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# ENERGY METABOLISM AND PERFORMANCE OF LOCAL SHEEP FED ON AMMONIATED RICE STRAW AND CONCENTRATE SUPPLEMENTED WITH WARU AND BAMBOO LEAF MEALS

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**ABSTRACT**: This study evaluated the effects of waru (*Hibiscus tiliaceus*) leaf meal (WLM) and bamboo (*Gigantochloa apus*) leaf meal (BLM) supplementation on energy metabolism and performance of local sheep fed on ammoniated rice straw and concentrate. Sixteen male local sheep aged 12-18 months with initial average body weight of  $27.1 \pm 2.11$  kg (7.78% coefficient of variation) were completely randomized according to a Completely Randomized Design (CRD) to receive one of the following treatments: TO (ammoniated rice straw + concentrate feed); T1 (T0 + 2.49 g WLM); T2 (T0 + 1.87 g WLM + 0.33 g BLM); and T3 (T0 + 1.25 g WLM + 0.65 g BLM). The concentrate was provided at 3% of live weight, and ammoniated rice straw was available on a controlled *ad libitum*. Analysis of variance indicated that WLM and BLM supplementation significantly increased digestible energy (DE), metabolizable energy (ME), energy retention (ER), ER to El efficiency, ER to DE efficiency, average daily gain (ADG), and consumption rate (CR), while significantly decreasing the feed conversion ratio (FCR) (P < 0.01). However, the treatments had no significant effect (P > 0.05) on dry matter intake (DMI) and energy intake (EI). In conclusion, dietary supplementation with 1.87 g of WLM and 0.33 g of BLM per kg concentrate was more effective than the other combinations in improving energy metabolism and performance of local sheep.



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# INTRODUCTION

The year-round availability of rice straw makes it a highly potential feed resource, particularly during the dry season. However, the low nutritional value of rice straw, due to its high silica content, low ruminal carbohydrate degradability, and low nitrogen content, limits its utilization (Peripolli et al., 2016). A widely adopted method to enhance its quality is urea ammoniation. An *in vitro* study by Ma et al. (2020) demonstrates that ammoniated rice straw can significantly increase acetate, butyrate, and volatile fatty acid (VFA) production.

The use of concentrate in the feedlot industry aims to increase productivity in a short period. A higher allocation of concentrates can improve energy intake and overall performance by increasing energy density (Schmitz et al., 2018), but a total ration made up of 60% to 80% concentrate can decrease ruminal pH to 6.0 and 5.6 at 6 hours post-feeding, respectively (Wanapat et al., 2014). The declining ruminal pH is due to lactate accumulation from starch fermentation, which reduces cellulolytic bacterial activity, decreases cellulase enzyme function, and lowers fiber digestibility as an energy source (Li et al., 2021). These disturbances negatively affect energy efficiency and animal performance. Given that smallholder farmers in the region predominantly raise local sheep breeds, addressing such nutritional challenges is crucial to sustain their production systems.

Rumen stability can be maintained by supplementing livestock diet with flavonoids. Flavonoids help modulate rumen conditions by stabilizing pH, reducing methane, increasing propionate proportions, and promoting the growth of lactate-utilizing bacteria, such as *Streptococcus ruminantium* and *Megasphaera elsdenii* (Balcells et al., 2012; Seradj et al., 2018). Flavonoids serve as buffers in cattle fed on high-concentrate diets (Bata et al., 2016). By maintaining rumen pH stability, flavonoids support cellulolytic bacterial activity, improve fiber digestion efficiency, and enhance energy availability for livestock growth.

Several studies have shown that waru (*Hibiscus tiliaceus*) and bamboo (*Gigantochloa apus*) leaves contain flavonoid compounds. Nasution et al. (2021) reported that the inclusion of 0.24% or 2.4 g/kg DM waru leaf meal was optimal for improving microbial protein synthesis efficiency in local sheep. Bata et al. (2016) demonstrated that supplementation with WLM increased body weight gain and improved feed efficiency in Sumba Ongole cattle. Li et al. (2021) reported that the supplementation of bamboo leaf extract at 0.13% or 1.3 g/kg DM enhanced milk production, rumen fermentation, and rumen microbial populations. It seems that, the combination of waru and bamboo leaves has the potential to produce synergistic effects in supporting rumen fermentation and improving feed digestion efficiency. Waru leaves

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Supporting Information

contribute by enhancing microbial protein synthesis and improving feed utilization efficiency, while bamboo leaves contribute by maintaining gastrointestinal health through their antibacterial properties that inhibit pathogenic bacteria, as well as their antioxidant and anti-inflammatory compounds.

This study aimed to evaluate the potential synergistic effects of waru leaf meal (WLM) and bamboo leaf meal (BLM) supplementation on energy metabolism and performance of local sheep fed on ammoniated rice straw and concentrate.

#### **MATERIALS AND METHODS**

This study was conducted in accordance with the provisions set forth in the Government Regulation of the Republic of Indonesia No. 95/2012 on Veterinary Public Health and Animal Welfare, particularly those related to the humane treatment and welfare of animals used in research. All procedures involving animals were performed with due regard to minimizing pain, suffering, and stress, and ensuring appropriate care throughout the research period. The research also followed internal guidelines established by Universitas Jenderal Soedirman for animal-based research activities.

This study was conducted from March 1 to June 15, 2024, at Amanah Farm, Datar Village, Sumbang District, Banyumas Regency. The Proximate analysis was done at the Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Jenderal Soedirman University. The gross energy analysis was conducted at the Ruminant Instrument Standard Testing Laboratory, Pasuruan, and the Tropical Livestock Research Center Laboratory, Gadjah Mada University, Yogyakarta. Rice straw was collected from various rice varieties in Banyumas Regency and surrounding areas. It was chopped and treated with a mixture of 10% water, 4% urea, and 2% cassava pulp, then ensiled in sealed plastic drums for 14 days. After fermentation, the ammoniated straw was air-dried and sprayed with molasses before feeding. The nutrient composition of the feed is presented in Table 1. Waru leaf meal (WLM) and bamboo leaf meal (BLM) was prepared from mature and young leaves. Both types of leaves were oven-dried until constant weight was achieved, then pulverized and incorporated into the concentrate according to the treatment dosage.

Table 1 - Nutrient composition of experimental feed								
Feed	DM (%)*	CP (% DM)**	CF (% DM)*	Ash (% DM)*	Energy (Mcal)**			
Ammoniated rice straw	36.33	8.64	26.97	23.68	3.47			
Concentrate TO	84.81	14.20	17.72	11.15	3.88			
Concentrate T1	84.22	13.97	17.60	11.34	4.03			
Concentrate T2	85.22	13.51	17.13	11.12	4.01			
Concentrate T3  DM= Dry Matter: CP= Crude Protein	85.67	13.47	17.74	11.35	3.94			

DM= Dry Matter; CP= Crude Protein; CF= Crude Fiber. \* Laboratory of Animal Nutrition and Feed, Faculty of Animal Science, Jenderal Soedirman University, 2024. \*\* Laboratory of Large Ruminant Standard Instrument Testing, Pasuruan.

A total of sixteen male local sheep, aged 12–18 months with an average initial body weight of  $27.1 \pm 2.11$  kg (coefficient of variation: 7.78%), were used. The sheep were dewormed prior to the experiments and housed individually in 50 cm × 100 cm pens. The sheep were completely randomized according to a Completely Randomized Design (CRD) to receive one of the following treatments: T0: Ammoniated rice straw + concentrate (control); T1: T0 + 2.49 g/kg concentrate WLM; T2: T0 + 1.87 g/kg concentrate WLM + 0.33 g/kg concentrate BLM; T3: T0 + 1.25 g/kg concentrate WLM + 0.65 g/kg concentrate BLM.

Each treatment was replicated four times. Concentrate was offered at 3% of body weight (on dry matter basis), ammoniated rice straw on a controlled ad libitum basis, and free access to drinking water. The concentrate was given twice daily (07:00 AM and 03:00 PM), followed by ammoniated rice straw. The experimental period was divided into three phases: adaptation (7 days), preliminary (14 days), and feeding trial (60 days). The 7-day adaptation phase allowed the animals to acclimate to the housing environment and basal diet, thereby minimizing stress-related variation before data collection. The subsequent 14-day preliminary phase was conducted to stabilize rumen fermentation patterns and feed intake under the experimental diet, ensuring that any transient effects from dietary change were eliminated prior to the main trial. The 60-day feeding trial was selected to cover a sufficient growth period for detecting differences in performance, feed efficiency, and nutrient utilization. Feed (consumed and residue), feces, and urine were collected during a five-day sampling period, in the middle of the feeding trial, using the total collection method (Cole and Ronning, 1974). A 100-gram feed sample was oven-dried at 60°C for 48 hours. Feces were collected using trays placed beneath the pens, sprayed every four hours with 10% formalin to prevent decomposition, then weighed, recorded, and sampled by 100 g to be oven-dried. Urine was collected buckets placed beneath the pens and pre-filled with sulfuric acid to maintain pH below 3 to prevent nitrogen volatilization; a 10-ml sample was frozen at -20°C for analysis. Proximate composition of feed, residues, and feces was analyzed using the AOAC (1990) method. Gross energy was determined using a bomb calorimeter (Dittmann et al., 2014).

Energy metabolism variables were calculated as follows: Energy intake (EI) = DMI  $\times$  Feed energy; Digestible energy (DE) = EI - Fecal energy; Methane energy (Hales et al., 2022) = 0.2433  $\times$  DMI; Metabolizable energy (ME) = DE - (Urinary energy + Methane energy); Energy retention (ER) = DE - Urinary energy; Efficiency of ER to EI (%) = (ER  $\div$  EI)  $\times$  100%; Efficiency of ER to DE (%) = (ER  $\div$  DE)  $\times$  100%

Sheep performance variables were calculated using the following formulas: Dry matter intake (DMI) = DM consumed – DM residue; Average daily gain (ADG) = (Final body weight – Initial body weight) ÷ Days of rearing; Feed conversion ratio (FCR) = DMI ÷ ADG; Consumption rate (CR) = Feed intake ÷ Feeding duration

The collected data were presented as mean  $\pm$  standard deviation (SD) and were analyzed using IBM SPSS Statistics (version 30). One-way ANOVA was used to test treatment effects (model: Yij =  $\mu$  + Ti + eij). Significant differences ( $\alpha$  = 0.05) were further examined by Tukey's HSD.

## **RESULTS**

Energy metabolism for each treatment is presented in Table 2. Analysis of variance showed that the treatment had a highly significant effect (P < 0.01) on digestible energy (DE), urinary energy, metabolizable energy (ME), energy retention (ER), ER to EI efficiency, and ER to DE efficiency. However, the treatment had no significant effect (P > 0.05) on energy intake (EI), fecal energy, or methane energy. Digestible energy (DE), metabolizable energy (EM), and energy retention (ER) in T2 were similar to those in T3, but higher than those in T0 (control) and T1. The efficiency of ER to EI in T2 was higher than in T0, T1, and T3. The efficiency of ER to DE in T3 was higher than in T0, T1, and T2. The lowest urinary energy was observed in T3, followed by T1, T0, and T2.

The performance of sheep under each treatment is presented in Table 3. Analysis of variance showed that the treatment significantly affected body weight gain, average daily gain (ADG), and feed conversion ratio (FCR) (P < 0.05), as well as consumption rate (CR) (P<0.01). However, the treatment had no significant effect (P>0.05) on dry matter intake (DMI) or final body weight. Body weight gain, average daily gain (ADG), and consumption rate (CR) of sheep in T2 were higher than those in T0 (control), T1, and T3. The feed conversion ratio (FCR) of sheep in T2 was similar to T1 but lower than T0 and T3.

Treatment	T0	T4	T2	Т3	
Variable	T0 (control)	T1 (2.49 g WLM)	(1.87 g WLM + 0.33 g BLM)	(1.25 g WLM + 0.65 g BLM)	P value
Energy intake (Mcal)	4.68±0.20	4.62±0.16	5.49±0.65	5.03±0.52	0.057
Fecal energy (Mcal)	1.51±0.08	1.45±0.02	1.37±0.10	1.52±0.25	0.452
Digestible energy (Mcal)	3.17±0.16b	3.17±0.16b	4.12±0.56a	3.51±0.34ab	0.007
Urinary energy (Mcal)	0.07±0.01b	0.07±0.01b	0.08 ±0.02a	0.04±0.01c	0.001
Methane energy (Mcal)*	0.31±0.02	0.29±0.01	0.35±0.04	0.31±0.05	0.152
Metabolizable energy (Mcal)	2.79±0.15b	2.82±0.09b	3.69±0.50a	3.16±0.30ab	0.005
Energy retention (Mcal)	3.10±0.16b	3.11±0.16b	4.03±0.54a	3.47±0.34ab	0.006
Efficiency ER to EI (%)	66.25±1.27b	67.24±1.04b	73.57±1.36a	68.96±2.96b	0.001
Efficiency ER to DE (%)	97.69±0.17b	97.77±0.08b	98.00±0.20b	98.72±0.26a	0.001
Microbial protein synthesis (mgN/day)	1298±716	1389±733	1734±691	480±145	0.142

Treatment	ТО	T1 (2.49 g	T2 (1.87 g WLM	T3 (1.25 g WLM	P value
Variable	(Control)	WLM)	+ 0.33 g BLM)	+ 0.65 g BLM)	
Dry matter intake (DMI) (kg/day)	1.19±0.10	1.11±0.05	1.27±0.33	1.20±0.21	0.734
Ratio of rice straw intake to concentrate intake	ke 22:78	20:80	25:75	21:79	
Initial body weight (kg)	27.00±1.78	25.95±1.53	27.25±3.13	28.18±2.45	0.607
Final body weight (kg)	37.55±2.91	36.70±1.39	41.78±5.45	39.28±5.47	0.370
Body weight gain (kg) in 45 days	6.18±0.89b	6.78±0.45ab	9.30±1.94ª	6.06±1.31b	0.018
Average daily gain (ADG) (kg/day)	0.14±0.02b	0.15±0.01ab	0.21±0.04a	0.15±0.03b	0.019
Feed conversion ratio (FCR)	8.80±1.06a	7.38±0.47ab	6.16±1.15b	8.26±0.94a	0.010
Consumption rate (kg/hour)	0.44±0.05b	0.39±0.05b	0.95±0.17a	0.43±0.14b	0.001

## DISCUSSION

## **Energy metabolism**

The analysis results indicated that the WLM and BLM supplementation did not affect (P > 0.05) dry matter intake (DMI), energy intake (EI), and, by extension, feed palatability. According to Parakkasi (1999), factors influencing feed intake include palatability, animal condition, feed type, and environmental factors. In this study, energy content was similar across treatments (Table 2), reflecting no differences in feed intake. Tahuk et al. (2021), stated that dietary energy content is one of the key factors influencing feed intake levels. The primary purpose of feed consumption in livestock is to meet energy requirements. When dietary energy is low, animals tend to consume more feed to fulfill their energy needs, but when dietary energy is high, feed intake tends to decline (Parakkasi, 1999)

Digestible energy in T1 was similar to T0, because ammoniated rice straw in T1, created an alkaline rumen environment, which moderated the significant impact of flavonoids derived solely from WLM. Ammonia derived from ammoniated rice straw acts as a buffer in the rumen and helps maintain microbial population balance, thereby supporting adequate fiber fermentation. A study by Utami (2020) reported that supplementing 0.24% Waru leaf meal (on a DM basis) in the concentrate was less effective in improving fiber digestibility in sheep fed ammoniated rice straw and concentrate. In contrast, the combined supplementation of WLM and BLM demonstrated higher energy digestibility. This improvement may be attributed to the higher flavonoid content of both ingredients, along with the ability of bamboo leaves to support digestive tract health, thereby optimizing digestion and nutrient absorption. According to a study by Bata and Rahayu (2017), the flavonoid content in bamboo leaves is 2.04 mg/mL, which is higher than that in waru leaves, at only 0.48 mg/mL. Flavonoids contribute to modulating rumen conditions, such as maintaining rumen pH, acting as anti-inflammatory agents, reducing methane production, increasing propionate proportion, and enhancing the population of lactate-utilizing bacteria in the rumen (Balcells et al., 2012). These conditions create a more efficient rumen environment, thereby supporting the fermentation process. Li et al. (2021) reported that the inclusion of bamboo leaf extract in the diet can improve rumen fermentation parameters, such as increasing total VFA, stabilizing rumen pH, and reducing the Firmicutes-to-Bacteroidetes ratio. Do et al. (2018) stated that a lower Firmicutes/Bacteroidetes ratio in the rumen is associated with more efficient fiber digestion, particularly of lignin. Additionally, flavonoids and phenolic acids in bamboo leaves exhibit high antioxidant activity (Shen et al., 2024). Antioxidants in the rumen can reduce protozoa populations, suppress pathogenic bacteria, and alter fermentation patterns by increasing the propionate-to-total VFA ratio, where propionate serves as a major energy source for ruminants through the process of gluconeogenesis (Wu et al., 2022).

Digestible energy in T3 (0.65 g BLM) showed a declining trend compared to T2 (0.33 g BLM), which may be related to the increased dosage of BLM. While supplementation with 0.33 g BLM (T2) had a positive effect on rumen fermentation, a higher dose of 0.65 g (T3) resulted in reduced fermentation performance. This effect is likely due to the antibacterial compounds present in bamboo leaves, which may have disrupted the microbial balance in the rumen. Bamboo leaves are rich in flavone C-glycosides such as orientin, isoorientin, vitexin, isovitexin, tricin, luteolin, and luteolin-6-C-arabinoside (Zhang et al., 2008; Su et al., 2019), with orientin, vitexin, and tricin known to exhibit antibacterial activity. These antibacterial compounds may inhibit carbohydrate-fermenting bacteria, thereby contributing to the reduction in total VFA (Crespo et al., 2012). Urinary energy reflects the amount of energy lost due to nitrogen excretion in the form of urea and other metabolites. Analysis of variance showed that the treatment had a highly significant effect on urinary energy (P = 0.001), with the lowest value observed in T3, which was significantly different from T0, T1, and T2. The higher urinary energy values in T0, T1, and T2 may indicate an asynchrony between energy and nitrogen availability in the rumen. Nitrogen derived from dietary protein that is not efficiently utilized due to limited fermentable energy is converted into ammonia (NH<sub>3</sub>). Unused ammonia is absorbed through the reticulorumen villi into the bloodstream and subsequently converted into urea by the liver. A portion of this urea returns to the rumen via saliva as recycled nitrogen, but the majority is excreted through urine (Getahun et al., 2019). In contrast, the reduction in urinary energy observed in T3 suggests better synchronization between energy and nitrogen, allowing more nitrogen to be utilized for microbial protein synthesis and tissue formation, with less being lost through urinary excretion. Metabolizable energy in T2 was similar to that of T3, but higher than T0 and T1, indicating that the combined supplementation of WLM and BLM enhanced energy utilization efficiency. Metabolizable energy reflects the amount of energy available to livestock to support production processes and tissue maintenance. According to Weiss (2019) stated that metabolizable energy is obtained by subtracting the energy lost through urine and methane gas from the amount of digestible energy. Although urinary energy loss in T2 was higher than in the control, and methane energy losses were relatively similar across all treatments, the digestible energy values in T2 and T3 was higher compared to T0 and T1. Energy retention in T2 was similar to that in T3 but higher than in TO and T1. High energy retention indicates that sheep can efficiently utilize the consumed energy for productive purposes, such as body weight gain. This increase is consistent with the higher values of digestible energy and metabolizable energy observed in T2 and T3. In contrast, T0 and T1 treatments showed lower energy retention, indicating that much of the consumed energy was not efficiently utilized, but rather lost through feces, urine, or methane gas. Ahmad et al. (2023) reported that incorporating 1% DM flavonoids in complete rumen modifier (CRM) improved energy retention in sheep.

The efficiency of ER to El in T0, T1, and T3 were similar results but increased in T2. This indicates that, in this treatment, the digestion and rumen fermentation processes were optimal, allowing most of the consumed energy to be utilized effectively for growth. However, the efficiency of ER to DE was highest in treatment T3, even though total digestible energy in T3 tended to be lower compared to T2. This condition suggests that, although rumen digestibility in T3 was not as high as in T2, post-rumen digestion in T3 was more efficient, allowing the energy that had been digested to be utilized optimally by the body and stored as body energy. This efficiency is supported by the lower energy loss through urine and methane in T3, as well as the antioxidant and anti-inflammatory compounds in bamboo leaves, which is contributing in maintaining intestinal mucosal integrity and supporting nutrient absorption in the post-rumen digestive tract.

#### Sheep performance

The energy produced from metabolic processes in livestock is harnessed for maintenance, and any energy excess will fuel livestock growth and production. This study found that the increased energy digestibility, metabolizable energy, energy retention, and energy efficiency in T2 sheep positively impacted their average daily gain (ADG). The supplementation of 1.87 g of WLM and 0.33 g of BLM enhanced the sheep's ability to utilize energy more efficiently, leading to optimal growth and a significant increase in body weight. The increase in body weight gain in T2 is consistent with the high energy retention, indicating that more of the consumed energy was retained as body tissue. This efficiency is supported by the presence of flavonoids, which enhance rumen fermentation efficiency and microbial protein synthesis, both of which were higher in T2, with microbial protein synthesis at 1734 mgN. The increase in body weight with flavonoid supplementation has been reported in several studies. Wang et al. (2018) found that body weight gain in calves fed on mulberry leaf flavonoids was higher than in those without supplementation. Du et al. (2019) reported that supplementing finisher lambs with 3.40 g and 10 g of A. mongolicum Regel flavonoid extract improved body weight gain. According to Dorantes-Iturbide et al. (2022), supplementation with a low-dose polyherbal additive (1 g/kg DM) in finisher sheep enhanced feed energy utilization efficiency for body weight gain by up to 23%. The supplementation of flavonoid-rich plants has enhanced muscle protein synthesis in sheep (Qin et al., 2020). Different results were reported by Balcells et al. (2012) that supplementing flavonoid-enriched plant extract into a high-concentrate diet did not affect the ADG of cattle but improved rumen fermentation and reduced acidosis effectively by increasing the population of lactate-utilizing bacteria. The body weight gain of sheep in T3 was not significantly different from that in T0. This may be attributed to the lower energy retention in T3 compared to T2, as well as the low microbial protein synthesis, which was only 480 gN. The low microbial protein synthesis indicates suboptimal rumen microbial activity, likely due to the high concentration of antibacterial compounds resulting from the increased dose of bamboo leaf meal. As a consequence, the availability of fermentation end-products for animal growth was limited. This became one of the main factors contributing to the poor growth performance observed in T3, despite the fact that urinary energy loss was the lowest (0.04 Mcal), indicating relatively efficient nitrogen utilization. This finding is in line with (Paixão et al., 2022), who reported that the addition of flavonoids in the form of red propolis extract could enhance average daily gain up to an optimal dose, but exceeding that dose led to a decline in growth performance.

The feed conversion ratio (FCR) in T2 was similar to that in T1 but lower than in T0 and T3. A lower FCR indicates higher feed efficiency. Supplementation with WLM and BLM has enhanced feed efficiency because the flavonoids in both supplements modulate rumen conditions, improving rumen fermentation and positively affecting feed efficiency. Similarly, incorporating mulberry leaf flavonoids in calves' feed has reportedly improved feed efficiency (Kong et al., 2019). Studies have demonstrated the positive effects of flavonoids and phenolic compounds on livestock productivity and health, including enhanced rumen fermentation and mitigated nutritional stress, such as bloating and acidosis, which ultimately promote animal performance (Kalantar, 2018). The consumption rate of sheep in T2 was higher than in T0, T1, and T3. This result is closely related to the rate of digesta flow in the rumen, as explained by Mulyana et al. (2022), who stated that feed consumption is influenced by how quickly digesta passes through the rumen. A higher level of digestibility accelerates the outflow of digesta, resulting in faster rumen emptying and increased feed intake due to greater stimulation of hunger. These findings suggest that the sheep in T2 had a faster digesta flow rate than those in other treatments. Similarly, Fita et al. (2024) reported that supplementation with WLM at certain levels can increase feed consumption, supporting the results observed in the present study.

# CONCLUSION

This study concluded the effectiveness of supplementing 1.87 g waru leaf meal (WLM) and 0.33 g bamboo leaf meal (BLM) into feed to improve energy metabolism and the performance of local sheep. The combination of these leaf meals enhanced digestible and metabolizable energy, increased energy retention, and supported better growth performance. Improvements in feed utilization efficiency were also observed, indicating better metabolic function and nutrient absorption. Incorporating WLM and BLM into smallholder feeding systems may serve as a low-cost and locally available strategy to improve sheep productivity.

## **DECLARATIONS**

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# **Data availability**

The dataset and/or analysed during the current study available form the corresponding author on reasonable request.

# **Ethical approval**

All procedures involving animals in this study were carried out humanely and without causing suffering, in accordance with the Government Regulation of the Republic of Indonesia No. 95/2012 on Veterinary Public Health and Animal Welfare, and complied with the internal ethical guidelines of Universitas Jenderal Soedirman for animal-based research.

## Authors' contribution

I. Aisyah was responsible for data collection, statistical analysis, result interpretation, and drafting the manuscript. M. Bata and S. Rahayu designed the study, determined the methodology, interpreted the results, and finalized the manuscript. E. A. Rimbawanto and A. Setyaningrum reviewed the manuscript and provided critical feedback.

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## **Competing interests**

The authors declare no competing interests in this research and publication.

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