Research Article

The Effect of Formaldehyde-Protected Soybean Meal in A Pelleted Ration on the Performance and Animal Behavior of Sheep

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Abstract |This study used agro-industrial by-products as fiber sources and formaldehyde-protected soybean meal to decrease rumen digestible protein and improve sheep production. This study used 18 rams aged 10 months with an initial body weight of 25.01 kg ± 1.57 kg. A completely randomized design assigned rams to three treatments: control feed (FPS0) with 100% soybean meal, FPS50 with 50% soybean meal + 50% formaldehyde-protected soybean meal, and FPS100 with 100% formaldehyde-protected soybean meal. Sheep receiving a diet containing both protected and unprotected soybean meal demonstrated similar dry matter intake (1245.16 to 1306.81 g/d), average daily gain (80.85 g to 113.09 g), feed conversion ratio (11.66 to 16.34), and feed cost per gain (Rp 54,756.26 to Rp 79,762.44). Daily nitrogen intake varied from 30.38 to 33.51 grams, with 6.3 to 7.43 grams eliminated by feces and 0.55 and 0.76 grams excreted through urine. Similar nitrogen absorption, digestibility, and retention were observed between treatments. All sheep ate for 273.33 to 292.92 minutes daily. Rumination was shorter in sheep fed protected soybean meal compared to the control group fed unprotected soybean meal (384.17-390.83 min/d vs. 437.50 min/d). All sheep spent mealtimes between 0.53 and 0.56 min/g DMI. Formulated ration containing formaldehyde-protected soybean meal exhibited similar average daily gain, feed conversion ratio, and feed cost per gain. The total chewing activity of sheep in the FPS100 group was similar to that of the FPS50 group but shorter than that of the FPS0 group.

Keywords: Animal Behavior, Cassava Stump, Corn Cob, Digestibility, Nitrogen Balance, Pelleted Feed.

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INTRODUCTION

In Indonesia, meat from sheep has become favorable because it is cheaper than beef; therefore, the production of sheep is essential to the agricultural economy. Enhancing the nutritional content of sheep feed is crucial to improve growth, health, and sheep production. Soybean meal is a common protein source in ruminant diets because of its high protein content. However, its quick breakdown in the rumen can reduce the amount of essential amino acids that are available for small intestine absorption, thus limiting animal performance.

Protection of soybean meal was an effective strategy to reduce ruminal protein degradation in the rumen, increase bypass protein for nutrient utilization in the intestines and reduce feed cost. Applying formaldehyde 1% to soybean meal in ruminants' feed has significantly lowered their protein degradation (Suhartanto *et al.*, 2014), and those ruminants enhanced their growth by achieving a more efficient conversion of feed (Kumar *et al.*, 2015; Bhatt and Sahoo, 2019; Chauhan *et al.*, 2024) and meat product (Adiwinarti *et al.*, 2019), indicating that the treatment did not impact the animals' health (Kumar *et al.*, 2015). When used wisely, formaldehyde-treated feed can be an effective

strategy for maximizing yield and lamb health (Chauhan *et al.*, 2024). Suhartanto *et al.* (2014) reported that incorporating formaldehyde at a 1% concentration is more effective for producing protein that remains undegraded. Formaldehyde will not accumulate in the environment, and its use in animal nutrition is unlikely to cause an environmental risk. When employed as a feed additive, the ingested percentage of formaldehyde is primarily eliminated as formic acid in urine, carbon dioxide, and water.

In addition to reducing feed cost, agricultural byproducts have been widely used for ruminants' rations. Corn cobs (He *et al.*, 2022; Ojediran and Olorunlowu, 2025) and cassava stumps (Animashahun *et al.*, 2022; Hossain *et al.*, 2025) were agricultural waste products used as fiber sources in ruminants' feed. Crude fiber content in corn cobs was 0.81% (Njideka *et al.*, 2020) to 33.09% (Kamlasi *et al.*, 2024), while those in cassava stumps was 12.53% (Animashahun *et al.*, 2022). Those agricultural waste can be used as a source of crude fiber in the dry season while grass is limited in Indonesia.

This research aims to study the impact of using agro-industrial by-products as fiber sources and formaldehyde-protected soybean meal to decrease rumen digestible protein in a completed total mixed ration on sheep production, with an emphasis on growth performance, feed intake, feed efficiency, nutrient digestibility, nitrogen balance, and animal behavior.

MATERIALS AND METHODS

The research took place at the Faculty of Animal Agricultural Science Farm, Universitas Diponegoro, Semarang, and Rojokoyo Farm, Tengaran, Salatiga, in Indonesia. Research flowchart can be seen in Figure 1.

Ethical approval

The Animal Ethics Committee of the Animal and Agricultural Science Faculty at Universitas Diponegoro, Indonesia, granted approval for the animal-related procedures in this study (number. 61-11/A-31/KEP-FPP, January 2025).

Materials

Eighteen heads of rams aged 10 months were used in this study. The initial body weight of those rams was 25.01 kg ± 1.57 kg (CV = 6.29%). Rams were fed pelleted total mixed ration consisting of 36% corn cob, 10% cassava stump/cassava root crown, 15% soybean meal (SBM), 10% local fish meal, 24% pollard, and 5% molasses, then it was added to mixed mineral at about 2%. Feeds were formulated based on dry matter in different treatments and mixed into pelleted total mixed ration.

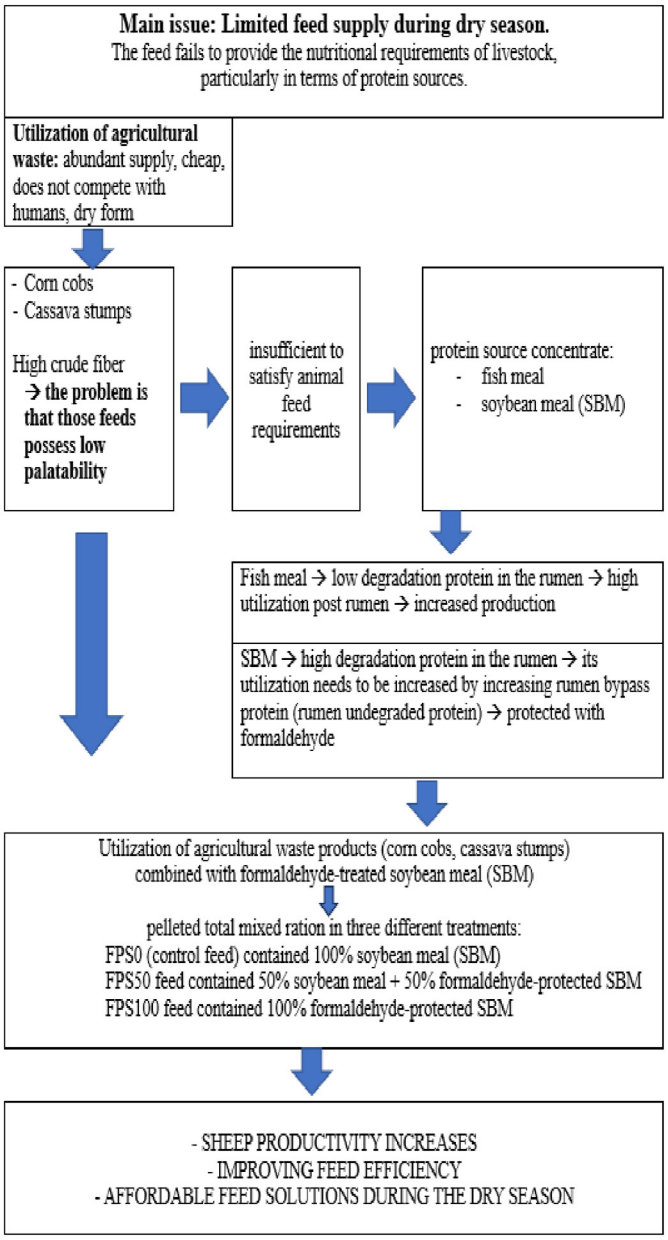

Figure 1: Research flowchart.

Method

Rams were randomly assigned in a completely randomized design using 3 different treatments, which were control (FPS0), FPS50, and FPS100. Control feed (FPS0) contained 100% soybean meal, FPS50 feed contained 50% soybean meal + 50% formaldehyde-protected soybean meal, and FPS100 feed contained 100% formaldehyde-protected soybean meal. The rations had the same nutrient contents: 90.04% dry matter, 18.40% ash, 14.14% crude protein, 2.62% crude fat, 12.80% crude fiber, and 52.05% nitrogen-free extract. The soybean meal was protected as stated by Adiwinarti *et al.* (2019). The SBM was protected with 1% formaldehyde. Formalin, a 37% formaldehyde solution diluted four times with water, was sprayed on the SBM, which was then fermented overnight. After that,

the SBM was aerated for one day and sun-dried for two. This study was held over 92 days and included 13 days of adaptation period, 16 days of pre-treatment period, and 63 days of treatment period. In the adaptation period, sheep were dewormed by albendazole and treated by Ivomec to protect from endo- and ectoparasites. In the pre-treatment period, sheep were fed based on their treatments. Feed and water were provided *ad libitum*. Each animal was weighed regularly (five times) during the treatment period before morning feeding and the average daily gain (ADG) was calculated using linear regression.

Feces and urine were collected during 7 days at the end of the experiment (El-Nomeary *et al*., 2021) to determine nutrient digestibility and nitrogen balance. The nutrient digestibility was calculated using the formula of Behan *et al*. (2019).

$$Digestibility\,\% = \frac{nutrient\ consumed - nutrient\ excreted\ in\ faeces}{nutrient\ consumed\ (g/day)} \times 100$$

Retained Nitrogen was determined by subtracting the amounts of fecal and urinary Nitrogen from the total Nitrogen intake. The method to collect fecal samples referred to Darlis *et al*. (2000), while urine sampling was conducted according to Elamin *et al*. (2012).

Table 1: Daily nutrient intake and performance of sheep fed a ration containing formaldehyde-protected soybean meal during experimental period.

Parameters	FPS0[1]	FPS50[2]	FPS100[3]	p-value
DMI (g/d)	1306.81±69.33	1291.25±22.81	1245.16±51.47	0.133
DMI in %BW (%)	4.58±0.34	4.47±0.25	4.49±0.16	0.719
DMI (g/kg $BW^{0.75}$)	105.87±2.51	103.64±4.49	103.02±2.98	0.56
OMI (g/d)	1066.35±56.57	1053.66±18.61	1016.05±42.00	0.133
Crude Protein Intake (g/d)	184.78±9.80	182.58±3.22	176.06±7.28	0.133
Crude Fiber Intake (g/d)	167.27±8.87	165.28±2.92	159.38±6.59	0.133
Ether extract intake (g/d)	34.24±1.82	33.83±0.60	32.62±1.35	0.133
Nitrogen Free Extract intake (g/d)	680.19±36.09	672.10±11.87	648.10±26.79	0.132
Initial body weight (kg)	25.07±2.07	24.96±1.76	25.00±0.99	0.994
Final body weight (kg)	32.19±3.15	32.92±1.32	30.5±2.01	0.206
ADG (g)	102.31±27.55	113.09±16.85	80.85±21.42	0.068
Feed conversion ratio	13.71±4.24	11.66±2.01	16.34±4.39	0.125
Feed cost/gain (IDR/kg)	63111.04±19549.16	54756.26 ±9578.38	79762.44±21345.67	0.073

Note: DMI = dry matter intake; OMI = organic matter intake; CPI = crude protein intake; CFI = crude fiber intake; ADG = average daily gain;[1]FPS0 (control) means a ration containing 100% soybean meal.[2]FPS50 means a ration containing 50% soybean meal + 50% formaldehyde-protected soybean meal.[3]FPS100 means a ration containing 100% formaldehyde-protected soybean meal.

Table 2: Nutrient digestibility of sheep fed a ration containing formaldehyde-protected soybean meal.

Parameters	FPS0[1]	FPS50[2]	FPS100[3]	p-value
DM digestibility (%)	63.07±9.22	58.89±4.42	59.78±8.06	0.609
OM digestibility (%)	68.45±8.12	64.86±3.38	65.05±7.44	0.55
EE digestibility (%)	62.36±11.00	58.20±5.92	63.54±9.33	0.57
CF digestibility (%)	58.63±12.23	55.23±5.31	53.96±10.21	0.696
CP digestibility (%)	79.52±6.41	77.74±3.86	79.28±4.69	0.807
NFE digestibility (%)	68.17±7.68	64.08±3.39	63.99±7.81	0.476
TDN (%)	57.91±6.98	54.85±2.89	55.17±6.32	0.603

[1]FPS0 (control) means a ration containing 100% soybean meal.[2]FPS50 means a ration containing 50% soybean meal + 50% formaldehyde-protected soybean meal.[3]FPS100 means a ration containing 100% formaldehyde-protected soybean meal.DM: dry matter; OM: organic matter; EE: ether extract; CF: crude fiber; CP: crude protein; NFE: nitrogen-free extract; TDN: total digestible nutrients.

During the last week of the experimental period, activities related to animal behaviors were documented every 5 minutes over a 48-hour period, with lighting provided at night. The total chewing time was determined by summing the durations of eating and ruminating (Mirzaei-Alamouti *et al*., 2021). Mealtime was calculated by dividing the difference between the eating duration and the total dry matter intake (DMI). The data were evaluated using a completely randomized design through a one-way ANOVA, utilizing SPSS statistics software version 27.

RESULTS AND DISCUSSION

Performance of sheep

Sheep fed rations containing protected and unprotected soybean meal demonstrated similar dry matter intake (1245.16 to 1306.81 g/d, Table 1), and therefore the nutrient intake and the nutrient digestibility were also the same (Table 1 and Table 2) because of similar nutrient content in the rations. This indicates that increasing the proportion of protected soybean meal to 100% did not interfere with consumption, despite potential concerns about smell or taste (Flis and Molik, 2024). The dry matter intake of sheep in this study was around 4.47 to 4.58% of the body weight Araújo *et al*. (2023) reported that sheep consumed 4.26 to 5.30% of live weight.

Descriptively, FPS0 sheep had higher dry matter intake (g/kg $BW^{0.75}$) than FPS50 and FPS100 sheep (Table 1). The decrease in DMI found in sheep fed protected soybean meal (FPS50 and FPS100 sheep) might be caused of a reduction in rumination (Table 4). Mirzaei-Alamouti *et al*. (2021) indicated increasing the amount of rumen undegradable protein reduced the overall time spent ruminating (min/d). According to Malik *et al*. (2023), reducing rumination resulted in lower rumen pH levels. Low rumen pH causes acidosis, which is deleterious to animal welfare. According to Bowen (2021), acidosis in livestock results in decreased feed consumption and body weight, depression, and even mortality. The dry matter intake of sheep has been reflected to the growth rate of sheep (Figure 2). In fact, the ADG of sheep, feed conversion ratio, and feed cost per gain between the treatments was similar between the treatments. Nutrient digestibility between treatments was not significantly different (Table 2) from that observed in feed intake (Table 1), due to the same feed composition. Pereira *et al*. (2021) reported that similar feed intake and chemical composition of diet resulted in similar digestibility.

Table 3: Nitrogen balance of sheep fed a ration containing formaldehyde-protected soybean meal.

Parameters	FPS0[1]	FPS50[2]	FPS100[3]	p-value
N intake (g/d)	33.44^{A}±1.80	33.51^{A}±0.92	30.38^{B}±1.06	0.001
Fecal N (g/d)	6.82±2.09	7.43±1.13	6.3±1.47	0.492
N absorbed (g/d)	26.61±2.90	26.07±1.91	24.08±1.54	0.145
N digestibility (%)	79.52±6.41	77.74±3.86	79.28±4.69	0.807
Urinary N (g/d)	0.76±0.63	0.65±0.31	0.55±0.17	0.735
N retention (g/d)	26.25±3.26	25.42±1.92	23.81±1.39	0.266
N retention (% N intake)	78.1±6.39	75.78±3.85	78.24±4.36	0.651

^{A,B}Means within a row with different superscripts differ significantly (P=0.001) [1]FPS0 (control) means a ration containing 100% soybean meal.[2]FPS50 means a ration containing 50% soybean meal + 50% formaldehyde-protected soybean meal.[3]FPS100 means a ration containing 100% formaldehyde-protected soybean meal.

Table 4: Feeding behavior of sheep fed a ration containing formaldehyde-protected soybean meal.

Parameters	FPS0[1]	FPS50[2]	FPS100[3]	p-value
Eating (min/d)	292.92±22.88	290±27.43	273.33±27.09	0.391
Rumination (min/d)	437.50±46.39	390.83±32.85	384.17±48.13	0.097
Chewing (min/d)	730.42^{A}±36.03	680.83^{AB}±36.35	657.5^{B}±26.12	0.005
Dry matter intake (g/d)	1306.81±69.33	1291.25±22.81	1245.16±51.47	0.133
Mealtime (min/g DMI)	0.56±0.04	0.53±0.03	0.53±0.03	0.210
Standing (min/d)	702.08±41.99	765.83±109.37	821.67±194.37	0.314
Laying (min/d)	729.58±40.48	670.00±107.04	618.33±194.37	0.359

^{A,B}Means within a row with different superscripts differ significantly (P=0.005)[1]FPS0 (control) means a ration containing 100% soybean meal.[2]FPS50 means a ration containing 50% soybean meal + 50% formaldehyde-protected soybean meal.[3]FPS100 means a ration containing 100% formaldehyde-protected soybean meal.

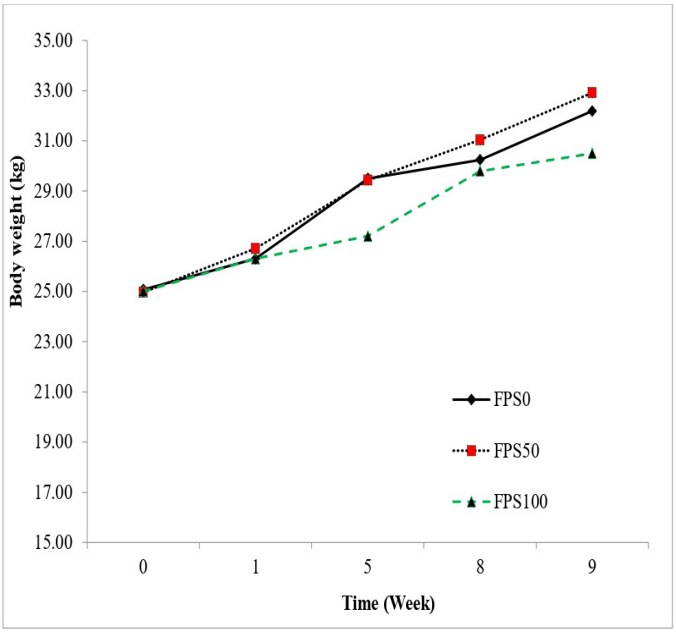

Figure 2: Growth rate of sheep fed a ration containing formaldehyde-protected soybean meal

Nitrogen intake of FPS50 sheep was higher (P=0.001) than those of FPS100 sheep, but it was similar to those of FPS0 (Table 3). Nitrogen excretion from feces and urine were not different between the treatments. The N absorbed, N digestibility, and N retention were also similar (Table 3).

The nitrogen intake of FPS100 sheep was lower than other sheep due to the impact of their dry matter intake. However, the nitrogen retention was similar between the treatments (Table 3). This could be due to the fact that FPS100 sheep expelled 22.55% of their feces and urine, which was lower than FPS50 sheep (24.11%) but similar to FPS0 sheep (22.67%). This indicated that the FPS100 sheep were more efficient in digesting and absorbing feed. Martins *et al.* (2021) indicated that nitrogen levels in feces and urine, as well as nitrogen balance, diminished linearly with reduced nitrogen intake. This study found that all sheep voided feces and urine at comparable rates. The comparable quantity of nitrogen excreted in feces and urine is attributable to the same diet formulation across all treatments, as noted by Pereira *et al.* (2021). In this study, the daily nitrogen intake ranged from 30.38 to 33.51 grams, while the nitrogen expelled through feces was between 6.3 and 7.43 grams per day, and that excreted via urine was 0.55 to 0.76 grams per day. The fact that nitrogen intake exceeded the amounts excreted in both feces and urine suggests that the diet was well-balanced. Pereira *et al.* (2021) stated that a diet is well-balanced when the intake of nitrogen exceeds the amount excreted in feces. This study was similar to Silva *et al.* (2023) research that reported nitrogen intake of lambs was about 31.7 to 43.3 g/day; absorb nitrogen was 24.3 to 35.1 g/day; and nitrogen balance was around 18.5 to 30.2 g/day. The retained nitrogen or nitrogen balance in all sheep examined in this study exceeded the levels reported by Costa *et al.* (2021), Martins *et al.* (2021) and Pereira *et al.* (2021). The experiment conducted by Costa *et al.* (2021) demonstrated that the nitrogen excretion by lambs, through both urine and feces, was higher than that observed in the present study.

Surprisingly, retained nitrogen conversion of FPS50 sheep (0.20±0.02) was better than those of FPS100 sheep (0.3±0.08) and the same as those of FPS0 sheep (0.25±0.05). This indicated that the replacement of 50% protected SBM was better than those of 100% protected SBM. Suhartanto *et al.* (2014) stated that the higher the level of formaldehyde, the greater the amount of dissolved formaldehyde in the rumen, which inhibits microbial activity and reduces protein synthesis in the rumen.

Feeding behavior of sheep

The incorporation of protected soybean meal in sheep diets may influence their feeding behavior in various ways. Numerous researchers have found that animal behavior can serve as an indicator of health, welfare, and feed management. Simões *et al.* (2021) indicated that the duration of eating and rumination can serve as indicators to monitor nutritional management. Rumination behavior may facilitate an objective evaluation of sheep health and welfare (Schneidewind *et al.*, 2023). However, the detection of rumination as an indicator of welfare remains insufficiently researched. Lying, eating, and drinking behaviors are significant parameters for assessing welfare (Schneidewind *et al.*, 2023).

The FPS100 sheep took faster to chew their food than the FPS0 sheep (P=0.005), but it was the same as the FPS50 sheep. There was a tendency (P=0.097) that rumination time of FPS50 and FPS100 sheep was shorter (P=0.097) than those of FPS0 sheep (Table 4). However, mealtime (min/g DMI) of all the treatments was relatively similar. Other animal behaviors (standing and laying) were not significantly different between the treatment (Table 4).

In this study, all of the sheep spent time eating at a similar time about 273.33 to 292.92 min/day or 4.56 to 4.88 hours (Table 4). Alba *et al.* (2021) observed that lambs ate for about 180 minutes (3 hours) per day, whereas Berthel *et al.* (2023) claimed that sheep ate for about 294 minutes (4.90 hours) daily. However, Alba *et al.* (2021) stated that feeding time may range from 1 to 12 hours per day depending on the diet type.

Sheep receiving protected soybean meal (FPS50 and FPS100) spent less time ruminating (384.17-390.83 min/d) than the control group (437.50 min/d, P=0.097, (Table 4). This shorter rumination time suggests that more feed bypassed the rumen without re-mastication, which

is crucial for enhancing growth in high-performance ruminants (Simões *et al*., 2021). However, it is important to note that reduced rumination can lower rumen pH, potentially leading to acidosis (Malik *et al*., 2023).

The rumination duration of sheep fed a ration containing formaldehyde-protected soybean meal in this study (384.17-390.83 min/day) was shorter than that reported by Mirzaei-Alamouti *et al*. (2021): 413-447 min/day, Costa *et al*. (2019): 502.75-582.75 min/day, Alba *et al*. (2021): 504.00 min/day, Almasi *et al*. (2022): 464.00 min/day, Berthel *et al*. (2023): 432 min/day, and Krone *et al*. (2024): 570-588 min/day. However, Malik *et al*. (2023) reported that the rumination time of sheep fed pelleted total mixed ration was 103 minutes daily. The differences in rumination time might be caused by different types of feed and different fiber content as Costa *et al*. (2019) reported that the particle size and fiber content influence the duration of retention in the rumen, hence sustaining mastication activity. However, increased feed consumption by the sheep correlates with reduced rumination time (Costa *et al*., 2019).

The chewing time of FPS100 sheep was shorter (P=0.005) than that of the control (FPS0 sheep). The chewing time (Table 4) was impacted by the time spent eating and ruminating. As previously stated, sheep fed protected soybean meal spent less time (P=0.097) ruminating than control sheep fed unprotected soybean meal, resulting in shorter chewing time. Other researchers reported that sheep chewing duration varied from 684.00 minutes/day (Alba *et al*., 2021), 671 to 708 minutes/day (Mirzaei-Alamouti *et al*., 2021), 726.00 minutes/day (Berthel *et al*., 2023), and 726.75 to 748.00 minutes/day (Costa *et al*., 2019).

Mealtime (min/g DMI) of sheep in all treatments was the same (0.53-0.56 min/g DMI) due to the similar composition of total mixed ration in all treatments. This finding is similar to Krone *et al*. (2024), who reported that the chewing effort of sheep was from 0.41-0.46 to 0.50-0.53 min/g DM depending on the fiber content of feed.

Adequate lying time is regarded as a significant factor in dairy cow welfare, as increased lying durations typically indicate enhanced cow comfort, while reduced lying times are associated with adverse conditions (Tucker *et al*., 2021). Further research should examine lying behavior from a perspective that emphasizes positive welfare (Muhammad *et al*., 2022). Lying behavior was observed by Williams *et al*. (2021) in pregnant ewes 14 days prior to parturition to identify signs of parturition or ill health. This study found no significant differences in laying and standing times between the treatments (Table 4). This suggests that all sheep experienced identical conditions. Researchers reported that laying time of sheep around 446.50 (Abdurrahman *et al*., 2024) to 918.94-1025.83 minutes per day, while standing time around 414.17 to 483.13 minutes per day (Nugroho *et al*., 2015).

CONCLUSION

Formulated ration containing formaldehyde-protected soybean meal exhibited similar average daily gain, feed conversion ratio, and feed cost per gain. Chewing activity of FPS100 was similar to those of FPS50, but shorter than those of FPS0. However, providing 100% protected soybean meal over an extended period may be detrimental, as the associated reduction in rumination time could lower rumen pH and potentially depress feed intake.

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NOVELTY STATEMENT

This study specifically investigates the effects of integrating formaldehyde-protected soybean meal and agricultural byproducts (corn cobs and cassava stumps) into pelleted sheep diets, focusing on both animal performance indicators (including growth and feed efficiency) and behavioral responses to elucidate the impact of protected protein sources and agricultural byproducts in pelleted diets on the overall welfare and productivity of sheep.

AUTHORS' CONTRIBUTIONS

RA designed the research, supervised the fieldwork, analyzed the data, prepared and wrote the manuscript. EDR, APA, ARF participated in designing the research, supervised the fieldwork, reviewed and edited the manuscript. EPW, STY, VR supervised the fieldwork, reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript

Conflict of interest

No conflicting interests are disclosed by the writers.

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