NEUTRAL DIGESTIVE FIBER DIGESTIBILITY OF GOATS FED DIETS WITH GRADED LEVEL OF CHITIN AND CHITOSAN FEED ADDITIVES FROM SNAIL SHELLS

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BENIN CITY, NIGERIA

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE BACHELOR OF AGRICULTURE HONOURS, DEGREE IN ANIMAL SCIENCE, OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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CERTIFICATION

This is to certify that this project was carried out by Favour Osetohamen OTAIGBE, with the Matriculation Number AGR2000111, of the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

PROF. M.A. BAMIKOLE **(PROJECT SUPERVISOR)**	**DATE**
MRS. B. O. ISAAC **(PROJECT CO-SUPERVISOR)**	**DATE**
DR. N.C. AKAEZE **(HEAD OF DEPARTMENT)**	**DATE**

DEDICATION

This project work is dedicated firstly to Almighty God, for guidance, strength and mercies all through the course of my study in the University of Benin, to my Parents (Dad and Mom) for the continued support and love.

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I want to appreciate God for His provision and supply for me and giving me the strength to finish this journey. I want to acknowledge quite a number of people for all the impact they've had at different points in my journey.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The study investigated the chemical composition of chitin and chitosan fed diets, and their effects on goat's neutral detergent fiber (NDF) digestibility.

Twelve (12) West African Dwarf goats were randomly assigned to six dietary treatments (control, chitin at 3% and 6%, chitosan at 0.5% and 1%, and 0.01% oxytetracycline), alongside guinea grass at a ratio of 50:50. Chitin and chitosan were extracted using chemical processes involving demineralization, deproteinization, and deacetylation, while diets chemical compositions were analyzed, The NDF value (%) range from 25.00 to 93% with control and 6% chitin having the least and highest values respectively and were significantly different ($P<0.05$) from each other. Results showed significant differences ($P<0.05$) in NDF intake and digestibility across treatments were not significantly different.

The study concludes that chitin and chitosan from snail shells had no negative influence on NDF intake and digestibility. This approach offers a sustainable and value-added use of snail shell waste in animal nutrition.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Ruminants require a minimum amount of fiber in the diet to maintain adequate ruminal fermentation and animal health. It is necessary that part of this fiber should come from forage, because neutral detergent fiber from forage (NDF stimulates the

chewing activity and saliva production, which maintain the buffering capacity of rumen (National Research Council. 2001). Roughage contains cellulose and hemicelluloses which potential as source of carbohydrate in the diet of goat. Goats have ability to utilize cell wall of the roughage which contains the cellulose and hemicelluloses (Ismartoyo *et al*., 2022). Production of goat depends on the quality of the diet consumed by goat. The diet of goat usually consists of about 80% roughage as basal diet and 20% concentrate. In Indonesia, goats are commonly fed roughages known as Elephant grass Mini elephant grass, *Panicum maximum* (PM), and *Brachiaria decumbens* (BD). These four grasses contain relatively high acid detergent fiber (ADF) and neutral detergent fiber (NDF). The high fiber content of the grasses will be degraded and fermented by rumen microbes in the rumen of goat (Ismartoyo *et al*., 2022). The ADF concentration were Elephant grass, Mini elephant grass, *Panicum maximum*, and *Brachiaria decumbens* were 38%, 34%, 40% and 44%, respectively, whereas NDF content of Elephant grass, Mini elephant grass, *Panicum maximum*, and *Brachiaria decumbens* were 70%, 54%, 63% and 64%, respectively (Ismartoyo *et*. *al*., 2022).

Feed with high ADF and NDF content is ideal for ruminant feeding for ruminant; however, it should be noted that high fiber in the dietary decrease the digestibility of the diet which contains not only cellulose and hemicelluloses but also lignin which is resistant to microbial fermentation in the rumen. Plant biomass is the most abundant resource in nature and is a key feed resource in livestock production. Ruminants, for

example, cattle, are adapted to survive and thrive on highly fibrous forages due to a mutual relationship with billions of microbes such as bacteria, fungi, and protozoa housed in their first compartment of the stomach, the rumen. While the host provides the necessary rumen conditions for the microbes' survival and proliferation, temperature of around 39°C, pH of 5.5 to 6, and feed substrate, the microbes ferment the fibrous feed, producing volatile fatty acids, the primary energy sources for the host. Dietary fiber plays a crucial role in producing volatile fatty acids (energy source), promoting overall rumen health, and also supporting milk fatty synthesis, rumen motility, rumination, and rumen pH stabilization (Gondo, 2025).

Fiber digestion also plays a critical role in the nutrition and productivity of ruminant animals such as goats. The inclusion of chitin and chitosan in goat diets may affect microbial activity in the rumen, leading to changes in fiber degradation efficiency. Understanding this effect is vital for developing cost-effective and sustainable feed strategies, especially using locally sourced materials like snail shells. Low fiber digestibility is a common challenge in small ruminant feeding, often leading to reduced feed efficiency and animal performance. Despite the promising role of chitin and chitosan as feed additives, limited data exist on their influence on fiber digestibility in goats. Moreover, the potential use of snail shells (waste product) as a source of these additives remains largely untapped in livestock nutrition research. Chitin and chitosan are naturally occurring biopolymers with bioactive properties. They are abundantly found in the exoskeletons of crustaceans, insects, and mollusks

like snails. While most studies have focused on chitin/chitosan derived from shrimp and crab shells, snail shells offer a locally available and underutilized alternative. Chitosan has been shown to influence gut microbiota, enhance nutrient absorption, and improve fiber digestion in non-ruminants; its potential in ruminant feeding, particularly in relation to fiber digestibility, is still being explored.

The giant African land snail, belonging to the family *Achatinidae*, is among the largest terrestrial gastropods, boasting an average lifespan of 5-7 years, though some individuals have been documented to live up to 10 years (Egbeneje *et al.,* 2024). *Achatina fulica*, a notable species of this genus, is renowned for its invasiveness, capable of consuming over 500 plant species. Despite being listed among the 100 most harmful invasive alien species globally, *A. fulica* continues to thrive, adapting easily to diverse environments beyond its natural habitat (Egbeneje *et al.,* 2024). Notably, snail shells offer a promising source for chitosan production, a valuable material with various industrial applications. The southern region of Nigeria harbors diverse snail species, with *Archachatina fulica* being a common delicacy (Egbeneje *et al.,* 2024). The shells of these snails, rich in carbohydrates, offer a higher yield of chitosan compared to seafood waste. However, despite their potential, there's limited research on utilizing snail shells for chitosan production. Chitosan production via chemical deacetylation of chitin presents a viable solution to mitigate environmental pollution while harnessing economic opportunities (Egbeneje *et al.,* 2024).

Chitin, a structural component found in arthropods, mushrooms, algae, coral, and nematodes, offers diverse applications across various industries. Its degree of acetylation is crucial for determining its physical properties and functional characteristics. Chitosan, a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine and N-acetyl-D- glucosamine (acetylated unit), is derived from the chitin shells of crustaceans like shrimp, through treatment with an alkaline substance such as sodium hydroxide (FAO, 2019). Despite its solubility challenges, higher molecular weight chitosan with increased degree of deacetylation (DD) forms stable complexes with DNA/RNA molecules, enhancing delivery efficiency (FAO, 2019). Commercial production of chitosan involves the deacetylation of chitin, primarily sourced from crustacean exoskeletons and fungal cell walls.

1.2 Justification of the Study

The increasing demand for sustainable livestock production has intensified the need for alternative, cost-effective, and locally available feed additives that can enhance nutrient utilization, particularly in small ruminants like goats (Mahgoub *et al.,* 2025). In regions where fibrous feed resources dominate, improving fiber digestibility is critical to maximizing productivity and maintaining animal health. Neutral detergent

fiber (NDF) digestibility represents the cell wall components (cellulose, hemicellulose, and lignin) whose digestibility determines voluntary feed intake and energy availability (Carrillo-Díaz *et al.,* 2022). Therefore, improving fiber digestibility remains an essential goal in ruminant feeding systems. Chitin and chitosan, biopolymers derived from snail shells, offer promising functional benefits due to their antimicrobial, prebiotic, and bioactive properties. Their potential to modulate rumen microbial populations and enhance fermentation makes them valuable candidates for improving fiber digestion in goats. However, while their use has been more explored in monogastric nutrition, there remains a significant gap in research regarding their specific impact on fiber degradation dynamics within the ruminant digestive system. The aim of this study is to investigate the neutral detergent fiber (NDF) digestibility in goats fed diets supplemented with graded levels of chitin and chitosan derived from snail shells. The overarching objective is to provide livestock farmers with practical, cost-effective, and environmentally sustainable feed strategies to improve productivity. By enhancing fiber digestibility and supporting animal health through the inclusion of natural bio-additives, this research promotes a holistic approach to animal nutrition and resource utilization. In the face of global challenges such as climate change and diminishing feed resources, this study highlights the importance of scientific innovation in developing adaptive feeding solutions that align with the nutritional and economic needs of both animals and producers.

1.3 Objectives of the Study

The objectives of this study were to determine the:

1. Chemical composition of diets containing different levels of chitin and chitosan from snail shells; and
2. Neutral detergent fiber intake, balance and retention of goats fed diets containing additives of chitin and chitosan from snail shells.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Ruminant Nutrition

In ruminant nutrition, many types of feed additives are used to improve production performance and to maintain the good health and metabolic condition of farm animals.

The generally used feed additives are organic acids, feed enzymes pro and prebiotics, and herb extracts on the other hand, chitosan is a new and relatively less used in the diet of the animals. Chitosan is a nontoxic polyglycosamine and is rarely present in nature (mushrooms) containing β-(1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino-D-glucose units. It is a deacetylated to varying degrees form of chitin, widespread in nature component of the exoskeleton of shrimps, crabs, and insects. Different from chitin, chitosan is soluble in acidic solutions (Świątkiewicz *et al.,* 2015), and it is moderately digested in the gastrointestinal tract of mono-gastric animals (Hirano *et al.,* 1990). Chitosan is commercially obtained from chitin through the deacetylation process; in this process chitin is treated with a strong solution of sodium hydroxide at a higher temperature (Singla and Chawla (2001). Chito-oligosaccharides are produced by chitosan depolymerization using acid hydrolysis, hydrolysis by physical methods, and enzymatic degradation. Chitosan and its oligosaccharide derivatives have reactive functional groups, that is, amino acids and hydroxyl groups, unlike chitin they have antimicrobial (Holappa *et al.,* 2006), anti-inflammatory (Yoon *et al.,* 2007) (Ma *et al.,* 2011), anti-oxidative, antitumor (Shen *et al.,* 2009), immunostimulatory, (Zaharoff *et al.,* 2007), and hypocholesterolemic properties. In the environment, particularly in the agriculture sector, the emission of enteric methane contributes significantly, and the production of methane by ruminants also characterizes a substantial feed energy loss. Proper provision of forage and choice of feed supplements can improve the total mixed ration quality and nutrient digestibility and alternatively improve the production

performance of the animals and decrease the methane emission in an animal farming system.

2.1 Fiber Components and Digestion Process

2.1.1 Understanding fiber and its role in ruminant nutrition

Fiber, a carbohydrate component of plant cell walls, is challenging to digest but plays a vital role in ruminant diets. It is classified into Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Hemicellulose, each contributing differently to digestion and overall animal health.

Neutral Detergent Fiber (NDF)

NDF represents the total fiber component within a feedstuff. It is crucial for providing the "scratch factor" in the rumen, which promotes contractions and rumination. This functional fiber is referred to as physically effective neutral detergent fiber (peNDF) (Kung, 2014). The benefits of peNDF are that it:

- Stimulates rumination and saliva production, which stabilizes rumen pH.
- Influences milk fat production by altering the volatile fatty acid (VFA) ratio in favor of acetate, essential for milk fat synthesis in the mammary gland.

The effectiveness of peNDF is influenced by particle size. For example, cows fed chopped alfalfa hay exhibited more stable rumen pH than those fed pelleted alfalfa hay, highlighting the impact of processing methods on fiber quality (Adesogan *et al.,* 2019).

2.1.2 Components of NDF

NDF is further divided into:

1. **Acid Detergent Fiber (ADF):** Composed of cellulose and lignin, ADF is the least digestible fiber component.

i. Cellulose: A linear polysaccharide of glucose molecules that forms the primary cell wall of plants.
ii. Lignin: A structural, woody component that provides rigidity to plants and is indigestible. Its content increases as plants mature.

2. Hemicellulose

i. A complex carbohydrate made of branched polysaccharides like xylose, mannose, galactose, and arabinose.
ii. Provides flexibility to plants and is more digestible than ADF.

2.1.3 Importance of NDF and ADF in digestion

i. NDF: Predicts feed intake and rumen fill, as it influences the physical bulk of the diet.

ii. ADF: Indicates fiber digestibility, with higher levels corresponding to lower digestibility.

2.2 Chitin and Chitosan: Sources, Structure and Properties

2.2.1 Brief History of Chitin

The first discovery of chitin was in mushrooms by Henri Bracannot (1811), a French botanist and director of botanical abundant at the academy of science in Nancy. In 1823, chitin was isolated from insects and was got its name glucans (Odier *et al.,* 1823) except for chitin of some centric diatom when another French scientist, A. Odier named it "chitine" which simply means "tunic" or "coverage". In 1859, C. Roughet a chemist discovered that form of chitin known in nature and unlinked with other chitin could be transferred into water soluble form through some chemical reactions. After that in 1870, this modified chitin was named chitosan (Muzzarelli *et al.,* 2013). Despite the early discovery of chitin, researches about it became more intensive only in the last 20 years, this can be attributed to the fact that chitin in its pure form is water insoluble; its uses are extended considerably after chemical treatment.

Chitin

In the world, chitin is the second most vital natural polymer and its *N*-deacetylated derivative chitosan has been known as a useful biopolymer that is used in the food

industry, medicine, and agriculture. Chitin is the second most plentiful natural polymer having structural polysaccharide (Joanna *et al.,* 2010). A chief constituent of the carapaces, crusts, and crustacean shells such as crabs, shrimps, and lobsters; it is also a constituent of the cell walls in yeast and fungi ((Brown *et al.,* 2012). Chitin (chemical formula ($C_8H_{13}1O_5N$) n) can only be soluble in concentrated mineral acids (El Knidri *et al.,* 2018)**.** The structure of the chitin is a linear polymer containing mostly β (1→4)- linked 2-acetamido-2-deoxy-β-D-glucopyranose units and partially β-(1→4)-linked 2-amino-2- deoxy-β-D-glucopyranose. In this structure, the chitin is insoluble in water and only soluble in common specific organic solvents such as N, N-dimethylacetamide (DMAc)-LiCl (Kumirska *et al.,* 2010) hexafluoroacetone or hexafluoro-2-propanol and the chemical structure of the chitin is presented in Figure 1 (Kurita, 2001). When the N-acetylation degree is lower than 50%, chitin can be soluble in an acidic solution having a pH less than 6 and later is called chitosan. Therefore, chitosan is a combined name of the partially and fully de-acetylated chitin, however, a firm nomenclature concerning the degree of N-deacetylation between chitin and chitosan has not been defined.

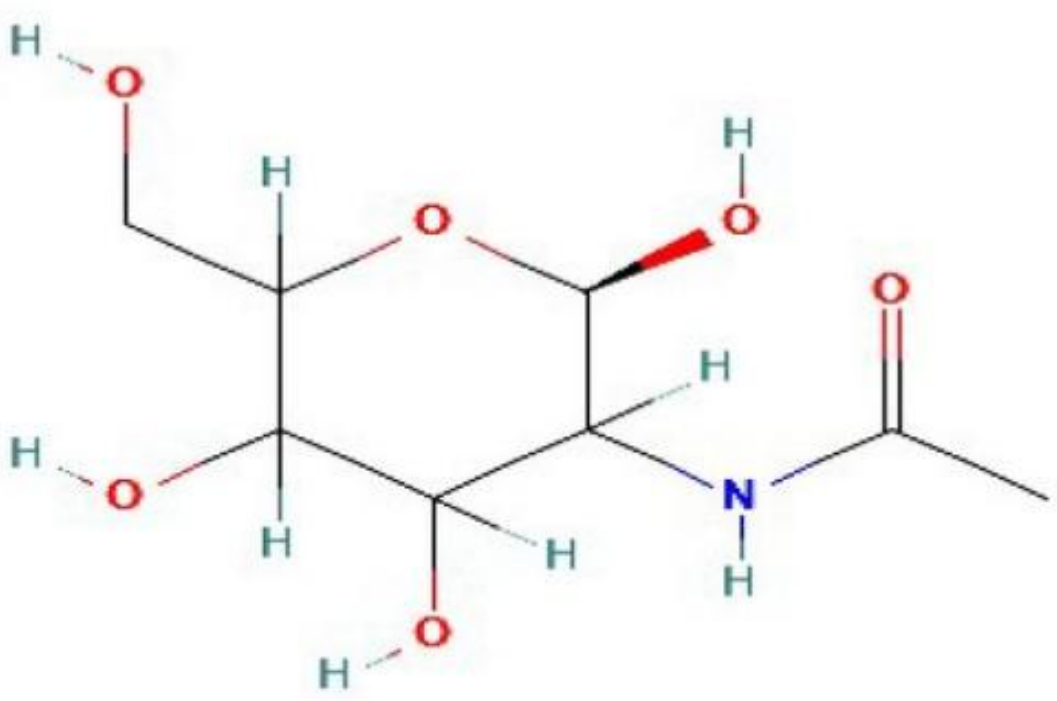

Figure 1: Chemical structure of the chitin; Source Pubchem

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In the nomenclature of the European chitin society (EUCHIS), chitosan and chitin should be divided by the solubility and insolubility in 0.1 M acetic acid ((Petri *et al.,* 2007). Chitosan is a soluble material, whereas chitin is insoluble. The molecular weight of the chitin and chitosan is up to numerous million g/mol. The average molecular weight of commercially obtainable chitosan ranges between 3800–500,000 g/mol, and it has 2%–40% degree of N-acetylation ((Tajik *et al.,* 2008). Chitosan and chitin have commercial importance because in these two compounds the nitrogen content is high (6.89%), and these compounds have greater abilities in biocompatibility, biodegradability, non-toxicity, and adsorption. In addition, chitin and chitosan have lower toxicity. Chitosan is highly insoluble and has a lesser chemical reactivity. The difference between chitin and chitosan depends on the degree of deacetylation. If the threshold of deacetylation is above 50%, it is referred to as chitosan. The deacetylation ranges from 44.1% to 98.0%, depending on the species. Grasshoppers, honeybee beetles, shrimp shells, and blowfly larvae all are examples of the highest deacetylation. In addition, commercial chitin is obtained from crustaceans and aquatic invertebrates and has similar products and futures that are in insects produced chitin with like qualities (Anggraeni *et al.,* 2022).

Chitosan

Chitosan is composed of two repeated units of D-glucosamine and N-acetyl-D-glucosamine and these units are linked together by β (1→4) linkage, while chitosan, a

linear polysaccharide composed of two repeated units, D-glucosamine and N-acetyl-D-glucosamine, linked by β-(1→4) linkages. Chitosan is categorized in terms of intrinsic properties such as molecular weight, viscosity, and degree of deacetylation, and the chemical structure of the chitosan is presented in Figure 2 (Jimnez *et al.,* 2019). Chitosan is considered a natural compound, biocompatible and non-toxic, biodegradable and bioactive mucoadhesive compound, and is commonly used as a food product in Japan in 1983 and Korea in 1995 and in 2012 food and in addition drug administration United Stated recommended chitosan as for food production. The chitosan is fully or partially de-acetylated biopolymer chitin. Chitosan is the second most polysaccharide in nature having a greater molecular weight and a polycationic polymer. Normally chitosan is found in the exoskeleton of insects, mollusks, crustaceans, and some algae, however, a large quantity of chitosan is obtained from marine crustaceans (Li *et al.,* 2018). In a year, shells of crustacean are generated through the extraction of chitin (106–107 tons), and from this extraction different protein and chitosan from this waste has added value (Teng *et al.,* 2001). Numerous studies proposed that chitosan has antimicrobial properties and worked against the killing of the bacteria and different fungi like filamentous fungi and yeast, it also studied that chitosan also has antiviral and anti-inflammatory, analgesic, anticholesteromic and homeostatic effects (Duffy, Ciara *et al.,* 2018). Chitosan can be used directly, or it can be mixed with other polymers such as inoculants of silage, for food processing and preservation, biotechnology, water treatment, tissue engineering,

the cosmetic industry, and the pharmaceuticals textile (El-Knidri *et al.,* 2018). Nowadays, in ruminants particularly in beef and dairy cattle chitosan is also used in animal feeding and it improved rumen fermentation and digestibility (Gandra *et al.,* 2006). The extraction of chitosan can be done either by biological or chemical methods. The industrial and chemical process starts with the removal of different minerals like calcium chloride and known as demineralization followed by deproteinization and decolorization like carotene and astaxanthin. Lastly, the deacetylation process is carried out through potassium or sodium hydroxide (Naveed *et al.,* 2019) while the biological method is considered environment friendly. In this method demineralization is done by using lactic acid and protease is used for deproteinization, discoloration acetone or organic solvents are used, and lastly, bacteria are used for deacetylation. Recently A new method of extraction, also used known as the microwave irradiation method, has been recently developed (Phibert *et al.,* 2017). The raw material (crustaceans' species), which is used for chitosan, method of extraction, and seasonal variation play a crucial role in the quality of the final product (Puvvada *et al.,* 2012).

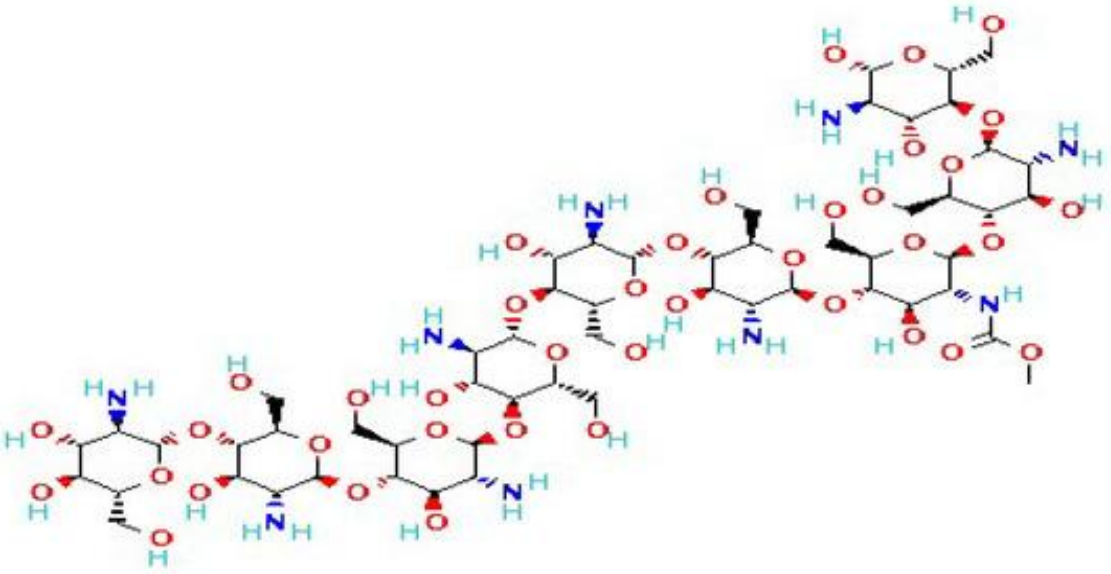

Figure 2: Chemical structure of the chitosan: Source Pubchem

Source: (https://pubchem.ncbi.nlm.nih.gov/;

2.3 Chitin and Chitosan Sources

Chitin

The main source of Chitin is the exoskeleton of mollusks, insects, fungi, and crustaceans. However, the chief chitin source is the shells of the shrimps and crabs. The chitin is normally present in two forms (allomorphs) contingent on the source, these two forms are α and β-forms. Furthermore, in γ-chitin, this form is commonly available in a combined form with either α or β forms than a different form (Kumirska *et al.,* 2010*)*. From these forms, α-Chitin is the most plentiful form which is available and is obtained from the crustacean's exoskeleton mainly crabs and shrimps. While the β-Chitin and γ-chitin can be received from the squid pens and yeast respectively (Campana-Filho *et al.,* 2007). The α-chitin can be obtained from β-Chitin through an alkaline process followed by passing through water (Noishiki *et al.,* 2003). Numerous methods and procedures are used to obtain the chitin from different sources and earlier research published. The composition of the crustacean shells is proteins (30%–40%), calcium carbonate (30%–50%), chitin (20%–30%), and pigments. The above-mentioned composition of crustacean shells is varying depends on the different species and also from season to season (Aranaz *et al.,* 2009). There are various steps like washing, grinding, and sieving followed by the removal of different minerals like calcium carbonate in dilute acidic acid and known as demineralization then deproteinization (NaOH or KOH) is used to obtain the chitin from the crustacean

shells chemically and deproteinization process, enzymatic hydrolysis is used (Kumirska *et al.,* 2010). In addition, it is also reported that different microorganisms are also used for demineralization and deproteinization (Kumirska *et al.,* 2010).

Chitosan

The chitin is radially converted into chitosan through partial or complete de-acetylation of the chitin in solid and dissolved under enzymatic hydrolysis (chitin deacetylase) or alkaline condition in industries. Chitosan is produced from the natural source of chitin, and it affects the production parameters and preparation. Different studies showed that the β-chitin form is higher reactive in N-deacetylation compared to α-chitin (Kurita *et al.,* 1993). The change in chitin morphology semi-crystalline is due to the chitosan received in a solid-state reaction having a heterogeneous distribution of N-acetyl groups along the molecular chains (Gandra *et al.,* 2016). The change in the process of chitosan production like temperature, alkali concentration, and alkali ratio to shell also means that the production of chitosan contains different chitosan in molecular weight and N-acetylation degree. Different types of impurities are added in the preparation of the chitosan from the chitin like heavy metals, protein, acid, and alkaline residues. Different studies proposed that the impurities content and different factors like weight-average molecular weight, polydispersity, degree of N-acetylation (DA), and pattern of acetylation (PA) in commercially available chitosan are unknown (Weinhold *et al.,* 2009). Chitosan microstructure knowledge is vital for

understanding the structural properties and activity relationships in them, and distinct importance in this respect is cited in the biomedical use of chitosan (Weinhold *et al.,* 2009). In addition, the biological, synthesized, active, and water-soluble derivatives of chitin and chitosan are also essential.

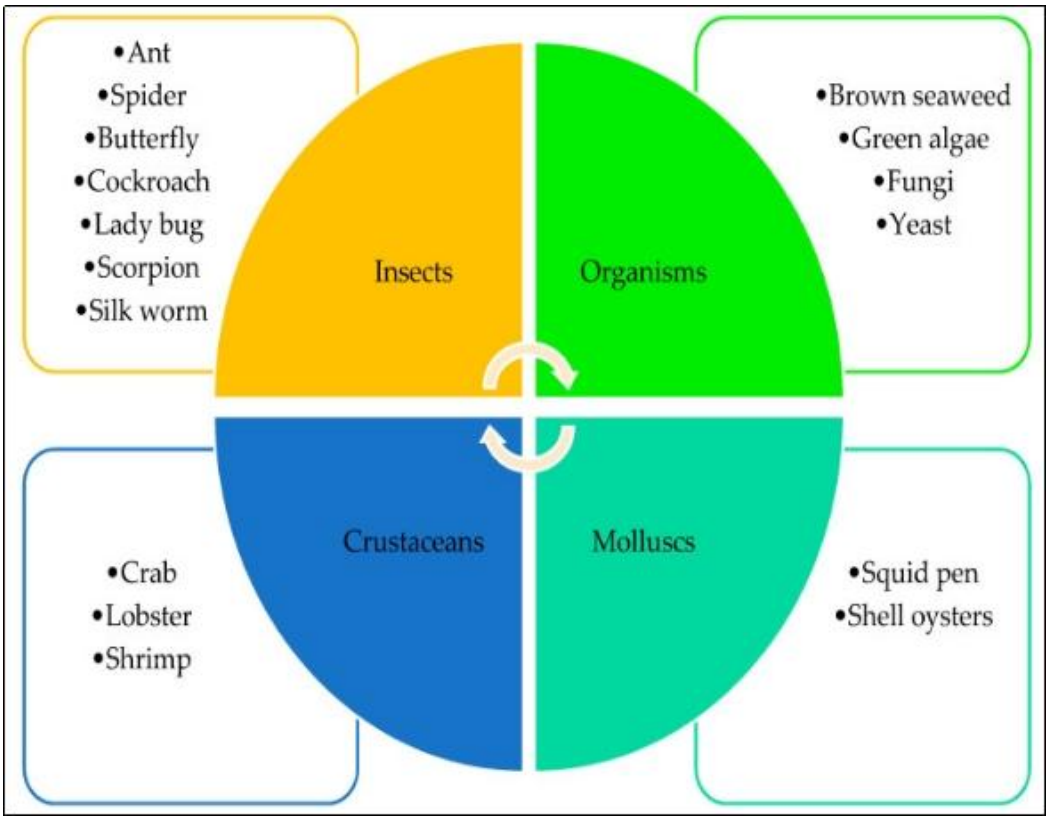

Figure 3: Sources of chitin production (Santos *et al.*, 2020).

2.4 Chitin and Chitosan Properties

2.4.1 Chitin/chitosan physico-chemical properties

Color and appearance: chitin is colorless to off-white, hard, inelastic, nitrogenous Chitin/Chitosan (Madhavan, 1992).

Degree of polymerization (DP): ranging between 2000 biomedical, agricultural, environmental and Industrial and 4000.

Degree of deacetylation (DDA): The degrees of deacetylation in chitin usually range from 5-15% and in chitosan from 70-95%, the higher degree of acetylation (DA) of chitin the lower solubility in common solvents (Kurita, 2001).

Crystallinity: is the hydrogen bounding formed between the arranged strands of chitin, the higher crystallinity the more stability for chitin molecules and the degree of crystallinity is a function of the degree of deacetylation (Rathke, 1993).

Molecular weight: the chitin molecular weight average ranges from 1.03×106 to 2.5×106 Dalton but the conversion of chitin to chitosan by deacetylation reduce it to 1 105 to 5×105 (Lee, 1974).

Solubility: chitin is hydrophobic (water insoluble) as well as in most organic solvents. In contrast chitosanss+ is soluble in dilute organic acids at low pH.

Chemical reactivity: because they have amino and hydroxyl groups which they easily substituted with other groups. Processability: chitin can easily processed into gels, beads, powders, fibers, membranes, cotton, flakes, sponges, colloids, films, spins.

Processability: chitin can easily processed into gels, beads, powders, fibers, membranes, cotton, flakes, sponges, colloids, films, spins.

Stability: they are remarkably stable in concentrated alkaline solutions even at high temperatures.

Chelating activity: because they are high basic polysaccharide.

2.4.2 Chitin and Chitosan Biological Properties

Biodegradability: eco-friendly and non-toxic or from seafood allergic.

Biocompatibility: they have no antigenic properties animal but also with plant tissues.

Bioactivity: bacteriostatic, haemostatic, immunologic, analgesic, cicatrizant, anti-ulcer, such as anticoliac-diseases, anti-inflammatory, hypeuricemic, hypocholesteroloemic, free radical scavenging activity, anticoagulant, anti-gastritis, anti-thrombogenic, antiviral, antibacterial, antifungal, anti-tumor, spermicidal etc (Okamoto *et al.,* Nagahama *et al.,* 2022).

2.5 Chitosan Production from Different Sources

2.5.1 Crustacean shells as a source of chitosan

Chitosan is traditionally extracted from the exoskeletons of crustaceans, such as periwinkle shells, Snails shells and crab shells, which represent a significant waste stream from the seafood processing industry (Sagheer *et al.,* 2009). This process involves acid treatment to dissolve the calcium carbonate, followed by alkaline treatment to remove proteins and pigments. However, the heavily mineralized exoskeletons make the extraction process arduous (Duan *et al.,* 2019).

2.5.2 Insect biomass as a source of chitosan

Chitin and chitosan can also be obtained from the cuticles of insects, such as beetles. The properties of insect-derived chitosan are generally similar to those obtained from crustaceans (Philibert *et al.,* 2017).

2.5.3 Fungal cell walls as a source of chitosan

Chitin and chitosan are also found in the cell walls of various fungi, including Zygomycetes, which can contain larger amounts of these polysaccharides compared to other fungal classes (Merzendorfer, 2011; Dhillon *et al.,* 2013). The chitin and chitosan in fungal cell walls are synthesized by chitin synthase and chitin deacetylase enzymes, respectively (Dhillon *et al.,* 2013). Fungal-derived chitosan has been found

to be free of the allergens present in crustacean-derived chitosan, making it a suitable alternative for biomedical and food applications (Li *et al.,* 2012).

2.6 Role of Chitin and Chitosan in the Digestibility of Ruminant Animals

Various research findings suggest that these biopolymers (Chitin and chitosan) play a significant role in enhancing digestion and overall production performance in ruminants (Shah *et al.,* 2022)

Several studies have demonstrated that supplementing chitin and chitosan improves feed intake and nutrient digestibility in dairy cows, lambs, and sheep. Research by Zhang *et al.* (2022) indicated that the inclusion of seleno-chitosan enhanced the growth rate and wool production in Chinese Marino sheep while also improving blood parameters. Similarly, the supplementation of chitosan in lamb diets resulted in increased feed intake, enhanced digestibility of neutral detergent fiber, dry matter, and crude protein, as well as improved nitrogen balance and microbial protein production (Pereira *et al.,* 2018). However, despite these benefits, no significant effect was observed on the overall production performance of feedlot lambs.

The impact of chitosan on rumen fermentation has been found to vary depending on the species and dietary conditions. Wencelova *et al.* (2014) reported that chitosan supplementation in sheep diets reduced total ruminal protozoa count and improved

rumen fermentation, which could contribute to enhanced nutrient breakdown and absorption.

2.7 Benefits of Chitin and Chitosan in Ruminants

2.7.1 Improvement in digestibility and rumen fermentation

Chitosan has demonstrated its potential as a rumen fermentation modulator, significantly enhancing nutrient digestibility and altering fermentation patterns. Supplementing beef cattle diets with chitosan improved the digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry matter (DM). Additionally, chitosan supplementation increased the production of total volatile fatty acids (VFAs), a key energy source for ruminants, in vitro batch cultures (Dias *et al.,* 2017).

Shah *et al.* (2022) reported that chitosan shifted the fermentation pattern in the rumen from acetate production toward propionate, improving energy efficiency. Similarly, Dias *et al.,* (2017) found that chitosan supplementation increased ruminal pH in fermentation trials with a concentrate-to-forage mixture of 20:80, indicating its ability to mitigate the acidifying effects of high concentrate diets.

2.7.2 Enhancing feed efficiency and animal performance

For dairy cows, chitosan supplementation has been linked to enhanced milk production due to its impact on energy utilization **(**Shah *et al.,* 2022**).** Lambs supplemented with chitosan exhibited increased feed intake, better digestibility of

crude protein and fiber, improved nitrogen balance, and enhanced microbial protein synthesis, although the effect on production performance was variable (Zhang *et al.,* 2022)

In sheep, the use of seleno-chitosan improved growth rates, wool production, and blood parameters, as observed by Zhang *et al.,* (2022). These findings highlight the potential of chitosan to support animal growth and production across different ruminant species.

2.7.3 Antimicrobial properties and methane reduction

Chitosan's antimicrobial activity, attributed to its interaction with negatively charged bacterial surfaces, leads to changes in membrane permeability and intracellular leakage, resulting in bacterial death (Dias *et al.,* 2017). This mechanism not only aids in rumen microbial modulation but also reduces the reliance on antibiotics, addressing concerns over antimicrobial resistance and residues in animal products (Barton *et al.,* 2000). Furthermore, chitin from black soldier flies has been shown to decrease methane emissions in vitro, as demonstrated by Jayanegara *et al.,* (2017). This property makes chitin and chitosan attractive options for reducing the environmental impact of ruminant production systems.

2.8 Snail Shells as a Source of Chitin and Chitosan

Figure 4: ***Achatina fulica***

Source: Egbeneje *et al.* (2024).

Waste generated from the shell of snails is the main precursor for the production of chitosan, but there is limited existing work on the use of the aforementioned shell during chitosan production processes. *Athena archatina* (tiger snail), *Archatina fulica* (land snail), *Archachatina marginata* (African giant), *Limcolana aurora* species, and other garden snails are distinct varieties of snails commonly found in the southern part of Nigeria, while the most commonly consumed as a delicacy is *A. fulica*, which belongs to the family *Achantinidae* (Oluyemisi *et al.,* 2021; Igbinosa *et al.,* 2016). The presence of a high content of carbohydrates (86.83/100 g–92.76/100 g) in snail shell is responsible for the high yield of chitosan in snail shell compared to the amount recorded in seafood waste (Ademolu *et al.,* 2018). The shell of crustaceans is one of the prominent sources of chitin and chitosan because of the biomaterial in abundance and the extraction process on a well-established industrial scale. The environmental pollution caused by snail shells was caused by the fact that snails are abundant during the rainy season (Ademolu *et al.,* 2018). Restaurants, eateries, hotels, and club houses are the sources of discarded shells of snails that are responsible for a severe degree of threat to the environment (Oluyemisi *et al.,* 2021). After removal of the edible portion of the snail, the shells are indiscriminately abandoned without proper disposal. In 2017, the waste generated from crustacean shells was 8.4 million tonnes (FAO, 2019). As a result of large amounts of shells littering the environment, they have become a nuisance to our immediate environment. Therefore, in a bid to have a clean environment and also to have an economy with massive and immense prosperity, there

is an urgent need to recycle, and the process of recycling results in the production of chitosan through chemical deacetylation of earlier formed chitin (Amoo *et al.,* 2019; Kolawole *et al.,* 2017). Despite the array of work done on chitosan production from different sources, there are limited or no studies on concurrent production as well as characterization of chitosan from snail shells. Also, the production of chitosan involves four sequential preparation processes; the steps involved can be interchanged in a bid to enhance the quantity and quality of chitosan produced. There is a little work on the production of chitosan through the alteration production process, while in most cases; characterization of produced chitosan obtained through the interchange of production processes has not been investigated.

2.8.1 Materials and methods

Figure 5: Sample of washed and sun-dried Snail shells obtained from Esa-Oke

Source: Adekanmi *et al.,* 2023

Materials

Snail shells (*A. fulica*) weighing 2,500 g utilized in this research were purchased from the Gboko Main Market, Benue State, Nigeria in January, 2023. Sodium hydroxide (NaOH 97% purity) from Fizmerk Company, India, nitric acid (HNO3 99% purity; Fizmerk Company, India), hydrochloric acid (HCl 97%) from May and Baker Ltd., Dagenham England, and acetic acid (99.8%) from May and Baker Ltd., Nigeria. Reagents were diluted to the appropriate concentrations required for the analytical procedures with deionized water.

Extraction of Chitosan

Chitosan was prepared according to the method described by Chawla *et al.* (2014). Initially, the snail shells were cleaned of loose tissue, washed thoroughly with distilled water, and dried in a hot air oven at 60 °C for 24 h. The dried shells were then crushed using a mortar and pestle, ground into a fine powder, and sieved through a 250 μm sieve. For demineralization, 400 g of the snail shell powder was weighed using an analytical balance and added to 4% HCl (1.3 N) in a ratio of 1:14 g/ml (w/v). This mixture was vigorously shaken and maintained at ambient temperature (32 °C) for 24 hrs. Afterward the mixture was thoroughly washed with distilled water to achieve neutrality and sieved through a 250 μm sieve. The residue was subsequently washed with deionized water and oven -dried in a tray dryer at 40 °C for 24 hrs. For deproteinization, the demineralized residue (400 g) was treated with 5% NaOH (1.25

N) at a ratio of 1 :12 g/ml (w/v), with constant stirring at 90 °C for 2 h to remove the proteins. The mixture was sieved through a 250 μm sieve and filtered under vacuum. The filtrate was washed with distilled water for 30 min until it reached a neutral pH (pH= 7). The deproteinized residue was then oven -dried at 60 °C for 24 h.

Production of Chitosan from Chitin

Chitosan was prepared according to the method described by Chawla *et al.* (2014). The extracted chitin (2,000 g) with a particle size of 40 μm was transferred to a ceramic mortar. A 50% (w/v) NaOH solution (20 L) was added to the chitin and mixed thoroughly. The ceramic mortar was then centered on a heater and the mixture was heated at 1,400 W for 8 h. Once heated, the mixture was filtered and the residue was rinsed with distilled water until a neutral pH was reached. The residue was then dried in a hot air oven at 40 °C to a constant weight and subsequently stored in high density polyethylene bags for further analysis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of Study

The experimental study was conducted at the University of Benin Farm Project, Benin city, Edo state. The area falls within latitudes 3°8' and 7°8'N and longitudes 3°2' and 9°8'E. The climate is humid and is located in the interface between the tropical rainfall and derived savanna zone of south-south Nigeria. It receives a mean annual temperature of about 31°C and annual rainfall of 2000–2300mm (Obaseki *et al.,* 2018)

3.2 Source of Snail Shells

The snail shells, which served as the primary source of chitin and chitosan for this experiment, were obtained from markets around Benin City. These were thereafter mixed to obtain a homogenous sample. The shells were washed with clean water to remove visceral, sand and other debris. The cleaned shells were air-dried, milled and stored in an air-tight container for further analysis.

3.3 Experimental Animals

Twelve (12) West African Dwarf (WAD) weaners goats were used for this study. Animals were given prophylactic treatment against ectoparasite dipping in solution of

ivermetin, and using tetracycline to treat against endoparasites. The metabolism cages and equipment's were thoroughly washed and disinfected.

3.4 Experimental Procedure and Design

The goats were randomly assigned to the six experimental diets, with two animals per dietary treatment. The experiment was carried out using a Completely Randomized Design (CRD).

3.5 Experimental Treatments

Six (6) experimental diets were formulated comprising of varying levels of additives. Each diet was fed at 50% supplementation level to guinea grass (*Meathyrsus maximus*) at 50% inclusion level

The six (6) experimental treatments are as follows:

T_1= 50% concentrate without additive (Control) +50% guinea grass

T_2=50% concentrate containing 3% chitin + 50% guinea grass

T_3= 50% concentrate containing 6% chitin +50% guinea grass

T_4= 50% concentrate containing 0.5% chitosan +50% guinea grass

T_5= 50% concentrate containing 1% chitosan +50% guinea grass

T_6= 50% concentrate containing 0.01 tetracycline +50% guinea grass

Table 3.1: Feed Ingredients Composition of Experimental Diets containing varying Levels of Chitin and Chitosan from Snail Shells

Ingredients	Snail					Antibiotics
	Chitin		Chitosan			
	T_1 (Chitin 3.0%)	T_2 (Chitin 6.0%)	T_3 (Chitosan 1.0%)	T_4 (Chitosan 0.5%)	T_5 (Control)	T_6 Antibiotics
Maize	21	21	21	21	21	21
SBM	2	2	2	2	2	2
Wheat bran	32.5	22	40	42	43	44
PKC	34	41.5	28.5	27	26.5	25.49
Chitin (Snail)	3	6	0	0	0	0
Chitosan (Snail)	0	0	1	0.5	0	0
Antibiotic (Tetracycline)	0	0	0	0	0	0.01
Bone meal	1	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix	1	1	1	1	1	1
Total	100	100	100	100	100	100
CP (16-18)	16.505	16.4435	16.515	16.5185	16.5665	16.50937
CF (10-12)	10.508	10.7705	10.233	10.2955	10.2453	10.16875
ME (2500kcal)	2651.12	2613.62	2576.12	2576.12	2651.12	2646.5652

3.6 Digestibility Study

The animals were confined in a metabolic cage for seven days period. Fecal and urine samples were collected daily in the morning before feeding from trays fixed into the cages. 1mL of 20% normality sulphuric acid was added to the urine sample collected daily to kill the microbes and prevent volatilization of ammonia. Faeces and urine collected were stored in the refrigerator for further study. 10% of faeces collected after the end of digestibility study was oven dried at 100°C, to constant weight for further analysis.

3.7 Chemical Analysis

Samples of concentrate diets, faeces and urine were analyzed for proximate composition using the AOAC (2010) procedures and cell wall components (neutral detergent fiber, acid detergent fiber, hemicellulose) were determined using Van soest *et al* (1991).

1. Determination of ash

Two grams of the samples was weighed into a known-weight crucible and ignited in the muffle furnace at 550 degrees Celsius for six hours. Following removal from the furnace, the crucibles were allowed to cool in a desiccator before being weighed. Organic matter was calculated by subtracting the value of ash from 100%.

2. Determination of crude protein

Kjeldahl's technique was utilized to determine the crude protein content. A conical flask was filled with 0.5 grams of the sample, 10 milliliters of 72% concentrated sulfuric acid, and 2 grams of mixed catalyst ($CuSO_4$:$NaSO_4$ at a ratio of 1:9). The materials were heated to 4200c in order to achieve a light green digest. The digest was added to a 120 mL universal reagent bottle and diluted to the 50 mL mark with distilled water. Distillation was performed by adding 5 milliliters of the digest to 10 milliliters of 40% NaOH and 30 milliliters of distilled water in a Kjeldahl flask. 5 milliliters of boric acid indicator were made from this. The distillate was collected in around 20 mL, and 0.1N HCL was used to titrate it. This was done for each treatment.

The Crude protein was calculated using the formulae below:

%CP = NA x 14 / 1000 x VA x 100/ W x 100 / 5 x 6.25

Where NA = Normality of acid

VA = Volume of acid

W = Weight of the sample

3. Determination of crude fiber

A known weight (1g) of the sample was placed in a beaker, and 100ml of 1.25% H2SO4 was added. The mixture was placed on a heating mantle at a temperature of

70^0C to 100^0C. After boiling, the sample was heated for 30 minutes and topped with hot water to maintain the 100ml mark. After heating, the mixture was filtered through a fiber cloth and the residue was rinsed with hot water until a clear solution was obtained. The residue was then returned to the beaker, and 100ml of 1.25% NaOH was added. The liquid was returned to the heating mantle at the same temperature and, once boiling, cooked for 30 minutes while maintaining the 100mll mark by topping with hot water. After heating, the mixture was filtered through a fiber cloth and the residue was rinsed with hot water until a clear solution was obtained. The sample was placed in a 70^0C laboratory oven and dried for 30 minutes. After drying, the samples were placed in crucibles and weighed before being heated in the muffle furnace at 550 degrees Celsius for 30 minutes. Following their removal from the furnace, the crucibles were allowed to cool in a desiccator before being weighed.

The formula for calculating crude fiber is:

$$\%CF = \frac{Weight\ of\ crude\ fiber}{Weight\ of\ sample}\ x\ 100$$

4. Determination of ether extract

The principle is based on the solubility of fats and fatty substances in organic solvents. A known weight (2g) of the sample was placed in a known weight filter paper, and the Crude e lipid was then extracted with petroleum ether using a Soxhlet extractor. After extraction, the solvent is distilled off and the oil residue is dried in an oven for 1 hour

and 30 minutes at 75°C. The dried residue is then cooled in a desiccator and weighed. The percentage of crude lipid is computed using the initial sample weight. Hydrolysis with boiling HCl is required for animal-derived materials that are high in fat prior to extraction. One sample is extracted at a time, but if crude lipid is required for additional examination. However, for crude lipid content only, many samples can be extracted. The weight of the crude lipid is calculated as the difference between the weight of the sample before and after extraction.

The formula for calculating Ether Extract is:

%EE = weight of oil / weight of sample x 100/

5. Determination of nitrogen free extract

NFE is made up of sugars, starch, and some hemicellulose. It is calculated by subtracting the ash, protein, fat, and crude fiber from the dry matter total is subtracted from 100. Therefore, it includes the cumulative mistakes of the other determinations (not a precise value).

NFE (%) = 100 - (ash% + CF% + EE% + CP%)

NB: The NFE fraction comprises not only critical nutrients, but also organic acids, resins, tannins, pigments, and water-soluble vitamins. It is utilized for the provision of energy since it contains non-structural carbohydrates.

3.8 Statistical Analysis

Data collected were analyzed using the Genstat 12th Edition. Mean separation was done using the Duncan Multiple Range Test (DMRT) in the same software.

CHAPTER FOUR

RESULT

4.1 Chemical composition of diets with inclusion of different levels of chitin and chitosan from snail shells

Table 1 shows the chemical composition of diets containing chitin and chitosan feed additives from snail shell. The chemical composition includes dry matter, ash content, crude fiber, crude protein, ether extract, organic matter and nitrogen free extract.

Dry matter

The dry matter content had a range of 91.09 - 91.85%. The highest dry matter was recorded in Control diet (T1) (91.85), while the lowest dry matter was recorded in 0.5% snail (91.09) (T4) The dry matter content of T1 was not significantly different from other diets except for diet with 0.5% chitosan (T4).

Ash content

The percentage of ash in the varying diets ranged from 8.50-17.00, with the highest percentage of ash found in 6% snail chitin diet (T3) (17. 00%) and the lowest concentration (%) present in 0.5% snail chitosan (8.50%) there is significant difference between them($P<0.05$).

Crude fiber

Crude fiber content (%) had a range of 18.50–26.00 with the highest concentration of crude fiber present in 6% snail chitin (T3) (26.00) and the lowest concentration present in control diet (T1) (18.50). There is significant difference between them ($P<0.05$).

Crude protein

The crude protein which is one of the most important components needed for proper growth and development had a range of 14.88 -19.25% with the lowest concentration present in 6%snail chitin (14.88%) and the highest present in 3% snail chitin (T2) (19.25%). There was no significant difference ($P>0.05$) between diets.

Ether extract content

The ether extract content (%) had a range of 8.50-12.00, with antibodies diet containing the lowest amount (8.50) 6% snail chitin (T3) the highest value of ether extract (12.00). There is significant difference ($P<0.05$) between diets

Nitrogen free extract

The nitrogen free extract (NFE) content (%) ranged from 30.12-42.31. The highest percentage of NFE was present control diet (42.31) while the lowest was present in 6% snail chitin (T3), there was significant difference ($P<0.05$) between diets.

Organic matter

The highest percentage (91.50%) of organic matter was present in 0.5% snail chitosan (T4) and the lowest amount (83.00%) was present in 6% snail chitin diet (T3). There was significant difference ($P>0.05$) between diets.

Table 4.1: Chemical composition of diets fed to goats with inclusion levels of chitin and chitosan

Composition %	T_1	T_2	T_3	T_4	T_5	T_6	SEM
DM	91.85a	91.47ab	91.84a	91.09b	91.55a	91.10b	0.123
CF	18.50b	22.00ab	26.00a	22.50ab	21.00ab	22.00ab	1.658
ASH	11.50b	15.50a	17.00a	8.50c	13.00b	11.50b	0.577
CP	17.94a	19.25a	14.88a	17.50a	15.75a	18.38a	1.526
EE	9.75bcd	10.75b	12.00a	9.25cd	10.25bc	8.50d	0.354
NFE	42.31a	32.50b	30.12b	42.25a	40.00a	39.62b	0.818
OM	88.50b	84.50c	83.00c	91.50a	87.00c	88.50b	1.260
NDF	25.00c	60.00b	93.00a	61.00b	69.50b	22.00c	4.17
HEMI	11.00b	40.70a	39.05a	45.70a	30.40a	14.15b	4.43
ADF	14.00d	19.30c	53.95a	15.30cd	39.10b	7.85e	1.233

SEM=Standard error of means, T1= 50% guinea grass + 50% concentrate without chitin and chitosan, T2=50% guinea grass + 50% concentrate with 3% snail chitin, T3=50% guinea grass + 50% concentrate with 6% snail chitin, T4-50% guinea grass + 50% concentrate with 0.5% snail chitosan, T5=50% guinea grass + 50% concentrate with 1% snail chitosan, 6=50% guinea grass + 50% concentrate with 0.01% oxytetracycline, OM= organic matter, CF= crude fiber, CP= crude protein, NFE= non-fiber extract, EE• Ether extract, ASH=Ash, NDF= neutral detergent fiber, HEMI = hemicellulose, ADF= acid detergent fiber

4.2 NDF utilization of diets fed to goats with inclusion levels of chitin and chitosan

Variable	T_1	T_2	T_3	T_4	T_5	T_6	SEM
CONC. DMI	186.3a	171.6a	205.6a	199.6a	208.2a	166.8a	14.16
GRASS DMI	168.1a	173.3a	181.8a	181.4a	179.2a	162.5a	13.48
TOTAL DMI	354.4a	344.9a	284.4a	381.0a	387.4a	329.3a	43.1
NDF CONC DMI	46.6d	103.0c	191.2a	117.8bc	131.2b	36.7d	7.77
NDF GRASS DMI	104.2a	107.4a	112.7a	112.5a	111.1a	104.2a	8.36
NDF TOTAL INTAKE	150.8c	210.4b	304.0a	230.2b	242.2b	137.4c	14.88
NDF DIGESTIBILITY%	66.47a	81.44a	81.12a	83.97a	69.81a	64.17a	7.07
INITIAL BODY WEIGHT	7.915a	7.225a	9.913a	9.145a	8.235a	7.920a	1.220

Means within the same row with different superscript different significantly ($P<0.05$),SEM= standard error of means,T1=50% Guinea grass +50% concentrate without chitin and chitosan,T2=50% Guinea grass+ 50% concentrate with 3% chitin,T3=50% Guinea grass+ 50% concentrate with 6% chitin,T4=50% Guinea grass+ 50% concentrate with 0.5% chitosan,T5=50% Guinea grass+ 50% concentrate with 1% chitosan,T6=50% Guinea grass+ 50% concentrate with 0.01% tetracycline, CONC DMI= concentrate dry matter intake, GRASS DMI= grass dry matter intake, NDF CONC DMI=neutral detergent fiber concentrate dry matter intake, NDF GRASS DMI= neutral detergent fiber grass dry matter intake, NDF TOTAL INTAKE= neutral detergent fiber total intake, NDF DIGESTIBILITY% = neutral detergent fiber digestibility

4.2.1. Concentrate DMI

Based on the results in table 2 the range of values was from 166.8 - 208.2. Although the animal feeds with 1% Snail chitosan (T5) and 6% snail chitin (T3) had the highest concentrate DMI. While the animal feeds with 0.01% tetracycline (T6) and 3% chitin (T2) had the lowest concentrate DMI. There was no significant differences ($p>0.05$) among treatments.

4.2.2 Grass DMI

Based on the results in table 2 the range of values for grass DMI was 162.5 - 181.8. There was no significant differences ($p>0.05$) among treatments.

4.2.3 Total dry matter intake (Total DMI)

From the results in table 2 the range of values for Total DMI was from 284.4 - 387.4. although the animal fed with diet containing 1% snail chitosan (T5) had the highest dry matter intake 387.4 g, while animals fed diet containing 6% snail chitin had the lowest dry matter intake (T3) 284.4 g. there was no significant differences ($p>0.05$) among treatments

4.2.4 NDF Concentrate DMI

From table 2 the range of values was 36.7- 191.2. The highest concentrate DMI 91.2g) was observed from goats fed with 6% snail chitin (T3) and the lowest Values (36.7g) and (46.6g) was observed from the goat fed with 0.01% tetracycline (T6) and control

(T1) respectively. It was noted that there was significant difference ($p<0.05$) among the treatments.

4.2.5 NDF Grass DMI

Based on the results in table 2 the range of values was 104.2–112.5. There was no significant differences ($p>0.05$) among treatments.

4.2.6 NDF total intake

From Table 2 the range of values for NDF total intake (g) was 137.4-304.0. The highest total intake (304.0g) was observed from goats fed with 6% snail chitin (T3) and the lowest (137.4g) was observed from the goat fed with 0.01% tetracycline (T6). It was noted that there was significant difference ($p<0.05$) among the treatments.

4.2.7 NDF digestibility

From Table 2 Digestibility values ranged from 64.17 - 83.97. The highest NDF digestibility (83.97%) was observed from goats fed with 0.5% snail chitosan and the lowest (64.17%) was observed from goats fed with 0.01% tetracycline (T6). there was no significant differences ($p>0.05$) among treatments.

4.2.8 Initial body weight (Kg)

Based on the results in Table 2, the range of values was 7.225 – 9.913. Although the animal fed with 6% snail chitin (T3) had the highest initial weight (9.913) and the animal fed with 3% snail chitin (T2) had the lowest initial weight (7.225). There was no significant difference ($P>0.05$) in the initial body weight of the animals

CHAPTER FIVE

DISCUSSION

This study evaluated the chemical composition of graded levels of chitin and chitosan derived from snail shells into the diets of goats on the neutral detergent fiber (NDF) digestibility and dry matter intake (DMI) of goats. The observed results are discussed in relation to the chemical composition, concentrate and grass dry matter intake (DMI), NDF intake from sources, total NDF intake, and NDF digestibility percentage.

5.1 Chemical Composition of Experimental Diets

The dry matter (DM) content of all diets was generally high (ranging from 91.09% to 91.85%), indicating good feed preservation and minimal moisture, which is essential for feed preservation and precise nutrient delivery. The values align with the findings of Okoye *et al.,* (2019), who reported that feed ingredients with DM above 90% ensure stable nutrient delivery in small ruminants. Crude fiber content was highest in T3= 6% chitin (26.00%) and lowest in T1= control (18.50%). The range reflects the inherent fibrous nature of chitin, which acts similarly to cellulose.

Ash values indicate the total mineral content, with T3= 6% chitin and T2=3% chitin showing the highest levels (17.00% and 15.50%, respectively) indicating the mineral-rich nature of snail shells, especially calcium and magnesium (Ademolu *et al*., 2018). While T4= 0.5% chitosan showing the lowest ash content (8.50%) indicating a lower-level bioavailable mineral input

Crude Protein (CP) levels ranged from 14.88% to 19.25%, did not differ significantly across treatments, suggesting that chitin and chitosan inclusion did not compromise protein availability. This indicates chitin as a useful additive without compromising the essential protein required for growth and maintenance (Oluyemisi *et al*., 2021).

Ether Extract (EE) was highest in Treatments T3= 6% chitin (12.00%) and lowest in T6=0.01% tetracycline (8.50%), high EE in T3= 6% chitin reflect better fat binding or absorption properties of chitosan at moderate inclusion levels, a trait supported by chitosan's known lipid interaction abilities (Lim *et al.,* 2015).

Nitrogen-Free Extract (NFE), which represents digestible carbohydrates, was highest in T4= 0.5% chitosan and T5=1% chitosan (above 40%), indicating more soluble carbohydrates available for rapid fermentation and energy while it was lowest in T2=3% chitin and T3=6% chitin as a result of higher fiber and ash contents, which diluted the proportion of digestible non-fiber carbohydrates

Organic matter content was highest in T4=0.5% chitosan (91.50%) and lowest in T3=6% chitin (83.00%). The high OM value observed in T4 reflects greater nutrient density and less inorganic residue.

Neutral Detergent Fiber (NDF) NDF values was highest in T3=6% chitin (93.00%) and lowest in T6=0.01% tetracycline (22.00%). However moderate NDF (like in T3=6% chitin) can improve rumination and saliva production.

Acid Detergent Fiber (ADF) representing less digestible fiber was highest in T3=6% chitin (53.95%), which might impair digestibility and T6= 0.01% tetracycline which had the lowest ADF (7.85%), potentially supporting better energy availability.

Hemicellulose (HEMI) content followed a similar trend was highest in T4=3% chitin (45.70%) and lowest in T1= control (11.00%). As hemicellulose is more fermentable than ADF, moderate inclusion (like in T4=0.5% chitosan) supports rumen microbial activity.

5.2 NDF Utilization

In both concentrate and grass DMI there was no significant differences ($p>0.05$) among the treatments. Values ranged from 166.8 g (T6=0.01% tetracycline) to 208.2 g (T5=1% chitosan) for concentrate DMI and from 162.5 g (T6=0.01% tetracycline) to 181.8 g (T3=6% chitin) for grass DMI. These results suggest that the inclusion of chitin and chitosan did not adversely affect the palatability or voluntary intake of the diets by the goats. According to (Zain 2007) who noted that fiber-rich diets supplemented with additives did not significantly influence feed intake in ruminants.

Total DMI varied across treatments, was significantly highest in T5=1% chitosan (387.4 g) and T4=0.5% chitosan (381.0 g) and lowest was T3= 6% chitin (284.4 g). This aligns with the findings of Permana *et al*. (2015), where high fiber-binding

properties of chitosan could interfere with rumen fermentation efficiency at higher doses.

NDF intake from concentrate was significantly different across diets ranging from 36.7 g (T6= 0.01% tetracycline) to 191.2 g (T3=6% chitin), while grass-derived NDF remained relatively constant across all treatments. Total NDF intake followed a similar trend, highest in T3=6% chitin (304.0 g) and lowest in T6= 0.01% tetracycline (137.4 g). This demonstrates that dietary fiber level from concentrates can substantially alter total fiber intake.

NDF digestibility showed notable but statistically non-significant trends, with T4=0.5% chitosan having the highest digestibility at 83.97%, followed closely by T2=3% chitin (81.44%) and T3=6% chitin (81.12%). The lowest digestibility was recorded in T6= 0.01% tetracycline (64.17%). These results indicate that moderate inclusion (like in T4) enhances fiber digestibility, possibly by stimulating rumen microbial activity and improving fiber breakdown. Excessive levels, however, may hinder microbial function due to antimicrobial properties of chitosan (Mulyani *et al*., 2019). Li *et al*. (2021) also found that bioactive compounds like chitosan could modulate rumen fermentation, improving digestibility when use in moderation.

Initial body weights were similar across treatments, ranging from 7.225 kg to 9.913 kg, confirming that all animals were of comparable baseline health and nutritional status, ruling out bias from pre-experimental differences

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The results of this study suggest that incorporating chitin and chitosan derived from snail shells improve fiber utilization and animal performance, contributing to cost-effective and eco-friendly livestock production systems. Goats fed 0.5% and 1% Snail chitosan feed additives provided the best overall balance between fiber content, digestibility, and nutrient intake.

6.2 Recommendation

Based on the findings of this study, it is recommended that optimal inclusion levels of chitin and chitosan in goat diets should be maintained around the levels used in 0.5% and 1% chitosan to enhance fiber digestibility and support rumen efficiency.

The utilization of snail shells as a source Chitin and chitosan can serve as effective natural feed additives in small ruminant diets, contributing to sustainable waste utilization and improved livestock nutrition.

REFERENCES

Adekanmi, A. A., Adekanmi, U. T., Adekanmi, A. S., Ahmad, L. K., and Emmanuel, O. O. (2023). Production and characterisation of chitosan from chitin of snail shells by sequential modification process. *African Journal of Biotechnology*, 22(2), 39-53.

Ademolu, K.O., Akintola, M.Y., Olalonye, A.O., and Adelabu, B.A. (2018). Traditional utilization and biochemical composition of six mollusc shells in Nigeria. *Revista de Biologia Tropical,* 63(2), 459-464.

Adesogan, A. T., Arriola, K. G., Jiang, Y., Oyebade, A., Paula, E. M., Pech-Cervantes, A. A., Romero, J. J., Ferraretto, L. F., and Vyas, D. (2019). Technologies for improving fiber utilization.

Amoo, K.O., Olafadehan, O.A., and Ajayi, T.O. (2019). Optimization studies of chitin and chitosan production from *Penaeus notialis* shell waste. *African Journal of Biotechnology*, 18(27), 670-688

Anggraeni, A.S., Jayanegara, A., Laconi, E.B., Kumalasari, N.R., and Sofyan, A. (2022). Marine by-products and insects as a potential chitosan source for ruminant feed additives. *Czech. Journal of Animal Science*, 67, 295–317.

Aranaz, I., Mengíbar, M., Harris, R., Paños, I., Miralles, B., Acosta, N., Galed, G., and Heras, Á. (2009). Functional characterization of chitin and chitosan. *Current Chemical. Biology*, 3, 203–230.

Barton, M. D. (2000). Antibiotic use in animal feed and its impact on human health. *Nutrition Research Reviews*, 13(2), 279–299

Campana-Filho, S.P., Britto, D.D., Curti, E., Abreu, F.R., Cardoso, M.B., Battisti, M.V., Sim, P.C., Goy, R.C., Signini, R., and Lavall, R.L. (2007). Extraction, structures and properties of alpha-and beta-chitin. *Química Nova*, 30, 644–650.

Carrillo-Díaz, M. I., Miranda-Romero, L. A., Chávez-Aguilar, G., Zepeda-Batista, J. L., González-Reyes, M., García-Casillas, A. C., Tirado-González, D. N., and Tirado-Estrada, G. (2022). Improvement of ruminal neutral detergent fiber degradability by obtaining and using exogenous fibrolytic enzymes from white-rot fungi. *Animals: an open access journal from MDPI*, 12(7), 843. https://doi.org/10.3390/ani12070843

Chawla, S., Cano, A. S., Goyal, P., and Khan, I. (2014). Extraction and characterization of chitin and chitosan from locally available crustaceans. *Journal of the Mexican Chemical Society*, 58(3), 273–277.

Dhillon, G. S., Kaur, S., and Brar, S. K. (2013). Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. *Renewable and Sustainable Energy Reviews*, 27, 789–805

Dias, A. M. M., Lopes, F. F., Resende, F. D. O. C., Lima, L. D., da Silva, J. A., Junior, H. M., and Pereira, R. A. N. (2017). Effects of chitosan and cashew nut shell liquid on in vitro ruminal fermentation and methane emission. *Animal Production Science*, 57(8), 1632–1638

Duan, W., Li, X., Wang, Q., Li, P., and Li, C. (2019). Purification, characterization and protective effect of Acaudina molpadioides polysaccharide against H_2O_2-induced oxidative stress in HaCaT cells. *Food Chemistry*, 277, 554–561.

Duffy, C., O'Riordan, D., O'Sullivan, M., Jacquier, J.C. (2018). In vitro evaluation of chitosan copper chelate gels as a multimicronutrient feed additive for cattle. *Journal of the Science of. Food and Agriculture*, 98, 4177–4183.

Egbeneje, V.O., Swande, P.I., Akpehe, A.A., Enyi, S.O., and Ofukwu, S.E. (2024). Extraction and characterization of chitosan from giant African land snail's shells. *International Journal of Pharmacognosy*, 11(10), 551-57.

El Knidri, H., Belaabed, R., Addaou, A., Laajeb, A., and Lahsini, A. (2018). Extraction, chemical modification and characterization of chitin and chitosan. *International Journal of Biological. Macromolecule*s, 120, 1181–1189.

FAO (2019). An estimated 3 million tonnes of shrimp entered the international trade in 2018. Retrieved from http://www.fao.org/inaction/globefish/market-reports/resource-detail/en /c/1199292/

Gandra, J.R., Takiya, C.S., Oliveira, E.R.D., Paiva, P.G.D., Goes, R.H.D.T., Gandra, É.R.D.S., and Araki, H.M.C. (2016). Nutrient digestion, microbial protein synthesis, and blood metabolites of Jersey heifers fed chitosan and whole raw soybeans. *Revista Brasileira De Zootecnia*, 45, 130–137.

Gondo, Takudzwa. (2025). The role of fiber in ruminant health and dairy productivity.

Hirano, S., Itakura, C., Seino, H., Akiyama, Y., Nonaka, I., Kanbara, N., and Kawakami, T. (1990). Chitosan as an ingredient for domestic animal feeds. *Journal of Agricultural Food Chemistry,* 38, 1214–1217.

Holappa, J., Hjálmarsdóttir, M., Másson, M., Rúnarsson, Ö., Asplund, T., Soininen, P., Nevalainen, T., and Järvinen, T. (2006). Antimicrobial activity of chitosan N-betainates. *Carbohydrates Polymers*, 65, 114–118.

Igbinosa, I.B., Isaac, C., Adamu, H.O., and Adeleke, G. (2016). Parasites of edible land snails in Edo State, Nigeria, *Helminthologia*, 53(4), 331-335.

Ismartoyo, I., Syahriani, S., and Sarwan, S. (2022). The feed ADF and NDF digestibility of goat fed four different diets. *Hasanuddin Journal of Animal Science (HAJAS)*, 4, 41–47. https://doi.org/10.20956/hajas.v4i1.20192

Jayanegara, A., Novandri, B., Yantina, N., and Ridla, M. (2017). Use of black soldier fly larvae (Hermetia illucens) to substitute soybean meal in ruminant diet: An in vitro rumen fermentation study. *Veterinary World*, 10, 1439–1446.

Jiménez-Ocampo, R., Valencia-Salazar, S., Pinzón-Díaz, C.E., Herrera-Torres, E., Aguilar-Pérez, C.F., Arango, J., and Ku-Vera, J.C. (2019). The role of chitosan as a possible agent for enteric methane mitigation in ruminants. *Animals*, 9, 942.

Kolawole, M.Y., Aweda, J.O., and Abdulkareem, S. (2017). *Archachatina Marginata* bio-shells as reinforcement materials in metal matrix composites. *International Journal of Automotive and Mechanical Engineering,* 14(1), 4068-4079

Kumirska, J., Czerwicka, M., Kaczyński, Z., Bychowska, A., Brzozowski, K., Thöming, J., and Stepnowski, P. (2010). Application of spectroscopic methods for structural analysis of chitin and chitosan. Marine. *Drugs*, 8, 1567–1636.

Kung, L. (2014). The role of fiber in ruminant ration formulation. http://ag.udel.edu/anfs/ faculty/kung/articles/role_of_fiber_in_ruminants

Kurita, K. (2001). Controlled functionalization of the polysaccharide chitin. Progress in Polymer Science, 26, 1921–1971.

Kurita, K., Tomita, K., Ishii, S., Nishimura, S.I., and Shimoda, K. (1993). β-chitin as a convenient starting material for acetolysis for efficient preparation of N-acetylchito oligosaccharides. *Journal of Polymer Science. Part A: Polymer. Chemistry*, 31, 2393–2395.

Lee, V. F. (1974). Solution and shear properties of chitin and chitosan (Publication No. 74-29, 446) [Ph.D. dissertation, University of Washington]. University Microfilms.

Li, J., Cai, C., Li, J., Li, J., Li, J., Sun, T., Wang, L., Wu, H., and Yu, G. (2018). Chitosan-based nanomaterials for drug delivery. *Molecules*, 23, 2661.

Li, R. T., Qin, C., Wang, Q., and Li, H. (2012). Chitin and chitosan from terrestrial organisms. In C. Qin, M. Li, and Q. Wang (Eds.), Chitin, chitosan, oligosaccharides and their derivatives: Characterization and production. *Apple Academic Press,* 57–68.

Ma, P., Liu, H.-T., Wei, P., Xu, Q.-S., Bai, X.-F., Du, Y.-G., and Yu, C. (2011). Chitosan oligosaccharides inhibit LPS-induced over-expression of IL-6 and TNF-α in RAW264. 7 macrophage cells through blockade of mitogen-activated protein kinase (MAPK) and PI3K/Akt signaling pathways. *Carbohydrates Polymers*, 84, 1391–1398.

Madhavan, P. (1992). Chitin, chitosan and their novels applications. In Science lecture series. CIFT.

Mahgoub, O., Osman, N. E. H. I. E., and Lu, C. D. (2025). Nutrient Utilization and Requirements in Sheep and Goats Raised Under Different Systems and Fed Low Nutritional Novel Feeds for Meat Production. *Animals: An Open Access Journal from MDPI*, 15(18), 2658. https://doi.org/10.3390/ani15182658

Merzendorfer, H. (2011). The cellular basis of chitin synthesis in fungi and insects: common principles and differences. *European Journal of Cell Biology*, 90(9), 759-769.

Muzzarelli, R. A. (2013). Chitin. Elsevier.

Nagahama, H., New, R., Jayakumar, S., Koiwa, T., Furuike, H., and Tamura, J. (2008). Chitosan and its derivatives for tissue engineering applications. *Biotechnology Advances*, 26(1), 1–21.

National Research Council (2001). Nutrient Requirements of Dairy Cattle: Seventh Revised Edition. The National Press.

Naveed, M., Phil, L., Sohail, M., Hasnat, M., Baig, M.M.F.A., Ihsan, A.U., Shumzaid, M., Kakar, M.U., Khan, T.M., and Akabar, M. (2019). Chitosan oligosaccharide (COS): An overview. *International Journal of Biological Macromolecules*, 129, 827–843.

No, H. K., Meyers, S. P., and Prinyawiwatkul, W.Z. (2007). Application of chitosan for improvement of quality and shelf life of foods: A review. *Journal of Food Science*, 72(5), R87–R100.

No, H. K., Park, N. Y., Lee, S. H., and Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology*, 74(1–2), 65–72.

Noishiki, Y., Takami, H., Nishiyama, Y., Wada, M., Okada, S., and Kuga, S. (2003). Alkali-induced conversion of β-chitin to α-chitin. *Biomacromolecules*, 4, 896–899.

Obaseki, E. E., Akpobo, K. I., and Eguavoen, O. I. (2018). Appraisal of soil quality parameters in rubber plantation land use type in some parts of Edo State, Nigeria. *Nigerian Journal of Applied Science*, 36(2), 245-253.

Okamoto, Y., Watanabe, M., Miyatake, K., Morimoto, M., Shigemasa, Y., and Minami, Y. (2002). Effect of chitin / chitosan and their oligomers/monomers on migration of fibroblast and vascular endothelium. *Biomaterials*, 23(8), 1975–1979.

Oluyemisi, A.B., Aishat, O.S., Ese, M.I., Olatundun, A., Olutayo, A.A. and Lateef, G.B. (2021). Characterization of Chitosan Obtained from Snail Shell (Achatina fulica) and its Excipient Potentials in Metronidazole Tablet Formulation. *Dhaka University Journal of Pharmaceutical Sciences*, 20(1): 31-39

Pereira, E. S., de Oliveira, R. L., de Araújo, M. J., de Carvalho, H. F. N., de Sá, H. C. A.,de Vasconcelos, A. M., and Bezerra, A. D. A. (2018). Effect of chitosan on production performance of feedlot lambs. *Journal of Agricultural Science*, 10(10), 405–415

Petri, D.F., Donegá, J., Benassi, A.M., and Bocangel, J.A. (2007). Preliminary study on chitosan modified glass ionomer restoratives. *Dental Materials*, 23, 1004–1010.

Philibert, A., Lavoie, G., and Fabien, N. H. (2017). Chitin and chitosan: production, properties, and applications in biomedical engineering, a review. *Engineering Journal*, 21(6), 135-163.

Philibert, T., Lee, B.H., and Fabien, N. (2017). Current status and new perspectives on chitin and chitosan as functional biopolymers. *Applied Biochemistry and Biotechnology*, 181, 1314–1337.

Puvvada, Y.S., Vankayalapati, S., and Sukhavasi, S. (2012). Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. *International Current Pharmaceutical. Journal*, 1, 258–263.

Rathke, T. (1993). Determination of the degree of N-deacetylation in chitin and chitosan as well as their monomer sugar ratios by near infrared spectroscopy. *Journal of Polymer Science Part A: Polymer Chemistry*, 31(3), 749–753.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. Progress in Polymer Science, 31, 603–632.

Sagheer, M. A., Shehata, M. R. N., and El-Kafrawy, K. M. H. (2009). Chitosan as a flocculant in wastewater treatment. *Applied Water Science*, 3(1), 1–13.

Shah, A. M., Qazi, I. H., Matra, M., and Wanapat, M. (2022). Role of Chitin and Chitosan in Ruminant Diets and Their Impact on Digestibility, Microbiota and Performance of Ruminants. *Fermentation*, 8(10), 549.

Shah, S. R., Mustafa, M., Farmanullah, K., Ali, S., Rahman, Z. U., Khan, M. A., and Akbar, H. (2022). Meta-analysis of dietary chitosan effects on performance, nutrient utilization, and product characteristics of ruminants. *Animals*, 12(3), 365.

Shen, K.-T., Chen, M.-H., Chan, H.-Y., Jeng, J.-H., and Wang, Y.-J. (2009). Inhibitory effects of chitooligosaccharides on tumor growth and metastasis. *Food and Chemical Toxicology*, 47, 1864–1871.

Singla, A., and Chawla, M. (2001). Chitosan: Some pharmaceutical and biological aspects-an update. *Journal of Pharmacy and Pharmacology*, 53, 1047–1067.

Swiatkiewicz, S., Swiatkiewicz, M., Arczewska-Wlosek, A., and Jozefiak, D. (2015). Chitosan and its oligosaccharide derivatives (chito-oligosaccharides) as feed supplements in poultry and swine nutrition. *Journal of Animal Physiology and Animal Nutrition*, 99, 1–12.

Tajik, H., Moradi, M., Razavi Rohani, S.M., Erfani, A.M., and Jalali, F.S.S. (2008). Preparation of chitosan from brine shrimp (*Artemia urmiana*) cyst shells and effects of different chemical processing sequences on the physicochemical and functional properties of the product. *Molecules*, 13, 1263–1274.

Teng, W.L., Khor, E., Tan, T.K., Lim, L.Y., and Tan, S.C. (2001). Concurrent production of chitin from shrimp shells and fungi. *Carbohydrate Research*, 332, 305–316.

Tertsegha, S., Akubor, P.I., Iordekighir, A.A., Christopher, K., and Okike, O.O. (2024). Extraction and characterization of chitosan from snail shells (*Achatina fulica*). *Journal of Food Quality and Hazards Control*. 11, 186 -196

Weinhold, M.X., Sauvageau, J.C., Keddig, N., Matzke, M., Tartsch, B., Grunwald, I., Kübel, C., Jastorff, B., and Thöming, J. (2009). Strategy to improve the characterization of chitosan for sustainable biomedical applications: SAR guided multi-dimensional analysis. *Green Chemistry*, 11, 498–509.

Wencelová, M., Pristaš, P., Ondruška, Ľ., Juhás, Š., and Javorský, P. (2014). Evaluating the effects of chitosan, plant oils, and different diets on rumen metabolism and protozoan population in sheep. *Veterinarni Medicina*, 59(4), 169–175.

Yoon, H.J., Moon, M.E., Park, H.S., Im, S.Y., and Kim, Y.H. (2007). Chitosan oligosaccharide (COS) inhibits LPS-induced inflammatory effects in RAW 264.7 macrophage cells. *Biochemical and Biophysical Research Communicatios*, 358, 954–959.

Zaharoff, D.A., Rogers, C.J., Hance, K.W., Schlom, J., and Greiner, J.W. (2007). Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination. *Vaccine*, 25, 2085–2094.

Zhang, Q., Han, Y., Yang, Y. and Shen, X. (2022). Effects of the seleno-chitosan on daily gain, wool yield, and blood parameter in the Chinese merino sheep. *Biol. Trace Element Research*, 200, 4704–4711.