NITROGEN BALANCE AND RETENTION OF GOATS FED DIETS CONTAINING CHITIN AND CHITOSAN FROM PERIWINKLE SHELLS

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

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CERTIFICATION

This is to certify that this project work was carried out by **Clinton Agbona AKWAGWA** with Matriculation Number **AGR2000062** 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 Almighty for his Grace all through the course of my programme in the University of Benin.

To my loving parents Mr. and Mrs. Solomon Akwagwa, to my dear siblings and entire family.

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I sincerely express my deepest gratitude to Almighty God for granting me the strength, wisdom, and perseverance to complete this research project. I am extremely grateful to my lecturers for their incessant supervision and successful completion of this project. My appreciation goes to the Dean of Faculty, Faculty of Agriculture, the Head of Department.

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ABSTRACT

This study was conducted to evaluate the nitrogen balance and retention in West African Dwarf (WAD) goats fed diets containing varying levels of chitin and chitosan extracted from periwinkle shells, an unconventional feed resource. A total of 12 weaner female WAD goats were randomly assigned to six dietary treatments: control (no additive), 3% chitin, 6% chitin, 0.5% chitosan, 1% chitosan and antibiotic (0.01% oxytetracycline). The trial lasted 15 days, with an 8-day digestibility and nitrogen balance trial using metabolic cages. Parameters assessed included feed intake, nitrogen intake, faecal and urinary nitrogen excretion, nitrogen balance, nitrogen digestibility, and nitrogen retention. Chemical analyses of the experimental diets were also conducted with ash ranging from 9% - 20%, crude fiber (17.50% - 24.50%), crude protein (15.75% - 22.75%), dry matter (91.10% - 91.85%), Nitrogen free extract (12.50%. - 42.31%), organic matter (80% - 91%) and ether extract (8.50% - 10%) . Results showed that chitin and chitosan-based diets, particularly at 6% Chitin and 0.5% chitosan inclusion, significantly improved nitrogen balance and retention compared to 1% chitosan and 0.01% oxytetracycline diets. Urinary nitrogen loss was significantly lower in chitin-fed goats, indicating better nitrogen utilization. There were no adverse effects on dry matter intake, and overall performance remained optimal. It is concluded that chitin and chitosan from periwinkle shells are viable, eco-friendly feed additives that can enhance nitrogen efficiency in goats. Chitosan, in particular, may serve as a natural alternative to antibiotics in small ruminant nutrition. The study recommends broader application and further research into their economic and microbial effects.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The livestock industry plays a crucial role in food security and economic development, particularly in developing countries where small ruminants like goats serve as vital sources of meat, milk, and income (Ogunbosoye *et al.,* 2020). Nutrition is a key factor affecting productivity, and protein is one of the most important nutrients required for growth, reproduction, and maintenance in goats (Akinmutimi and Essien, 2019). To meet rising feed costs and reduce competition between humans and animals for conventional feed additives, there has been growing interest in unconventional feed resources. Among these are chitin and chitosan, bioactive compounds extracted from marine waste such as periwinkle shells (Nworgu *et al.,* 2021). These compounds are known for their potential in enhancing nutrient utilization and immune response in animals (Ibrahim *et al.,* 2022). Chitin is a fibrous substance found in the exoskeletons of crustaceans, insects, and some fungi, while chitosan is a deacetylated derivative of chitin with more bioactive properties (Shah *et al.,* 2022). Studies suggest that chitosan can reduce ammonia production in the rumen, improve nitrogen retention, and promote better feed efficiency (El-Zaiat *et al.,* 2024).

Nitrogen balance studies are important tools for assessing protein utilization and retention in animals. By monitoring nitrogen intake, faecal and urinary nitrogen losses,

researchers can determine the efficiency of dietary protein use, which is critical for formulating cost-effective rations (Ajayi *et al.,* 2019). This study investigates the effects of diets containing chitin and chitosan from periwinkle shells on nitrogen balance and retention in West African Dwarf (WAD) goats, aiming to explore the potential of these marine-based additives as sustainable feed ingredients.

1.2 Justification of the Study

Periwinkle shells are often discarded as waste, yet they are rich in chitin, representing a low-cost, underutilized resource (Okafor *et al.,* 2020). Chitosan has shown promising effects in improving nitrogen retention and rumen fermentation efficiency in ruminants (Shah *et al.,* 2022). Understanding nitrogen balance will help in determining the effectiveness of chitin and chitosan as dietary protein enhancers in goat nutrition. The study supports sustainable livestock production by reducing feed cost, managing waste, and improving animal performance. It contributes to the ongoing efforts to optimize ruminant feeding strategies in tropical regions.

1.3 Objectives of the Study

The objectives of this study were to determine;

1. The chemical composition of experimental diets containing feed additives of chitin and chitosan from periwinkle shell.
2. The nitrogen intake, balance, retention, and digestibility of goat fed diets of chitin and chitosan from periwinkle shells.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutrient Requirement of Goats

Goats are small ruminants with unique nutritional needs that vary according to age, physiological status, and production goals (NASEM, 2016). Their nutrient requirements include energy, protein, minerals, vitamins, and water, all of which must be supplied in adequate amounts for maintenance, growth, reproduction, and lactation (Ndukwu *et al.,* 2019). Energy is a primary nutrient needed to support metabolic processes and physical activity, typically supplied through carbohydrates and fats in the diet (Sikosana *et al.,* 2018). The energy requirement is influenced by factors such as breed, feed quality, and environmental conditions, especially in tropical regions where heat stress can affect intake (Adeyemi and Olaniyi, 2017).

Protein requirements in goats are critical as they influence tissue repair, growth, milk production, and immune function. Ruminant protein metabolism is complex, involving rumen degradable and undegradable protein fractions, with microbial protein synthesis playing a vital role (Moseley *et al.,* 2019). Dietary protein deficiency can lead to reduced growth rates and poor reproductive performance (Osei-Amponsah *et al.,* 2021). Minerals such as calcium, phosphorus, magnesium, and trace elements like zinc, copper, and selenium are essential for skeletal development, enzyme function, and overall metabolism (Ndukwu *et al.,* 2019). Deficiencies or imbalances can cause metabolic disorders and reduced productivity (Akinola *et al.,* 2020). Vitamins, especially fat-soluble vitamins A, D, and E, are necessary for physiological functions including vision, bone growth, and antioxidant defense (Shah *et al.,* 2022). Water intake must also be sufficient to maintain hydration and support digestion, especially under tropical conditions where dehydration risk is higher (Adewuyi *et al.,* 2020).Understanding and meeting these nutrient requirements through appropriate feed formulation is crucial for optimizing goat performance, especially when unconventional feed resources like chitin and chitosan are introduced (Ibrahim *et al.,* 2019). Research indicates that supplements derived from marine by-products can improve nitrogen utilization and overall nutrient balance in goats (Okafor *et al.,* 2020).

2.2 Uses of Unconventional Feed Resources (UFRs) in Goat Nutrition

Unconventional feed resources (UFRs) refer to non-conventional materials not typically used in standard animal feed, often due to limited availability, low awareness, or processing challenges (Oloruntola *et al.,* 2018). These resources, however, can offer substantial nutritional value, especially in regions facing high feed costs or seasonal feed shortages, such as tropical Africa (Agbede *et al.,* 2019). In goat production, particularly with West African Dwarf (WAD) goats, UFRs have proven valuable in improving feed intake, nutrient utilization, and reducing feed costs (Akinola and Oloruntola, 2020). Examples include agro-industrial by-products like cassava peels, palm kernel cake, brewery waste, and marine residues like periwinkle shells (Okafor *et al.,* 2020).

Chitin and chitosan derived from periwinkle shells are among the emerging UFRs being investigated for their nutritional and functional roles in small ruminant diets (El-Zaiat *et al.,* 2024). These compounds offer not only protein-binding and nitrogen-retention capacities but also improve gut health due to their antimicrobial properties (Shah *et al.,* 2022). One major advantage of UFRs is their local availability, which supports feed sustainability and reduces dependence on imported ingredients (Ogunbosoye *et al.,* 2020). Additionally, their utilization helps in waste management by converting food processing by-products into valuable feed inputs (Adeyemi *et al.,* 2023). However, inclusion of UFRs requires careful consideration of anti-nutritional factors, palatability, and digestibility (Akinfemi and Olatunji, 2017). Proper processing such as drying, grinding, or chemical treatment is often needed to improve nutrient availability and reduce risks. Overall, integrating unconventional feed resources like chitin and chitosan into goat diets aligns with sustainable livestock feeding strategies and offers potential to improve nitrogen retention and productivity in goats under tropical conditions (Ibrahim *et al.,* 2019).

2.3 Chitin and Chitosan: Sources and Characteristics

Chitin is a natural biopolymer found predominantly in the exoskeletons of crustaceans, insects, and the cell walls of fungi (Kumar *et al.,* 2018). It is the second most abundant organic polymer in nature after cellulose, and it serves as a structural material providing strength and protection (Ogunbosoye *et al.,* 2020). In West Africa, periwinkle shells (e.g., *Tympanotonus fuscatus*) are a rich and underutilized source of chitin (Okafor *et al.,* 2020). Chitosan is a deacetylated derivative of chitin, produced through alkaline or enzymatic deacetylation processes (Shah *et al.,* 2022). This transformation gives chitosan improved solubility in acidic solutions, making it more biologically active and suitable for use in animal feed (Nworgu *et al.,* 2021). Both chitin and chitosan are biodegradable, non-toxic, and possess antimicrobial and antioxidant properties that contribute to improved animal health and feed efficiency (El-Zaiat *et al.,* 2024). The chemical structure of chitin is composed of β-(1→4)-linked N-acetyl-D-glucosamine units, while chitosan consists of a mix of glucosamine and N-acetyl glucosamine units depending on the degree of deacetylation (Ibrahim *et al.,* 2019). These structures allow them to bind with proteins, lipids, and minerals, affecting digestion and nutrient absorption (Zhang *et al.,* 2020). In ruminants, chitin and chitosan can influence microbial fermentation, nitrogen metabolism, and gut microbial balance (Adeyemi *et al.,* 2023). Their positive effects on nitrogen retention are attributed to their ability to inhibit proteolytic and ureolytic bacteria in the rumen, reducing ammonia production and nitrogen loss (Patra and Saxena, 2019).

The use of periwinkle shells as a source of chitin/chitosan not only provides a cost-effective and sustainable feed additive but also helps reduce environmental waste from seafood processing (Agboola *et al.,* 2021). Incorporating these by-products into goat diets reflects a circular economy approach and contributes to sustainable livestock nutrition in the tropics (Osei-Amponsah *et al.,* 2021).

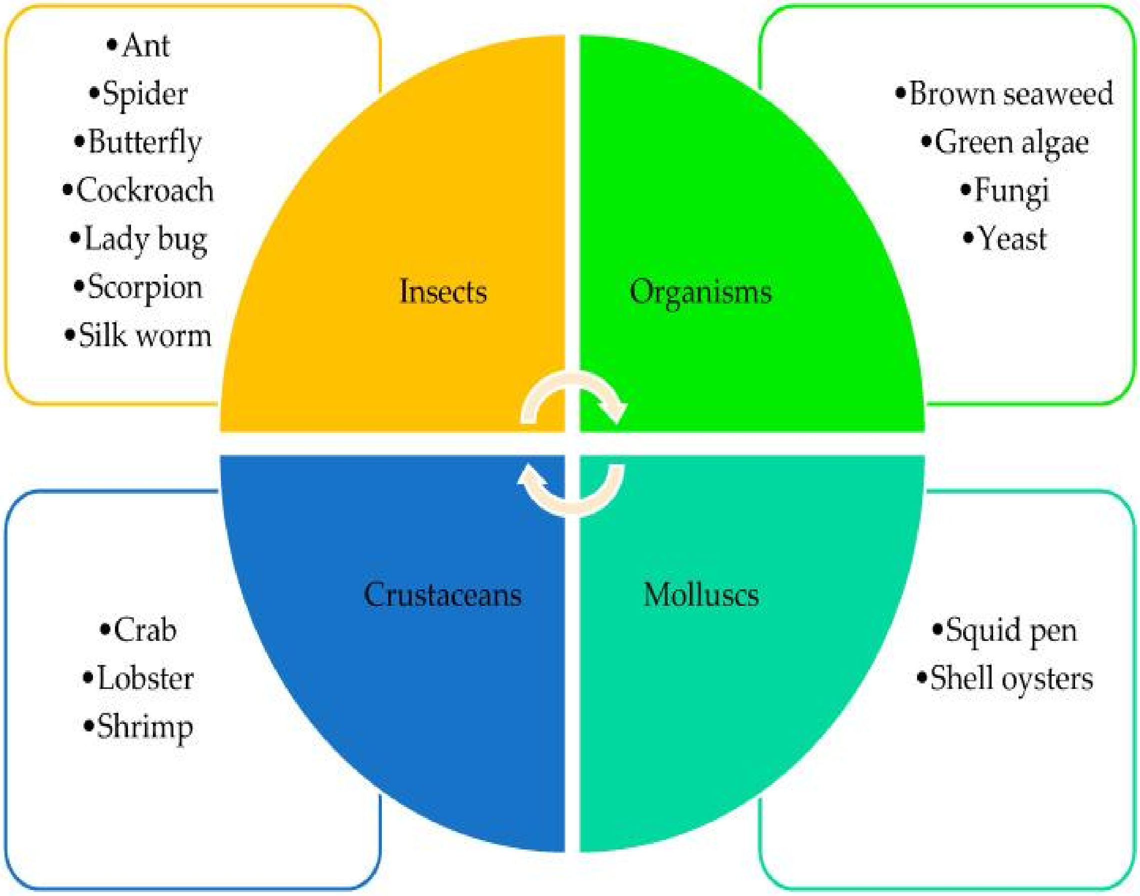

Figure 1: Sources of chitin production (Santos *et al.,* 2020)

2.4 Performance of Goats Fed Diets containing Chitin and Chitosan

The inclusion of chitin and chitosan in goat diets has shown promising effects on growth performance, nutrient utilization, and health parameters in both experimental and field settings (Okafor *et al.,* 2020). These compounds, when properly processed and included at appropriate levels, can enhance feed efficiency, weight gain, and overall productivity of goats (Agboola *et al.,* 2021). Studies conducted on West African Dwarf (WAD) goats have demonstrated that chitosan supplementation can lead to improved average daily gain and feed conversion ratio compared to control groups fed conventional diets (Olaniyi *et al.,* 2022). This is largely attributed to chitosan's capacity to reduce ammonia accumulation in the rumen, promoting more efficient microbial protein synthesis (Shah *et al.,* 2022). Furthermore, chitin has been observed to act as a functional fiber, improving gut motility and supporting a healthy gut environment, which aids in better nutrient absorption (El-Zaiat *et al.,* 2024). This fiber-like behaviour also helps reduce enteric pathogens, lowering the incidence of gastrointestinal disturbances in goats (Patra and Saxena, 2019).

In terms of nitrogen metabolism, both chitin and chitosan have been shown to positively influence nitrogen retention by reducing urinary nitrogen losses and enhancing nitrogen digestibility (Ibrahim *et al.,* 2019). Improved nitrogen retention not only supports muscle development and growth but also reduces nitrogen-related environmental pollution from livestock waste (Adeyemi *et al.,* 2023). Additionally, performance improvements have been linked to chitosan's ability to modify rumen fermentation patterns, particularly by reducing methane production and increasing propionate formation, which supports better energy utilization (Zhang *et al.,* 2020). This shift in volatile fatty acid production enhances energy availability for maintenance and growth. Despite these benefits, the level of inclusion must be optimized, as excessive amounts of chitosan may negatively affect feed palatability or interfere with the absorption of certain nutrients (Nworgu *et al.,* 2021). Therefore, careful formulation and controlled trials are essential when using these additives in goat diets. Overall, the performance of goats fed chitin and chitosan-based diets supports their inclusion as sustainable feed additives that enhance productivity and reduce dependence on conventional protein sources (Ogunbosoye *et al.,* 2020).

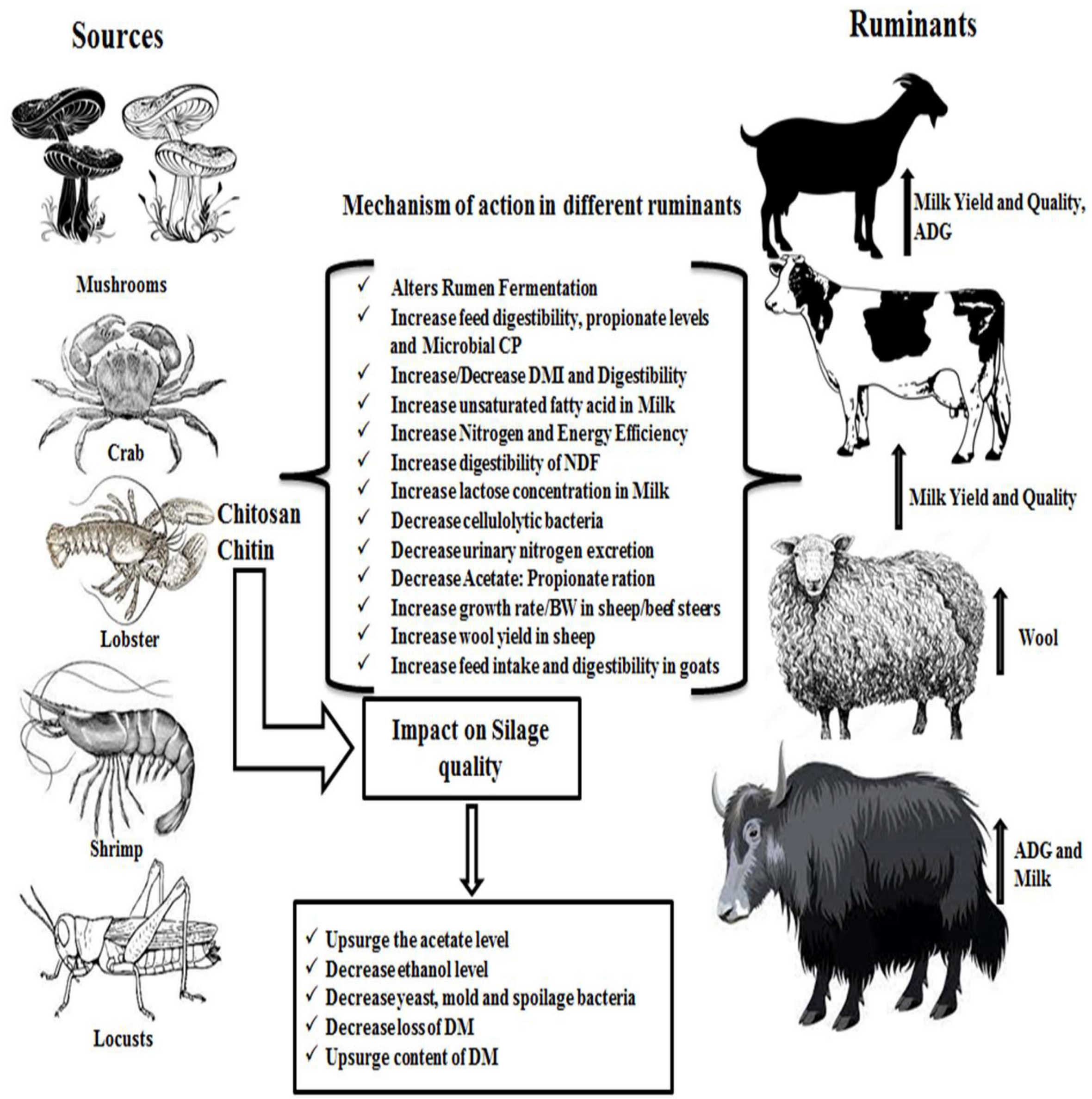

Figure 2: Mechanism of action of chitin and chitosan (Shah *et al.,* 2022)

2.5 Nitrogen Metabolism in Ruminants

Nitrogen metabolism in ruminants is a central aspect of protein nutrition and plays a key role in determining the efficiency of feed utilization and animal productivity McDonald *et al.,* 2018). In the rumen, dietary protein is degraded by microorganisms into peptides, amino acids, and ultimately ammonia, which is either used for microbial protein synthesis or absorbed into the bloodstream (Wang *et al.,* 2021). The efficiency of this process depends on the balance between the degradability of protein in the rumen and the availability of fermentable energy to support microbial growth (Khan *et al.,* 2017). When fermentable energy is inadequate, excess ammonia is not utilized by microbes and is instead absorbed, converted into urea in the liver, and excreted in urine, leading to nitrogen loss (Adeyemi *et al.,* 2023). A well-balanced diet that synchronizes nitrogen and energy release improves nitrogen capture by rumen microbes, enhancing microbial protein output, an essential protein source for ruminants (Patra and Saxena, 2019). Microbial protein is then digested in the small intestine, where amino acids are absorbed for tissue growth and maintenance (El-Zaiat *et al.,* 2024). Chitin and chitosan, as feed additives, have shown potential to modulate nitrogen metabolism by inhibiting proteolytic and ureolytic bacteria in the rumen, thereby reducing ammonia production and enhancing nitrogen retention (Ibrahim *et al.,* 2019). Chitosan's cationic nature allows it to bind with negatively charged microbial cell walls, altering microbial population dynamics in favour of more efficient fermenters (Shah *et al.,* 2022).

The net nitrogen balance, the difference between nitrogen intake and total nitrogen excreted in faeces and urine is a key indicator of nitrogen utilization efficiency (Okafor *et al.,* 2020). Positive nitrogen balance reflects efficient protein utilization and is directly linked to animal growth, milk production, and reproductive performance (Agboola *et al.,* 2021). Improving nitrogen metabolism through strategic feeding, including the use of unconventional protein-rich additives like chitin and chitosan, is critical in enhancing sustainable goat production in the tropics (Ogunbosoye *et al.,* 2020). This not only boosts animal performance but also reduces environmental pollution from nitrogen-rich waste (Adeyemi and Olaniyi, 2017).

2.6 Nitrogen Balance and Retention

Nitrogen balance is a fundamental concept in ruminant nutrition that measures the difference between nitrogen intake and nitrogen losses through faeces and urine (McDonald *et al.,* 2018). It is used to evaluate how efficiently dietary protein is being utilized for maintenance, growth, reproduction, or production (Khan *et al.,* 2017). A positive nitrogen balance indicates that the animal is retaining nitrogen, which is essential for tissue development and productivity, while a negative balance reflects catabolism or protein deficiency (Wang *et al.,* 2021). In goats, particularly under tropical feeding conditions where forage quality is often poor, achieving positive nitrogen balance is a critical performance target (Adeyemi *et al.,* 2023). Nitrogen retention is the portion of ingested nitrogen that remains in the body after accounting for faecal and urinary losses, and it is a direct reflection of how much nitrogen is available for growth or productive functions like milk or muscle synthesis (Olaniyi *et al.,* 2022). The measurement of nitrogen balance involves collecting data on feed intake, nitrogen content of feed, faeces, and urine over a defined period using metabolic cages (Ibrahim *et al.,* 2019). Chitin and chitosan supplementation has been reported to enhance nitrogen retention by modifying rumen fermentation and reducing nitrogen excretion in urine (Shah *et al.,* 2022). These compounds slow down proteolysis and ammonia formation in the rumen, allowing more nitrogen to be incorporated into microbial protein (El-Zaiat *et al.,* 2024). This microbial protein is eventually digested and absorbed in the intestines, contributing significantly to the animal's protein requirements (Patra and Saxena, 2019). In goats fed chitin/chitosan-supplemented diets, studies have shown improved nitrogen digestibility and retention, leading to better growth rates and feed conversion efficiency compared to goats on conventional diets (Agboola *et al.,* 2021). Moreover, improving nitrogen retention not only supports animal productivity but also reduces the environmental burden of nitrogenous waste in intensive systems (Okafor *et al.,* 2020). As a performance indicator, nitrogen balance plays a vital role in evaluating the suitability of novel feed ingredients such as marine-derived chitin and chitosan in sustainable goat nutrition, particularly for indigenous breeds like the West African Dwarf (WAD) goat (Adeyemi and Olaniyi, 2017).

2.7 Chitin and Chitosan from Periwinkle Shells

Periwinkle shells (*Tympanotonus fuscatus*), commonly found along West African coastal regions are rich in chitin, a structural polysaccharide that can be extracted and converted into chitosan through deacetylation (Okafor *et al.,* 2020). These shells, often discarded as waste from seafood processing, represent an abundant and underutilized resource for sustainable feed input in animal production (Agboola *et al.,* 2021). Chitin from periwinkle shells is primarily located in the exoskeleton matrix, composed of N-acetyl-D-glucosamine units bonded in a β-(1→4) configuration, giving it strength and resistance to degradation (Ibrahim *et al.,* 2019). Through chemical treatment involving demineralization, deproteinization, and deacetylation, chitin is transformed into chitosan, a more soluble and functionally active derivative (Shah *et al.,* 2022). The chitosan derived from periwinkle shells possesses several biological properties including antimicrobial, antioxidant, and immunomodulatory effects, which are beneficial when used in livestock diets (El-Zaiat *et al.,* 2024). Its ability to bind toxins and pathogens within the gastrointestinal tract supports gut health and improves nutrient absorption (Patra and Saxena, 2019). As a feed additive, periwinkle shell-derived chitin and chitosan have been shown to enhance nitrogen retention and feed efficiency in small ruminants such as goats (Olaniyi *et al.,* 2022). Their inclusion in diets reduces urinary nitrogen excretion by modulating rumen microbial activity, especially by inhibiting ureolytic bacteria that convert protein to ammonia (Adeyemi *et al.,* 2023).

In regions where conventional feed resources are costly or scarce, such as in many parts of sub-Saharan Africa, the valorization of seafood waste into functional feed ingredients offers economic and environmental advantages (Ogunbosoye *et al.,* 2020). Utilizing periwinkle shells aligns with sustainable agriculture goals, promoting circular resource use and reducing feed input costs (Osei-Amponsah *et al.,* 2021).Furthermore, chitosan from periwinkle shells can be blended with forages like guinea grass to improve crude protein content and nitrogen digestibility in goat diets, especially in dry seasons when forage quality declines (Nworgu *et al.,* 2021). This makes periwinkle-derived chitin and chitosan suitable as unconventional feed supplements for improved goat performance and nitrogen metabolism.

Plate 1: Perwinkle shell (Aimikhe *et al.,* 2021)

2.8 Effect of Chitosan on Rumen Fermentation and Nitrogen Efficiency

Chitosan, a natural polysaccharide derived from chitin, has gained significant attention as a functional feed additive in ruminant nutrition due to its ability to modulate rumen fermentation and enhance nitrogen utilization (Ibrahim *et al.,* 2019). Its cationic nature allows it to interact with the negatively charged microbial cell membranes, leading to selective inhibition of undesirable rumen microbes such as ureolytic and methanogenic bacteria (Shah *et al.,* 2022). This antimicrobial action alters the rumen microbial population, favoring the proliferation of beneficial microbes involved in fiber degradation and microbial protein synthesis (Adeyemi *et al.,* 2023). As a result, chitosan supplementation has been shown to reduce ammonia-N concentrations in the rumen by limiting protein degradation and enhancing ammonia incorporation into microbial biomass (El-Zaiat *et al.,* 2024). Chitosan also influences the production of volatile fatty acids (VFAs), the primary source of energy in ruminants. It tends to increase the molar proportion of propionate while reducing acetate and methane, improving energy efficiency and reducing greenhouse gas emissions from livestock (Patra and Saxena, 2019). By improving microbial protein synthesis and reducing nitrogen losses through urine, chitosan enhances overall nitrogen efficiency, allowing more dietary nitrogen to be retained for tissue growth and production (Agboola *et al.,* 2021). This is particularly beneficial in small ruminant production systems where protein sources are expensive or scarce.

In goats, dietary inclusion of chitosan has led to higher nitrogen retention values and improved feed conversion efficiency, especially when combined with fibrous feeds such as guinea grass (Olaniyi *et al.,* 2022). These outcomes suggest that chitosan can serve as a strategic tool to improve rumen function, reduce nitrogen waste, and support sustainable production in tropical regions (Ogunbosoye *et al.,* 2020). Furthermore, using chitosan from agro-industrial by-products like periwinkle shells contributes to circular economy goals and adds value to seafood waste, enhancing the sustainability of ruminant feeding systems (Osei-Amponsah *et al.,* 2021).

Table 2.1: Proximate composition (% dry matter basis) of chitin feed and chitosan feed from periwinkle shell

Component	Chitin feed (%)	Chitosan feed (%)
Moisture	7.10	6.85
Crude Protein	5.40	6.75
Ether Extract (Fat)	1.15	1.30
Crude Fibre	28.65	14.10
Ash	17.35	9.25
Nitrogen-Free Extract	40.35	61.75

Source: Onosakponome *et al.,* (2021); Ugbogu *et al.,* (2016); Laboratory values may vary slightly based on method of extraction and drying.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site and Unit

The study was conducted at the Ruminant Unit of the University of Benin Farm Project, Benin City, Edo State, Nigeria. The location lies within the humid tropical rainforest zone of Nigeria, characterized by a warm, wet climate and dense vegetation. Geographically, the site is located at latitude of 6.3349° N, longitude of 5.6191° E, and an altitude of approximately 77 meters above sea level. The average annual rainfall ranges from 1,500 mm to 2,000 mm, with relative humidity between 70% and 90%, depending on the season (NIMET, 2023; Ojeh *et al.,* 2021).

3.2 Experimental Equipment

The study utilized metabolic cages specially designed for individual goats, which allow for separate collection of faeces and urine. Additional equipment includes weighing scales, feeding troughs, drinking troughs. Others are hot plates, ovens, muffle furnace, Soxhlet extractor, Kjeldahl apparatus, and desiccators used in laboratory analysis (AOAC, 2019).

3.3 Experimental Animals and Management

Twelve (12) West African Dwarf (WAD) weaner goats with average initial body weights of 5–11 kg were sourced locally. The goats were quarantined for 14 days during which they were dewormed, vaccinated, and monitored for health status. They were housed individually in metabolic cages and fed ad libitum and served clean water throughout the eight days trials.

3.4 Experimental Diets

Six experimental diets were formulated and fed to goat at 50% supplemental level alongside Guinea grass. The six diets formulated were:

T1 (Control): concentrate without chitin/chitosan

T2: concentrate + 3% Chitin

T3: concentrate + 6% chitin

T4: concentrate + 0.5% chitosan

T5: concentrate + 1% chitosan

T6 (Antibiotics): concentrate + 0.01 oxytetracycline

Diets were formulated to meet NRC (2007) nutrient requirements for growing goats.

Table 3.1: Feed ingredients composition of experimental diets containing varying levels of chitin and chitosan from periwinkle shells

Ingredients	Periwinkle					Antibiotics
	T1 (Control)	T2 (3% Chitin)	T3 (6% Chitin)	T4 (0.5% Chitosan)	T5 (1% Chitosan)	T6 Antibiotics
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 (Periwinkle)	0	3	6	0	0	0
Chitosan (Periwinkle)	0	0	0	0.5	1	0
Antibiotic (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	10.2955	10.1688
ME(2500kcal)	2651.12	2613.62	2576.12	2641.66	2636.48	2646.57

3.5 Collection and Preparation of Chitin and Chitosan

Periwinkle shells were washed, sun-dried, ground, and extracted for chitin and chitosan using standard procedures of Varun *et al.* (2017).

3.6 Experimental Procedure and Design

The study followed a Completely Randomized Design (CRD). Twelve WAD goats were randomly assigned to six dietary treatments (T1–T6) with two goats per treatment group. The feeding trial lasted for 15 days, preceded by a 7-day adaptation period. Animals were housed in metabolic cages for a digestibility and nitrogen balance trial. Feed was served twice daily (8:00 am and 3:00pm) and feed leftovers were collected and weighed each morning to determine daily feed intake. Water was provided ad libitum. Total Faeces and urine were collected daily from each goat.

3.7 Variables Monitored

3.7.1 Feed intake trial

Daily feed intake was calculated by subtracting feed leftover from feed served.

3.7.2 Chemical analysis

Samples of feed, faeces, and urine was analyzed using standard AOAC (2019) Neutral Detergent Fibre (NDF) and Acid Detergent Fibre were analysed using standard procedures of Van Soest *et al.* (1991). Hemicellulose is difference between NDF and ADF values

3.8 Nitrogen Balance Study

Total nitrogen intake, faecal nitrogen, and urinary nitrogen were determined.

Nitrogen Intake (NI)= Feed intake × % nitrogen

Faecal Nitrogen (FN) = Daily faecal output × % nitrogen

Urinary Nitrogen (UN)= Daily urine output × % nitrogen

Nitrogen Retained (NR) = NI – (FN + UN)

Nitrogen Digestibility (%) = [(NI – FN) / NI] × 100

Nitrogen Retention (%) = [NR / NI] × 100

Urine was collected in cans and Six drops of sulfuric acid was added to prevent nitrogen loss through volatilization.

3.9 Statistical Analysis

Data collected were analyzed using the Genstat 12th edition. Analysis of variance was determined and Mean separation was done using the Duncan Multiple Range Test (DMRT) in the same software (Steel and Torrie, 1980).

CHAPTER FOUR

RESULTS

4.1 Chemical Analysis of Experimental Diet

4.1.1 Dry matter

Control diet (91.85%) had the highest dry matter (DM) and was significantly higher than 0.5% chitosan diet (90.75%). 3% chitin diet, 6% chitin diet , 1% chitosan diet and 0.01% oxytetracycline diet had intermediate values (91.10%–91.70%) and were not significantly different from each other.

4.1.2 Crude fibre

6% chitin diet (24.50%) had the highest fibre content, significantly higher than all others. 1% chitosan diet (17.50%) had the lowest fibre, while 3% chitin diet, 0.5% chitosan diet and 0.01% oxytetracycline diet were intermediate (18.50%–22.00%).

4.1.3 Crude protein

0.5% chitosan diet (22.75%) had significantly the highest crude protein (CP), making it the most protein-rich diet. All others (control diet, 3% chitin diet, 6% chitin diet, 1% chitosan diet, 0.01% oxytetracycline diet) ranged from 15.75% –18.38% and were not significantly different, suggesting moderate protein supply. High CP in 0.5% chitosan diet may enhance growth and nitrogen retention.

4.1.4 Ether extract

No significant differences were found among treatments ($P > 0.05$) as all values (8.50%–10.00%) shared the same superscript. Indicate similar fat content, hence similar energy contribution from lipids across diets.

4.1.5 Nitrogen free extract

1% chitosan diet (42.50%) and control diet (42.31%) had the highest Nitrogen free extract (NFE), indicating more readily available carbohydrates (sugars/starches). 3% chitin diet (31.50%) had the lowest, significantly different from others. 6% chitin, 0.5% chitosan and 0.01% oxytetracycline diet were moderate. High NFE suggests better energy availability for maintenance and production.

4.1.6 Ash

3% chitin diet (20.00%) had the highest mineral content, followed by 1% chitosan diet (15.50%) and 6% chitin diet (14.00%). 0.5% chitosan diet (9.00%) had the lowest ash content, indicating fewer minerals. Control diet and 0.01% oxytetracycline diet (11.50%) were similar and significantly lower than 3% chitin diet and 1% chitosan diet. 3% chitin diet may offer better mineral supply, essential for metabolic and structural functions.

4.1.7 Organic matter

0.5% chitosan diet (91.00%) had the highest organic matter (OM) , significantly greater than all others. 3% chitin diet (80.00%) had the lowest, indicating high ash (mineral) content. Control diet and 0.01% oxytetracycline diet (88.50%) were similar. High OM reflects better digestible organic nutrient concentration.

Table 4.1: Chemical composition (%) of experimental diets (on dry matter basis) containing different levels of chitin and chitosan

Composition (%)	T1	T2	T3	T4	T5	T6	SEM
DM	91.85a	91.40ab	91.70ab	90.75c	91.55ab	91.10bc	0.146
CF	18.50d	21.00bc	24.50a	19.00cd	17.50d	22.00b	0.677
ASH	11.50d	20.00a	14.00c	9.00e	15.50b	11.50d	0.354
CP	17.94b	17.50b	17.94b	22.75a	15. 75b	18.38b	0.838
EE	9.75a	10.00a	9.00a	10.00a	8.75a	8.50a	0.45
NFE	42.31a	31.50c	34.56bc	39.25ab	42.50a	39.62ab	1.458
OM	88. 50b	80.00e	86.00c	91.00a	84.50d	88.50b	0.354

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

Source: Laboratory analysis (2025)

4.2 Nitrogen Utilization of Diets Fed to Goats

4.2.1 Concentrate dry matter intake

All treatments (T1–T6) had statistically similar concentrate DMI, as indicated by the same superscript 'a' across the board. This suggests that the inclusion of chitin and chitosan at varying levels did not significantly affect the goats' willingness or ability to consume concentrate feed. The highest DMI was observed in control group (186.3%), while the lowest was in 3% chitin (156.8%), but these differences were not statistically significant (P>0.05).

4.2.2 Grass dry matter intake

All treatments have the same superscript letter "a", indicating that there is no significant difference (P>0.05) in grass dry matter intake among the different treatment groups. The highest grass intake was observed in T5 (1% Chitosan = 172.5%), while the lowest was in T4 (0.5% Chitosan = 145.6%), but these variations are not statistically significant.

4.2.3 Nitrogen intake from concentrate

All treatments are marked with the same superscript "a", indicating that there is no significant difference (P>0.05) in nitrogen intake from concentrate across all treatment groups. The highest nitrogen intake was recorded in T4 (0.5% Chitosan = 6.280%), while the lowest was in T5 (1% Chitosan = 4.305%). However, the difference is not statistically significant.

4.2.4 Nitrogen intake from grass

All treatment groups are assigned the same superscript "a", meaning there is no statistically significant difference ($P > 0.05$) in nitrogen intake from grass among the treatments. T5 (1% Chitosan) recorded the highest nitrogen intake (1.999%), while T4 (0.5% Chitosan) had the lowest (1.690%). However, the difference is not statistically significant.

4.2.5 Total nitrogen intake

All treatments are labeled with the same superscript "a", meaning there is no statistically significant difference ($P > 0.05$) in total nitrogen intake among all treatment groups. T4 (0.5% Chitosan) recorded the highest total nitrogen intake (7.970%), and T2 (3% Chitin) the lowest (6.095%), but the differences are not statistically significant.

4.2.6 Faecal output of nitrogen

All treatments share the same superscript "a", indicating no statistically significant difference ($P > 0.05$) in faecal nitrogen output across treatments. Numerically, T5 (1% Chitosan) showed the highest faecal N loss (2.655%), while T3 (6% Chitin) showed the lowest (1.730%), but the variation is not significant.

4.2.7 Urinary output of nitrogen

Urinary nitrogen output differed significantly ($P < 0.05$) among treatments. The highest urinary N was recorded in T5 (1% chitosan=1.3950%), indicating greater nitrogen loss through urine, possibly due to poor nitrogen utilization or excess protein catabolism. The lowest urinary N in T3 (6% Chitin=0.2850%) suggests more efficient nitrogen utilization, with less nitrogen lost via urine. Treatments T1(control), T2(3% Chitin), T4(0.5% chitosan) and T6(0.01% oxytetracycline) were intermediate, showing moderate nitrogen excretion.

4.2.8 Total nitrogen output

All treatments share the same superscript "a", indicating that there were no statistically significant differences ($P > 0.05$) in total nitrogen output among the treatments. The total nitrogen output, which includes both faecal and urinary nitrogen losses, was statistically similar across all diets. Despite numerical variations (T5=1% chitosan) being the highest at 4.049% and T3= 6% chitin the lowest at (2.016%), these differences are not significant enough to suggest that any treatment had a stronger or weaker effect on nitrogen excretion. This implies that the inclusion of chitin and chitosan from periwinkle shells at varying levels in the diet did not significantly affect total nitrogen loss in goats compared to control or antibiotic treatments.

4.2.9 Nitrogen balance

All treatments have the same superscript “a”, meaning there is no statistically significant difference ($P > 0.05$) in nitrogen balance among the treatments. Nitrogen balance reflects the difference between nitrogen intake and total nitrogen output (urine + faeces), indicating how much nitrogen is retained in the animal’s body. The values suggest positive nitrogen balance in all treatments, which means the goats were retaining nitrogen (a sign of effective protein utilization and potential tissue growth). Although T4 (0.5% chitosan) showed the numerically highest nitrogen balance (5.090%) and T5 (1% chitosan) the lowest (2.255%), the differences were not statistically significant. This indicates that none of the diets, including those with chitin/chitosan or antibiotics, significantly outperformed the others in terms of nitrogen retention.

4.2.10 Nitrogen retention

Nitrogen retention differed significantly ($P < 0.05$) among treatments. T3 (6% Chitin=70.92%) recorded the highest nitrogen retention, indicating superior protein utilization efficiency, likely due to an optimal inclusion level of chitin/chitosan enhancing microbial protein synthesis and nitrogen assimilation. T5 (1% chitosan=37.68%) had the lowest retention, suggesting that excessive chitin/chitosan might have impaired protein digestion or increased nitrogen loss. Other treatments (T1=control, T2= 6% Chitin, T4=0.5% chitosan, T6= 0.01% oxytetracycline) were statistically similar and intermediate in performance.

4.2.11 Nitrogen digestibility

There is no statistically significant difference ($P>0.05$) in nitrogen digestibility across treatments. Numerically, T3 (6% chitin) had the highest nitrogen digestibility (75.03%), suggesting slightly improved digestion and absorption of nitrogen. T5 (1% chitosan) had the lowest value (61.42%), though not significantly different. All treatments recorded digestibility above 60%, which reflects generally good nitrogen digestion in the animals.

Table 4.2: nitrogen utilization of diets fed to goats with different inclusion levels of chitin and chitosan

Variables	**T1**	**T2**	**T3**	**T4**	**T5**	T6	SEM
Conc DMI	186.3a	156.8a	174.3a	172.6a	170.7a	166.8a	27.2
Grass DMI	168.1a	147.2a	165.3a	142.6a	172.5a	162.5a	26.6
N Conc Intake	5.345a	4.385a	5.005a	6.280a	4.305a	4.905a	0.795
N Grass Intake	1.950a	1.710a	1.920a	1.690a	1.999a	1.885a	0.311
N Total Intake	7.295a	6.095a	6.925a	7.970a	6.304a	6.790a	1.091
N Faecal Output	2.275a	1.745a	1.730a	2.320a	2.655a	1.940a	0.643
N Urinary Output	0.9331abc	0.9746abc	0.2850c	0.5623bc	1.3950a	1.1735ab	0.2124
N Total Output	3.208a	2.720a	2.016a	2.883a	4.049a	3.116a	0.698
N Balance	4.090a	3.380a	4.905a	5.090a	2.255a	3.670a	1.005
N Retention	55.62ab	54.98ab	70.92a	60.49ab	37. 68b	53.80ab	7.87
N Digestibility	68.83a	71.29a	75.03a	67.52a	61.42a	71.28	7.83

Means within the same row with different superscript different significantly (P<0.05), SEM= standard error of means, T1(Control) = concentrate without chitin/chitosan, T2= concentrate + 3% Chitin, T3 = concentrate + 6% chitin, T4 = concentrate + 0.5% chitosan, T5= concentrate + 1% chitosan, T6 (Antibiotics) =concentrate + 0.01 oxytetracycline, DMI= dry matter intake, conc = concentrate and N = nitrogen.

Source: Laboratory Analysis (2025).

CHAPTER FIVE

DISCUSSION

This study evaluated the effects of incorporating chitin and chitosan extracted from periwinkle shells into the diets of West African Dwarf (WAD) goats on their nitrogen balance, retention, and overall nutrient utilization. The observed results are discussed in relation to the chemical composition, nutrient intake, nitrogen utilization, and the performance of the animals.

5.1 Chemical Composition of Experimental Diets

The dry matter (DM) content of all diets was generally high (ranging from 90.75% to 91.85%), indicating good feed preservation and minimal moisture, which is essential for preventing spoilage and ensuring consistent nutrient intake. 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 fibre (CF) content was significantly highest in T3= 6% chitin (24.50%) and lowest in T5 =1% chitosan (17.50%). The higher CF in T3 (6% chitin) could be attributed to the fibrous nature of chitin, which may limit digestibility when included excessively (Akinfala *et al.,* 2021). However, moderate fibre levels (like in T5= 1% chitosan) could enhance rumen motility and microbial activity, promoting better nutrient digestion (Aro *et al.,* 2018).

Crude protein (CP) was highest in T4= 0.5% chitosan (22.75%), suggesting enhanced protein concentration in the chitosan-supplemented diet. This aligns with observations by Ukanwoko and Ibeawuchi (2017), who stated that chitosan may support nitrogen retention through its antimicrobial properties that reduce protein-degrading bacteria in the rumen. Treatments T1 (control), T2 (3% chitin) , T3(6% chitin), T5 (1% chitosan), and T6(0.01% oxytetracycline) had relatively similar CP values (15.75–18.38%), showing that the inclusion of chitin and chitosan at moderate levels did not compromise dietary protein.

Ether extract (EE) did not differ significantly across treatments, maintaining values between 8.50% and 10.00%, which is consistent with expected lipid levels in goat rations. High EE may promote energy supply, but excessive levels can impair microbial fermentation (NRC, 2007).

Ash content, a measure of mineral presence, was significantly highest in T2= 3% chitin (20.00%) and lowest in T4= 0.5% chitosan (9.00%). The variations may reflect the mineral contribution from periwinkle-based additives. Organic matter (OM) followed similar trends, indicating inversely proportional changes to ash contents.

5.2 Feed and Nitrogen Intake

Dry matter intake (DMI) of both concentrate and grass showed no significant differences across treatments. This suggests that inclusion of chitin and chitosan did not negatively affect palatability or feed acceptance. According to Onwuka *et al.* (2020), feed acceptability is crucial in nutritional trials, especially when using unconventional feed ingredients.

Nitrogen intake (from both concentrate and grass) was highest in T4 (0.5% chitosan = 6.280%) and T5 (1% chitosan=1.999%) respectively, indicating improved protein availability in those diets. Despite the structural rigidity of chitin, its gradual breakdown in the rumen may provide a sustained nitrogen release (Esonu *et al.,* 2016).

5.3 Faecal and Urinary Nitrogen Losses

Faecal nitrogen output did not significantly differ among treatments, but numerically, T5 (1% chitosan = 2.655%) recorded the highest faecal nitrogen, suggesting possible incomplete protein digestion or microbial loss. This may also reflect reduced digestibility at certain inclusion levels. However, urinary nitrogen output differed ($P < 0.05$), with 1% chitosan diet showing the highest loss (1.3950%) and 6% chitin diet the lowest (0.2850%). High urinary nitrogen indicates inefficient nitrogen utilization, possibly due to increased amino acid catabolism (Balcells *et al.*, 2012). Conversely, lower urinary nitrogen in 6% chitin-fed goats reflects better nitrogen conservation, implying that optimal chitin levels may enhance microbial protein synthesis and nitrogen retention.

5.4 Nitrogen Balance, Retention, and Digestibility

Nitrogen balance was significantly highest in T4 (0.5% chitosan=5.090%), T3 (6% chitin=4.905%), and T1 (control=4.090%), indicating better nitrogen utilization. These treatments suggest that chitosan and chitin enhanced nitrogen retention, possibly through antimicrobial effects that reduce proteolysis in the rumen (Binta and Omage, 2021). T5(1% chitosan= 2.255%) and T2 (3% Chitin= 3.380%) had significantly lower nitrogen balance values, suggesting that the inclusion levels may have been suboptimal or led to inefficiencies.

Nitrogen retention differed significantly ($P < 0.05$), with 6% chitin diet showing the highest retention (70.92%). This suggests superior protein utilization efficiency and effective nitrogen assimilation into body tissues. The improvement could be attributed to chitin's prebiotic effects, enhancing rumen microbial growth and nitrogen recycling (Yun *et al.,* 2020). The lowest retention (37.68%) in 1% chitosan diet might indicate that excessive deacetylated chitosan interfered with digestion, possibly due to binding effects that reduced protein availability. These results imply that chitin at 6% inclusion level offers the most balanced benefit for nitrogen metabolism.

Nitrogen digestibility ranged from 61.42% to 75.03% across treatments, with no significant differences. This indicates that all diets supported reasonable protein digestibility, even with chitin or chitosan presence. It supports the view by Oduguwa *et al.* (2020) that chitosan does not interfere with nitrogen absorption at moderate levels.

Overall, the study revealed that diets supplemented with chitin and chitosan from periwinkle shells did not negatively affect nitrogen intake or utilization. Diets showed promising improvements in nitrogen balance and reduced urinary nitrogen loss. This indicates the potential of chitin and chitosan as effective unconventional feed resources in improving nitrogen efficiency and possibly growth performance in WAD goats.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study evaluated the chemical composition and nitrogen utilization of goat diets containing varying inclusion levels of chitin and chitosan derived from periwinkle shells. Results showed that the inclusion of these bioactive polysaccharides influenced feed composition and nitrogen metabolism without adverse effects on intake. Chitin increased fibre and mineral content, while chitosan improved crude protein and organic matter levels. Feed intake and nitrogen intake were not significantly affected by treatment, indicating good palatability and acceptance of all diets. Nitrogen retention and urinary nitrogen output were the most responsive parameters, 6% chitin diet recorded the highest nitrogen retention, indicating superior protein utilization efficiency, while excessive 1% chitosan led to higher nitrogen loss through urine. Overall, all goats maintained positive nitrogen balance, suggesting effective nitrogen utilization and adequate protein supply. The study concludes that chitin and chitosan from periwinkle shells can be safely incorporated into goat diets as sustainable feed additives, with 6% chitin and 0.5% chitosan showing optimal results.

6.2 Recommendations

1. 6% chitin and 0.5% chitosan are recommended for improved nitrogen retention and overall feed efficiency in goats.
2. Further studies should explore long-term feeding trials and growth performance metrics (e.g., weight gain, feed conversion) for comprehensive evaluation.
3. Economic analysis should be conducted to determine the cost-benefit ratio of using chitin/chitosan-based feeds.
4. Studies should assess the effects on rumen microbiota and health markers to support their use as natural feed additives.
5. Training programs should educate local farmers on extraction techniques for chitin and chitosan from seafood waste to promote on-farm sustainability.

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