hematological parameters examined were significantly influenced by the increasing dosage of prebiotic blend. The PCV, WBC increased with the increasing dosage of prebiotic blend and ESR values decreased with increasing dosage of prebiotic blend.
The WBC values observed in the snail's *haemolymph* had enough potential to fight any invading pathogens in the body of snail. The results of the haemocyte cell counts, morphological differentiation and identification showed two cell types; Haemocyte type 1 and Haemocyte type 2, (which was less in population). Haemocyte type 1 was believed to be the major principle for snail internal defense system.

Haemolymph serum lipid profile of grower AGLS showed that all the parameters, HDL, LDL, VLDL and triglycerides were significantly affected by increasing dosage of prebiotic blend. The results revealed decreased levels of LDL, VLDL, triglycerides, but increased levels of HDL as the inclusion levels of prebiotic blend in the diets increased. This was a positive influence. The ratio LDL: HDL was significantly decreased across the treatments as the amount of prebiotic preparation increased. This was a proof of good health status of the snails and no mortality observed. FI of grower AGLS on Lactobacillus plantarum-supplemented diets (11.47) and Saccharomyces cerevisiae-supplemented diets (11.49) did not differ significantly. However, the body weight gain observed in Saccharomyces cerevisiae-supplemented diet (225.18) was significantly greater relative to the Lactobacillus plantarum-supplemented diet (141.37). These results showed that probiotics significantly affected the FI and the BWG. The main effects of inclusion levels of probiotic types showed that a 1.0% increase in inclusion level of probiotics enhanced FI, shell configuration and BWG of grower AGLS. Probiotics also enhanced appetite in grower AGLS at 1.0% to 3.0% inclusion levels. The interaction effect of probiotics and inclusion levels on performance of grower AGLS also revealed that *A.marginata* responded negatively in terms of feed efficiency to increasing levels of Lactobacillus plantarum-supplementation but Saccharomyces cerevisiae supplementation positively influenced efficiency in grower AGLS.

The main effect of inclusion levels of probiotic type revealed that a 1.0% increase in inclusion level of probiotic improved DM, CP, CF, and EE digestibility in grower AGLS (*A.marginata*). The live weight of grower AGLS on Saccharomyces cerevisiae-

supplemented diet significantly differed from Lactobacillus plantarum-supplemented diet. This indicated that yeast was more effective a growth promoter than Lactobacillus plantarum. The dressing percentage followed the same trend. The result showed that a 3.0% yeast inclusion in the diets of grower AGLS enhanced carcass dressing percentage.
Again, the results of interaction effect of probiotics and inclusion levels on the gut sections of grower AGLS showed that the supplementation of the diets of snails with probiotics significantly increased the gut length and each gut section.

Concerning the microbial load, A 1.0% - 3.0% increase in inclusion levels of probiotics depressed the microbial load in oesophagus section of the GIT of grower AGLS and a 3.0% inclusion of probiotic reduced the fungi load to zero in the oesophagus. The total bacteria count (TBC) observed in the stomach section of grower AGLS on Lactobacillus plantarum-supplemented diet was significantly greater relative to yeast-supplemented diet, implying that *Saccharomyces cerevisiae* is more effective in reducing bacteria load in the stomach. However, Lactobacillus plantarum was more effective in removing the fungi in the stomach. A 1.0% to 3.0% increase in inclusion of probiotics reduced the microbial load in the stomach section of the GIT of AGLS and a 2.0% to 3.0% probiotics inclusion levels decreased the total fungi load in the stomach.

The total bacteria count observed in the small intestine of grower AGLS on Lactobacillus plantarum-supplemented diet was significantly lower compared to yeast-supplemented diet indicating that yeast was more effective in reducing the bacteria load in the small intestine. Similar observation was made on the total fungi count. A 1.0% to 3.0% increase in inclusion levels of probiotics depressed the bacteria load in the small intestine. Lactobacillus spp on 1.0%-3.0% probiotic inclusion level increased the Lactobacillus counts in the small intestine. This was beneficial to the snails. For fungi counts, 1.0% - 3.0% probiotic inclusion levels resulted in the reduced total fungi count along the GIT of the snails.

In the large intestine, the total bacteria count observed on Lactobacillus planetarum-supplemented diet and yeast-supplemented diet differed significantly. The value was higher in Lactobacillus plantarum-supplemented diet. This meant that yeast was more effective as a growth enhancer than Lactobacillus planetarum. Similar result was observed for total fungi count. Generally, the results of this study portrayed positive influence of the

probiotics on the health of the animals and increased inclusion levels of probiotics led to the depression in the microbial load in the GIT of grower AGLS (*A.marginata*), Diverse bacteria and fungi species were observed in the GIT of gower AGLS. It was also observed that there were more bacteria species than fungi species.

6.2 Conclusion

From the result of the studies carried out, it could be concluded that;

Pineapple fruit, among other tropical fruits assessed had the highest performance. However, pawpaw fruit was the best alternative to pineapple fruit. High fibre contents of the fruits resulted in poor feed intake and in turn, reduced growth performance. The results of the chemical analysis of the fibre fractions of the feedstuffs revealed pineapple as the best fruit with the best values for NDS, NDF, ADF, ADL, etc. The results of the qualitative and quantitative phytochemical analysis revealed that tannins, phenols, glycosides, photo steroids, and flavonoids were only present in trace amount but saponins, and alkaloids were predominantly present across the feedstuffs administered to the snails.

The performance of hatchling AGLS fed basal diet supplemented with varying levels of *β-D-mannanase* revealed that the body weight gain increased with increasing dosage of fibre degrading enzyme (*β-D-mannanase*), showing a positive influence. However, the best FCR value was observed in treatment H_3 (0.15 g *β-D-mannanase* /kg feed). The nutrient digestibility increased with increasing dosage of *β-D-mannanase*, demonstrating the efficacy of the enzyme in degrading *galactomannans* and *mannans* in the feed, converting feed to flesh. The cost analysis revealed that the feed cost increased with increasing levels of *β-D-mannanase* in the feed. However, the cost/g body weight gain (₦/g) decreased as the level of fibre degrading enzyme in the feed increased. Based on this observation, it is more economical to add 0.15 g *β-D-mannanase* /kg feed to the diet of AGLS hatchlings.

The total body weight gain of grower AGLS across the treatments, were significantly affected by increasing dosage of *β-D-mannanase.* The FCR was at its best in the diet containing 0.20 g *β-D-mannanase* /kg feed. The digestibility of feed by grower AGLS increased as the level of *β-D-mannanase* increased, with the highest value in the treatment with 0.20 g *β-D-mannanase* /kg feed. The inclusion of the enzyme in the diet of grower

AGLS increased the total feed cost and cost per weight gain. It is therefore not economical to add *β-D-mannanase* to the diet of grower AGLS and if it must be added, it should not exceed 0.15 g *β-D-mannanase* /kg feed. Addition of *β-D-mannanase* to *monogastric* animals, diets would enhance their digestibility of feeds resulting in better performance. These studies involving the use of fibre degrading enzyme (B-D-*mannanase*) had demonstrated that the enzyme possessed potential beneficial effects on the health and nutrient utilisation.

Dietary supplementation of prebiotic preparation enhanced the growth performance of grower AGLS due to the synergistic effects of its components. Supplementation of grower AGLS with prebiotic mixture significantly increased the body weight gain compared to the negative control treatment. All the serum biochemical parameters measured such as serum total protein, serum albumin, globulin, albumin-globulin ratio, creatinine etc. were significantly influenced by the supplementation of the basal diets with varying levels of prebiotic-organic acid blend. All *hematological* indices examined followed the same trend with the biochemical indices.

Haemocytes, one of the constituents of *haemolymph* in *A.marginata* were observed to be of two types: *Haemocyte* type 1 and *haemocyte* type 2. *Haemocyte* type1 were larger in population and were believed to be involved in cellular immune activities in *A.marginata*. All parameters analyzed in *haemolymph* serum lipid profile; Total cholesterol, triglycerides, HDL, LDL, VLDL, LDL: HDL, and risk ratio revealed that the grower AGLS were quite healthy. The result of study four suggested that prebiotic blend could be incorporated into the corn-soya bean meal to feed grower AGLS without any harmful effects on growth, haematology and biochemistry.

Inclusion of Lactobacillus plantarum to the diets of grower AGLS (*A.marginata*) at the level of $2x10^8$ CFU/kg feed enhanced their growth performance. Supplementation of the diets of grower AGLS (*A.marginata*) with *Saccharomyces cerevisiae* (Yeast) up to the level of $3x10^8$ CFU/kg feed increased the growth performance and enhanced the health status of the snails and exhibited greater efficiency than *Lactobacillus plantarum* at the same level of concentration. The main effect of inclusion levels of probiotic type, showed that a 1.0% increase in inclusion level of probiotics enhanced feed intake, body weight gain and shell

configuration of grower AGLS. The results of the microbial load at different sections of the GIT of grower AGLS's stomach has highest value ($6.3x10^6$ CFU), followed by the small intestine ($6.1x10^6$ CFU) while the lowest bacteria count was observed in oesophagus ($3.7x10^6$ CFU). From the result above it could be suggested that probiotics strains modified the intestinal micro flora of grower AGLS with beneficial effects on the host animals which were reflected in their positive performances. Therefore, probiotic should be considered as potential beneficial elements in feeding and management system of snail production (heliculture) without the use of antibiotic feed additive.

6.3 Recommendations

From the foregoing results, it could be recommended that:

- In *Archachatina marginata*, addition of in-feed fibre degrading enzyme (*β-D-mannanase*) to the diets of the hatchling AGLS would enhance their growth performance and if the same enzyme is to be administered to the feed of grower AGLS, the level should not exceed 0.15 g *β-D-mannanase*/kg feed for optimum profit.
- Diet supplementation of grower AGLS with organic acid-prebiotic blend up to the level of 2.50 g prebiotic blend/kg feed would help to promote the growth and the health status of the snails.
- Supplementation of the basal diet of grower AGLS (*A.marginata*) with *Lactobacillus plantarum* at the inclusion level of $2x10^8$ CFU/kg feed enhances its growth performance as well as its health status.
- Inclusion of *Saccharomyces cerevisiae* (Yeast) to the diet of grower AGLS (*A.marginata*) up to the level of $3x10^8$ CFU/kg feed would enhance its growth performance and it has a greater efficiency than Lactobacillus plantarum at the same concentration.
- Maintaining hygienic conditions and proper intensive management of snails (from day-old hatchlings) and dietary supplementation of appropriate growth promoters such as fibre degrading enzyme (*β-D-mannanase*), Lactobacillus plantarum or *Saccharomyces cervisiae* (Yeast), would greatly enhance the performance of snails.

- Snails procured directly from the wild/bush, roadside, backyard or markets should be thoroughly purged and the meat after harvesting properly processed before consumption because of the possibility of environmental contamination and their natural mode of feeding.
- Further research is required to investigate the possibility of eliminating (or minimize) aestivation from African giant land snail's life cycle, knowing that there appears to be a correlation between the period of aestivation and the growth of snail and consequently the table size and for the snail farmer, aestivation implies the loss of valuable growing time and invariably loss of money.

More research is also required into the area of establishing internationally acceptable standard nutrient requirements for different phases of growth in snail. This would help to enhance productivity and availability of snails in all seasons, since the demand for snails is increasing daily because of its peculiar usefulness in nutrition, medicine and cosmetic industry.

6.4 Contributions to Knowledge

- The study revealed that African giant land snail development is limited by feed resources that are high in fibre content up to $12.93 \pm 2.98\%$.
- The study also showed that enzyme (*β-D-mannanase*) supplementation was very effective in enhancing digestibility of nutrients and in turn promoting performance in snails.
- Dietary supplementation of organic acid-prebiotic preparation improved the performance of African giant land snails (*A.marginata*).
- All the serum biochemical, haematological parameters and lipid profile of *haemolymph* in grower African giant land snail were significantly influenced by the supplementation of the diets with varying inclusion levels of prebiotic blend.
- Probiotics supplementation resulted in enhanced nutrient digestibility and better performance in grower AGLS with greater efficiency using *Saccharomyces cerevisiae*.
- A 1.0% to 3.0% increase in inclusion levels of probiotics depressed the microbial load (bacteria and fungi) in the *gastrointestinal* tract of grower AGLS (*A.marginata*)

in addition to promoting the growth of the beneficial microorganisms and ultimately improved the health status of the animals.

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