IN VITRO NUTRITIVE VALUE OF DIET CONTAINING DIFFERENT INCLUSIONS LEVELS OF CHITIN, AND CHITOSAN FROM PERIWINKLE SHELLS

BY

Samuel Oghenevwede MUKORO(master)
AGR2000091

DEPARTMENT OF ANIMAL SCIENCE
FACULTY OF AGRICULTURE
UNIVERSITY OF BENIN
BENIN CITY, NIGERIA

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF AGRICULTURE HONORS (B. AGRIC. IN ANIMAL SCIENCE)

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CERTIFICATION

This is to certify that this Project work was carried out by **Samuel Oghenevwede MUKORO(master)** with Matriculation Number, **AGR2000091** under the guidance of the Project Supervisors approved by the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

PROFESSOR M. A. BAMIKOLE **DATE**

(Project Supervisor)

MRS. B. ISAAC **DATE**

(Co-Project Supervisor)

DR. N. C. AKAEZE **DATE**

(Head of Department)

DEDICATION

This Project work is dedicated to God Almighty, for His provisions, grace, favour, wisdom, understanding and never ending love throughout the course of this Research and Study.

And to my incredible Parents, Mr. and Mrs. Mukoro, my amazing Siblings, other family and special friends who through their financial, spiritual, emotional, physical, psychological, physiological support and continuous encouragement saw me through the duration of this study.

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ABSTRACT

This study evaluated the in vitro nutritive value of diets containing different inclusion levels of chitin and chitosan extracted from periwinkle shells (*Tympanotonus fuscatus*). The experiment aimed to determine the effects of these biopolymers on gas production, nutrient digestibility, and fermentation characteristics using in vitro rumen incubation techniques. Chitin and chitosan were extracted from processed periwinkle shells and incorporated into diets at varying levels (0.5–6%), alongside control and antibiotic-based treatments. Chemical composition analysis showed significant variations in crude fibre, ash, and protein content, with the 0.5% chitosan diet recording the highest crude protein (22.75%) and organic matter (91.00%). Gas production increased progressively with incubation time, peaking at 24 hours. Diets containing 0.5% chitosan produced the highest cumulative gas volume (30.00 ml/200 mg DM), indicating enhanced microbial fermentation, while higher chitin levels (6%) suppressed fermentation activity. Post-fermentation parameters showed that moderate chitosan inclusion (0.5%) improved dry matter digestibility (67.77%), organic matter digestibility (64.86%), metabolizable energy (7.58 MJ/kg DM), and short-chain fatty acid production (0.657 mmol/L) without significantly affecting methane output. Excessive inclusion levels reduced digestibility and fermentation efficiency.

CHAPTER ONE

1.0 INTRODUCTION

Chitin and its deacetylated derivative, chitosan, are naturally occurring N-acetylated polysaccharides found in the exoskeletons of crustaceans, insects, and certain molluscs. In recent years, they have attracted growing interest as alternative feed ingredients and functional additives in livestock nutrition due to their bioactive, antimicrobial, and immunomodulatory properties as well as their potential to valorize shell wastes within a circular bioeconomy framework (Shah *et al*., 2022). Valorization of marine wastes such as periwinkle shells not only mitigates environmental pollution but also provides a low-cost renewable source of biopolymer for feed applications (Amanzougarene *et al*., 2020).

Marine mollusc shells and other crustacean by-products are a major source of the structural polysaccharide chitin and its deacetylated derivative chitosan. Chitin is a linear polymer of N-acetyl-D-glucosamine that occurs naturally in exoskeletons and shells of marine organisms, while chitosan is derived through partial deacetylation and possesses unique solubility and biological properties that make it useful in food, agriculture, biomedical, and animal-nutrition applications (Odili *et al*., 2021; Gbenebor *et al*., 2017). Extraction and characterization protocols involving demineralization, deproteinization, decolorization, and deacetylation have been successfully applied to various shell wastes such as shrimp, crab, and molluscs including periwinkle shells to obtain chitin and chitosan suitable for research and industrial applications (Odili *et al*., 2021; Onosakponome *et al*., 2025).

In Nigeria, the technical feasibility of obtaining high-quality chitin and chitosan from periwinkle shells has been extensively demonstrated (Gbenebor *et al.,* 2017; Odil*i et al.,* 20210).

Researchers have reported that optimized acid and alkali treatments can yield high-quality chitin with desirable degrees of deacetylation and crystallinity (Gbenebor *et al*., 2017; Odili *et al*., 2021). Analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Thermogravimetric Analysis (TGA) are commonly used to confirm the product's identity and degree of deacetylation in Nigerian laboratories (Odili *et al*., 2021; Onosakponome *et al*., 2025). These findings establish periwinkle shells as a viable local source of chitin and chitosan for potential use in feed formulation and other industrial applications.

In the quest to enhance animal feed efficiency and sustainability, novel feed additives derived from agro or marine industry by-products are of increasing interest. One promising class of such additives is the structural polysaccharides chitin and chitosan, extracted from shell wastes of marine organisms.

In animal nutrition, chitin and chitosan are of interest for several functional roles: modulation of gut microflora, binding of nutrients or bile acids, improving immune status, and even influencing fermentation in ruminant systems. For instance, chitosan inclusion in ruminant diets has been reported to alter rumen fermentation patterns (more propionate, less acetate), potentially reducing methane emissions and improving energetic efficiency (Shah *et al*., 2022). However, the effects on nutrient digestibility vary widely: while some studies show improved digestibility or feed efficiency, others report decreased digestibility at higher inclusion levels, possibly due to microbial inhibition or nutrient binding (Shah *et al*., 2022).

Given the dependence of biological effects on source, physicochemical properties (degree of deacetylation, molecular weight, particle size), and inclusion level, it becomes essential to evaluate chitin/chitosan derived specifically from periwinkle shells and to assess their nutritive value in in vitro systems before proceeding to in vivo trials. The in vitro approach offers a relatively rapid, cost-effective screening of inclusion levels, digestibility and fermentation behaviour under controlled conditions.

Thus, this study will examine diets containing graded inclusion levels of chitin and chitosan extracted from periwinkle shells, and evaluate their in vitro nutritive value (e.g., digestibility, fermentation indices) to establish safe and effective inclusion rates for subsequent animal feeding trials.

1.2 Justification of the Study

Periwinkle (*Tympanotonus fuscatus*) shells are abundant along the coastal areas of Nigeria, especially in states such as Rivers, Akwa Ibom, Cross River, and Bayelsa, where the mollusc serves as a common delicacy and source of livelihood (Akpan, 2018). The processing and consumption of periwinkles generate large quantities of shell waste, which pose serious environmental and sanitation problems when discarded indiscriminately (Odili, 2021). These shells, however, are rich in chitin, a biopolymer that can be converted into chitosan, both of which have valuable industrial and agricultural applications (Gbenebor, Akpan, and Adeosun, 2017). Therefore, valorizing periwinkle shell waste into useful feed additives presents an eco-friendly and economically viable solution to waste management and feed improvement in Nigeria (Onosakponome *et al*., 2025).

Chitin and chitosan have attracted increasing attention in animal nutrition due to their functional properties, such as antimicrobial activity, immunostimulation, cholesterol-lowering effects, and their ability to modify gut microflora and fermentation processes (Shah *et al*., 2022). In ruminant nutrition, studies have shown that chitosan supplementation can alter rumen fermentation patterns, reduce methane production, and enhance propionate formation, thereby improving feed efficiency and reducing greenhouse gas emissions (Rey *et al*., 2023). However, the effects of these polymers depend largely on their source, level of inclusion, and physicochemical characteristics, such as molecular weight and degree of deacetylation. Since the majority of available chitosan is derived from shrimp and crab shells, its evaluation from periwinkle shells within Nigerian feed contexts remains largely unstudied.

Local studies have demonstrated the feasibility of extracting and characterizing chitin and chitosan from periwinkle shells using chemical methods suitable for small- to medium-scale production (Akpan, 2018; Onosakponome *et al*., 2025). For example, Akpan (2018) reported that periwinkle shells yielded chitin with similar structural properties to that of shrimp-derived chitin, while Gbenebor *et al*. (2017) and Odili (2021) confirmed that Nigerian periwinkle shells contain sufficient chitin content to support industrial-scale recovery. These findings highlight the technical potential of using periwinkle shells as a local source of chitin and chitosan for agricultural and feed applications.

Despite this progress, there is limited information on the nutritive implications of including chitin and chitosan derived from periwinkle shells in livestock diets, particularly under in vitro digestibility and fermentation conditions relevant to feed formulations. Existing studies use commercially refined chitosan from crustaceans, which may differ chemically from locally sourced materials due to variations in extraction methods and mineral content (Shah *et al*., 2022; Rey *et al*., 2023). Thus, there is a pressing need for Nigeria specific studies that quantify how varying inclusion levels of periwinkle derived chitin and chitosan influence the digestibility, gas production, and nutrient utilization potential of animal feeds.

Furthermore, the high cost of imported feed additives and the scarcity of locally available functional ingredients contribute to the rising cost of animal production in Nigeria (Adebayo *et al*., 2020). If periwinkle derived chitosan demonstrates favorable nutritive or functional effects, it could serve as a low cost, locally sourced feed additive that improves the profitability and sustainability of livestock farming (Akpan, 2018; Onosakponome *et al*., 2025).

Finally, employing in vitro methods provides a cost effective and ethically acceptable preliminary step in evaluating the nutritive value and fermentation characteristics of diets containing chitin and chitosan before proceeding to animal (in vivo) trials (Blümmel *et al*., 1997; Shah *et al*., 2022). This approach will allow researchers to determine optimal inclusion levels that maximize nutrient digestibility and minimize potential negative effects such as nutrient binding or reduced palatability.

1.3 Objectives of the Study

The main objectives of this study was to determine:

4. The chemical composition of diets with varying inclusion levels of chitin and chitosan from periwinkle shells
5. The in vitro gas production, dry matter digestibility, and post in vitro fermentation characteristics of diets containing different levels of chitin and chitosan.

CHAPTER TWO

2. 0 LITERATURE REVIEW

2.1 Overview of Chitin and Chitosan

Chitin is a naturally occurring linear polysaccharide composed of β-(1→4)-linked N-acetyl-D-glucosamine units, ranking second only to cellulose as the most abundant biopolymer in nature (Rinaudo, 2006). Its partially deacetylated derivative, chitosan, consists of repeating units of D-glucosamine and N-acetyl-D-glucosamine linked by β-(1→4) glycosidic bonds (Kumar, 2000). Chitosan is distinguished from chitin by the presence of free amino groups that confer cationic properties, allowing it to dissolve in dilute organic acids and exhibit antimicrobial, antioxidant, and metal-chelating activities (Dutta *et al*., 2004; Shah *et al*., 2022).

Chitin occurs mainly in two crystalline forms: α-chitin (antiparallel chains, typical of crustaceans) and β-chitin (parallel chains, often found in mollusks such as periwinkle), which differ in their hydrogen bonding and physicochemical properties (Minke and Blackwell, 1978). The degree of deacetylation (DD) and molecular weight (Mw) significantly influence chitosan's solubility, viscosity, and functional behavior in biological systems (Rinaudo, 2006).

In animal nutrition, these physicochemical properties determine how chitosan interacts with feed components, microbes, and digestive enzymes (No *et al*., 2007). For instance, low molecular weight chitosan is more soluble and can enhance nutrient absorption or gut health, while high molecular weight or poorly deacetylated chitin can reduce digestibility due to its structural rigidity (Razdan and Pettersson, 1994).

2.2 Periwinkle Shells as a Sources of Chitin and Chitosan

Periwinkle (*Tympanotonus fuscatus*) is a common marine mollusc in West African coastal communities. The shells, often discarded after consumption, are rich in calcium carbonate and contain extractable organic fractions of chitin (Akpan *et al*., 2018). Several Nigerian studies have demonstrated that periwinkle shells can yield high-quality chitin and chitosan suitable for industrial and feed applications (Odili *et al*., 2021; Onosakponome *et al*., 2025). Proximate analyses show that the shells possess substantial ash and organic matter contents, making them a viable raw material for chitin production (Elegbede *et al*., 2023).

Globally, chitin and chitosan are mainly sourced from shellfish processing wastes shrimp, crab, lobster, and molluscan shells (Kurita, 2001). In Nigeria, periwinkle (*Tympanotonus fuscatus*), a common estuarine mollusc, provides a significant but underutilized source of chitin. Periwinkle shells, which constitute over 60% of the organism's dry mass, are often discarded as waste, contributing to environmental pollution in coastal areas (Odili, 2021).

Several Nigerian studies have demonstrated the feasibility of extracting chitin and chitosan from periwinkle shells using conventional acid-base methods. Gbenebor, Akpan, and Adeosun (2017) reported that periwinkle shells yielded chitin with high crystallinity and thermal stability, comparable to shrimp shell chitin. Similarly, Odili (2021) successfully extracted and characterized chitin and chitosan from Nigerian periwinkle shells, confirming the β-chitin structure through FTIR and XRD analyses.

Onosakponome *et al*. (2025) reported that adjusting acid and alkali concentrations during extraction affected yield and degree of deacetylation, suggesting the importance of optimization for industrial scalability. In addition, Elegbede *et al*. (2023) analyzed periwinkle shell composition, showing high calcium carbonate ($CaCO_3$) and significant chitin content suitable for chitosan conversion.

Periwinkle shells' availability in Niger Delta states such as Rivers, Akwa Ibom, and Bayelsa makes them a locally sustainable raw material for chitin and chitosan production, offering both economic and environmental benefits (Gbenebor *et al*., 2017; Odili, 2021).

2.3 Extraction Methods and Physicochemical Characterization

Chitin and chitosan extraction generally involves demineralization, deproteinization, and deacetylation steps (Kumar, 2000). In periwinkle shells, demineralization is commonly performed with hydrochloric acid (HCl) to dissolve calcium carbonate, while deproteinization uses sodium hydroxide (NaOH) to remove protein residues (Odili, 2021). The resulting chitin is then deacetylated with concentrated NaOH at elevated temperatures (80–100 °C) to yield chitosan (Gbenebor *et al*., 2017).

Physicochemical characterization typically employs Fourier Transform Infrared Spectroscopy (FTIR) for functional group identification, X-ray Diffraction (XRD) for crystallinity, and Scanning Electron Microscopy (SEM) for surface morphology (Onosakponome *et al*., 2025). Thermal analyses such as Thermogravimetric Analysis (TGA) further confirm material stability.

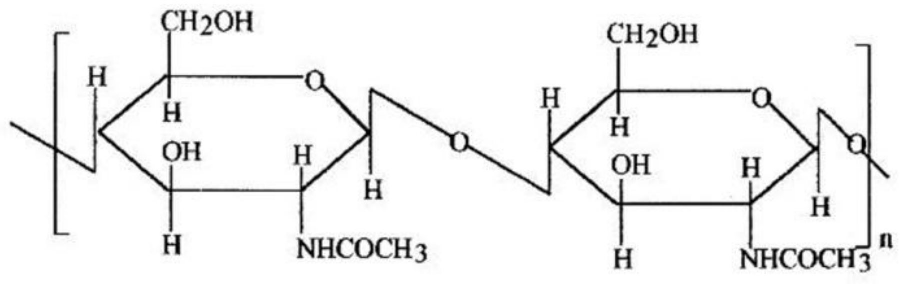
Variations in acid/base concentration and reaction time significantly influence yield, color, and degree of deacetylation (Odili, 2021). Nigerian periwinkle shells often yield β-chitin, which is more reactive and easier to deacetylate than α-chitin from crustaceans, making them advantageous for biological applications (Elegbede *et al*., 2023).

2.4 Properties and Characteristics of Chitin and Chitosan

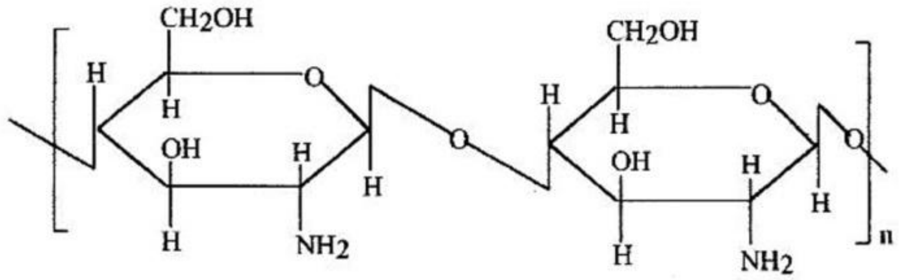
Chitin is a natural, linear polysaccharide composed primarily of β-(1→4)-linked N-acetyl-D-glucosamine units, and it is the second most abundant biopolymer in nature after cellulose (Aranaz *et al*., 2021). It occurs widely in the exoskeletons of crustaceans such as crabs, shrimps, lobsters, and in the cell walls of fungi and insects. Chitosan, on the other hand, is a deacetylated derivative of chitin obtained through chemical or enzymatic removal of acetyl groups, resulting in a polymer of D-glucosamine and N-acetyl-D-glucosamine units (Ke *et al*., 2021). The degree of deacetylation differentiates chitin from chitosan and largely determines their solubility, charge density, and functional applications (Pellis *et al*., 2022).

2.4.1 Chemical Structure and Composition

Both chitin and chitosan are linear polysaccharides, but they differ in the functional groups attached to their C2 position. Chitin contains an acetamido group ($–NHCOCH_3$), while chitosan contains a free amino group ($–NH_2$) after deacetylation (Aranaz *et al*., 2021). The presence of free amino groups in chitosan confers cationic properties under acidic conditions, making it a unique natural polycation among polysaccharides (Rinaudo, 2006). This cationic nature is the foundation for many of its biological activities and applications in food, medicine, and water treatment (Guarnieri *et al*., 2022).



Chitin



Chitosan

Figure 1: Chemical structure of Chitin and Chitosan

Source: Chawla and Kanatt, 2015

2.4.2 Biological and Functional Properties of Chitin and Chitosan in Animal Nutrition

Chitin and chitosan possess multiple biological properties relevant to feed science, including antimicrobial, hypocholesterolemic, antioxidant, and immune-enhancing effects (Dutta *et al*., 2004; Shah *et al*., 2022).

Chitin is generally insoluble and resistant to enzymatic hydrolysis, whereas chitosan exhibits better solubility and biological activity. Functionally, chitosan can: bind lipids, bile acids, and microbial cell surfaces, influencing nutrient digestibility; exert antimicrobial and antiparasitic actions that modify rumen microbial populations; and act as a prebiotic or immunomodulatory compound (Uyanga *et al.*, 2023). This binding can reduce cholesterol absorption and influence intestinal microflora, improving gut health and nutrient absorption in animals (Razdan and Pettersson, 1994). These properties can alter rumen fermentation patterns, particularly by decreasing methane emission and changing volatile-fatty-acid (VFA) profiles. However, outcomes depend on the polymer's DD, molecular weight, and inclusion level (Rey *et al.*, 2023).

In ruminants, chitosan has been shown to alter rumen fermentation profiles, decreasing methane production while increasing propionate formation, which enhances energy efficiency (Rey *et al.*, 2023). However, excessive inclusion can reduce fiber digestibility due to antimicrobial suppression of fibrolytic microbes (Goiri *et al.*, 2009). In monogastrics, moderate inclusion levels of chitosan improved lipid metabolism and immune status, but high levels impaired feed conversion (Razdan and Pettersson, 1994).

Research evidence indicates that supplementation with chitosan in ruminant diets can modify rumen fermentation. Rey *et al.* (2023) observed reductions in acetate:propionate ratio, methane production, and rumen ammonia concentration, suggesting enhanced energy efficiency. Conversely, excessive inclusion levels may suppress fibrolytic bacterial activity, leading to reduced fibre digestibility (Zanferari *et al.*, 2018). Positive effects on nitrogen utilization and feed conversion have been documented at moderate doses (Shah *et al.*, 2022). Most available data, however, come from studies using crustacean-derived chitosan; mollusc-derived materials such as those from periwinkle shells remain largely unexplored (Odili *et al.*, 2021).

2.4.3 Degree of Deacetylation (DDA)

The degree of deacetylation (DDA) or degree of acetylation (DA) represents the percentage of acetylated or deacetylated units in the polymer chain. It is a crucial parameter influencing solubility, viscosity, charge distribution, and biological interactions (Baxter *et al*., 2022). Chitin generally has a high degree of acetylation (>50%), while chitosan has a DDA greater than 50% (Ke *et al*., 2021). Higher DDA enhances the presence of free amino groups, leading to improved solubility in acidic media and greater capacity for ionic interactions (Pellis *et al*., 2022).

2.4.4 Solubility and Ionization

Chitin is insoluble in water, organic solvents, and dilute acids due to its high crystallinity and extensive hydrogen bonding (Aranaz *et al*., 2021). Conversely, chitosan is soluble in dilute acidic solutions such as acetic, lactic, or formic acid because its amino groups are protonated to form $–NH_3^+$, resulting in a positively charged polymer chain (Ke *et al*., 2021). Solubility is influenced by molecular weight, degree of deacetylation, and distribution of acetyl groups (Pellis *et al*., 2022).

2.4.5 Molecular Weight and Crystallinity

The molecular weight (MW) of chitin and chitosan varies depending on the source and extraction process. Typical MWs range from <10 kDa (for oligomeric chitosans) to >1,000 kDa for high-molecular-weight polymers (Baxter *et al*., 2022). The MW affects viscosity, mechanical strength, and biodegradation rate (Aranaz *et al*., 2021). Chitin is more crystalline and thermally stable than chitosan due to its acetylated structure, whereas deacetylation decreases crystallinity and alters thermal properties (Rinaudo, 2006).

2.4.6 Chemical Reactivity and Functional Modifications

The amino groups in chitosan are highly reactive and enable chemical modifications such as alkylation, acylation, crosslinking, and grafting, which can tune solubility, hydrophobicity, and bioactivity (Ke *et al*., 2021). Chitin is less reactive because of its acetamido groups, but partial deacetylation can improve its reactivity. Modified chitosans have been developed for applications in drug delivery, wastewater treatment, and antimicrobial coatings (Guarnieri *et al*., 2022).

2.4.7 Antimicrobial Properties

Chitosan exhibits significant antimicrobial activity against bacteria, fungi, and some viruses (Guarnieri *et al*., 2022). This property is attributed to the interaction between its protonated amino groups ($–NH_3^+$) and the negatively charged microbial cell membranes, leading to membrane disruption, leakage of intracellular contents, and inhibition of microbial growth (Ke *et al*., 2021). The antimicrobial efficiency depends on molecular weight, degree of deacetylation, and environmental pH (Aranaz *et al*., 2021).

2.4.8 Physical Appearance and Practical Use

Chitin typically appears as a white, crystalline powder, while chitosan is slightly yellowish and amorphous (Rinaudo, 2006). Both materials can be processed into films, beads, hydrogels, fibers, and nanoparticles, making them versatile in pharmaceuticals, food coatings, and agriculture (Ke *et al*., 2021).

2.5 Evidence from Feed and In Vitro Studies

Beyond ruminants, in vitro experiments and reviews on monogastrics (poultry, fish) show chitin/chito-oligosaccharides (COS) affect gut microbial metabolites (short-chain fatty acids), intestinal barrier markers, and in some cases in vitro measures of digestibility or enzymatic activity. There is growing evidence that chitin or COS may act as a prebiotic or immunomodulatory ingredient in poultry and fish, but responses depend on particle size, degree of deacetylation, and dose. Extrapolating rumen results to monogastrics is not straightforward.

2.5.1 Poultry and Monogastrics

Razdan and Pettersson (1994) reported that broilers fed diets containing 3% chitosan exhibited lower plasma cholesterol but slightly reduced protein digestibility. More recent studies (Onifade *et al*., 2023) suggest that small inclusions (<1%) enhance immune response and gut health. However, effects vary with chitosan's molecular characteristics, species, and diet formulation.

2.5.2 Ruminants

Ruminant in vitro studies have demonstrated chitosan's role in modulating fermentation. Rey *et al*. (2023) found that chitosan supplementation (2–6 g/L) reduced methane and increased propionate proportion in rumen fluid, indicating improved energy utilization. Similarly, Goiri *et al*. (2009) reported that chitosan altered microbial population structure, lowering methanogenic archaea abundance.

2.5.3 Fish and Aquatic Species

In aquaculture, chitin and chitosan have been used as functional feed additives. Incorporation of chitosan at 0.5–1% in catfish diets enhanced immune response and growth performance (Harikrishnan *et al*., 2020). For tilapia and shrimp, chitosan nanoparticles improved feed conversion efficiency and disease resistance (Pan *et al*., 2021).

Given that periwinkle is a marine organism, its chitosan may possess unique bioactivities due to β-chitin structure and trace mineral content, which warrant evaluation in in vitro systems before animal feeding trials.

2.6 In Vitro Evaluation of Nutritive Value

In-vitro assays provide rapid, inexpensive, and ethically preferable screening of feed ingredients and complete diets by simulating digestion or microbial fermentation under controlled laboratory conditions. They are used to estimate digestibility, fermentability, metabolizable energy, and fermentation end-products (Volatile Fatty Acid profiles, ammonia, gas and methane production) before committing to costly in-vivo trials (Boga et al., 2014). In-vitro results are particularly valuable for: Comparing many treatments or inclusion levels (e.g., graded levels of an additive), elucidating mechanism (microbial shifts, VFA changes), and narrowing candidate treatments for follow-up animal experiments.

Chitin and chitosan inclusion in in vitro diets have shown varying results. Lower inclusion (0.5–2%) generally enhances microbial fermentation, while higher inclusion (>4%) tends to suppress gas production and total digestibility (Rey *et al*., 2023). Therefore, determining the optimal inclusion level for periwinkle-derived chitosan through in vitro assays is crucial to establish safe and beneficial usage rates in animal feed.

2.7 The nutritional and industrial potential of Nigerian periwinkle derived chitin and chitosan

The nutritional and industrial potential of chitin and chitosan derived from periwinkle shells in Nigeria has gained increasing attention due to the abundance of this marine resource and the environmental burden posed by shell waste in coastal communities. Periwinkle shells (Tympanotonus fuscatus) are readily available in large quantities across the Niger Delta region and other coastal states such as Bayelsa, Rivers, and Akwa Ibom, making them a sustainable raw material for chitin and chitosan production (Elegbede *et al*., 2023). These shells contain significant amounts of calcium carbonate, proteins, and chitinous materials, which can be transformed into valuable biopolymers for various industrial and agricultural uses (Gbenebor *et al*., 2017; Odili *et al*., 2021).

In animal nutrition, the use of chitin and chitosan has shown promise as functional feed additives that can enhance feed efficiency, immune response, and gut health, particularly in monogastric animals such as poultry and fish (Ogunleye et *al*., 2022). Nigerian studies have highlighted that the bioactive properties of locally extracted chitosan—such as its antimicrobial and antioxidative effects—can contribute to reducing the need for antibiotic growth promoters in livestock feed (Onosakponome *et al*., 2025). The cationic nature of chitosan, resulting from the presence of amino groups, enables it to interact with negatively charged microbial membranes, thereby exerting antimicrobial action and improving intestinal microflora balance (Odili *et al*., 2021).

From an industrial perspective, chitosan from periwinkle shells has shown potential in water purification, biodegradable film production, and pharmaceutical formulations, indicating its wide applicability beyond feed use (Gbenebor *et al*., 2017; Onosakponome *et al*., 2025). Furthermore, the valorization of periwinkle shell waste into chitin and chitosan products aligns with Nigeria's circular economy goals and waste-to-wealth initiatives. This approach supports both environmental sustainability and economic empowerment for coastal communities, where shell waste accumulation has been a longstanding ecological concern (Elegbede *et al*., 2023).

Hence, the continued exploration of periwinkle shells as a bioresource for chitin and chitosan production in Nigeria not only promotes waste reduction but also adds value through the development of locally sourced feed additives and biopolymer materials. Such innovations are crucial for advancing sustainable livestock production systems and promoting self-reliance in feed ingredient sourcing (Ogunleye *et al*., 2022).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Site and unit for the experiment

The experiment will be carried out at the small ruminant unit of the University of Benin farm project, and the Department of Animal Science research laboratory of the same University of Benin, Benin City, Edo State, Nigeria.

Benin City Lies between latitude 6o and 301N of the Equator and longitude 5o 401 and 6oE of the Greenwich meridian in the rain forest zone, with mean monthly temperature of 27.6oC. The area has an average annual rainfall and relative humidity of 2162 mm and 72.5 % respectively (CNES/Airbus, 2016). While the experimental location lies between latitude 6ºS and 30ºN of the equator and longitude 5.4ºW and 6ºE of the Greenwich meridian with a temperature of 27.6ºC (NAA, 2014; Google Earth, 2021).

The unit will be a conventional dwarf walled house, which will allow cross ventilation and roof that is made of asbestos which will prevent direct sun and rain from getting into the pens and the floor which will be made of concrete will be laid with wood shaving as bedding materials. The unit will be cleaned and disinfected with izal (active ingredient: Phenol) few days before the animals arrived to the farm.

3.2 Experimental materials

Periwinkle [Tympanotonus fusatus (L.)] shells amongst others.

3.2.1 Source of Periwinkle shell

Periwinkle [Tympanotonus fusatus (L.)] shells Were source from different markets in Benin. The shells were mixed to obtain a homogeneous sample. Shells were washed with clean water to remove viscera and other sand debris, crushed, milled and stores for further analysis.

3.3 Chitin and chitosan was extracted from ground periwinkle shell using standard procedure of Varun et al. (2017)

3.4 Management and feeding of animals for *In-vitro* study

Rumen liquor was collected from eight (8) West African dwarf goats were restricted in group pens for one week at the University of Benin Farm project, Ugbowo Campus, Benin City. Animals were fed grass and concentrate supplement for the duration restricted. Water was also provided *ad libitum, collection was done in the early hours of the morning via a stomach tube into a pre-warmed flask to keep the microorganism alive.*

3.5 Substrate collection and preparation for the *In-vitro* incubation study

The substrate for incubation was prepared with 40% concentrate (growers mash), 30% legume (Centrosema molle) and 30% grass (Panicum maximum).

3.6 Buffer Preparation

The buffer was prepared a day before rumen liquor collection and maintained at a pH of 6.2 (Navaro-villa et al., 2011) A phosphate bicarbonate buffer (Mould et al., 2005) used (g/l) consist of the following composition: Na2HPO4. 12H2O =1.985, KH2PO4 = 1.302, MgCl2.6H2O = 0.105, NH4HCO3 = 1.407, NaHCO3 = 5.418, NaOH = 0.100l.

3.7 Rumen liquor collection for incubation

Rumen liquor was collected from the goats housed at the University of Benin Teaching and research farm, Ugbowo Campus, Benin City. The collection was via stomach tube. The liquor was collected in the early hours of the morning into a pre-warmed flask prior to feeding the animals.

The flask containing the rumen liquor was taken to the laboratory where it was strained through four layers of cheese cloth. The strained liquor was mixed with a buffer solution in a ratio of 1:2.

This mixture was put in a water bath and gassed with CO_2 to maintain anaerobic condition and a temperature of 39°C to keep the microorganisms alive.

3.8 Incubation

The *In-vitro* fermentation was carried out using 100ml calibrated syringes filled with 30ml of the buffered inoculum (rumen liquor: buffer, 1:2). The weighed dacron bags containing the substrate were placed inside the syringes before the inoculum was introduced into the syringes. The syringes were tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The syringes were fitted with silicon tube and clipped to prevent escape of gas before placing them in the incubator at 39^{o}C.

The syringes containing only the inoculum served as the blank while the bags containing only the substrate served as the control. The time for the commencement of incubation was noted and the syringes were monitored and readings taken at 3hours intervals for 24hours. For each incubation time, the head space of the syringes was measured and gas production volume recorded. At 24hours of incubation, the final readings were taken and the syringes put on ice to stop further gas production.

3.9 Determination of methane and Dry matter digestibility

40% NaOH solution was prepared and 4ml was injected into each incubation syringes upon the completion of the 24hours incubation period. The NaOH solution absorbed the CO_2 leaving behind methane (Fievez *et al.,* 2005). The syringes were well agitated before readings were taken and recorded.

For the Dry matter degradability (DMD), the sealed dacron bags containing the samples was removed from the syringes and washed under running tap water until it became clean. The bags were then dried in a lab oven at 100^{o}C to constant weight and the DMD was calculated as

$$\%DMD = \frac{\text{Wt. of sample before incubation - Wt. of sample after incubation}}{\text{Wt. of sample before incubation}} \times \frac{100}{1}$$

Methane gas volume (CH_4 GV) were calculated using the following formulas

$$\frac{\text{Methane gas volume}}{\text{total gas volume}} \times \frac{100}{1}$$

3.10 Determination of Post *In-vitro* parameters

The post *In-vitro* parameters such as Organic matter digestibility (OMD), Metabolizable energy (ME), Short Chain Fatty Acid (SCFA) were estimated using the equations:

ME = 2.20 + 0.136GV + 0.057CP + 0.00029CF (Menke and Steingass 1988)

OMD = 14.88 + 0.88GV + 0.45CP + 0.651XA (Menke and Steingass 1988)

SCFA = 0.0239GV – 0.0601 (Getachew *et al.,* 1999)

Where:

GV, CP, CF, and XA are total gas volume, crude protein, crude fiber, and ash of the incubated samples respectively.

The Fermentation Efficiency and effect of methane reduction is calculated as

$$FE = \frac{\text{Dry matter digestibility (mg/kg)}}{\text{Total gas volume (ml/g)}}$$

$$CH_4red\ (\%) = \frac{\text{Average } CH_4 \text{ of the control} - CH_4 \text{ of treated samples}}{\text{Average } CH_4 \text{ of the control}} \times \frac{100}{1}$$

3.11 Statistical Analysis

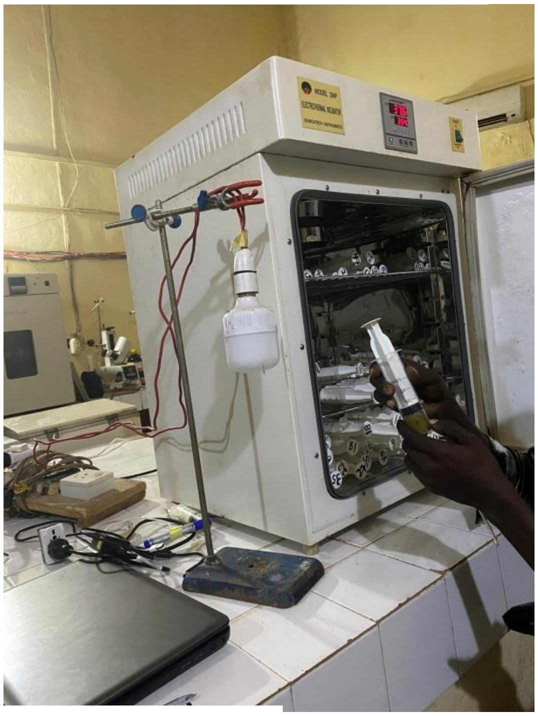
Data collected will be analyzed using SAS (2014) and separation of mean will be done using the Duncan multiple range test (DMRT) in the same SAS (2014) software.

Plate 1: Restraining the goat.

Plate 2: Rumen liquor Collection

in vitro

Plate 4: The incubation process



e 5: In vitro incubation system

CHAPTER FOUR

4.0 RESULT

4.1 Chemical Composition of Experimental Diets Containing Periwinkle Chitin and Chitosan

The table 1 presents the Chemical composition of experimental diets containing different inclusion levels of chitin and chitosan derived from periwinkle shells, compared with a control and an oxytetracycline-supplemented diet. The parameters evaluated include crude fibre (CF), ash, crude protein (CP), ether extract (EE), dry matter (DM), nitrogen-free extract (NFE), and organic matter (OM). Superscripts within each row indicate significant differences ($p<0.05$) among treatments.

Crude fibre values ranged from 17.50% to 24.50%, with the highest value observed in the 6% chitin diet (T3) and the lowest in the 1% chitosan diet (T5). This shows that increasing the inclusion level of chitin led to higher fibre content, which may be attributed to the fibrous nature of chitin as a structural polysaccharide. The lower fibre content in the chitosan diets, particularly at 1%, indicates better deacetylation and solubility of chitosan compared to chitin, which could enhance digestibility.

Ash content ranged from 9.00% to 20.00%, with the 3% chitin diet (T2) recording the highest value, suggesting higher mineral content possibly due to residual minerals in the periwinkle shells. The lowest ash content was observed in the 0.5% chitosan diet (T4), indicating that higher chitosan solubility might reduce inorganic residues.

Crude protein (CP) values ranged between 15.75% and 22.75%, with the 0.5% chitosan diet (T4) showing the highest protein value. This implies that moderate inclusion of chitosan may enhance microbial activity and nitrogen retention, leading to better protein synthesis. The relatively lower CP values in the 3% chitin and 1% chitosan diets may suggest that excessive inclusion could interfere with protein availability or digestion.

Ether extract (EE) values ranged from 8.50% to 10.00%. The 3% chitin (T2) and 0.5% chitosan (T4) diets had the highest EE content, which could indicate improved lipid retention and energy value. The lowest EE in the antibiotic-supplemented diet (T6) suggests that natural additives like chitosan may promote better lipid metabolism than synthetic antibiotics.

Dry matter (DM) content remained relatively constant across treatments (91.40–91.85%), indicating uniform feed moisture content and sample stability during analysis.

Nitrogen-free extract (NFE), which represents the carbohydrate fraction, ranged from 31.50% to 42.50%. The 1% chitosan diet (T5) recorded the highest NFE value, implying enhanced carbohydrate availability and potential energy supply. In contrast, the 3% chitin diet (T2) had the lowest NFE, possibly due to the high fibre content that inversely affected carbohydrate proportions.

Organic matter (OM) values ranged from 80.00% to 91.00%, with the 0.5% chitosan diet (T4) showing the highest value. This indicates that moderate chitosan inclusion improved organic matter utilization, likely due to its positive effect on nutrient digestibility and microbial fermentation. Conversely, the lowest OM was found in the 3% chitin diet (T2), which may be due to reduced digestibility associated with high fibre levels.

Table 1: Chemical Composition of Experimental Diets Containing Periwinkle Chitin and Chitosan

parameters	Control (T1)	3% Chitin periwinkle (T2)	6% Chitin periwinkle (T3)	0.5% Chitosan periwinkle (T4)	1 Chitosan periwinkle (T5)	0.01 oxytetracycline (T6)	SEM
Cf(%)	18.59^{d}	21.00^{bc}	24.50^{a}	19.00^{cd}	17.50^{d}	22.00^{b}	0.68
Ash(%)	11.50^{d}	20.00^{a}	14.00^{c}	9.00^{c}	15.50^{b}	11.50^{d}	0.35
Cp(%)	17.94^{b}	17.50^{b}	17.94^{b}	22.75^{a}	15.75^{b}	18.38^{b}	0.84
EE(%)	9.75^{a}	10.00^{a}	9.00^{a}	10.00^{a}	8.75^{b}	8.50^{a}	0.45
Dm(%)	91.85^{a}	91.40^{ab}	91.70^{a}	90.75^{b}	91.55^{ab}	91.10^{ab}	0.15
NFE(%)	42.31^{a}	31.50^{b}	34.56b^{c}	39.25_{bc}	42.50^{b}	39.62^{b}	1.46
OM(%)	88.50^{b}	80.00^{c}	86.00^{c}	91.00^{a}	84.50^{d}	88.50^{b}	0.35

SEM: Standard error of Mean

CF - Crude Fibre

CP - Crude Protein

EE - Ether Extract

DM - Dry Matter

NFE - Nitrogen Free Extract

OM - Organic Matter

Superscripts within a row having different letters differ significantly ($p<0.05$).

4.2 Gas Production Characterization of Different Levels of Chitin and Chitosan from Periwinkle Shells

Table 2 show the hourly gas production of chitin and chitosan from periwinkle shell at different level of inclusion for an in vitro digestibility study.

At 3 Hours

At the early stage of incubation (3 hours), gas production values were low and statistically similar across all treatments ($p > 0.05$).Values ranged from 4.000 to 4.667 ml/200 mg Dm, showing no significant differences.

At 6 Hours

At 6 hours, gas production slightly increased across all treatments as microbial activity intensified. Values ranged from 6.667 ml/200 mg Dm in the 0.01 Oxytetracycline group to 8.667 ml in the 0.5% chitosan treatment, but the differences were still not statistically significant ($p > 0.05$).

The modest rise shows the onset of active fermentation, where rumen microbes begin utilizing the soluble carbohydrates and easily degradable fractions of the substrates. Chitin-supplemented treatments (3% - 6%) showed a small but noticeable increase compared to control, suggesting a gradual stimulation of microbial fermentation.

At 9 Hours

By the 9th hour, fermentation became more pronounced, and differences among treatments emerged ($p < 0.05$). The highest gas production was observed in chitosan 0.5% (16.00 ml/200 mg Dm) and 1% (15.33 ml/200 mg Dm), while control (10.67 ml/200 mg Dm) and chitin 6% (10.67 ml/200 mg Dm) recorded the lowest values.

This indicates that low-level chitin supplementation (0.51%) promoted microbial growth and feed degradability, enhancing fermentation efficiency. The higher gas volumes imply better substrate utilization at these levels, while the low output at Chitin 6% inclusion suggests possible microbial inhibition at high chitin concentrations.

At 12 Hours

At 12 hours, gas production continued to increase significantly across all treatments. The 0.5% chitosan treatment produced the highest gas volume (19.33 ml/200 mg Dm), followed by 1% (18.00 ml/200 mg Dm), while the control (14.67 ml/200 mg Dm) and 6% chitin (13.33 ml/200 mg Dm) remained the lowest. This stage represents the peak of active fermentation, where microbial populations are fully established and vigorously degrading complex carbohydrates. The results confirm that moderate chitosan inclusion (0.5 - 1%) sustained microbial activity more effectively than both control and antibiotic treatments.

At 15 Hours

At 15 hours, fermentation activity remained high, but the rate of increase in gas production started to vary among treatments. 0.5% chitosan had the highest gas production (22.00 ml/200 mg Dm), closely followed by 1% Chitosan (21.33 ml/200 mg Dm) and 3% Chitin (20.00 ml/200 mg Dm). The control (16.67 ml/200 mg Dm) and 6% chitin (16.67 ml/200 mg Dm) had the lowest values, showing a significant difference ($p < 0.05$).

This suggests that while low to moderate chitosan levels enhanced fermentation, excessive inclusion Chitin (at 6%) may have limited substrate breakdown due to reduced microbial efficiency or enzyme inhibition.

At 18 Hours

At the 18-hour mark, gas production continued to rise, indicating that fermentation was still ongoing. Chitosan at 0.5% (24.67 ml/200 mg Dm) and 1% (24.00 ml/200 mg Dm) maintained their superiority, while control (20.67 ml/200 mg Dm) and 0.01% Oxytetracycline (20.00 ml/200 mg Dm) were significantly lower. 6% chitin produced only 18.67 ml/200 mg Dm, again showing suppressed microbial activity.

These results confirm that low inclusion levels Chitosan (0.5 - 1%) sustained fermentation for longer durations, while higher inclusion (6%) limited the extent of gas production.

At 21 Hours

At 21 hours, gas production approached its maximum in most treatments. 0.5% chitosan yielded the highest gas value (27.33 ml/200 mg Dm), followed by 1% chitosan (26.00 ml/200 mg Dm) and 3% Chitin (24.67 ml/200 mg Dm). The control and antibiotic treatments produced 22.67 ml/200 mg Dm each, while 6% chitin remained lowest at 19.33 ml/200 mg Dm. This stage marks the stabilization phase, where most fermentable components had been utilized. The sustained gas production in 0.5% and 1% chitin treatments indicates a prolonged and efficient microbial fermentation, while 6% chitin appeared to restrict gas evolution.

At 24 Hours

At the final incubation period (24 hours), total cumulative gas production was reached. Chitosan 0.5% recorded the highest overall gas production (30.00 ml/200 mg Dm), followed by 3% Chitin (29.33 ml/200 mg Dm) and 1% Chitosan (28.00 ml/200 mg Dm). The control (24.00 ml/200 mg Dm) and antibiotic (26.00 ml/200 mg Dm) were notably lower, while 6% chitin (23.33 ml/200 mg Dm) had the least gas output.

This final result confirms that low levels of chitosan (0.5 - 1%) significantly enhanced microbial fermentation and feed degradability throughout the 24-hour incubation. The high chitin level (6%), however, had an inhibitory effect, likely due to reduced microbial efficiency or limited nutrient accessibility at excessive inclusion.

Table 2: Gas production characterization of diet containing different levels of chitin and chitosan from periwinkle shells

Treatment	3 hours	6 hours	9 hours	12 hours	15 hours	18 hours	21 hours	24 hours
Control	4.667^{a}	7.333^{a}	10.67^{b}	14.67^{bc}	16.67^{bc}	20.67^{ab}	22.67^{b}	24.00^{ab}
0.01% Oxytetracycline	4.667^{a}	6.667^{a}	11.33^{b}	15.33^{abc}	18.67^{abc}	20.00^{ab}	22.67^{b}	26.00^{ab}
Chitosan periwinkle 0.5%	4.000^{a}	8.667^{a}	16.00^{a}	19.33^{a}	22.00^{a}	24.67^{a}	27.33^{a}	30.00^{a}
Chitosan periwinkle 1%	4.000^{a}	8.000^{a}	15.33^{a}	18.00^{ab}	21.33^{a}	24.00^{a}	26.00^{a}	28.00^{ab}
Chitin periwinkle 3%	4.667^{a}	8.000^{a}	12.67^{b}	15.33^{bc}	20.00^{ab}	22.67^{ab}	24.67^{ab}	29.33^{ab}
Chitin periwinkle 6%	4.000^{a}	7.333^{a}	10.67^{b}	13.33^{c}	16.67^{c}	18.67^{b}	19.33^{c}	23.33^{b}
SEM	0.816	1.011	0.807	1.211	1.004	1.515	0.886	1.882

^{a,b,c,d} Superscript represent the level of significant difference between treatments. Mean in the same column with different superscript are significantly different ($p>0.05$)

Control= diet with 0% chitin and chitosan

4.3 Post *in Vitro* Gas Production of Diets Containing Different inclusion Levels of Chitin and Chitosan From Periwinkle Shells

The table 3 presents the effect of varying inclusion levels of chitin extracted from periwinkle shells on post in vitro gas production parameters, including methane (CH_4), carbon dioxide equivalent (CH_4GV), dry matter digestibility (DMD), fermentation efficiency (FE), metabolizable energy (ME), organic matter digestibility (OMD), and short-chain fatty acids (SCFA). Methane production (CH_4) ranged from 9.33 to 13.33 ml, with the highest value observed in the 0.5% chitosan treatment (13.33 ml) and the lowest in the control (9.33 ml). However, the differences were not statistically significant ($p>0.05$). Similarly, CH_4GV (total gas volume) varied from 38.31 to 45.83 ml/200 mg Dm, with no significant difference across treatments. DMD values ranged from 63.70 to 68.63%, with 0.01% antibiotics group recording the highest digestibility (68.63%), followed closely by the 0.5% chitosan treatment (67.77%). This implies that chitin at lower inclusion levels (≤0.5%) may enhance feed degradability comparable to antibiotic supplementation. However, digestibility declined slightly at higher inclusion levels (1% chitosan - 6% chitin), possibly due to the inhibitory effect of excessive chitin on rumen microbes. FE showed minor variations among treatments (2.20-2.81 ml/mg DM), with the control group having the highest value (2.81 ml/mg DM). The values did not differ significantly ($p>0.05$), from other treatment indicating that the addition of chitin and chitosan did not negatively influence microbial fermentation efficiency. ME ranged from 6.40 to 7.58 MJ/kg DM, with the highest value recorded at 0.5% chitosan inclusion (7.58 MJ/kg DM). This suggests that moderate chitin inclusion could enhance energy availability from the substrate, likely due to optimal microbial fermentation. At higher Chitin levels (6%), ME decreased, indicating a possible threshold beyond which chitin suppresses fermentation efficiency.

OMD followed a similar pattern to ME, with values between 47.82 and 64.86%. The 0.5% chitin treatment achieved the highest OMD, showing improved fermentation and nutrient release at moderate chitin inclusion levels. The control and antibiotic treatments had lower OMD, further confirming that low levels of chitin may serve as an effective feed additive to improve organic matter utilization. SCFA production ranged from 0.497 to 0.657 mmol/L, peaking at the 0.5% chitosan level (0.657 mmol/L). SCFA are important indicators of fermentation activity and energy supply to the host. The increase at moderate chitin levels suggests enhanced fermentation, while the decline at 6% chitin diet may be attributed to microbial inhibition or reduced substrate digestibility.

Table 3: Post in vitro gas production of diets containing different inclusion levels of chitin and chitosan from periwinkle shells

Treatment	**CH4 (mL)**	**CH4GV (%)**	**DMD (%)**	**FE**	**ME**	**OMD (%)**	**SCFA**
Control	9.33^{a}	39.30^{a}	67.20^{a}	2.810^{a}	6.492^{b}	47.82^{d}	0.5135^{ab}
0.01% Oxytetracycline	10.00^{a}	38.31^{a}	68.63^{a}	2.648^{a}	6.788^{ab}	56.77^{c}	0.5135^{ab}
Chitosan periwinkle 0.5%	1a3.33^{a}	44.42^{a}	67.77^{a}	2.282^{a}	7.583^{a}	64.86^{a}	0.6569^{a}
Chitosan periwinkle 1%	12.67^{a}	45.83^{a}	67.20^{a}	2.429^{a}	6.911^{ab}	58.00^{bc}	0.6091^{ab}
Chitin periwinkle 3%	12.67^{a}	42.79^{a}	63.93^{a}	2.205^{a}	7.193^{ab}	63.22^{ab}	0.6410^{ab}
Chitin periwinkle 6	10.00	43.12^{a}	63.70^{a}	2.754^{a}	6.403^{b}	59.44^{abc}	0.4976^{b}
SEM	1.193	3.72	3.90	0.2202	0.256	1.656	0.0450

^{a,b,c,d} represent the level of significant difference between treatments. Mean in the same column with different superscript are significantly different ($p>0.05$)

SEM= Standard Error of Means

Control= diet with 0% Chitin and Chitosan

CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical Composition of Experimental Diets Containing Periwinkle Chitin and Chitosan

The results of this study demonstrate distinct effects of chitin and chitosan derived from periwinkle shells on the Chemical composition of experimental diets. Increasing chitin inclusion notably increased crude fibre (CF), with the highest values observed in the 6% chitin diet, reflecting the fibrous and insoluble nature of chitin. This aligns with the findings of Pascon *et al*. (2025), who reported that excessive dietary chitin can negatively affect protein and lipid digestibility in aquaculture species. In contrast, lower CF in chitosan diets, particularly at 1% inclusion, indicates enhanced solubility and digestibility, supporting improved nutrient utilization (Andriani *et al*. 2023).

Ash content was highest in the 3% chitin diet, likely due to residual inorganic material from the periwinkle shells, which are rich in calcium carbonate and other minerals. Conversely, the lowest ash observed in the 0.5% chitosan diet suggests that deacetylation and purification processes reduce inorganic residue, thereby improving feed quality (Rosidah and Mulyani, 2022). Crude protein (CP) levels were highest in the 0.5% chitosan diet, suggesting that moderate chitosan inclusion can enhance nitrogen retention, microbial activity, and digestive efficiency (Andriani *et al*., 2023). Diets with higher chitin or higher chitosan (1%) showed lower CP values, likely due to structural interference from excessive fibre, which reduces nutrient availability (Pascon *et al*., 2025). These results underscore the importance of both the type and inclusion level of shell-derived biopolymers in diet formulation. Ether extract (EE) was maximized at moderate inclusion levels of both chitin (3%) and chitosan (0.5%), suggesting improved lipid retention and energy value in these diets. Chitosan may enhance lipid metabolism through stimulation of digestive enzymes and improved gut morphology, consistent with prior reports on aquaculture species (Andriani *et al*., 2023). The lowest EE in the antibiotic-supplemented diet indicates that natural additives like chitosan may outperform synthetic antibiotics in supporting lipid utilisation.

Dry matter (DM) remained consistent across all treatments (91.40–91.85%), confirming that variations in proximate composition were not influenced by moisture content and that observed differences reflect genuine treatment effects. Nitrogen-free extract (NFE), representing digestible carbohydrate content, was highest in the 1% chitosan diet and lowest in the 3% chitin diet. This demonstrates that moderate chitosan inclusion enhances carbohydrate availability, whereas high chitin inclusion reduces the NFE fraction due to elevated fibre content (Pascon *et al*., 2025). Organic matter (OM) was highest in the 0.5% chitosan diet (91.00%) and lowest in the 3% chitin diet (80.00%). Higher OM in moderate chitosan diets indicates improved digestibility and nutrient utilisation, while high chitin inclusion reduces the bioavailable nutrient fraction due to elevated fibre and ash levels (Pascon et al., 2025; Zynudheen *et al*. 2011).

5.2 *In vitro* Gas Production of Diets Containing Different Inclusion Levels of Chitin and Chitosan From Periwinkle Shells

The trend in gas production observed in Table 2 reflects the progressive nature of rumen microbial fermentation, where cumulative gas volume increases over time as microorganisms degrade fermentable substrates. The inclusion of chitosan derived from periwinkle shells significantly influenced both the rate and extent of gas production, indicating its modulatory effect on rumen microbial activity and feed degradability.

At the early incubation stages (3 - 6 hours), gas production was low and statistically similar across treatments. This reflects the lag phase of microbial adaptation, where rumen microbes attach to the substrate surface and initiate enzyme synthesis. Such early-phase similarities are consistent with findings by Getachew and Makkar (2019), who explained that initial gas production is typically slow as microbial colonization begins. At this point, chitin had not yet exerted a noticeable effect on microbial fermentation.

Adegbeye *et al*. (2019) and Patra and Yu (2022) reported that low concentrations of chitosan and chitin stimulate beneficial rumen bacteria and improve fermentation efficiency without disrupting microbial balance. The improved gas yield suggests that small amounts of chitin act as prebiotics, promoting microbial growth and enzymatic hydrolysis of fibrous feed materials.

Mohammed *et al*. (2022) noted that chitin from periwinkle shells enhances nutrient utilization and supports microbial proliferation due to its biocompatible nature.

Between 18 and 21 hours, the fermentation process began to stabilize as most readily fermentable substrates were utilized. Nevertheless, 0.5% and 1% chitosan treatments maintained significantly higher gas production than the control and antibiotic treatments. This sustained activity suggests that chitin supported prolonged microbial efficiency in the rumen. According to Elghandour *et al*. (2020), chitosan and related polysaccharides enhance the persistence of fibrolytic microbes and prolong fermentation duration, thereby improving feed conversion efficiency. The antibiotic treatment, in contrast, showed moderate fermentation but did not outperform the chitin-supplemented diets—likely because antibiotics suppress both harmful and beneficial microbes (Patra and Yu, 2022).

At 24 hours, cumulative gas production peaked, confirms that low inclusion levels of chitosan (0.5–1%) optimize rumen fermentation, while higher levels (≥3%) tend to inhibit microbial activity. Excess chitin may form complexes with nutrients or increase the viscosity of the fermentation medium, reducing substrate accessibility (Oboh *et al*., 2023). Adegbeye *et al*. (2019) similarly observed that excessive chitosan supplementation can reduce gas production due to partial inhibition of cellulolytic bacteria and limited nutrient solubilization.

5.3 Post *In Vitro* Gas Production of Diets Containing Different Inclusion Levels of Chitin and Chitosan from Periwinkle Shells

Methane (CH_4) and gas volume (CH_4GV) are key indicators of fermentation activity and microbial metabolism in the rumen. The non-significant variations observed in CH_4 and CH_4GV across treatments suggest that the inclusion of chitin, even up to 6%, did not adversely affect methanogenesis. However, a slight increase in methane production at lower inclusion levels (0.5 - 1%) indicates that chitin might have provided additional fermentable substrates that of Chitosan supported microbial growth and fermentation. This observation aligns with the findings of Binta *et al.* (2022) and Abdalla *et al*. (2020), who reported that moderate levels of chitin or chitosan can enhance microbial activity without causing excessive gas accumulation.

Dry matter digestibility (DMD) improved slightly with 0.5% chitosan. This implies that small amounts of chitosan may enhance microbial degradation of feed materials, possibly by stimulating cellulolytic bacteria or stabilizing rumen pH. The slight reduction in DMD at higher inclusion levels (1% chitosan –6% chitin) suggests that excessive chitin may reduce the accessibility of fibrous feed components to microbial enzymes. Similar trends were reported by Goiri *et al*. (2010) and Tan *et al*. (2021), who noted that chitosan supplementation at low doses enhances feed degradability, but higher doses may suppress microbial fermentation due to its antimicrobial characteristics.

Fermentation efficiency (FE) varied insignificantly among treatments, indicating that the overall efficiency of microbial fermentation was not compromised by the inclusion of chitin. This stability suggests that chitin acts as a mild modulator of rumen fermentation rather than a disruptor. The slightly higher FE observed in the control group may reflect normal microbial balance without external influence, while similar FE values among chitin treatments show that the additive did not hinder microbial conversion of feed to end products.

Metabolizable energy (ME) increased with 0.5% chitosan inclusion, enhanced energy availability from improved fermentation efficiency and nutrient digestibility. However, higher inclusion levels (chitin at 6%) led to a decline in ME, likely due to microbial inhibition or reduced fermentation intensity. This observation supports the findings of Vargas-Bello-Pérez *et al.* (2019), who reported that optimal chitin supplementation can increase energy utilization, but excessive levels may interfere with rumen microbial activity.

Organic matter digestibility (OMD) followed a similar pattern to ME, with the highest value recorded at 0.5% chitosan inclusion. This indicates that moderate levels of chitin enhanced the breakdown of organic matter and improved nutrient availability. Reduced OMD at higher inclusion levels may be due to the antimicrobial action of chitin, which can suppress certain rumen microorganisms responsible for fiber degradation.

Short-chain fatty acids (SCFA) are major end products of carbohydrate fermentation and serve as the main source of energy for ruminants. The highest SCFA concentration was observed at 0.5% chitosan indicating optimal fermentation activity and energy production. This result implies that chitosan at low levels promotes microbial fermentation efficiency, enhancing volatile fatty acid synthesis. However, SCFA concentration decreased at 6% inclusion, suggesting reduced microbial activity or substrate utilization. Similar findings were reported by Zhou *et al*. (2020), who explained that while chitin enhances fermentation at optimal concentrations, higher doses may inhibit microbial proliferation.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

This study investigated the in vitro nutritive value of diets containing different inclusion levels of chitin and chitosan derived from periwinkle shells (Tympanotonus fuscatus). The research aimed to assess how varying levels of these biopolymers affect gas production, digestibility, and fermentation characteristics under controlled laboratory conditions. Periwinkle shells, a common marine waste in Nigeria, were processed to extract chitin and chitosan. These compounds were added to experimental diets at different inclusion levels.

Results showed that gas production increased over time across all treatments, but the highest values were obtained at 0.5% chitosan diet. These levels improved microbial activity, feed degradability, and energy yield, while 3% and 6%cl chitin reduced fermentation efficiency due to possible microbial inhibition.

Post-fermentation results revealed that 0.5% chitin diet enhanced dry matter digestibility (DMD), organic matter digestibility (OMD), metabolizable energy (ME), and short-chain fatty acids (SCFA) production without increasing methane output. This indicates that moderate chitin inclusion supports efficient rumen fermentation and nutrient utilization.

6.2 Conclusion

This study showed that Chitin and chitosan can help improve feed quality, support animal health, and reduce harmful gases from digestion. When used in the right amounts, they can improve nutrient use and fermentation in the rumen. However, too much of them can reduce digestibility because of their strong antimicrobial effects. Adding chitin and chitosan from periwinkle shells to animal feed affected rumen fermentation and gas production.

Chitin and chitosan from periwinkle shells can serve as natural, eco-friendly feed additives. Using small inclusion levels (0.5–1%) can improve fermentation efficiency and nutrient use, reduce dependence on antibiotics, and help manage shell waste sustainably in livestock feed production.

6.3 Recommendations

1. Moderate levels of chitin and chitosan from periwinkle shells should be used in animal diets to improve digestibility and fermentation without negative effects.
2. The extraction process for chitin and chitosan should be standardized to ensure consistent quality.
3. Further in vitro and in vivo studies should be carried out to determine the best inclusion levels for different livestock.
4. 0.5% chitosan diet had the best post in vitro result.

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