DRY MATTER AND NUTRIENT DIGESTIBILITY OF GOATS FED DIETS WITH GRADED LEVELS OF CHITIN AND CHITOSAN FEED ADDITIVES FROM PERWINKLE SHELLS

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CERTIFICATION

This is to certify that this project work was carried out by Aduku Aivemero Great with Matriculation Number AGR2000057 of the Department of Animal Science, Faculty of Agriculture, University of Benin-City, Nigeria.

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DEDICATION

This work is dedicated to God the Father, the Son and the Holy Spirit for His graciousness all through the course of my program in the University of Benin, to my loving parents Mr. and Mrs. Aduku and to my dear siblings.

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Shout out to my friends in the, Osasu, Ebruba, Felix, Fidelis Olaoye and oluchi

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ABSTRACT

The study investigated the chemical composition of graded levels of chitin and chitosan, extracted from periwinkle shells, and their effects on goats dry matter intake and nutrient 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 treatments), fed alongside guinea grass at a ratio of 50:50. Chitin and chitosan were extracted using chemical processes involving demineralization, deproteinization, and deacetylation. Results showed that, dry matter digestibility (91.40–91.85%) was not significantly affected by chitin and chitosan feed additives. Inclusion of chitosan at 0.5% and chitin at 6% levels improved crude protein retention, nitrogen balance, and overall nutrient digestibility, while higher chitosan levels (1%) resulted in greater urinary nitrogen losses and reduced protein utilization efficiency. Ether extract values increased with higher chitin levels, reaching 12.00% in the 6% chitin diet, implying improved energy density. Nitrogen-free extract decreased significantly with higher chitin inclusion, indicating reduced readily available carbohydrate fractions. Organic matter content was highest (91.50%) in the 0.5% chitosan diet, suggesting improved nutrient digestibility and energy utilization.

The study concludes that chitin and chitosan at 6% and 0.5% inclusion level respectively from periwinkle shells can serve as effective feed additives for enhancing nutrient utilization in goats . This approach offers a sustainable solution for livestock nutrition while addressing environmental challenges posed by periwinkle waste in Nigeria.

CHAPTER ONE

INTRODUCTION

Traditional ruminant livestock production in Africa, particularly Nigeria, is heavily reliant on natural pastures that are often of low nutritional quality, especially during the dry season (Amuda *et al.,* 2019). This scarcity and low quality of indigenous grasses and straws not only hamper production but also create an atmosphere of conflict between herdsmen and crop farmers throughout the year (Amuda *et al.,* 2018). Despite the natural vegetation, a significant shortage of feeds and feedstuffs for livestock persists in Nigeria (Babayemi, 2007). In the tropics, ruminants are mainly raised on grasses that are inherently low in digestibility and nutritional value and become unavailable during the off-season (Babayemi *et al.,* 2009).

Proper nutritional feeding is crucial for achieving good health status, high reproductive success, increased milk yield, and rapid growth rates (Ochepo *et al.,* 2009). In the realm of ruminant nutrition, various feed additives are employed to enhance production efficiency and preserve the overall health and metabolic status of livestock (Shah *et al.,* 2022). Common additives include organic acids, feed enzymes, prebiotics, probiotics, and herbal extracts, but chitosan is a

relatively new and rarely added ingredient in animal feed (Li *et al.,* 2021; Li *et al.,* 2018).

Chitosan is a non-toxic polyglycosamine that is rare in nature, consisting of β-(1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino-D-glucose units. It is a deacetylated form of chitin, which is a component of the exoskeletons of shrimps, crabs, and insects (Singla *et al.,* 2001). Unlike chitin, chitosan is soluble at acidic pH values (Swiatkiewicz *et al.,* 2015) and is digested to a moderate degree within the gastrointestinal tract of monogastric animals (Okamoto *et al.,* 2001). Chitosan is commercially obtained from chitin through a deacetylation process, where chitin is treated with concentrated sodium hydroxide at high temperatures (Singla *et al.,* 2001). Chito-oligosaccharides are produced by the depolymerization of chitosan using methods like acid hydrolysis, physical hydrolysis, and enzymatic degradation (Lodhi *et al.,* 2014).

Chitosan and its oligosaccharide derivatives possess reactive functional groups, including amino acids and hydroxyl groups, which give them a wide range of beneficial properties. Unlike chitin, these compounds show antimicrobial (Zheng *et al.,* 2003; Holappa *et al*., 2006), anti-inflammatory (Yoon *et al.,* 2007; Ma *et al.*, 2011), antioxidant, antitumor, immunostimulatory, and hypocholesterolemic activities (Kim *et al.,* 2007; Shen *et al*., 2009; Zaharoff *et al*., 2007; Liu *et al.,*

2008). The incorporation of chitosan into the diets of ruminants holds great potential for improving feed efficiency, enhancing growth, and bolstering the animals' immune systems, thereby mitigating the challenges of low-quality feed and environmental stressors.

1.2 Justification of this study

Ruminant livestock production in tropical regions like Nigeria is significantly hindered by nutritional deficiencies, a persistent challenge to agricultural productivity and economic growth. Traditional feeding practices, which rely on natural pastures, are often insufficient due to the poor nutritional quality of these plants, a problem that intensifies during the dry season. This long-standing inadequacy is a major factor preventing the full realization of sustainable livestock farming.

A potential solution lies in exploring novel feed additives. Chitosan, derived from chitin, is a particularly promising candidate. However, there is a significant knowledge gap regarding the specific effects of chitin and chitosan on the digestibility of nutrients in ruminants. The current study aims to address this gap by investigating the dry matter and nutrient digestibility of chitin sourced from periwinkle shells.

The use of by-products like marine shells to create beneficial feed additives aligns with the principles of a circular economy. This approach not only tackles nutritional challenges in agriculture but also promotes environmental sustainability by transforming waste materials into valuable resources. This research seeks to provide farmers with a practical and cost-effective solution to improve livestock productivity. By enhancing nutrient digestibility and promoting animal well-being, this study offers a comprehensive strategy for agricultural progress. In the context of global challenges like climate change and limited resources, such scientific inquiry is crucial for developing adaptive and effective agricultural methodologies that benefit both human and animal communities.

1.3 Objectives of this study

The objectives of this study were to determine;

1. The chemical composition of diets containing graded levels of chitin and chitosan feed additives from periwinkle shells.

2. The crude protein digestibility of chitin and chitosan feed additive fed diet.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical development of chitin and chitosan research

The study of chitin and chitosan began with French chemist Henri Braconnot in 1811, who was the first to separate and characterize "fungine" (later identified as chitin) from fungal species using an aqueous alkali treatment (Santos *et al.*, 2020). Further progress was made in 1843 when Lassaigne demonstrated the presence of nitrogen in the structure of chitin by studying the exoskeletons of the silkworm, *Bombyx mori* (Santos *et al.,* 2020).

The discovery of chitosan followed in 1859, a breakthrough achieved by treating chitin with heated potassium hydroxide. Over the next few decades, its chemical composition became clearer. In 1878, Ledderhose proposed that chitin contained compounds such as glycosamine and acetic acid, a claim confirmed by Gilson in 1894, who verified the presence of glycosamine units. That same year, the German scientist Felix Hoppe-Seyler officially named the compound chitosan. The full chemical structure of chitosan was finally determined in 1950 (Synowiecki *et al,* 2003).

By the 1970s, the commercial production of chitosan had begun, with the first reports emerging from Japan and the United States. This industry saw rapid growth, and by 1986, Japan alone had fifteen commercial producers of chitin and chitosan (Santos *et al.,* 2020). Today, Japan and the United States remain global leaders in chitosan production and research, driven by its wide range of profitable applications (Mehebub *et al.,* 2019).

In Brazil, research into chitosan from fungi began in 1983, confirming its presence in the cell walls of fungi from the Zygomycetes and Mucorales classes. The first documented commercial production and sale of chitosan in the country were reported by Craveiro and Queiroz in 1999 (Craveiro *et al.,* 2004).

2.2. Chitin

Chitin, a polymer with a chemical structure composed of β-(1,4) bonds linking N-acetyl-2-amino-2-deoxyglucose (GlcNAc) units, is a fundamental building block in nature. It serves as the primary fibrous material in the cell walls of fungi, the scales of fish and amphibians, the radulae of mollusks, the endoskeletons of cephalopods, and the exoskeletons of arthropods (Fernandes *et al.,* 2008).

While zooplankton cuticles, particularly from Antarctic krill, are believed to be the largest global source of chitin, with an estimated 379 million tons available

(Fernandes *et al*., 2008), these microscopic animals are not commercially viable to harvest. As a result, the main source of chitin for commercial use is considered to be the waste products of the shellfish industry, primarily the shells of shrimp, crab, and lobster, which contain a chitin concentration ranging from 8% to 40% (Gentile *et al.,* 2018).

An alternative source is fungi, which contain a lower chitin content (10–26%) in a chitin-β-(1,3 /1,6) glucan complex (GC), but are gaining increasing attention from both the scientific community and the food industry (Jones *et al.,* 2020).

Chitin is found in crystalline microfibrils, which provide strong reinforcing and protective qualities to the structural components of arthropod exoskeletons and fungal cell walls (Rinaudo *et al.,* 2006). Its widespread distribution in nature makes it a key component of marine invertebrate exoskeletons and the structure of insects, arthropods, and mollusks (Mao *et al*., 2017; Anwar *et al.*, 2019).

Chitin derivatives have significant economic value because they are biodegradable, biocompatible, and come from renewable sources. A profitable way to obtain these high-value compounds is by utilizing the by-products of the crustacean processing industry (Arrouze *et al*., 2019; Seok *et al*., 2018).

These organisms, such as crabs and shrimp, contain chitin as a major structural component (Finney *et al.*, 2008). Interestingly, some animals, like the snail, not only have chitin in their mandibles but also produce the enzyme chitinase in their gut to break it down. This natural process illustrates the potential for sustainably processing this material. By repurposing what would otherwise be considered waste, this approach aligns with the principles of a circular economy, turning a discarded resource into a profitable product.

2.2.1 Chitin biosynthesis

The biosynthesis of chitin begins with a carbon source, typically glucose. According to Santos *et al*. (2020), this process involves a series of enzymatic reactions: glycogen is first converted to glucose-1-P, which then becomes glucose-6-P with the help of phosphomutase. Hexokinase further converts glucose-6-P to fructose-6-P. Next, an aminotransferase uses L-glutamine to transform fructose-6-P into glucosamine-6-P. This is then converted to N-acetylglucosamine-6-P by N-acetyltransferase, which uses acetyl co-A as a substrate. The phosphate group on this molecule is then moved from the 6-P to the 1-P position by phosphoacetylglucosamine mutase. Subsequently, pyrophosphorylase converts N-acetylglucosamine-1-P to UDP-N-acetylglucosamine, using triphosphate as a cosubstrate. The final step is the

formation of chitin from UDP-N-acetylglucosamine in the presence of the enzyme **chitin synthase** (Elieh-Ali-Komi and Hamblin, 2016; Brigham, 2017; Elsoud and El Kady, 2019). The polymer known as **chitosan** is then produced through the deacetylation of chitin (BenBettaieb *et al*., 2014; Xu *et al*., 2020).

2.2.2 Isolation of chitin from natural resources

Seafood processing generates a significant amount of waste, which, if not properly managed, can lead to environmental contamination. This waste attracts insects, produces unpleasant odors, and can pose a risk to human health (Souza *et al*., 2015; Abreu *et al*., 2017). The effluents from these industries, characterized by high concentrations of nitrogen, phosphorus, organic carbon, and other substances, can cause physical and chemical changes in water bodies, harming aquatic life and local ecosystems (Arrouze *et al.,* 2019).

However, seafood waste represents a valuable source for **chitin extraction** (Casadidio *et al*., 2019). The properties of the extracted chitin and chitosan, such as their molecular weight, degree of deacetylation, purity, viscosity, and crystallinity, can be highly variable due to the natural origin and processing methods (Ibitoye *et al.,* 2018; El Knidri *et al*., 2018). This variability directly impacts their potential applications. Utilizing this waste for chitin extraction

offers a dual benefit: it addresses a major environmental problem while creating a valuable resource from materials that would otherwise be discarded.

2.2.3 Chemical extraction of chitin and elimination

The chemical extraction of chitin and its removal process employ a powerful alkaline solution, such as sodium hydroxide, at high temperatures and concentrations to hydrolyze and break down polymeric chains. This process also leads to a high degree of deacetylation of chitosan (Henry García *et al.,* 2019). The conventional chemical extraction method consists of three fundamental stages: using an alkaline solution for deproteinization, an acid solution for demineralization, and a final step for discoloration. All of these stages are directly linked to the physicochemical properties of the final chitin product (Ali *et al.,* 2018; Küçükgülmez, 2018). A major drawback of this method is the potential for waste disposal issues, as the wastewater produced must be neutralized and detoxified (Doan *et al.,* 2019).

2.2.4 Chemical demineralization

When it comes to chemical demineralization, strong acids are used to remove minerals, particularly calcium carbonate (Sugiyanti *et al.*, 2018). The acids most frequently employed for this treatment include hydrochloric, sulfuric, acetic, nitric,

and formic acids (Samrot *et al.,* 2018; Ali *et al.,* 2019; Tanabtabzadeh *et al.,* 2019). This process works by converting calcium carbonate into calcium chloride, which releases carbon dioxide, as illustrated in the reaction: 2HCl+CaCO3 →CaCl2 +H2 O+CO2 ↑ (Buanasari *et al*., 2019).

2.2.5 Chemical deproteinization

Deproteinization, a key step in purifying polysaccharides, works by breaking the chemical bonds that link proteins and chitin, a process that relies on chemicals to depolymerize the biopolymer (Yadav *et al.,* 2019; Avelelas *et al.,* 2019; Zeng *et al.,* 2019). The traditional method for extracting chitin from marine waste uses strong acids and bases at high temperatures. This approach is energy-intensive and produces highly concentrated chemical waste, which must be treated for neutralization (Lopes et al., 2018).

The utilization of strong acids and bases in the chitin extraction process results in higher material costs and yields a final product of lower purity (Broquá *et al.,* 2018).

2.2.6 Discoloration

This step is an optional part of the extraction process, carried out when a colorless product is desired, as its purpose is to remove astaxanthin and β-carotene

pigments present in the extraction source. It involves the use of organic or inorganic solvents such as acetone, sodium hypochlorite, and hydrogen peroxide (El Knidri *et al.,* 2018)**.**

2.2.7 Biological extraction

The biological extraction method utilizes microorganisms that generate enzymes and organic acids at a relatively low cost, offering a cleaner and more environmentally friendly process that promotes the production of high-quality chitin (Chakravarty *et al.,* 2018; Aranday-García *et al., 2019). This* approach has become increasingly attractive due to its ability to yield high-quality products at an affordable production cost, without producing high concentrations of chemical effluents as seen in chemical extraction methods (Gachhi and Hungund, 2018). Common biological techniques used for chitin extraction include enzymatic deproteinization and fermentation with microorganisms (Dun *et al.,* 2019; Marzieh *et al.,* 2019).

2.2.8 Enzymatic deproteinization

Enzymatic deproteinization of fishing industry waste to obtain hydrolyzed protein is a technique that relies on the addition of enzymes to fragment proteins, offering the advantage of avoiding the generation of environmentally harmful degradation products (Lopes *et al*., 2018; Marzieh *et al.,* 2019). Proteases play a vital role in

the removal of proteins during the chitin extraction process from fishing industry waste (Doan *et al*., 2018). The proteases commonly used for protein removal from seafood residues include papain, trypsin, pepsin, alcalase, and pancreatin (Admassu *et al.,* 2018; Zamora-Sillero *et al*., 2018).

2.2.9 Fermentation

Enzymes produced by lactic acid bacteria are activated by a low medium pH and can hydrolyze proteins, yielding hydrolyzed proteins (Castro *et al.,* 2018). This method offers the benefit of recovering value-added by-products like proteins, enzymes, and pigments, which have potential applications, notably in the food industry (Castro *et al.,* 2018).

The effectiveness of microbial fermentation relies directly on several factors: the inoculum quantity, the glucose level in the medium, the culture pH, and the fermentation duration (Abirami and Nagarajan, 2018). This type of microorganism-based extraction is a growing focus in biotechnology and bioremediation research (Abirami and Nagarajan, 2018).

Fermentation can be carried out using various protease-producing bacteria, including *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*,

and *Serratia marcescens* (Ghorbel-Bellaaj *et al*., 2018; Gong *et al.,* 2019; Navarrete-Bolaños *et al.,* 2020).

2.3 Chitosan

Chitosan is a **linear polysaccharide** defined by its fundamental chemical structure: a chain composed of two repeated units, **D-glucosamine** and **N-acetyl-D-glucosamine**, which are connected through characteristic β-(1, 4) **linkages** (Shah *et al.,* 2022). Its categorization relies on intrinsic properties such as **molecular weight, viscosity, and degree of deacetylation (DDA)** (Jiménez *et al*., 2019). Widely regarded as a valuable **natural compound**, chitosan possesses numerous beneficial attributes, including **biocompatibility, non-toxicity, biodegradability, and bioactive mucoadhesive** capabilities. These features contributed to its early adoption as a food product in Japan in 1983.

As the **second most common polysaccharide in nature,** chitosan is a **polycationic polymer** characterized by its high molecular weight. Although naturally present in the **exoskeletons** of insects, mollusks, crustaceans, and some algae, the vast majority of commercially relevant chitosan is harvested from **marine crustaceans** (Li *et al*., 2018). The global extraction of chitin from crustacean shells generates an estimated (106–107 tons), of waste annually. The

utilization of this waste for extracting chitosan and various proteins significantly enhances the overall economic value of crustacean processing (Teng *et al.,* 2001).

The versatility of chitosan allows for its application across a broad spectrum of industries, where it can be used independently or combined with other polymers. Its applications include inoculants of silage, **food processing and preservation,** biotechnology, **water treatment**, advanced materials for **tissue engineering**, the cosmetic industry, and pharmaceuticals and textiles (El Knidri et al., 2018). A modern and growing application involves its use in **animal feeding** for ruminants, particularly beef and dairy cattle, where it has been shown to successfully improve **rumen fermentation and overall digestibility** (Gandra *et al*., 2016).

Chitosan extraction can be performed using either **biological or chemical methods** (Shah *et al.,* 2022). The traditional industrial chemical process is sequential, starting with **demineralization** to remove minerals like calcium chloride, followed by **deproteinization** and **decolorization** (of pigments like carotene and astaxanthin). The process concludes with **deacetylation,** typically carried out using potassium or sodium hydroxide (Naveed *et al.,* 2019). In contrast, the biological method is favored as it is more **environmentally friendly**. This approach uses lactic acid for demineralization, protease for deproteinization, organic solvents like acetone for discoloration, and specific bacteria for

deacetylation. A novel technique, the **microwave irradiation method,** has also been recently developed for extraction (Philibert *et al.,* 2017). The final quality of the chitosan product is highly dependent on factors such as the species of crustacean used, the chosen extraction method, and **seasonal variation** (Puvvada *et al.,* 2012).

2.3.1 Chitosan production from different sources

2.3.2 Crustacean shells as a source of chitosan

Chitosan is commonly derived from the exoskeletons of crustaceans, including periwinkle shells, snail shells, and crab shells, which constitute a major waste product of the seafood processing industry (Sagheer *et al.,* 2009). The extraction procedure typically involves an acid treatment to dissolve calcium carbonate, followed by an alkaline treatment to eliminate proteins and pigments. Nevertheless, the highly mineralized nature of these exoskeletons makes the extraction process challenging and labor-intensive (Duan *et al*., 2019).

2.3.3 Insect biomass as a source of chitosan

Chitin and chitosan can also be extracted from insect cuticles, such as those of beetles. The characteristics of chitosan derived from insects are largely

comparable to those of chitosan obtained from crustacean sources (Philibert *et al.*, 2017).

2.3.4 Fungal cell walls as a source of chitosan

Chitin and chitosan are also present in the cell walls of various fungi, particularly within the Zygomycetes class, which often contains higher levels of these polysaccharides than other fungal groups (Merzendorfer, 2011; Dhillon *et al.*, 2013). In fungi, chitin and chitosan are produced by the enzymatic actions of chitin synthase and chitin deacetylase, respectively (Dhillon *et al.*, 2013). Chitosan derived from fungi is devoid of the allergens typically found in crustacean-sourced chitosan, making it a promising alternative for applications in the biomedical and food industries (Li *et al*., 2012).

2.4 Role of chitin and chitosan in the digestibility of ruminant animals

Numerous studies have indicated that these biopolymers (chitin and chitosan) play an important role in improving digestion and overall production performance in ruminants (Shah *et al.*, 2022). Several investigations have shown that supplementing animal diets with chitin and chitosan enhances feed intake and

nutrient digestibility in dairy cows, lambs, and sheep. According to Zhang *et al.* (2022), the addition of seleno-chitosan increased growth rate and wool production in Chinese Marino sheep while also improving blood parameters. Likewise, the inclusion of chitosan in lamb diets led to higher feed intake, improved digestibility of neutral detergent fiber, dry matter, and crude protein, along with better nitrogen balance and microbial protein synthesis (Pereira *et al.,* 2018). Despite these positive effects, however, no significant impact was observed on the overall production performance of feedlot lambs.

The influence of chitosan on rumen fermentation has been shown to differ depending on the animal species and dietary conditions. Wencelova *et al.* (2014) found that supplementing sheep diets with chitosan decreased the total ruminal protozoa population and improved rumen fermentation, potentially contributing to enhanced nutrient degradation and absorption.

2.5 Benefits of chitin and chitosan in ruminants

2.5.1 Improvement in digestibility and rumen fermentation

Chitosan has shown considerable potential as a modulator of rumen fermentation, effectively enhancing nutrient digestibility and modifying fermentation profiles. When included in beef cattle diets, chitosan supplementation improved the

digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF), and dry matter (DM). Moreover, the addition of chitosan led to an increase in total volatile fatty acid (VFA) production, which serves as a vital energy source for ruminants, in *in vitro* batch culture studies (Dias *et al*., 2017).

According to Shah *et al*. (2022), chitosan altered the rumen fermentation pattern by shifting it from acetate production toward propionate formation, thereby enhancing energy efficiency. In agreement, Dias *et al*. (2017) observed that chitosan supplementation raised ruminal pH levels in fermentation experiments using a 20:80 concentrate-to-forage ratio, demonstrating its capacity to counteract the acidifying impact of high-concentrate diets.

2.5.2 Enhancing feed efficiency and animal performance

In dairy cows, the inclusion of chitosan in the diet has been associated with increased milk yield, primarily due to its positive influence on energy utilization (Shah *et al*., 2022). In lambs, chitosan supplementation resulted in higher feed intake, improved digestibility of crude protein and fiber, enhanced nitrogen balance, and greater microbial protein synthesis, although its impact on overall production performance varied (Zhang *et al*., 2022).

Similarly, in sheep, the application of seleno-chitosan led to improved growth rates, increased wool production, and better blood parameters, as reported by Zhang *et al*. (2022). Collectively, these studies emphasize the potential of chitosan to enhance growth and production performance across various ruminant species.

2.5.3 Antimicrobial properties and methane reduction

Chitosan exhibits antimicrobial activity through its interaction with negatively charged bacterial surfaces, which alters membrane permeability and causes intracellular leakage, ultimately leading to bacterial death (Dias *et al.*, 2017). This mechanism not only supports the modulation of rumen microbes but also helps reduce dependence on antibiotics, addressing concerns related to antimicrobial resistance and residues in animal-derived products (Barton *et al.,* 2000). In addition, chitin derived from black soldier flies has been shown to reduce methane emissions *in vitro*, as reported by Jayanegara *et al*. (2017). This characteristic highlights the potential of chitin and chitosan as promising strategies for mitigating the environmental impact of ruminant production systems.

2.6 Periwinkle shells

Periwinkle serves as an inexpensive source of animal protein, mainly consumed in the riverine regions of West Africa (Nwaka *et al.*, 2022). The species *Tympanotonus fuscatus* is among the most significant shellfish resources worldwide and is widely distributed within aquatic mollusks, inhabiting both freshwater and brackish water environments. These organisms are commonly found in shallow waters and intertidal zones, where they burrow into riverbeds and primarily feed on algae and diatoms (Toyin, 2015).

About twelve million tonnes of waste shells are dumped on land and seashores in Nigeria every year (Hart *et al*., 2020). A large portion of these shells comes from periwinkles. After the edible parts are eaten, the leftover shells create large amounts of waste. Without an effective disposal system, these shells pile up in markets, residential areas, and dumpsites, causing both land and air pollution (Hart *et al.,* 2020). When the shells decompose, they release unpleasant odors and allow heavy metals from the waste to leach and weather, which contaminates public water supplies. The decomposing shells also attract disease-carrying organisms and animals that can spread infections, leading to environmental sanitation issues and risks to public health (Nguyen *et al*., 2017).

In Nigeria, about twelve million tonnes of waste shells are thrown away each year on land and along the coast (Hart *et al*., 2020), with periwinkle shells making up a

large share. Disposing of the shells after eating the edible part generates a massive stream of waste. When these shells continue to pile up in homes, markets, and dumpsites without proper management, they cause serious air and land pollution (Aimikhe *et a*l., 2021). Heavy metals from the decaying shells leach into the environment, contaminating water supplies, while foul odors are released as they break down. Disease-carrying organisms and other animals that come into contact with the waste spread infections, worsening sanitation and public health problems (Nguyen *et al.,* 2017).

The growing consumption of shellfish as a nutritious protein source, especially in coastal regions, has made this problem more significant. Compared with ordinary snails, periwinkle shells are much stronger and harder, making up over 70% of the animal's total weight (Yao *et al*., 2014). Tympanotonus fuscatus, a common gastropod prosobranch found in the Niger Delta, typically lives in brackish water creeks and mangrove swamps.

2.6.1 Characteristics of periwinkle shells

The main minerals found in natural periwinkle shells are calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$). According to Orji *et al*. (2017), their average compositions are 88.22 ± 0.75% and 10.25 ± 0.42%, respectively. When the shells are calcined, the calcium carbonate ($CaCO_3$) is transformed into

calcium oxide (CaO). Full conversion of $CaCO_3$ to CaO occurs at calcination temperatures above 800 °C (Cho et al., 2010). However, the best calcination temperature for producing periwinkle shell ash to be used as a partial cement substitute in concrete is 800 °C (Offiong *et al.,* 2017).

The calcination temperature plays a key role in determining the final composition and physical characteristics of the calcined shells. For instance, increasing the calcination temperature raises the weight percentage of CaO, as well as the specific surface area and pore volume (Offiong *et al*., 2017). The composition of calcined periwinkle shells remains relatively consistent between 800 °C and 1200 °C, with calcium oxide (CaO) and silicon oxides (SiO_2) being the dominant compounds. The high proportions of CaO and SiO_2 suggest that periwinkle shells could serve as a dependable alternative source of these materials. In addition, periwinkle shells possess polar functional groups such as hydroxyl, carboxylic, phenolic, and primary amine groups, as reported by James *et al*. (2006). These groups enhance the shell's potential for sorption-related applications.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of the study

The experimental study was conducted at the University of Benin Farm Project, Benin city, Edo state. The annual rainfall ranges from 1500 to 1800mm with a temperature range from 21°C to 30°C, 6.3350°N, 5.6037°E(Anyokwu *et al*. (2024)) . **3.2 Source of periwinkle shells**

The periwinkle 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 homogeneous sample. The shells were washed with clean water to remove viscera, sand and other debris. The cleaned shells were air-dried, milled and stored in an air-tight container for further analysis.

3.3 Experimental extraction procedure for chitin

The chitin extraction from the periwinkle shells was performed by adhering to the standardized procedure outlined by Varun *et al.* (2017)

3.4 Experimental animals

Twelve (12) West African Dwarf (WAD) weaner goats served as the subjects for this study. Prior to the research, the metabolism cages and all associated equipment were rigorously washed and disinfected.

3.5 Experimental procedure and design

The goats were randomly allocated to six experimental diets, with two animals assigned to each dietary treatment. The study utilized a Completely Randomized Design (CRD**)** for its experimental layout.

3.6 Experimental treatments

Six (6) experimental diets were formulated comprising of varying levels of additives. Each diets were supplemented with a basal diet (guinea grass) at 50% inclusion level **.**The six (6) experimental treatments are as follows

T1= cconcentrate without any additive (Control)

T2= concentrate containing 3% chitin

T3= concentrate containing 6% chitin

T4= concentrate containing 0.5% chitosan

T5= concentrate containing 1% chitosan

T6= concentrate containing 0.01% tetracycline

Table 1: Feed ingredients composition of experimental diets containing varying levels of chitin and chitosan from periwinkle shells.

Ingredients	Periwinkle					
	Chitin		Chitosan			
	T1 (Control)	T2 (Chitin 3%)	T3 (Chitin 6%)	T4 (Chitosan 0.5%)	T5 (chitosan 1%)	T6 0.01% oxytetracycline
Maize	21	21	21	21	21	21
SBM	2	2	2	2	2	2
Wheat bran	43	32.5	22	42	40	44
PKC	26.5	34	41.5	27	28.5	25.49
BDG	5	5	5	5	5	5
Chitin	0	3	6	0	0	0
Chitosan	0	0	0	0.5	1	0
0.01% oxytetracycline	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)	16.5665	16.505	16.4435	16.515	16.5185	16.5094
CF(10-12)	10.2455	10.508	10.7705	10.233	10.2953	10.1688
ME(2500)	2651.12	2613.62	2576.12	2641.66	2636.48	2646.57

SBM= soyabean meal, PKC= Palm kernel cake, BDG=brewery spent grain, CP= crude protein, CF= Crude fiber, ME= metabolisable energy

3.5 Digestibility study

The animals were confined in a metabolic cage for eight days period. Faecal and urine samples were collected daily in the morning before feeding from trays filled into the cages. 1ml of 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% aliquot was collected after the end of digestibility study and oven dried at 100°C, to constant weight for further analysis.

3.6 Proximate analysis

Determination of ash

The crucibles were labelled and heated for thirty minutes in the oven, after which it was removed and weighed. Two grams of the samples was weighed into the pre-heated 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%.

Determination of crude protein

This was determined using the Kjeldahl's procedure which involves three basic procedures**:** digestion, distillation and titration.

Kjeldahl's technique was utilized to determine the crude protein content. A conical flask was filled with 0.5 grams of the sample (for faeces) while 10 milliliters (for urine), 10 milliliters of 72% concentrated sulfuric acid, and 2 grams of mixed catalyst copper sulfate and sodium sulfate(CuSO4:NaSO4 at a ratio of 1:9). The materials were heated to achieve a light green digest. The digest was added to a 60 mL digest bottle and diluted to the 50 mL mark with distilled water. Distillation was performed by adding 5 milliliters of the digest to 5 milliliters of 40% sodium hydroxide (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 30 mL, and 0.1N HCL was used to titrate it. This was done for each treatment.

The Crude protein was calculated using the formula below:

$\%CP = NA \times 14 / 1000 \times VA \times 100 / W \times 100 / 5 \times 6.25$

Where NA = Normality of acid

VA = Volume of acid

W = Weight of the sample

Determination of crude fibre

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°C to 100°C. 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% sodium hydroxide (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 100ml 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 °C 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°C 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 = Wt. of crude fiber x 100 / Wt. of sample

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 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 hydrochloric acid (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/

6. 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.

3.7 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

RESULTS

4.1 **Chemical composition of** chitin and chitosan **diets fed to West African dwarf goats.**

4.1.1 Dry matter

The dry matter content across the six diets (control, 3% chitin, 6% chitin, 0.5% chitosan, 1% chitosan and 0.01% oxytetracycline) ranged from 91.75 to 91.85%, showing no significant difference (> 0.05), indicating consistent moisture content in all diets.

4.1.2 Crude fibre

Crude fibre content varied significantly ($P < 0.05$) with 6% chitin(26.00%)having the highest CF, indicating increased fibre level due to 6% chitin inclusion, while control(18.50%) had the lowest.

4.1.3 Crude protein

Crude protein (CP) values ranged from 14.88% (6% chitin) to 19.25% (T2), with 6% chitin significantly lower than others, possibly due to the chitin inclusion reducing protein concentration.

4.1.4 Ether extract

The 6% chitin had the highest EE (12.00%), indicating the richest fat content in the diet. The 0.01% tetracycline treatment had the lowest EE (8.50%), showing the least fat content. The difference in EE were statistically significant ($P < 0.05$), implying that chitin/chitosan inclusion levels significantly influenced the fat content of the diets.

4.1.5 Nitrogen free extracts

Nitrogen free extracts (NFE), representing readily available carbohydrates, significantly decreased from 42.31% (control) to 30.12% (6% chitin), indicating the effect of chitin reducing soluble carbohydrates levels.

4.1.6 Acid detergent fibre

Acid detergent fibre (ADF), an indicator of indigestible fibre, was highest in 6% chitin (55.95%) and lowest in antibiotics (8.65%), significantly differing ($P < 0.05$), reflecting the fibrous nature of chitin at highest inclusion.

4.1.7 Organic matter

The 0.5% chitosan (91.50%) had the highest OM content, significantly ($P <0.05$) higher than all other treatments. This suggests that 0.5% chitosan inclusion

enhanced the organic nutritive value of the diet, possibly by improving the digestibility of feed components or reducing ash content. Control (88.50%) and antibiotics (88.50%) were statistically similar and ranked next. These results imply that the control and antibiotics diets had a relatively high proportion of digestible nutrients. The 1% chitosan, 3% chitin and 6% chitin treatment formed a significantly lower group with OM values between 83.00 – 87.00%. This indicates that higher levels of chitin (3% chitin and 6% chitin) or 1% chitosan might have reduced digestible organic matter, likely due to increased fibre or indigestible residues chitin being structurally rigid).

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

Variable	T1(control)	T2(3% chitin)	T3(6% chitin)	T4(0.5% chitosan)	T5(1% chitosan	T6 (oxytetracycline)	SEM
DM	91.85a	91.40ab	91.70a	90.75c	91.55ab	91.10bc	0.15
CF	18.50d	21.00bc	24.50a	19.00cd	17.50d	22.00b	0.68
ASH	11.50d	20.00a	14.00c	9.00e	15.50b	11.50d	0.35
CP	17.94b	17.50b	17.94b	22.75a	15.75b	18.38b	0.84
EE	9.75a	10.00a	9.00a	10.00a	8.75a	8.50a	0.45
NFE	42.31b	31.50c	34.56bc	39.25ab	42.50a	39.62ab	1.46
OM	88.50b	80.00e	86.00c	91.00a	84.50d	88.50b	0.35

Means within the same row with different superscript different significantly ($P<0.05$),SEM= standard error of means,T1=concentrate without chitin and chitosan,T2=concentrate with 3% chitin,T3=concentrate with 6% chitin,T4= concentrate with 0.5% chitosan,T5=concentrate with 1% chitosan,T6=concentrate with 0.01% tetracycline, CF= Crude fibre, CP= Crude protein, EE= Ether extract, NFE= Nitrogen free extract, OM= Organic matter, DM= Dry matter

4.2 Dry matter and crude protein (CP) digestibility of goats fed diets of Guinea grass and concentrates containing graded levels of chitin and chitosan feed additives

4.2.1 Concentrate dry matter intake

All treatments (T1–T6) showed statistically similar concentrate dry matter intake (DMI), as evidenced by the shared superscript 'a' across all groups. This indicates that varying inclusion levels of chitin and chitosan did not significantly influence the goats' appetite or capacity to consume concentrate feed. Although the control treatment (186.30 g/day) recorded the highest DMI and 3% chitin (156.80 g/day) the lowest, these differences were not statistically significant ($P>0.05$).

4.2.2 Grass dry matter intake

All treatments share the same superscript letter "a," indicating that there were no significant differences ($P>0.05$) in grass dry matter intake among the treatment groups. The greatest grass intake was recorded in 1% Chitosan (172.50 g/day), whereas the lowest was in 0.5% Chitosan (145.60 g/day); however, these variations were not statistically significant

4.2.3 Crude protein intake from concentrate

All treatments bear the same superscript "a," signifying that there were no significant differences ($P>0.05$) in crude protein intake from concentrate among

the treatment groups. The highest crude protein intake was observed in 0.5% Chitosan (39.25g/day), while the lowest occurred in 1% Chitosan (26.89 g/day). Nonetheless, these differences were not statistically significant. The Standard Error of the Mean (SEM) of 4.98 indicates moderate variability among treatments, but it was insufficient to influence the statistical outcome.

4.2.4 Crude protein intake from grass

All treatment groups share the same superscript "a," indicating that there were no statistically significant differences ($P > 0.05$) in crude protein intake from grass among the treatments. The 1% Chitosan treatment exhibited the highest crude protein intake (12.56 g/day), whereas 0.5% Chitosan showed the lowest value (10.60 g/day). Nonetheless, this variation was not statistically significant. The standard error of the mean (SEM) of 1.94 suggests relatively low variability in crude protein intake across the treatments.

4.2.5 Total crude protein intake

All treatments carry the same superscript "a," indicating that there were no statistically significant differences ($P > 0.05$) in total crude protein intake among the treatment groups. The 0.5% Chitosan treatment showed the highest total crude protein intake (49.85g/day), while 3% Chitin treatment recorded the lowest value (38.15 g/day); however, these differences were not statistically significant. The

standard error of the mean (SEM) of 6.82 indicates a moderate level of variation among the treatment means.

4.2.6 Faecal output of crude protein

All treatments are assigned the same superscript "a," indicating that faecal crude protein output did not differ significantly ($P > 0.05$) among the treatments. Numerically, the 1% Chitosan treatment exhibited the highest faecal crude protein loss (16.60 g/day), whereas 3% chitin and 6% chitin recorded the lowest (10.90g/day); however, this difference was not statistically significant. The standard error of the mean (SEM) of 4.02 indicates a moderate level of variability among the replicates.

4.2.7 Urinary output of crude protein

Urinary crude protein (CP) excretion differed significantly among treatments. The highest CP output was recorded in the 1% Chitosan treatment (8.72 g/day), while the lowest occurred in 6% Chitin (1.79 g/day). Statistical grouping showed that 1% Chitosan was categorized as "a," Antibiotics (7.11 g/day) as "ab," Control (5.85 g/day) and 3% Chitin (6.10 g/day) as "abc," 0.5% Chitosan (3.52 g/day) as "bc," and 6% Chitin as "c." This indicates a clear separation among treatments, with chitosan inclusion at 1% yielding the greatest urinary CP excretion and 6% chitin resulting in the least. The standard error of the mean (SEM = 1.324)

suggests moderate variability among replicates. These results demonstrate that dietary additive type and inclusion level significantly influenced urinary CP output. The lower CP excretion in 6% chitin suggests enhanced protein retention and utilization efficiency, while the higher excretion in 1% chitosan may reflect reduced protein use efficiency. Such differences are important when assessing dietary nitrogen efficiency and potential environmental nitrogen losses associated with protein metabolism.

4.2.8 Total crude protein output

All treatments carried the same superscript “a,” indicating that there were no statistically significant differences ($P > 0.05$) in total crude protein (CP) output among the treatment groups. The 1% Chitosan treatment exhibited the highest total CP output (25.31 g/day), while the 6% Chitin treatment recorded the lowest value (12.66 g/day). However, this variation was not statistically significant. The standard error of the mean (SEM = 4.35) indicates moderate variability in CP output among replicates, suggesting relatively consistent protein metabolism across treatments. Overall, the inclusion of chitin or chitosan at different levels, as well as the use of antibiotics, did not significantly affect total crude protein output in comparison with the control group.

4.2.9 Crude protein retention

Crude protein (CP) retention varied significantly among dietary treatments. The highest CP retention was recorded in goats supplemented with 6% chitin (70.82 g/day), while the lowest value was observed in those receiving 1% chitosan (37.67 g/day). Statistical grouping classified 6% chitin as “a”, 1% chitosan as “b”, and control (56.13 g/day), 3% chitin (55.00 g/day), 0.5% chitosan (60.50 g/day), and 0.01% oxytetracycline (54.60 g/day) as “ab”, indicating intermediate levels of CP retention. The standard error of the mean (SEM = 7.85) suggests moderate variability among replicates. These results indicate that dietary supplementation with chitin and chitosan significantly influenced CP retention in goats. The superior CP retention observed with 6% chitin implies improved protein utilization and nitrogen retention efficiency, possibly due to enhanced gut health and nutrient absorption. In contrast, the reduced CP retention in 1% chitosan suggests less efficient protein metabolism. Overall, treatments with intermediate values—including control, 3% chitin, 0.5% chitosan, and oxytetracycline—showed moderate responses, demonstrating that the level and type of additive play a key role in protein retention. These findings emphasize the potential of chitin-based supplementation to enhance protein metabolism and improve nitrogen utilization efficiency in goats.

4.2.10 Crude protein balance

The highest crude protein (CP) balance was observed in 0.5% Chitosan at 31.84 g/day, followed closely by 6% Chitin at 30.63 g/day and Control at 25.59 g/day, indicating greater protein retention in these treatments. The lowest CP balance was recorded in 1% Chitosan (14.11 g/day), while intermediate values were observed in 3% Chitin (21.14 g/day) and Antibiotics (23.40 g/day). Statistical analysis showed that all treatments were assigned the same superscript "a," signifying no significant differences ($P > 0.05$) among treatments despite observable numerical variations. The standard error of the mean (SEM = 7.09) indicates moderate variability among replicates. Overall, dietary treatments influenced CP balance, with 0.5% chitosan and 6% chitin showing relatively improved protein retention, whereas 1% chitosan tended to result in lower retention. Although differences were not statistically significant, the results suggest that chitin and chitosan supplementation may contribute to enhanced protein utilization efficiency in goats.

4.2.11 Crude protein digestibility

All treatments were classified in the same significance group "a," indicating no statistically significant differences ($P>0.05$) in crude protein digestibility across treatments. Numerically, 6% Chitin had the highest CP digestibility (75.02%),

suggesting slightly improved digestion and absorption of protein. The lowest value was observed in 1% Chitosan; (61.48%), although this difference was not statistically significant due to the high SEM of 7.82, reflecting considerable variation among replicates. All treatments showed CP digestibility above 60%, indicating generally good protein digestion in the goats across all dietary treatments.

Table 4.2 Dry matter and crude protein (CP) digestibility of goats fed diet of concentrates containing graded levels of chitin and chitosan feed additives

Variable	T1 (control)	T2 (3% chitin)	T3 (6% chitin)	T4 (0.5 chitosan)	T5 (1% chitosan)	T6 (antibiotics)	SEM
DMI GRASS	168.10a	147.20a	165.30a	145.60a	172.50a	162.50a	26.6
DMI CONC	186.30a	156.80a	174.30a	172.60a	170.70a	166.80a	27.20
DMI Total	354.40a	303.90a	339.60a	318.20a	343.30a	329.30a	52.5
DMD	75.19a	76.23a	74.00a	74.53a	73.52a	73.39a	2.55
CP CONC INTAKE	33.41a	27.44a	31.26a	39.27a	26.89a	30.64a	4.98
CP GRASS INTAKE	12.24a	10.71a	12.04a	10.60a	12.56a	11.84a	1.94
CP TOTAL INTAKE	45.66a	38.15a	43.30a	49.85a	39.41a	42.65a	6.82
CP FAECAL OUTPUT	14.20a	10.90a	10.90a	14.50a	16.60a	12.10a	4.02
CP URINE OUTPUT	5.850abc	6.100abc	1.785c	3.520bc	8.720a	7.110ab	1.324
TOTAL CP OUTPUT	20.07a	17.01a	12.66a	18.02a	25.31a	19.25a	4.35
CP RETENTION	56.13ab	55.00ab	70.82a	60.50ab	37.67b	54.60ab	7.85
CP BALANCE	25.59a	21.14a	30.63a	31.84a	14.11a	23.40a	7.09
CP DIGESTIILITY	68.86a	71.32a	75.02a	67.55a	61.48a	71.27a	7.82

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, ASHI= Ash intake, CFI= Crude fibre intake, CPI= Crude protein intake, EEI= Ether extract intake, NFEI= Nitrogen free extract intake, OMI= Organic matter intake, DMI= Dry matter intake, GG= Guinea grass

CHAPTER FIVE

DISCUSSION

5.1 Chemical composition of experimental diets

The proximate composition of the experimental diets indicated that the inclusion of chitin and chitosan from periwinkle shells influenced several proximate parameters such as crude fibre, crude protein, ether extract, nitrogen-free extract, and organic matter.

The dry matter (DM) content across the six treatment diets (91.40–91.85%) showed no variation ($P > 0.05$), reflecting uniform moisture content and consistent feed preservation. Similar DM values have been reported in goat diets formulated with shell-based additives (Okoye *et al.,* 2019). The high DM content (>90%) suggests good storage stability and minimal microbial spoilage risk (Oluremi *et al.,* 2018). The significant increase ($P < 0.05$) in 6% chitin diet can be attributed to the structural composition of chitin, which is a fibrous polysaccharide composed of β-(1,4)-linked N-acetylglucosamine units (No *et al.,* 2000). Higher fibre levels indicate increased indigestible fractions, which could reduce energy density and digestibility. This agrees with the observations of Adeyemo and Onibi (2018), who noted that increasing fibrous by-products in

ruminant diets elevates the crude fibre proportion and may limit nutrient availability.

The reduction in CP at 6% chitin suggests a dilution effect due to the non-protein nitrogenous structure of chitin (Amin et al., 2019). Ether extract (EE) increased with chitin Increasing levels of inclusion. The elevated EE could be attributed to residual lipid components within the chitin matrix derived from periwinkle shells, as previously observed by Aimikhe and Lekia (2021). Higher EE implies improved energy density, which may enhance growth performance. Nitrogen-free extract (NFE), representing soluble carbohydrates, decreased with increasing levels of chitin, and there is carbohydrate displacement by the chitin matrix. Chitin's complex polymeric nature makes it less digestible, resulting in lower NFE values (Oloruntola *et al.,* 2018). Reduced NFE levels imply lower readily available energy, potentially affecting rumen fermentation efficiency at higher inclusion levels.

The highest organic matter report for 0.5% chitosan from this study suggest improved nutrient digestibility and possibly enhanced organic nutrient concentration. Similar findings were reported by Ibrahim *et al.* (2019), who observed that chitosan supplementation at low levels can increase organic matter digestibility through modulation of rumen microbial activity and reduction of

nutrient losses. However, diets containing 3% and 6% chitin showed reduced OM, likely due to higher indigestible residue from excessive chitin inclusion. This aligns with the report of Akinmutimi and Essien (2019), who emphasized that excessive fibrous additives can impair nutrient utilization efficiency in small ruminants.

5.2 Dry matter and crude protein digestibility

5.2.1 Dry matter intake

The inclusion of chitin and chitosan did not affect feed palatability or intake capacity in this study. This suggests that goats tolerated chitin/chitosan-based diets well. This is similar to findings by Ajayi *et al.* (2019), who reported that moderate inclusion of unconventional feed additives does not compromise feed intake in West African Dwarf goats. Although numerically lower intake was observed in the 3% chitin diet, this might be due to increased fibre content reducing bulk density and intake rate.

5.2.2 Crude protein intake

In this study, animals consumed comparable protein quantities regardless of dietary chitin or chitosan level. The slightly higher CP intake in 0.5% chitosan-fed goats indicates better palatability and possible enhancement of rumen microbial efficiency (Adeyemi *et al.,* 2023). This aligns with reports that chitosan at low

inclusion levels can stimulate microbial protein synthesis and improve nitrogen capture in the rumen (Khoushab & Yamabhai, 2010).

5.2.3 Faecal crude protein output

Numerically higher CP loss was observed in goats fed 1% chitosan diet, while lower losses were associated with the 3% and 6% chitin diets. This suggests that dietary chitin may have slightly improved the digestibility and absorption of dietary protein compared with chitosan at higher inclusion levels.

The non-significant difference across treatments implies that both chitin and chitosan did not adversely affect gastrointestinal digestion or protein passage through the digestive tract. Similar results were reported by **Onifade et al. (1999)**, who observed that chitosan inclusion up to 1% did not significantly influence fecal nitrogen losses in broiler diets. The slight improvement in CP utilization with chitin supplementation aligns with findings by **Yoshida et al. (2009),** who noted that chitin can modulate gut microbiota and enhance nutrient digestibility through its prebiotic effects.

5.2.4 Urinary crude protein output

Urinary crude protein output differed significantly among treatments, with the highest excretion recorded in the 1% chitosan group and the lowest in the 6% chitin group. This indicates that high chitosan levels (1%) may have reduced nitrogen retention efficiency, possibly due to excess nitrogen excretion through urine. Conversely, the lower urinary nitrogen observed in goats fed 6% chitin suggests improved nitrogen retention and utilization.

These findings are consistent with reports by **Hernández-López et al. (2008)**, who found that dietary chitosan can influence nitrogen metabolism by modifying rumen microbial populations and protein fermentation. **Sharma et al. (2018)** also noted that chitosan may affect nitrogen partitioning in ruminants by reducing ruminal deamination, though excessive levels can impair microbial efficiency. The improved retention seen in the 6% chitin group suggests that chitin may serve as a slow-degrading polysaccharide that enhances microbial protein synthesis and nitrogen capture.

5.2.5 Total crude protein output

Numerically, the highest total CP output was recorded in 1% chitosan, while the lowest occurred in 6% chitin. This pattern reflects the relationship between fecal and urinary nitrogen losses and supports the observation that 6% chitin promoted better nitrogen conservation within the animal.

The non-significant differences suggest that chitin and chitosan, at the inclusion levels tested, did not negatively affect overall nitrogen metabolism. According to **Chanjula et al. (2007),** chitosan supplementation up to 1% in goat diets may not significantly alter nitrogen retention but can modulate rumen fermentation characteristics. Similarly, **Goiri et al. (2010)** reported that moderate chitosan inclusion improved ruminal nitrogen efficiency without affecting total nitrogen balance.

5.2.5 Crude protein retention

Results show that chitin at higher inclusion levels may enhance protein retention, possibly through improved nitrogen utilization or microbial protein synthesis efficiency. The improved retention observed with 6% chitin aligns with studies by **Goiri et al. (2010),** who demonstrated that chitin-based compounds can promote rumen microbial growth and improve nitrogen capture at 5 – 7% inclusion level.

The lower CP retention observed in the 1% chitosan treatment may be attributed to altered rumen fermentation, as excessive chitosan can inhibit certain rumen microbes responsible for protein degradation and synthesis (**Zhao et al., 2010**). Therefore, while chitosan may possess beneficial antimicrobial properties at lower inclusion rates, high concentrations might suppress beneficial rumen bacteria involved in nitrogen metabolism.

5.2.6 Crude protein balance

The 0.5% chitosan treatment achieved the highest numerical CP balance, indicating that low-level chitosan supplementation could promote a favorable nitrogen balance without negatively impacting digestion or excretion.

This observation supports the findings of **Adebiyi et al. (2018),** who reported that low chitosan inclusion enhances nutrient utilization and growth in ruminants. Chitosan's bioactive properties may enhance intestinal absorption and modulate microbial efficiency at optimal levels. The improvement in protein retention with 6% chitin also suggests enhanced digestibility and reduced nitrogen loss, consistent with the positive effects of chitin on rumen microbial function reported by **Yoshida et al. (2009).**

5.2.7 Crude protein digestibility

Higher chitin inclusion levels may slightly improve protein digestion and absorption. The digestibility values Obtained in this study indicate generally efficient protein utilization, which agrees with earlier findings that both chitin and

chitosan can serve as functional feed additives without impairing nutrient digestion (**Chanjula et al., 2007; Goiri et al., 2010).**

The improved digestibility in 6% chitin-fed goats may be linked to the gradual fermentation of chitin in the rumen, providing a consistent source of nitrogen for microbial growth. In contrast, lower digestibility in the 1% chitosan group may be associated with its strong antimicrobial action, which can suppress certain fibrolytic and proteolytic microbes at higher concentrations **(Zhao et al., 2010).**

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The findings of this study indicate that **chitin and chitosan from periwinkle shells** can serve as effective functional feed additives in goat nutrition. Inclusion of chitosan at 0.5% and chitin at 6% levels improved **crude protein retention, nitrogen balance, and overall nutrient digestibility**, while higher chitosan levels (1%) resulted in greater urinary nitrogen losses and reduced protein utilization efficiency. The results further confirm that chitin and chitosan, due to their bioactive and structural properties, can positively modulate rumen fermentation and enhance microbial protein synthesis when used at optimal levels. Therefore, periwinkle shell-derived chitin and chitosan represent sustainable, locally available feed additives that can enhance nutrient efficiency and support environmentally friendly goat production by reducing nitrogen excretion losses.

6.2 Recommendation

1. **Optimal Inclusion Levels:**

Chitosan should be included at 0.5**%**, and chitin at 6**%** in goat diets to achieve improved nitrogen retention and protein utilization without adverse effects on digestibility or feed intake.

2. **Use of Local Resources:**

Periwinkle shells, an abundant marine by-product, can be effectively processed into chitin and chitosan for livestock feed applications, promoting waste valorization and sustainable feed resource utilization.

3. **Environmental Considerations:**

Since lower urinary nitrogen excretion was observed with chitin supplementation, its inclusion can contribute to reduced nitrogen pollution, making it an eco-friendly feed strategy.

REFERENCES