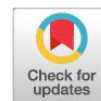


Research Article



Effects of Papain or Bromelain Administration on Rumen Fermentation, Blood Metabolites, and Meat Quality in Sheep

DILLA MAREISTIA FASSAH¹, YUSTI PUJIAWATI^{1,2}, KOKOM KOMALASARI¹, BACHTAR BAKRIE², SALFINA NURDIN AHMAD², ENI SITI ROHAENI², DEWI APRI ASTUTI^{1*}

¹Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Jl. Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia; ²Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency of Indonesia, Cibinong Science Center, Jalan Raya Jakarta-Bogor, Cibinong, Bogor 16915, Indonesia.

Abstract | Papain and bromelain are plant-derived proteolytic enzymes that may enhance protein degradation and nutrient availability in livestock. This study aimed to evaluate the effects of oral administration of papain or bromelain on rumen fermentation characteristics, blood metabolite profiles, and meat quality of thin-tailed sheep. Fifteen female thin-tailed sheep (8–10 months old; body weight 12.75 ± 0.30 kg) were randomly assigned to three groups ($n = 5$ per group): a control group, a papain-treated group (36 mg/kg body weight), and a bromelain-treated group (36 mg/kg body weight), with treatments administered orally. Sheep were fed a diet consisting of 70% starter concentrate and 30% *Pennisetum purpureum* for 12 weeks. The parameters measured included rumen fermentation characteristics, blood metabolites profile, feedlot performance, and meat quality. Oral administration of papain or bromelain tended to reduce nutrient intake ($P < 0.10$) and increase the molar proportion of propionate ($P = 0.08$). In addition, bromelain administration tended to increase blood albumin concentration compared with the control ($P = 0.06$). No differences were observed in average daily gain, final body weight, and carcass traits by papain or bromelain oral administration. The administration of papain significantly increased ($P < 0.05$) the meat moisture content compared to bromelain, but in general, no changes were observed in the quality of lamb meat with the administration of papain or bromelain. In conclusion, oral administration of papain or bromelain at 36 mg/kg BW may modify rumen fermentation characteristics but is insufficient to enhance growth performance and meat quality.

Keywords | Bromelain, Feedlot performance, Thin-tailed sheep, Meat quality, Papain, Rumen fermentation

Received | September 02, 2025; **Accepted** | December 17, 2025; **Published** | February 09, 2026

***Correspondence** | Dewi Apri Astuti, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Jl. Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia; **Email:** dewiaa@apps.ipb.ac.id

Citation | Fassah DM, Pujiawati Y, Komalasari K, Bakrie B, Ahmad SN, Rohaeni ES, Astuti DA (2026). Effects of papain or bromelain administration on rumen fermentation, blood metabolites, and meat quality in sheep. *J. Anim. Health Prod.* 14(1): 342-350.

DOI | <https://dx.doi.org/10.17582/journal.jahp/2025/14.1.342.350>

ISSN (Online) | 2308-2801



Copyright: 2026 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

The sheep population in Indonesia has shown a consistent increase over the years. This condition is due to the increasing demand for mutton and lamb chops, especially in Java and Sumatra islands, which are rich in traditional cuisine. Improving the feeding efficiency is a key to

fulfilling the growing demand in sheep production. The efficient digestion process is essential for supporting growth performance and product quality in intensive sheep production systems. Among various strategies, feed additives, particularly exogenous enzymes, offer a great potential to modulate the rumen microbial activity and enhance nutrient digestibility (Chung *et al.*, 2012).

Papain and bromelain, plant-derived proteases that are widely known for their application in meat tenderization through the breakdown of both myofibrillar and connective tissue fractions postmortem (Ramli *et al.*, 2018; Maqsood *et al.*, 2018). Both proteases were reported to improve the tenderness and nutritive value by decreasing shear force and increasing amino acid content in the treated meat (Malek *et al.*, 2016; Singh *et al.*, 2018; Xu *et al.*, 2020). Many studies have reported the effectiveness of papain and bromelain applied at post-mortem by meat marination or injection on meat tenderization (Maqsood *et al.*, 2018; Habtu *et al.*, 2020; Marino *et al.*, 2023). Their proteolytic activities are stable across a wide range of pH and temperature conditions (Pavan *et al.*, 2012). Papain has an optimal temperature of 50–60 °C (Kusumadjaja and Dewi, 2010; Malle *et al.*, 2015). In acidic conditions, Bromelain enzyme activity is optimum at the temperature range of 10–20 °C, while in alkaline conditions, the temperature for optimum bromelain activity is 30–40 °C. Besides that, at neutral pH values, a temperature of 40–60 °C is required to achieve the optimum activity (Manzoor *et al.*, 2016). Despite its application postmortem, both proteases gained interest as an oral supplement to livestock. Those enzymes have been demonstrated to have antibacterial properties against various pathogens (Ahamed *et al.*, 2016; dos Anjos *et al.*, 2016), which could promote a beneficial microbial population in the rumen. The proteolytic activity of bromelain could break down feed components and enhance the availability and digestibility of nutrients (Pimentel *et al.*, 2022). Therefore, they may influence rumen fermentation and nutrient digestibility. These changes may be reflected in blood metabolite profiles such as glucose, albumin, and lipid parameters.

Several studies in poultry reported that bromelain can improve intestinal morphology, enhance nutrient absorption, modulate immune systems, and improve meat quality (Akit *et al.*, 2019; Yenice *et al.*, 2023). Bromelain inclusion has shown a positive effect on rumen fermentation characteristics and nutrient digestibility in cattle (Khongpradit *et al.*, 2022). Only a few studies have examined protease supplementation in ruminants (Contreras *et al.*, 2009; Putriana *et al.*, 2020), while comparative trials evaluating the oral administration of papain and bromelain in ruminants remain scarce. The present study aimed to evaluate the effects of oral papain and bromelain administration on rumen fermentability, blood metabolite profiles, feedlot performance, and meat quality of thin-tailed sheep. Our study may provide new insights into the potential use of papain and bromelain in sheep production systems.

This research got approval from Directorate of Management for Research and Innovation Permit and Scientific Authorities, National Research, and Innovation Agency of Indonesia with number of Ethical Clearance: 192/KE.02/SK/10/2023 and was conducted in accordance with animal experimental guidelines provided by Directorate of Management for Research and Innovation Permit and Scientific Authorities, National Research and Innovation Agency of Indonesia.

ANIMALS AND DIETARY TREATMENTS

The feeding trial was conducted for 12 weeks (84 days), which was preceded by 7-day adaptation period. Fifteen female thin-tailed lambs (8–10 months old, BW 12.75 ± 0.30 kg) were used in this study, located at the Pamoyanan Local Farm, Bogor, West Java, Indonesia. The treatments were with no administration (control=C; n=5), 36 mg/kg BW papain administration (n=5), and 36 mg/kg BW bromelain administration (n=5). The number of animals was selected based on comparable sheep trials and chosen to ensure statistical reliability, while also considering ethical considerations for animal use. The papain (100,000 u/g activity) was obtained from Nanning Pangbo Bioengineering Service Co., Ltd (China), while bromelain (50,000 u/g activity) was obtained from Ningbo Dingyuan Biotechnology (China). Previous oral bromelain study in dairy goats applied 185 mg/kg BW for 7 months (Contreras *et al.*, 2009). In this study, the dosage was selected to evaluate safety and metabolic responses in meat-type small ruminants. Although enzyme activities differed, equal supplementation dosing was used to ensure comparable administration volumes and handling under practical farm conditions. This study was designed to assess the relative enzyme response rather than absolute activity equivalence. The enzyme was offered by top dressing at morning feeding, while the control treatment received a placebo.

FEEDING MANAGEMENT

All animals were individually housed and fed concentrate and *Pennisetum purpureum* cv. Thailand in the ratio of 70:30. The formulation followed the requirements of sheep (NRC, 2007). The ingredients and chemical composition ratio of the concentrate mixed with grass are presented in Table 1. Animals were fed three times a day based on 4% DMI of their BW for 12 weeks. Individual daily intake, including concentrate, forage, and supplements, was recorded, while the water was available *ad libitum*. The forage and concentrate were collected as a sample for proximate analysis by using an FT-NIR Spectrometer solid cell (NIRFlex N-500, BUCHI, Switzerland). Every two weeks, the body weight was measured at 09.00 a.m. before morning feeding.

Table 1: Nutrient content of forage and concentrate of the basal diet.

Nutrient (%)	<i>Pennisetum purpureum</i> cv. Thailand	Concentrate
Dry matter	16.10	88.30
Ash	8.59	6.18
Crude protein	11.10	16.30
Crude fat	1.39	2.28
Crude fiber	33.00	10.20
Nitrogen-free extract	45.90	65.00
Total digestible nutrient ¹	67.30	75.40

¹Wardeh (1981).

RUMEN FLUID AND BLOOD SAMPLES COLLECTION

Individual blood samples were collected 2 hours after morning feeding on the last day of the proteases administration through the jugular vein using an EDTA collection vial for plasma metabolite measurements. The EDTA plasma was separated by centrifugation (3000 rpm for 15 min) and stored at -20 °C until analysis. After the last blood collection, rumen fluid was collected using an oral stomach tube method (Shen *et al.*, 2012). The pH measurement was done from fresh rumen fluid, and about 0.5 mL of rumen fluid was kept on glycerol medium for total bacteria population, and 1 mL on trypan blue formalin saline for total protozoa analysis. A total of 2 drops of HgCl₂ were added to the rest of the rumen fluid sample before being stored at -20 °C until further analysis.

RUMEN FERMENTATION ANALYSIS

Rumen fluid pH was immediately measured with a portable pH meter (HI99163, Hanna Instrument, Romania). Concentration of total and partial VFA was analyzed using gas chromatography Scion 436 GC-MS (Bruker Corp., Germany). Ammonia concentration was analyzed using the Conway micro-diffusion method following Jayanegara *et al.* (2016). Total bacteria were counted using the roller tube method, while total protozoa population was counted using the Fuchs-Rosenthal counting chamber (Ogimoto and Imai, 1981).

BLOOD METABOLITE ANALYSIS

All metabolite profiles, including glucose, triglyceride, cholesterol, total protein, and albumin, were measured using colorimetric kits number ETI11210400-6, ETI11630200-2, ETI10150200-5, ETI115700400-5, and ETI15600400-4 from Human Diagnostic worldwide (Weisbaden, Germany), respectively.

SLAUGHTER PROCEDURES AND MEAT QUALITY MEASUREMENTS

On day-85, lambs were weighed and slaughtered according to Halal protocols. The hot carcass weight and initial meat

pH were recorded immediately post-slaughter. The left leg of each carcass was removed, vacuum-sealed, and aged at 2°C for 7 days. The physicochemical analysis of leg meat was evaluated at 7 days. The leg meat was cut, and then the rest of the samples were stored at -20 °C. Samples for cooking loss, shear force, and meat color were prepared unblended, and results were obtained promptly.

At seven days of aging, about 200 g samples of minced leg meat were divided to analyze the chemical composition, calcium content, and cholesterol. The meat's chemical composition (moisture, crude protein, and crude fat) was analyzed using the standard AOAC method (AOAC, 2019). The physicochemical parameters of meat were analyzed 7 days post-mortem using a calibrated portable pH meter (HI99163, Hanna Instrument, Romania). Cooking loss (%) was assessed by weight difference before and after cooking at 75 °C for 50 minutes in a water bath. Then, samples for cooking loss were utilized to assess shear force with a Warner-Bratzler shear force equipment. The greater force required to cut through the muscle fibers indicates the enhanced toughness of the meat. The water-holding capacity was determined by the centrifugation method described by Fassah *et al.* (2023). The water-holding capacity (WHC) was calculated by quantifying the moisture retained in the meat sample relative to the initial moisture content.

STATISTICAL ANALYSIS

All data were assessed for deviations and mean using an analysis of variance by SPSS Statistics software (version 26.0; IBM Corp, Armonk, NY, USA). Standard errors of the mean (SEM) were calculated as the standard deviation divided by the square root of the sample size, based on the raw experimental data. Orthogonal contrast (control vs. papain, control vs. bromelain, and papain vs. bromelain) was used to compare the effects of papain and bromelain administration. Statistical significance level was set at $P \leq 0.05$, and tendencies were designated at $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

NUTRIENT INTAKE

Protease oral administration tended to reduce ($P < 0.10$) nutrient intake (dry matter, crude protein, crude fat, ether extract, and nitrogen-free extract) compared to the control group, while a significant reduction ($P < 0.05$) was observed for bromelain compared to the control (Table 2). Papain administration showed a tendency to lower intake of ether extract ($P = 0.09$) and nitrogen-free extract ($P = 0.10$) compared to the control group.

The reduction in nutrient intake due to proteases administration may be associated with changes in nutrient

Table 2: Effect of oral Papain or Bromelain administration on nutrient intake of thin-tailed lambs.

Parameters	Control	Papain	Bromelain	SEM	P-Value			
					P-Value	Control vs Papain	Control vs Bromelain	Papain vs Bromelain
Total DM intake/BW ^{0.75} (g/d)	69.3	63.0	60.7	1.68	0.08	0.11	0.03	0.53
Total nutrient intake/BW ^{0.75}								
Crude protein (g/d)	10.6	9.59	9.25	0.26	0.09	0.10	0.04	0.55
Crude fiber (g/d)	10.3	9.42	8.98	0.25	0.08	0.13	0.03	0.43
Ether extract (g/d)	1.46	1.32	1.27	0.03	0.08	0.09	0.03	0.55
Ash (g/d)	4.62	4.21	4.04	0.11	0.08	0.11	0.03	0.50
Nitrogen-free extract (g/d)	42.4	38.5	37.1	1.03	0.09	0.10	0.03	0.55

SEM = standard error mean, BW = body weight; DM = dry matter.

Table 3: Effect of oral Papain or Bromelain administration on rumen fermentation characteristics of thin-tailed lambs.

Parameters	Control	Papain	Bromelain	SEM	P-Value			
					P-Value	Control vs Papain	Control vs Bromelain	Papain vs Bromelain
pH	6.83	6.79	6.58	0.07	0.33	0.82	0.18	0.25
Total bacteria, log CFU/mL	12.2	12.3	11.2	0.33	0.33	0.84	0.24	0.18
Total protozoa, log CFU/mL	5.33	5.31	5.27	0.02	0.61	0.83	0.36	0.48
NH ₃ , mM	17.8	20.6	20.6	0.63	0.11	0.07	0.07	1.00
Total volatile fatty acid, mM	204.3	203.6	198.6	4.20	0.87	0.95	0.64	0.68
C2, %	50.1	45.9	44.4	1.34	0.22	0.20	0.10	0.65
C3, %	38.4	43.3	42.4	1.01	0.08	0.04	0.08	0.65
isoC4, %	0.98	1.10	1.09	0.05	0.60	0.38	0.42	0.94
C4, %	9.29	8.52	10.8	0.52	0.21	0.52	0.24	0.09
isoC5, %	0.78	0.73	0.81	0.02	0.48	0.46	0.65	0.25
C5, %	0.51	0.49	0.47	0.03	0.92	0.84	0.69	0.84
C2/C3 ratio	1.31	1.07	1.05	0.06	0.12	0.09	0.07	0.85

SEM = standard error mean.

utilization and metabolic efficiency (Zheng *et al.*, 2020; Rao *et al.*, 2022; Wang *et al.*, 2023; Rodriguez-Carias *et al.*, 2018). In this study, proteases increase the molar proportion of propionate (Table 3). As a major gluconeogenic substrate in ruminants, propionate provides more energy to meet metabolic demands. Although digestibility was not measured in this study, an improvement in nutrient utilization may promote earlier satiety, leading to lower feed intake while maintaining adequate nutrient requirements. In comparison to the control, bromelain administration showed a significant reduction in nutrient intake compared to papain; however, no significant effect was observed in the nutrient intake between the two proteases. Bromelain and papain are proteolytic enzymes, but they may have different specific activities in the digestive process. In this study, the enzyme activity of the bromelain used was lower than that of papain, which reflects the broader range of proteolytic activity compared to papain. Proteolytic activity can be influenced by environmental factors such as temperature and pH. Recently, there has been no direct

comparison between bromelain and papain on their effects on nutrient intake in lambs. Previously reported, bromelain showed a broader optimal pH and temperature ranges, lower activation energy, and higher catalytic efficiency, which make it exhibit more digesting proteins and lower nutrient intake (Manzoor *et al.*, 2016; Corzo *et al.*, 2012; Ansari *et al.*, 2025). In line with our study, the addition of exogenous proteolytic enzymes to dairy cattle has been shown to improve nutrient digestibility while feed intake was decreased (Eun and Beauchemin, 2005). Our results suggest that papain and bromelain may support nutrient efficiency by acting on the digestion process, enabling lambs to maintain performance despite reduced nutrient intake. Bromelain provides a greater impact on feed utilization efficiency, reflected in lower nutrient intake.

RUMEN FERMENTATION

Oral administration of papain or bromelain did not change the rumen pH and microbial population, while protease administration showed an increasing trend (P=0.08) in

the propionate molar proportion (Table 3). Lambs offered both individual proteases showed an increasing trend ($P=0.07$) in NH_3 concentrations compared to the control. Papain administration significantly increased ($P<0.05$) propionate molar proportion compared to control, while bromelain showed a tendency ($P=0.08$) to increase propionate and have lower ($P=0.10$) acetate. Compared to papain, bromelain administration showed a tendency to produce more butyrate ($P=0.09$). Individual protease administration showed a tendency to have a lower ($P < 0.10$) acetate/propionate ratio.

Our study revealed a potential shift in rumen fermentation patterns toward more efficient energy utilization following the administration of papain or bromelain. Papain or bromelain, as a proteolytic enzyme, may improve protein degradation, increase the availability of amino acids and peptides, which are then rapidly deaminated by ruminal microbes, releasing ammonia (Xu *et al.*, 2019). In addition, bromelain has been shown to improve dry matter digestibility and crude protein digestibility, which may be reflected in higher rumen ammonia concentrations (Putriana *et al.*, 2020).

An increase in propionate molar proportion was observed as a trend due to papain or bromelain administration in comparison to the control. Volatile fatty acids are produced from microbial fermentation of carbohydrates in the rumen. These results were in line with our ammonia data, which was elevated by proteases administration. Ammonia is a critical nitrogen source for microbial protein synthesis in the rumen, which may influence microbial population (Xu *et al.*, 2019), including propionate-producing bacteria, even without changing total bacteria numbers (Sparks *et al.*, 2011). Furthermore, this change might downregulate the activity of acetate-associated fibrolytic bacteria such as *Fibrobacter succinogenes*, which shifts the microbial balance (Gleason *et al.*, 2021).

Although there is no change in bacterial and protozoal populations, the enhanced propionate production suggests a shift in rumen microbial fermentation due to the enzymatic effect of the proteases. Oral administration

of proteases is expected to contribute to initial stage of protein degradation within the rumen before they are degraded. Despite their generally low bioavailability in ruminants, a partial enzyme activity may persist long enough to hydrolyze dietary proteins and alter the microbial fermentation pathways (Lorkowski, 2012; Song *et al.*, 2022). Although methane was not measured in this study, previous studies suggested that increased propionate formation may indicate improved hydrogen utilization and lower methane production, leading to a more energy-efficient fermentation process favorable for growth (Ungerfeld 2020; Palangi and Lackner 2022; Sun *et al.* 2015; Nguyen *et al.* 2018). Overall, papain and bromelain administration can modulate rumen fermentation by enhancing nutrient availability, favoring propionate over acetate production without major changes in the total microbial population.

BLOOD METABOLITES

As shown in Table 4, bromelain administration showed a tendency to have higher blood albumin compared to the control. However, no significant differences ($P>0.05$) were observed in any measured parameters, including glucose, triglycerides, and total protein, by papain or bromelain administration (Table 4). The lack of changes suggests that the proteases administration may have a minimal impact on modulating nutrient metabolism in this dosage. However, our results suggest that proteases are metabolically safe and do not disrupt the blood metabolites homeostasis in lambs.

In this study, bromelain administration tended to result in higher serum albumin levels compared to the control ($P= 0.06$), while no change was observed with papain administration. This result may reflect the changes in protein synthesis, possibly linked to the stronger proteolytic action of bromelain to improve nutrient digestibility and absorption. In this study, an increase in serum albumin was observed alongside the numerical increase in ruminal ammonia levels. This result may suggest more deamination process of dietary protein or microbial turnover, resulting to greater nitrogen availability for protein utilization

Table 4: Effect of oral Papain or Bromelain administration on the blood metabolites of thin-tailed lambs.

Parameters	Control	Papain	Bromelain	SEM	P-Value				Reference ¹
					P value	Control vs Papain	Control vs Bromelain	Papain vs Bromelain	
Glucose (mg/dL)	85.6	78.8	86.8	2.89	0.51	0.36	0.88	0.29	33.00-98.10
Triglyceride (mg/dL)	18.0	18.3	23.5	1.58	0.31	0.94	0.18	0.20	5.00-78.00
Total protein (g/dL)	6.51	6.68	6.68	0.11	0.76	0.53	0.53	1.00	3.10-11.40
Albumin (g/dL)	3.43	3.59	3.69	0.06	0.17	0.24	0.06	0.44	1.12-5.38

SEM = standard error mean. ¹Varanis *et al.* (2021).

Table 5: Effect of oral Papain or Bromelain administration on physicochemical meat quality of thin-tailed lambs.

Parameters	Control	Papain	Bromelain	SEM	P-Value			
					P-Value	Control vs Papain	Control vs Bromelain	Papain vs Bromelain
Moisture (%)	74.3	76.9	72.6	0.85	0.11	0.20	0.39	0.04
Protein (%)	18.7	19.9	20.4	0.44	0.28	0.27	0.13	0.66
Fat (%)	3.60	4.47	4.03	0.55	0.73	0.55	0.89	0.46
pH	5.43	5.43	5.50	0.02	0.29	0.92	0.17	0.19
Water holding capacity (%)	86.2	83.0	84.0	0.99	0.44	0.22	0.38	0.71
Cooking loss (%)	30.3	30.2	31.3	1.87	0.97	0.98	0.86	0.84
Shear force (kg/cm ²)	2.65	2.48	2.27	0.14	0.53	0.61	0.27	0.55

SEM = standard error mean

(Tan *et al.*, 2021; Xu *et al.*, 2019). Previously reported, elevated ruminal ammonia has been associated with changes in blood parameters, including blood urea nitrogen and albumin levels (Hare *et al.*, 2023). Additionally, enhanced microbial activity induced by enzymatic treatments has been linked to elevated ammonia concentrations and shifts in blood nitrogen indicators (Fomichev *et al.*, 2019). Although the rumen microbial population remained unchanged, bromelain administration may have improved protein digestion efficiency and amino acid absorption, thereby supplying more substrates for albumin synthesis. The numerical trends of albumin increased may indicate the potency of the metabolic benefits of bromelain by increasing dosage for administration.

FEEDLOT PERFORMANCE

No significant changes ($P>0.05$) in average daily gain and final body weight were observed with papain or bromelain administration (Figure 1a,b). Neither protease significantly affected ($P>0.05$) hot carcass weight or carcass percentage of female thin-tailed lambs (Figure 1c, d).

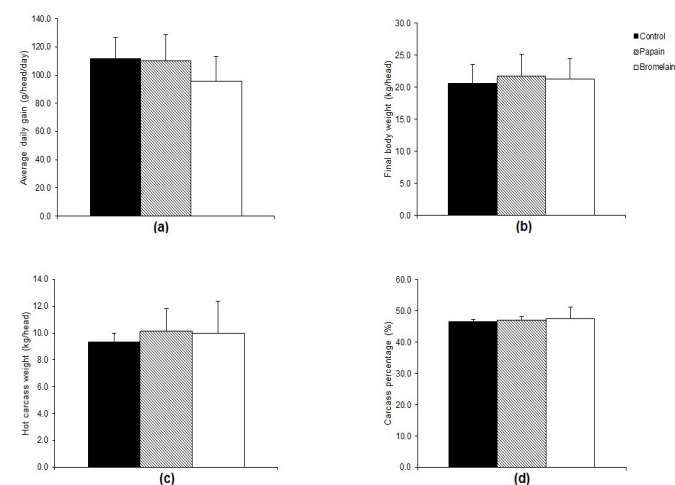


Figure 1: Effect of papain or bromelain oral administration on feedlot performance of female thin-tailed sheep. Average daily gain (a); final body weight (b); hot carcass weight (c); carcass percentage (d).

In this study, protease administration showed a tendency to increase the molar proportion of propionate. However, using current dosages, this improvement did not translate into measurable gains. This is especially relevant in ruminants, since the muscle accretion changes require longer adaptation periods to dietary modifications (Thanh *et al.*, 2022; Wang *et al.*, 2024). However, the improvement in fermentation and nutrient digestibility observed in this study could support enhanced growth if they were administered in higher dosages or over a longer period. While current dosages of protease administration did not show significant improvements in growth and carcass quality, a trend in the shift of rumen fermentation pattern showed a promising result.

MEAT QUALITY

In this study, chemical and physical meat properties were not different ($P>0.05$) between treatments (Table 5), whereas papain administration exhibited higher moisture content ($P= 0.04$) compared to bromelain. The comparable physical traits between treatments ($P>0.05$) indicate that protease administration did not adversely affect meat physical quality. Proteases, such as papain and bromelain, can degrade muscle proteins and reduce meat toughness when applied exogenously in the meat tenderization process (Manohar *et al.*, 2016). In this study, both proteases were administered orally, resulting in enhancement of nutrient digestion and absorption. The tendency to increase ammonia and serum albumin levels by protease administration supports its effect in enhancing digestion and absorption; however, it has not had a significant impact on the chemical composition of lamb meat. Papain administration contained approximately 10% more intramuscular fat compared to bromelain, although the difference was not significant. The numerical higher fat may partially explain the higher moisture content observed, as fat acts as a physical barrier that limits the water loss and interacts with muscle proteins to stabilize water within the tissue (Daszkiewicz *et al.*, 2005). It has been reported that intramuscular fat helps preserve

muscle fiber integrity during cooking, reducing structural shrinkage and moisture evaporation, which can result in greater apparent water retention (Im *et al.*, 2024).

The lack of a substantial shift in these meat components affects the physical quality parameters. Water holding capacity and cooking loss are strongly influenced by the stability and structure of muscle proteins, while shear force reflects the connective tissue content and myofibrillar integrity (Al-Moadhen *et al.*, 2024). Protein structure and its interaction with water molecules are critical for water holding capacity, influencing juiciness and tenderness (Hughes *et al.*, 2014). Higher fat content in meat is associated with lower cooking loss since it retains more moisture during cooking, resulting in less weight loss, and lower shear force values indicating more tender meat (Jung *et al.*, 2016). Thus, no significant enhancement in the chemical composition of meat showed a similar structure and functional properties of meat. These findings would likely result in minimal variation in sensory characteristics, which reflect consumers' perception of lamb meat (Monaco *et al.*, 2015). Overall, our study suggests that the current dosage and administration duration were not sufficient to improve the quality of lamb meat. A longer feeding duration or higher dosages of administration are required to confirm the effectiveness of papain or bromelain administration to enhance the meat quality of lambs.

CONCLUSION

Oral administration of papain or bromelain at a 36 mg/kg body weight effectively modulates rumen fermentation, notably by increasing the molar proportion of propionate and reducing nutrient intake. However, the current administration period did not significantly improve feedlot performance or meat quality characteristics. These findings suggest that oral papain or bromelain administration shows a promising positive effect on influencing rumen fermentability. A longer-term administration period and higher dosages are recommended to clarify its effects on feedlot performance and meat quality improvements in thin-tailed sheep production.

ACKNOWLEDGEMENTS

This research was supported by the RIIM LPDP Grant and BRIN, grant number: 4/IV/KS/05/2023. The authors would like to extend their appreciation to Edi Kalvin Don-ta Perangin Angin, Fadhlia Novita Ramadhani Aulia, Fiona Syafira Oktaviani, Rif'ani Amalia Rahmadani Hantoro, and Mercy Slait for their valuable technical assistance throughout the study.

NOVELTY STATEMENT

This study offers new insights into the oral administration of papain or bromelain in meat-type sheep, a feeding strategy that is rarely evaluated in ruminant production. This work demonstrates that oral protease supplementation modulates rumen fermentation by increasing propionate availability, thereby supporting gluconeogenic energy supply without compromising animal growth or meat quality. The results emphasize a rumen-driven physiological response for improving nutrient utilization in the sheep production system.

AUTHOR'S CONTRIBUTION

DMF conceptualized, investigated, analyzed the data, wrote the original draft, and edited. YP and KK conceptualized, investigated, wrote, and reviewed the original draft. SNA, ESR, and BB wrote and reviewed the original draft. All authors reviewed and approved the final manuscript. DAA conceptualized, funded the acquisition, wrote, and reviewed the original draft.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

We acknowledge the use of Grammarly and ChatGPT in the preparation of this manuscript for language editing and refinement. All scientific content, interpretation, and conclusion were determined and verified by the authors.

CONFLICT OF INTEREST

The authors have declared no conflict of interest regarding the publication of this study.

REFERENCES

- Ahamed TS, Priya VV, Gayathri R, Geetha RV (2016). Evaluation of anti microbial activity of pineapple extract against selected oral pathogen. *J. Pharm. Sci. Res.*, 8: 491–492.
- Akit H, Zainudin NNMN, Wahid NAA, Zakaria S, Foo H, Loh T (2019). Dietary bromelain improves nutrient digestibility, digesta viscosity and intestinal villus height as well as reduces intestinal *E. coli* population of broiler chickens. *Mal. J. Anim. Sci.*, 22: 1–16.
- Al-Moadhen H, Lees JC, van der Werf JHJ, McGilchrist P (2024). The impact of genetic and non-genetic factors on lamb loin shear force. *Animals*, 14: 2628. <https://doi.org/10.3390/ani14182628>
- Ansari M, Prajapati KP, Mittal S, Mishra S, Gupta V, Anand BG, Kar K (2025). High-low temperature switch induces formation of catalytic and cytotoxic amyloid aggregates of papain. *Food Chem.*, 479: 143657. <https://doi.org/10.1016/j.foodchem.2025.143657>
- AOAC (2019). Official methods of analysis of AOAC International. 21st Edition. Latimer GW, editor. AOAC International, Washington DC.

- Chung YH, Zhou M, Holtshausen L, Alexander TW, McAllister TA, Guan LL, Oba M, Beauchemin KA (2012). A fibrolytic enzyme additive for lactating Holstein cow diets: Ruminal fermentation, rumen microbial populations, and enteric methane emissions. *J. Dairy Sci.*, 95: 1419–1427. <https://doi.org/10.3168/jds.2011-4552>
- Contreras A, Paape MJ, Miller RH, Corrales JC, Luengo C, Sánchez A (2009). Effect of bromelain on milk yield, milk composition and mammary health in dairy goats. *Trop. Anim. Health Prod.*, 41: 493–498. <https://doi.org/10.1007/s11250-008-9213-7>
- Corzo CA, Waliszewski KN, Welti-Chanes J (2012). Pineapple fruit bromelain affinity to different protein substrates. *Food Chem.*, 133(3): 631–635. <https://doi.org/10.1016/j.foodchem.2011.05.119>
- Daszkiewicz T, Bąk T, Denaburski J (2005). Quality of pork with a different intramuscular fat (IMF) content. *Pol. J. Food Nutr. Sci.*, 14: 31–36
- dos Anjos MM, da Silva AA, de Pascoli IC, Mikcha JMG, Machinski M, Peralta RM, de Abreu Filho BA (2016). Antibacterial activity of papain and bromelain on *Alicyclobacillus* spp. *Int. J. Food Microbiol.*, 216: 121–126. <https://doi.org/10.1016/j.ijfoodmicro.2015.10.007>
- Eun JS, Beauchemin KA (2005). Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, and milk production. *J. Dairy Sci.*, 88(6): 2140–2153. [https://doi.org/10.3168/jds.S0022-0302\(05\)72890-3](https://doi.org/10.3168/jds.S0022-0302(05)72890-3)
- Fassah DM, Kang HJ, Beak SH, Jung DJS, Jeong I, Na SW, Yoo SP, Hong SJ, Kim HJ, Haque MN, Lee HJ, Jo C, Baik M (2023). Effects of dietary glycerol supplementation on meat quality, palatability, and lipid metabolism gene expression in the longissimus thoracis of Hanwoo steers. *Meat Sci.*, 198. <https://doi.org/10.1016/j.meatsci.2022.109093>
- Fomichev YP, Bogolyubova NV, Mishurov AV, Rykov RA (2019). Bio correction of enzymatic and microbial processes in rumen and intermediary metabolism in sheep with the use of antioxidant and organic iodine dietary supplements. *Russ. Agric. Sci.*, 45: 464–469. <https://doi.org/10.3103/S1068367419050069>
- Gleason CB, Settlege RE, Beckett LM, White RR (2021). Characterizing effects of ingredients differing in ruminally degradable protein and fiber supplies on the ovine rumen microbiome using next-generation sequencing. *Front. Anim. Sci.*, 2: 745848. <https://doi.org/10.3389/fanim.2021.745848>
- Habtu E, Mekonnen B, Kiros H, Fasseha H, Getachew B (2020). Meat tenderization of efficiency of papain, bromelain and zingiber officinale on old aged beef carcass of local Zebu cattle. *TTSR*. 4(1): 9–15.
- Hare KS, Lambert K, Chagas AC, Watanabe DHM, Penner GB (2023). PSXII-30 changes in ruminal digesta and ruminal ammonia-N concentration with differing durations feed restriction for sheep. *J. Anim. Sci.*, 101: 637–638. <https://doi.org/10.1093/jas/skad281.742>
- Hughes JM, Oiseth SK, Purslow PP, Warner RD (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Sci.*, 98: 520–532. <https://doi.org/10.1016/j.meatsci.2014.05.022>
- Im C, Song S, Cheng H, Park J, Kim GD (2024). Changes in meat quality and muscle fiber characteristics of beef striploin (*M. longissimus lumborum*) with different intramuscular fat contents following freeze-thawing. *LWT*, 198: 116081. <https://doi.org/10.1016/j.lwt.2024.116081>
- Jayanegara A, Dewi SP, Ridla M (2016). Nutrient content, protein fractionation, and utilization of some beans as potential alternatives to soybean for ruminant feeding. *Med. Pet.*, 39: 195–202. <https://doi.org/10.5398/medpet.2016.39.3.195>
- Jung EY, Hwang YH, Joo ST (2016). The relationship between chemical compositions, meat quality, and palatability of the 10 primal cuts from Hanwoo steer. *Korean J. Food Sci. Anim. Resour.*, 36: 145–151. <https://doi.org/10.5851/kosfa.2016.36.2.145>
- Khongpradit A, Boonsaen P, Homwong N, Buaphan S, Maitreejit W (2022). Effect of pineapple stem starch in concentrate diet on rumen fermentation in beef cattle and in situ dry matter degradability. *Agric. Nat. Res.*, 56(2): 277–286. <https://doi.org/10.34044/j.anres.2022.56.2.06>
- Kusumadaja AP, Dewi RP (2010). Determination of optimum condition of papain enzyme from papaya var Java (*Carica papaya*). *Indones. J. Chem.*, 5(2): 147–151. <https://doi.org/10.22146/ijc.21822>
- Lorkowski G. (2012). Gastrointestinal absorption and biological activities of serine and cysteine proteases of animal and plant origin: Review on absorption of serine and cysteine proteases. *Int. J. Physiol. Pathophysiol. Pharmacol.*, 4(1): 10–27.
- Malek K, Norazan M, Ramaness P, Othman Z, Malek R, Aziz R, Aladdin A, Enshasy HEI (2016). Cysteine Proteases from *Carica papaya*: An important enzyme group of many industrial applications. *IOSR J. Pharm. Biol. Sci.*, 11(2): 11–16.
- Malle D, Tellusa I, Lasamahu AA (2015). Isolation and characterization of papain from the latex of papaya (*Carica papaya* L). *Indones. J. Chem. Res.*, 2(2): 182–189. <https://doi.org/10.30598/ijcr.2015.2-dom>
- Manohar J, Gayathri R, Vishnupriya V (2016). Tenderisation of meat using bromelain from pineapple extract. *Int. J. Pharm. Sci. Rev. Res.*, 39: 81–85.
- Manzoor Z, Nawaz A, Mukhtar H, Haq I (2016). Bromelain: Methods of therapeutic applications extraction, purification and therapeutic application. *Braz. Arch. Biol. Technol.*, 59: 1–16. <https://doi.org/10.1590/1678-4324-2016150010>
- Maqsood S, Manheem K, Gani A, Abushelaibi A (2018). Degradation of myofibrillar, sarcoplasmic and connective tissue proteins by plant proteolytic enzymes and their impact on camel meat tenderness. *J. Food Sci. Technol.*, 55(9): 3427–3438. <https://doi.org/10.1007/s13197-018-3251-6>
- Marino R, della Malva A, Caroprese M, de Pilli T, Alessandrino O, Picariello G, Sevi A, Albenzio M (2023). Proteomics in bovine semitendinosus muscle to assess emerging strategies based on papain injection and ultrasounds on meat tenderization process. *Meat Sci.*, 200: 109147. <https://doi.org/10.1016/j.meatsci.2023.109147>
- Monaco CA, Freire MTA, Melo L, Rosa AF, Carrer CC, Trindade MA (2015). Eating quality of meat from six lamb breed types raised in Brazil. *J. Sci. Food Agric.*, 95(8): 1747–1752. <https://doi.org/10.1002/jsfa.6894>
- Nguyen SH, Nguyen HDT, Bremner G, Hegarty RS (2018). Methane emissions and productivity of defaunated and refaunated sheep while grazing. *Small Rum. Res.*, 161: 28–33. <https://doi.org/10.1016/j.smallrumres.2018.02.004>
- NRC (2007). Nutrient requirements of sheep. National Academics Pr, Washington DC.
- Ogimoto K, Imai S (1981). Atlas of rumen microbiology. Japan Scientific Societies, Tokyo.

- Palangi V, Lackner M (2022). Management of enteric methane emissions in ruminants using feed additives: A review. *Animals*, 12: 3452. <https://doi.org/10.3390/ani12243452>
- Pavan R, Jain S, Shraddha, Kumar A (2012). Properties and therapeutic application of bromelain: A review. *Biotechnol. Res. Int.*, 2012:1–6. <https://doi.org/10.1155/2012/976203>
- Pimentel PRS, dos Santos Brant LM, de Oliveira Lima AGV, Cotrim DC, Nascimento T, Oliveira RL (2022). How can nutritional additives modify ruminant nutrition? *Rev. Fac. Cienc. Agrar. UNCuyo*, 54(1): 175–189. <https://doi.org/10.48162/rev.39.076>
- Putriana L, Bachruddin Z, Hanim C, Kurniawati A, Yusiati LM, Widayati O (2020). The effect of bromelain from pineapple (*Ananas comosus*) on increasing protein digestibility of milk replacer for lamb. *IOP Conf. Ser. Earth Environ. Sci.*, 478: 012030. <https://doi.org/10.1088/1755-1315/478/1/012030>
- Ramli ANM, Manas NHA, Hamid AAA, Hamid HA, Illias RM (2018). Comparative structural analysis of fruit and stem bromelain from *Ananas comosus*. *Food Chem.*, 266: 183–191. <https://doi.org/10.1016/j.foodchem.2018.05.125>
- Rao SVR, Raju J, Srilatha T, Nagalakshmi D, Raju MVLN, Paul SS, Prakash B (2022). Effect of supplementing papaya (*Carica papaya*) latex on performance, carcass traits and nutrient digestibility in broiler chicken fed recommended and sub-optimal levels of dietary protein. *Anim. Feed Sci. Technol.*, 285: 115226. <https://doi.org/10.1016/j.anifeedsci.2022.115226>
- Rodríguez-Carias AA, Quintana BA, Solorzano LC, Randel PF (2018). Evaluation of α -amylase and protease on diet nutrient intake and digestibility, and physiological parameters in lambs. *J. Agric. Univ. P.R.*, 101: 63–78. <https://doi.org/10.46429/jaupr.v101i1.14294>
- Shen JS, Chai Z, Song LJ, Liu JX, Wu YM (2012). Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. *J. Dairy Sci.*, 95: 5978–5984. <https://doi.org/10.3168/jds.2012-5499>
- Singh TA, Sarangi PK, Singh NJ (2018). Tenderisation of meat by bromelain enzyme extracted from pineapple wastes. *Int. J. Curr. Microbiol. Appl. Sci.*, 7: 3256–3264. <https://doi.org/10.20546/ijcmas.2018.709.404>
- Song M, Kim B, Cho JH, Kyoung H, Choe J, Cho JY, Kim Y, Kim HB, Lee JJ (2022). Modification of gut microbiota and immune responses via dietary protease in soybean meal-based protein diets. *J. Microbiol. Biotechnol.*, 32(7): 885–891. <https://doi.org/10.4014/jmb.2205.05033>
- Sparks M, Huang Y, Baldwin R, Li W, Connor E, Li C, Sonstegard T, Schroeder S, Bequette B, Li R (2011). Detection of functional shifts in the rumen microbiota in response to propionate intake in cattle. In: *Metagenomics and its applications in agriculture, biomedicine and environmental studies*. Nova Science Publishers Inc., New York. pp. 165–183.
- Sun X, Henderson G, Cox F, Molano G, Harrison SJ, Luo D, Janssen PH, Pacheco D (2015). Lambs fed fresh winter forage rape (*Brassica napus* L.) emit less methane than those fed perennial ryegrass (*Lolium perenne* L.), and possible mechanisms behind the difference. *PLoS One*, 10: e0119697. <https://doi.org/10.1371/journal.pone.0119697>
- Tan P, Liu H, Zhao J, Gu X, Wei X, Zhang X, Ma N, Johnston LJ, Bai Y, Zhang W, Nie C, Ma X (2021). Amino acids metabolism by rumen microorganisms: Nutrition and ecology strategies to reduce nitrogen emissions from the inside to the outside. *Sci. Total Environ.*, 800: 149596. <https://doi.org/10.1016/j.scitotenv.2021.149596>
- Thanh LP, Jiang Q, Wichasit N, Batistel F, Parys C, Guyader J, Looor JJ (2022). Alterations in skeletal muscle mRNA abundance in response to ethyl-cellulose rumen-protected methionine during the periparturient period in dairy cows. *Animals*, 12: 1641. <https://doi.org/10.3390/ani12131641>
- Ungerfeld EM (2020). Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Front. Microbiol.*, 11. <https://doi.org/10.3389/fmicb.2020.00589>
- Varanis LFM, Schultz EB, Oliveira KA, Sousa LF, da Cruz WFG, Junior GDLM (2021). Serum biochemical reference ranges for lambs from birth to 1 year of age in the tropics. *Semina: Cienc. Agrar.*, 42: 1725–1739. <https://doi.org/10.5433/1679-0359.2021v42n3Supl1p1725>
- Wang J, Shen C, Zhao G, Hanigan MD, Li M (2024). Dietary protein re-alimentation following restriction improves protein deposition via changing amino acid metabolism and transcriptional profiling of muscle tissue in growing beef bulls. *Anim. Nutr.*, 19: 117–130. <https://doi.org/10.1016/j.aninu.2024.04.028>
- Wang J, Wu P, Wang S, Xu Y, Qiu X, Gao Z, Zhao Z (2023). Effects of dietary fucoidan on growth performance, intestinal digestive enzyme activities and microbial flora of lambs. *Chinese J. Anim. Nutr.*, 35: 2417–2430.
- Wardeh MF (1981). Models for estimating energy and protein utilization for feeds. Utah State University, Utah.
- Xu J, Cao H, Zhang B, Yao H (2020). The mechanistic effect of bromelain and papain on tenderization in jumbo squid (*Dosidicus gigas*) muscle. *Food Res. Int.*, 131: 108991. <https://doi.org/10.1016/j.foodres.2020.108991>
- Xu Y, Li Z, Shen J, Zhu W (2019). Microbe-mediated ruminal ammonia production in ruminants and its impacts on rumen function. *Acta Microbiol. Sin.*, 59:781–788.
- Yenice G, Atasver M, Kara A, Özkanlar S, Gelen SU, İskender HA, Gür C, Gedikli S (2023). Effects of bromelain on growth performance, biochemistry, antioxidant metabolism, meat quality, and intestinal morphology of broilers. *Braz. Arch. Biol. Technol.*, 66. <https://doi.org/10.1590/1678-4324-2023220852>
- Zheng W, Hao X, Zhang H, Xiang B, Zhang W, Zhang C (2020). Effects of *Saccharomyces cerevisiae* and *Bacillus licheniformis* on growth performance and rumen fermentation in sheep. *Sci. Agric. Sin.*, 53(16): 3385–3393.