MAKERERE UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF ANIMAL AND RANGE SCIENCES

ASSESSING THE EFFECT OF VARYING LEVELS OF POULTRY MANURE AND FERMENTATION PERIODS ON THE CHEMICAL COMPOSITION, DIGESTIBILITY, AND RUMEN FERMENTATION KINETICS OF COFFEE HUSKS

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A SPECIAL PROJECT RESEARCH REPORT SUBMITTED TO THE DEPARTMENT OF ANIMAL AND RANGE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE IN AGRICULTURE OF MAKERERE UNIVERSITY

NOVEMBER 2025

DECLARATION

I MUSIIMENTA SARAH declare that this research titled "**ASSESSING THE EFFECT OF VARYING LEVELS OF POULTRY MANURE AND FERMENTATION PERIODS ON THE CHEMICAL COMPOSITION, DIGESTIBILITY, AND RUMEN FERMENTATION KINETICS OF COFFEE HUSKS**" is my original work and it has not been submitted to any institution of higher learning for any award.

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APPROVAL

The special project report titled **ASSESSING THE EFFECT OF VARYING LEVELS OF POULTRY MANURE AND FERMENTATION PERIODS ON THE CHEMICAL COMPOSITION, DIGESTIBILITY, AND RUMEN FERMENTATION KINETICS OF COFFEE HUSKS** has been written by MUSIIMENTA SARAH under my supervision as the university academic supervisor.

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DEDICATION

I dedicate this research to my parents for their endless spiritual, emotional and financial support, my siblings for their love and my supervisor, Dr. Pius Lutakome for guiding me throughout the research process.

ACKNOWLEDGEMENT.

I want to thank God that has given me the wisdom and strength to reach this far, for the life, good health and for His grace that has been sufficient to me and my family. I want to thank my supervisor, Dr. Pius Lutakome for his time, guidance and mentorship throughout the research period.

I also appreciate the support and encouragement from my friends Mary, Atiika and Jordan. Thank you so much.

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ABSTRACT

This study evaluated the effect of poultry manure inclusion and fermentation periods on the chemical composition, in vitro digestibility, and rumen fermentation kinetics of coffee husks for the development of ruminant feed. Coffee husks, though abundant in Uganda, are underutilized due to their high fiber content and anti-nutritional compounds such as tannins, caffeine, and polyphenols, which limit digestibility and nutrient absorption. While microbial fermentation has been shown to improve feed quality, access to commercial inoculants remains limited in rural areas where livestock are produced. Poultry manure offers a low-cost, locally available source of fermentative microbes, yet its optimal inclusion levels in coffee husk silage remain undefined. A 4×2 factorial experiment was conducted in a completely randomized design at Makerere University's Animal Science Laboratory. Coffee husks and poultry manure were mixed at 0%, 5%, 10%, and 25% PM (Poultry Manure) inclusion levels and fermented for 28 and 38 days. Post-fermentation, samples were dried, ground, and analyzed for dry matter, ash, crude protein, fiber fractions (NDF, ADF, ADL), calcium, and phosphorus using standard AOAC and Van Soest procedures. In vitro gas production was assessed using rumen liquor from freshly slaughtered cattle, and fermentation kinetics were modelled using Groot's equation. Organic matter digestibility and short-chain fatty acid production were estimated from gas volumes and chemical composition. Results showed that crude protein and phosphorus content increased significantly with poultry manure inclusion, while ash content declined. Fiber fractions varied, with ADL increasing at higher PM levels. Gas production kinetics revealed enhanced microbial fermentation at moderate PM inclusion, with significant increases in asymptotic gas volume and gas at the point of inflection. However, in vitro digestibility and SCFA production declined at higher PM levels, indicating a trade-off between nutrient enrichment and fermentability. The findings suggest that moderate inclusion of poultry manure (5–10%) in coffee husk improves nutritional value and fermentation efficiency, offering a sustainable feed alternative for ruminant production in Uganda

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Uganda's dairy industry has achieved remarkable growth since liberalization in 1993, with exports reaching 0.16 million tons of cattle dairy products valued at over US$120.74 million in 2019, compared to just 412 tons of imports worth US$1.69 million in the same year. Current milk production stands at 2.04 million tons, ranking Uganda third in the East African region after Kenya and Tanzania (Waiswa & Günlü, 2023). Milk production in Uganda is primarily driven by small-scale farmers, who own over 90% of the country's cattle herd. In rural areas, where 96% of poor Ugandans live, about 60% of households keep mainly indigenous cattle. (Gebremichael, 2021). However, the Uganda's dairy industry still faces challenges like high production costs, low milk yields, and low prices, leading to low profits. This has driven some farmers to replace dairy cattle with more profitable beef herds, affecting the industry's viability (Waiswa & Günlü, 2023). Therefore, the dairy sector continues to face feed scarcity due to climatic changes affecting forage quality and availability (Ekou et al., 2014). Click or tap here to enter text. To cope with feed scarcity, farmers have adopted several strategies, including changing feed resources based on availability and cost (Constantine Katongole, 2012). The most widely used feed resources are agro-industrial by-products, commercial ingredients, and crop supply residues, respectively (HL & EK Ndyomugyenyi, 2015). Maize bran and dairy meal are the most utilized agro-industrial by-products, while Maize stover and banana peels are reported to be the most utilized crop residues for animal nutrition in the three agro- ecological zones of Uganda(Mugerwa et al., 2012) Conventional alternative feeds developed for dry seasons include non-conventional options in Uganda such as animal wastes (chicken manure, cattle dung, pig dung), food wastes, and by-products like blood meal, bone meal, fish meal, and meat offal. Insect-based feeds, like black soldier fly larvae and maggots, are also used. Additionally, dairy farmers often utilize crop wastes including sweet potato vines, cassava peels, brewers' waste, and cocoyam leaves. (Gado et al., 2011).

Coffee husks have also been used by some farmers due to their availability in Uganda because of the increasing coffee production. Coffee husk is the main residue obtained in dry coffee cherry processing, which consists of outer skin, pulp, and parchment (Muzaifa et al., 2021). Studies have shown that coffee husks contain 93%dry matter,13.15% crude protein, 2.08 % ether extract,43.63 % crude fibre,29.62% nitrogen-free extracts (Permata Sari et al., 2021). While Coffee husks have been used for other uses such as biochar production, an amendment for replenishing soil nutrients (Kiggundu & Sittamukyoto, 2019), their use as livestock feed is still limited because of the anti-nutrients, such as some forms of polyphenols, tannins and caffein which hinder digestion and absorption of nutrients, affecting nutrition (Soares Gomes et al., 2024). The high fibre content of coffee husks makes them less palatable to animals.

To solve this challenge, coffee husk fermentation is done to enhance nutrient quality. Different materials have been used to ferment coffee, including *Aspergillus niger* (Milawarni et al., 2020), higher fungi such as *Pleurotus ostreotus* (Badarina et al., 2013), among other commercial microbes. However, due to the high costs and scarcity of commercial sources of fermentation microbes, farmers require cheap and readily available alternative sources of microorganisms to inoculate the coffee husks, making poultry manure an alternative. Ruminants can use non-protein nitrogen (NPN) sources to partially replace crude protein in cereal-based diets, enhancing the ruminal environment and providing ammonia for microbial protein synthesis. Chicken manure, with its high nitrogen content, serves as a cost-effective nutritional alternative for substituting conventional feeds in ruminant diets. (Rosa et al., 2019). Poultry manure contains microorganisms that could improve the nutritional value of CH (Kadam et al., 2024).

In the study by Wan et al., (2020), cellulose-degrading microbes, *including Bacillus licheniformis, Bacillus amyloliquefaciens, Ureibacillus thermosphaericus, Bacillus megaterium, Geobacillus pallidus, Bacillus pumilus, Geobacillus sp.,* and *Paracoccus denitrificans,* were identified from poultry manure and maize straw compost, demonstrating their potential role in enhancing cellulose breakdown and improving composting efficiency. In light of these challenges, this study sets out to explore how poultry manure can be harnessed as a simple, affordable, and locally available inoculant to improve the nutritive value of coffee husk silage. This research, therefore, aimed at determining the optimal inclusion levels of poultry manure (0, 5, 10, 15, and 20%) and fermentation periods (28 and 38 days) for coffee husk silage to improve its nutritive value and digestibility for dairy cattle. In doing so, it seeks to provide smallholder farmers with a practical, low-cost feeding strategy that enhances milk production, reduces feed costs, and promotes sustainable use of agricultural by-products in Uganda's dairy sector.

1.2 STATEMENT OF THE PROBLEM

The problem to be addressed through this study is the low digestibility of coffee husks (CH) when used as a feed resource for dairy cattle in Uganda. Uganda produces about 2.04 million tons of milk annually from 4.14 million cows, averaging 492.8 liters per cow. Roughly 80% of this milk is marketed, earning US$835.9 million in 2019, with 34% processed into products and 66% sold raw. Exports reached 0.16 million tons worth US$120.74 million, while imports were just 412 tons valued at US$1.69 million (Waiswa & Günlü, 2022). Despite the growth of Uganda's dairy industry, feed shortages remain a major challenge. Urban farms face high costs, with feed accounting for over 60% of expenses, while resource-poor farmers rely on roadsides, swamps, and industrial by-products for fodder. These constraints limit productivity and highlight the need for sustainable feeding solutions (M. et al., 2025).

Coffee husks, a major agro-industrial byproduct in coffee-producing regions, are often discarded or underutilized, despite their potential as a feed resource for dairy cattle (Cangussu et al., 2021). These could be utilised as non-conventional feed resources for dairy cows. However, their direct use in dairy rations is limited by poor digestibility and nutrient imbalance, which restricts milk yield and quality (Marew et al., 2024). This presents both an environmental challenge and a missed opportunity for dairy farmers who face high feed costs and seasonal shortages.

Poultry manure, rich in nitrogen and non-protein nitrogen, has been shown to improve microbial protein synthesis, cellulose digestion, and balance the nutrient profile of fibrous residues, offering a low-cost supplement for dairy diets (Rosa et al., 2019). Yet, the optimal inclusion levels of poultry manure in coffee husks remain unclear, raising concerns about excess nitrogen, palatability, and potential toxicity (Rosa et al., 2019).

Additionally, the inoculum levels used in rumen fermentation studies significantly influence microbial activity, fermentation kinetics, and variability in digestibility estimates (Kang et al., 2024). Determining effective inoculum concentrations is essential for reliable evaluation of coffee husks supplemented with poultry manure in dairy feeding systems (Rosa et al., 2019). Similarly, controlled fermentation periods are important for improving fiber degradation and stabilizing coffee husks. Short fermentation periods may fail to sufficiently alter the feed, while excessively long durations risk nutrient losses and undesirable microbial activity (Nkosi et al., 2024; Kang et al., 2024).

Despite these promising approaches, there is limited literature on how poultry manure inclusion levels, inoculum concentrations, and fermentation durations interact to improve the chemical composition, digestibility, and rumen fermentation kinetics of coffee husks for dairy cattle. This knowledge gap limits the ability to transform coffee husks into a safe, consistent, and cost-effective feed resource.

1.3 JUSTIFICATION

Uganda's agricultural sector generates substantial quantities of coffee husks as a by-product of coffee processing, particularly in central regions such as Mukono and Kampala (UCDA, 2024). Despite their abundance, coffee husks remain underutilized in dairy feeding due to their high fiber content, which reduces palatability and hinders nutrient absorption in ruminants (Tripathi et al., 2025). These limitations contribute to feed inefficiency and discourage farmers from adopting coffee husks as a reliable feed resource.

Microbial fermentation has been demonstrated to enhance the nutritional value of fibrous agricultural residues by reducing anti-nutritional factors and improving digestibility (Esther & José, 2020). However, the use of commercial microbial inoculants is often impractical for smallholder farmers due to high costs and limited availability in rural livestock-producing areas. This creates a gap between scientific recommendations and on-the-ground feasibility, leaving farmers vulnerable to seasonal feed shortages and high production costs.

Poultry manure presents a promising alternative inoculant. It is widely available in peri-urban and rural areas and contains fermentative microbes and nutrients that can enhance the ensiling process. When combined with coffee husks, poultry manure has the potential to improve crude protein content, stimulate microbial activity, and reduce fiber fractions, making the final product more digestible and nutritionally balanced (Sagni et al., 2024). Nevertheless, there is limited empirical data on the optimal inclusion levels of poultry manure in coffee husk silage, particularly regarding its effects on chemical composition, in vitro digestibility, and rumen fermentation kinetics.

This study is therefore justified by the need to provide practical, low-cost, and locally adaptable solutions for feed scarcity in Uganda. Animal feeds account for approximately 70% of total livestock production costs (Uganda, 2023). Improving access to affordable, nutritious feed resources is essential for enhancing milk yields, productivity, and resilience among smallholder dairy farmers. Moreover, Uganda's dairy sector plays a critical role in household income, food security, and national development. According to the Uganda Bureau of Statistics, 72.8% of households keep at least one type of livestock, with dairy cattle being a major contributor to rural livelihoods. Importantly, 34.1% of these households are headed by women and 20.1% by youth, making dairy farming a key driver of gender equity and youth empowerment (Statistics, 2024).

By identifying the optimal poultry manure inclusion levels and fermentation durations for coffee husk silage, this study addresses a critical knowledge gap and contributes to sustainable livestock production, waste valorization, and climate-smart agriculture. It aligns with Uganda's development priorities and the Millennium Development Goals (MDGs), particularly those focused on eradicating extreme poverty and hunger, promoting gender equality, and ensuring environmental sustainability (United Nations, 2015). Furthermore, it supports the transition toward the Sustainable Development Goals (SDGs), which emphasize inclusive growth, food security, and climate action.

1.4 OBJECTIVES

1.4.1 General objectives

- To enhance the utilization of coffee husks as alternative non-conventional feed resource for improved dairy production.

1.4.2 Specific objectives

1. To evaluate the effect of varying inclusion levels of poultry manure and fermentation duration on the chemical composition of coffee husks.
2. To evaluate the effect of varying inclusion levels of poultry manure and fermentation duration on rumen kinetics of coffee husks.

1.4 HYPOTHESIS

- Treating coffee husks with poultry manure at varying inclusion levels enhances the chemical composition of coffee husks.
- Poultry manure inclusion increases rumen degradability of coffee husks

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Current status of the feed industry

Uganda's population is expected to reach 102 million by 2050, driving a sharp rise in demand for animal-source foods. Meat and milk production must increase significantly to meet this need, with beef, poultry, and pork consumption projected to more than triple. Nearly 60% of households rely on livestock for their livelihoods, with cattle, goats, pigs, sheep, and poultry being the most commonly farmed animals. Feed quality is the most critical factor in boosting productivity, especially in intensive systems, as it accounts for about 70% of production costs (Kugonza, 2025)

Rapid urbanization in developing nations is shrinking pasturelands and limiting space for forage cultivation. At the same time, climate change and variability are degrading the availability and quality of feed. Smallholder livestock producers face persistent hurdles—scarcity of compound feeds, limited access to improved forages, and rising costs of supplements. These pressures have pushed farmers to explore alternative feed options, including agricultural by-products like coffee husks (Marew et al., 2024)..

Across the plant-based food supply chain, harvesting and processing for human consumption often result in perishable and potentially harmful byproducts that are not properly managed, potentially harming the environment. These by-products, however, offer various practical uses. A particularly beneficial option in animal nutrition is repurposing them as alternative feed sources, especially for herbivorous livestock Garc et al., 2019).

2.2 Origin and distribution of coffee.

Coffee is one of the world's most valuable commodity crops, second only to petroleum in global trade, and it significantly supports the economies and livelihoods of millions in over 50 countries across Asia, Latin America, and Africa (Funlayo et al., 2017). Coffee originated in Ethiopia, where the rich biodiversity and cultural heritage gave rise to the world's first cultivation of Arabica beans (Duressa, 2018). Coffee contributes 15% of Uganda's export earnings, with production dominated by Robusta and Arabica in a 4:1 ratio. Robusta includes *Nganda, Erecta,* and high-yielding Clonal types, while Arabica varieties—SL 28, SL 14, KP 423, and Nyasaland—are grown in mountainous regions like Elgon, Rwenzori, and Zeu in Zombo District (UCDA, 2016).

Coffee thrives as a plantation crop in tropical highlands for C. Arabica and lowlands *for C. canephora,* requiring 15–30°C temperatures, well-distributed rainfall, and a dry season of under five months. Its shade tolerance and forest-like growth needs make it suitable for agroforestry, with ideal soils being deep, permeable, well-drained, and aerated (Pohlan, n.d.).

2.3 Feeding in ruminants

Managing diet composition is a practical way to support animal health and product quality. While cattle industries have advanced in genetics and nutrition, intensive systems may harm welfare and raise disease risks. Climate change adds pressure by reducing forage availability and exposing animals to extreme temperatures. Rising demand for animal products calls for sustainable nutritional strategies that align with ethical and environmental standards (McGrath et al., 2018)

Ruminants can convert plant fiber into valuable products like milk and meat. Incorporating by-products from crop production into their diets can lessen environmental waste and promote a circular economy by recycling biomass. Additionally, using these by-products as feed can help alleviate competition for food and land between ruminants and humans or non-ruminant animals, thereby reducing the demand for traditional feed resources (Garc et al., 2019). In ruminant metabolism, the generation of acetate and butyrate results in the release of free hydrogen, whereas propionate synthesis offers an alternative route for hydrogen utilization within the rumen. Methane, a potent greenhouse gas, contributes to energy loss in ruminants and significantly impacts global warming. Therefore, strategies aimed at reducing enteric methane emissions not only help mitigate greenhouse gas output but also improve the overall productivity of livestock systems (Palangi & Lackner, 2022).

2.4 The use of poultry manure as feed

Pollution from pig, cattle, and poultry farms has become one of the most pressing environmental challenges, with poultry manure emerging as a major contributor due to its volume and disposal difficulties. As modern poultry farms grow in scale, the management of manure —linked to odour, soil and water contamination, and air pollution— demands innovative solutions. One such solution is the use of poultry manure as livestock feed, particularly for ruminants, which can efficiently utilize its high protein and mineral content. This approach not only reduces environmental waste but also aligns with "environmentally balanced feed management systems" that aim to minimize nutrient output from animals. By converting manure into a nutritional resource, farmers can address both ecological concerns and feed cost pressures, promoting sustainable livestock production (Henuk & Dingle, 2003).

Dried poultry manure has long been recognized as a viable feed resource for ruminants, offering both nutritional and economic advantages. Its incorporation into cattle diets can significantly reduce feed costs by recycling nutrients that would otherwise contribute to environmental waste. The savings achieved through this approach are often sufficient to offset the cost of drying, making it a practical solution for dairy and beef producers (Ghaly & Macdonald, 2012). Poultry wastes are an important source of energy as well as an unconventional non-protein nitrogen source for ruminants (Narasimha et al., 2013)

Ruminants possess a unique digestive system that allows them to efficiently utilize the nutrients in DPM for productive functions such as growth, milk production, and reproduction. When properly processed and incorporated into balanced diets, DPM not only reduces feed costs but also promotes sustainable agriculture by recycling poultry waste into useful livestock feed (Näsi, 1979).

2.5 Use and nutrient composition of coffee husks

Nutrition plays a vital role in confined cattle production, where high feed costs drive up overall expenses. As a solution, agro-industrial residues—especially coffee husks—offer a promising, sustainable alternative for ruminant feeding (Permata Sari et al., 2021). The use of agro-industrial by-products as animal feeds has grown in importance due to their potential to substitute conventional feedstuffs. Energy-rich by-products can replace grains, fibre-rich ones can substitute roughage, and nitrogen-rich materials may serve as protein supplements. Understanding their chemical composition and nutritive value is essential before recommending their inclusion in rations (Garc et al., 2019). Coffee husks (CHs) and coffee pulp are the solid residues obtained after dehulling coffee cherries during dry or wet processing, respectively (Kouamé et al., 2011). Coffee husk and pulp, despite their caffeine and tannins that hinder degradation, are rich in lignocellulosic materials, making them ideal for microbial processes. These by-products can be used to produce biogas, enzymes, mushrooms, and compost, offering sustainable waste management solutions. Their inclusion in composting, often with animal manures and agricultural residues, promotes organic waste recycling and enhances soil fertility, turning an environmental challenge into a valuable resource. (Dzung et al., 2013)

The use of CHs in animal feed is still limited because CHs contain antinutrients such as some forms of polyphenols, tannins, and caffeine, which hinder the digestion and absorption of nutrients, affecting nutrition (Gomes et al., 2024). Tannins play a significant role in modulating ruminal microbial activity, particularly by suppressing proteolytic bacteria, which in turn inhibits nitrogen metabolism in the rumen. Their selective action on ruminal bacteria also reduces bio-hydrogenation, allowing a greater flow of unsaturated fatty acids to the duodenum. This microbial shift contributes to increased levels of conjugated linoleic acid in meat and milk, although the effect is dosage-dependent. Overall, tannins enhance nitrogen utilization efficiency by promoting nitrogen retention within the animal's body (Besharati et al., 2022).

Coffee husk and coffee pulp have proven to be valuable substrates for agricultural recycling, particularly in mushroom cultivation, composting, and bioprocessing. Despite containing caffeine and tannins that can slow natural degradation and pose disposal challenges, their richness in lignocellulosic materials makes them ideal for microbial activity. These by-products have been successfully used to produce biogas, enzymes, and compost, contributing to sustainable waste management. When mixed with animal manures and phosphate rock, coffee husk can be composted through anaerobic and aerobic fermentation methods, yielding high-quality compost suitable for soil application after six months (Dzung et al., 2013).

2.6 Fermentation

In vitro techniques, including chemical composition analysis and digestibility assays, are effective for assessing the nutritional value of agro-industrial by-products. These methods are more cost-efficient than in vivo experiments, offering better control over conditions and allowing for quick screening of various feed options. However, the high variability in the nutritional content of by-products, such as coffee husks, complicates the creation of universal feeding guidelines for ruminants. This highlights the need for targeted studies to assess the digestibility and fermentation properties of individual by-products as potential alternative feed sources. (Garc et al., 2019).

The method of *in vitro* degradability is simpler and more economical to obtain the feed digestibility and aims to mimic the fermentation of rumen-reticulum using rumen liquid and/or fibrolytic enzymes. The technique has applications and advantages, such as the determination of digestion rate and digestion extension, which is related to digestibility and affects the passage rate and consumption (Moura et al., 2017)

White-rot fungi, particularly *Pleurotus* species, are renowned for their ability to degrade lignin in plant biomass through the action of extracellular lignin-modifying enzymes. These enzymes catalyze non-specific oxidation reactions that break down lignin, thereby enhancing access to cellulose, making these fungi highly effective in processing lignocellulosic materials. Compared to bacteria, white-rot fungi exhibit superior efficiency in lignin degradation, which facilitates the utilization of cellulose for nutritional and metabolic purposes. *Pleurotus* species thrive on a wide range of lignocellulosic substrates, including decaying wood, wood residues, and agricultural wastes such as coffee husks, making them valuable agents in organic waste transformation and sustainable feed development (Adewole & Olanrewaju, 2017)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site.

The experiment was conducted in the animal science laboratory, Department of Animal and Range Sciences, College of Agricultural and Environmental Sciences, Makerere University, located in Kampala, central Uganda. The laboratory is located approximately 5Km northwest of Kampala city at a latitude of 0.3343°N and 32.5675°E, approximately 1240 m above sea level.

The area experiences a tropical savanna climate with temperatures between 18 °C and 28°C and two distinct rainy seasons with annual rainfall of about 1200mm (Jury, 2018).

3.2 Collection of materials and experimental design.

3.2.1 Collection of materials

Fresh poultry manure was collected from the KOICA (Korea International Cooperation Agency) farm found in MUARIK (Makerere University Agricultural Research Institute Kabanyoro, located along Gayaza -Zirobwe road, Wakiso District. The farm rears kuroiler birds in a battery cage system for mainly egg production and the birds are fed on locally sourced feeds majorly maize bran and soybean meal, mineral supplements including salt and premixes and additives including antibiotics and vitamins. Poultry manure was collected on 16th May, 2025 in a polyethene bag and transported and stored at room temperature and then mixed homogeneously before use.

Coffee husks were collected from Redcherry Holdings limited located on Kampala-Jinja Road express way, Kyetume, Mukono about 22.5km from Kampala. The factory uses semi wet processing in peeling of the skin and fermentation.

Experimental design

The experiment was a 4x2 factorial design comprised of four treatments made by mixing randomly different proportions of coffee husks and poultry manure in a completely randomised design and ensiled for 28 and 38 days, as shown in Table 1.Samples were ensiled for 28 days because similar studies done to to use coffee husks for biomethanation show that 28 days were required to produce methane after inoculation of coffee husks with mycophyta fungi(Jayachandra et al., 2011). Also other studies done to produce reducing sugars from coffee husksby pre-treatment with white rot fungi show that 21 days are required to produce high yieds(Sabogal-Otálora et al., 2022).

During ensiling,the fresh poultry manure (used as an inoculant) and coffee husks were mixed homogeneously in required proportions and molasses added to support growth of microbes. The mixtures were then packed and compacted in airtight plastic containers and then covered and sealed with seal tape to provide anaerobic conditions required for fermentation. The containers were then stored at room temperature in the laboratory for the required period of time.

Treatment	PM (%)	CH (%)	Fermentation Days	Biological replicates
1	0	100	28	3
2	5	95	28	3
3	10	90	28	3
4	20	80	28	3
1	0	100	38	3
2	5	95	38	3
3	10	90	38	3
4	20	80	38	3

Treatment formulation

Four dietary treatments were formulated by varying the proportions of poultry manure (PM) and coffee husk (CH). Treatment 1 consisted of 0% PM and 100% CH, serving as the control. Treatment 2 contained 5% PM and 95% CH, Treatment 3 included 10% PM and 90% CH, while Treatment 4 comprised 25% PM and 75% CH. Each of the 4 treatments was replicated 6 times to enhance statistical reliability and minimize experimental error, resulting in a total of 24 samples. 3 samples per treatment were then fermented for 28 days and the other 3 samples for 38 days respectively.

Following the formulation, the 24 samples (4 treatments × 3 replicates × 2 durations) were divided equally into two groups based on fermentation duration. Twelve samples were subjected to anaerobic fermentation for 28 days, while the remaining twelve were fermented for 38 days. Upon completion of the fermentation process, all samples were air-dried for five days to reduce moisture content and stabilize the material. The dried samples were then ground uniformly using FOSS CT293 Cyclotec ™ and sieved through 5mm sieve to obtain a homogeneous powder suitable for subsequent analyses. During grinding, the mill was cleaned every before putting the next sample with a towel to prevent cross contamination between treatments.

The processed samples were utilized for chemical composition analysis and in vitro gas production studies to evaluate their nutritional and fermentative characteristics.

3.3 Data collection

Data on crude protein, ash, dry matter, fibre, calcium and phosphorus in percentage and in vitro gas in ml/200gDM, and fermentation kinetics were collected in the laboratory, while other parameters were calculated using formulae. Crude protein, phoshorus, calcium and in vitrogas were measured in duplicates. Quality control meaures such as grinding samples to uniform size, drying samples to a constant weight weight were done to reduce errors.

3.4. Chemical composition analysis

The samples of CH and PM were analysed for dry matter (DM), ash, crude protein (CP), fibre (NDF, ADF, ADL), calcium, and phosphorus using standard procedures.

3.3.1 Dry matter

Dry matter was analysed using the oven dry method at 105°C for 24 hours
2.0 g of the sample was weighed into oven-dried and cooled crucibles, and the weights of the sample and crucibles were noted. The crucibles with the sample were then placed in the oven at 105° C for 24 hours. After 24hours, samples were removed and dry matter determined as,
Dry matter= (weight of oven - dry sample / original sample weight) × 100

3.3.2 Ash

Ash content was analyzed using the 924.05 method of AOAC (2009). 2.0g of the sample was weighed into oven-dried and cooled crucibles, and the weights of the sample and crucibles were recorded. The crucibles containing the sample were then placed in a muffle furnace at 550°C for 8 hours, and the ash content was calculated as Ash = (Ash weight/sample weight) × 100.

3.3.3 Crude protein

The protein content of the sample was analyzed using the Kjeldahl method. A duplicate of 0.3 g of the sample was weighed into oven-dried and cooled digestion tubes, to which 5 mL of concentrated sulfuric acid and a selenium catalyst were added. The mixture was then placed in a block digester at 360°C for 3 hours. After digestion, the mixture was diluted with distilled water to a final volume of 100 mL.

Next, 30 mL of boric acid was measured into a conical flask and positioned at the steam distiller outlet. A further 30 mL of the sample was measured into a distillation bottle, and 40 mL of sodium hydroxide was added. Ammonia gas released during the distillation was collected in the boric acid solution and subsequently titrated against 0.1 M hydrochloric acid until a pink color appeared, at which point the titre value was recorded. The crude protein content was then calculated by multiplying the nitrogen content by 6.25.

3.3.4 Neutral Detergent Fibre

Neutral Detergent Fibre (NDF) was analyzed following the method outlined by (Van Soest et al., 1991). Initially, 0.5 to 0.52 grams of each sample were weighed in duplicates and placed into a glass beaker. A spoonful of sodium sulfate was added to minimize frothing during the process. Subsequently, 100 ml of neutral detergent solution was introduced, and the mixture was boiled under reflux conditions for one hour. After this duration, the mixture was filtered using hot water to collect the residue. The residue was then oven-dried overnight at 105°C, weighed, and ashed at 550°C for eight hours to determine the NDF content.

3.3.5 Acid Detergent Fibre

0.5-0.52g of each sample was weighed in duplicates into a glass beaker, and 1 spoonful of sodium sulphite was added to reduce frothing. Then 100ml of the Acid detergent solution was added, and then the mixture was boiled under reflux conditions for 1 hour. After 1 hour, the mixture was filtered using hot water to obtain a residue, which was oven dried overnight at 105 °C and then weighed and ashed at 505°C for 8 hours to calculate ADF.

3.3.6 Acid Detergent Lignin

A duplicate weighing of 0.5-0.52g of each sample was placed in a glass beaker, and a spoonful of sodium sulphite was added to minimize frothing. Subsequently, 100ml of Acid Detergent Solution was introduced, and the mixture was boiled under reflux for one hour. After this duration, the mixture was filtered using hot water to obtain the residue, which was then oven-dried overnight at 105°C and weighed to determine the Acid Detergent Fiber (ADF).

The ADF residue was weighed again, and 15ml of sulfuric acid was added. The mixture was then allowed to sit at room temperature for three hours, with occasional stirring. After three hours, it was filtered through a porous crucible base and rinsed with distilled water to eliminate any remaining acid. The residue was then oven-dried at 105°C for four hours, followed by ashing at 550°C for eight hours to calculate the Acid Detergent Lignin (ADL).

3.5. In vitro gas production

In vitro gas production was determined following the procedures of Menke and Steingass in (1998)(Nasser et al., 2009). The syringes were previously well washed, dried, and pre-warmed at 39 °C. Rumen liquor used to ferment the coffee husks was collected from a freshly slaughtered cow at a local abattoir at Kalerwe, Kampala, Uganda. The rumen of the freshly slaughtered animal was sectioned into lengths with a knife, and rumen contents were sampled with a hand (Fortina et al., 2022). The middle part of the rumen collected was filled in a pre-warmed thermos flask, which was tightly sealed and transported to the animal science laboratory within one hour. The rumen contents were pooled and macerated to make a homogenous slurry. The slurry was then filtered through 4 layers of cheesecloth into a 500 mL glass flask under continuous flushing with carbon dioxide gas to produce rumen liquor inoculum. Flasks with the rumen liquor were kept at 39°C in a shaking water bath at all times. Approximately 200mg of the inoculated ground coffee husks were weighed and carefully introduced into 100ml graduated gas syringes. Syringes were then filled with 30ml of inoculum containing 10ml of rumen liquor and 20ml of buffer solution. In vitro incubation of samples was carried out in duplicates. Three blank gas syringes containing only 30ml of rumen liquor-buffer mixture with no substrate were also incubated alongside the coffee husk samples to correct for GP. The syringes were placed in a shaking water bath maintained at 39°C and incubated and incubated for 120 hours. Cumulative gas volume was recorded at regular intervals, and net gas was obtained as the difference between cumulative gas volume and average blank volume.

3.6 Gas profile and Gas production kinetics

Groote's model was used to determine the gas produced during substrate degradation. Cumulative gas volumes produced from breakdown of the samples were read and noted at 0,2,4,6,12,24,48,72,96, and 120 hours and fitted into the model.

$G(t)= A/[1+(B/t)]^{C}$

Where G= Gas produced at a given time, A = Asymptotic gas production(mg/g), B= time taken for half of the asymptotic gas to be produced(h), t= in vitro incubation time, C= sharpness parameter that determines the shape of the curve.

T1= Point of inflection, TRmax= Time at which maximum substrate is degraded, Rmax= maximum fractional rate of substrate degradation, were also derived using formulae described by Groot et al (1996) as follows,

- $TI = B[(C-1/c+1)]^{1/c}$

$R=Ct^{c-1}/[B^{c}+t^{c}]$

$tRM=B(c-1)^{1/c}$

In vitro Organic matter digestibility (IVOMD) of the samples was estimated from the gas volumes produced upon incubation of the 200mg for 10, 24,48, 72, and 96 hours, levels of crude protein and ash content of the sample according to Menke and Steingass as follows.

IVOMD(g/kgDM) = 14.88+0.8893×gas produced +0.0448×CP(g/kgDM) +0.0651×ash(g/kgDM)

Short-chain fatty acids (SCFA) were calculated according to the equation from Getachew et al. (2002) as follows

SCFA = 0.0222GP-0.00425

Where GP is net gas produced(ml/200mgDM)

3.7 Data analysis

One-way analysis of Variance (ANOVA) was performed using the R 4.3.1analytical software for Windows to check whether the values of the treatments obtained were statistically significantly different from each other. Polynomial contrasts were also obtained to identify linear, quadratic, and cubic variations between treatments at 95% confidence level following the equation,

$$Y_{ij}=\mu+T_i+e_{ij}$$

Where μ is the grand mean, T_i is the treatment effect, and e_{ij} is the error effect

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical compositions of Coffee husks with graded levels of poultry manure ensiled for 28 and 38 days

Ensiling duration of 28 and 38 represents the length of time the inoculated coffee husks were exposed to anaerobic environment to allow breakdown of the feed to release nutrients. Linear contrast, L represents the linear relationship between the variables, quadratic contrast, Q represents the curvilinear relationship while cubic contrast, C represents S shaped relationship between the variables.

There was no significant difference in the levels of crude protein obtained from the fermented coffee husks after increasing poultry manure levels and fermentation days. However crude protein was higher with manure inclusion with the highest as 10.9g/kg at 20%manure inclusion compared to coffee husks fermented with no manure which was 8.91g/kg. Dry matter generally increased with increasing levels of poultry manure after fermenting for 28 days with highest as 95g/kg at 20% manure inclusion. However, the increase was not significant therefore, poultry manure did not affect significantly the overall moisture content of coffee husks.

ADF levels were generally higher when fermentation period was 38 days with ADF reaching 24.7g/kg at than when the coffee husks were fermented for only 28 days where the highest was 22.6g/kg. The values were however lower than when manure was not included. The NDF levels showed a significant linear increase as inclusion levels increased after 38 days of fermentation, ADL levels increased significantly with increasing manure inclusion and fermentation days. NDF levels did not show a particular trend in variations as the manure inclusion levels and fermentation varied. However, coffee husks with no poultry manure contain higher ADF and NDF compared to when poultry manure was included. Also, inclusion of poultry manure resulted in significant linear increase calcium and phosphorus levels with the highest 20% manure inclusion reaching 0.349g/kg P and 0.688g/kg Calcium.

Table 1 Chemical composition of coffee husks with varying levels of chicken manure ensiled for 28 and 38 days

Variables	Ensiling duration	Poultry manure inclusion				SEM	*P*-values		
		0	5	10	20		L	Q	C
Dry matter (g/kg DM)	28	93.0	94.3	94.0	95	3.75	0.116	0.267	0.548
	38	91.7	91.3	87.3	86.0		0.219	0.284	0.610
Ash (g/kg DM)	28	92.0	90.5	90.6	87.6	0.753	0.048	0.559	0.405
	38	91.2	91.1	87.3	86.7		0.064	0.825	0.348

Crude protein (g/kg DM)	28	8.91	10.1	9.71	10.6	1.43	0.234	0.862	0.477
	38	12.14	15.9	11.2	10.9		0.671	0.635	0.515
Neutral detergent fibre (g/kg DM)	28	32.1	35.6	31.5	33.3	1.35	0.970	0.790	0.343
	38	31.1	34.4	24.3	40.2		0.0385	0.934	0.828
Acid detergent fibre (g/kg DM)	28	27.62	22.3	16.2	22.6	1.46	0.452	0.657	0.570
	38	24.3	23.3	24.7	23.3		0.835	0.877	0.454
Acid detergent lignin (g/kg DM)	28	9.52	8.57	10.9	12.8	0.88	0.043	0.254	0.471
	38	10.9	11.4	10.0	10.0		0.469	0.855	0.570
P (g/kg DM)	28	0.209	0.223	0.243	0.331	0.011	0.0078	0.1645	0.5904
	38	0.212	0.236	0.206	0.349		0.0056	0.0319	0.0582
Ca (g/kg DM)	28	1.52	2.73	3.49	3.36	0.716	0.268	0.202	0.442
	38	2.92	3.11	1.96	0.688		0.185	0.161	0.274

[1]L= linear contrast, Q = quadratic contrast, C = cubic contrast

4.2 Gas production kinetics

Time to reach half the asymptotic gas volume increased from 15hours to 21.6hours when 10% manure was included in coffee husks. The gas produced at point of inflection linearly increased significantly when poultry manure was included with the highest as 60.7ml/g at 20%poultry manure inclusion. The fractional rate of degradation was different among treatments and did not show any treend but showed the same values of 1.56 at 5% and 20% manure inclusion

1

Table 2 Gas production kinetics of Coffee husks with varying levels of chicken manure ensiled for 28 and 38 days

Variables (units)	Ensiling duration	Poultry manure inclusion				SEM	*P*-value		
		0 (Treat 1)	5	10	20		L	Q	C
A (ml/200 g DM)	28	35.1	34.5	38.7	34.3	3.05	0.8640	0.4380	0.2430
	38	37.3	35.8	41.0	31.9		0.5110	0.3240	0.2240
B (hr)	28	16.5	16.2	17.4	17.8	1.12	0.0775	0.5701	0.4197
	38	15.0	14.8	21.6	19.3		0.0175	0.4960	0.0390
C	28	1.63	1.56	1.35	1.56	0.156	0.5740	0.4020	0.4380
	38	1.63	1.37	1.33	1.84		0.4409	0.0384	0.6395
T1 (hr)	28	2.31	2.2	1.51	2.49	0.404	0.9410	0.2490	0.2870
	38	2.14	1.69	2.21	2.90		0.1230	0.1520	0.6380
TRmax (ml/h)	28	6.16	5.72	3.86	6.37	1.34	0.8460	0.3160	0.3750
	38	5.72	4.07	5.18	8.46		0.1444	0.0927	0.9180
Rmax (ml/hr)	28	0.0291	0.0315	0.0377	0.0279	0.00603	0.913	0.308	0.459
	38	0.0317	0.0426	0.0283	0.0210		0.1440	0.1970	0.2930
Gas at T1 (ml/g)	28	39.7	45.7	51.7	57.7	0.577	1.25e-08	1.000	1.000
	38	42.7	48.7	54.7	60.7		1.25e-08	1.000	1.000

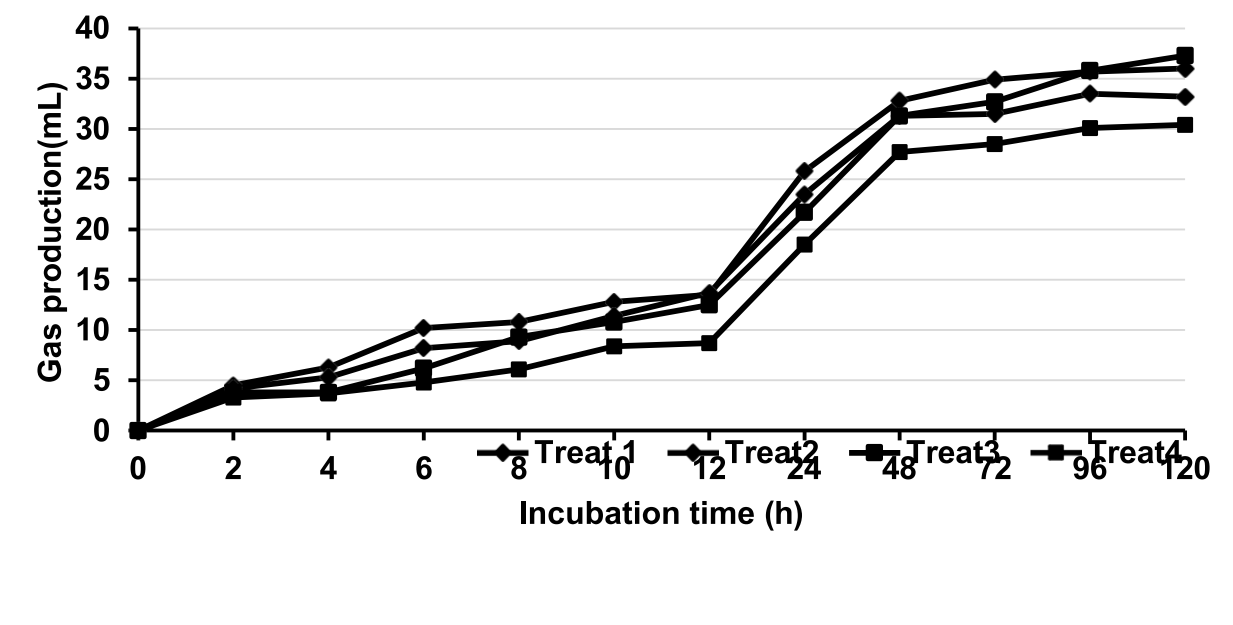

Figure 1Gas production after 38 days ensiling

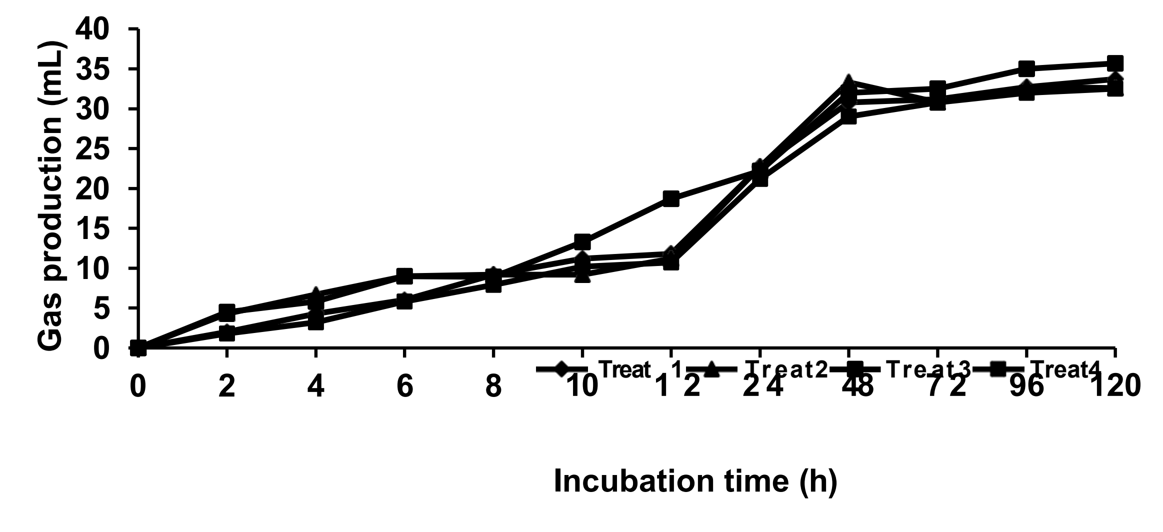

Figure 2 Gas production after 28 days ensiling

There was no significant difference in the gas produced in the the first three hours of feed degradation. There rapid gas production in the first 6 hours of feed degradation. The highest gas production rate was observed when poultry manure was included compred to when it was not included in the coffee husks.

Generally, gas production increased non linearly with initial lag phase taking 12 hours followed by rapid fermentation up to 48 houra and the curve then flattens.

Fermentation for 28 and 38 days show similar trends, however fermentation for 28 days shows a steeper slope than 38 days

In-vitro organic matter digestibility(IVOMD) and Short chain fatty acids(SCFA).

IVOMD decreased as the poultry manure inclusion increased from 87.9g/kg in the control to 82.4g/kg at 25%PM inclusion.. SCF production decreased as poultry manure inclusion rate increased with a production of 82mmol/l at 25% PM inclusion compared to 87mmol/l when no manure is included.

Table 3 In vitro organic matter digestibility and short-chain fatty acid production of Coffee husks with varying levels of chicken manure ensiled for 28 and 38 days

		Poultry manure inclusion			
Variables	Ensiling duration	0	5	10	20
IVOMD (g/kg OM)	28	87.96587	87.91069	85.24723	82.42300
	38	88.94584	89.11392	87.76758	83.26178
SCFA (mmol/l)	28	1.70515	1.66075	1.59415	1.52755
	38	1.72735	1.68295	1.66075	1.54975

[2]

[2] IVOMD= In-vitro organic matter digestibility, SCFA= Short chain fatty acids

CHAPTER FIVE

5.0 DISCUSSION

5.1 Crude Protein (CP)

Crude protein (CP) content increased with PM inclusion, especially after 38 days of fermentation. The highest CP value was recorded at 5% PM (15.9 g/kg DM), compared to 12.14 g/kg DM in the control. Although the p-value (P = 0.671) was not statistically significant Crude protein was higher when coffee husks were inoculated due increase in mirobial protein in poultry manure.. The crude protein levels were however still low because fermentation did not provide the aerobic condtions required by the microbestherefore hydrolysis of complex proteins to simpler proteins was reduced.(Chang et al., 2022)the numerical increase is biologically meaningful for ruminant nutrition. This trend is supported by a review in the *European Journal of Biology* that highlights coffee husk's protein enrichment protein-enrichment potential when combined with poultry waste (Kouamé et al., 2011b). Additionally, the Co-ordinated Research Project on poultry breeding, College of Veterinary Science, Sri Venkateswara Veterinary University, foundational work on utilisation of poultry manure as an unconventional protein source in small ruminant rations, feeding poultry waste to sheep confirms its value as a non-protein nitrogen source for ruminants (Narasimha et al., 2013) .

5.2 Ash and Phosphorus (P)

Ash content decreased significantly at 28 days (P = 0.048), from 92.0 g/kg DM in the control to 87.6 g/kg DM at 25% PM. This may reflect dilution of mineral concentration due to increased organic matter. Phosphorus (P) content, however, increased significantly at both 28 and 38 days (P = 0.0078 and P = 0.0056), rising from 0.209 g/kg DM to 0.331 g/kg DM.

5.3 Fiber Fractions (NDF, ADF, ADL)

Fiber fractions responded variably. Acid Detergent Lignin (ADL) increased significantly at 28 days (P = 0.043), from 9.52 g/kg DM in the control to 12.8 g/kg DM at 25% PM, indicating higher indigestible fiber.This is because lignin was not brokendown by microbes in poultry manure sice the microbes that breakdown lignin operate in aerobic conditions(Iram et al., 2021). Although Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) did not show statistically significant differences, ADF dropped to 16.2 g/kg DM at 10% PM after 28 days, suggesting partial fiber breakdown. Therefore some of the hemicellulose and cellulose in NDf were broken down by the microbes in poultry manure to simpler compounds such as simple sugars. Fibre fractions were however generally lower in inoculated coffee husks than when coffee husks were not inoculated. This is because cellulose and hemicellulose bonds were eliminated by cellulase and hemicellulose enzymes into simple bond(Suwignyo et al., 2015).These trends are supported by recent studies in *BMC Veterinary Research*, which examined coffee husk fiber dynamics in poultry litter and found similar shifts in fiber composition (Narasimha et al., 2013).

5.4 Gas Production Kinetics

Gas production kinetics revealed important fermentation dynamics. Time to half-max gas production (B) increased significantly at 38 days ($P = 0.0175$), from 15.0 hours in the control to 21.6 hours at 10% PM, indicating slower microbial fermentation. The sharpness parameter (C) also varied significantly ($P = 0.0384$), reflecting changes in fermentation curve shape. Most notably, gas volume at the point of inflection (T1) increased extremely significantly at both 28 and 38 days ($P = 1.25e-08$), rising from 39.7 ml in the control to 57.7 ml at 25% PM. This is because prolonged fermentation resulted in prolonged anaerobic conditions which are not suitable for the microbes therefore fibre breakdown was reduced resulting in subsequent reduction in gas production rate and inconsistent gas production patterns.

5.5 In Vitro Organic Matter Digestibility (IVOMD) and SCFA Production

In vitro organic matter

In vitro organic matter digestibility (IVOMD) declined with increasing PM inclusion. At 28 days, IVOMD dropped from 87.9 g/kg OM in the control to 82.4 g/kg OM at 25% PM. Although no p-values were provided, the trend suggests reduced digestibility due to increased lignin and fibre-bound nitrogen. Short-chain fatty acid (SCFA) production followed a similar pattern, decreasing from 1.66 mmol in the control to 1.53 mmol at 25% PM.This is because of the increased fibre content which reduced digestibility of the substrate by the microbes.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

- It is concluded that there was no significant difference among the different poultry manure inclusions in terms of chemical composition and gas production. There was no significant diffrerence when coffee husks were fermented for 28 days and 38 days. Fermenting of coffee husks with poultry manure did not show a significant effect on fibre degradation therefore fermentation may not be the best alternative to treat coffee husks as a carbohydrate feed source indairy cattle. These results, however, need to be confirmed by in vivo studies before commercial use of poultry manure as an inoculant is used in dairy diet formulations.
- There is no Significant difference in crude protein, ash, and dry matter with increasing poultry manure inclusion.
- Increasing poultry manure increased the availability of calcium and phosphorus.
- Treating coffee husks with poultry manure at varying inclusion levels enhances the chemical composition but its digestibility and rumen degradability reduces with maximum gas production at 48hrs.
- Poultry manure at the level of 20% in coffee husks husk rations positively influenced ruminal microbial fermentation and digestion of the carbohydrates in coffee husks, suggesting the possibility of using inoculated and fermented coffee husks as a potential alternative feed in dairy nutrition

6.2 RECOMMENDATION

- According to the results obtained, poultry inclusion levels above 20% are optimum for the fermentation of coffee husks to utilise the carbohydrates in fibre
- Researchers should consider increasing poultry manure levels and reducing fermentation days to determine the best inclusion levels and fermentation period required to obtain optimum nutrients from coffee husks.

Future research should focus on in vivo experiments to confirm the above results and observe any deviation from the data obtained from in vitro trials

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