P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.031

Digestibility of Feed Nutrients and Characteristics of Rumen Fluid Supplemented with Garlic Peel Antioxidants (*Allium sativum*) and Organic Minerals

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Article History: 24-654 Received: 03-Oct-24 Revised: 04-Mar-25 Accepted: 07-Mar-25 Online First: 21-Mar-25

ABSTRACT

This research aimed to determine the effect of adding garlic peel antioxidant supplementation and organic minerals to rations prepared with an RDP: RUP ratio of 60:40, on digestibility, rumen fermentation characteristics, microbial protein synthesis, protozoa population, and methane gas production. This research used an *in vitro* method, which used goat rumen fluid, with a completely randomized design with 4 treatments and 4 replications. Treatment consisted of P0=no supplementation; P1=3% organic mineral supplementation; P2=Supplementation 3% garlic peel; P4=Supplementation 1.5% organic minerals + 1.5% garlic peel. The results show that feed supplementation using garlic peel antioxidants and organic minerals significantly affects ($P<0.05$) on dry matter digestibility, organic matter digestibility, crude fiber digestibility, and crude fat digestibility. However, there was no significant ($P>0.05$) effect on pH values, VFA and NH3 concentrations. Furthermore, has a real effect on reducing protozoa populations and methane gas. This research concluded that rations with the addition of 3% garlic peel antioxidant supplementation and the addition of 1.5% garlic peel supplementation and 1.5% organic minerals can improve rumen fermentation characteristics and nutrient digestibility. However, further research needs to be done on in vivo methods directly on livestock.

Key words: Supplementation, Antioxidants, Garlic Peel, Organic Minerals, Digestibility.

INTRODUCTION

To meet the needs of young livestock, livestock in the growth period, and for livestock with high production, it is necessary to improve feed quality. Feed improvements are expected to optimize rumen performance so nutrient absorption can run optimally. Rumen microbes can function to break down feed into a simpler form so that it is more easily absorbed. Generally, fibrous feed will produce higher levels of acetic acid and CH4 (methane) than grain-based feed. Feeding supplementation with garlic peel flour (Allium sativum) and organic minerals contains allicin and organic minerals (Se, Cr and Zn). Addition of several plant extracts to the rumen resulted in inhibition of deamination and methanogenesis, resulting in lower ammonia, CH4 and acetate, as well as higher concentrations of propionate and butyrate. Research shows that administering garlic peel extract can increase propionate production, reduce acetate or CH4 production, and modify proteolysis, peptidolysis, or deamination in the rumen (Tuwaidan et al. 2024). Efficiency of a mixture of secondary metabolites from garlic extract and oranges on CH4 production, rumen fermentation, feed efficiency, and digestibility in various feeding methods, *in vitro* at a level of 20%/substrate has the potential to reduce CH4 production effectively in all feeding methods. This treatment shows reducing power as high as 69% if the amount of concentrate composed reaches 800 g/kg ration, increases 20% VFA production and does not interfere with fiber digestibility (Ahmed et al. 2021).

Antioxidants play a great role in the health and production of livestock. Antioxidants play a crucial role in safeguarding the body by capturing free radicals, thereby lowering the risk of various diseases (Pruteanu et al. 2023). According to Prodanović et al. (2023), antioxidants enhance overall health, improve reproductive function, and support milk quality in dairy cows. These compounds, molecules, or substances are stable enough to donate electrons or hydrogen to neutralize free radicals. Various food sources serve as the primary providers of antioxidants (Ibroham et al. 2022). Chauhan et al. (2021) found that supplementation with plant extracts rich in antioxidants can mitigate oxidative stress in the blood of sheep raised in high-temperature environments. Furthermore, Wang et al. (2022)

Cite This Article as: Sari RM, Astuti T, Akbar SA and Nurhaita, 2025. Digestibility of feed nutrients and characteristics of rumen fluid supplemented with garlic peel antioxidants (*Allium sativum*) and organic minerals. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2025.031

reported that polyphenol- and antioxidant-rich extracts can enhance antioxidant levels in meat while inhibiting lipid oxidation during storage in sheep. Additionally, Yanfen et al. (2021) highlighted that green tea polyphenol supplementation functions as an antioxidant, playing a role in increasing milk production and reducing stress in lactating cows.

Garlic peel (*Allium sativum*) is a source of natural antioxidants which are rich in sulfur compounds and allicin. These compounds have the potential to protect cells from oxidative damage that can be caused by free radicals, which often increase in the digestive system of livestock due to the fermentation process. Ranjan et al. (2020) stated that garlic peel supplementation can increase positive microbial activity in the rumen, while reducing the number of pathogenic microbes, thereby increasing feed digestibility. Molho-Ortiz et al. (2022) added that the use of garlic and cinnamon essential oil 900 mg/L rumen inoculum effectively reduced CH_4 emissions by 64.7%; however, it can suppress dry matter digestibility *in vitro*.

Meanwhile, organic minerals have been recognized as a more efficient alternative to conventional inorganic minerals. Organic minerals have a higher level of availability, so they are more easily absorbed by the livestock's body. By adding organic minerals to feed, nutrient digestibility can be significantly increased. Kincaid et al. (2000) stated that organic mineral supplementation improves rumen function and helps in regulating rumen pH, which is very important for digestive health. Various studies have highlighted the positive effects of Se and vitamin E supplementation on antioxidant activity and overall health improvement in dairy cattle (Salles et al. 2022). Another study found that administering Se and vitamin E significantly enhances calf growth rates by reducing perinatal oxidative stress (Xiao et al. 2021). Specifically, Se is recognized as an essential element for ruminants, contributing to normal growth (Jamali et al. 2022), fertility and the prevention of health issues such as retained placenta, abortion, mastitis (Wang et al. 2021) and diarrhea in calves (Schinwald et al. 2022).

If studied further, providing antioxidants from garlic peel and organic minerals can be used as a supplement in animal feed. The hope is that there will be an increase in feed digestibility and absorption with optimal rumen fluid characteristics. This study aims to examine the effect of providing garlic peel antioxidants and organic minerals in ruminant feed on rumen fluid characteristics and feed digestibility.

MATERIALS AND METHODS

Ethical approval

Ethical approval was not required because this study did not use any live animals.

Study period and location

This research was taken place at Ruminant Laboratory in the Faculty of Animal Science, Andalas University, Padang, Indonesia from July to September 2024.

Sample preparation and experimental diets

Forage samples (field grass, lamtoro, indigofera, titonia) were collected from the area around the UMMY campus. Each sample was dried using sunlight in a greenhouse, then continued drying using a 60°C oven for 24 hours until the samples could be cut and ground using a grinder machine. Samples that have been ground are filtered using a 1mm sieve. Concentrate samples, namely rice bran, ground corn, minerals were obtained at one of the poultry shops, and tofu dregs were obtained from one of the tofu factories, then dried and ground. Garlic peel supplement ingredients are dried and ground, while organic minerals are made using rice media.

Experimental design

This study used an *in vitro* approach, completely randomized plan with 4 treatments and 4 replications. Treatment consisted of P0=no supplementation; P1=3% organic mineral supplementation; P2=Supplementation 3% garlic peel; P4=Supplementation 1.5% organic minerals + 1.5% garlic peel. The variables observed were the nutrient digestibility, pH value of rumen fluid, total VFA concentration, NH_3 concentration, microbial protein synthesis, protozoa population and methane production. The chemical composition of each diet can be seen in Table 1.

Table 1: Ingredients and chemical composition of experimental diets

Component	Diet
Ingredient composition (%)	
Native grass	28
Tithonia diversifolia	10
Leucaena leucocephala	17
Indigofera zollingeriana	15
Tofu West	8
Rice Bran (*Oroza sativa)*	10
Mays (*Zea mays*)	11
Mineral	1
	100
Chemical Composition (%)	
Organic matter	89.17
crude protein	17.40
Crude fiber	17.98
Crude fat	4.04
Nitrogen Free Extract	45.73
Total Digestible Nutrient (TDN)	66.98
Neutral Detergent Fiber (NDF)	36.40
Acid Detergent Fiber (ADF)	23.50
Cellulose	16.47
Hemicellulose	12.72
Rumen Degradable Protein (RDP)	60.00
Rumen Undegradable Protein (RUP)	40.00

In vitro method

Samples of diets were incubated with buffered rumen according to Tilley and Terry method (Tilley and Terry 1963). Rumen liquor was obtained from a slaughterhouse in the city of Padang from four Ettawa goats which were fed *ad libitum* course and concentrate. The rumen content was filtered with four layers of nylon (100m strainer size) and then placed into thermos flasks after the slaughter (39°C). As suggested by McDonald et al. (2010), filtered rumen liquor was diluted with buffer solution at a ratio of 1:4 (rumen fluid:buffer solution). An amount of 2.5g sample was mixed with 250mL of mixed solution (rumen liquor and buffer) in each Erlenmeyer flask and incubated anaerobically by injecting CO_2 gas into the Erlenmeyer, which was then immediately sealed with a rubber lid. Each sample was replicated three times. Each Erlenmeyer was kept in an orbital shaking incubator at 39°C and 100rpm for 48h. The pH of the Erlenmeyer was tested using a pH meter after it was immersed in ice water for 48h to cease microbial activity.

The supernatant and residue were then separated using a centrifuge at 3000rpm for 5min at 4°C. The NH3, total VFA, microbial protein synthesis and number of protozoa analyzes supernatant was stored in bottles in a -18°C freezer. The Conway and O'Malley (1942) method was used to determine the NH3 concentration. Steam distillation method was used according to Abdurachman and Askar (2000) to determine total VFA concentration. Lowry's technique (Lowry et al. 1951) was used to determine microbial protein production. The number of protozoa was determined by means of Ogimoto and Imai (1981). Methane production was determined by using partial VFA components followed by Abdurachman and Askar (2000) procedures. Moss et al. (2000) equation was used to calculate *in vitro* methane (CH4) emissions based on partial VFA component concentrations. Meanwhile, the residue was filtered through Whatman paper No.41 and dried in an oven at 60°C in 48h for nutrient digestibility. Nutrient digestibility was determined by subtracting nutrient residue from their initial amounts before the incubations, respectively. The *in vitro* was conducted in five replications and each replication was served by two bottles containing only a mixed solution of rumen liquor and buffer. However, no samples were incubated as blanks or as a correction factor in the calculation of nutrient digestibility. These formulas were used to compute *in vitro* nutrient digestibility:

$$\text{IVDMD} = \frac{\text{DM samples} - (\text{DM residual} - \text{OM blanks})}{\text{DM samples}} x100\%$$

$$\text{IVOMD} = \frac{\text{OM samples} - (\text{OM residual} - \text{OM blanks})}{\text{OM samples}} x100\%$$

$$\text{IVCPD} = \frac{\text{CP samples} - \text{CP residual}}{\text{CP samples}} x100\%$$

$$\text{IVEED} = \frac{\text{EE samples} - \text{EE residual}}{\text{EE samples}} x100\%$$

$$\text{IVCFD} = \frac{\text{CF samples} - \text{CF residual}}{\text{CF samples}} x100\%$$

$$\text{IVNFED} = \frac{\text{NFE samples} - \text{NFE residual}}{\text{NFE samples}} x100\%$$

Where:
IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic matter digestibility, IVCPD: *In vitro* crude protein digestibility, IVEED: *In vitro* extract ether digestibility, IVCFD: *In vitro* crude fiber digestibility, IVNFED: *In vitro* nitrogen-free extract digestibility, DM: dry matter, OM: organic matter, CP: crude protein, EE: extract ether, CF: crude fiber, NFE: Nitrogen-Free Extract.

Making garlic: Peel Flour (*Allium sativum*) was prepared by peeling the garlic to separate the peel and flesh. The peel then dried at a temperature of 80°C for 2x24 hours and then ground using a blender (Prayitno et al. 2013).

Making organic chromium and selenium minerals: The base solution used to make Chromium was 2.5481g of $CrCl_3 \cdot 6H_2O$ in 500mL of aquadest (1000 ppm Chromium). Then 200 ppm Chromium was made by taking 200mL of Chromium stock and adding it to 800mL of aquadest. The medium used was rice. The method for making it is as follows: (1) weigh 200 grams of rice; (2) rice mixed with 100mL of 200ppm Cr solution and 25mL of aquadest, then stirred thoroughly; (3) the material is placed in a glass jar and sterilized using an autoclave at 121°C for 20 minutes; (4) the material is spread in a plastic tray that has been sterilized with alcohol; (5) adding 5g of dry starter *Saccharomyces cerevisiae* by placing it flat on the surface of the media and covering it with plastic; (6) the culture is left for 3-5 days in a sterilized room, maintaining the sterilization of the room by spraying alcohol; (7) When they are big, immediately put them in the oven at 70°C until dry, then blend until they become flour. Organic selenium used 0.7025g of SeO2 material with manufacturing stages such as Cr organic. To obtain a concentration of Cr 1.5ppm and Se 0.3ppm, the substrate was mixed with a carrier (yellow corn flour) to reduce its concentration according to the treatment. Whereas, the manufacture of Zinc Lysinate Mineral was carried out according to Prayitno and Widyastuti (2010). A total of 0.099g of ZnSO4 was mixed with 0.198g of Lysine-HCl, then dissolved in 50mL of water and mixed into 1kg of starch. After that, it was dried at a temperature of 60°C for 24 hours. (Prayitno and Widyastuti 2010)

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software was used to analyze the collected data using analysis of variances (IBM SPSS Statistics, USA) version 21.0.

RESULTS AND DISCUSSION

Nutrient digestibility

The addition of garlic peel antioxidant supplementation and organic minerals in the ration gave results that tended to increase the digestibility of the ration ($P<0.05$). The highest digestibility was obtained in feed supplementation with the addition of 1.5% garlic peel and 1.5% organic minerals. The nutrient digestibility of this study can be seen in Table 2.

Feed supplemented with garlic peel can improve digestibility. This can be caused by the addition of antioxidants and antibacterials contained in garlic peel. Garlic contains flavonoids and organosulfur which act as antioxidants and antibacterials. Hedges and Lister (2007) stated that flavonoids, fructans, organosulfur and saponins are phytochemical components found in onions (*Allium*). Hanen et al. (2012) added that the largest antioxidant components in onions come from phenolics, flavonoids and vitamin C, while organosulfur has an important role as an antibacterial. The high antibacterial content of garlic peel flour can inhibit the growth of pathogenic bacteria so that the absorption of nutrients and antioxidants is better. Hedges and Lister (2007) stated that the antimicrobial content of garlic can inhibit *Clostridioum, Bacillus, Escherichia, Micrococcus, Mycobacterium, Pseudomonas, Salmonella, Staphylococcus, Streptococcus* and several others.

Table 2: Nutrient digestibility of experimental diets

Digestibility (%)	Treatments			
	No Supplementation	3% Organic minerals	3% garlic peel	1.5% organic minerals + 1.5% garlic peel
IVDMD	55.26±1.41b	56.87±0.83b	62.23±1.05a	59.48±1.22ab
IVOMD	52.87±2.58b	54.75±1.08b	61.32±1.35a	58.07±5.96ab
IVCFD	56.24±2.91b	60.87±1.95b	66.14±3.99a	65.82±4.68b
IVCPD	59.26±0.77a	60.43±1.68a	62.54±1.62a	61.02±1.95a
IVEED	51.39±4.20c	53.20±2.21c	61.41±2.41a	59.06±1.52b
IVNDFD	56.24±2.82c	57.08±3.15c	64.32±3.81a	60.21±4.40b
IVADFD	52.35±1.40b	54.12±1.50b	57.45±1.65a	55.34±1.91b
IVRUPD	58.12±2.44b	60.07±2.08a	62.02±3.45a	60.09±1.50a

Values (mean±SD) bearing different letters in a row differ significantly (P<0.05); *IVDMD, *in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; IVCFD, *in vitro* crude fiber digestibility; IVCPD, *in vitro* crude protein digestibility; IVEED, *in vitro* ether extract digestibility; IVNDFD, *in vitro* neutral detergent fiber digestibility; IVADFD, *in vitro* acid detergent fiber digestibility; IVRUPD, *in vitro* rumen undegradable protein digestibility/by pass protein.

Table 3: Rumen fermentation characteristics of experimental diets.

Variables	Treatment			
	No supplementation	3% organic minerals	3% garlic peel	1.5% organic minerals + 1.5% garlic peel
pH	7.05±0.17	6.98±0.10	6.95±0.13	6.94±0.09
Total VFA (mM)	97.42±8.54b	98.7±6.29b	117.49±6.45a	106.86±6.29b
NH_3	11.49±2.78b	12.67±1.89b	15.63±1.50a	13.35±1.78b

Values (mean±SD) bearing different letters in a row differ significantly (P<0.05).

Table 4: Microbial protein synthesis (MPS), total protozoa, and methane production of dietary treatments

Variables	Treatment			
	No supplementation	3% organic minerals	3% garlic peel	1.5% organic minerals + 1.5% garlic peel
MPS (mg/100mL)	97.42±2.28c	98.75±1.11bc	117.49±2.01a	106.86±4.49b
Total Protozoa (cell x $10^{5)}$	5.60±0.89a	4.44±0.87ab	2.56±0.69b	3.06±0.45b
Methane Production (mM)	13.30±4.10a	12.86±3.56a	10.41±3.45b	11.92±4.22b

Values (mean±SD) bearing different letters in a row differ significantly (P<0.05).

The antibacterial content of onion peel inhibits the growth of pathogenic bacteria in the digestive tract, so that digestive nutrients can be absorbed properly. The antioxidants absorbed will help prevent oxidation in digestive tract cells so that the cells in the digestive tract become healthier and nutrient absorption improves. High nutrient absorption in the digestive tract increases the digestibility of crude protein in feed. This is in accordance with the opinion of Sukaryana et al. (2019) who stated that digestibility can be influenced by the level of feeding, type of feed, animal species, how food is processed and the health of the digestive tract. By-pass protein digestibility (RUPD) is an important parameter in the updated protein evaluation system for ruminant production, and will have an effect on ruminant productivity. If RUP is not digested, this will result in no supply of protein that can be metabolized by livestock. The results of this study showed that the digestibility of RUP varied in each treatment ration but was consistently in the range of 58-62%. These results are in line with research by Buckner et al. (2013) who reported that RUP digestibility varied greatly from 25-60%.

Rumen fermentation characteristics

The addition of garlic peel antioxidant supplementation and organic minerals in the ratio gave significantly different results (P<0.05) to the synthesis of microbial protein, methane gas and total protozoa. However, it has no real influence on the pH, VFA and NH3 values formed. The characteristics of rumen fermentation in this study can be seen in Table 3. Table 3 shows that the effect of supplementation does not affect the pH value of the rumen fluid, but numerically the pH value of the rumen fluid supplemented using garlic peel shows a decrease in value. This could be because feed supplemented with garlic peel can affect the fermentation process in the rumen. However, the pH value produced in this study was still within normal limits, namely 6.94-7.05. Viennasay and Wanapat (2020) reported that the ideal pH to support normal cellulolytic bacteria activity ranges from 6.3 to 6.8.

Dietary supplementation using garlic peel can can increases the concentration of propionate and butyrate and reduces the concentration of acetate in the rumen, so that garlic peel has the potential to increase the energy supply of ruminant livestock, propionate is converted into glucose in the liver as a precursor to milk lactose (Suhendra et al. 2015). Added high propionate has an impact on methane emissions, because propionate is a hydrogen consumer, thus reducing hydrogen produced in the rumen which also reduces methane synthesis (Vyas et al. 2018).

From Table 4, it can also be seen that the methane gas produced has a significant effect (P<0.05) in reducing the methane gas produced. This is because various organosulfur compounds, such as allicin, contained in garlic or its products have the potential to inhibit membrane lipid synthesis in the archaea community, thereby reducing the population of methanogenic archaea and resulting in decreased methane emissions (Mbiriri et al. 2015). Methane production is positively correlated with protozoa growth, due to the symbiosis between methanogenic bacteria and protozoa so that the number of protozoa will increase the growth of methanogenic bacteria (Machmüller et al. 2003). High methane production and concentration can indicate that a lot of feed energy is wasted during the fermentation process, thereby reducing the efficiency of feed use (Widiawati et al. 2010). Methane formation affects the final fermentation product, especially the number of ATP moles, which then affects the efficiency of hexose conversion into energy sources (Wahyuni et al. 2014).

Therefore, garlic onion peel supplementation mainly reduces methane emissions by changing the proportion of important short-chain volatile fatty acids in the rumen, especially propionate and acetate (Suybeng et al. 2020).

Conclusion

This research concluded that rations with the addition of 3% garlic peel antioxidant supplementation could improve rumen fermentation characteristics and nutrient digestibility. However, further research needs to be done on *in vivo* methods directly on livestock.

Acknowledgement: The author would like to thank the Directorate of Research and Community Service, Ministry of Research and Technology, which has funded this research with the Basic Research scheme with contract number 112/E5/PG.02.00.PL/2024.

Funding: The research was funded by Directorate of Research and Community Service, Ministry of Research and Technology, with the Basic Research scheme in 2024.

Conflict of interest: The authors have declared that no competing interests exist.

Author's contribution: RM, NT, TA, SAA designed the experimental work in the laboratory. RM prepared the manuscript and performed data analysis. TA and NT assisted in laboratory analysis under the supervision of RM and SAA. All authors read and approved the final version of the manuscript.

Data Availability Statement: The data supporting this study's findings are available in this article. There is no other supporting data.

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