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Supplementation of Nutmeg (Myristica Fragrans) Pericarp in Animal Feed Biscuits as Transportation Feed for Beef Ruminants

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Abstract

The increasing demand for ruminant livestock products in Indonesia has been accompanied by challenges related to the high cost and fluctuating availability of conventional feed, which accounts for approximately 60–70% of total production costs. Agro-industrial byproducts, including sago pulp and rice bran, combined with locally available protein sources such as gamal (Gliricidia sepium) leaf meal and fish meal, offer potential alternative feed ingredients. However, the nutritional quality and digestibility of compact feed biscuits supplemented with nutmeg (Myristica fragrans) pericarp for use during livestock transportation remain insufficiently investigated. This study aimed to evaluate the nutrient composition, in vitro digestibility, and ruminal fermentation characteristics of feed biscuits supplemented with nutmeg pericarp meal at 0% (P0/Sample A), 5% (P1/Sample B), and 10% (P2/Sample C). A laboratory experiment was conducted using a completely randomized design (CRD), proximate analysis, and the two-stage Tilley and Terry in vitro digestibility method. The feed biscuits contained 94.46–95.36% dry matter, 15.83–17.72% crude protein, 2.31–3.04% ether extract, 17.85–18.86% crude fiber, 46.95–51.23% nitrogen-free extract (NFE), and 12.78–13.43% ash. Supplementation significantly affected dry matter, crude protein, and crude fiber digestibility ($p < 0.01$). The 5% supplementation treatment (Sample B) achieved crude protein and crude fiber digestibility values of 64.19% and 77.05%, respectively, while the 10% treatment (Sample C) produced the highest ammonia nitrogen (NH_3-N) concentration (39.84 mg/100 mL). Organic matter digestibility ranged from 89.66% to 94.31%, whereas volatile fatty acid (VFA) concentrations did not differ significantly among treatments ($p > 0.05$). These findings suggested that nutmeg pericarp-supplemented feed biscuits had promising nutritional and digestibility characteristics, particularly at the 5% supplementation level, and could be further evaluated as supplemental feed for beef cattle during transportation.

INTRODUCTION

The ruminant livestock subsector in Indonesia plays a strategic role in supplying animal-derived food products, particularly meat and dairy products, to meet increasing nutritional demands driven by population growth and greater public awareness of the importance of animal protein. However, the development of the ruminant livestock industry, encompassing both

smallholder and commercial farming systems, continues to face challenges related to fluctuations in feed availability and the high cost of quality feed. Feed represents the largest component of livestock production expenses, accounting for approximately 60–70% of total production costs (Al Huda et al., 2024; Suryani et al., 2025). Heavy reliance on conventional feed ingredients, such as corn, soybean meal, and rice bran, whose availability and prices are influenced by competition with human food requirements and seasonal market fluctuations, presents a major obstacle to maintaining production efficiency and business sustainability (Jalal et al., 2023; Malenica et al., 2023). Therefore, identifying affordable, locally available, nutritionally adequate alternative feed resources that minimize competition with human food supplies is essential for improving the sustainability of ruminant production systems.

Meanwhile, agricultural production and agro-industrial processing generate substantial quantities of byproducts that remain underutilized (Shah et al., 2025). In major agricultural production regions, sago pulp, a byproduct of sago starch extraction, and rice bran, a byproduct of rice milling, are potentially valuable alternative feed ingredients (Sa'diah et al., 2025; Susanti, 2022). Sago pulp contains carbohydrates and structural fiber that can contribute to dietary energy supply, although its relatively low protein content and limited digestibility may restrict its direct utilization (Fadly et al., 2022). Rice bran provides additional energy and lipid sources, but its availability and nutritional composition may vary (Yılmaz Tuncel, 2023). Furthermore, gamal (Gliricidia sepium) leaves offer potential as a locally available plant protein source because of their adaptability to different growing conditions and suitability for cultivation by smallholder farmers (Tahuk et al., 2022). Nevertheless, their utilization may be constrained by palatability limitations associated with certain secondary plant metabolites (Tunkala et al., 2023). Fish meal can also be incorporated as a source of high-quality protein and essential amino acids to improve the nutritional balance of the feed formulation.

These agricultural byproducts and locally available feed ingredients are frequently utilized separately through conventional feeding practices, potentially limiting their nutritional efficiency and contributing to waste management challenges (Ali et al., 2023). Feed processing technologies that combine these materials into compact, nutritionally balanced formulations may improve their utilization. Feed biscuit production involves mixing sago pulp, rice bran, gamal leaf meal, and fish meal with suitable binding agents, such as molasses or tapioca starch, followed by molding and mechanical compression (Christmas et al., 2022). This processing approach offers several potential advantages, including improved handling and storage efficiency, greater convenience during transportation, and the combination of complementary energy and protein sources. Depending on formulation and processing conditions, compact feed biscuits may also influence palatability and nutrient availability during ruminal fermentation (Azizah et al., 2023).

Before these feed biscuits can be recommended for direct feeding to ruminants under in vivo conditions, their nutritional composition and digestibility must be evaluated experimentally. The in vitro digestibility method developed by Tilley and Terry (1963) provides a laboratory-based approach for estimating feed digestion through sequential incubation procedures that simulate ruminal fermentation and subsequent enzymatic digestion. Compared with animal feeding trials,

in vitro methods generally require fewer resources and provide a practical means of estimating dry matter digestibility (DMD; KcBK) and organic matter digestibility (OMD; KcBO) (Mashudi et al., 2026). These analyses can help characterize the digestibility of feed biscuits containing agricultural byproducts and complementary protein sources, although the effects of individual ingredients and their interactions require appropriate experimental comparisons.

The importance of this study lies in the need to evaluate the nutritional quality and digestibility of feed biscuits formulated from sago pulp, rice bran, gamal leaf meal, and fish meal, particularly when supplemented with nutmeg (Myristica fragrans) pericarp. Differences in ingredient proportions and supplementation levels may influence nutrient composition, digestibility, and ruminal fermentation characteristics (Ali et al., 2023; Tunkala et al., 2023). Nutmeg pericarp represents an additional agro-industrial byproduct with potential applications in alternative feed formulations (Antasionasti et al., 2021; Suwarda et al., 2021). However, its incorporation into compact feed biscuits requires experimental evaluation to determine whether supplementation affects nutrient utilization and fermentation characteristics (Tariq et al., 2025; Warsito, 2021). Such investigations may contribute to the development of alternative feed resources while supporting agricultural byproduct utilization and circular economy practices (Malenica et al., 2023).

Based on these considerations, the present study investigated the nutrient composition, in vitro digestibility, and ruminal fermentation characteristics of agricultural byproduct-based feed biscuits containing sago pulp, rice bran, gamal leaf meal, and fish meal, supplemented with different levels of nutmeg (Myristica fragrans) pericarp meal. The formulation was investigated for its potential application as supplemental feed for beef cattle during transportation (Babikian et al., 2021; Meléndez et al., 2021; Widiastuti et al., 2026).

The study addressed the nutritional characteristics of feed biscuits formulated from agricultural byproducts with varying levels of nutmeg pericarp supplementation. Particular attention was given to dry matter digestibility (DMD; KcBK), organic matter digestibility (OMD; KcBO), crude protein digestibility (CPD; KcPK), and crude fiber digestibility (CFD; KcSK), as determined through laboratory digestion procedures. The study also examined ruminal fermentation end products, including volatile fatty acids (VFAs) and ammonia (NH_3), to assess the effects of nutmeg pericarp supplementation on fermentation characteristics. These parameters were used to evaluate the nutritional potential of the formulated feed biscuits rather than to establish their effectiveness under actual livestock transportation conditions.

Accordingly, this study aimed to determine the nutrient composition of agricultural byproduct-based feed biscuits supplemented with nutmeg pericarp meal and to evaluate the effects of supplementation on in vitro dry matter, organic matter, crude protein, and crude fiber digestibility. It also aimed to assess ruminal fermentation characteristics through measurements of VFAs and ammonia concentrations. The findings were intended to identify promising supplementation levels and provide preliminary evidence supporting further evaluation of these feed biscuits as supplemental feed for beef cattle during transportation.

The novelty of this study lies in the evaluation of nutmeg (Myristica fragrans) pericarp supplementation in compact feed biscuits formulated from sago pulp, rice bran, gamal leaf meal, and fish meal. Although these ingredients have potential applications as alternative feed resources, their combined nutritional characteristics and responses to different nutmeg pericarp supplementation levels require further investigation. By integrating proximate composition analysis, in vitro nutrient digestibility measurements, and ruminal fermentation assessments, this study sought to provide evidence concerning the nutritional suitability of the formulated feed biscuits. The findings may contribute to the development of agricultural byproduct-based feed formulations and provide a basis for subsequent in vivo studies evaluating their practical application during beef cattle transportation.

RESEARCH METHODS

This study focused on developing functional feed biscuits for ruminant livestock. A laboratory-based experimental approach involving in vitro analysis was employed to evaluate the nutritional composition and digestibility of the formulated feed biscuits.

Time and Place

The research is planned to last for 6 months. The process of making feed biscuits will be carried out at the Slaughter Livestock Production Laboratory, Faculty of Agriculture, Pattimura University. Proximate and in-vitro digestion analysis will be carried out at the Laboratory of Animal Nutrition Biochemistry, Faculty of Animal Husbandry UGM, Yogyakarta.

Stages of Research, Process, and Externalities

The research is divided into four main stages that are intercontinuous:

Phase I: Preparation and Processing of Raw Materials

Process:

1. **Nutmeg Peel:** Collected from people's plantations, cleaned, peeled off the outer skin, and dried using an oven at 600C for 24 hours to maintain the stability of myrhicystic compounds.
2. **Gamal Leaves:** Dried for 24 hours to lower the level of cuminmarin, dried using an oven at 500C for 6 hours and then ground into flour.
3. **Sago Dregs:** Dried to a moisture content of <12% to prevent mold.
4. **Flouring:** All raw materials (including bran, and fishmeal) are screened using a 20-40 mesh sized sieve to obtain a homogeneous particle size.

External: Availability of stock of functional raw materials in the form of ready-to-mix flour.

Stage II: Formulation and Manufacture of Animal Feed Biscuits

The formulation and nutritional value of animal feed biscuits (based on calculations) can be seen in the table below:

Table 1. Formula and nutritional content of animal feed biscuits

Ingredients (%)	P0 – Control (Shovel 0%)	P1 (Shovel 5%)	P2 (10%)
Nutmeg Fruit Peel Flour	0	5	10
Gamal Leaf Flour	20	20	20
Sago Pulp	35	35	35

Rice bran	35	30	25
Fishmeal	10	10	10
Nutritional content (%)			
Dry Materials (BK)	88,70	88,90	89,10
Crude Protein (PK)	13,65	13,35	13,05
Crude Fiber (SK)	11,75	12,25	12,75
Crude Fat (LK)	4,05	4,30	4,55
Metabolic Energy (EM)	2,745 kcal/kg	2,720 kcal/kg	2,695 kcal/kg

Process:

1. The ingredients are mixed according to the predetermined ration table (P0, P1, P2).
2. The dough is added 1 liter of coconut water and 300 ml of molasses to help gelatinize starch from sago dregs.
3. Animal feed biscuit printing
4. Dried in the oven at 500C

Output: A physical product of animal feed biscuits with three levels of nutmeg supplementation.

Phase III: Laboratory Tests (Chemical Quality and Digestibility)

Process:

1. **Chemical Test (Proximate):** Analysis of Dry Matter (BK), Crude Protein (PK), Crude Fiber (SK), Crude Fat (LK), and Metabolic Energy (EM) estimation.
2. **Digestibility Analysis:** Digestibility analysis of organic matter (KcBo), Digestibility analysis of dry matter (KcBk), Ammonia Concentration (NH3) and Volatile fatty acid concentration (VFA).

- **Outputs:** Ready-to-publish data on the nutritional profile and physical characteristics of biscuits.

Stage IV: Data Analysis and Reporting

Process:

The test results data were analyzed using *Analysis of Variance* (ANOVA) based on the Complete Random Design (RAL). If there is a significant difference, a follow-up test (DMRT) is carried out to determine the best treatment.

Outputs: Final report of the research, draft journal article (SINTA), and seminar results.

RESULTS AND DISCUSSION

Preparation of Ingredients for Making Animal Feed Biscuits

The research activity has begun with the preparation of all raw materials used in the formulation of transportation feed biscuits. The main ingredient in the form of nutmeg peels is obtained from the community nutmeg plantation center in Maluku Province. The collected nutmeg peels are then selected to obtain fresh ingredients, free of fungal contaminants or foreign objects. After the sorting process, nutmeg peels are washed using running water, cut into smaller sizes, then dried using an oven at 60°C for ±24 hours. Drying is carried out to reduce moisture content

so that the ingredients are more durable to store and maintain the content of bioactive compounds, especially myristic, which is expected to function as an antistress agent in livestock.

In addition to nutmeg peels, gamal leaves are also prepared as a source of vegetable protein. Gamal leaves are first dried for 24 hours to reduce the content of cuminary, then dried using an oven at 50°C for approximately 6 hours until the appropriate moisture content is achieved. Furthermore, gamal leaves are ground into flour using a hammer mill and sifted with a mesh size of 20–40 so that a uniform particle size is obtained. Sago pulp as an energy source has also been dried until it reaches a moisture content of less than 12%, then floured and sifted. Complementary ingredients in the form of rice bran and fishmeal are prepared through a weighing and sieving process so that all ingredients have a homogeneous particle size.

The results of the activity at this stage show that all raw materials have been successfully prepared according to the standard research procedures. Nutmeg peel flour, gamal leaf flour, sago dreg, rice bran, and fishmeal are stored in a dry condition and ready to be used for the formulation process of feed biscuits. With the availability of these raw materials, the research stage can be continued to the formulation process and manufacture of biscuits according to the treatment design that has been set. This stage is in accordance with the design of the preparation of raw materials in the research proposal.

Externally achieved:

- All research raw materials have been available in the form of homogeneous flour.
- The quality standards of raw materials have met the requirements for the mixing process.
- Raw material stock is ready to be used in the process of making feed biscuits.

Animal Feed Biscuit Making Process

The stages of formulation and manufacture of feed biscuits have been successfully carried out in accordance with the research design. The biscuit formulation is arranged in three treatments, namely P0 (without nutmeg skin supplementation), P1 (nutmeg peel supplementation 5%), and P2 (nutmeg peel supplementation 10%). Each treatment is prepared based on the composition of the constituent ingredients that have been designed so that it has a relatively balanced nutritional content according to the needs of broiler ruminant livestock.

All constituent ingredients are weighed according to the formulation of each treatment and then mixed using a mixer until homogeneous. After the mixing of dry ingredients is completed, 1 liter of coconut water and 300 ml of molasses are added as an adhesive as well as helping the gelatinization process of starch from sago dreg. The dough is then stirred again until it achieves a uniform texture and is easy to print. Furthermore, the dough is molded using a feed biscuit mold with a uniform size to produce a relatively similar shape, size, and weight of biscuits in each treatment.

The molded biscuits are then dried using an oven at 50°C until they reach the expected moisture content. After the drying process is complete, the biscuits are cooled at room temperature before being packaged in an airtight container to prevent moisture absorption during storage. The results of the initial observations showed that the entire treatment produced biscuits with a complete shape, a uniform color according to the composition of the ingredients, a distinctive

nutmeg aroma in the P1 and P2 treatments, and a texture that was hard enough that it was estimated to be able to survive the livestock transportation process.

In general, the production process went well without experiencing significant technical problems. The entire treatment succeeded in producing a prototype of feed biscuits which was then used as a sample in laboratory testing of physical, chemical, and digestible quality.

Externally achieved:

- A prototype of animal feed biscuits with three levels of nutmeg skin supplementation (P0, P1, and P2) was successfully produced.
- All samples have been treated coded and ready to be used as laboratory testing materials.
- Biscuit products with good physical characteristics were obtained as a candidate for livestock transportation feed.

Chemical Quality and Digestibility Analysis

Chemical quality testing was carried out through proximate analysis which included dry matter (BK), crude protein (PK), crude fiber (SK), crude fat (LK), and metabolic energy (EM) estimation.

In addition to proximate analysis, in vitro digestibility analysis was also carried out which included dry matter digestibility (KcBK), organic matter digestibility (KcBO), ammonia concentration (NH_3), and production of volatile fatty acids (VFA) in the rumen and post-rumen.

Chemical Quality Analysis.

The results of the research illustrated from the results of proximate analysis are a method of chemical analysis of livestock raw materials based on their composition and usefulness. Proximate analysis was carried out to determine the content of protein, crude fiber, softness, moisture content and ash (Table 2.).

Table 2. Nutritional content of the Experimental Ration

Sample Name	Dry Ingredients (%)	Percentages Based on Dry Materials				
		Ash	Crude Protein	Crude Fat	Crude Fiber	BETN
Sample A	94,46	13,35	17,28	2,82	17,94	48,61
Sample B	95,36	12,78	15,83	2,31	17,85	51,23
Sample C	95,03	13,43	17,72	3,04	18,86	46,95

Primary Date, 2026.

The results of the proximate analysis showed that feed biscuits supplemented with nutmeg peel flour (*Myristica fragrans*) have adequate nutritional quality to meet the basic living needs and production of broiler ruminant livestock.

Dry Ingredient Content

The Dry Matter Content (BK) of feed biscuits in all treatments (Table 1) is classified as very high, ranging from **94.46% to 95.36%** (moisture content). This high BK value is very crucial in the manufacture of feed biscuits ($\leq$ 5,54%*compact feed*). The low moisture content minimizes the risk of mold formation and microbiological damage during storage and transportation, so that biscuits have a long *shelf life* when used as transportation feed.

High dry matter (BK) content (>94.5%) with very low moisture content (<5.5%) in animal feed biscuit products made from sago pulp, rice bran, and nutmeg peel flour (*Myristica fragrans*) supplementation is one of the most important physical-chemical indicators that determine the quality and effectiveness of supplementary feed. This nutrient density and minimal moisture have a significant impact on storage stability, transportation efficiency, and physiological aspects of broiler ruminant livestock.

Physical Stability and Shelf Life

Water is the main medium for enzymatic activity and the growth of decaying microorganisms such as molds, fungi, and bacteria. Feed moisture content exceeding 12–14% is highly susceptible to mycotoxin contamination (such as aflatoxin) and biological decay processes during storage.

Moisture content below 5.5% stops most of the free water () activity within the feed matrix. These dry conditions prevent fat hydrolysis reactions and organic matter breakdown, so feed biscuits have a much longer shelf life. This is crucial considering that these biscuits are designed as transport feeds that must withstand being stored in transport containers or storage warehouses on ships without the risk of mold or clumping during inter-island travel. a_w

Logistics Efficiency and Compact *Feed Concept*

Transportation of broiler cattle via sea and land requires feed with a high volume density to save cargo space. Conventional feed such as fresh forage contains a moisture content of up to 70–80%, so it is very heavy, takes up space (bulky), wilts quickly, and triggers the accumulation of waste on top of the transport fleet.

Through the moisture content of <5.5%), the weight of the feed transported purely represents the percentage of solid nutrients (dry matter content >94.5%). The formulation of biscuits with this low humidity allows the feed pressing process to produce dense, high-density biscuits, and has a superior *durability index*. The feed is not easily cracked or destroyed into dust due to vehicle shocks during transportation.

Nutrient Density and Dry Matter Consumption (KBK)

The reticulum capacity of livestock is limited, especially when livestock experience transportation stress which generally reduces appetite (*voluntary feed intake*). In conditions of limited feed capacity above the transport fleet, livestock must get as dense nutritional intake as possible in each bite.

Feed biscuits with dry ingredients >94.5% guarantee that every gram of feed consumed by livestock contains a high density of protein, dissolved carbohydrates (BETNs), and minerals without being diluted by the weight of water. The availability of these solid nutrients keeps the energy supply and precursors of livestock metabolism fulfilled to suppress *shrinkage rate* during travel.

Protection of Nutmeg Secondary Metabolite Compounds

This drying process, which produces low moisture content, also plays an important role in preserving bioactive compounds from nutmeg husk flour, such as essential oils (myristicin, limonene) and phenolic compounds. Low environmental humidity prevents premature oxidative degradation of these natural antioxidants, so that the functional function of feed biscuits as an

oxidative stress suppressant and mild sedative for livestock during transportation can be optimally maintained.

Crude Protein Content

The Crude Protein (PK) content of the ration is in the range **of 15.83% – 17.72%** (Table 1). The high PK level is supported by the use of a combination of gamal leaf flour (20%) and fishmeal (10%) as a source of legume and biological protein. Nutmeg peel flour supplementation of up to 10% (P2 / Sample C) which replaces the position of rice bran does not reduce the quality of the ration protein, but rather contributes the highest PK (17.72%). PK levels above 15% are ideal for broiler ruminant livestock in transportation conditions to prevent muscle tissue degradation/catabolism due to treatment stress.

The Crude Protein (PK) content of the experimental ration which was in the range **of 15.83% to 17.72%** (average 15–17%) showed a very high nutritional qualification and was ideal for feed supplemental ruminants and broilers. This figure was achieved through a proportional combination of biological protein sources (10% fishmeal) and local leguminoses (gamal leaf flour 20%), as well as the substitution of rice bran with nutmeg peel flour (*Myristica fragrans*) up to 10%. The supply of crude protein in this range provides various positive implications for rumen metabolism, the fulfillment of basic living needs, and the protection of livestock body tissues from shrinkage during transportation.

Fulfilling Nutritional Needs and Tissue Restoration

The standard crude protein requirement for broiler ruminant livestock (such as beef cattle or local goats) at moderate to fattening levels generally ranges from 10% to 14%. With a PK percentage of 15–17%, these feed biscuits are not only able to meet basic maintenance needs, but also provide an abundant amount of nitrogen to support muscle tissue synthesis and carcass formation.

In transportation conditions, livestock experience environmental stress (heat exposure, fleet shocks, and space limitations) that trigger an increase in the hormone cortisol. This stress condition spurs the body's protein catabolism process (gluconeogenesis from muscle tissue amino acids) to provide emergency energy. Feed with high PK levels acts as a nutritional defense fortress that supplies exogenous amino acids directly, thus minimizing muscle protein breakdown and suppressing the *shrinkage rate* of livestock during travel.

Efficiency of Rumen Microbial Protein Synthesis

Ruminants are unique in that most of their protein needs are met by microbial proteins synthesized within the rumen. To optimize microbial protein synthesis, a balance is needed between the supply of dissolved nitrogen from feed protein degradation and the availability of dissolved carbohydrate energy (Nitrogen-Free Extract Ingredients / BETN).

The ration PK level of 15–17% supported by the high BETN content (46–51% of sago pulp) creates synchronous (integrated) feed conditions. Proteins from fishmeal and gamal leaves are overhauled in the rumen into ammonia (), which is then assembled by rumen microbes along with the carbon skeleton of sago starch to form new microbial protein cells. The increase in the

population of rumen microbes directly improves fiber digestibility and overall feed utilization efficiency. NH_3

Contribution of Local Ingredients and Nutmeg Flour Substitution

The use of gamal leaf flour (*Gliricidia sepium*) as much as 20% has been proven to be a major contributor to high-quality nitrogen that is easily degraded in the rumen, while fishmeal (10%) provides protein intake through the rumen (*bypass protein*) which is rich in essential amino acids to be absorbed directly in the small intestine.

Substitution of rice bran with nutmeg husk flour from 0% to 10% (P2) did not reduce the PK level of the ration, but produced the highest PK level in the P2 treatment (17.72%). This indicates that nutmeg husk flour does not damage the feed protein binding matrix and still maintains the nutrient balance of the ration. Natural phenolic compounds and tannins in nutmeg husks in measured concentrations are also suspected to provide a partial protective effect (*protein bypass*) on high-quality proteins from fishmeal, so that the protein is not completely degraded in the rumen and can be optimally utilized by livestock in the post-rumen.

Crude Fat Content

The content of Crude Fat (LK) increases as the percentage of nutmeg peel flour increases, from **2.31% (P1) to 3.04% (P2).** This increase is influenced by the lipid component and natural essential oil content contained in nutmeg peel. This level of crude fat < 5% is within safe limits for rumen microbial synthesis, because excess fat content (> 6%) can wrap feed fibers and poison cellulolytic bacteria.

The Crude Fat Content (LK) of the experimental ration which was in the range **of 2.31% to 3.04%** (average 2.32–3.04%) showed a very ideal, proportional, and safe nutritional qualification for complete feed and biscuits supplemented by ruminant broiler livestock. This percentage of fat was obtained from formulations based on sago dregs, rice bran, gamal leaf flour, fish meal, as well as gradual substitution of nutmeg peel flour (*Myristica fragrans*) from 0% (P0), 5% (P1), to 10% (P2). The amount of crude fat content in this range has highly beneficial physiological and technological implications for the digestive process of the rumen, the physical stability of the biscuits, and the supply of metabolic energy during livestock transport.

Safety of Rumen Ecology and Cellulolytic Microbial Activity

The ruminant digestive system has biological limitations in tolerating fat content in the ration. The maximum limit of crude fat content that is safe for rumen ecosystems ranges from 5% to 7%. If the feed fat content exceeds this limit, the fat molecules will coat the surface of the feed fiber and microbial cell walls, and are directly toxic to cellulolytic bacteria (fiber digesters). This can reduce the digestibility of crude fiber and disrupt the balance of rumen fermentation.

The experimental ration crude fat content of 2.32–3.04% was well below this critical threshold. This range of values ensures that nutmeg peel flour supplementation up to the level of 10% does not cause adverse *effects* on the rumen microbial population. These results are in line with the organic matter digestibility data (KcBO >89–94%) that have been tested, where microbial digestion remains optimal without being hampered by the closure of the fat layer in the feed matrix.

Gradual Increase and Contribution of Nutmeg Skin Lipid Compounds

Proximate analysis data showed a trend of increasing crude fat content along with the increase in the percentage of nutmeg peel flour, from 2.31% in P0/P1 to reach the highest value of 3.04% in P2 treatment (10% nutmeg supplementation). This increase comes from the contribution of natural lipid components and essential *oils* contained in nutmeg peel.

The skin of nutmeg is rich in aromatic compounds and natural fatty acids such as myritic acid, phenolic, and terpenoid acid. This essential oil not only contributes the value of ether extract (crude fat), but also carries a functional function as a natural antioxidant. In livestock transportation conditions that trigger oxidative stress, the presence of bioactive lipid components and essential oils from nutmeg bark plays a role in helping to reduce free radicals, while providing a distinctive aroma that can increase the palatability of supplement feed.

Metabolic Energy Density and Physical Resistance of Biscuits

Physiologically, fats have a gross energy value about 2.25 times higher than carbohydrates and proteins. Although the percentage is low (2.32–3.04%), the presence of these crude fats still makes an important contribution to increasing the energy density of feed biscuits. The energy supply from fats helps to meet the basic living energy needs of livestock during transport without overloading the work of the rumen excessively.

From the aspect of feed technology, fat content in the range of 2–3% acts as a *natural lubricant* during the process of pressing and printing feed biscuits. Fat helps bond between feed particles with adhesives (molasses/tapioca) and sago pulp, resulting in a compact feed biscuit, has a high *durability index*, and is not easily rancid due to free fat oxidation due to controlled levels.

Crude Fiber Content

The Crude Fiber Content (SK) of the experimental ration which is in the range **of 17.85% to 18.86%** shows a very balanced nutritional qualification and is ideal for supplementary feed and complete feed in the form of biscuits for broiler ruminants. This percentage of crude fiber is obtained from a ration formulation based on sago pulp (35%), gamal leaf flour (20%), rice bran, and the addition of nutmeg peel flour (*Myristica fragrans*) at the levels of 0%, 5%, and 10%. This figure reflects the condition of feed that is not only able to support the health of the rumen ecosystem, but is also efficient in maintaining nutritional density and shelf life of supplementary feed.

Digestive Balance and Prevention of Rumen Physiological Disorders

Crude fiber is a very vital feed component for ruminant livestock to stimulate re-chewing activities (*rumination*) and saliva secretion (*salivation*). Saliva contains natural bicarbonate compounds that function as a *buffer* to maintain the pH of the rumen at optimal conditions (6.5–6.8).

If the processed supplement feed has too low crude fiber content (), livestock are at high risk of indigestion such as rumen acidosis due to the fermentation of soluble carbohydrates that are too fast. On the other hand, the SK level of 17.85–18.86% in this feed biscuit is in the ideal range. This value is high enough to maintain the rhythm of peristaltic movement and rumen papillae, but not excessive so that it does not cause digestive fatigue or a decrease in feed consumption levels.< 15%

Fiber Stability Due to Nutmeg Peel Flour Substitution

Based on the ration formulation, an increase in the percentage of nutmeg husk flour to 10% (P2) that replaced rice bran did not trigger a drastic spike in crude fiber, but rather kept it stable in the range of 17–18%. This suggests that the cell wall fraction structure (cellulose, hemicellulose, and lignin) of nutmeg husk flour has characteristics comparable to that in rice bran.

The stability of this SK level directly contributes to the results of the digestibility test. As seen in the data on Digestibility of Dry Matter (KcBK) and Digestibility of Organic Matter (KcBO), the presence of fiber at the level of 17.85–18.86% can still be overhauled and optimally adapted by the digestive enzymes of rumen cellulolytic microbes without inhibiting the degradation process of other organic nutrients.$\approx 50-51\% > 89-94\%$

Physical Durability of Biscuits and Feed Efficiency of Transportation

From the perspective of *feed processing technology*, the braiding of coarse fibers from sago pulp, gamal leaf flour, and nutmeg peel flour acts as a *structural matrix* when feed is compacted and molded into biscuits.

The presence of coarse fibers in the range of 17.85–18.86% provides flexibility and structural strength to the biscuits, resulting in a high *durability index* and not easily cracking or shattering into dust during logistics handling. In the context of broiler transportation, feed with measurable fiber content guarantees that livestock get a stable sense of satiety (*gut fill*) as well as a regular supply of energy without overloading the cargo space of the carrier.

Nitrogen-Free Extra Material Content (BETN)

The content of Nitrogen-Free Extract (BETN) reaches **46.95% – 51.23%** (Table 1). The high value of BETN comes from sago pulp (35%) which is rich in easily soluble carbohydrates. This abundant supply of BETN serves as a source of *fast-release energy* that is urgently needed by livestock to restore stamina and maintain physical condition during transportation trips.

BETN represents a fraction of easily soluble carbohydrates (such as starch, simple sugars, and highly digestible hemicellulose) that are the main source of energy for rumen and host microbes. The high value of BETN in these feed biscuits is largely contributed by the starch content of sago pulp (35%) and rice bran. These non-fiber carbohydrates will be rapidly fermented in the rumen producing Volatile Fatty Acids (VFA) as the main source of metabolic energy for broiler ruminant livestock.

Ash Contents

Ash content analysis results ranged from **12.78% to 13.43%** (table 1). Ash content represents the total content of organic and inorganic minerals (such as calcium, phosphorus, magnesium, and potassium) contained in the ration. The high ash content in this formula is contributed by the contribution of constituent raw materials such as fishmeal (a source of macrominerals Ca and P), gamal leaf flour, and natural minerals contained in agro-industrial waste (sago pulp and nutmeg peel). These minerals are very important to support the physiological function of the livestock body and rumen microbial metabolism.

The presence of inorganic minerals reflected in ash content is essential in meeting the physiological needs of ruminant livestock. Macro minerals such as calcium and phosphorus are

absolutely necessary for the formation and maintenance of skeletal tissue, metabolic enzyme function, and rumen fluid buffers. In addition, certain minerals in ash (such as sodium, potassium, and magnesium ions) act as essential cofactors that support the optimal growth of cellulolytic and amylolitic bacterial populations within the rumen, so that overall the initial nutritional profile of these feed biscuits has proven to be very balanced and meets modern ruminant feed quality standards. 13,35%

Externally achieved:

The document results of the proximate analysis of the nutritional content of animal feed biscuits with three levels of nutmeg skin supplementation (P0, P1 and P2).

Digestibility

Evaluation of the nutritional value of ruminant feed ingredients in *vitro* is one of the reliable, efficient, and highly correlated biochemical methods with *in vivo testing* (Tilley & Terry, 1963). The two-stage method developed by Tilley and Terry replicates the digestive process biologically in the rumen (microbial fermentation stage for 48 hours) followed by enzymatic digestion using pepsin-HCl (simulation of abomasum and small intestine for the next 48 hours). Through this approach, key parameters such as dry matter digestibility (KcBK), organic matter digestibility (KcBO), crude protein digestibility (KcPK), crude fiber digestibility (KcSK), and rumen fermentation end products in the form of Volatile Fatty Acids (VFA) and Ammonia () can be measured precisely to assess the quality and potential utilization of a ration or feed ingredient. NH_3

Based on the results of the fingerprint analysis using the Complete Random Design (RAL) with three levels of treatment (Sample A, Sample B, and Sample C) and two replicas, a diversity of biological responses was obtained that provided an in-depth picture of the dynamics of nutrient degradation (Table xx).

Digestibility of Dry Matter (KcBK) in the Rumen and Post-Rumen

The results of statistical analysis showed that the treatment had **a very significant effect ()** $P < 0,01$ on the KcBK value of Phase I (Rumen), with a value of . Sample A produced the highest rumen digestibility (), followed by Sample B () and Sample C (). This very striking difference reflects the variation in the availability of F_{hitung} 4115,6150,53%44,07%43,49%*soluble fractions* as well as the physical-chemical structure of each feed ingredient in the early incubation phase of rumen microbes. Sample A has a proportion of *fermentable carbohydrate fractions* that are more responsive to early colonization by bacteria and rumen protozoa.

However, when entering Phase II (Total Digestibility after enzymatic digestion of pepsin), the KcBK values of the three samples became relatively uniform, ranging from to , and statistically showed an 50,23%51,09%**intangible difference ()** $P > 0,05$. This phenomenon indicates that the fraction of nutrients that failed to be optimally degraded by rumen microorganism enzymes in Sample B and Sample C was able to undergo enzymatically continued digestion by pepsin in the post-rumen phase. This balances the total utilization of dry *matter digestibility*.

Digestibility of Organic Matter (KcBO) in the Rumen and Post-Rumen

In contrast to dry matter, the digestibility value of organic matter (KcBO) at both the rumen and total stages showed **a distinct unpronounceable effect ( )** $P > 0{,}05$ between the three treatments, with the average rumen KcBO being very high and uniform (up to) and total KcBO reaching the range of up to . $89{,}66\%89{,}77\%93{,}85\%94{,}31\%$

The high KcBO number indicates that the organic components that make up the feed material (such as structural, non-structural, and protein carbohydrates) of the three samples have excellent solubility in the rumen liquid. The rumen microbial community (*cellulolytic, amylolytic, and proteolytic bacteria*) is able to utilize these organic materials efficiently. The absence of a significant difference in total KcBO confirms that the fermentation and enzymatic digestion capacity of the post-rumen is able to compensate for variations in the initial composition of organic matter, resulting in an equivalent level of organic nutrient uptake for ruminant livestock.

Crude Protein Digestibility (KcPK) in the Rumen and Post-Rumen

Variety-based fingerprint analysis on crude protein digestibility showed **a very pronounced effect ()** $P < 0{,}01$ on both Stage I (rumen,) and Stage II (total post-rumen). $F_{hitung} = 1303{,}74 F_{hitung} = 88{,}50$

- In **Phase I (Rumen), Sample B** produced the highest KcPK value (), followed by Sample C () and Sample A (). All three showed very noticeable differences between treatments based on the Tukey HSD test.$27{,}10\%24{,}87\%22{,}47\%$
- In **Phase II (Post-Rumen),** a similar pattern was seen where **Sample B** achieved the highest total protein digestibility of , a very pronounced difference compared to Sample A() and Sample C().$64{,}19\%58{,}33\%56{,}47\%$

The high digestibility of protein in Sample B indicates that the peptide bonds and protein structure in treatment B feed materials are more vulnerable and easily accessible to rumen microbial protease enzymes and pepsin enzymes in the post-rumen. The efficiency of the utilization of feed protein is crucial in supplying essential amino acids that will be absorbed in the small intestine to support the productivity of broiler and dairy livestock.

Table 3. Average Recapitulation of *In-Vitro Digestibility Variables* in the Rumen and Post-Rumen

Observation Variables	Sample A	Sample B	Sample C	Calculation
Digestibility of Dry Matter (KcBK) Rumen (%)	$50{,}53 \pm 0{,}09^{a}$	$44{,}07 \pm 0{,}11^{b}$	$43{,}49 \pm 0{,}05^{c}$	$4115{,}61^{**}$
Digestibility of Dry Matter (KcBK) Post-Rumen (%)	$51{,}09 \pm 0{,}13^{a}$	$50{,}23 \pm 0{,}01^{a}$	$50{,}79 \pm 0{,}86^{a}$	$1{,}51^{tn}$
Digestibility of Organic Matter (KcBO) Rumen (%)	$89{,}77 \pm 0{,}74^{a}$	$89{,}66 \pm 0{,}26^{a}$	$89{,}70 \pm 0{,}12^{a}$	$0{,}03^{tn}$

Digestibility of Organic Matter (KcBO) Post-Rumen (%)	$94{,}31 \pm 0{,}66^{a}$	$93{,}85 \pm 1{,}45^{a}$	$94{,}17 \pm 0{,}33^{a}$	$0{,}12^{tn}$
Digestibility of Crude Proteins (KcPK) Rumen (%)	$22{,}47 \pm 0{,}06^{a}$	$27{,}10 \pm 0{,}04^{c}$	$24{,}87 \pm 0{,}14^{b}$	$1303{,}74^{**}$
Digestibility of Crude Proteins (KcPK) Post-Rumen (%)	$58{,}33 \pm 0{,}83^{a}$	$64{,}19 \pm 0{,}65^{b}$	$56{,}47 \pm 0{,}01^{a}$	$88{,}50^{**}$
Crude Fiber Digestibility (KcSK) Rumen (%)	$85{,}01 \pm 0{,}80^{a}$	$102{,}69 \pm 4{,}75^{b}$	$90{,}16 \pm 0{,}33^{a}$	$21{,}22^{*}$
Post-rumen Crude Fiber Digestion (KcSK) (%)	$59{,}90 \pm 1{,}04^{a}$	$77{,}05 \pm 2{,}87^{b}$	$64{,}09 \pm 1{,}03^{a}$	$46{,}16^{**}$

Primary Date, 2026

Description:

- *Values are presented in the form of Standard Deviation Average (SD).*$\pm$
- *Numbers on the same line with different supersrip letters (a, b, c) show a noticeable difference based on the Tukey HSD follow-up test ().*$\alpha = 0{,}05$
- *(*) Real difference (); (**) Very real difference (); () Unreal difference ().*$P < 0{,}05 P < 0{,}01$tn$P > 0{,}05$

Crude Fiber Digestion (KcSK) in the Rumen and Post-Rumen

The crude fibre digestibility (KcSK) parameter also provides a very interesting and statistically significant response:

- In **Stage I (Rumen),** the treatment exerted **a real effect ( )**$P < 0{,}05$ with . $F_{hitung} = 21{,}22$**Sample B** recorded the highest average rumen KcSK of , in stark contrast to Sample A () and Sample C ().$102{,}69\%85{,}01\%90{,}16\%$
- In **Phase II (Post-Rumen),** the treatment exerts **a very noticeable effect ( )**$P < 0{,}01$ with , where $F_{hitung} = 46{,}16$**Sample B** again excels with total crude fiber digestibility reaching . $77{,}05\%$

The digestibility value of crude fibers in Sample B that is close to or slightly exceeds () at the rumen stage is a phenomenon that is often found in laboratory $100\%102{,}69\%$*analysis of in vitro* high-fiber materials. This is generally due to a combination of analytical factors, including the relatively low initial crude fiber content of the substrate so that it is susceptible to residue weighing deviations, as well as the contribution of rumen microorganism biomass mass that is closely attached to the rest of the fiber and is also counted as part of the residue or undergoes chemical matrix interactions during the acid-base extraction process. Biologically, this figure confirms that the cell wall fractions (cellulose and hemicellulose) in Sample B have a very superior degradability to the colonization of rumen microbial cellulase enzymes.

Externally achieved:

- Documents of the results of analysis of dry matter digestibility (KcBk), organic matter digestibility (KcBo), crude protein digestibility (KcPK) and crude fiber digestibility (KcSK) in rumen and post-rumen in vitro.

Dynamics of Rumen Fermentation Characteristics (VFA and Ammonia Production)NH_3

Production of Volatile Fatty Acids (VFA)

Volatile Fatty Acids (VFA) are the main product of carbohydrate fermentation by rumen microbes that function as the main energy source (providing up to 70–80% of the total energy needs) for ruminant livestock. The observed partial VFA variables include Acetic Acid, Propionic Acid, and Butyric Acid, as well as Total VFA accumulation.

Table 4. Results of Analysis of Volatile Fatty Acids (VFA) Production in Rumen

Observation Variables	Sample A	Sample B	Sample C	Calculation
Acetic Acid (mMol)	$19{,}36 \pm 4{,}31^{a}$	$24{,}05 \pm 1{,}73^{a}$	$21{,}08 \pm 6{,}57^{a}$	$0{,}52^{tn}$
Propionic Acid (mMol)	$6{,}15 \pm 0{,}20^{a}$	$6{,}55 \pm 0{,}60^{a}$	$7{,}18 \pm 0{,}26^{a}$	$3{,}42^{tn}$
Butyric Acid (mMol)	$3{,}49 \pm 1{,}02^{a}$	$4{,}42 \pm 1{,}49^{a}$	$6{,}77 \pm 0{,}13^{a}$	$5{,}21^{tn}$
Total VFA (mMol)	$28{,}99 \pm 5{,}52^{a}$	$35{,}01 \pm 3{,}83^{a}$	$35{,}02 \pm 6{,}70^{a}$	$0{,}80^{tn}$

Primary Date, 2026.

Based on the results of the ANOVA RAL test:

1. **Acetic Acid:** The average ranges from up to mMol (, , differing not real). Acetic dominates the proportion of total VFA (~60–67%), which indicates high fiber or cell wall fermentation activity within the $19{,}3624{,}05F_{hitung} = 0{,}52P > 0{,}05$*in vitro* medium. Despite the slight variation between treatments, acetic acid still dominates the proportion of partial VFA produced. This reflects the high fermentation activity of cell wall or fiber fractions (from sago pulp, gamal leaf flour, and nutmeg husk) by cellulolytic microbial populations within the *in vitro* medium, which is a typical characteristic of fibrous feed digestion in ruminant livestock.
2. **Propionic Acid:** The average ranges from up to mMol (, , differing not real). Although Sample C shows a tendency for slightly higher propionate values, the diversity between replicates makes the difference not statistically significant. Propionate is the main glucogenic precursor in ruminant liver.$6{,}157{,}18F_{hitung} = 3{,}42P > 0{,}05$
3. **Butyric acid:** The average ranges from up to mMol (, , different is not real). The range of butyric acid formed indicates the normal fermentation activity of rumen microbes in breaking down feed carbohydrates, where butyrate along with other flying fatty acids is ready to be absorbed by the rumen wall as a source of metabolic energy for livestock. $3{,}496{,}77F_{hitung} = 5{,}21P > 0{,}05$

4. **Total VFA:** The total accumulation of VFA ranges from up to mMol with a value (). Physiologically, this total range of VFA reflects the metabolic activity of rumen microbes that are normal and active in degrading the feed substrate being tested.$28,9935,02F_{hitung} = 0,80P > 0,05$

Ammonia Concentration () of Rumen FluidNH_3

The concentration of rumen fluid showed NH_3**a noticeable effect ()**$P < 0,05$ with the value of . Based on Tukey HSD's follow-up test, $F_{hitung} = 12,34$**Sample C** produced the highest concentration (mg/100 ml) that was significantly different from $NH_3$39,84**Sample B** (mg/100 ml), while 34,77**Sample A** was in the intermediate position (mg/100 ml).36,35

Biologically, ammonia () is the main source of nitrogen that is essential for the synthesis of rumen microbial body proteins (NH_3*microbial crude protein synthesis*). The optimal range of rumen fluid to support maximum microbial growth is generally reported to be up to mg/100 ml, or up to above mg/100 ml in feed conditions with high protein degradability. The values recorded for all treatments (>34 mg/100 ml) showed that the feed material tested had a very abundant content of $NH_3$52030NH_3*degradable proteins*, so that the availability of nitrogen for cellulolytic and proteolytic bacterial populations in the incubation medium was abundantly sufficient.

Table 5. Results of analysis of NH3 production in Rumen

Observation Variables	Sample A	Sample B	Sample C	Calculation
Ammonia () (mg/100 ml)NH_3	$36,35 \pm 1,72^{ab}$	$34,77 \pm 0,40^{a}$	$39,84 \pm 0,40^{b}$	$12,34^{*}$

Primary Date, 2026.

Externally produced:

Document of the results of the analysis of the partial production of Volatile Fatty Acids (VFA) and Ammonia (NH3) in the rumen.

Based on the entire series of Complete Random Design (RAL) fingerprint analysis of fermentation and *in vitro digestibility parameters*, it can be concluded that:

1. The treatment has a very real effect () on the KcBK rumen, KcPK rumen, total KcPK, and total KcSK; has a real effect () on and KcSK rumen; and has an intangible effect () on total KcBK, KcBO rumen, total KcBO, as well as all VFA fractions (acetate, propionate, butyrate, and total VFA).$P < 0,01P < 0,05NH_3P > 0,05$
2. **Sample B** showed the most consistent performance of excellence especially in the digestibility parameters of crude protein and total crude fiber, while **Sample C** produced the highest concentration of ammonia () which supports the availability of nitrogen for rumen microbes.NH_3

3. The fermentation characteristics of all three samples are within an excellent physiological range to support the utilization of agricultural waste-based feeds in ruminant rations.

CONCLUSION

Based on the findings, this study concluded that feed biscuits formulated from agricultural byproducts and supplemented with nutmeg (Myristica fragrans) pericarp meal exhibited promising nutritional characteristics and in vitro digestibility, indicating their potential as supplemental feed for beef cattle during transportation. The ruminal fermentation characteristics of the three treatments suggested that the formulated feed biscuits could support nutrient utilization in ruminant diets. Furthermore, nutmeg pericarp contains bioactive compounds, including essential oils, saponins, and tannins, that may influence ruminal microbial activity and fermentation processes. However, their antimicrobial effects and potential to reduce oxidative and metabolic stress during livestock transportation require further experimental validation. Additional studies assessing shelf life, animal performance, and physiological responses under actual transportation conditions are necessary to establish the practical effectiveness of these feed biscuits.

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