UNIVERSITY FOR DEVELOPMENT STUDIES

NUTRITIONAL VALUE OF DRIED RUMEN DIGESTA CONCENTRATE FOR RUMINANT

BY

HALIDU AGOLISI MAMUDU

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THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE AND CONSUMER SCIENCE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN ANIMAL NUTRITION

JULY, 2023

DECLARATION

I hereby declare that this thesis is the result of my original work and that no part of it has been presented for another degree in this University or elsewhere.

Candidate' Signature:….. ………… Date: 24/10/2023

Halidu Agolisi Mamudu

Supervisors

I hereby declare that the preparation and presentation of the thesis were supervised by the guidelines on supervision of the thesis laid down by the University for Development Studies.

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ABSTRACT

An evaluation of nutritional value of rumen digesta in ruminant diets was investigated in 5 Experiments. Experiment I, the effect of four different treatment methods (sun-dried, oven-dried, fermented, and urea-fermented) on chemical composition, *in vitro* digestibility and microbial quality of Rumen digesta from the Bolgatanga abattoir collected in four climatic seasons (early wet season [EWS], main wet season [MWS], early dry season [EDS], and late dry season [LDS]. The experiment was conducted as a 4*4 factorial in a completely randomized design. The two-way interaction effect of season and processing methods had a significant effect on DM, CP, EE, Ash, NDF, and ADF. The crude protein values obtained were 17.58, 22.26, 15.25 and 8.42% for early wet, main wet, early dry and late dry seasons respectively. The crude protein values obtained for the methods were 14.72, 14.36, 13.96 and 20.45 for sun-dried, oven-dried, fermented and urea-fermented methods respectively. The main effect of processing method and season had a significant effect on digestible organic matter. The urea-fermented processing method consistently recorded a higher IVDOM. The processing methods all resulted in a significant reduction of microbial population in the dried rumen digesta. The decline in *Salmonella spp.* and *E. coli* concentration was in the range of 90-100% for all the processing methods with oven drying and urea fermented methods recording a 100% reduction in both microorganisms. In experiment II, two rumen digesta processing methods (Unpelleted and Pelleted) and different inclusion levels (0, 5, 10 and 15%) were used to assess the effects on the chemical composition and microbial load on dried rumen digesta. The experiment was conducted as a 2*4 factorial in a completely randomized design. Processing methods and DRD inclusion levels had a significant ($P < 0.05$) interaction effect on CP, Ash, LAB, and E. coli. The mash method of the 15% inclusion level of dried rumen digesta (DRD) had the highest CP (14.22%). Method and inclusion levels had a significant interaction effect on *E. coli*. In experiment III, rice straw was supplemented with urea-ferment-dried rumen digesta using four dietary inclusion levels of (0, 5, 10 and 15%) in a completely randomized design. The highest (P<.001) digestible organic matter (DOM) was recorded in the 15% DRD pellet concentrate supplemented with 50% rice straw. Dried rumen digesta pellet significantly enhanced the *in vitro* organic matter digestibility of the processed rumen digesta. In Experiment IV, the effect of urea-fermented dried rumen digesta pellets (UFDRDP) concentrate on the apparent digestibility and growth performance of Djallonké sheep in the savanna agroecological zone of Ghana was examined. Sixteen Djallonké rams with an average initial weight of 9.90 kg were used for the study. The diets consisted of four levels of DRD (0%, 5%, 10%, and 15%) and were combined with rice straw as the basal diet. This was replicated four times in a completely randomized design (CRD) over 84 days. The concentrate diets had a crude protein (CP) content ranging from 101.0 to 131.4 g/kgDM. The neutral detergent fibre (NDF) content varied between 447.6 and 543.9, while the ADF ranged from 198.7 to 235.0. The DM intake was similar among the rams, crude protein intake was significantly higher in rams fed 15% DRD. The digestibility coefficient for DM did not differ significantly, but there was a significant difference in the crude protein digestibility coefficient, with the highest values observed in rams fed 10% and 15% DRD. Final live weight gain showed a significant variation with rams fed 15% DRD gaining twice as much weight compared to the control diet. The trend was similar for average daily weight gain, with rams on 15% DRD achieving significantly higher gains compared to the control group (48.93 vs 28.89 g/day/head). Experiment V, focused on the effects of urea-fermented dried rumen digesta pellets (UFDRDP) on the blood profile of Djallonké sheep, the haematological parameters of rams were not significantly affected by the dietary treatments. However, the albumin and blood urea nitrogen concentrations were significantly influenced by the dietary treatment. Feeding young rams with UFDRDP showed improvements in final live weight gain and average daily weight gain in Djallonké sheep in the Savanna agro-ecological zones. Urea-fermented dried rumen digesta

pellets can be used as a supplement for small ruminants to enhance their growth and performance.

Keywords: Season, method, *E. coli*, *in vitro gas production*, *rumen digesta*, *Sheep*

LIST OF PUBLICATION AND CONFERENCE PAPERS

Journal articles

1. Halidu Mamudu Agolisi, Terry Ansah and Frederick Adzitey (2023). Effects of season and processing methods on chemical composition, microbial load and in vitro gas production of dried rumen digesta. Trop. Agric. (Trinidad) Vol 100 (3), 221–230.

2. Halidu Mamudu Agolisi and Terry Ansah (2023). Effects of urea-treated rumen digesta pellet concentrate on digestibility, growth performance and blood profile of Djallonk´e sheep. Scientific African 21 (2023) e01864.

Conference paper and Exhibition

1. Halidu Mamudu Agolisi, Terry Ansah and Frederick Adzitey (2023). Chemical Compositions, Microbial Quality and in Vitro Gas Production of Rice Straw Supplemented with Processed Dried Rumen Digesta. Conference processing, 2nd Joint Annual Conference of GSAP and GASA on 28th August – 1st September 2023, KNUST, Ghana.

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DEDICATION

This thesis is dedicated to the entire Agolisi family.

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LIST OF ABBREVIATIONS

ADF: Acid detergent fibre

ADG: Average daily gain

ANOVA: Analysis of variance

AOAC: Association of Analytical Chemists

CP: crude protein

DMD: Dry Matter Digestibility

DMI: Dry Matter Intake

DRD: Dried Rumen Digesta

EDS: Early Dry Season

EWS: Early Wet Season

IVOMD: In vitro Organic Matter Digestibility

LDS: Late Dry Season

ME: Metabolizable Energy

MWS: Main Wet Season

PDF: Neutral Detergent fibre

UFDRD: Urea-fermented dried rumen digesta

WAD: West African Dwarf

CHAPTER ONE

1.1 Introduction

Livestock holds immense value in the global market, estimated at a minimum of $1.4 trillion. This industry forms a crucial part of extensive market chains and employs a staggering 1.3 billion people worldwide (Max and Esteban, 2017). According to Thornton (2010b), approximately 600 million small-scale farmers in developing countries, who are living in poverty, depend directly on cattle for their means of sustenance. Ruminants, apart from providing draft power, milk, meat, and manure, also fulfil various other functions. Animal protein plays a significant role in the average person's diet, contributing to meeting approximately 33% of the daily protein requirement through dairy, meat, and eggs sourced from animals. With the continuous growth of the global population and increasing urbanization, the demand for livestock products continues to rise steadily (Thornton, 2010a). Livestock species play social-economic and cultural roles in rural households in developing nations, contributing to increased farm revenue and overall well-being. Moyo *et al.* (2010) emphasize the diverse importance of livestock, which spans a wide range of advantages.

Smallholder farmers contribute 35% of agricultural GDP, support 70% of the rural population, and produce 90% of agricultural goods in Sub-Saharan Africa. Karbo and Agyare (2002) highlight livestock's crucial role in providing dietary protein, accounting for up to 18% of the local population's carbohydrate-based diets. In Ghana, livestock holds significant importance for households, offering benefits such as meat consumption, manure for soil fertility enhancement, and income generation through animal sales. Moreover, it plays a vital role in Ghanaian cultural and religious customs (MoFA, 2011).

Livestock farmers in sub-Saharan Africa, including Ghana, face a significant challenge in terms of feed and feeding. Over the years, the cost of feed ingredients has skyrocketed, causing serious concern among livestock producers (Kassam *et al*., 2009). Currently, the exorbitant prices of feedstuffs present a critical issue in livestock management. This is particularly alarming because feed expenses typically make up approximately 60-80% of the recurring costs in intensive animal production (Onifade, 1993). In several developing countries, including Ghana, the nutrition of ruminant livestock heavily relies on natural pasture with limited supplementation (Konlan *et al*., 2017; Ansah and Issaka, 2018). Ghana faces feed constraints for smallholder ruminant production. Ruminants heavily rely on crop residues and unimproved pastures, which are deficient in essential nutrients such as nitrogen, minerals, and energy which affect animal productivity (Antwi *et al*., 2014).

The absence of quality natural pasture during the dry season causes a significant reduction in animals' weight and their market value. This situation presents a significant challenge for livestock farmers in Sub-Saharan Africa. Nitrogen supplementation is crucial for maximizing fermentable structural polysaccharides in crop residues, particularly during dry seasons. However, competition, import costs, and uncertainty in protein feed production have increased protein concentrate prices, necessitating exploring alternative sources for economical and long-term animal production (Merry *et al*., 2001). To tackle the obstacles associated with feed resources in livestock production, one potential solution is the utilization of rumen digesta. This can be a valuable protein source that supports the growth and overall health of ruminants. Embracing such alternatives allows livestock producers to overcome feed-related obstacles and maintain a profitable and sustainable animal production system.

Rumen digesta is a by-product generated daily at abattoirs (Odunsi *et al*., 2004). It constitutes material from the rumen, the first stomach compartment of ruminant animals, and accounts for approximately 80% of an adult ruminant's stomach (Church, 1993). Rumen digesta is plant material and is high in microbial protein, energy, protein, and vitamins, especially vitamin B complex (Emmanuel, 1978; McDonald *et al.*, 1990; Agolisi *et al*., 2022). Rumen digesta is an economical feed resource that presents disposal challenges at abattoirs (Adeniji and Oyeleke, 2008). In the case of dried rumen digesta obtained from the Tamale abattoir, it contains approximately 14.01% crude protein in terms of dry matter, making it a viable protein source for animal nutrition when appropriately processed (Agolisi *et al.*, 2022). Rumen digesta protein composition may change based on plant material, microbe activity, forage intake and slaughter time (Sakaba *et al.*, 2017).

Cattle rumen digesta is abundant in high-quality crude protein, which includes essential amino acids essential for animal nutrition (Esonu *et al*., 2006; Kamalu *et al.*, 2010; Agbabiaka *et al.*, 2012). The high amino acid content of rumen digesta makes it an exceptional feed source for livestock (Jovanovic *et al*., 1997; Gohl, 1982). Furthermore, Agbabiaka *et al.* (2012) and Elfaki and Abdelatti (2015) have highlighted the significant levels of calcium, phosphorus, and magnesium found in CRD, making it a valuable source of minerals. Rumen digesta is used as feed for ruminant and monogastric animals in some countries (Okere, 2016).

Numerous research studies have highlighted the potential of dried rumen digesta (DRD) from ruminants as a cost-effective protein supplement in livestock nutrition. Al-Wazeer (2016) suggested that incorporating DRD into the diet of ruminants, as a partial replacement for barley grain and soybean meal, can enhance economic returns compared to conventional feed ingredients. DRD has proven effective in various experiments involving fish, poultry, rabbits, cattle, goats, and sheep (Odunsi, 2003; Esonu *et al*., 2006; Okpanachi *et al*., 2010; Agbabiaka *et al*., 2011; Agolisi *et al*., 2022).

In specific cases, the inclusion of 10% DRD in the diet of Awassi lambs did not negatively impact their health or nutrient digestibility (Al-Wazeer, 2016). Similarly, in another study, Djallonke sheep fed with 12% DRD showed no signs of health issues or digestibility problems (Agolisi *et al.*, 2022). These findings further support the suitability and potential benefits of incorporating DRD as a protein supplement in livestock feed. The increasing demand for meat has led to a rise in the slaughter of ruminant animals, consequently generating a larger amount of waste in the form of rumen digesta at slaughterhouses. The increase in waste generation raises concerns about urban pollution (Ristianto *et al*., 2016). Rumen digesta, often discarded as waste, causes environmental problems for residents and contributes to unregulated disposal, including water source contamination (Agbabiaka *et al*., 2011; Uddin *et al*., 2018). Improper handling of slaughterhouse waste not only poses significant health risks to individuals but also affects the wider community, leading to a growing public concern (Fearon *et al*., 2014).

Relying solely on the scavenging behaviour of livestock is insufficient to meet their nutritional requirements for optimal production. Therefore, it is essential to ensure an adequate supply of feed in terms of both quality and quantity to achieve optimal livestock performance (Maigandi and Owanikin, 2002). Unfortunately, the northern part of Ghana, where a substantial number of cattle, sheep, and goats are located, faces challenges with feed shortage and insufficient supply, especially during the dry season. Additionally, the costs of feed ingredients, which are either imported or in high demand for human consumption, have been escalating.

Ghana produces a significant amount of rumen digesta annually, which has the potential to be a valuable resource for livestock feeding. However, due to a lack of technological know-how, it is underutilized. Given that feeding is a significant issue in ruminant production in Ghana, it is critical to consider the use of Rumen digesta as feed ingredients to reduce production costs and maximise profit for improved livestock enterprises. It contains a significant amount of nutrients that support animal performance (Boda, 1990). Some works have been done on feeding dried rumen digesta on different species of animals in some countries including Ghana (Odunsi, 2003; Esonu *et al.*, 2006; Okpanachi *et al*., 2010; Agbabiaka *et al*., 2011; Agolisi *et al*., 2022). However, research works on the relationship between season and multiple processing methods' effect on nutritive value and microbial quality of rumen digesta are limited especially in Ghana. Therefore, this research sought to fill a knowledge gap on the relationship between season and multiple processing methods' effect on the nutritive value and microbial quality of rumen digesta. Also, the effect of feeding dried rumen digesta concentrate pellet with crop residues and native pastures in ruminants needs to be investigated in the savannah zone of Ghana. This could help livestock farmers increase livestock productivity and reduce waste in most of the abattoirs in the savannah zone. This research sought to use processed rumen digesta as the sole source of protein for ruminants in northern Ghana.

1.2 Research Questions

1. Can change in season affect the nutritive value of rumen digesta?
2. Does pelleting affect the chemical composition and microbial quality of dried rumen digesta?
3. Can dietary supplementation of pelleted dried rumen digesta concentrate improve digestibility in the ruminant?

4. Can pelleted dried rumen digesta concentrate improve the performance of ruminants?
5. Does dried rumen digesta pellets have an adverse effect on haematology and serum profile of ruminants?

1.3 Main Objective

The main objective of this study was to assess the nutritional value of processed dried rumen digesta as a supplement for ruminants.

1.4 The objectives of this study were as follows:

1. To determine the effect of season and processing methods on chemical compositions, microbial quality (*Lactic acid bacteria, Salmonella and E. coli*) and *in vitro* gas production of dried rumen digesta.
2. To determine the effect of pelleting on the chemical composition and microbial quality of dried rumen digesta concentrates.
3. To estimate the effect of supplementing rice straw with graded levels of pelleted dried rumen digesta on *in vitro* digestibility.
4. To determine the effect of varying levels of urea-treated dried rumen digesta pellets supplementation on apparent nutrient digestibility and growth performance of young Djallonké rams.
5. To assess the effect of urea-treated dried rumen digesta pellets supplementation on haematology and serum biochemistry of young Djallonké rams.

CHAPTER TWO

2.0 Literature Review

2.1 Livestock Production

In many Sub-Saharan African (SSA) nations, livestock production has been a key factor in economic growth, contributing around 35% of the agricultural GDP and providing the majority of jobs for the rural population (FAO, 2012). Approximately 90% of the agricultural products in SSA are produced by smallholder farmers (FAO, 2012). Livestock are raised by subsistence farmers to meet their multiple needs (Onyango *et al*., 2015). Apart from the monetary benefits of raising animals, they are managed for their socio-economic benefits such as savings for the medium-term, insurance against crop failure, hide, manure, a way of diversifying investment and performing social and cultural activities (Weyori *et al*., 2018).

According to Hatab *et al*. (2019), several livestock development strategies and programmes for sub-Saharan African economies tend to have less of an impact on livestock output and productivity for reducing poverty and ensuring food security for rural families. Covarrubias *et al.* (2012) asserted that traditional livestock systems (Verpoorten, 2009) in which the animals search for nourishment, water, and a place to live with little to no veterinary care are still used to produce livestock in arid areas of sub-Saharan Africa. On the other hand, animals are raised to help subsistence farmers achieve a variety of goals (Onyango *et al*., 2015).

The average number of small ruminants kept by smallholder farmers in northern Ghana ranges from 6 to 10 (Karbo and Agyare, 2002; Oppong-Anane, 2010; Baah *et al*., 2012). However, in the Upper West of Ghana, Amankwah *et al.* (2012) noted a comparatively significant flock size of 21% per family. These animals are typically raised to sell during trying times to help with household food and monetary needs.

According to Oppong-Anane (2010), the majority of the animals are raised in semi-intensive systems with little year-round feed supplementation. The dry season is typically when they are not housed (Duku *et al*., 2010).

In many places, sheep and goats are often tethered throughout the growing season to reduce crop devastation because of this, animals have less access to green forage (Awuma, 2012). The practice causes miscarriages, weight loss, weakened immune system, infections, and rainy season death in animals (Ottere *et al*., 2002). During the dry season, animals are free to roam, but the availability and quality of the diet are typically poor, which causes delayed growth (Oppong-Anane, 2013). Animals that are managed under an open grazing system undergo significant fluctuations in growth during the dry season resulting in weight loss (MoFA, 2010; Baah *et al*., 2012). Small ruminants like Djallonkè sheep, Djallonkè Sahelian crossbred sheep, and West African dwarf goats all show weight gains of between 10.2 and 57.4 g/d and between 0.7 and 51.5 g/d during the dry season, respectively (ITC, 2014). This demonstrates the variability in weight gain among these animals under such conditions. These variations in growth can be attributed to different management techniques employed by farmers and fluctuations in feed availability, which can sometimes lead to weight losses (Annor *et al*., 2007; Karbo and Agyare, 2002; MoFA, 2010).

2.1.2 Challenges of Ruminant Production in Ghana

One of the primary challenges faced in ruminant production is inadequate access to feed resources, especially during the dry season. This shortage poses a pressing concern for ruminant management and productivity (Tolera, 2007). While forage is available during the wet season, farmers often struggle to provide adequate access to it due to the need for tethering or stall-feeding their animals during the cropping period (Awuma, 2012). In Ghana, the primary sources of feed for ruminants are natural pastures and crop residues, while agro-industrial by-products play a relatively minor role. However, in urban areas of Ghana, the availability of natural pastures has decreased due to infrastructure development. As a result, urban farmers are increasingly compelled to explore alternative feed sources such as crop residues (Amankwah *et al*., 2012). This shift is driven by the need to adapt to the changing agricultural landscape and ensure a sustainable supply of feed for livestock in urban settings. The increasing urbanization rate, as indicated by the 2010 population census, has resulted in a 51% decrease in grazing lands for livestock (MoFA, 2011; ADB, 2014).

A significant cause of Ghana's low ruminant livestock output is the absence of appropriate nourishment during the dry season. The scarcity of green and high-quality forage during this period inhibits livestock productivity, leading to weight loss in animals (Ball, 2001). The northern region of Ghana plays a crucial role in the country's livestock sector, housing a diverse range of livestock species. However, livestock production in the region faces multiple constraints, such as the absence of improved breeds, limited access to affordable quality feed, weak livestock extension services, inadequate technology adoption, and a lack of robust veterinary services (Bukhari *et al*., 2019).

During the wet season, forage growth is vigorous, but as the rainy season ends, the forage becomes fibrous and less nutritious due to high lignification rates common in tropical regions. Additionally, bushfires further exacerbate the problem in many areas. Although crop residues are abundantly available for supplementation, they often have high fibre and lignin content, resulting in poor nutritional value (Derso, 2009).

Overall, the unavailability and poor quality of feed resources, particularly during the dry season, inadequate livestock management practices, and limited access to improved breeds and veterinary services are the primary constraints facing ruminant production in Ghana (Karbo *et al*., 2002).

2.1.3 Difficulties associated with accessing protein feed resources

One of the primary challenges in modern ruminant production is the need to lower feeding expenses while improving the quality of livestock products. However, the availability and affordability of animal feed pose significant obstacles for average farmers (Ruzic-Muslic *et al*., 2014). The cost of protein and diet formulation is a critical concern in livestock production and performance. The high prices of soybeans, import dependency, and production fluctuations have created barriers for livestock farmers, limiting their access to protein sources (Ruzic-Muslic *et al*., 2014). Farmers relying on imported protein sources face unstable prices, currency fluctuations, and supply shortages, further hindering their access to protein for livestock production (Merry *et al*., 2001). The cost of importing protein concentrates has had a substantial impact on supply, demand, and overall market dynamics (Ruzic-Muslic *et al.*, 2014).

One approach to reduce feeding costs and minimize environmental impact is to utilize alternative feed sources such as feedstuffs, browse, and shrubs. However, these materials often contain varying levels of anti-nutritional factors (ANFs). These anti-nutritional elements may make it more difficult for animals to consume and utilize the fodder that comes from trees. Nevertheless, it has been discovered that some legume sources have a good impact on the way rumen bacteria work, reducing the harmful effects of ANFs (Yacout, 2016).

2.2 Ruminant Feed Resources

2.2.1 Pasture

Pasture is land used for grazing livestock, primarily grasses and legumes (Addo, 2007). Good pasture management significantly impacts animal production components like performance, milk output, and conception rates (Arseneau, 2010). Addo (2007) emphasizes the importance of specific traits in pasture plants to ensure optimal animal performance and productivity. Both wild pasture and developed grassland can be roughly classified as pasture. Rangelands covered in forage crops, either annual or perennial, that have grown on their own in a natural setting are referred to as pastures (Addo, 2007). According to Estell *et al.* (2012), these rangelands support about 30% of the world's population and make up roughly 54% of the land-based environment. The Coastal savanna, Guinea savanna, and Derived savanna are notable examples of natural pastures in Ghana. These pastures play a crucial role in supporting livestock production and contribute to the agricultural diversity of the country. Proper management and conservation of these pastures are vital for ensuring their sustainable use and continued contribution to Ghana's livestock sector (Addo, 2007).

On the other hand, man-made pastures are deliberately created and managed by humans through a range of practices, including controlling weeds, applying fertilizers, providing irrigation, reseeding, implementing rotational grazing, and carefully regulating stocking rates. This group includes a variety of pasture types, including ley/rotational pastures, irrigated pastures, and annual or perennial pastures (Addo, 2007).

Natural pastures are the main source of feed for ruminant cattle in Northern Ghana (Ansah *et al.*, 2014). Ghana has a significant pasture production potential of 107,000 km2, including 71,000 km2 of unreserved savannah forest and 360,000 km2 of permanent pasture (Oppong-Anane, 2006), and dominant grass species like Guinea grass, Bahama grass, elephant grass, giant star grass, and carpet grass are crucial for grazing animals, providing essential forage for pasture systems (Arseneau, 2010).

2.2.2 Crop Residues and Agro-Industrial By-Products (AIBP) as Ruminant Feed

According to Correddu *et al.* (2020), there are multiple advantages to integrating by-products into animal diets. These advantages include lowering feeding expenses for farmers, enhancing the value of animal products, and promoting better animal health. Furthermore, Branciari *et al*. (2021) suggest that specific by-products, such as olive mill waste, can be utilized to extract phenol metabolites, which have the potential to improve the microbial quality of meat. Another study by Branciari *et al*. (2020) indicates that incorporating such by-products can also increase the presence of antioxidant molecules in milk and dairy products. After the harvest of crops, there are residual materials known as crop residues. These residues include maize stover, cassava tops, maize cobs, and rice straws. Cereal straws, sugarcane tops, bagasse, cocoa pod husks, and pineapple trash are just a few examples of fibre crop leftovers that are often used as ruminant animal feed in underdeveloped nations. Livestock farmers often use crop wastes like maize, sugar cane, grain sorghum, soybeans, wheat, and vegetables as animal feed. In the Yendi District of Ghana, cereal and legume residues are the most commonly used crop leftovers. Primary ruminant feeds in the Northern Region of Ghana are primarily composed of groundnut haulms, cowpea haulms, and pigeon pea residue. Maize, millet, and sorghum are the primary crop residues in sub-Saharan Africa, with average utilization of 1.5 tons per year as livestock feed (Kossila, 1984). Crop residue generation in the West African subregion varies between 0.07 and 70.57 million tons, accounting for 1% to 82% of national feed resources (Fleischer, 1991). Ghana produces 9.38 million metric tons of crop residues annually, including roots, tuber, and cereal stalks for animal feed (Ampadu-Agyei *et al*., 1994; Oppong-Anane, 2010). In northern Ghana, the annual generation of crop residues surpasses 5 million tons (Karbo and Agyare, 2002; MoFA, 2011). Notably, sorghum straw yields in Northern Ghana are estimated to be 8.5 tons of dry matter per hectare, surpassing other crop residues (Konlan *et al.*, 2017). In the Yendi District of Ghana, 94% of crop residues are used as ruminant livestock supplements, with groundnut haulms accounting for 40%. Cereal straws, like sorghum straw, are less commonly sold as feed (Ansah *et al.*, 2006; Konlan *et al*., 2015). Agro-industrial by-products serve as sources of energy, protein, or a combination of both (Aregheore, 2000; Sindhu *et al*., 2002).

The growing global concern regarding food waste has led to the implementation of the UN's Agenda 2030, which aims to mitigate the environmental consequences of human activities (Duque-Acevedo *et al.*, 2020). To address this issue, new production strategies are being developed, with a focus on the circular model. This model aims to create a more efficient system by minimizing the use of natural resources and waste products, emphasizing the utilization of waste as valuable co-products (Murray *et al.*, 2017; Toop *et al*., 2017). However, co-products have limitations, including the diverse nutritional composition resulting from various processing techniques. Additionally, crop residues and agro-industrial by-products require preservation methods to ensure product stability, overcome seasonal availability challenges, and extend shelf life, particularly for co-products with high moisture and lipid content (Salami *et al.*, 2019).

2.3 Improving the nutritional content of crop leftovers and agro-industrial by-products in animal feed

To enhance the quality of crop residues, it is important to employ efficient storage methods that consider the physical properties of the residue. The transportation of crop residues to storage locations requires extra manpower. Bulky crop wastes like maize, millet and sorghum stovers are typically heaped or bunched in the field with the option of later transporting them to the homestead or directly feeding animals from the stack. The type of residues has an impact on these procedures (Suttie, 2000).

Ruminants can improve the utilization of crop residues by using chemical, biological, and physical methods to degrade **the** cellulose-lignin complex, making structural carbohydrates more accessible to rumen bacteria (Mahesh, 2013). Mahesh (2013) recommends adding brans, millings, oilseed cakes, legumes, urea, and fodder from multifunctional trees to crop wastes. Processing techniques can increase the metabolizable energy and digestibility of agricultural residues.

2.3.1 Processing Crop Residues Using Physical Techniques

To improve the utilisation of crop wastes, several physical techniques can be used, including soaking, chopping, grinding, pelleting, boiling, gamma irradiation, and high-pressure steaming. According to research (Ibrahim, 2012), these treatments cause animals to consume more agricultural residue. For instance, it has been discovered that grinding and cutting straws increase the amount of straw consumed daily by animals (Malik, 2015). This improvement is due to the feed taking less time to be broken down into a size suitable for rumen microbial digestion. Additionally, it has been shown that pelleting increases consumption, presumably because grinding reduces dustiness (Chaturvedi *et al*., 1973). Soaking straw in water has been reported to increase the digestible organic matter and intake of straw by Van Soet (2006), although the nitrogen retention varied. Soaking has also been found to increase the dry matter intake of the treated material.

2.3.2 Processing Crop residues using chemical techniques

Alkalis, acids, or oxidizing agents can be used to weaken cell wall components, solubilize constituents, and increase cell wall swelling, facilitating the entry of microbial enzymes and improving the digestibility of crop residues (Mood *et al.*, 2013). Sodium hydroxide (NaOH) is commonly employed to enhance the digestibility of crop residues. Chapple (2014) found that NaOH treatment significantly increased both in vitro (up to 38% units) and in vivo digestibility (24-30% units) of straw, resulting in a substantial increase in intake by approximately 30%. Urea is another alkali used for treating crop residues. Scotties (1997) conducted an observation on the ensiling process, noting that treating straw with 4% urea and allowing it to ferment for 3-6 weeks resulted in doubled intake and digestibility of straw, oat straw, and mixed legume haulms. This improvement in feed quality led to higher weight gain in sheep when compared to animals fed untreated straw. Furthermore, other studies, such as the research conducted by Egyir (1994), have demonstrated that ensiling straw with urea (at concentrations of 3-5%) can increase digestibility by 10-12%. However, the adoption of chemical treatment among smallholders in Africa is limited due to challenges related to availability, cost, and handling (Erenstein, 2003).

2.3.3 Processing crop residues through biological methods

The utilization of fungi-derived metabolized lignocelluloses provides a biological approach to enhancing the nutritional value of straw through selective delignification (Jalc, 2002). This method is particularly relevant in developing countries where challenges arise in producing sufficient quantities of fungi or their enzymes due to limited technology. However, challenges include the potential production of toxic substances by fungi and difficulties in controlling pH, temperature, pressure, and oxygen and carbon dioxide concentrations for optimal fungal growth (Jalc, 2002).

According to Beauchemin *et al.* (2004), as fermentation technology and alternative enzyme production systems continue to advance, it is anticipated that the processing costs associated with crop residues will decrease in the future. This decrease in costs could pave the way for the emergence of new commercial products that could play substantial roles in future ruminant production systems. Gupta *et al.* (2013) observed a 50% increase in nitrogen and protein content in rice straw cultivated with *Pleurotus sajor-cajun*, indicating the potential for dual benefits of improving feed quality. Langar *et al*. (1980) cultivated *Agaricus bisporus* and *Volvariella dysplasia* on wheat and paddy straw, respectively, resulting in increased crude protein, soluble cell content, and lignin in the post-fungal harvested straw compared to untreated straw. Zafar *et al.* (1981) reported a 43% in vivo digestibility of paddy straw biodegraded by *Pleurotus sajor-cajun*, compared to 28.3% for non-biodegraded rice straw.

2.3.4 Pelleting

Pelleting has remained a popular processing technique in the field of feed manufacturing, and its use continues to prevail. In essence, pelleting involves transforming a finely ground mixture of ingredients into compact, freely flowing agglomerates known as pellets. This process incurs substantial costs in terms of both initial investment and ongoing expenses. However, the expense is typically justified by the subsequent benefits observed in plant profitability and animal performance (Lecznieski *et al.*, 2001). For maximizing animal growth, pelleting stands as the most extensively employed thermal processing method in the preparation of animal diets (Dozier *et al*., 2010). The advantages associated with pelleting encompass a reduction in ingredient segregation, enhanced handling convenience, improved feed flow within the equipment, diminished selective feeding behaviour, increased bulk density, elimination of harmful organisms, and the potential to reduce formulation costs by incorporating alternative ingredients (Fairfield, 2003). Furthermore, pelleting allows for the reduction of dietary energy content (Lecznieski *et al.*, 2001).

2.4 Protein Sources for Ruminants

A variety of plant-based protein sources can be utilized in livestock diets. Sources include oilseeds, by-products, legumes, and waste from food production (Crawshaw, 2001). Breweries and bioethanol production by-products such as maize gluten feed can also be used as good sources of proteins for animals (Ruzic-Muslic *et al.*, 2014; Fernandez, 2017). Brewers' grain has an average crude protein of 240 g/kg (Crawshaw, 2002) while maize gluten feed has higher levels between 600-700 g/kg (Fernandez, 2017).

Animal byproducts and oilseeds that have undergone the oil extraction process are valuable sources of protein for livestock diets. Cakes and meals, obtained from various oilseed crops, serve as excellent sources of protein for livestock rations, providing a good quality protein source. These are by-products of oil extraction with optimal sources of proteins; groundnut cake (40- 48%), Soybean meal (48-50%), cottonseed cake (45%), sunflower meal (35%), oil palm kernel expeller (18%), rape seed meal (40%) and copra meal (23%) (Fernandez, 2017; Sindhu *et al.*, 2002). The main protein source in animal nutrition is soybean meal (SBM), as widely recognized (Zagorakis *et al.*, 2018). Sunflower meal (SFM) is considered valuable for supplementing low-degradable protein feedstuffs (Molina *et al.*, 2003; NRC, 2001; Ruzic-Muslic *et al.*, 2014). Furthermore, rapeseed meal (RSM) is widely utilized as a protein source in animal nutrition and has demonstrated favourable results, particularly in dairy cow diets (Mulrooney *et al*., 2009). Pea seeds (PS) and fababean seeds (FBS), known for their high crude protein (CP) content (Larsen *et al*., 2009), are also recognized as significant protein sources. Lupin seeds and groundnut meal are valuable protein sources in dairy cow diets due to their higher nitrogen and ether extract content, making them high-quality feedstuff for ruminants (Froidmont and Bartiaux-Thill, 2004; Weiss, 2000). In addition to the aforementioned options, forage legumes and straws can be utilized as protein sources. Cassava leaves and legumes are examples of forage legumes that can serve this purpose (Ruzic-Muslic *et al*., 2014; Fernandez, 2017). Groundnut straw, in particular, contains a higher protein content compared to cereals (Ruzic-Muslic *et al*., 2014; Fernandez, 2017). Furthermore, several tropical legumes such as Aeschynomene, Arachis, Centrosema, Desmodium, Leucaena, Macroptilium, and Stylosanthes show promise as protein feed sources (Quesenberry and Wofford, 2001).

2.5 Protein Nutrition of ruminant animals

Ruminants require protein feeding for efficient carbohydrate digestion and microbial protein production. The primary objective is to meet rumen microorganisms' demands for ammonia, amino acids, and peptides. The second objective is to satisfy the host animal's maintenance, growth, health, and reproduction needs. Protein nutrition aims to minimize dietary crude protein while meeting MP and amino acid needs for desirable yields with precise protein and fat amounts (Das *et al*., 2014).

Ruminant animal protein is split into two categories: Rumen Degradable Protein (RDP) and Undegradable Dietary Protein (UDP), depending on the intended yields. The percentage of dietary protein that is degraded in the rumen is known as RDP, whereas the smaller, more variable part of the protein that avoids rumen degradation is known as UDP. The host animal obtains amino acids from microbial protein in the rumen and UDP in the lower digestive tract. Low-yielding animals typically rely on microbial protein, while a combination of both can meet their protein needs (Mayank and Tanuj, 2008).

2.5.1 Ruminant Digestion System

Ruminants have a notable advantage compared to non-ruminants because of their digestive system, which exhibits various functional and anatomical adaptations. These adaptations enable ruminants to efficiently extract energy from fibrous plant materials, particularly cellulose and other complex carbohydrates that are typically difficult to digest (Van Soest, 2006). Ruminants' digestive system involves microbial fermentation before gastric and intestinal processes, a crucial characteristic (Niwiska, 2012). This unique system involves a symbiotic relationship between the ruminant and a large population of microorganisms integrated within its digestive tract. The rumen, the first chamber of the ruminant's four-compartment stomach, serves as the primary site for microbial fermentation. Rumen contains a diverse microbial population with over 200 bacteria and 20 protozoa species (McDonald *et al*., 2002). Protozoa can be retained in the rumen, potentially locking up protein and impeding its utilization by the host animal (McDonald *et al*., 2002). Bacteria in the rumen play a crucial role in ruminal fermentation (Kamra, 2005). These bacteria thrive in an acidic environment with a pH range of 5.5 to 7.0, living without oxygen at temperatures around 39-40^{o}C, while relying on a moderate concentration of fermentation products provided by the ingested material from the ruminant (Hungate, 1966). The filtered Rumen fluid contains 1 billion bacteria and 1 million protozoa per ml, with a distribution uneven due to solid digesta (McDonald *et al.*, 2002). According to Hungate (1966), rumen bacteria can range from

16.2 to 40.8 billion per ml. Krause and Oetze (2006) estimate 4 to 88 billion bacteria per ml of rumen content and attributed the differences to factors like diet, feeding schedule, sampling time, and individual animal differences.

According to Tamminga *et al.* (2007), some protozoa play a role in ingesting and digesting various food particles, including bacteria and smaller protozoa, leading to the remodelling of bacterial protein into a higher-quality protein with around 80% biological value. This phenomenon could be advantageous in utilizing rumen content as animal feed.

The digestive system of ruminants comprises specialized compartments with distinct functions. The reticulum and omasum filter feedstuffs, while the abomasum acts as the enzymatic stomach (Niwiska, 2012). Feedstuffs undergo microbial fermentation in the rumen, producing volatile fatty acids, microbial cells, and methane and carbon dioxide (McDonald *et al.,* 2011). The rumen constitutes a complex environment consisting of microbes, feed, gases, and rumen fluid. Microorganisms in the rumen attach to feed particles, forming biofilms that degrade plant material. This process is facilitated by a diverse microbial ecosystem comprising bacteria, ciliate protozoa, anaerobic fungi, and bacteriophages (Kamra, 2005).

Volatile fatty acids are primarily absorbed through the rumen wall, while gases are expelled through eructation. Microbial cells and undigested food components pass to the abomasum and small intestine, where enzymes facilitate digestion. A second phase occurs in the large intestine (McDonald *et al.*, 2011). Undegradable Dietary Protein (UDP) in the lower digestive tract is primarily absorbed as amino acids after enzymatic digestion. Rumen Degradable Protein (RDP) contains a significant portion of nitrogen

(N) and ammonia, which is recycled back to the rumen as urea through saliva and excreted through urine (Mayank and Tanuj, 2008).

2.5.2 Metabolism of Protein in Ruminants

The protein that is absorbed by the gut is called metabolizable protein (MP), and it is made up of microbial protein and protein that resists rumen breakdown. According to Das *et al*. (2014), it serves a variety of functions for animals, including maintenance, growth, foetal development during gestation, and milk production. Both microbial and dietary sources contribute to the protein available to ruminants (Das *et al.*, 2014), with dietary protein, endogenous protein, and microbial protein being the primary sources utilized for maintenance, growth, and production in ruminant animals (McDonald *et al*., 2011).

Ruminants use 70% of their metabolic energy from microbial fermentation of meal components, with microbial proteins providing 90% of amino acids (Niwinska, 2012). Carbohydrates and proteins are converted into intermediate compounds, including sugars and amino acids. Ruminants' diets consist of microbial protein and protein broken down in the rumen. Microorganisms in the rumen synthesize microbial protein by dissolving food protein into peptides, amino acids, and ammonia, which they use to synthesize proteins (Niwiska, 2012). The rumen breaks down protein, producing microbial biomass, carbon dioxide, methane, ammonia, and volatile fatty acids (VFAs). These VFAs, including acetate, propionate, butyrate, and branched-chain VFAs, are absorbed by the host animal in the small intestine. The efficiency and rate of dietary protein degradation by microbes depend on the amount of microbial protein entering the intestine (McDonald *et al.*, 2011). Fermentation in ruminants is influenced by protein, vitamins, and organic acids (Koenig *et al.*, 2003). The small intestine receives

VFAs, digested proteins, lipids, carbohydrates from microbes, and feed residues, supporting animal maintenance and meat/milk production. Choosing the right protein source can affect rumen degradation (Dijkstra *et al*., 2005). In lamb production, using low-degradability animal-based nutrients in the reticulum-rumen is important. These nutrients provide essential amino acids for lamb growth, unlocking their production potential (Ruzic-Muslic *et al.*, 2014).

2.6 Impact of Abattoir Waste on the Environment

The abattoir, which is involved in obtaining edible portions of slaughtered animals for human consumption, produces considerable waste materials comprising organic substances like fat, grease, hair, feathers, undigested feed, as well as processed water and other by-products. This waste accounts for a substantial percentage of the animal's weight, with approximately 35% generated per slaughtered animal (Coker *et al*., 2001; Nafarnda *et al*., 2006). For every 1000 kg carcass weight, 6 kg of manure is generated, and slaughtered beef can yield 100 kg of partially digested feed, excluding rumen or stockyard manure (Coker *et al*., 2001). Countries like Thailand and Ghana have produced significant quantities of dry rumen digesta and other waste materials from their abattoirs (FAO, 2012; Fearon *et al*., 2014), which ultimately find their way into the environment as waste. Disposing of abattoir waste has been a significant challenge, especially in developing countries like Ghana (Fearon *et al.*, 2014). Improper waste disposal practices near abattoirs in Nigeria and Ghana pose significant environmental concerns, including water bodies and air pollution (Weobong, 2001; Adelegan, 2002; Osibanjo and Adie, 2007).

These practices are attributed to inadequate waste recovery and treatment facilities (Adeyemo *et al*., 2009). Research studies have identified abattoir activities as major contributors to surface and underground water pollution, as well as air pollution, with potential indirect impacts on nearby residents' health. A study in Ghana revealed highly polluted effluent water discharged from slaughterhouses exceeding acceptable standards set by the EPA (Weobong and Adinyira, 2011; Fearon *et al*., 2014). This improper discharge of blood and animal faeces into streams can result in the depletion of oxygen levels and the accumulation of excess nutrients, leading to the accumulation of toxins (Nwachukwu *et al*., 2011). Consequently, primary producers in affected water bodies endure direct harm, causing a decline in fish yield and disruptions within the food chain (Islam and Tanaka, 2004). Furthermore, there is an added risk of waterborne diseases and respiratory ailments among humans residing in areas affected by abattoir pollution (Mohammed and Musa, 2012).

2.6.1 Utilization of Rumen Digesta

Rumen digesta, which is the waste material derived from the digestive system of ruminant animals in slaughterhouses, presents a significant challenge in the urban areas of developing nations. This waste consists of partially digested forage primarily located in the rumen, a specialized chamber in the animal's stomach where microbial fermentation takes place (Okere, 2016). Within the rumen, the composition of the digesta is stratified, comprising gases, liquids, and particles that exhibit variations in size, density, and other physical characteristics (Awodun, 2008). The fermentation process within the rumen digesta involves the activity of diverse microorganisms, including bacteria, protozoa, fungi, and archaea (Awodun, 2008).

When a single bovine animal is slaughtered, it is estimated to yield approximately 24.5 kilograms of fresh rumen contents or 3.8 kg of dry matter, with a dry matter content of 15.5% (Muslimah *et al*., 2017). In Owerri, Nigeria, it has been approximated that the collective annual rumen digesta output from cows, sheep, and goats amounts to 2,952,720 kg, equivalent to approximately 295.27 tons (Okere, 2016). If this entire yearly yield were packed into bags weighing 50 kilograms each, the result would be an astonishing 59,054 bags (Okere, 2016). Furthermore, Fearon *et al.* (2014) have estimated that the Tamale metropolis in Ghana produces a substantial quantity of rumen digesta annually, reaching 822,900 tons.

According to Okere's (2016) evaluation of the economic value of rumen digesta generated from slaughterhouses, selling processed rumen digesta at an average market price of ₦600 resulted in a gross annual income of ₦35,432,640.00. The annual handling cost for the digesta was ₦5,905,400.00, leading to an annual profit of ₦29,527,240.00. This total income would be sufficient to employ 681 graduates for one month and support a regular monthly salary of ₦50,000.00 for 49 graduates. The enterprise can offer higher earnings than the Nigerian minimum wage and steady employment for 123 secondary school leavers (Okere, 2016).

Although the majority of rumen digesta produced in slaughterhouses worldwide is typically discarded as waste (Ristianto *et al.*, 2016), it holds great potential as an organic fertilizer to combat soil nutrient depletion, especially in Sub-Saharan Africa (Schobery, 2002; Ristianto *et al.*, 2016). The application of rumen digesta to soil enriches it with additional nutrients (Chinkuyu, 2002; Ekpe, 2012), and researchers have also explored its thermal recycling in power plants (Arvanitoyannis and Ladas, 2008). Moreover, rumen digesta serves as a valuable feed ingredient for both ruminant and non-ruminant animals in various regions (Okere, 2016), offering an alternative source of nutrients to alleviate feed shortages in the livestock industry (Amata, 2014; Adedipe *et al.*, 2005).

Significant efforts are being made to process rumen digesta from slaughterhouses effectively, aiming to enhance its nutritional value and economic significance in livestock production (Amata, 2014). While studies have demonstrated that dried rumen digesta can be used as animal feed at different levels, it is recommended to supplement it with other feed ingredients to ensure a balanced diet (Ra and Iliyasu, 2017; Elfaki *et al.*, 2014; Togun *et al.*, 2010; Osman and Elimam, 2015). Numerous countries, including Cameroon, Egypt, Sudan, Ethiopia, Nigeria, Saudi Arabia, Thailand, and India, have researched the utilization of dried rumen digesta as animal feed (Ra and Iliyasu, 2017).

2.6.2 Rumen Digesta as an Alternative Feed for Livestock

Rumen digesta, a type of livestock waste, can be used as a substitute for forage basal feed in livestock nutrition (Ristianto *et al.*, 2016). Before incorporating rumen digesta into animal feed, it needs to be properly treated, such as through light heat or sun-drying. The processing temperature is important for ensuring the availability of amino acids and other compounds in the digesta (Makinde and Sonaiya, 2007). Yitbarek *et al.* (2016) found that adding dried rumen digesta to animal feed has no adverse effects on growth performance. Nutritionists recognize its nutritional value as a cost-effective feed component (Togun *et al.*, 2010; Elfaki *et al.*, 2014; Osman and Elimam, 2015). Studies show that feeding blood and dried rumen digesta to various species, including lamb, cattle, quail, catfish, and poultry, does not have negative consequences (Osman and Elimam, 2015; Dairo *et al.*, 2005; Mishra *et al.*, 2015).

Dried rumen digesta contains essential crude protein (18.52%), fungus, protozoa, and bacteria (Agbabiaka *et al.*, 2011). Its efficiency and palatability improve when mixed with other feed substances (Esonu *et al.*, 2006). Agbabiaka *et al.* (2011) found that it contains 18.4% ash, 24.81% nitrogen-free extract, 18.58% crude protein, 3.77% crude fat, and 34.44% crude fibre. Its use in cattle feed formulations offers flexibility and reduces environmental risks from abattoir waste. Its use in cattle feed compositions provides flexibility and lowers environmental risks from abattoir waste (**O**skov, 2007).

2.7. Effect of dietary supplementation on the intake of dry matter and nutrient digestibility in ruminants

Dietary supplements are vital for enhancing the nutritional quality of ruminant diets. They provide essential nutrients in small amounts, address deficiencies in soluble nitrogen and minerals, boost protein or energy levels, promote diet intake, and ultimately enhance animal productivity. Popular supplement types include energy concentrates (such as cereal and rice bran), protein concentrates (like soybean meal and groundnut cake), molasses, non-protein nitrogen (such as urea), and minerals (Gatenby, 2002).

To enhance microbial nitrogen in the rumen, feed with crude protein levels below 6% requires supplementation with concentrates (Pathak, 2008). Adult ruminants can maintain their bodies if their feed contains 6-7% crude protein and 50-55% digestibility, but most crop residues fall short of these requirements. To support a healthy rumen ecosystem, meet the animal's needs, and optimize the use of crop residues, it is recommended to provide nutritional supplements that offer fermentable energy, nitrogen, and micronutrients like B vitamins, roughage, bypass protein, and bypass energy (Preston and Leng, 1981).

Ruminants have adapted to utilize low-quality forage for their maintenance, growth, and reproductive needs, thanks to the microbial population in their forestomach that digests fibrous and soluble parts of plants they consume. Supplementing with nitrogen can positively impact the rumen's ecosystem and facilitate the digestion of fibrous portions in animals, particularly since many forages lack nitrogen and are high in fibre content (Matthews et al., 2019). To enhance livestock production, it is necessary to provide feed supplementation. In Ghana, the effectiveness of supplementation for sheep and goats depends on the quality of the natural pasture and the specific supplement utilized (Korir, 2008). The period from January to April is critical for supplementation due to the scarcity of forages and water (Amoko, 2008). Incorporating a feed supplement with a minimum protein content of 7% in poor-quality diets has demonstrated the ability to increase both feed intake and animal productivity (Lazzarini *et al.*, 2009).

Supplementary feeding using readily available agricultural by-products is essential for higher turnover in ruminant livestock production (Shamsuddoha and Edward, 2000). In Ghana, farmers use crop residues, urea-treated straws, agro-industrial by-products, browse plants, and forage tree legumes for supplementation during the dry season (Ansah et al., 2010; Issaka, 2014). However, these methods face limitations due to labour scarcity and insufficient nutritive value of range grasses. Insufficient feedstuff availability also hinders their effectiveness (Makkar, 2003). To improve animal diet quality, it is essential to supplement with resources with higher energy, protein content, or superior digestibility (Wales and Doyle, 2003; Jamie et al., 2009). Concentrates can improve forage or straw intake, while energy or nitrogen supplementation is crucial for animal survival and improved body status (Osredkar and Sustar, 2011).

Various studies have shown positive effects of supplementation on livestock performance. Sheep and goats fed sorghum stover and wheat straw with supplements showed satisfactory results (Todini *et al.*, 2007). Adding legumes to the diet improves growth performance in goats (Marsetyo *et al*., 2017). Additionally, replacing soybean meal with dried rumen digesta resulted in improved straw intake and nutrient digestibility in beef cattle (Cherdthong *et al*., 2014). Similar benefits were observed in Black Bengal goats fed dried rumen digesta (Uddin *et al*., 2018), and sheep fed high levels of concentrate diet (Dessie *et al*., 2010). Overall, supplementation with suitable feedstuffs can enhance livestock productivity, improve weight gain, and optimize feed utilization, leading to better animal performance and higher turnover in livestock production

2.7.1 Effect of dried rumen digesta on feed intake of ruminant animals

Mondal et al. (2013) research on Bengal goats found no significant differences in dry matter and organic matter intake when DRD was included at a 10% level compared to the control diet. Cherdthong *et al.* (2014) studied Thai cattle's diets replacing soybean meal with DRD, finding no significant differences in DM and OM intakes among different DRD levels. However, Salinas-Chavira *et al.* (2007) had a contrasting finding in their study involving Pelibuy×Dorper lambs. They noted a significant increase in DM intake when the lambs were fed a diet containing 4% DRD compared to those on the control diet. Another study by Osman and Abass (2015) explored the effects of feeding Sudan desert lambs at different levels of DRD, namely 0%, 10%, and 20%. The researchers reported a significant increase in DM intake when the lambs were fed a diet comprising 20% DRD, as compared to the groups receiving 0% and 10% DRD.

Olafadehan *et al.* (2014) stated that up to 40% inclusion of DRD in Yankasa lamb diets increased DM and OM intake, while a 60% inclusion level caused a decrease in feed consumption. Meanwhile, Nasser *et al.* (2012) observed a reduction in DM intake in calves fed a 16% DRD diet compared to those fed lower levels of DRD.

In summary, the studies mentioned present varying results regarding the effect of incorporating DRD into animal diets. While some studies found no significant differences in DM and OM intake when DRD was included, others reported increased intake with higher levels of DRD inclusion. Additionally, there were cases where DM intake decreased or feed consumption decreased when higher levels of DRD were fed. The outcomes may vary depending on the species, level of DRD inclusion, and other factors specific to each study.

2.7.2 Effect of dried rumen digesta on nutrient digestibility on ruminants

Al-Wazeer (2016) found that replacing soybean meal with dried rumen digesta did not significantly impact the digestibility of dry matter, organic matter, crude protein, neutral detergent fibre, and acid detergent fibre in lambs. Mondal et al. (2013) found no significant effects on the digestibility of DM, OM, CP, and NDF in Black Bengal goats, and Cherdthong *et al.* (2014) found similar outcomes in cattle diets when DRD was added at different inclusion levels. Fajemisin *et al.* (2010) found that a diet with 25% dried rumen content increased crude protein digestibility in West African Dwarf sheep, but decreased dry matter, neutral detergent fibre, and acid detergent fibre digestibility. Olafadehan *et al.* (2014) found that lambs fed diets with 20% and 40% DRC improved DM, organic matter, and CP digestibility compared to those fed 0% and 60% DRC. However, no significant effects were observed on NDF and ADF digestibility. These findings suggest that the inclusion of DRC in the diet can influence the digestibility of specific nutrients, indicating that the effects of DRC on digestibility may vary depending on the particular nutrient. In a separate study by Rios-Rincon *et al*. (2010), replacing alfalfa hay with DRC in cattle feed led to a decrease in the digestibility of OM and ADF in both the rumen and the entire digestive tract. Suggesting that using DRD instead of alfalfa hay had a negative impact on the digestibility of these nutrients in cattle. In a study by Dey *et al.* (1992), Black Bengal goats were fed diets in which rice straw was replaced with dried rumen contents mixed with molasses. Dey et al. (1992) found a significant increase in nutrient digestibility in goats-fed rumen digesta, affecting dry matter, organic matter, crude protein, crude fibre, neutral detergent fibre, and hemicellulose. The authors concluded that the digestibility of the diets was further enhanced when the rumen contents were either ensiled or ensiled after impregnation with 1% urea. However, Patra and Ghos (1990) study found no significant difference in organic nutrient digestibility coefficients between fed dried rumen digesta and 10% molasses in goats. This suggests that the inclusion of molasses did not have a significant impact on the digestibility of these nutrients in the goats. Ghosh and Dey (1993) investigated the utilization of a mixture of dried rumen digesta (DRD), dried poultry droppings, and urea in Black Bengal goats fed complete rations at different ratios. The authors concluded that this mixture could be well utilized by goats without any significant effect on nutrient digestibility when fed along with a concentrated mixture at a ratio of 50:50.

These studies highlight that the effects of including dried rumen digesta in animal diets can vary depending on the specific composition of the diet and the inclusion of other additives such as molasses or dried poultry droppings. While some studies reported significant improvements in nutrient digestibility with the inclusion of dried rumen digesta, others did not observe significant differences. The overall impact on nutrient digestibility may depend on factors such as the animal species, the specific composition and processing of the diet, and the interactions between different feed components. Overall, the effects of including DRD in animal diets on nutrient digestibility can vary depending on the specific nutrient, animal species, and the level of DRD inclusion.

While some studies reported no significant effects on digestibility, others observed changes in the digestibility of certain nutrients when DRD was included in the diet.

2.7.3 Effect of dried rumen digesta on growth performance of ruminant animals

Mondal *et al.* (2013) and Osman *et al.* (2015) conducted studies with kids and lambs, respectively, and found that including dried rumen digesta up to 10% in the diet did not have any significant impact on the final live weight and average daily gain (ADG) of the animalsOsman and Abass (2015) found that incorporating up to 20% dried rumen digesta in Sudan desert lambs did not affect their final body weight, overall weight gain, or average daily gain (ADG). Salinas-Chavira et al. (2007) found no differences in ADG and feed efficiency among Pelibuy×Dorper lambs fed with dried rumen digesta. Al-Wazeer (2016) found no significant differences in final body weight. The study showed that lambs fed diets containing 10% and 20% dried rumen digesta had similar total gain and ADG compared to those on a control diet with 0% dried rumen digesta. Messermith (1973) found that dried rumen digesta in rations up to 15% did not affect ADG, feed consumption, feed efficiency, or feed conversion. Bolsen *et al*. (1996) found weight gain on control diets similar to those fed different types of ensiled digesta. Abouheif *et al.* (1999) evaluated the effect of dried rumen digesta on dietary inclusion and found similar final body weight gain.

Olafadehan *et al.* (2014) observed that body weight gain and ADG increased in Yankasa lambs with higher dried rumen content (DRC) but decreased at 60% DRC. Fajemisin *et al.* (2010) reported no effect of replacing cassava peels with DRD on ADG in West African Dwarf sheep. Limited information exists on pelleting dried rumen digesta effect on feed intake and digestibility, and further research is needed. Overall, the findings suggest that the inclusion of dried rumen digesta in animal diets can have varying effects on growth performance, depending on the species, level of inclusion, and other factors specific to each study.

In conclusion, while the studies discussed provide valuable insights into the effects of dried rumen digesta on growth performance, it is important to critically assess their findings. The variation in results across studies emphasizes the need for additional research, considering different animal species, levels of inclusion, and experimental conditions. By addressing these gaps, future studies can contribute to a more robust understanding of the effect of dried rumen digesta on animal growth and performance.

2.7.4 Haematological and Blood Biochemical Components of Ruminants

Blood serves as a vital indicator of both physiological and pathological changes within an organism (Erhunmwunse and Ainerua, 2013). Its primary role involves the transportation of oxygen from respiratory organs to body cells, thereby facilitating the distribution of essential nutrients and enzymes to cells while simultaneously eliminating waste products (Zaccone *et al.*, 2006; Slaker and Suverton, 1982). This intricate process plays a crucial role in maintaining the internal environment's homeostasis (Bentrick, 1974). To execute these diverse functions, blood relies on its constituents, namely the haematological and biochemical components, which work individually and collectively (Akinmutimi, 2004).

The composition of an animal's diet, including both the amount and quality of the food, as well as the presence of anti-nutritional elements, can have an impact on both the haematological (related to blood) and biochemical components of the blood (Akinmutimu, 2004). The biochemical components, in particular, are highly responsive to the presence of potentially harmful elements present in the feed. Esonu *et al*. (2006) have emphasized that haematological parameters play a significant role in reflecting the physiological condition of an animal, offering valuable information about its response to different physiological circumstances. Furthermore, it has been widely observed by researchers that the blood cell profile undergoes distinct changes throughout an animal's lifespan (Khan and Zafar, 2005). These changes are indicative of the natural physiological development and maturation processes occurring within the animal's body.

2.8.0 INFERENCES FROM LITERATURE REVIEW

- Livestock production by smallholder farmers in sub-Saharan Africa has substantial economic and socio-cultural significance.
- Traditional livestock systems and challenges related to feed availability during the dry season impact animal health and productivity.
- The high costs and limited availability of protein sources pose challenges in ruminant production.
- Abattoir waste disposal poses significant environmental and health challenges in many developing countries.
- Rumen digesta, if properly processed and utilized, holds the potential for economic and agricultural benefits, including income generation, employment opportunities, soil fertility improvement, and alternative fuel sources.
- Dried rumen digesta offers a viable and cost-effective option as a feed ingredient, contributing to livestock nutrition while addressing waste management concerns.
- Dried rumen digesta inclusion in feed formulations can provide benefits for both animal performance and environmental sustainability.
- Supplementing ruminant diets with appropriate feedstuffs is essential to optimize animal performance, meet nutritional requirements, and overcome the limitations of basal diets, especially during periods of scarcity.

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Experimental site

Two sites in Ghana: the Forage Evaluation Unit (FEU) of the University for Development Studies in Nyankpala, Tamale and the Ecological Agriculture Laboratory at the Bolgatanga Technical University (BTU) in Sumbrungu, Bolgatanga were used for the chemical composition and *in vitro* gas analysis.

3.2 Material Collection and Processing

Data collection was divided into four seasons for the rumen digesta. The seasons were as follows: early wet (May July 2021), main wet (August-October 2021), early dry (November 2021-January 2022), and late dry (February-April 2022).

Rumen digesta from cattle was obtained from the Bolgatanga abattoir. The rumen digesta was collected from cattle examined by veterinary staff to ensure that they were healthy. The rumen digesta were carefully collected into containers (Plate 1) and transported to the experimental site, where they were placed in a sack and tied. A weight was placed on it for 3 hours to expel the liquid (Plate 2). This reduced the moisture content in the rumen digesta and divided it into four equal portions for processing.

Plate 1: Collecting rumen digesta into containers

Plate 2: Expelling water out of the fresh digesta

3.2.1 Open sun-drying

After squeezing out fluid the fresh digesta was open-sun-dried by spreading it on polythene sheets for 3 days under the sun (Plate 3).

Plate 3: Sun drying the digesta

3.2.2 Oven drying

After water in the rumen digesta was squeezed out of the fresh digesta 20 kg was placed in an electric oven and dried at a constant temperature of 60 C for 48 hours in the laboratory (Plate 4).

Plate 4: Oven drying the digesta

3.2.3 Fermentation

After the water was initially squeezed out of the fresh digesta, 20 kg of the rumen digesta was packed and sealed in polythene bags for fourteen (14) days (Plate 5). The fermented product was further sun-dried for 3 days by spreading it on polythene sheets under the sun.

Plate 5: Fermentation of digesta

3.2.4 Urea fermentation

After the water was initially squeezed out of the fresh digesta, 20 kg was thoroughly mixed with 100 g urea fertilizer packed and sealed in polythene bags for fourteen (14) days as in plate 5. The fermented product was further sun-dried for 3 days by spreading it on polythene sheets.

3.4 Proximate analysis

The dried rumen digesta was ground in a centrifugal mill and passed through a 1 mm sieve (Retseh GmbH, Hann, Germany) for chemical analysis and *in vitro* gas production. Dried rumen digesta (DRD) was analysed for ash and crude protein (CP) using the procedures of the AOAC (2000).

3.4.1 Dry matter determination

Two grams of each of the samples were weighed into a previously oven-dried crucible and in a vacuum oven (FISTREEM, OVA031.XX3.5, UK) at 60ºC for 48 hours (AOAC, 2000).

Dry matter was calculated as dry matter (g/kg) = $\left(\frac{Dry\ sample\ weight}{Wet\ sample\ weight}\right) * 1000$ = Equation 3.1

3.4.2 Ash Determination

Ash was determined according to the procedure of AOAC (2000). Approximately 2 g of dried sample was weighed into a preheated cooled crucible and heated to 550°C in a muffle furnace (Carfbolite Gero, CWF 1100, UK) for 4 hours. Samples were allowed to cool in a desiccator and reweighted.

The ash content was calculated as $\left(\frac{weight\ of\ ash}{Weight\ \ of\ sample}\right) * 1000$ = Equation 3.2

The organic matter (g/kg DM) was calculated as 1000 - ash = Equation 3.3

After heating, the crucible was removed and the sample was allowed to cool in a desiccator to room temperature, ensuring minimal moisture absorption. The cooled samples were reweighed meticulously using precise techniques. By comparing the weight before and after heating, the ash content of the sample was accurately determined. This adherence to the AOAC procedure ensured reliable and precise results, contributing to a comprehensive understanding of the sample's composition.

The ash content was calculated as $\left(\frac{weight\ of\ ash}{Weight\ \ of\ sample}\right) * 1000$ = Equation 3.2

The organic matter (g/kg DM) was calculated as 1000 - ash = Equation 3.3

3.4.3 Crude protein

The crude protein content of the samples was determined according to the method of AOAC (2000). A gram (1g) of each dried sample was weighed and placed into the Kjeldahl digestion tube and blank determination was done by digesting filter paper in each set of digestion. Approximately 15 ml of concentrated sulphuric acid (H_2SO_4) and two Kjeldahl tabs were added to the content of each digestion tube. The Kjeldahl tabs contained potassium sulphate (K_2SO_4) and copper sulphate ($CuSO_4$) which increase the boiling point and acted as catalysts respectively. The tubes were mounted on the Kjeldahl digestion block with a fume exhaust set (J.P Selecta) heated gradually to

420°C and maintained for 3 hours. The tubes were removed and allowed to cool to room temperature after which, 50mls of distilled water was added and distilled using an automated Kjeldahl distillation apparatus (Pro-Nitro, J.P. Selecta, s.a Spain). The apparatus draws 50mls of previously prepared 355 sodium hydroxide (35% NaOH) into the digestion tubes and 25 ml of 4% Boric acid (4% H3BO3) into 25mls Erlynmeyer flasks to trap the liberated ammonia during the distillation period of 9 min per sample. The distillate was collected and titrated against 0.1N HCL (hydrochloric acid). The average titre values were recorded and the percentage nitrogen (%N), as well as the per cent crude protein (% CP), were calculated using the formula: % Nitrogen = (T-B) × N × 1.4/weight of the sample (g) % crude protein = % nitrogen × 6.25

Where:

T – Sample titre value

B – Blank titre value

N – Concentration of HCL

3.5 *In vitro* Gas digestion

The dried rumen digesta was ground in a centrifugal mill and passed through a 1 mm sieve (Retseh GmbH, Hann, Germany) for *in vitro* gas production. The technique and procedure of Theodorou *et al*. (1994) were adopted for the *in vitro* gas production at 24 h and 72 h. Dried rumen digesta samples (200 mg) were incubated in 50 ml test tubes containing buffered rumen fluid under anaerobic conditions. Fresh rumen fluid was obtained from slaughtered cattle at the Bolgatanga Abattoir and filtered under continuous flushing with carbon dioxide. The filtered rumen fluid was then mixed with McDougall's solution.

The gas reading was then fitted to the exponential curve of Orskov and McDonald (1979) without an intercept using GraphPad Prism 7.9 edition. The degradation parameters (b and c) were derived from the exponential model: $Y = b\ (1 - exp^{-ct})$

Where:

Y = gas volume at time t (ml/200 mg)

b = asymptote gas production (ml/200 mg)

t = time (h)

c = fractional rate of gas production (ml/h)'.

3.6 Assessment of microbial load in both sole-dried Rumen Digesta (DRD) and DRD-based concentrate.

The sole DRD and DRD-based concentrates were analysed for total microbial load, lactic acid bacteria, *Escherichia coli* and *Salmonella species*. Enumeration of microbial load and lactic acid bacteria was done using a modified method as described by Maturin and Peeler (2001) and Adzitey *et al*. (2019). Briefly, 10 g of each diet was added to 90 ml of 1% Buffered Peptone Water (BPW) to obtain the 'Neat'. Serial dilutions (10^{-1}-10^{-5}) were made in 9 ml BPW using 1 ml of the 'Neat'. After which, 100 ul of each serially diluted aliquot were spread plated unto duplicate Plate Count Agar (PCA) and de Man, Rogosa and Sharpe (MRS) plates, for microbial load and lactic acid bacteria, respectively. The plates were then incubated at 37^{o}C for 24 h and colonies were counted using a colony counter.

3.8 Statistical Analysis

The data obtained from the study were analysed using a one and two-way analysis of variance (ANOVA) in GenStat (18.2 edition). A significance level of 5% was considered, and any observed differences were deemed statistically significant. To further examine the differences between means, the Tukey test was applied to separate and compare the individual means. This post-hoc test allows for a comprehensive comparison of the means and helps identify significant differences among the groups.

CHAPTER FOUR

4.0 EXPERIMENT 1: EFFECT OF SEASON AND PROCESSING METHODS ON CHEMICAL COMPOSITIONS, MICROBIAL QUALITY (Lactic acid bacteria, *Salmonella* and *E. coli*) AND *IN VITRO* GAS PRODUCTION OF DRIED RUMEN DIGESTA

4.1.0 Introduction

This study aimed to investigate the variations in chemical composition and microbial quality, including *lactic acid bacteria*, *Salmonella*, and *E. coli*, of dried rumen digesta under different seasons and processing methods. Several authors have highlighted the presence of significant levels of crude protein in dried rumen digesta, along with other microorganisms such as protozoa, fungi, and lactic acid bacteria. These findings have emphasized the potential of dried rumen digesta as a valuable feed source for livestock, particularly ruminants (Adeniji and Balogun, 2002; Dairo *et al.*, 2005; Esonu *et al.*, 2006; Agbabiaka *et al.*, 2011; Sakaba *et al.*, 2017; Agolisi *et al.*, 2020; Agolisi *et al.*, 2022).

The nutrient composition of dried rumen digesta can vary depending on several factors, including the quality and diversity of the consumed herbage, the population and activity of rumen microorganisms, the season, the processing method employed, and the duration between forage ingestion and animal slaughter (Sakaba *et al.*, 2017). The level of heat applied during processing plays a crucial role in determining the availability of amino acids and other compounds within the digesta (Makinde and Sonaiya, 2007). However, limited research has been conducted to explore the relationship between season, processing methods, and the nutritive value of dried rumen digesta, especially within the context of Ghana.

Therefore, this experiment aims to bridge this knowledge gap by investigating the influence of season and processing methods on the nutritive value and microbial quality of dried rumen digesta.

4.1.1 Objectives

The objectives of this experiment were to:

The objectives of this experiment were to determine the:

- Chemical composition (CP, EE, Ash, NDF and ADF) of dried rumen digesta affected by season and processing methods
- *In vitro* gas production of dried rumen digesta affected by season and processing methods
- Microbial quality (Total microbial count, Lactic acid bacteria, *E. coli* and *Salmonella spp.*) of dried rumen digesta affected by processing methods.

4.1.2 Hypothesis

1. Ho = the proximate composition, microbial quality and *in vitro* gas production of dried rumen digesta will not differ with seasons and methods of processing.
2. Ha = the proximate composition, microbial quality and *in vitro* gas production of dried rumen digesta will differ with seasons and methods of processing.

4.2.0 Materials and Methods

4.2.1 Location and experimental design

The study was conducted in two separate places: the Forage Evaluation Unit (FEU) at the University for Development Studies in Nyankpala, Tamale, Ghana, and the Ecological Agriculture Laboratory at the Bolgatanga Technical University, Bolgatanga, Ghana, respectively. The study adopted a 4*4 factorial layout and used a completely randomized design. Seasons (4) and processing techniques (4) were the deciding considerations. The digesta was processed using four different techniques: open-air drying in the sun, drying in an oven, fermentation with urea, and fermentation without urea.

4.2.2 Material Collection and Processing

The collection of rumen digesta was done across four distinct seasons, namely early wet (May-July 2021), main wet (August-October 2021), early dry (November 2021-January 2022), and late dry (February-April 2022), as outlined by Konlan *et al*. (2017). The rumen digesta was obtained from cattle at the Bolgatanga abattoir as outlined in subsection 3.2, plate 1. The rumen digesta was collected from cattle examined by veterinary staff to ensure that they were healthy. After draining the fluid from the digesta it was then divided into four equal portions for further processing, with each portion assigned to each of the specified treatments as outlined in subsection 3.2.

4.2.3 Chemical analyses

The dried rumen digesta was ground in a centrifugal mill and passed through a 1 mm sieve (Retseh GmbH, Hann, Germany) for chemical analysis and *in vitro* gas production. Dried rumen digesta was analysed for ash and crude protein (CP) using the procedures of the AOAC (2000). The Kjeldahl method was used to obtain the nitrogen concentration multiplied by 6.25 to get the crude protein. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the addition of sodium sulphite and alpha-amylase (Van Soest, 1991) using the Ankom^{200} fibre analyser. All nutrient composition results were reported on a dry matter basis.

4.2.4 Method for in vitro gas production

The procedure of Theodorou *et al*. (1994) was adopted for the *in vitro* gas production at 24 h and 72 h. Dried rumen digesta samples (200 mg) were incubated in 50 ml test tubes.

The McDougall salivary buffer was prepared a day before the incubation. McDougall's salivary buffer solution was prepared from solutions A and B. Solution A was made by dissolving 19.60 g $NaHCO_3$, 9.28 g $Na_2HPO_4 \cdot 2H_2O$, 1.14 g KCl, 0.94 g NaCl and 0.26 g of $MgCl \cdot 6H_2O$ in 2 L of distilled water. Solution B was made by dissolving 2.65 g of $CaCl_2 \cdot 2H_2O$ in 50 ml of distilled water. A complete salivary buffer was prepared by adding 2 ml of solution B to solution A, which was then warmed to 39 ^{C} with continuous stirring and flushing with carbon dioxide (CO_2) and samples were incubated.

Fresh rumen fluid was obtained from slaughtered cattle at the Bolgatanga Abattoir. The rumen fluid was placed into a thermos flask that was pre-warmed to a temperature of 39 ºC after the animals had been slaughtered and rumen taken out. The rumen fluid was filtered through a four-layer cheesecloth whilst being warmed at 39 ºC and then mixed with McDougall's buffer in a ratio of 1:4 (1 part of rumen fluid, 4 parts of buffer) under continuous flushing with carbon dioxide (Ansah *et al*., 2018). 30 ml of the buffer and rumen fluid was dispensed into the test tube containing the feed sample. They were then placed in a water bath (Clifton, England) set to a temperature of 39 ºC. The pressure as a result of fermentation in the incubation tubes was measured using a digital manometer over 48 hours at regular intervals.

The IVDOM was computed from the 24-hour gas production using the equation:

IVDOM (%) = 14.88 + 0.8893 GP + 0.0448 CP + 0.651% Ash (Menke, 1988).

The metabolizable energy (ME) for the DRD concentrate was estimated using the concentrate equation:

ME (MJ/Kg DM) = 1.06 GP + 0.157 CP + 0.22 CF 0.081 Ash (Menke, 1988).

Where:

GP = 24-hour in vitro gas production

CP stands for crude protein.

CF stands for crude fat.

SCFA (mmol) = 0.0239 * GP * 0.0601

Where CP = crude protein

The gas reading was then fitted to the exponential curve of Orskov and McDonald (1979) without an intercept using GraphPad Prism 7.9 edition. The degradation parameters (b and c) were derived from the exponential model: $Y = b(1 - exp^{-ct})$, where, Y = gas volume at time t (ml/ 200 mg), b = asymptote gas production (ml/ 200 mg), t = time (h), c = fractional rate of gas production (ml/h).

4.2.5 Microbial load analysis of rumen digesta

The fresh and processed rumen digesta were analysed for total microbial load, lactic acid bacteria, *Escherichia coli* and *Salmonella species*. Enumeration of microbial load and lactic acid bacteria was done using a modified method as described by Maturin and Peeler (2001), Andrews *et al*. (2018), Adzitey *et al*. (2019) and Feng *et al*. (2020). Briefly, 10 g of each diet was added to 90 ml of 1% Buffered Peptone Water (BPW) to obtain the 'Neat'. Serial dilutions (10^{-1}-10^{-5}) were made in 9 ml BPW using 1 ml of the

'Neat'. After which, 100 µl of each serially diluted aliquot were spread plated unto duplicate Plate Count Agar (PCA), de Man, Rogosa and Sharpe (MRS), Salmonella Shigella (SS) and Levine Eosin Methylene Blue (LEMB) plates for microbial load, lactic acid bacteria, *Salmonella species* and *E. coli*, respectively. The plates were then incubated at 37^{o}C for 24 h and colonies were counted using a colony counter.

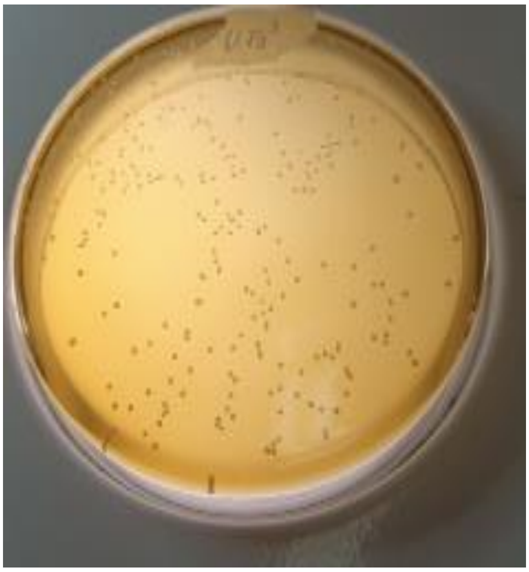

Plate 7: Lactic acid bacteria

Plate 6: E. coli

4.2.6 Statistical analysis

The data was subjected to statistical analysis of variance using GenStat 18.2 edition with the following model: $y_{ijk} = \mu + \alpha_i + \beta_i + (\alpha + \beta)ij + \varepsilon_{ijk}$ where Yijk: observed variation, µ: population means α_i: effect of season, βi: effect of processing method; α+β interaction effect of season and processing method and εijk: error. Significant differences among treatment means were tested by using the Bonferroni test at 5%.

4.3 Results

The chemical composition of the rumen digesta evaluated at different seasons and processing methods is shown in Table 4.1. Season and processing methods had a significant influence interaction effect on dry matter, crude protein, ether extract, ash, NDF and ADF. The dry matter ranged between 95.63 and 97.88.0%. The late dry season (LDS) rumen digesta (97.88%) and oven-dried method had the highest DM (97.50%).

Table 4.1: Effect of season and processing method on proximate compositions of rumen digesta

Factors		Parameters (%/gDM)					
		DM	CP	ASH	EE	NDF	ADF
Season	Early Wet	95.63^{c}	17.58^{b}	18.50^{a}	6.18^{c}	72.90^{a}	47.63^{b}
	Main Wet	96.25^{b}	22.26^{a}	14.50^{b}	7.81^{a}	64.26^{c}	37.4^{d}
	Early Dry	97.87^{a}	15.25^{c}	12.78^{c}	5.59^{d}	70.13^{b}	42.2^{c}
	Late Dry	97.88^{a}	8.42^{d}	12.75^{c}	7.42^{b}	74.75^{a}	53.73^{a}
	S.e.d	*0.198*	*0.053*	*0.2165*	*0.099*	*0.085*	*0.155*
	p. value	*<.001*	*<.001*	*<.001*	*<.001*	*<.001*	*<.001*
Method	Sun-Dried	96.75^{bc}	14.72^{b}	13.50^{c}	5.89^{d}	70.48b	45.65^{c}
	Oven-Dried	97.50^{a}	14.36^{c}	15.88^{a}	6.44^{c}	73,43^{a}	46.33^{b}
	Fermented	97.13^{ab}	13.96^{d}	14.75^{b}	6.96^{b}	73.58^{a}	47.10^{a}
	Urea-Fermented	96.25^{c}	20.45^{a}	14.38^{b}	7.70^{a}	64.58^{c}	41.88^{c}
	S.e.d	*0.198*	*0.053*	*0.2165*	*0.099*	*0.085*	*0.155*
	p. value	*<.001*	*<.001*	*<.001*	*<.001*	*<.001*	*<.001*
m×s	*S.e.d*	*0.395*	*0.106*	*0.4330*	*0.199*	*0.17*	*0.31*
	p. value	*<.001*	*<.001*	*<.001*	*<.001*	*<.001*	*<.001*

DM = dry matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre. ^{a,b,c,d} *Means within the same column with different superscripts are significantly different (p< 0.05).*

The CP ranged from 8.42 to 22.26%. The highest crude protein content in the rumen digesta was observed in the main wet season (22.26%) and the urea-fermentation (20.45%) method. The ether extract (EE) ranged between 5.59 and 7.81% for the sun-dried method and the main wet season respectively. Generally, the late dry season and fermented method had the highest NDF and ADF concentrations.

Table 4. 2: effect of season and processing method on *in vitro* gas production profile of rumen digesta

Factors		Parameters (%/gDM)				
		IVDOM (% DM)	B (ml/)	c (ml/h)	SCFA (mmol/l)	ME (g/DM)
Season	Early Wet	43.79^{a}	8.97ab	0.12^{a}	0.148	3.78^{b}
	Main Wet	40.04^{b}	9.53^{a}	0.11^{a}	0.149	4.85^{a}
	Early Dry	39.32^{b}	8.06^{b}	0.16^{a}	0.129	3.78^{b}
	Late Dry	37.19^{c}	9.91^{a}	0.09^{b}	0.194	4.04^{b}
	S.e.d	0.975	*0.314*	0.013	*0.013*	0.141
	p. value	*<.001*	*<.001*	*<.001*	0.003	*<.001*
Method	Sun-Dried	39.19^{b}	8.46^{b}	0.14^{a}	0.151	3.89^{b}
	Oven-Dried	42.53^{a}	8.48^{b}	0.13^{a}	0.134	3.67^{b}
	Fermented	41.33^{ab}	9.12^{ab}	0.16^{a}	0.148	3.94^{b}
	Urea-Fermented	43.45^{a}	10.39^{a}	0.06^{b}	0.188	4.94^{a}
	S.e.d	0.975	*0.314*	0.013	*0.013*	0.141
	p. value	*<.001*	*0.003*	*<.001*	0.013	*<.001*
m×s	*S.e.d*	1.949	*0.628*	*0.026*	*0.023*	0.282
	p. value	0.051	*0.339*	*<.001*	0.395	0.015

VDOM = digestible organic matter, SCFA= short-chain fatty acid, ME = metabolizable energy. *^{a,b,c,d} Means within the same column with different superscripts are significantly different ($p< 0.05$).*

Table 4.2 shows the results on the IVDOM, asymptote gas production (b), rate of degradation (c) and short-chain fatty acids (SCFA) of rumen digesta at different seasons and processing methods. There was a significant main effect of season and processing method on IVDOM, rate of degradation and SCFA. The main wet season rumen digesta had the highest IVDOM (40.04%) with the late dry season samples recording the least (37.19%). The fastest rate of gas production (0.09 ml/hr) was recorded in the late dry season and urea-fermented method (0.06 ml/hr) of the rumen digesta.

Table 4.3: Effect of processing method on Microbial population of dried rumen digest

Parameter (log cfu/g)	Fresh rumen digesta	Fermented rumen digesta	Oven dried rumen digesta	Sun dried rumen digesta	Urea fermented rumen digesta
Total microbial count	12.64	6.23	6.26	6.29	6.03
Lactic acid bacteria	8.06	4.88	4.96	5.11	4.64
Salmonella spp.	4.51	0.00	0.00	1.47	0.00
E. coli	6.62	0.94	0.00	2.20	0.00

The fresh rumen digesta had an average total microbial count of 12.64 log cfu/g but after processing, this was reduced by about 50% in all the processing methods (Table 4.3). The similar trend was observed in the other parameters. *Salmonella spp* was reduced by 100% in the fermented, oven dried and urea-fermented rumen digesta whilst oven-dried and urea fermented recorded a 100% reduction in *E. coli*.

4.4 Discussion

Variations in seasons and processing methods relative to the chemical composition in the current study are attributed to the nutritive quality of the forage ingested, microorganism reaction and population in the rumen and length of time the animal takes before slaughter after consuming the forage. In this study, season and processing methods significantly influenced the crude protein content of the rumen digesta.

The processing method significantly enhanced the crude protein content of the processed rumen digesta. An average CP of 15.86% was obtained for the DRD for the entire period under study which was above the 12% CP recommended for sheep growth and maintenance (NRC, 2007). The average CP of 15.86% was slightly higher than the

12.8%, 11.4%, 12.57% and 14.01% CP observed by Froidmont (2004), Togun *et al*. (2010), Mondal *et al*. (2013) and Agolisi *et al*. (2022), respectively. The growth of fermentation microbes during the processing methods may have accounted for the higher CP in those treatments compared to the drying methods. Generally, the urea-fermented method improved the NDF and ADF content.

Rumen digesta has been found by previous authors to contain a high concentration of fibre and semi-fermented feed ingredients (Al-Wazeer, 2016). Higher ADF concentration tends to lower nutrient digestibility and feed intake. Cell wall fractions of forage affect feed degradation in the rumen due to the indigestible nature of lignin that acts as a barrier limiting access of microbial enzymes to the structural polysaccharides of the wall. According to Mathison *et al.* (1999) and Agbagla-Dohnani *et al*. (2001), environmental, seasonal effects and proportion of morphological fractions namely stem, leaf and seed ratios affect degradability. The seasonal effect on the nutrient composition can be explained by the high consumption of low-quality forage in the dry season periods. The nutrient composition of dry season feed resources has been reported to contain low protein and high cell wall carbohydrates (Konlan *et al.*, 2018).

In vitro gas production, which is an indirect measure of microbial fermentation of feed and digestibility, was consistently higher in the urea-fermented method in all seasons. This is a result of the urea application which breaks down the recalcitrant cell wall components of the rumen digesta. Generally, the IVDOM and asymptote gas production were high in the urea-fermented processing method compared to the sun, oven and fermented methods. Higher gas production from microbial fermentation with a corresponding lower digestible organic matter suggests a potential efficiency in the digestibility of the rumen digesta in this study. The high crude protein content in the urea-fermented method could have supplied the cellulolytic microbes with the needed degradable protein to digest the dry matter. The low IVDOM and asymptote gas production (b) in the sun and oven drying methods are attributable to the cell walls in the rumen digesta since the digesta is partially digested forage in the rumen. Lignin acts as a barrier, limiting access of rumen microbes to the structural polysaccharides during rumen fermentation (Agbagla-Dohnani *et al*., 2001). According to Ammar *et al*. (2000), fibre levels are negatively correlated with *in vitro* digestibility.

Rumen microorganisms play different roles in feed digestion and fermentation of plant structure and non-structural nutrients (Durand and Ossa, 2014). In this study, the total microbial load for the fresh rumen digesta was lower than the 17.8 log cfu/g reported by Mondal *et al.* (2013) for dried rumen digesta but lower than the figure recorded by Agolisi et al. (2022) in their earlier study for rumen digesta. The low LAB recorded in the urea-fermented process is attributed to the inclusion of the urea which suppressed the growth of lactic acid bacteria through its buffering capacity. The presence of LAB in diets is expected to improve gut health, digestion and fermentation (Pessione, 2012). Except for the sun-dried method, all the processing methods recorded no presence of Salmonella spp. Escherichia coli was found in the fermented and sun-dried methods but absent in the oven-dried and urea fermented. This may be attributed to the high temperature in the processing methods.

The *in vitro* gas production method is an important technique used to indirectly estimate microbial fermentation of feed and its digestibility. In the present study, the observed gas production consistently exhibited higher values in the urea-fermented method, regardless of the season. These findings shed light on the potential benefits of the urea-fermented method as a viable option for enhancing microbial fermentation and improving feed digestibility, highlighting its relevance in optimizing animal diets and promoting sustainable livestock production.

Generally, the IVDOM and asymptote gas production were higher in the urea-fermented processing method compared to the sun, oven and fermented methods. The higher gas production resulting from microbial fermentation, coupled with the presence of highly digestible organic matter, indicates a potential improvement in the digestibility of the rumen digesta observed in this study. The high crude protein content in the urea-fermented method may have provided the necessary degradable protein to support the activity of cellulolytic microbes, facilitating the digestion of the dry matter. Conversely, the lower gas production and asymptote values in the sun and oven drying methods can be attributed to the presence of cell walls in the rumen digesta, which act as a barrier, limiting the access of rumen microbes to the structural polysaccharides during fermentation (Agbagla-Dohnani *et al*., 2001). The level of fibre in the rumen digesta negatively correlates with *in vitro* digestibility (Ammar *et al*., 2000).

Rumen microorganisms play different roles in feed digestion and fermentation of plant structure and non-structural nutrients (Durand and Ossa, 2014). In this study, the total microbial load for the fresh rumen digesta was lower than the 17.8 log cfu/g reported by Mondal et al. (2013) for dried rumen digesta. Similarly, it was lower than the figure reported by Agolisi et al. (2022) in their earlier study for rumen digesta. The low LAB recorded in the urea-fermented process is attributed to the inclusion of the urea which suppressed the growth of lactic acid bacteria through its buffering capacity. The presence of *lactic acid bacteria* in diets is expected to improve gut health, digestion and fermentation (Pessione, 2012). Except for the sun-dried method, all the processing methods recorded no presence of Salmonella spp. Escherichia coli was found in the fermented and sun-dried methods but absent in the oven-dried and urea fermented. This may be attributed to the high temperature in the processing methods.

4.5 Conclusion

This study evaluated the effects of season and processing methods on the chemical composition and *in vitro* gas production, the microbial load of fresh and processed rumen digesta. The crude protein of urea-fermented rumen digesta increased by almost 100% compared to sun-dried rumen digesta. Crude protein content and IVDOM of the rumen digesta were adversely affected by season. The total microbial count was reduced by about 50% with the application of the processing methods. *Salmonella spp.* and *E. coli* were reduced following the introduction of the different processing methods.

CHAPTER FIVE

5.0 EXPERIMENT 2: CHEMICAL COMPOSITION AND MICROBIAL QUALITY OF PELLET-DRIED RUMEN DIGESTA CONCENTRATES

5.1.0 Introduction

It was confirmed from Experiment 1 that the urea-fermented method was effective in improving the nutritional and microbial quality of the dried rumen digesta in the samples evaluated. Hence the need for Experiment II to determine the effect of pelleting urea-fermented dried rumen digesta on the chemical composition and microbial quality of dried rumen digesta concentrate.

Rumen digesta, despite its potential as a feed resource, is not commonly used in livestock feeding due to several limitations. It is characterized by low palatability, high moisture content, and the presence of indigestible fibre, making it less appealing for animals to consume (Khattah *et al.*, 2011; Mabrouk *et al.*, 2016). To address these challenges and enhance its usability, various processing methods have been employed. Processing methods such as oven drying, sun drying, and mixing rumen digesta with substances like blood, barley grain, and molasses have been used to reduce the limitations associated with its use (Khan *et al.*, 2014). These methods aim to improve the palatability and nutrient composition of rumen digesta, making it more suitable for inclusion in animal diets.

By incorporating rumen digesta into complete diets and processing them into pellets, the palatability and acceptance of this feed resource can be significantly enhanced (Seankamsorn and Cherdthong, 2019). Pelleting offers several advantages, such as reducing ingredient segregation, improving ease of handling, ensuring better flow in feeding equipment, and enabling cost-effective formulation by incorporating alternative ingredients (Behnke, 1994; Fairfield, 2003). Therefore, pelleting can be considered a viable approach for improving the palatability and utilization of dried rumen digesta.

5.1.1 Objectives

- To determine the effect of urea-fermented dried rumen digesta pellet diet at different inclusion levels (0, 5, 10 and 15%) on the chemical composition.
- To determine the effect of urea-fermented dried rumen digesta pellet diet at different inclusion levels (0, 5, 10 and 15%) on microbial quality.

5.1.2 Hypothesis

H_O: Chemical composition and microbial population will not differ among the two (2) processing methods on dried rumen digesta concentrate when included at different inclusion levels.

H_A: Chemical composition and microbial population will differ among the two (2) processing methods on dried rumen digesta concentrate when included at different inclusion levels.

5.2.0 Materials and Methods

5.2.1 Experimental design

The experimental design was 2^4 factorial design; 2 processing methods (pellet and un-pelleted DRD) and 4 inclusion levels (0, 5, 10 and 15%) in a completely randomized design.

5.2.2 Experimental samples

The maize bran, cassava peels and rice bran were purchased from the livestock feed market, Bolgatanga in the Upper East region.

The treated rumen digesta was prepared as outlined in Experiment 1 (section 4.2). Previously, urea-fermented dried rumen digesta was ground (and sieved through 1 mm using a centrifugal mill, Retseh 200 GmbH, Hann, Germany). The ground urea-fermented DRD was mixed with other ingredients in levels at 0, 5, 10 and 15% (Table 1) and divided into two portions. One portion of the formulated diets was left in the form and labelled mash, while the other portion was pelleted using a machine (figure 8) and also labelled pellet.

Plate 8: Pelleting the formulated diet

Table 5.1: Ingredients for the experimental diet

	DRD inclusion levels (%)			
Ingredients (% As fed basis)	0	5	10	15
Maize bran	65	60	55	50
Cassava peels	13.5	13.5	13.5	13.5
Rice bran	15	15	15	15
Shea nut cake	5	5	5	5
Urea-fermented DRD	0	5	10	15
Premix	1	1	1	1
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100

The sole Urea-fermented DRD and Urea-fermented DRD-based concentrates were analysed for total microbial load, lactic acid bacteria, *Escherichia coli* and *Salmonella species*. Enumeration of microbial load and lactic acid bacteria was done using a modified method as described by Maturin and Peeler (2001) and Adzitey *et al*. (2019). Briefly, 10 g of each diet was added to 90 ml of 1% Buffered Peptone Water (BPW) to obtain the 'Neat'. Serial dilutions (10^{-1}-10^{-5}) were made in 9 ml BPW using 1 ml of the 'Neat'. After which, 100 ul of each serially diluted aliquot were spread plated unto duplicate Plate Count Agar (PCA) and de Man, Rogosa and Sharpe (MRS) plates, for microbial load and lactic acid bacteria, respectively. The plates were then incubated at 37^{o}C for 24 h and colonies were counted using a colony counter.

5.2.3 Statistical analysis

The data was subjected to statistical analysis of variance using GenStat 18.2 edition with the following model: $y_{ijk} = \mu + \alpha_i + \beta_i + (\alpha + \beta)ij + \varepsilon_{ijk}$ where Yijk: observed variation, μ: population means α_i: effect of season, βi: effect of processing method; α+β interaction effect of season and processing method and εijk: error. Means were compared using the Bonferroni test at 5% (P<0.05) least significant differences.

5.4 Results

The dried rumen digesta (DRD) concentrate, which was processed using various techniques and inclusion levels, was subjected to a chemical composition analysis. The findings are shown in Table 5.2. The dry matter content of the unpelleted and pelleted diets showed notable variations (P0.001) in the dry matter content. Comparatively speaking, the unpelleted feed had the largest dry matter percentage (98.12%). At a 10% inclusion level, the pelleted diet, on the other hand, had the lowest dry matter content (93.82%). For the crude protein content of the DRD-based concentrate, a significant interaction impact between the processing technique and inclusion levels was also noted (P0.005).

Table 5.2: Chemical composition of urea-fermented DRD-based concentrate diet

Method	DRD Inclusion Level (%)	Parameters (%/gDM) DM	CP	EE	ASH	NDF	ADF
UNPELLETED	0	98.12	10.34^{ab}	12.51	7.20^{b}	52.9^{ab}	17.22^{abc}
	5	97.55	11.78^{c}	12.47	8.61^{b}	57.7^{ab}	19.01^{abc}
	10	98.06	12.88^{d}	13.36	10.45^{a}	59.3^{ab}	23.24^{ab}
	15	97.19	14.22^{e}	13.43	10.69^{a}	64.2^{a}	25.18^{a}
PELLETED	0	95.27	10.06^{e}	11.54	7.20^{b}	44.6^{b}	13.52^{c}
	5	95.00	10.94^{d}	12.19	8.16^{b}	50.4^{ab}	15.39^{bc}
	10	93.82	11.85^{c}	12.55	8.34^{b}	51.0^{ab}	16.99^{bc}
	15	94.27	12.94^{d}	12.57	8.66^{b}	54.0^{ab}	19.82^{abc}
SED		0.613	0.075	0.20	0.43	4.10	1.741
P value	Method	<.001	<.001	<.001	<.001	0.003	<.001
	Level	0.173	<.001	<.001	<.001	0.526	0.003
	Method*Level	0.248	0.005	0.284	0.012	0.064	0.233

DM= dry matter, CP= crude protein, EE= ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre. [a,b,c,d] Means within the same row with different superscripts are significantly different ($p < 0.05$).

The CP content increased with increasing levels of the urea-fermented dried rumen digesta (UFDRDP). The highest CP (14.22%) was obtained in the mash diet at a 15% inclusion level while the least (10.06%) was recorded in the pelleted diet at a 0% inclusion level.

The EE was significantly affected by methods and inclusion levels. The highest EE (13.43%) was obtained in the mash diet at 15% inclusion levels for UFDRD. NDF was significantly influenced by the processing method, with the unpelleted mash obtaining the highest at the 15% inclusion level and the least at 0% inclusion. The ADF was significantly affected by method and inclusion level, with the unpelleted diet recording the highest at 15% inclusion of UFDRDP and the least in the mash at 0% inclusion. The ADF ranged from 13.52 to 25.18 for the pelleted and unpelleted urea-fermented DRD diets.

Table 5.3 shows the results on TBC, LAB and *E. coli* of the urea-fermented DRD-based concentrate processing method and at different inclusion levels of the dried rumen digesta. There was no significant interaction effect between the method and inclusion levels on TBC. The highest total bacteria count (TBC) was obtained in the Mash diet at a 15% inclusion level and the least in the Pellet diet at a 0% inclusion level. There was a significant interaction effect between the method and inclusion level on lactic acid bacteria. Population counts on lactic acid bacteria increased as the levels of urea-fermented DRD increased in the diet.

Table 5.3: Microbial quality assessment of unpelleted and pelleted urea-fermented DRD diets at different inclusion levels

Method	Level (%)	Parameter (log cfu/g)		
		TBC	LAB	*E. coli*
UNPELLETED	0	5.15^{d}	4.40^{de}	2.53^{a}
	5	5.25^{d}	4.59^{c}	2.13^{b}
	10	5.92^{b}	4.69^{bc}	1.65^{c}
	15	6.16^{a}	5.58^{a}	1.39^{d}
PELLETED	0	4.77^{e}	4.09^{f}	0.00^{e}
	5	4.84^{e}	4.32^{e}	0.00^{e}
	10	5.59^{b}	4.54^{cd}	0.00^{e}
	15	5.78^{b}	4.78^{b}	0.00^{e}
SED		0.04	0.049	0.050
P value	Method	<.001	<.001	<.001
	Level	<.001	<.001	<.001
	Method*Level	0.518	<.001	<.001

TBC = Total microbial count (TBC), Lactic acid bacteria (LAB). ^{a,b,c,d} Means within the same row with different superscripts are significantly different ($p < 0.05$).

The highest LAB was recorded in the Mash at a 15% inclusion level and the least in Pelleted at 0% inclusion levels. Method and inclusion levels had a significant interaction effect on *E. coli*. Generally, the Mash method recorded the highest *E. coli* at 0% inclusion level while no *E. coli* was detected in the pelleted diet at all levels. There was a decreasing trend of *E. coli* as the levels of DRD increased in the mash concentrate diet.

5.5 Discussion

The analysed nutrient composition of the urea-fermented DRD-based concentrate diet revealed a significant difference between the pelleted and unpelleted diets. The analysis revealed an increase in the moisture content of the pelleted diet compared to the unpelleted diet. This rise in moisture content can be attributed to the steam generated during the pelleting process, which adds moisture to the final product. This decrease in nutrient content may be attributed to the thermal processing involved in the pelleting process. A study conducted by Amdt *et al.* (1999) reported a reduction in the solubility of the protein in soy flour when exposed to high cooking temperatures. This suggests that the heat generated during the pelleting process might have led to the denaturation or alteration of certain proteins, resulting in a decrease in the overall crude protein content of the pelleted diet.

Interestingly, it is theoretically expected that thermal processing should not significantly affect the concentration of crude nutrients. According to Landon (2014), the process of pelleting, which involves heat and pressure, is not intended to cause significant nutrient loss or degradation. However, the observed decrease in crude protein, EE, NDF, and ADF in the pelleted diet suggests that some nutritional changes occurred during the pelleting process. These findings emphasize the importance of considering the potential effects of thermal processing methods, such as pelleting, on the nutrient composition of animal feed formulations.

Interestingly, Landon (2014) observed an increase in crude fat and protein when pig diets were pelleted, which was difficult to explain. It has been suggested that Fourier). The improvement in NDF and ADF values indicates that pelleting feedstuff enhances the availability of nutrients (Medel *et al.*, 2004). It is widely recognized that pelleting improves feed quality; however, there is some debate regarding the source of this improvement, as the processing involves both cooking and compressing the mash through a die (Millsr, 2012).

The microbial load for the urea-fermented DRD-based concentrates was higher than the average 3.20 cfu/gDM reported by Agolisi et al. (2022). The unpelleted diet had a higher microbial load than the pelleted diet. A similar trend was obtained in the lactic acid bacteria and this was attributed to the thermal processing of the diet. The microbial load increased as levels of the urea-fermented DRD increased in the concentrate diet which agreed with Agolisi et al. (2022). E. coli decreased with increasing urea-fermented DRD in the unpelleted diet and were not detected in the Pelleted diet.

5.6 Conclusion

In conclusion, the unpelleted and pelleted urea-fermented DRD-based-concentrate diets showed higher nutrient values. The chemical composition values in both the Mash and Pellet concentrate suggest that urea-fermented DRD can be used as a source of protein to enhance the utilization of poor-quality feed resources during the dry season to improve the growth performance of ruminants.

CHAPTER SIX

6.0 EXPERIMENT 3: *IN VITRO* GAS PRODUCTION OF RICE STRAW SUPPLEMENTED WITH GRADED LEVELS OF PELLETED UREA-FERMENTED DRIED RUMEN DIGESTA

6.1.0 Introduction

The previous experiment (Experiment 2) demonstrated high nutrient availability in processed dried rumen digesta. The absence of *E. coli* and *Salmonella spp*. was used as a basis to select the pelleted dried rumen digesta to concentrate for experiment 3. Pelleting was effective in reducing the microbial population in the dried rumen digesta-based concentrate. this necessitated the need to conduct experiment III to determine the effect of varying levels of pelleted dried rumen digesta pellet on *in vitro* gas production supplemented with 50% rice straw.

The use of rice straw as ruminant feed in the dry season is a common practice in Ghana (Ansah *et al.,* 2014). Pasturelands continue to experience heavy grazing, climate and variability in land use leading to a decrease in available forage for livestock (Kassahum *et al*., 2009; Tessema *et al.,* 2011). The situation is further worsened by seasonal variation which reduces forage quality resulting in low protein, energy, minerals and vitamin contents during the dry season (Tessema and Baars, 2004; Murthy *et al.*, 2011). Globally, finding an affordable source of protein has been a major issue for livestock owners due to increased animal and human competition, rising import costs, and unstable production and distribution of protein feedstuffs (Merry *et al*., 2001; Ruzic-Muslic *et al.,* 2014).

the incorporation of supplements in animal diets is crucial for addressing nutrient deficiencies and enhancing animal production. Particularly, nitrogen supplementation becomes essential for improving the rumen ecosystem, enabling better digestion of fibrous components in forages that are naturally low in nitrogen and high in fibre. This approach ensures a balanced and nutrient-rich diet, leading to improved animal health and productivity (Ruzic-Muslic *et al*., 2014). Pelleting is a widely used thermal method in animal feed processing aimed at maximizing feed utilization and enhancing growth performance in livestock (Dozier *et al*., 2010). Surprisingly, despite its potential to improve livestock productivity, pelleting has not received as much research attention as it deserves.

6.1.1 Objectives

- To determine the chemical composition of rice straw supplemented with a urea-fermented dried rumen digesta pelleted diet at different inclusion levels.
- To determine *in vitro* organic matter digestibility of rice straw supplemented with urea-fermented dried rumen digesta pelleted diet at different inclusion levels.

6.1.2 Hypothesis

Ho*: In vitro* organic matter digestibility of rice straw will not differ when supplemented with a pelleted dried rumen digesta diet at different inclusion levels.

$H_{A:}$ *In vitro* organic matter digestibility of rice straw will differ when supplemented with a pelleted dried rumen digesta diet at different inclusion levels.

6.2.0 Materials and Methods

6.2.1 Processing Experimental Feed

The pelleted diet was prepared using ingredients in Table 1. Rice straw within the area was harvested, chopped into pieces with a straw chopper, treated with urea fermented for 21 days and milled through a 2 mm centrifugal sieve (Retseh® ZM 200). Four dietary inclusion levels were formulated. The dried rumen digesta was incorporated into the concentrate diets at 0, 5, 10 and 15% (T0, T1, T2 and T3) at the expense of maize bran.

Table 6.1: Percentage composition of experimental diets

	Urea-fermented DRD inclusion levels (%)			
Ingredients (% DM fed basis)	T0	T1	T2	T3
Maize bran	32.5	30	27.5	25
Cassava peels	6.75	6.75	6.75	6.75
Rice bran	7.5	7.5	7.5	7.5
Shea nut cake	2.5	2.5	2.5	2.5
Urea-fermented DRD	0	2.5	5	7.5
Premix	0.5	0.5	0.5	0.5
Salt	0.25	0.25	0.25	0.25
Rice straw	50	50	50	50
Total	100	100	100	100

DRD = dried rumen digesta

6.2.2 Experimental design

There were four treatments (T0, T1, T2 and T3). A total of 4 runs of separate incubation were conducted for all the treatments. The study adopted a completely randomized design.

6.2.3 Method for *in vitro* gas studies

The in vitro gas production experiments were conducted according to Theodorou *et al*. method (1994), with minor adjustments to the rumen fluid source as described by Ansah *et al*. (2018) as explained in section 4.2.4.

6.2.4 Statistical analysis

The data was subjected to statistical analysis of variance using GenStat 18.2 edition with the following model: Yij = μ + Ti + eij**,** where Yij: observed variation, μ: population means Ti: effect of treatments levels, i the diets and eij error. Means were compared using the Tukey test at 5% ($P<0.05$) least significant differences.

6.3 Result

Table 6.2 presents the chemical composition of both the sole rice straw and rice straw combination with the urea-fermented DRD concentrate. The lowest dry matter content (92.3%), was obtained in the sole rice straw while the other four diets had dry matter content exceeding 90%.

Table 6.2: Chemical composition of the experimental diet (% DM)

Diet	DM	OM	CP	ASH	NDF	ADF
Sole RS	92.3±0.4	86.3±0.5	7.4±0.1	13.7±0.1	68.9±3.2	36.0±0.8
T0	98.6±0.6	91.1±0.5	11.7±1.2	8.9±0.5	57.4±3.2	29.2±2.7
T1	97.6±0.3	90.3±0.1	12.8±0.2	9.7±0.1	60.1±0.1	30.0±2.1
T2	97.8±0.1	90.0±0.3	13.3±0.3	10.0±0.3	64.9±0.5	33.3±1.0
T3	97.7±0.2	89.9±0.8	14.0±1.1	10.1±0.8	66.0±.03	35.0±0.5

DM = dry matter, OM = organic matter, CP = crude protein, NDF = Neutral detergent fibre, ADF = acid detergent fibre, RS = rice straw, T0 = 0% DRD, T1 = 5% DRD, T2 = 10% DRD and T3 = 15% DRD.

The organic matter content was higher in the 0% DRD-based concentrate diet (91.1%) and the lowest in the sole rice straw (86.3%). The crude protein content of the 15% DRD-based concentrate was relatively higher than other diets. The NDF was higher for sole rice straw (68.9%) compared to the other 4 diets. There was a trend of increased NDF content as levels of DRD increased in the diet, with the 15% DRD inclusion level having the highest NDF content and the lowest at the 0% inclusion level. A similar trend was observed for ADF.

Table 6.3: Effect of supplementing rice straw with graded levels of pelleted Urea-fermented dried rumen digesta on *in vitro* digestibility

	Urea-fermented DRD inclusion levels (%)					
Parameters	T0	T1	T2	T3	SED	P. value
IVDOM (%)	47.33^{c}	51.04^{a}	49.36^{b}	52.14^{a}	0.716	<.001
SCFA (mmol/l)	0.44	0.50	0.43	0.50	0.123	0.234
b (ml/gDM)	35.3	31.8	34.3	28.3	4.15	0.342
c (m/h)	0.02	0.03	0.02	0.02	0.004	0.201
ME (MJ/g/DM)	7.00^{c}	7.79^{b}	6.98^{d}	7.86^{a}	0.005	<.001

T0 = 0% DRD, T1 = 5% DRD, T2 =. 10% DRD and T3 = 15% DRD. [a,b,c,d] Means within the same row with different superscripts are significantly different ($p < 0.05$).

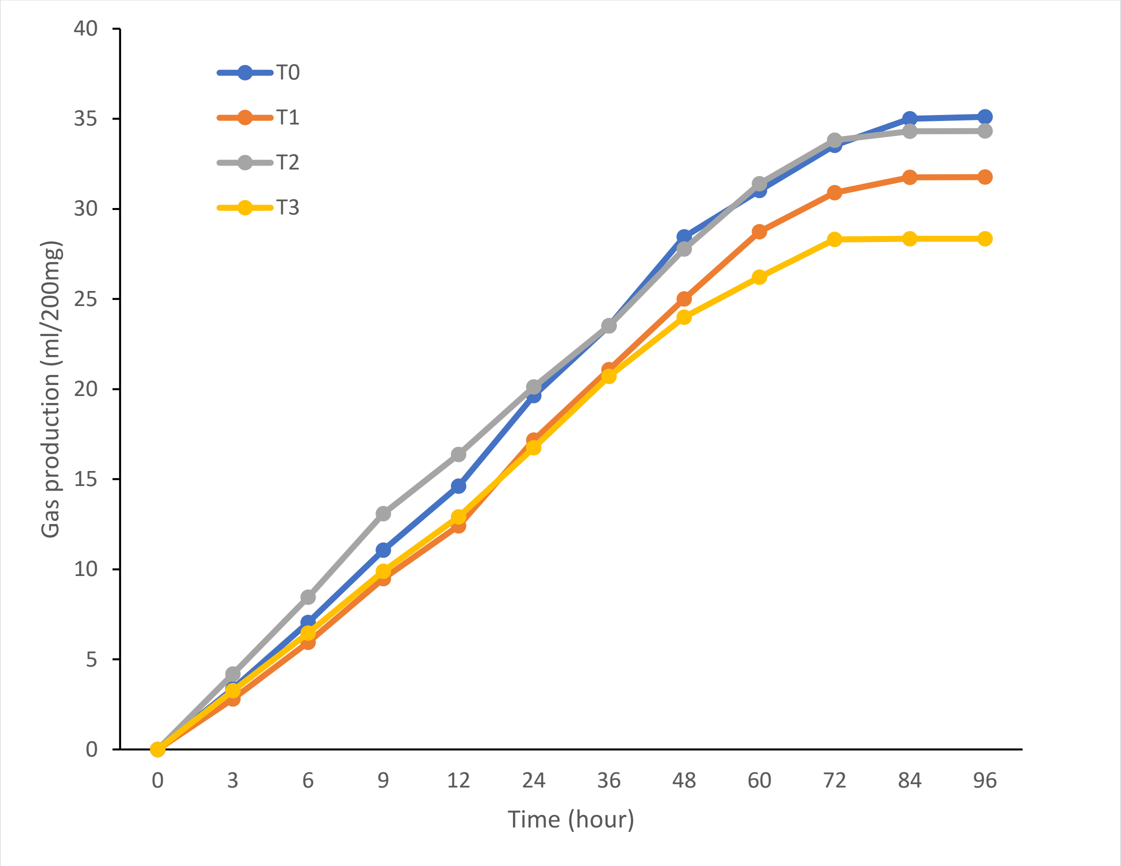

Figure 1: The effect of urea-fermented dry rumen digesta supplementation levels with rice straw on cumulative gas production at different times of incubation

Table 6.3 presents the outcomes of in vitro analysis on the digestible dry matter and fermentation characteristics of a pelleted concentrate based on urea-fermented DRD, supplemented with 50% rice straw. The results reveal that the 15% DRD concentrate exhibited the highest ($P < 0.001$) in vitro digestible organic matter (IVDOM), whereas the control had the lowest IVDOM. There were no significant differences ($P > 0.05$) in the levels of short-chain fatty acids (SCFA) among the different dietary treatments. The asymptote gas production (b) and the rate of gas production (c) also did not show significant variations ($P > 0.05$) among the treatments. However, the 15% concentration displayed the highest ($P < 0.001$) metabolizable energy, while the 10% concentration had the lowest metabolizable energy.

6.4 Discussion

The experimental diets analyzed in the study had sufficient dry matter content to support a reasonable intake of dry matter. This is particularly beneficial for sheep, as it enhances microbial activities and improves the digestion and utilization of nutrients (Oni et al., 2008; McDonald *et al*., 2011). Furthermore, the crude protein (CP) content of the rice straw used in the diets met the minimum requirement of 6-8% dry matter for successful microbial growth in ruminant animals. This indicates that the rice straw provided a suitable source of protein for the microbial population in the rumen, supporting their growth and activity (Van Soest, 1982). Supplementing 50% of graded levels of pelleted urea-fermented DRD-based concentrate increased the CP content for all the supplemented diets as levels of DRD-based concentrate increased and were within the NRC (2007) recommended 12% CP of total DMI for sheep growth and maintenance. Supplementation of graded levels of DRD-based concentrate decreased the NDF and ADF fractions for all the supplemented diets. However, both NDF and ADF fractions increased as the urea-fermented DRD incorporation increased and this was due to the fibrous nature of the DRD. The observed gradual increments in the NDF and ADF fractions align with the findings of Olafadehan *et al*. (2014), who reported similar increases in NDF and ADF values when incorporating urea-fermented DRD into cattle diets.

The in vitro gas technique is commonly used to assess feed degradation, with higher gas production indicating greater fermentation of the substrates. This technique provides a valuable means of measuring the extent of microbial activity and the breakdown of feed components. In the present study, the in vitro digestible organic matter was found to be higher in the 15% urea-fermented DRD concentrate supplemented with 50% rice straw. This can be attributed to the relatively higher crude protein (CP) content in the diet, which supported the activity of cellulolytic microbes in digesting the dry matter. Previous research has shown a positive correlation between CP and the rate of gas production (Nsahlai *et al*., 1994). The asymptote gas production (b) did not exhibit significant variation among the diets. However, the control diet recorded higher asymptote gas production (b), but also the lowest digestible organic matter (DOM), suggesting inefficiency in the digestibility of the feed. The inefficiency observed in the digestibility of the diet in this study could lead to the release of methane gas into the atmosphere when consumed by ruminant livestock. On a positive note, the metabolizable energy (ME) recorded for the supplemented diet in this study falls within the recommended range provided by NCR (2007). This suggests that the supplemented diet provides an appropriate level of metabolizable energy, which is crucial for supporting the nutritional needs and productivity of animals.

6.5 Conclusion and Recommendation

In conclusion, *in vitro* digestible organic matter (IVDOM) was increased by supplementing 50% of rice straw with a graded quantity of pelleted urea-fermented DRD-based concentrate. The in vitro gas generation of rice straw supplementation values in the pellet concentrate leads to the conclusion that the DRD pellet may be utilised as a source of protein to increase the utilisation of low-quality feed resources during the dry season to improve ruminant growth performance**.**

CHAPTER SEVEN

7.0 EXPERIMENT 4: EFFECT OF VARYING LEVELS OF PELLETED DRIED RUMEN DIGESTA SUPPLEMENTATION ON FEED PREFERENCE, APPARENT NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF DJALLONKÉ SHEEP

7.1.0 Introduction

In order to determine the effect of supplementing graded levels of pelleted urea-fermented DRD on feed preference and apparent nutrient digestibility, Experiment 4 was carried out as a continuation of previous experiments. The motivation behind this follow-up was the significant variation observed in the *in vitro* gas production of the supplemented diets during Experiment 3. The objective of Experiment 4 was to assess the effects of these graded levels on sheep when fed. Hence, the need to conduct this study to provide further information on the apparent nutrient digestibility of the pelleted dried rumen digesta.

The growth and productivity of small ruminants in most developing countries are often hampered by the inadequate supply of nutrition, especially during the dry season. Due to the high cost and scarcity of conventional concentrate for small ruminants, there has been a surge in the search for locally available feed resources to halt the decline in the growth of small ruminants, especially during the dry season (Merry *et al*., 2001).

Rumen digesta is a major waste from abattoirs with the potential to be recycled into animal feed. Unfortunately, due to the lack of technological know-how, rumen digesta is underutilized in Ghana. Rumen digesta, which typically contains about 9 to 20% crude protein (CP) of dry matter, holds significant potential as a valuable protein source for animal nutrition when appropriately processed (Adeniji and Balogun, 2002; Esonu *et al*., 2006; Agbabiaka *et al*., 2011; Sakaba *et al*., 2017; Agolisi *et al*., 2022).. Numerous studies have demonstrated the efficacy of DRD in the diets of various animals, including fish, poultry, rabbits, cattle, goats, and sheep (Odunsi, 2003; Esonu *et al*., 2006; Okpanachi *et al*., 2010; Agbabiaka *et al*., 2011; Agolisi *et al*., 2022). Furthermore, feeding Awassi lambs with 10% dried rumen digesta (DRD) did not cause any adverse effects on animal health or nutrient digestibility (Al-Wazeer, 2016). Similarly, Djallonké sheep fed with 15% DRD showed no signs of health issues or digestibility problems (Agolisi *et al*., 2022). The present study aimed to investigate the apparent nutrient digestibility of a pelleted DRD-based concentrate supplemented with rice straw for sheep, building upon the in vitro gas production experiment conducted in Experiment 3.

7.1.1 Objective

- To determine the effect of dried rumen digesta supplementation on feed preference, apparent nutrient digestibility and growth performance of Djallonké sheep.

7.1.2 Hypothesis

- Feed preference and apparent nutrient digestibility of rice straw sheep will not differ when fed to Djallonké sheep with pelleted dried rumen digesta-based concentrate.
- Feed preference and apparent nutrient digestibility of rice straw sheep will differ when fed to Djallonké sheep with pelleted dried rumen digesta-based concentrate.

7.2 Materials and Methods

7.2.1 Experimental site

The analysis of chemical composition was carried out at the Forage Evaluation Unit (FEU) of the University for Development Studies in Nyankpala, Ghana, as well as the Ecological Agriculture Research Laboratory at Bolgatanga Technical University, Ghana. On the other hand, the feeding trial took place at the Livestock Unit of Ecological Agriculture at Bolgatanga Technical University, Bolgatanga, from June 2022 to August 2022.

7.2.2 Experimental feed

Fresh rumen digesta was collected from cattle at the Bolgatanga abattoir in the Upper East Region of Ghana. Approximately 50 kg of rumen digesta was carefully gathered and placed in a jute bag, which was then subjected to a weight of about 50 kg for 3 hours to remove the liquid and reduce the moisture content. Following this, 100 kg of fresh rumen digesta was thoroughly mixed with 500 g of urea and sealed in an airtight container for 21 days to undergo fermentation. The resulting urea-fermented rumen digesta was sun-dried for 2 days and subsequently milled using a centrifugal mill with a 1 mm sieve (Retseh 200 GmbH, Hann, Germany).

The milled dried rumen digesta (DRD) was then mixed with other ingredients at different levels: 0%, 5%, 10%, and 15% (as presented in Table 1). All the formulated diets were pelleted and sun-dried for 2 days prior to being fed to the rams. All nutrient composition results were reported based on dry matter. information regarding the ingredients and chemical composition are shown in Table 7.1.

Table 7.1: Ingredients and chemical composition of the urea-fermented dried rumen digesta pellets (DRDP) concentrates and sole dried rumen digesta

	DRD inclusion levels				
Ingredients (% As fed basis)	0%	5%	10%	15%	Sole DRD
Maize bran	65	60	55	50	-
Cassava peels	13.5	13.5	13.5	13.5	-
Rice bran	15	15	15	15	-
Shea nut cake	5	5	5	5	-
Dried rumen digesta	0	5	10	15	-
Premix	1	1	1	1	-
Salt	0.5	0.5	0.5	0.5	-
Total	**100**	**100**	**100**	**100**	-
Chemical Composition (g/kg)					
Dry matter	953.20	950.50	948.20	943.70	962.50
Crude protein	101.00	110.40	119.50	131.40	174.50
Ether extract	81.20	83.30	86.80	85.60	43.20
Ash	72.00	81.60	83.40	86.60	144.00
Neutral detergent fibre	447.60	503.40	511.00	543.00	645.80
Acid detergent fibre	135.00	154.10	168.70	198.70	418.80

7.2.3 Sheep, treatments and feeding management.

Sixteen (16) young Djallonké rams (5-6 months old) with 9.9 ± 0.37 kg initial live body weight (BW) were randomly allocated to four dietary treatment groups in a Completely Randomized Design (CRD) and replicated four times. The rams were offered a daily ration of 3% of their live body weight every week. The rams received urea-fermented DRD pellets concentrate and rice straw in a ratio of 1:1 (50% straw diet and 50% concentrate). The concentrate diet and rice straw were offered twice daily at 7:00 am and 4:00 pm allowing a 30-minute lag between the offer of concentrate and rice straw. Each animal was individually housed in cages with a concrete floor that was covered with rice husk bedding. They had unrestricted access to water. A period of 14 days was allocated for the animals to acclimate to both the feed and the environment.

Samples of the concentrate and rice straw diets offered to the animals were collected on a daily basis and stored in a refrigerator until the end of the experiment. At the conclusion of the feeding trial, the sampled diet for each treatment replicate was combined into a bulk sample, from which a subsample was extracted for oven drying. Duplicate subsamples from each treatment were weighed and dried in an oven at 60°C for 48 hours. The percentage of dry matter (DM) was then calculated using the dried samples, and this value was used to estimate the total DM intake.

7.2.4 Feed digestibility

During the fifth week of the experiment, an important aspect under investigation was the digestibility of the feed. In order to evaluate this, faecal collection bags were securely attached to the rams, enabling the collection of their faeces for the subsequent digestibility trial. Every day, the output of faeces from each ram was meticulously collected, carefully weighed, and accurately recorded for further analysis. To specifically examine the digestibility of the urea-fermented faeces, samples were taken from the collected faecal output and stored in a refrigerator to preserve their integrity until the conclusion of the experiment. As the experiment came to an end, the faecal samples obtained from each treatment replicate were combined to create a bulk sample that represented the respective treatment group. From this combined bulk sample, a smaller subsample was extracted to undergo the process of oven drying.

To ensure reliable and consistent results, duplicate subsamples were taken from each treatment group. These duplicate subsamples were meticulously weighed and subsequently placed in an oven set at a temperature of 60°C. Over the course of 48 hours, the subsamples underwent thorough drying in the oven, allowing for the removal of any remaining moisture. After the drying process was complete, the dry matter percentage of each subsample was calculated based on their weight, providing a measure of the proportion of dry matter present.

Utilizing the calculated dry matter percentage, an estimation of the total dry matter digestibility for each treatment group was derived. This calculation involved assessing the difference between the daily intake of dry matter and the output of dry matter, which was obtained from the faecal samples collected earlier. The digestibility coefficient, a significant indicator of the efficiency of nutrient utilization, was then estimated by dividing the aforementioned difference by the daily intake of dry matter for each individual animal within the experiment.

$$\text{Nutrient digestibility coefficient} = \frac{Nutrien\ \ in\ feed - nutrient\ in\ faeces}{Nutrient\ in\ feed} \quad \text{=Equation 7.1}$$

7.2.7 Statistical analysis

The data was subjected to statistical analysis of variance using GenStat 18.2 edition with the following model: Yij = μ + Ti + eij**,** where Yij: observed variation, μ: population means Ti: effect of treatments levels, i the diets and eij error. The initial weight of the animals was used as a covariate in the analysis of growth parameters.

A significant difference was declared at 5% and the means were separated using the Tukey test.

7.3 Results

The chemical composition of the concentrate and sole urea-fermented DRD is shown in Table 1. The CP was in the range of 101.0 to 131.4 g/kgDM. The NDF ranged between 447.6 and 543.0g/kgDM whilst the ADF was in the range of 135 to 198.7 g/kg DM. The inclusion of urea-fermented DRD in the concentrate diets increased both CP and NDF concentrations.

Table 7. 2: Effect of graded levels of urea-fermented dried rumen digesta pellet concentrate on feed intake and apparent digestibility and growth performance of Djallonké sheep

Parameters	Urea-Fermented **DRD inclusion levels (%)**					
	T0	**T1**	**T2**	**T3**	**SEM**	***p*-value**
DMI(g/h/d)	313.6	317.0	310.4	308.9	8.680	0.796
CPI (g/h/d)	25.37^{c}	28.84^{b}	30.82^{b}	33.87^{a}	0.981	<.001
DM digested	0.70	0.71	0.71	0.70	0.018	0.258
CP digested	0.87^{b}	0.88^{b}	0.90^{a}	0.91^{a}	0.003	<.001
Initial live weight (kg)	9.96	10.02	10.46	9.15	1.102	0.694
Final live weight (kg)	11.71^{b}	12.15^{ab}	12.29^{a}	12.90^{a}	0.256	0.002
Live weight gain (kg)	1.75^{c}	2.27^{bc}	2.82^{ab}	3.75^{a}	0.254	0.001
Daily live weight gain (g)	28.89^{c}	36.07^{bc}	44.80^{ab}	48.93^{a}	4.04	0.001
FCR (gDMI/gADG)	12.71^{a}	9.13^{ab}	7.36^{b}	5.73^{b}	1.386	0.002

The intake of dry matter was similar among the dietary treatment, however, T1 recorded higher dry matter intake (317.0 g/DM). The daily CP intake differed significantly ($p<0.05$) with the highest obtained in rams fed 15% urea-fermented DRD concentrate (33.87 g/DM). The DM digested was similar ($P<0.05$) across the dietary treatments. The DM digestibility coefficient was in the range of 0.70 to 0.71. The CP digestibility differed ($P<0.001$) between the control and the other dietary treatments. The apparent CP digestibility coefficient increased with an increase in urea-fermented DRD with the highest obtained in rams fed 15% urea-fermented DRD.

The response of the rams fed with supplemented varying levels of pelleted urea-fermented DRD concentrate observed significant differences in all the growth parameters measured. The final live weight (kg), live weight gain (kg), daily live weight gain (g) and FCR (gDMI/gADG) were significantly different ($p<0.05$) among the treatments. The highest FCR was recorded in 15% DRD and the lowest in the control (0%).

7.4 Discussions

The CP content of the experimental diets exceeded the minimum range of 60-80 g/kg DM required for optimal microbial growth, as stated by Van Soest (1982). Including urea-fermented DRD in the experimental diets increased the CP content (131.40 g/kg DM), surpassing the 120 g/kg DM requirement reported by NRC (2007). Moreover, the inclusion of urea-fermented DRD in the concentrate diets elevated both CP and NDF concentrations, indicating that urea-fermented DRD performed better than maize in terms of CP and NDF. The presence of semifermented and unfermented dietary feed, microbial protein, and metabolic by-products of the rumen in rumen digesta (Elfaki and Abdelatti, 2015) contributed to the relatively higher crude protein and NDF levels in the DRD-based diets.

The comparable dry matter intake values between the control diet and the diet containing up to 15% dried rumen digesta suggest that the current levels of urea-fermented DRD in the diet were equally palatable to the control diet and did not negatively affect consumption. This finding also indicates that pelleting the experimental diets prevented selective feeding by the rams, which aligns with the results reported by Cherdthong *et al*. (2014) regarding the urea-fermented DRD-based concentrate diet. Likewise, in the study conducted by Mondal *et al.* (2013), the inclusion of DRD at a 10% level in the diet of Bengal goats did not yield any significant variances in terms of dry matter intake. Similarly, Fajemisin *et al.* (2010) observed that the incorporation of fermented rumen digesta mixed with poultry droppings into the diet of Djallonké sheep did not result in any significant impact on dry matter intake.

The daily intake of crude protein differed significantly, with the highest intake observed in rams fed the concentrate containing 15% urea-fermented DRD. The high CP content of the urea-fermented DRD-based concentrate diet contributed to the elevated CP intake recorded in treatment T3.

Conversely, Fajemisin *et al*. (2010) discovered a noteworthy increase in dry matter digestibility when Djallonké sheep were fed a diet comprising 25% urea-fermented DRD as the primary component.

The apparent digestibility coefficient of crude protein increased with higher levels of urea-fermented DRD, with the highest value obtained in rams fed 15% urea-fermented DRD. The final live weight (kg), live weight gain (kg), daily live weight gain (g), and feed conversion ratio (gDMI/gADG) exhibited significant differences ($p<0.05$) among the treatments. Rams fed 15% urea-fermented DRD had significantly higher live weight gain (3.75 vs. 1.75 kg) compared to the control group. The average daily live weight gain followed a similar trend, with the rams on the 15% urea-fermented DRD diet recording twice the gain compared to the control rams. The feed conversion ratio was significantly higher in the control group relative to the 15% urea-fermented DRD concentrate. The availability of fermentable carbohydrates and rumen ammonia nitrogen hydrolyzed from non-protein nitrogen and dietary protein by rumen microbes influence rumen digestion of dry matter. The presence of dead rumen microbes and other digestive enzymes in the DRD ensured protein degradation in the rumen was not restricted. This may have led to the supply of the needed ammonia nitrogen which is required for the synthesis of rumen microbial cells. This is supported by the high CP digestibility reported for the urea-fermented DRD-based concentrate in the present study. The relatively higher protein digestion by rams in the DRD pellets concentrates may have led to the buffering of the rumen environment to support the activities of cellulolytic bacteria responsible for the fermentation of cell wall carbohydrates in the rice straw. This could have accounted for the improved dry matter digestibility in the urea-fermented DRD-based pellet concentrate.

Other researchers have reported improvement in apparent nutrient digestibility with the inclusion of DRD in the diets of beef cattle (Cherdthong and Wanapat, 2014; Seankamsrn and Cherdthong, 2020) and sheep (Agolisi *et al.*, 2022) when compared to diets without DRD.

The significant difference in the final live weight gain of sheep on urea-fermented DRD compared to the 0% DRD is an indication of the practical effect of the superior digestibility of the nutrients in the urea-fermented DRD pellet concentrate. The present daily weight gains compared closely with the 16-48 g/d observed by Adu *et al.* (1992) and 48.21-56.47 g/d recorded by Agolisi *et al.* (2022), but lower than the 79-91 g/d reported by Baiden *et al.* (2007) for Djallonké sheep.

7.5 Conclusion and Recommendation

Feeding young rams with urea-fermented DRDP up to 15% gained about 53.33% final live weight and the average daily weight of Djallonké rams in the Savanna agro-ecological zones and could therefore be used as a supplement for small ruminants.

CHAPTER EIGHT

8.0 EXPERIMENT 5: EFFECT OF VARYING LEVELS OF PELLET- UREA-FERMENTED DRIED RUMEN DIGESTA SUPPLEMENTATION ON HAEMATOLOGY AND SERUM PROFILE OF DJALLONKÉ SHEEP

8.1.0 Introduction

Experiment 5 was conducted to determine the effect of rice straw and pelleted urea-fermented DRD-based concentrate on the haematology and serum profile of Djallonké sheep. Experiments 3 and 4 showed the potential of pelleted urea-fermented DRD-based concentrate to improve nutrient digestibility in ruminant animals. Hence the need for experiment 5 to measure the haematology and serum profile of Djallonké sheep.

Due to the high cost and scarcity of conventional concentrate for small ruminants, there has been a surge in the search for locally available feed resources to halt the decline in the growth of small ruminants, especially during the dry season (Merry *et al.*, 2001). DRD has been proven effective as a dietary component in various animal species, including fish, poultry, rabbits, cattle, goats, and sheep, as indicated by several studies (Agbabiaka *et al*., 2011; Agolisi *et al*., 2022).

Both the amount and the quality of feed, as well as the presence of anti-nutritional elements or factors, can exert significant influence on both the haematological and biochemical constituents of the blood (Akinmutimu, 2004). Notably, the biochemical components of blood are particularly susceptible to the effects of toxic elements present in the feed. As emphasized by Esonu *et al.* (2000), the haematological parameters play a crucial role as valuable indicators of an animal's physiological status, and any alterations in these parameters aid in evaluating the animal's response to various physiological conditions. Researchers, including Al-Wazeer (2016), have consistently observed distinct changes in blood cell profiles throughout an animal's life. The present study aimed to examine the impact of a pelleted rumen digesta-based concentrate on the blood profile of Djallonké rams.

8.1.1 Objective

- To determine the effect of pelleted urea-fermented dried rumen digesta-based concentrate on haematology and serum biochemistry of Djallonke sheep on urea-treated rice straw.

8.1.2 Hypothesis

- The haematology and serum profile of Djallonke sheep fed urea-treated rice straw supplemented with graded levels of pelleted dried rumen digesta will not differ among the experimental animals.
- The haematology and serum profile of Djallonke sheep fed urea-treated rice straw supplemented with graded levels of pelleted dried rumen digesta will differ among the experimental animals.

8.2.0 Materials and Methods

8.2.1 Collection of blood for haematological and serum analyses

In the morning before feeding, blood samples were collected from the animals both at the beginning and end of the study. Using a sterilized needle and syringe, approximately 10 ml of blood was drawn from the jugular vein. These blood samples were then carefully transferred into sample bottles containing anticoagulants, while another set of plain bottles without anticoagulants was used for a separate exercise. The full blood count using A 3-part blood analyzer is a diagnostic instrument used in clinical laboratories and healthcare settings to analyse blood samples. A 3-part blood analyzer plays a vital role in diagnosing and monitoring various medical conditions by providing essential information about the cellular components of blood. Its automation and precision help in saving time, improving efficiency, and aiding healthcare professionals in making informed decisions for patient care.

The blood samples collected for serological analysis were allowed to coagulate, followed by centrifugation at 1000 rpm for 10 minutes. The serum was carefully collected using a clean pipette and transferred into serum bottles.

For the measurement of total protein, the principle of serum proteins reacting with cupric ions in an alkaline solution was employed, as outlined by Gornall *et al*. (1949). This reaction leads to the formation of a blue-coloured complex, allowing for the quantification of total protein levels in the serum. The analysis of blood urea nitrogen (BUN) followed the procedures established by Fawcett and Scott (1960) and Chaney and Marbach (1962). These methods provide reliable guidelines for accurately measuring BUN levels, which are indicators of kidney function and overall health.

8.2.6 Statistical analysis

The data was subjected to statistical analysis of variance using GenStat 18.2 edition with the following model: Yij = μ + Ti + eij**,** where Yij: observed variation, μ: population means Ti: effect of treatments levels, i the diets and eij error. The initial weight of the animals was used as a covariate in the analysis of growth parameters. Significant difference among treatment means were tested by using Tukeys at 5%.

8.3 Results

The haematological parameters measured for all rams fed the urea-fermented DRD concentrate in this study did not differ among the dietary treatments in Table 8.1. The haemoglobin (Hb) ranged between 10.79 to 14.05 g/dl. However, there was a marginal increase in haemoglobin between the initial and the final. The final haemoglobin ranged between 34.4 to 39.54% and 16.8 – 34.42 g/dl for initial. The WBCs count was in the range of 6.90 and 8.82 whilst the RBC was 13.0.0 and 15.7. The Mean corpuscular volume ranged from 44.03.51 to 47.16 /dL for 5% and 10% urea-fermented DRD.

Table 8.1: Effect of graded levels of urea-fermented dried rumen digesta pellet supplementation on haematology of Djallonké sheep

	Urea-fermented **DRD inclusion levels (%)**					
Parameters	**T0**	**T1**	**T2**	**T3**	**SEM**	***p*-value**
Hb (g/dl)						
Initial	8.15^{a}	7.95^{ab}	5.28^{b}	6.00^{ab}	0.929	0.021
Final	10.79	10.84	13.16	14.05	1.712	0.206
Packed cell volume (%)						
Initial	32.5^{a}	34.4^{a}	21.7^{ab}	16.8^{b}	4.90	0.010
Final	36.76	34.42	36.77	39.54	5.21	0.843
WBC ($X10^{9}$/dl)						
Initial	4.53	4.40	4.60	6.25	1.071	0.312
Final	8.82	8.45	7.33	6.90	2.711	0.884
RBC ($X10^{12}$/dl)						
Initial	7.58^{a}	8.74^{ab}	*5.25^{ab}*	4.10^{b}	1.233	0.010
Final	14.7	13.0	13.6	14.1	3.49	0.775
Mean corpuscular volume (fl)						
Initial	42.92	42.20	41.90	41.30	1.109	0.547
Final	44.03	47.16	45.14	45.85	1.247	0.377
Mean corpuscular haemoglobin (pg)						
Initial	11.05^{ab}	9.05^{b}	9.95^{b}	14.98^{a}	1.584	0.014
Final	12.02	12.73	12.88	12.12	1.361	0.956

Hb = Haemoglobin; WBC = white blood cell; RBC = red blood cell

The serological parameters measured for all rams fed the urea-fermented DRD concentrate in this study did not differ among the dietary treatments (Table 2) except the final concentration of albumin and initial concentration of blood urea nitrogen (mg/dL) in the plasma which was significantly influenced (P<0.005) by dietary treatment. There was a marginal increase in the final albumin concentration in the dietary treatments except for the 15% urea-fermented DRD diet compared to the initial results. The rams on the urea-fermented DRD dietary treatment had a marginal increase in the total protein compared to the 0% dietary treatments, the values ranged between 64.42 and 77.34 g/d L whilst the aspartate aminotransferase (AST) ranged from 75.1 – 86.7 g/d L a marginal decrease from for the initial values. Most of the serum indices measured showed no significant (*p*>0.05) difference except for final albumin and blood urea nitrogen concentrations.

Table 8.2: Effect of graded levels of urea-fermented dried rumen digesta pellet supplementation on serum profile of Djallonké sheep

	Urea-fermented **DRD inclusion levels (%)**					
Parameters	**T0**	**T1**	**T2**	**T3**	**SEM**	***p*-value**
Albumin g/dL)						
Initial	29.02	26.73	24.98	25.50	1.41	0.059
Final	26.82^{a}	25.65^{ab}	24.74^{b}	27.10^{a}	1.07	0.005
Total protein (g/dL)						
Initial	75.6^{a}	60.5^{b}	60.9^{b}	60.5^{b}	5.62	0.048
Final	66.36	68.66	64.42	77.34	5.36	0.093
Aspartate aminotransferase (AST) (units/L)						
Initial	110.0	109.1	89.5	99.8	10.83	0.250
Final	85.1	80.2	86.7	75.1	7.83	0.434
Creatine						
Initial	97.8	105.5	86.5	103.1	21.88	0.826
Final	108.66	44.24	74.72	95.21	31.2	0.235
Total cholesterol (mg/dL)						
Initial	2.31	1.65	1.58	1.79	0.42	0.336
Final	2.22	2.08	1.87	1.96	0.14	0.126
Blood urea nitrogen (mg/dL)						
Initial	7.87	8.10	7.21	8.50	1.50	0.866
Final	7.63^{ab}	6.51^{ab}	4.72^{b}	8.44^{a}	1.11	0.033

^{a,b,c,d} Means within the same row with different superscripts are significantly different (*P*< 0.05).

8.3 Discussion

The comparable concentrations of haematological parameters observed in the sheep fed different dietary treatments indicate that the inclusion of pelleted urea-fermented DRD-based concentrate did not have any adverse effects on their health. The values of haemoglobin (10.16 - 14.05 g/dL) and packed cell volume (PCV) (34.42 - 39.54%) obtained in this study were within the normal physiological range for sheep, which is typically 8-16 g/dL and 27-45%, respectively (Pampori, 2003; Orheruata et al., 2004; Merck Veterinary Manual, 2010). This suggests that the urea-fermented DRD pellet diets effectively provided the necessary amino acids and iron to maintain a healthy haemoglobin concentration. In a previous study by Agolisi et al. (2022) involving the same breed of sheep fed different levels (0, 4, 8, and 12%) of DRD-based concentrate, the reported ranges for haemoglobin and PCV were 8.50-8.95 g/dL and 32.55-33.20%, respectively. These findings further support the adequacy of the urea-fermented DRD-based diets in maintaining normal haematological parameters. The white blood cell (WBC) counts were also similar among the rams in this study. The total WBC counts (6.90-8.82 x 109/L) observed fell within the normal range of 5 x 109/L and 4-12 x 109/L reported for sheep (Scott et al., 2006; Merck Veterinary Manual, 2010). In a study conducted by Konlan et al. (2012), Djallonké sheep fed a basal diet of rice straw and groundnut haulms supplemented with graded levels of shea nut cake concentrate showed a white blood cell (WBC) count range of 8.37 to 9.30 x 109/L. Similarly, Agolisi et al. (2022) conducted a study with Djallonké sheep fed graded levels (0, 4, 8, and 12%) of DRD-based concentrate and reported a WBC count range of 5.78 to 7.70 x 109/L.

The study's findings regarding the total red blood cell (RBC) counts, ranging from 13.0 to 14.70 x 1012/L, align with the normal physiological range of 9 to 15 x 1012/L as reported in the Merck Veterinary Manual (2010). Similarly, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values obtained in the study were within the normal range for healthy sheep, indicating efficient synthesis of haematological indices across the different dietary treatments. Packed cell volume, haemoglobin, and MCH are important indicators used to evaluate circulating red blood cells, and they are particularly helpful in diagnosing anaemia and assessing the bone marrow's capacity to produce red blood cells in mammals (Chineke *et al*., 2006).

The nutritional status of animals significantly affects haematological traits, and these traits serve as indicators of their nutritional well-being (Adeyele et al., 2017; Olorruntola *et al*., 2018). Alterations in haematological parameters provide valuable information about the animals' responses to various physiological and disease conditions (Yadav *et al*., 2002; Khan, 2005). Regarding albumin levels, the inclusion of urea-fermented DRD in the diet resulted in significant impact, with values ranging from 22.74 to 27.10 g/dL. These values were comparable to those reported for Djallonké rams on supplementation in studies conducted by Konlan *et al*. (2012), Ansah *et al*. (2016), and Agolisi *et al*. (2022), but higher than the range reported by Pampori (2003). Nevertheless, the albumin values fell within the normal physiological range of 24 to 30 g/dL as reported by Jackson and Cockcroft (2002) for sheep. Albumin concentration serves as an indicator of protein metabolism and constitutes approximately 50-65% of the total blood protein (Contreras *et al*., 2000).

The serum protein levels did not differ significantly among the diets but remained within the normal range of 60 to 93 g/dL reported for sheep (Contreras *et al*., 2000). This finding aligns with previous studies that reported no significant effect on serum protein levels in lambs fed DRD-based concentrate diets (Aruwayo *et al*., 2007; Agolisi *et al*., 2022). As for the aspartate aminotransferase (AST) levels (ranging from 75.1 to

86.7 U/L), creatine levels (ranging from 74.72 to 108.66 mg/dL), and cholesterol levels (ranging from 1.87 to 2.22 mg/L), all fell within the normal range for sheep The blood urea nitrogen obtained in the study all fell below the normal range of 10 – 26mg/dL as reported by Plumb (2005) for sheep. The values were lower than earlier values obtained by Agolisi *et al.* (2022) when Djallonke lambs were fed DRD-based concentrate diets and also lower than the value reported by Konlan *et al.* (2012) and Ansah *et al*. (2016) for Djallonke sheep under similar climatic conditions but different feed supplement. Serological parameters provide vital information on the physiological well-being of animals (Bellows *et al*., 1963). The relatively normal ranges even at the highest level of DRD inclusion for most of the serological parameters measured in the rams in the present study suggest that the secretion of enzymes and health status was not adversely affected in animals indicating the potential DRD pellets in the sheep.

8.4 Conclusion

In inclusion of urea-fermented dried rumen digesta in the diets of lambs to replace 15% of maize bran had no adverse effects on the health and physiological well-being of the rams. The haematological parameters, such as haemoglobin, PCV, WBC counts, and RBC counts, were within the normal physiological range for sheep, indicating that the diets provided the necessary amino acids and iron to maintain healthy haemoglobin concentrations. The values of the serum profile were within the normal range for sheep, indicating that the metabolic processes and overall health of the rams were not negatively affected.

These results support the potential use of DRD pellets as a nutritional supplement for sheep, providing a valuable alternative feed source without compromising their health status.

CHAPTER NINE

9.1 General Discussion

The study found that the processing method significantly impacted the crude protein content of rumen digesta, with an average CP content of 15.88%. This exceeded the recommended (NRC, 2007) 12% CP for sheep growth and maintenance. The average CP content was slightly higher than other studies, with dry season feed resources containing lower protein and higher cell wall carbohydrates. Seasonal effects on nutrient composition were evident.

The urea-fermented processing method showed higher in vitro digestible organic matter (IVDOM) and asymptote gas production compared to the sun, oven, and fermented methods. This higher gas production from microbial fermentation and corresponding higher digestible organic matter suggests greater efficiency in the digestibility of the rumen digesta in this study. The higher crude protein content in the urea-fermented method likely supplied cellulolytic microbes with the required degradable protein for better dry matter digestion. In contrast, the sun and oven drying methods resulted in lower IVDOM and asymptote gas production (b), which can be attributed to the presence of cell walls in the rumen digesta. The partially digested forage in the rumen contains lignin, which acts as a barrier, limiting rumen microbes' access to structural polysaccharides during fermentation (Agbagla-Dohnani *et al.*, 2001). According to Ammar (2000), fibre levels are negatively correlated with in vitro digestibility, indicating that higher fibre content in the rumen digesta would likely result in lower digestibility.

Generally, the urea-fermented processing method led to significantly increased crude protein content and improved digestibility of the rumen digesta compared to other processing methods tested in the study. Seasonal variations in nutrient composition were observed, with the dry season feed resources containing lower protein and higher cell wall carbohydrates. The presence of lignin in sun and oven-dried rumen digesta limited digestibility due to reduced microbial access to structural polysaccharides. Higher fibre levels were associated with lower in vitro digestibility, reinforcing the importance of the processing method in improving nutrient utilization.

In this study, the total microbial load of fresh rumen digesta was found to be lower than the reported value of 17.8 log cfu/g for dried rumen digesta by Mondal et al. (2013), but higher than the figure reported by Agolisi *et al*. (2022) in their previous study on rumen digesta. The application of urea in the processing method had a suppressive effect on lactic acid bacteria (LAB) due to its buffering capacity. LAB presence in diets is typically associated with improved gut health, digestion, and fermentation (Pessione, 2012). Except for the sun-dried method, all the processing methods used in this study showed no presence of Salmonella spp. Escherichia coli was found in the fermented and sun-dried methods but was absent in the oven-dried and urea-fermented methods. This difference in microbial presence could be attributed to the high temperature utilized in the processing methods, which likely exerted a bactericidal effect.

The analysed nutrient composition of the urea-fermented DRD-based concentrate diet revealed a significant difference between the urea-fermented DRD pellets and urea-fermented DRD unpelleted diets. The moisture content of the pelleted diet increased. However, the crude protein, EE, NDF and ADF contents decreased in the pelleted diet compared to the unpelleted diet. This is attributed to the thermal processing as a result of the steam produced which increased the moisture content. Amdt *et al.* (1999), observed decreased protein solubility of soy flour when exposed to high cooking temperatures. Pelleting ingredients can improve the availability of some nutrients such as starch gelatinization (Medel *et al*., 2004). The improvement in NDF and ADF values is an indication that pelleting feedstuff improves nutrient availability, it is globally, known that pelleting improves feed quality (Medel *et al*., 2004).

The unpelleted urea-fermented DRD diet recorded Escherichia coli species even though there were no Escherichia coli species in DRD for the urea-fermented method which was selected for this study. *Escherichia coli* species was present in the other feed ingredients used. The unpelleted urea-fermented DRD diet had a higher microbial load than the pelleted diet. A similar trend was obtained in the lactic acid bacteria and this was attributed to the thermal processing of the diet. The microbial load for the urea-fermented DRD-based concentrates was higher than the average 3.20 cfu/gDM reported by Agolisi *et al.* (2022). The microbial load increased as levels of the DRD increased in the concentrate diet which agreed with Agolisi *et al*. (2022).

Supplementing 50% of graded of levels pelleted urea-fermented DRD-based concentrate increased the CP content for all the supplemented diets as levels of urea-fermented DRD based concentrate increased and were within the NRC (2007) recommended 12% CP of total DMI for sheep growth and maintenance. Supplementation of graded levels of urea-fermented DRD-based concentrate decreased the NDF and ADF fractions for all the supplemented diets. The gradual increases in NDF and ADF fractions are in line with the report of Olafadehan *et al*. (2014) who reported an increase in NDF and ADF values when DRD was incorporated into the diets of cattle.

The digestible organic matter was higher in the 15% urea-fermented DRD concentrate supplemented with 50% rice straw. This is attributed to the relatively higher crude protein content in the diet which supported cellulolytic microbes to digest the dry matter. The asymptote gas production (b) showed no significant variation. However, the control diet recorded the higher asymptote gas production (b) but recorded the lowest DOM suggesting inefficiency in the digestibility of the feed. This could lead to the emission of methane into the atmosphere when fed to ruminant livestock. Higher gas production from microbial fermentation with a corresponding low digestible organic matter suggests a potential inefficiency in the digestibility of the diet in this study. The ME recorded in this study for the supplemented diet fell within the ME recommended by NCR (2007).

The study found comparable dry matter intake values between the control diet and the diet containing dried rumen digesta up to 15%. This suggests that the processed rumen digesta, at the given dietary levels, was equally palatable as the control diet and did not have any negative impact on consumption. The inclusion of pelleted experimental diets helped prevent selective feeding by the rams. This finding is consistent with the results reported by Cherdthong *et al*. (2014), who observed no significant difference in the intake of diets containing rumen digesta-based concentrate. In addition, Mondal et al. (2013) also reported no significant differences in dry matter intake when incorporating dried rumen digesta into the diet of Bengal goats at a 10% inclusion level. Similarly, Fajemisin *et al.* (2010) found no significant effect on dry matter intake when Djallonké sheep were fed a diet containing fermented rumen digesta mixed with poultry droppings. Overall, these findings indicate that including dried or fermented rumen digesta in the diets of ruminant animals did not negatively impact their dry matter intake, suggesting that these processed forms of rumen digesta were well-accepted by the animals and did not hinder their overall feed consumption.

Significant differences were observed in the daily crude protein (CP) intake among the treatments, with the highest intake recorded in rams fed a 15% urea-fermented DRD concentrate. This higher CP content in the DRD-based concentrate diet contributed to the increased CP intake observed in the T3 treatment group. The apparent digestibility coefficient of CP also showed an increase with higher levels of urea-fermented DRD, reaching its highest value in rams fed a 15% urea-fermented DRD concentrate.

Various parameters related to live weight and growth performance, including final live weight (in kilograms), live weight gain (in kilograms), daily live weight gain (in grams), and feed conversion ratio (FCR, in grams of dry matter intake per gram of average daily gain), exhibited significant differences among the treatments. Rams fed a 15% urea-fermented DRD concentrate had a significantly higher live weight gain (3.75 kg) compared to the control group (1.75 kg). The average daily live weight gain followed a similar trend, with the rams on the 15% urea-fermented DRD concentrate recording twice the gain observed in the control rams. The feed conversion ratio was also significantly lower in the group fed the 15% urea-fermented DRD concentrate compared to the control group. Similar improvements in apparent nutrient digestibility have been reported in previous studies that included DRD in the diets of beef cattle (Cherdthong and Wanapat, 2014; Seankamsrn and Cherdthong, 2020) and sheep (Agolisi *et al.*, 2022) when compared to diets without DRD.

The significant difference in the final live weight gain of sheep on urea-fermented DRD compared to the 0% urea-fermented DRD is an indication of the practical effect of the superior digestibility of the nutrients in the urea-fermented DRD pellet concentrate. The present daily weight gains compared closely with the 48.21-56.47 g/d recorded previously by Agolisi et al. (2022), but lower than the 79-91 g/d reported by Baiden *et al.* (2007) for Djallonké sheep.

The nutritional status of animals can significantly impact their haematological traits, and these traits serve as indicators of their overall nutritional health (Adeyele *et al.*, 2017; Olorruntola *et al.*, 2018). The haematological parameters evaluated in the rams fed the different dietary treatments in this study indicated no adverse effects on the health of the sheep. The observed values of haemoglobin and packed cell volume (PCV) were within the normal physiological range for sheep, as reported by previous studies (Pampori, 2003; Orheruata *et al.*, 2004; Merck Veterinary Manual, 2010). These parameters, along with mean corpuscular haemoglobin (MCH), are important indices for assessing circulating erythrocytes and are valuable in diagnosing anaemia and evaluating the bone marrow's capacity for red blood cell production in mammals (Chineke *et al.*, 2006). In an earlier study, Agolisi *et al.* (2022) reported haemoglobin and PCV ranges of 8.50 to 8.95g/dL and 32.55 to 33.20%, respectively, for the same breed of sheep fed various levels (0, 4, 8, and 12%) of DRD-based concentrate. These findings align with the results observed in the present study. Changes in haematological parameters hold significance in assessing animal responses to various physiological and disease conditions (Yadav *et al.*, 2002; Khan, 2005).

White blood cells (WBCs) play a crucial role in the immune response by combating infections and foreign substances entering the body. The similarity in WBC counts among the rams in this study suggests that their ability to fight infections was not compromised with the inclusion of urea-fermented DRD in the concentrate diet. The WBC counts observed were also within the normal range reported for sheep, which typically falls between 5 x 10^9/L and 4 to 12 x 10^9/L (Scott et al., 2006; Merck Veterinary Manual, 2010). Similar findings were reported by Konlan *et al.* (2012), who observed a WBC range of 8.37 to 9.30 x 10^9/L in Djallonké sheep fed a basal diet of rice straw and groundnut haulms supplemented with graded levels of shea nut cake concentrate. These results indicate that the inclusion of urea-fermented DRD in the concentrate diet did not negatively impact the WBC counts of the rams, and the observed values remained within the normal range for healthy sheep.

Serological "parameters provide vital information on the physiological well-being of animals (Bellows *et al.*, 1963). The relatively normal ranges even at the highest level of urea-fermented DRD inclusion for most of the serological parameters measured in the rams in the present study suggests that the secretion of enzymes was not adversely affected in animals indicating the potential of urea-fermented DRD pellets as diets for sheep. The inclusion of urea-fermented DRD in the diet had a significant impact on the albumin levels, which ranged from 22.74 to 27.10 g/dL. These values were similar to those reported by Konlan *et al.* (2012), Ansah *et al.* (2016), and Agolisi *et al.* (2022) for Djallonké rams on supplementation but higher than the range reported by Pampori (2003). However, these values fell within the normal physiological range of 24 to 30 g/dL as reported by Jackson and Cockcroft (2002) for sheep". Albumin concentration serves as an indicator of protein metabolism and accounts for about 50-65% of total blood protein (Contreras *et al*., 2000).

The serum protein levels did not show significant differences among the diets but remained within the normal range of 60 to 93 g/dL reported for sheep (Contreras *et al*., 2000). This finding aligns with previous studies that reported no significant effect on serum protein levels in lambs fed DRD-based concentrate diets (Aruwayo *et al*., 2007; Agolisi *et al*., 2022).

9.1.0 General Conclusions and Recommendations

9.2 Conclusions

- Dry season rumen digesta were generally inferior to wet season digesta relative to CP and DOM. Urea-treated dried rumen digesta contained an adequate amount of crude protein to serve as a dietary source for sheep.
- The total microbial count was reduced by about 50% with the application of the processing methods.

- Pelleting depressed microbial population in urea-fermented dried rumen digesta.
- Supplementing 50% of rice straw graded level of pelleted dried rumen digesta increased digestible organic matter and metabolizable energy
- Dietary inclusion of graded level of urea-fermented dried rumen digesta had no adverse effect on feed intake feed, live weight gain and apparent nutrient digestibility of the supplemented animals.
- The inclusion of urea-fermented dried rumen digesta in the diets of lambs to replace 15% of maize bran did not compromise the health status of lambs.

9.3 Recommendations

- Urea could be used to treat rumen digesta to enhance the crude protein content to serve as a dietary source of protein for ruminant animals.
- Urea-fermented Dried rumen digesta can be used to replace 15% of dietary maize bran in the diets of lambs without compromising the growth performance and health status of lambs.
- Further studies could explore the long-term effects and potential benefits of DRD-based concentrate diets on sheep productivity and performance.

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APPENDIX A

Plate 9: Fresh Rumen Digesta in Containers

Plate 10: Expelling water to reduce the moisture content

Plate 11: Sun-drying fresh rumen digesta

Plate 12: Oven drying fresh rumen digesta

Plate 13: Fermentation of fresh rumen digesta

Plate 14: Processed rumen digesta

Plate 15: Pellet urea-fermented DRD-based concentrate

Plate 16: Drying the pellets

Plate 17: Packaged DRD-based concentrate

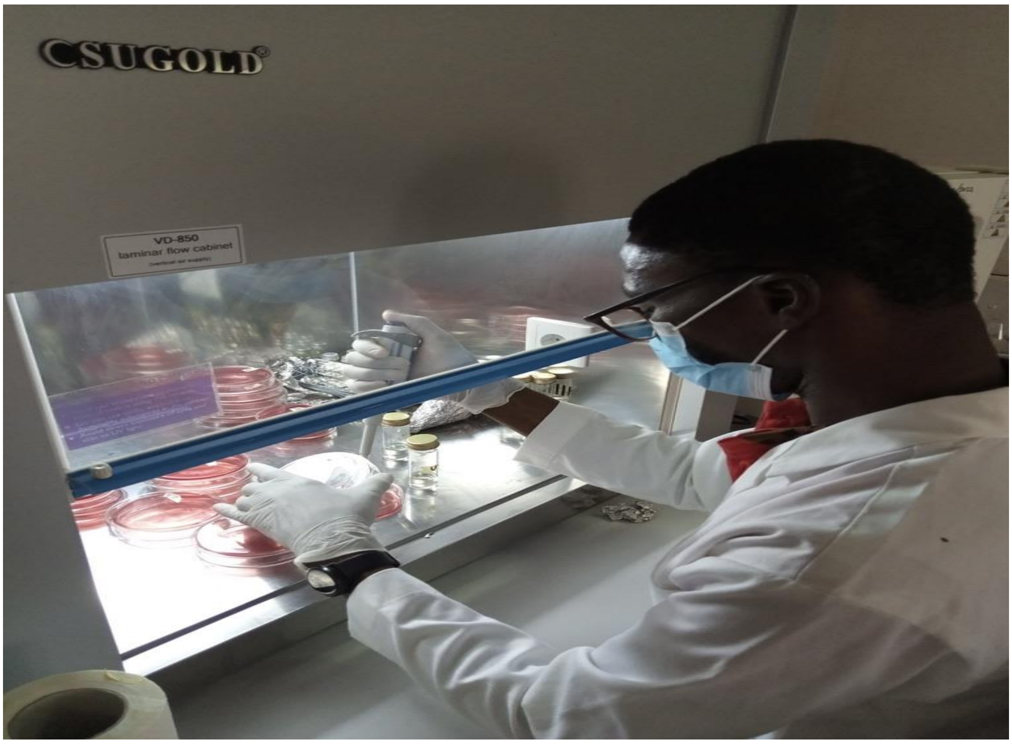

Plate 18: Culturing for microbial

Plate 19: *In vitro* gas production

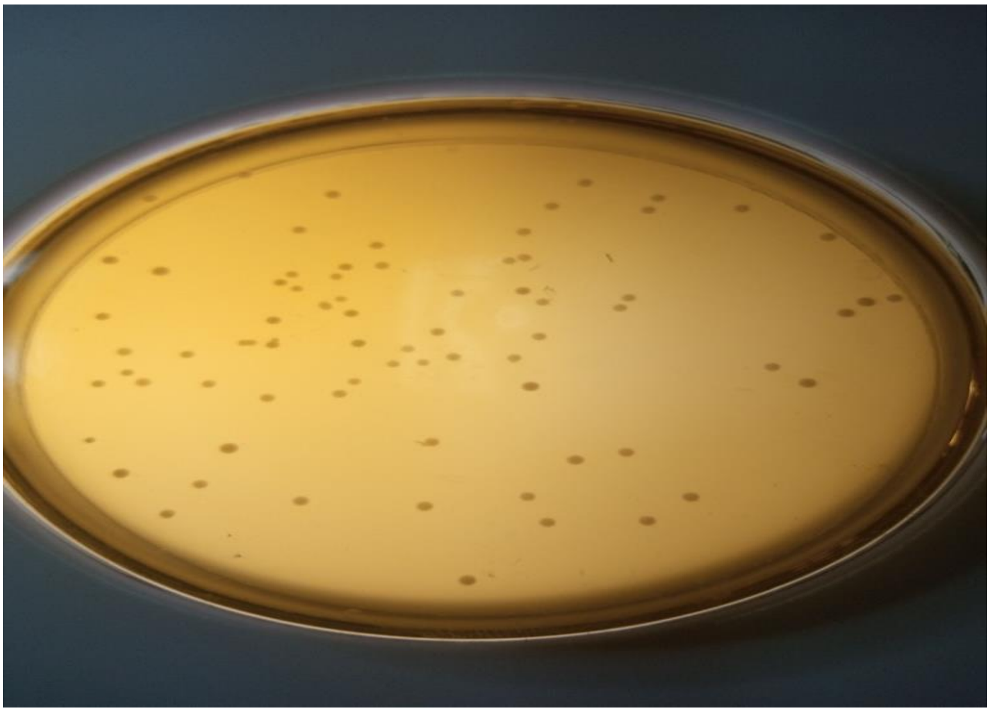

Plate 20: *Lactic acid bacteria*

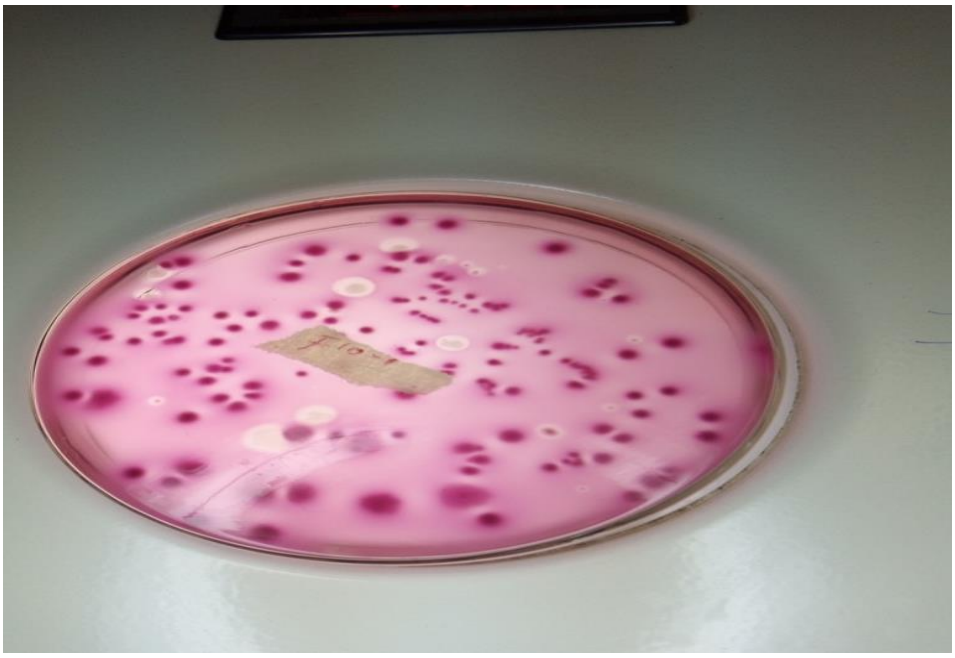

Plate 21: *E. coli*

APPENDIX B

Analysis of variance

Variate: % Dry Matter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	10.2656	3.4219	14.60	<.001
SEASON	3	47.3906	15.7969	67.40	<.001
METHOD.SEASON	9	28.1719	3.1302	13.36	<.001
Residual	32	7.5000	0.2344		
Total	47	93.3281			

Variate: %_Crude Protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	338.87238	112.95746	6737.25	<.001
SEASON	3	1196.55744	398.85248	23789.20	<.001
METHOD.SEASON	9	140.42139	15.60238	930.59	<.001
Residual	32	0.53652	0.01677		
Total	47	1676.38773			

Variate: %_Crude_Fat

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	21.43779	7.14593	120.37	<.001
SEASON	3	38.74623	12.91541	217.56	<.001
METHOD.SEASON	9	43.99682	4.88854	82.35	<.001
Residual	32	1.89966	0.05936		
Total	47	106.08051			

Variate: %ASH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	34.8750	11.6250	41.33	<.001
SEASON	3	264.7500	88.2500	313.78	<.001
METHOD.SEASON	9	16.1250	1.7917	6.37	<.001
Residual	32	9.0000	0.2812		
Total	47	324.7500			

Variate: IVOMD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	41.663	13.888	5.87	0.003
SEASON	3	272.021	90.674	38.32	<.001
METHOD.SEASON	9	46.490	5.166	2.18	0.051
Residual	32	75.710	2.366		
Total	47	435.885			

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	29.362	9.787	8.28	<.001
SEASON	3	23.329	7.776	6.58	0.001
METHOD.SEASON	9	12.582	1.398	1.18	0.339
Residual	32	37.833	1.182		
Total	47	103.106			

Variate: c

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	0.072450	0.024150	23.31	<.001
SEASON	3	0.033077	0.011026	10.64	<.001
METHOD.SEASON	9	0.075804	0.008423	8.13	<.001
Residual	32	0.033157	0.001036		
Total	47	0.214487			

Variate: ME_g_DM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	3	9.9357	3.3119	26.30	<.001
SEASON	3	9.5704	3.1901	25.33	<.001
METHOD.SEASON	9	3.1789	0.3532	2.80	0.015
Residual	32	4.0304	0.1260		
Total	47	26.7153			

Variate: %CP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	3.08740	3.08740	45.35	<.001
GRADED_LEVEL	3	21.89054	7.29685	107.18	<.001
METHOD.GRADED_LEVEL	3	0.85428	0.28476	4.18	0.023
Residual	16	1.08925	0.06808		
Total	23	26.92147			

Variate: IVOMD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	6.146	6.146	2.48	0.135
GRADED_LEVEL	3	28.409	9.470	3.82	0.031
METHOD.GRADED_LEVEL	3	9.197	3.066	1.24	0.329
Residual	16	39.617	2.476		
Total	23	83.370			

Variate: ME_g_D

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	0.19658	0.19658	3.85	0.068
GRADED_LEVEL	3	1.26664	0.42221	8.26	0.002
METHOD.GRADED_LEVEL	3	0.05668	0.01889	0.37	0.776
Residual	16	0.81800	0.05112		
Total	23	2.33790			

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	15.585	15.585	5.90	0.027
GRADED_LEVEL	3	40.241	13.414	5.08	0.012
METHOD.GRADED_LEVEL	3	4.632	1.544	0.58	0.634
Residual	16	42.241	2.640		
Total	23	102.700			

Variate: Total_Bacteria_Count

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Method	1	0.853450	0.853450	373.98	<.001
Graded_level	3	4.620651	1.540217	674.92	<.001
Method.Graded_level	3	0.005398	0.001799	0.79	0.518
Residual	16	0.036513	0.002282		
Total	23	5.516011			

Variate: LAB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Method	1	0.861044	0.861044	236.74	<.001
Graded_level	3	2.875492	0.958497	263.54	<.001
Method.Graded_level	3	0.364300	0.121433	33.39	<.001
Residual	16	0.058192	0.003637		
Total	23	4.159028			

Variate: E_coli

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Method	1	22.198872	22.198872	5816.99	<.001
Graded_level	3	1.148535	0.382845	100.32	<.001
Method.Graded_level	3	1.148535	0.382845	100.32	<.001
Residual	16	0.061059	0.003816		
Total	23	24.557001			

Variate: %DM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	59.1262	59.1262	104.97	<.001
GRADED_LEVEL	3	3.1857	1.0619	1.89	0.173
METHOD.GRADED_LEVEL	3	2.5684	0.8561	1.52	0.248
Residual	16	9.0127	0.5633		
Total	23	73.8930			

Variate: %Crude_Protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	4.38615	4.38615	102.37	<.001
GRADED_LEVEL	3	37.36203	12.45401	290.67	<.001
METHOD.GRADED_LEVEL	3	0.82242	0.27414	6.40	0.005
Residual	16	0.68553	0.04285		
Total	23	43.25613			

Variate: %ADF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	89.593	89.593	29.55	<.001
INCLUSION_LEVEL	3	108.009	36.003	11.87	0.003
METHOD.INCLUSION_LEVEL	3	15.984	5.328	1.76	0.233
Residual	8	24.258	3.032		
Total	15	237.844			

Variate: %NDF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	290.06	290.06	17.27	0.003
INCLUSION_LEVEL	3	40.45	13.48	0.80	0.526
METHOD.INCLUSION_LEVEL	3	183.70	61.23	3.65	0.064
Residual	8	134.35	16.79		
Total	15	648.56			

Variate: %NDF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	290.06	290.06	17.27	0.003
INCLUSION_LEVEL	3	40.45	13.48	0.80	0.526
METHOD.INCLUSION_LEVEL	3	183.70	61.23	3.65	0.064
Residual	8	134.35	16.79		
Total	15	648.56			

Variate: %Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	9.7155	9.7155	28.35	<.001
GRADED_LEVEL	3	17.2021	5.7340	16.73	<.001
METHOD.GRADED_LEVEL	3	5.1763	1.7254	5.04	0.012
Residual	16	5.4824	0.3426		
Total	23	37.5764			

Variate: %_CRUDE_FAT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	3.1745	3.1745	30.64	<.001
GRADED_LEVEL	3	4.1223	1.3741	13.26	<.001
METHOD.GRADED_LEVEL	3	0.4293	0.1431	1.38	0.284
Residual	16	1.6578	0.1036		
Total	23	9.3840			

Variate: IVOMD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	5.446	5.446	1.70	0.210
GRADED_LEVEL	3	16.749	5.583	1.75	0.198
METHOD.GRADED_LEVEL	3	41.772	13.924	4.36	0.020
Residual	16	51.116	3.195		
Total	23	115.082			

Variate: b

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	16.187	16.187	4.60	0.048
GRADED_LEVEL	3	16.851	5.617	1.59	0.230
METHOD.GRADED_LEVEL	3	7.692	2.564	0.73	0.550
Residual	16	56.356	3.522		
Total	23	97.085			

Variate: c

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
METHOD	1	0.001352	0.001352	0.68	0.422
GRADED_LEVEL	3	0.005351	0.001784	0.90	0.464
METHOD.GRADED_LEVEL	3	0.000685	0.000228	0.11	0.950
Residual	16	0.031807	0.001988		
Total	23	0.039194			

Variate: Final_MCV

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
TREATMENT	3	19.960	6.653	1.14	0.94	0.377
Covariate	1	17.486	17.486	2.98		0.112
Residual	11	64.462	5.860		1.17	
Total	15	98.139				

Variate: Final_MCH

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
TREATMENT	3	1.601	0.534	0.10	0.69	0.956
Covariate	1	0.813	0.813	0.16		0.697
Residual	11	56.132	5.103		0.93	
Total	15	58.098				

Variate: Final_Neut

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
TREATMENT	3	27.188	9.063	2.40	0.86	0.123
Covariate	1	4.372	4.372	1.16		0.305
Residual	11	41.561	3.778		1.01	
Total	15	68.749				