

# Feeding protected soybean groat to sheep: Effects on growth performance, nutrient digestibility, and carcass traits

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**ABSTRACT.** This study evaluated the effects of replacing soybean meal with rumen-protected soybean groat (PSBG) in the diets of thin-tailed sheep on growth performance, nutrient digestibility, and carcass quality. A total of 15 sheep (12 months old,  $23.43 \pm 1.40$  kg initial body weight [BW]) were randomly assigned to three dietary treatments: a control diet (CON), 10% PSBG substitution (PSBG10), and 20% PSBG substitution (PSBG20) with five animals per group. After 30 days of acclimatisation and 90 days of feeding trial, no differences ( $P > 0.05$ ) were observed in final BW, average daily gain, or feed conversion ratio. However, dry matter intake was higher ( $P = 0.028$ ) in the PSBG20 group compared to others. PSBG inclusion had no effect on overall nutrient digestibility, except for a significant increase in ether extract digestibility ( $P < 0.001$ ) in PSBG-fed animals. Carcass traits, organ weights, and the physical properties of *longissimus dorsi* and *triceps brachii* muscles were not influenced by dietary treatments. Sheep fed 10% or 20% PSBG had lower ( $P < 0.05$ ) cholesterol levels in *biceps femoris* and *longissimus dorsi* muscles compared to the CON. In *pectoralis profundus*, cholesterol content was also reduced ( $P < 0.05$ ) in the PSBG20 group. Conversely, the *triceps brachii* muscle had a higher ( $P < 0.05$ ) cholesterol level in the PSBG20 group compared to other treatments. In summary, while PSBG inclusion did not improve growth performance or protein digestibility, it favourably affected ether extract digestibility and meat lipid profiles, suggesting potential benefits due to reduced cholesterol content in specific muscles. Further research should focus on elucidating the underlying mechanisms behind these effects.

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## Introduction

Small ruminant (sheep and goats) farming plays a key economic role in supporting the livelihoods for majority of small-scale farmers in Indonesia. These animals are also an integral part of sustainable agricultural systems which contribute to food security. However, traditional production systems often rely on limited resources, i.e., low-quality feed, insufficient nutrient content, and environmental stressors, which compromise production efficiency (Azmi et al., 2021; Rahmatillah et al., 2024). Therefore,

researchers have focused on developing practical and cost-effective nutritional strategies to address these constraints and improve productivity and profitability for smallholder operations (Gading et al., 2020; Azmi et al., 2021).

In ruminants, an adequate supply of balanced protein is essential for optimal growth, product quality, and overall performance (Jayanegara et al., 2017; McGrath et al., 2018; Omphalius et al., 2019). Soybean meal (SBM) is a widely used protein source due to its high protein content and favourable amino acid (AA) profile (Suprayogi et al., 2022).

In ruminants, however, a major concern is the extensive degradation of high-quality protein in the rumen because it leads to nitrogen-carbon imbalance and subsequent nitrogen losses (Brake and Swanson, 2018; Gao et al., 2023; Irawan et al., 2023). This reflects low nitrogen use efficiency due to reduced AA absorption when high-quality protein sources such as SBM are included in ruminant diets. Protecting SBM protein from ruminal degradation by using rumen-protected protein (RPP) has been proposed as an effective strategy to improve AA utilisation (Adiwinarti et al., 2019; Pramono et al., 2019; Wulandari et al., 2019). By minimising ruminal degradation, more intact protein can pass the rumen and reach the small intestine, where it can be enzymatically digested into AA and peptides, increasing absorption and improving overall production performance and carcass quality (Azmi et al., 2021; Hidayat et al., 2025).

Over the past three decades, a large number of *in vivo* studies have evaluated the effectiveness of rumen-protected amino acids (RPAA) in improving nitrogen use efficiency (NUE) and productivity in small ruminants, beef cattle, and dairy cows, as summarised in several meta-analyses (Vyas and Erdman, 2009; Wei et al., 2022; Irawan et al., 2023). The cumulative evidence indicates that RPAA can overcome the metabolic inefficiency characteristic of rumen fermentation (Zanton et al., 2014; Omphalius et al., 2019; Räsänen et al., 2020). Unlike free amino acids, RPAA or RPP resist microbial deamination, allowing them to reach the abomasum for enzymatic digestion and absorption, thereby increasing AA availability for tissue growth and milk synthesis (Omphalius et al., 2019; Räsänen et al., 2020; Wang et al., 2022). In addition, protecting AA or proteins reduces nitrogen excretion as ammonia, thereby lowering environmental impact (Wang et al., 2010; Pereira et al., 2017). These advantages demonstrate the potential of RPP to support more sustainable ruminant nutrition and feeding. Nevertheless, most studies on RPAA have focused on large ruminants such as lactating dairy cows or beef cattle, and primarily used commercial RPAA products, with limited investigation of alternative protein sources like soybean groats (SBG). Unlike SBM, SBG is a lower-grade by-product unsuitable for human consumption and remains underutilised despite being widely produced (Riyanto et al., 2024a). Protecting SBG could increase AA availability and help improve production performance, particularly in lambs raised in tropical regions. Our prior *in vitro* study suggested that PSBG undergoes higher protein degradation in the abomasum than in the rumen

(Pramono et al., 2019). Earlier works have also showed that PSBG effectively protects protein from ruminal degradation *in vitro* and *in situ* (Wulandari et al., 2019; Widyobroto et al., 2022). Moreover, *in vivo* trials demonstrated improved production efficiency in goats and sheep fed PSBG (Adiwinarti et al., 2019; Riyanto et al., 2024a; 2024b).

To our knowledge, no studies have examined the effects of PSBG on thin tail sheep under tropical conditions. We hypothesised that PSBG supplementation would increase protein passage to the abomasum, reduce ruminal deamination, and increase amino acid absorption, ultimately improving production efficiency in lambs. Therefore, our study aimed to examine the effects of dietary PSBG on growth performance and carcass quality in 12-month-old thin tail sheep reared in a tropical environment. The findings are intended to support the development of efficient and practical nutritional strategies that promote the sustainability of small-scale sheep farming in these regions.

## Material and methods

This study was conducted at the Jatikuwung Experimental Farm of the Faculty of Animal Science, Universitas Sebelas Maret from December to February 2020. The procedures were conducted according to the local and national regulations on the care and use of laboratory animals and were approved by the Ethics Committee of the Graduate School of Universitas Sebelas Maret (Record No. 253/UN27.14/TA/00.03).

## Experimental design

The experiment was conducted using a complete randomised block design with 15 thin-tailed sheep of similar genetic background, approximately 12 months old, with an initial body weight of  $23.43 \pm 1.40$  kg. The animals were randomly assigned by body weight to three experimental groups: CON (control, fed a basal diet), PSBG10 (basal diet substituted with 10% PSBG), and PSBG20 (basal diet substituted with 20% PSBG), with five replicates per group. The basal diet consisted of a commercial complete feed (Semar OMEGA, Sekar Mendho Farm, Wonogiri, Indonesia), composed of copra meal (8.3%), pollard bran (8.3%), rice bran (8.3%), palm kernel meal (4.2%), soybean meal (8.3%), coffee bean husk (12.5%), maize gluten feed (8.3%), dried distillers' grain with solubles (8.3%), mung bean husk (8.3%), maize grain (8.3%), maize cob meal (4.2%), cassava meal and other agro-industrial byproducts (8.4%), available for purchase

**Table 1.** Ingredients and chemical composition of dietary treatments

Item	Ingredients		Dietary groups		
	CF <sup>1</sup>	PSBG	CON	PSBG10	PSBG20
CF			100.0	90.00	80.00
Protected soybean meal			0.00	10.00	20.00
Mineral-vitamin premix			1.00	1.00	1.00
Total			100.0	100.0	100.0
Chemical composition, % DM					
organic matter	87.16	92.49	87.16	87.69	88.23
crude protein	15.12	36.39	15.12	15.72	16.53
crude fibre	20.91	7.01	20.91	19.52	18.13
ether extract	2.55	11.65	2.55	3.46	4.37
nitrogen free extract	48.58	37.44	48.58	47.47	46.35
ash	12.84	7.51	12.84	12.31	11.77
Total digestible nutrients <sup>2</sup>	58.20	93.93	59.16	60.36	61.71

CF – complete feed, PSBG – rumen protected soybean groat, DM – dry matter; CON – basal diet (control group), PSBG10 – basal diet substituted with 10% PSBG, P2 – PSBG20 – basal diet substituted with 20% PSBG; <sup>1</sup> complete feed composition: copra meal (8.3%), pollard bran (8.3%), rice bran (8.3%), palm kernel meal (4.2%), soybean meal (8.3%), coffee bean husk (12.5%), maize gluten feed (8.3%), dried distillers' grain with solubles (8.3%), mung bean husk (8.3%), maize grain (8.3%), maize cob meal (4.2%), cassava meal and other agro-industrial byproducts (8.4%); <sup>2</sup> total digestible nutrients (TDN): calculated using the equation from Tillman et al. (1998), developed specifically for estimating the TDN content of Indonesian feed ingredients for small ruminants:  $TDN = 37.937 - 1.018 (CF) - 4.886 (EE) + 0.173 (NFE) + 1.042 (CP) + 0.015 (CF)^2 - 0.058 (EE)^2 + 0.008 (CF) \times (NFE) + 0.119 (EE) \times (NFE) + 0.038 (EE) \times (CP) + 0.003 (EE)^2 \times (CP)$

(Semar OMEGA, Sekar Mendho Farm, Wonogiri, Indonesia). The detailed composition and nutritional content of the experimental diets are presented in Table 1.

### Animal management and sample collection

The PSBG was produced through a sequential process involving sun-drying, grinding, and spraying with a mixture of 37% formaldehyde solution and water at a ratio of 1:5 until a homogenous mixture was obtained. This product was subsequently incubated at room temperature (approximately 25 °C) for 25 h under anaerobic conditions and aerated for 8 h before being offered to the animals. Following this step, PSBG was incorporated into the complete feed at 10% and 20% (dry matter basis), thoroughly mixed and fed individually to each animal (Riyanto et al., 2024a).

The animals were housed in separate cages and fed twice daily at 08:00 and 16:00 with clean water available *ad libitum*. The housing facilities were disinfected and cleaned before the arrival of the animals, with regular maintenance throughout the trial. A 30-day acclimatisation period preceded the 90-day feeding trial. Orts were collected daily before morning feeding and weighted. Feed was offered daily to allow for 10% refusals, based on the previous day intake. Body weight (BW) was measured every 2 weeks, and average daily gain (ADG) was calculated accordingly. For digestibility measurements, total faeces and orts were collected

daily during the last 10-days of the experiment using the total collection method. Total faecal output was weighed, recorded, and 10% of each defecation was sampled and sun-dried for further analysis (Hanim et al., 2020). Faecal and ort samples were pooled per animal and analysed for dry matter (DM; method 973.18), organic matter (OM; method 942.05), crude protein (CP; method 984.13), crude fibre (CF), and ether extract (EE; method 920.39) following AOAC protocols (AOAC International, 2005). Nutrient digestibility was calculated according to Cochran and Galyean (2015) as:

$$\text{digestibility (\%)} = \frac{[\text{intake (g/day)} - \text{fecal output (g/day)}]}{\text{intake (g/day)}} \times 100.$$

At the end of the experiment, the animals were slaughtered at the Jatikuwung Experimental Farm, Faculty of Animal Science, Universitas Sebelas Maret Surakarta. Prior to slaughter, the sheep were rested and fasted for 12 hours to minimise variation in slaughter weight caused by gastrointestinal content and to facilitate the slaughtering process. Following slaughter, all organs, non-carcass components, and carcass parts were separated and weighed. Samples of the *longissimus dorsi* (LD) and *triceps brachii* (TB) muscles were collected according to the Australian Meat and Livestock Corporation (AMLC, 1991) method, with LD and TB taken from the forequarter and hindquarter, respectively. The meat samples were wrapped in polypropylene plastic, placed in ice-cooled containers, and transported to the laboratory for analysis.

Meat colour was assessed according to the guidelines of the Australian Meat and Livestock Cooperation (AMLC, 1993) using 1–9 scale based on meat brightness. Water-holding capacity (WHC) was determined using the filter paper press method described by Warner (2014) and calculated as:

$$\text{WHC (\%)} = [1 - (W_a - W_b) / (W_a \times M)] \times 100,$$

where:  $W_a$  and  $W_b$  – meat weight before and after pressing, respectively;  $M$  – moisture content (g). Meat tenderness was measured using a Warner-Bratzler shear force device (C-LM3B, Beijing, China). Meat pH was recorded approximately 4 h post-slaughter upon arrival at the laboratory. Cooking loss was calculated as the percentage difference between initial and final weights after cooking:

$$\text{cooking loss (\%)} = [\text{initial weight before cooking (g)} - \text{final weight after cooking (g)} \times 100] / \text{initial weight before cooking (g)}.$$

The meat samples were then analysed for moisture, fat, and protein content using approximately 20 g of sample. The analysis was performed with a FoodScan instrument (Near Infrared Reflectance Spectroscopy Type 78810, Foss Electric A/S 69 DK, Slangerupgade, Denmark) (Kempen, 2001), applying wavelengths of 1186–1189 nm for moisture, 1414–1442 nm for protein, and 2310–2323 nm for fat content. For cholesterol determination, the samples were first ground

using a blender and quantified using a Microlab 200 UV-visible spectrophotometer (Merck Vital Scientific, Darmstadt, Germany).

## Statistical analysis

Analysis of variance was performed in R Studio (version 2024.12.1 + 563) using the *lme4* package, following confirmation of normal data distribution using the Shapiro-Wilk. Experimental diets (treatments) and measurement time points for BW and FI treated as fixed effects, while individual animals were considered as random effects. The statistical model applied was:

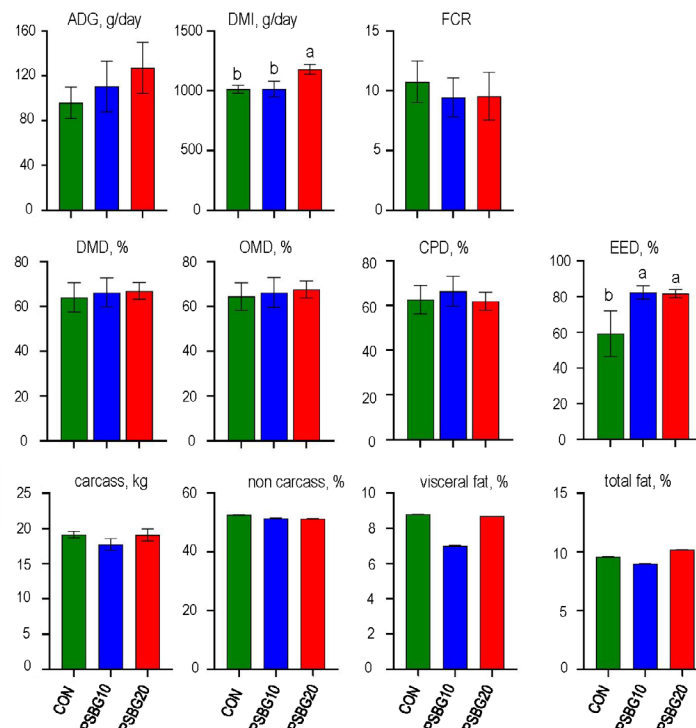
$$Y_{ijl} = \mu + D_i + T_j + A_l + e_{ijl},$$

where  $Y$  – variable (outcome);  $\mu$  – overall mean;  $D$  – fixed effect of diets (treatment);  $T$  – fixed effect of collection time;  $A$  – random effect of animal, and  $e$  – residual error. Post-hoc comparisons were conducted using Duncan's multiple comparison test. Statistical significance was declared at  $P < 0.05$  and tendencies discussed at  $P < 0.01$ .

## Results

### Production performance

Figure 1 presents the production performance parameters of thin-tailed sheep fed diets containing two levels of PSBG for three months. Average daily gain (ADG) and feed conversion ratio did



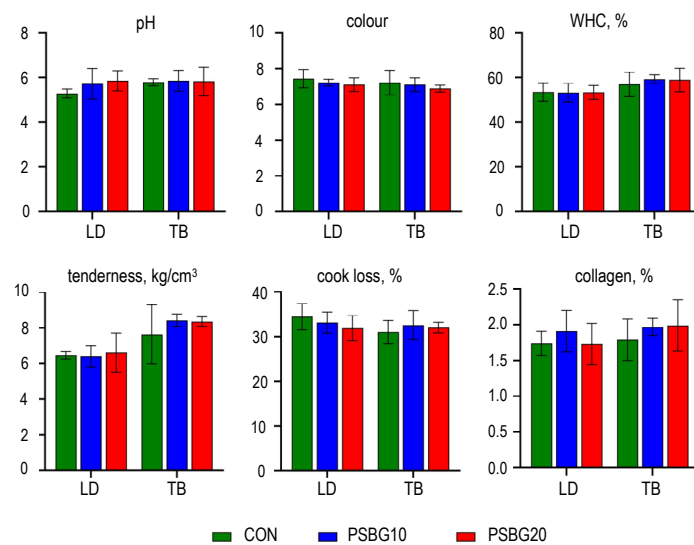
**Figure 1.** Production parameters of thin-tailed sheep fed protected soybean groats

CON – control, PSBG10 – protected soybean groat 10%, PSBG20 – protected soybean groat 20%; ADG – average daily gain, DMI – dry matter intake, FCR – feed conversion ratio, DMD – dry matter digestibility, OMD – organic matter digestibility, CPD – crude protein digestibility, EED – ether extract digestibility; values with different superscripts are significantly different at  $P < 0.05$

not differ significantly between the treatment groups ( $P > 0.05$  and  $P = 0.914$ , respectively). However, dry matter intake (DMI, g/day) differed significantly ( $P = 0.028$ ), with the PSBG20 group demonstrating higher intake compared to other groups. PSBG substitution had no significant effect on the digestibility of dry matter (DMD), organic matter (OMD), or crude protein (CPD) ( $P > 0.10$ ), but significantly improved ( $P < 0.001$ ) ether extract digestibility (EED) compared to the CON group. No significant differences were observed in carcass percentage, non-carcass components, visceral fat, and total fat content (Figure 1).

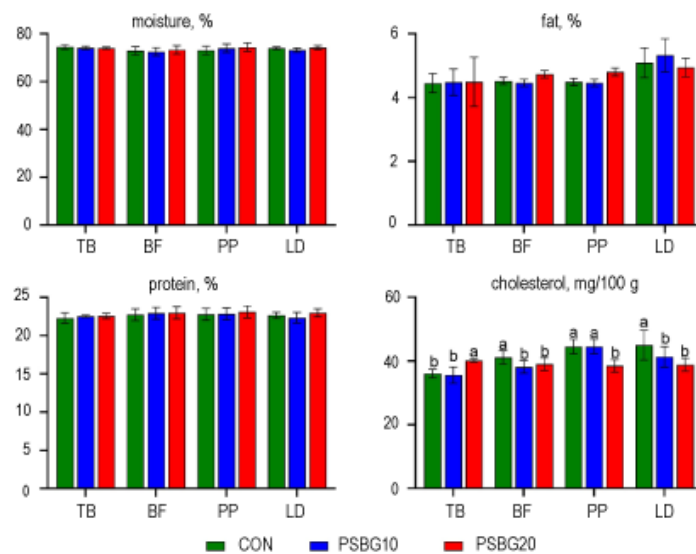
**Carcass components and quality attributes**

The study found no significant effects of PSBG dietary substitution on organ weights or the physical quality of LD and TB muscles in thin-tailed sheep (Figure 2). However, dietary treatment influenced muscle cholesterol concentrations. Sheep fed diets containing 10% or 20% PSBG had lower ( $P < 0.05$ ) cholesterol levels of *biceps femoris* (BF) and LD muscles compared to the control group (Figure 3). In the *pectoralis profundus* (PP) muscle, only the PSBG20 sheep showed reduced cholesterol levels ( $P < 0.05$ ). Conversely, cholesterol content in the TB muscle was higher ( $P < 0.05$ )



**Figure 2.** Physical quality of muscles of thin-tailed sheep fed protected soybean meal

CON – control, PSBG10 – protected soybean groat 10%, PSBG20 – protected soybean groat 20%, LD – *longissimus dorsi*, TB – *triceps brachii*, WHC – water holding capacity



**Figure 3.** Chemical parameters of muscles in thin-tailed sheep fed protected soybean meal

CON – control, PSBG10 – protected soybean groat 10%, PSBG20 – protected soybean groat 20%; TB – *triceps brachii*, BF – *biceps femoris*, PP – *pectoralis profundus*, LD – *longissimus dorsi*, values with different superscripts are significantly different at  $P < 0.05$

in the PSBG20 group than in other treatments. Moisture, fat, and protein content of all muscle tissues were unaffected by dietary treatments ( $P < 0.05$ ).

## Discussion

Protecting high-quality AA in soybean is a common strategy in animal nutrition to reduce deamination by rumen microbes, thereby increasing the flow of rumen-undegradable protein into small intestine for absorption. The protective effect of a very low formaldehyde doses is attributed to its ability to form stable-reversible chemical bonds with proteins with AA side chains (Pramono et al., 2019). This reaction results in a highly resistant SBM protein to microbial proteolysis in the rumen. Consequently, a greater proportion of intact, high-quality protein bypasses ruminal degradation and becomes available for enzymatic digestion and absorption in the small intestine, improving protein utilisation efficiency in ruminants. In the abomasum, the acidic environment and enzymatic activity facilitate the dissociation of formaldehyde-protein complexes, releasing AA for absorption (Brake and Swanson, 2018; Adiwintarti et al., 2019).

The present study demonstrated only minor effects of PSBG supplementation, primarily on ether extract digestibility and muscle cholesterol levels, with limited impacts on overall production performance, nutrient digestibility, or carcass traits. These findings contrast with previous reports showing that protected AA (e.g., methionine, lysine, histidine) in dairy cow diets increased plasma AA concentrations (Windschitl et al., 1988; Lee et al., 2015; Lobos et al., 2021; Van den Bossche et al., 2023) and post-ruminal AA flow in lambs (Liu et al., 2021), leading to improved production efficiency (Grassi et al., 2024). Similar benefits were observed in beef cattle, with reported improvements in both production and meat quality (Cabezas et al., 2023). However, many of these positive effects were observed in animals fed diets with lower metabolizable protein content (5–10% below requirements), as confirmed by a recent meta-analysis (Irawan et al., 2023). In contrast, studies employing iso-nitrogenous diets often reported no significant effects on production performance (Girma et al., 2019; Lee et al., 2019; Zang et al., 2021). Additionally, the response appears to depend on physiological status, with benefits largely limited to early lactation periods (Robinson et al., 2010; 2011). A limitation of our study is the absence of plasma AA measurements, which prevents con-

clusions regarding the degree of ruminal protection and systemic availability of AA from PSBG.

Several plausible explanations may account for the absence of significant effects observed in this study. First, the protection method applied to SBG may not have sufficiently preserved proteins or AA from ruminal degradation to influence growth performance, nutrient digestion, carcass percentage, or feed efficiency. Without measuring ruminal escape or post-ruminal absorption, the extent of protection, if any, remains unclear. Future studies should assess ruminal degradation kinetics and intestinal digestibility to verify protection efficacy. Second, the basal diets likely met or surpassed protein requirements. The average CP intake ranged from 11.39 to 13.08 g/kg BW<sup>0.75</sup>, which exceeds the reported requirement for Indonesian local sheep (>6.27 g/kg BW<sup>0.75</sup>) (Jayanegara et al., 2017). This aligns with findings from a recent meta-analysis showing that the positive effects of protected AA on production, nitrogen use efficiency, and AA supply were mainly observed under protein-deficient conditions (Irawan et al., 2023). Additionally, it is possible that the formaldehyde-based protection technique used in this study did not provide adequate resistance to ruminal degradation, thereby limiting the delivery of bypass protein to the small intestine. Future studies should evaluate the ruminal degradation kinetics and intestinal digestibility of the protected soybean meal to confirm whether the treatment successfully increased the proportion of protein delivery to the small intestine. As discussed above, if the basal diet contained already sufficient protein and AA content, the extra bypass protein may not have provided any additional growth advantage. A detailed analysis of the dietary AA profile and identification of potentially limiting nutrients would help clarify whether protein supply was adequate and whether any specific nutrient deficits may have constrained the response to supplementation.

Another relevant consideration is the efficiency of microbial protein synthesis in the experimental animals. Rumen microbes synthesise high-quality protein that often meets the AA requirements of the host animal (Irawan et al., 2024). If microbial protein production was sufficient in all dietary treatments, the additional protected protein may not have resulted in improved growth performance (Robinson et al., 2010). Furthermore, the balance between energy and protein availability is crucial for optimal nutrient utilisation (Jayanegara et al., 2017a; McGrath et al., 2018; Barbizan et al., 2020). If energy intake was limited, additional protein, whether

protected or not, may not have been effectively utilised for tissue synthesis or growth. In the present study, the actual bioavailability and post-ruminal flow of AA, as well as their balance, were not determined. Therefore, it is not possible to conclude whether the diets containing PSBG resulted in greater AA absorption.

While DM, OM, and CP digestibility were not statistically affected, a marked increase in EE digestibility was observed which could be due to the protective effects on dietary fat, in addition to the linear increase in dietary EE content resulting from PSBG inclusion. The increased EE digestibility likely contributed to greater energy intake. However, the difference in energy availability may not have been biologically sufficient to significantly improve ADG. Formaldehyde treatment is known to reduce ruminal fatty acid degradation or biohydrogenation (Gulati et al., 2005; Koivunen et al., 2015). This protective mechanism may explain the improved lipid intake, absorption, and metabolism observed in the PSBG groups. In the rumen, lipids (and protein) from diets treated with formaldehyde are protected from microbial fermentation due to cross-linking with the protein matrix, which renders them insoluble at rumen pH. These complexes are then dissociated under the acidic conditions of the abomasum (pH 2–3), allowing for lipid release and subsequent absorption (Gulati et al., 2005). As a result, the higher lipid intake in the PSBG10 and PSBG20 groups may have contributed to increased cholesterol synthesis (Gross et al., 2021). However, the lack of significant effects on growth performance suggests either that the additional energy from improved lipid digestion was insufficient to influence average daily gain under these experimental conditions, or that other factors such as individual animal variability, sample size, or trial duration limited the detection of treatment effects. Increasing the sample size or extending the feeding period in future studies may help clarify the potential benefits of replacing SBM with PSBGt.

## Conclusions

In summary, these findings indicate that the specific protection method and diet formulation applied in this study did not significantly improve performance parameters in small ruminants. Further research should focus on evaluating alternative protein protection strategies, quantify post-ruminal protein flow, and assess blood metabolites related to protein metabolism to better understand the impact

of protected soybean groat on nutrient utilisation and animal performance. Nevertheless, the observed reduction in cholesterol concentrations in selected muscles suggests potential benefits for improving meat quality and the underlying mechanisms behind these effects should be elucidated.

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## Conflict of interest

The Authors declare that there is no conflict of interest.

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