NITROGEN BALANCE AND RETENTION OF GOAT FED DIETS CONTAINING CHITIN AND CHITOSAN FROM SNAIL SHELLS.

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NOVEMBER, 2025.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF AGRICULTURE (B. AGRIC) DEGREE IN ANIMAL SCIENCE

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CERTIFICATION

This is to certify that this project work was carried out by **Iyengunmwena Favour Etinosa** with Matriculation Number **AGR2000087** of the Department of Animal Science, Faculty of Agriculture, University of Benin-City, Nigeria.

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DEDICATION

This project is dedicated first to Almighty God for His grace and strength throughout this journey. I also dedicate it to my loving parents, Mr. and Mrs. Iyengunmwena, whose prayers, encouragement, and sacrifices continue to inspire me. To my uncle and aunts for their constant support and guidance, I say thank you from the depths of my heart.

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ABSTRACT

The rising cost of feed additives has made livestock production increasingly expensive, especially for small ruminants. This has created the need to explore cheaper alternative feed sources that can still support efficient growth and nutrient utilization. Chitin and chitosan, obtained from snail shell waste, are potential feed additives known to enhance nutrient digestion and nitrogen use in animals. This study was conducted to evaluate the effects of diets containing chitin and chitosan on nitrogen balance and retention in goats. A total of twelve (12) goats were randomly assigned to six different dietary treatments.The trial lasted 15 days, with an 8-day digestibility and nitrogen balance trial using metabolic cages. Data on feed intake, fecal and urinary nitrogen excretion, and nitrogen balance were collected and analyzed statistically.

The results showed that goats fed diets chitosan retained more nitrogen compared to those on the control diet. There were no adverse effects on dry matter intake. The study concludes that chitosan derived from snail shell can serve as useful feed additives at 0.5% inclusion level for improving nitrogen utilization in goats.

CHAPTER ONE

1.1 Background of the study.

Goats are important to the livelihood of smallholder farmers across Nigeria, offering meat, milk, and income even in challenging environments (Devendra *et al.*, 2018; Adedeji *et al.*, 2019. Their low upkeep and resilience make them vital contributors to rural food security and economic stability.

Nutrition especially protein supply,strongly influences goat productivity. Protein supports growth, reproduction, and maintenance (McDonald *et al.*, 2016). .

A practical way to assess how well goats utilize dietary protein is through nitrogen balance, which is the difference between nitrogen intake and nitrogen excreted. A positive nitrogen balance signifies efficient protein utilization, which ultimately supports growth and production (Storm *et al.*, 2017).

Chitin is a naturally occurring polysaccharide second only to cellulose and is commonly found in the shells of crustaceans and snails. When chitin undergoes deacetylation, it becomes chitosan, a biopolymer valued for its biodegradability, biocompatibility, and functional properties (Younes & Rinaudo, 2015; Kaya *et al.*, 2016). In snail farming systems, shells are often wasted so turning them into a useful feed additive is both sustainable and cost-efficient.

Studies in dairy cattle, sheep, and beef heifers have shown that chitosan can positively influence rumen fermentation by increasing propionate production, decreasing the acetate to propionate ratio, and improving protein digestibility (de Paiva *et al.*, 2016; Dias *et al.*, 2017; Kirwan *et al*., 2021). A meta-analysis across various ruminants also confirmed improved crude protein digestibility and more favorable fermentation profiles (Harahap *et al*., 2022). In mid-lactation dairy cows, including chitosan in the diet improved nitrogen utilization and feed conversion (de Paiva *et al.,* 2017).

Although these benefits have been observed in larger ruminants, there's still limited research focused specifically on goats. Given how promising these findings are, there's a real opportunity to test whether similar advantages such as improved nitrogen balance and retention extend to goats when fed diets containing chitin and chitosan derived from snail shells. Goats, being efficient ruminants commonly used in smallholder systems, serve as an ideal model for evaluating the effects of feed additives such as chitosan on nutrient utilization and nitrogen balance.

1.2 Justification of the study

Goat production plays a major role in the livelihood of many households in Nigeria and other developing countries, providing meat, income, and social value (Devendra *et al.,* 2018; Adedeji *et al*., 2019). However, one of the biggest challenges in goat nutrition is the poor efficiency of protein utilization, as a large portion of the nitrogen consumed is lost through urine and feces instead of being retained for growth and productivity (McDonald *et al*., 2016; Rumen *et al*., 2021). This not only raises feeding costs for farmers but also increases environmental concerns from nitrogen waste (Patra, 2018). Exploring low-cost and sustainable feed additives that can improve nitrogen retention is therefore very important. Chitin and chitosan, natural biopolymers obtained from snail shells, have shown promising effects in improving nutrient utilization and reducing nitrogen losses in ruminants (Amin *et al*., 2021; O'Callaghan *et al*., 2022). Since snails are commonly available in Nigeria, using their shells as a source of chitin and chitosan could provide a cheap and locally available alternative feed resource (Iheanacho *et al*., 2020). Despite these potentials, there is limited research on how snail-derived chitin and chitosan affect nitrogen balance in goats (Patra, 2018; Amin *et al*., 2021). Generating reliable data in this area will not only provide useful information for farmers and researchers but also support the drive for more sustainable livestock systems. This study is therefore justified because it seeks practical solutions to reduce feed costs, improve goat productivity, and promote the efficient use of locally available resources. This provides the basis for evaluating the effects of snail-derived chitin and chitosan on nitrogen balance and retention in goats

1.3 Objectives of the study

The main objectives of this study were to determine;

1.The proximate composition of the experimental diets containing chitin and chitosan from snail shells

2. The effect of chitin and chitosan diets from snail shells on nitrogen intake, balance and retention.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of chitin and chitosan

The discovery and study of chitin began as far back as 1811, when a French chemist named Henri Braconnot first explored how to extract a substance he referred to as "fungine" from certain fungi. He achieved this by treating them with alkaline solutions, unknowingly opening the door to the modern study of chitin (Santos *et al*., 2020). Later, in 1843, Lassaigne analyzed insect shells specifically from Bombyx mori (the silkworm) and found that this compound contained nitrogen, which further supported the idea that it had a unique structure (Santos *et al*., 2020).

Chitosan was first obtained in 1859 by treating chitin with hot alkaline solution. Early studies in the late 19th century clarified its structure, identifying glucosamine and acetic acid as components. The term "chitosan" was later introduced by Hoppe-Seyler in 1894. Today, chitosan is recognized as a versatile biopolymer with a wide range of applications (Kaur & Dhillon, 2015).

By the 1970s, Japan started producing chitosan commercially (Santos *et al*., 2020). In Brazil, the story took a slightly different path. Early research into chitosan from fungal sources started around 1983, with discoveries about its presence in the cell walls of fungi belonging to the Zygomycetes and Mucorales classes. Eventually, in 1999, Craveiro and Queiroz were credited with the first documented commercial production and sale of chitosan in the country (Craveiro & Queiroz, as cited in Campos-Takaki *et al*., 2021)

https://www.mdpi.com/2073-4360/16/7/995.Department of Food Science and Engineering, Moutai Institute, Renhuai 564502, China.Polymers 2024, 16(7), 995; https://doi.org/10.3390/polym16070995

Figure 1. Chemical structure of chitin and chitosan

2.2 Snail shells as a source of chitin and chitosan

Snail shells are an abundant by-product of snail farming and consumption in Nigeria and other tropical regions. They are often discarded as waste, contributing to environmental pollution despite their rich composition of valuable biomaterials. These shells serve as an excellent alternative source of chitin and chitosan, which are natural biopolymers with wide applications in agriculture, biomedicine, and animal nutrition. Utilizing snail shells for chitosan extraction not only helps reduce waste management problems but also promotes sustainable resource use through value addition.

https://www.shells-of-aquarius.com/snail-shells.html

Figure 2: Snail shells

2.2.1 Characteristics of snail shells

Snail shells are hard, calcareous exoskeletons primarily composed of calcium carbonate ($CaCO_3$), organic matrix proteins, and chitin. The calcium carbonate provides rigidity and strength, while chitin forms the fibrous structural network that can be deacetylated to produce chitosan. Although the chitin content in snail shells is generally lower than in crustacean shells, it is still adequate for extraction using chemical or biological processes. Snail shells are typically lightweight, porous, and exhibit high mechanical strength. Their chemical composition and structural properties make them a viable, low-cost, and environmentally friendly raw material for the production of chitin and chitosan (Mohammed *et al*., 2023; Aranaz *et al*., 2021).

2.3 CHITIN

Chitin is a natural, fibrous polymer made up of repeating units of N-acetylglucosamine (GlcNAc) connected by β-(1→4) glycosidic bonds. It is considered the second most abundant biopolymer in nature after cellulose and serves as a key structural component in many living organisms. Chitin provides rigidity and strength to the exoskeletons of arthropods (such as crabs, lobsters, shrimp, and insects), cell walls of fungi, and structural parts of mollusks, annelids, and some algae (Younes & Rinaudo, 2015; Aranaz *et al.*, 2009).

Although chitin is found in a wide variety of organisms, the most widely exploited commercial sources are crustacean shells particularly shrimp, crab, and lobster which typically contain between 8–40% chitin by dry weight (Gentile *et al.*, 2018).Antarctic krill are also a rich source of chitin—with their global biomass estimated at around 380 million tonnes. (Atkinson *et al*., 2009; Elieh-Ali-Komi and Hamblin, 2016). Fungal sources offer an alternative, especially for industries interested in sustainable or vegetarian-friendly chitin. (Jones *et al.,* 2020).

Chitin exists in nature as microfibrils. It appears in three main forms: α-chitin, β-chitin, and γ-chitin. These forms vary in how their chains are arranged, which influences how easily they dissolve and how reactive they are during processing (Ifuku & Saimoto, 2012; Rinaudo, 2006).

In summary, chitin is a versatile biomolecule with widespread occurrence and growing commercial importance. Its strong and biodegradable nature makes it an attractive starting material for producing value-added derivatives like chitosan, which has wide applications in agriculture, medicine, water treatment, and more.

https://link.springer.com/rwe/10.1007/978-981-19-0710-4_25.S., H., U. Chandran, G., P. R., J., Sambhudevan, S. (2023). Biomedical Applications of Chitin. In: Thomas, S., AR, A., Jose Chirayil, C., Thomas, B. (eds) Handbook of Biopolymers . Springer, Singapore. https://doi.org/10.1007/978-981-19-0710-4_25

Fig 3: Sources of chitin production

2.3.1 Extraction methods of chitin

Chemical extraction remains the most common approach due to its simplicity and high yield. It involves two key steps: demineralization and deproteination.

In demineralization, acids such as HCl or organic acids (e.g., citric and acetic acids) are used to dissolve minerals like calcium carbonate (Pohling *et al.*, 2013; Trung *et al.*, 2018). Newer studies have explored eco-friendly alternatives such as CO_2-based carbonic acid, which removes minerals effectively but requires large amounts of water (Yuan *et al.*, 2023).

Deproteination removes proteins using alkalis like NaOH or KOH, though high alkalinity can degrade chitin and generate harmful waste (Ravi Kumar, 2000; Synowiecki & Al-Khateeb, 2003). Alternative methods such as hot-water treatment and DBD plasma have shown promise in reducing chemical use and waste generation (Yuan *et al.*, 2023; Borić *et al.*, 2021).

2.3.3 Biological extraction of chitin

Microbial fermentation uses bacteria such as Lactobacillus to produce acids and enzymes that remove minerals and proteins simultaneously. Enzymatic extraction relies on purified proteases from microbial or plant sources for controlled deproteination. These processes generate minimal waste but are slower and more expensive compared to chemical methods (Kozma *et al.*, 2022).

2.4 CHITOSAN

Chitosan is a naturally derived linear polysaccharide composed predominantly of β-(1→4)-linked D-glucosamine units, with occasional N-acetyl-D-glucosamine residues. It is produced through the partial deacetylation of chitin, and when the degree of deacetylation exceeds 50%, the material is typically referred to as chitosan rather than chitin. This threshold influences its solubility and biological activity, distinguishing it functionally.(Younes & Rinaudo, 2015; Shahidi & Abuzaytoun, 2005).

Both chitin and chitosan are recognized for their non-toxic, biodegradable, and biocompatible nature, which underpins their value in sustainable technologies, particularly in agriculture, medicine, and material science. Chitosan, in particular, has attracted substantial research interest due to its unique physicochemical and biological properties. Its inherent antibacterial and hemostatic features have enabled its use in biomedical fields, including the development of wound dressings, hydrogels, and biodegradable scaffolds. Furthermore, it is frequently blended with other biopolymers or engineered into nanocomposites to enhance its mechanical strength, improve stability, and promote cell adhesion in tissue engineering systems (Younes & Rinaudo, 2015; Mohammed *et al*., 2023; Kozma *et al*., 2022).

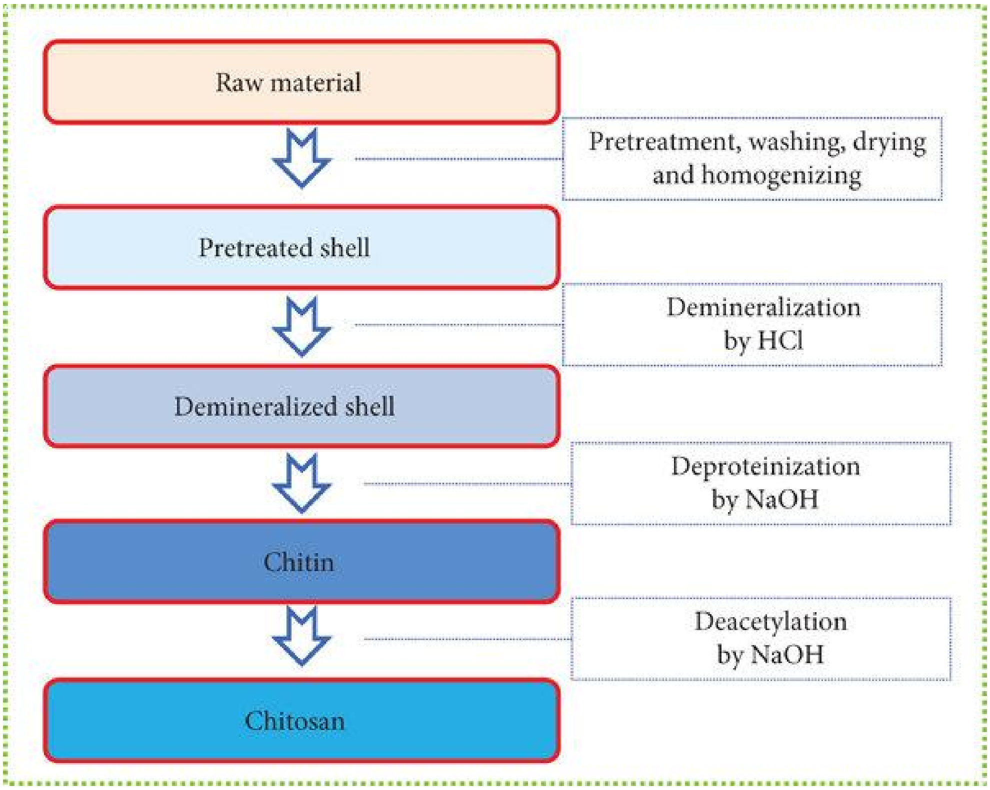
In food science, chitosan is used as a natural food packaging material due to its antimicrobial and barrier properties. It is also useful in environmental cleanup, especially for removing heavy metals from water, since it can be chemically modified with substances like humic acid or algae to boost its absorption capacity. Beyond that, chitosan derived porous carbon materials are being used in developing advanced supercapacitor electrodes(Mohammed *et al*.,2023)

In agriculture, chitosan offers numerous benefits. It helps trigger plant defense mechanisms by enhancing enzyme activity and stimulating the production of protective compounds like phytoalexins and callose. When applied to crops, it improves drought tolerance, nutrient uptake, and seed performance by altering membrane permeability and increasing protective sugars and proline content. Chitosan also serves as a biopesticide with antifungal, antiviral, and nematode-repellent properties. Moreover, it can improve soil water retention, act as a slow-release fertilizer carrier, and function as a soil conditioner and heavy metal binder(Mohammed *et al.*,2023).

2.4.1 Extraction methods of chitosan

Deacetylation is the process used to convert chitin into chitosan by removing acetyl groups from the chitin molecule. This is a very important step because it gives chitosan its useful properties like solubility in acids, and better reactivity for different applications.The most common method is the chemical method, where chitin is treated with a strong alkaline solution, usually sodium hydroxide (NaOH), at high temperatures (above 100°C). T(Younes & Rinaudo, 2015)

There's also a biological method, which uses a special enzyme called chitin deacetylase. It's more environmentally friendly but not widely used yet because the enzyme is hard to get in large amounts and it works slowly. Some researchers have tried using ionic liquids to help the enzyme work better or to assist chemical deacetylation. For example, some ionic liquids can boost enzyme activity or improve DD while reducing harmful by-products.(Younes & Rinaudo, 2015; Mohammed *et al*., 2023)



https://www.mdpi.com/2673-6209/5/2/15.Article: Chitin, (Scarvelli *et al.,* 2025)

Fig 4: Process of deactylation to form chitosan

2.5 Properties of chitin and chitosan

Chitosan is valued for its biodegradability, non-toxicity, and functional bioactivity. It has antimicrobial, antioxidant, and chelating properties, and can bind to proteins and fats in feed, thereby influencing digestion and metabolism (Younes & Rinaudo, 2015; Aranaz *et al.,* 2021).

2.6 Performance of oats fed diets containing chitin and chitosan

Feeding goats with diets that include chitin and chitosan has shown really positive results on growth performance, nutrient use, and overall health, both in research trials and on farms (Okafor *et al*., 2020). When these compounds are well processed and added in the right amounts, they can help improve feed efficiency, weight gain, and general productivity (Agboola *et al*., 2021).

Research with West African Dwarf (WAD) goats has shown that adding chitosan to their diet can boost average daily weight gain and feed conversion ratio compared to goats on regular diets (Olaniyi *et al*., 2022). This is mostly because chitosan helps reduce ammonia buildup in the rumen, which allows microbes to make protein more efficiently (Shah *et al*., 2022).

Chitin, on the other hand, behaves like dietary fiber—it supports better gut movement and maintains a healthy digestive system, helping the animal absorb nutrients more effectively (El-Zaiat *et al*., 2024). Its fiber-like properties also help cut down harmful gut bacteria, reducing the chances of stomach issues (Patra & Saxena, 2019).

When it comes to nitrogen metabolism, both chitin and chitosan help goats retain more nitrogen by lowering urinary nitrogen loss and improving digestibility (Ibrahim *et al*., 2019). Better nitrogen retention supports muscle growth and performance while also minimizing nitrogen-related pollution from animal waste (Adeyemi *et al*., 2023).

Performance benefits have also been tied to chitosan's ability to influence rumen fermentation—particularly by reducing methane production and increasing propionate levels, which helps goats make better use of the energy in their feed (Zhang *et al*., 2020). This change in fermentation pattern makes more energy available for growth and body maintenance.

In general, including chitin and chitosan in goat diets has proven to be a sustainable and effective way to boost performance while cutting down reliance on conventional protein sources (Ogunbosoye *et al*., 2020).

2.7 Effects of chitin and chitosan on ruminant nutrition

Chitosan inclusion in ruminant diets enhances nutrient digestibility, particularly crude protein and fiber, by improving nutrient utilization efficiency (Harahap *et al*., 2022). It promotes better nitrogen retention by reducing urinary nitrogen losses and supporting microbial protein synthesis in the rumen (Dias *et al*., 2017).

Additionally, chitosan modifies rumen fermentation reducing protozoan populations, increasing propionate production, and lowering methane emissions thereby improving both productivity and environmental sustainability (Kirwan *et al*., 2021).

Its antimicrobial properties further support gut health and feed efficiency, reducing disease risk in livestock (Younes & Rinaudo, 2015).

2.8 Concept of nitrogen balance and retention

Chitin and chitosan have gained attention in animal nutrition not only for their antimicrobial and environmental benefits but also for their potential role in improving nitrogen utilization efficiency in ruminants. Understanding nitrogen balance and retention is therefore critical to evaluating their effects in goat nutrition.

2.8.1 Definition

Nitrogen balance is the difference between the nitrogen an animal consumes and the nitrogen it loses (mainly in feces and urine). When intake exceeds losses, the animal is in a positive nitrogen balance, meaning it is retaining nitrogen for growth or production. Conversely, when intake is less than losses, the animal is in a negative nitrogen balance. Nitrogen retention is usually expressed in grams per day or as a percentage of nitrogen intake, and it serves as a measure of how efficiently dietary protein is converted into animal tissue (Hristov *et al.*, 2019). 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).

2.8.2 Importance of nitrogen balance

Nitrogen balance is a direct indicator of protein utilization efficiency. Higher nitrogen retention implies that dietary protein is being directed into productive functions such as muscle accretion and milk production, rather than being wasted. This has economic implications for farmers by reducing feed cost per unit of production, and environmental implications by lowering nitrogen excretion into soil and waterways. For this reason, feed additives such as chitin and chitosan are often evaluated to determine whether they improve nitrogen retention (Hristov *et al.*, 2019; Vyas *et al.*, 2021).

2.8.3 Measurement of nitrogen balance

This involves precise measurement of feed intake (dry matter), and total collection of feces and urine during a defined balance period. Nitrogen contents of feed, feces, and urine are analyzed, and balance is calculated as:

Nitrogen Balance= Nitrogen Intake - (Fecal N + Urinary N+ Other minor losses)

Laboratory analysis of nitrogen is typically carried out using either the Kjeldahl or Dumas method. Both are widely accepted, but the Kjeldahl method (digestion, distillation, titration) remains most common (McClements *et al.*, 2021).

2.8.4 Factors affecting nitrogen balance in goats

These include dietary protein level, energy supply, feed additives, and animal characteristics. Low-protein diets or excess degradable protein without adequate energy reduce nitrogen efficiency, while sufficient fermentable energy promotes microbial use of ammonia and improves nitrogen retention. Feed additives like chitosan can modify rumen fermentation to favor propionate production and enhance nitrogen utilization. Additionally, factors such as age, body weight, physiological status, and breed also affect nitrogen retention capacity (Hristov *et al.*, 2019; dos Santos *et al.*, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The study was carried out at the University of Benin Farm Project, Ugbowo Campus, Benin City, Edo State. The farm lies within the rainforest zone of Southern Nigeria and is suitable for small ruminant production.The annual rainfall ranges from 1500 to 1800mm with a temperature range from 21°C to 30°C.

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 extraction procedure for chitin and chitosan

Snail shells was washed, sun-dried,and ground.The extraction of chitin from snail shells was carried out following the standard procedure described by Varun *et al*. (2017). The process involved demineralization, deproteinization, and deacetylation steps to obtain chitosan.

3.4 Experimental animals

A total of 18 West African Dwarf (WAD) goats were used for the experiment. On arrival, the animals were acclimatized for two weeks, during which they were dewormed, and given prophylactic treatments against external parasites. The goats were housed in well ventilated pens, cleaned daily, and provided with fresh water ad libitum.

3.5 Experimental design

A Completely Randomized Design (CRD) was adopted to allocate treatments. There were a total of six dietary treatments, consisting of control, antibiotic diet, and varying inclusion levels of chitin and chitosan obtained from snail shells.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.

3.6 Experimental treatments

Six (6) experimental diets were formulated comprising of varying levels of additives. Each diets were fed at 50% supplement level to Guinea grass at 50% inclusion level.

The six (6) experimental treatments are as follows:

T1= 50% concentrate without additive (Control)

T2=50% concentrate containing 3% chitin

T3= 50% concentrate containing 6% chitin

T4= 50% concentrate containing 0.5% chitosan

T5= 50% concentrate containing 1% chitosan

T6= 50% concentrate containing 0.01 oxytetracycline

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

Ingredients	Snail					Antibiotics
	Chitin		Chitosan			
	T1 (Chitin 3%)	T2 (Chitin 6%)	T3 (Chitosan 0.5%)	T4 (Chitosan 1%)	T5 (Control)	T6 Antibiotics
Maize	21	21	21	21	21	21
SBM	2	2	2	2	2	2
Wheat bran	32.5	22	42	40	43	44
PKC	34	41.5	27	28.5	26.5	25.49
Chitin (snail)	3	6	0	0	0	0
Chitosan (snail)	0	0	0.5	1	0	0
Antibiotic (Tetracycline)	0	0	0	0	0	0.01
Bone meal	1	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix	1	1	1	1	1	1
Total	100	100	100	100	100	100
CP(16-18)	16.505	16.4435	16.515	16.5185	16.5665	16.50937
CF(10-12)	10.508	10.7705	10.233	10.2955	10.2453	10.16875
ME(2500KCAL)	2613.62	2576.12	2641.66	2636.48	2651.12	2646.57

3.7 Chemical analysis

Samples of the experimental diets, feces, and urine were analyzed for their proximate composition using the standard methods of the AOAC (2010).

Determination of ash

Two grams of each sample were weighed into pre-weighed crucibles and ashed in a muffle furnace at 550°C for six hours. The crucibles were cooled in a desiccator and reweighed. Ash content was expressed as a percentage of the sample, while organic matter was calculated by subtracting the ash percentage from 100.

Determination of crude protein

Crude protein content was determined using the Kjeldahl method. About 0.5 g of the sample was digested with 10 ml of concentrated sulfuric acid (72%) and 2 g of mixed catalyst ($CuSO_4$:$NaSO_4$, 1:9) at 420°C until a clear green digest was obtained. The digest was diluted to 50 ml with distilled water.

For distillation, 5 ml of the digest was mixed with 10 ml of 40% NaOH and 30 ml of distilled water in a Kjeldahl flask. The distillate was collected into 5 ml of boric acid indicator and titrated with 0.1N HCl.

Crude protein was calculated using the formula:

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

NA = Normality of acid

VA = Volume of acid used

W = Weight of sample

Determination of crude fibre

One gram of each sample was boiled in 100 ml of 1.25% H_2SO_4 for 30 minutes at 70–100°C, maintaining the volume with hot water. The mixture was filtered and rinsed until clear. The residue was then boiled in 100 ml of 1.25% NaOH for another 30 minutes and rinsed again. The residue was oven-dried at 70°C for 30 minutes, weighed, and later ashed in a muffle furnace at 550°C for 30 minutes.

Crude fibre was calculated as:

% CF = Wt. of crude fiber x 100 / Wt. of sample

Determination of ether extract (Crude Fat)

About 2 g of the sample was wrapped in filter paper and extracted with petroleum ether using a Soxhlet apparatus. After extraction, the solvent was evaporated, and the residue was oven-dried at 75°C for 1 hour 30 minutes, cooled, and weighed. The percentage of ether extract was determined as:

% EE = Weight of oil/Weight of sample × 100

Determination of Nitrogen-Free Extract (NFE)

The nitrogen-free extract (mainly sugars and starch) was estimated by difference:

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

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

All data collected were analyzed using Genstat 12th Edition. Differences between treatment means were separated using the Duncan Multiple Range Test (DMRT) at a 5% significance level.

CHAPTER FOUR

RESULTS

4.1 Proximate composition of experimental diets fed to West African dwarf goats.

Table 4.1 shows the proximate composition of the experimental diets containing different levels of chitin and chitosan. The dry matter (DM) content (%) ranged from 91.09 - 91.85. For crude protein(%) content, the range was 14.88-19.45 , the highest value was reported in diet with 3% chitin and the lowest was reported in diet with 6% chitin. However, no significant difference ($P>0.05$) was observed between diets.

The Ether extract (EE) content (%) ranged from 8.50 - 12.00 with antibodies diet containing the lowest amount 8.50 and 6% chitin containing the highest amount 14.88%

The ash content (%) ranged from 8.50 - 17.00 among diets and are significantly different ($P<0.05$) from each other.

The crude fibre (CF) content (%)of the experimental diets ranged from 18.50 - 26.00. The CF content of diets 6% inclusion level of chitin and had highest concentration and lowest concentration present in control diet. There was significant difference($P>0.05$) between diets. The nitrogen free extract (NFE) values (%) ranged from 30.12 - 42.31. There was significant difference ($P>0.05$) between diets

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

Variable	T1	T2	T3	T4	T5	T6	SEM
DM	91.85a	91.47ab	91.84a	91.09ab	91.09ab	91.55a	0.123
CF	18.50b	22.00ab	26.00a	22.50ab	17.50d	21.00ab	1.658
ASH	11.50b	15.50a	17.00a	8.50c	15.50b	13.00b	0.577
CP	17.94a	19.25a	14.88a	17.50a	15.75b	15.75a	1.526
EE	9.75bcd	10.75a	12.00a	9.25cd	8.75a	10.25bc	0.354
NFE	42.31a	32.50b	30.12b	42.25a	42.50b	40.00a	0.818
OM	88.50b	84.50c	83.00c	91.50a	84.50	87.00c	1.260

Means within the same row with different superscript different significantly (P<0.05),SEM= standard error of means,T1=concentrate without chitin and chitosan,T2=concentrate with 3% chitin,T3=concentrate with 6% chitin,T4= concentrate with 0.5% chitosan,T5=concentrate with 1% chitosan,T6=concentrate with 0.01%

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 containing chitin and chitosan.

4.2.1 Concentrate dry matter intake

All treatment groups (T1–T6) had statistically similar concentrate dry matter intake (DMI), as indicated by the same superscript “a” across treatments. This means that the inclusion of chitin and chitosan from snail shells at different levels did not significantly (P>0.05) affect the goats’ intake of concentrate feed. The highest concentrate DMI was recorded in the control group (227.5 g/day), while the lowest occurred in the 0.5% snail chitin group (148.6 g/day). However, these differences were not statistically significant, suggesting that the additives did not influence feed palatability or consumption.

4.2.2 Grass dry matter intake

There was no significant difference (P>0.05) in grass dry matter intake among all treatments, as all had the same superscript “a”. Goats fed the 3% snail chitin diet had the highest grass DMI (199.7 g/day), while those on 0.5% snail chitin had the lowest (152.5 g/day). The variations were numerical and not statistically different, implying that chitin and chitosan inclusion did not alter the animals’ forage intake.

4.2.3 Total dry matter intake

Total DMI showed no significant (P>0.05) differences among treatments. Numerically, goats on the control diet recorded the highest total DMI (416.7 g/day), while the lowest value was seen in the 0.5% snail chitin group (196.5 g/day). This indicates that the inclusion of snail chitin and chitosan did not negatively affect overall feed consumption.

4.2.4 Nitrogen intake from concentrate

All treatments had the same superscript "a", showing no significant difference (P>0.05) in nitrogen intake from concentrate feed. Goats on the 3% snail chitin diet recorded the highest nitrogen intake (7.84 g/day), while the lowest intake (4.45 g/day) occurred in the 0.5% snail chitin group. This suggests that the level of chitin or chitosan inclusion did not influence the protein intake from concentrate sources.

4.2.5 Nitrogen intake from grass

No significant difference (P>0.05) was observed in nitrogen intake from grass among the treatments. The 6% snail chitin group had the highest nitrogen intake from grass (2.32 g/day), while the lowest was recorded in the 0.5% snail chitin group (1.77 g/day). This shows that chitin or chitosan inclusion did not affect nitrogen contribution from forage.

4.2.6 Total nitrogen intake

There were no significant (P>0.05) differences in total nitrogen intake among the diets. The control group had the highest total N intake (10.16 g/day), while the lowest was observed in the 0.5% snail chitin group (6.40 g/day). This indicates that dietary treatments did not significantly influence the goats' nitrogen consumption levels.

4.2.7 Faecal nitrogen output

Faecal nitrogen output did not differ significantly (P>0.05) among treatments. The 6% snail chitin group had the highest faecal nitrogen loss (2.41 g/day), while the lowest value (1.16 g/day) was recorded in the 0.5% snail chitin group. Although there were numerical differences, they were not statistically significant, implying similar nitrogen digestibility across all diets.

4.2.8 Urinary nitrogen output

Urinary nitrogen output differed significantly (P<0.05) among treatments. The lowest urinary nitrogen loss was observed in goats fed the 0.5% snail chitin diet (0.45 g/day), while the highest was in the 3% snail chitin group (1.54 g/day). Lower urinary nitrogen losses in the 0.5% chitosan and 6% chitin groups suggest improved nitrogen utilization and retention. Conversely, higher urinary nitrogen loss in the mid-level chitin diets may indicate less efficient protein metabolism. These findings show that the level of chitin or chitosan inclusion can influence nitrogen excretion through urine.

4.2.9 Total nitrogen output

Total nitrogen output (faecal + urinary) did not differ significantly (P>0.05) among all treatment groups. Numerically, goats on the 3% snail chitin diet had the highest total N output (3.68 g/day), while those on the 0.5% snail chitin diet had the lowest (1.77 g/day). The results indicate that the overall nitrogen excretion was not significantly affected by the inclusion of snail chitin or chitosan.

4.2.10 Nitrogen balance

Nitrogen balance was positive across all treatments, indicating that the goats were retaining nitrogen and efficiently utilizing dietary protein. There was significant difference ($P<0.05$) among treatments. Numerically, goats fed the 0.5% snail chitosan diet had the highest nitrogen balance (7.445g/day),This was followed by 3% Snail chitin diet (6.27 g/day). These were not significantly different from each other. The aforementioned was significantly different ($p<0.05$) from other tretment group.The positive nitrogen balance across all treatments reflects adequate dietary protein and favorable nitrogen retention irrespective of chitin/chitosan inclusion level.

4.2.11 Nitrogen retention

No significant difference ($P>0.05$) was observed in nitrogen retention among the treatments. However, goats fed 6% snail chitin had the highest nitrogen retention (81.79%), followed closely by those on 1% chitosan (63.05%). The 0.5% chitosan group had the lowest retention (48.96%), though still within a positive range. This trend suggests that higher chitin inclusion may enhance nitrogen utilization efficiency.

4.2.12 Nitrogen digestibility

Nitrogen digestibility did not differ significantly ($P>0.05$) across treatments. The 0.5% snail chitin diet had the highest digestibility (82.80%), while the lowest (68.85%) was observed in the control treatment. All values above 60% indicate generally good nitrogen digestion and absorption among all diets.

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

Variables(g/day)	T1	T2	T3	T4	T5	T6	SEM
Conc DMI	186.3a	171.6a	205.6a	199.6a	208.2a	166.8a	14.6
Grass DMI	168.1a	173.3a	181.8a	181.4a	179.2a	162.5a	13.4
N Conc Intake	5.350bc	6.725ab	4.890c	7.825a	5.245bc	4.905c	0.467
N Grass Intake	1.950a	2.010a	2.110a	2.105a	2.075a	1.885a	0.1559
N Total Intake	7.300b	8.735ab	7.000b	9.930a	7.320b	6.790b	0.576
N Faecal Output	2.275a	1.640a	1.865a	1.695a	1.790a	1.940a	0.318
N Urinary Output	0.9350a	0.8250a	0.8600a	0.7900a	1.095a	1.1400a	0.280
N Total Output	3.210a	2.465a	2.725a	2.485a	2.885a	3.080a	0.523
N Balance	4.090b	6.270ab	4.275b	7.445a	4.435b	3.710b	0.750
N Retention (%)	56.12a	71.67a	61.45a	74.81a	59.90a	54.34a	7.19
N Digestibility (%)	68.85a	80.96a	73.56a	82.80a	75.46a	71.28a	3.96

Means within the same row with different superscript different significantly ($P<0.05$), SEM= standard error of means, T1(Control) =50% Guinea grass +concentrate without chitin/chitosan, T2= 50% Guinea grass +concentrate with 3% Chitin, T3 =50% Guinea grass + concentrate with 6% chitin, T4 = 50% Guinea grass +concentrate with 0.5% chitosan, T5= 50% Guinea grass +concentrate with 1% chitosan, T6 (Antibiotics) =50% Guinea grass + concentrate with 0.01 oxytetracycline, DMI= dry matter intake, conc = concentrate and N = nitrogen. Source: Laboratory analysis (2025)

CHAPTER FIVE

DISCUSSION

5.1 Proximate composition of experimental diets

The chemical composition of the experimental diets fed to West African Dwarf (WAD) goats is presented in Table 4.1. The dry matter (DM) content ranges concentrate diets were similar indicating that all diets were well-preserved and contained minimal moisture. This suggests good feed stability and reduced chances of spoilage. The DM values obtained were consistent with reports by Okoye *et al*. (2019), who stated that dry matter above 90% ensures better feed preservation and stable nutrient intake in small ruminant diets.

Crude protein (CP) values were the lowest in 6% chitin diet . The variations suggest that moderate inclusion of chitin may enhance protein content, while excessive inclusion could slightly reduce protein availability due to the fibrous nature of chitin.

Ether extract (EE) content observed in this study were within acceptable limits for goat diets, providing sufficient energy without inhibiting rumen fermentation, as supported by NRC (2007).

Low ash content in 0.5% snail chitosan may be attributed to differences in mineral content contributed by the chitin and chitosan sources used.

The increase in fibre content with higher chitin inclusion may be due to the inherent fibrous nature of chitin, which can affect digestibility at higher levels (Akinfala *et al*., 2021). Moderate fibre levels, however, are beneficial for maintaining healthy rumen function and improving nutrient utilization (Aro *et al*., 2018).

The control (T1) and 1% chitosan (T5) diets had the highest NFE values, suggesting higher soluble carbohydrate content that could enhance energy availability for rumen microbes.

5.2 Feed and nitrogen intake

The absence of significant variation in feed and nitrogen intake indicates that inclusion of chitin and chitosan in the diets did not adversely affect feed palatability or acceptability among the goats. This aligns with Onwuka *et al*. (2020), who emphasized that feed intake consistency is key in trials involving unconventional feed additives.

The higher nitrogen intake observed in 0.5 chitosan diet suggests that moderate chitosan inclusion enhanced protein availability and utilization efficiency, possibly due to its prebiotic and antimicrobial properties that support rumen microbial growth and nitrogen capture (Esonu *et al*., 2016).

5.3 Faecal and urinary nitrogen losses

The highest faecal nitrogen in the control diet (T1) may suggest slightly less efficient protein utilization compared to diets supplemented with chitin or chitosan. Similar findings were reported by Aro *et al.* (2018), who observed reduced nitrogen loss in faeces when goats were fed chitosan-based diets due to improved rumen microbial efficiency.

However, goats fed the 0.5% chitosan diet (T4) recorded the lowest urinary nitrogen excretion, suggesting more efficient nitrogen retention. Conversely, the relatively higher urinary nitrogen observed in the antibiotic diet (T6) and 1% chitosan diet (T5) indicates possible loss of nitrogen that was not utilized for tissue synthesis. Chitosan's ability to bind ammonia in the rumen could explain the reduced urinary nitrogen levels in T4, leading to better nitrogen utilization (Salgado-Cruz *et al*., 2020).

5.4 Nitrogen balance, retention, and digestibility

The higher nitrogen balance in T4 and T2 suggests improved nitrogen retention and utilization efficiency, likely due to the positive effects of chitosan and chitin on rumen fermentation and microbial protein synthesis. This agrees with the findings of Binta and Omage (2021), who reported that chitosan inclusion enhances nitrogen retention by reducing proteolysis and promoting microbial growth.

In general high retention values across diets indicate that the goats efficiently utilized dietary nitrogen for growth and maintenance. This observation aligns with Nworgu *et al*. (2019), who stated that unconventional feed additives can improve nitrogen retention when used within optimal levels.

The high digestibility observed, particularly in T4 and T2, suggests that moderate inclusion of chitosan or chitin did not hinder protein digestion. This supports the report of Oduguwa *et al*. (2020), who noted that chitosan inclusion in ruminant diets at moderate levels enhances nitrogen utilization without interfering with absorption.

Overall, the study revealed that supplementation with chitin and chitosan from snail shells did not negatively affect feed intake, nitrogen intake, or utilization. Instead, diets containing moderate levels, particularly the 0.5% chitosan diet (T4) showed improved nitrogen balance, retention, and digestibility. This indicates the potential of chitin and chitosan as beneficial feed additives for enhancing nitrogen efficiency and overall productivity in West African Dwarf goats.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The findings of this study indicate that chitin and chitosan extracted from snail shells can be incorporated into West African Dwarf goat diets without adverse effects on feed intake or nitrogen utilization.

Chitosan, particularly at 0.5% inclusion (T4), significantly improved nitrogen balance, retention, and digestibility, demonstrating its potential as a natural alternative to antibiotic growth promoters.

Chitin at 3% inclusion, though less effective than chitosan, still supported adequate nitrogen retention and digestibility, suggesting it can serve as a safe supplementary feed component.

Inclusion of both additives did not negatively affect dry matter intake or overall feed acceptance, highlighting their suitability for goat diets.

Overall, chitin and chitosan from snail shells are safe, cost-effective, and sustainable feed additives that can enhance nitrogen efficiency in WAD goats at 3% and 1% inclusion levels respectively.

6.2 Recommendations

1. Chitosan at 0.5% inclusion should be prioritized in WAD goat diets to improve nitrogen utilization and retention followed by 3% chitin.

2. Economic analyses should be conducted to evaluate cost-benefit ratios for practical farm applications.

3. Future studies should explore the impact of chitin and chitosan on rumen microbiota.

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