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Optimizing Sheep Productivity: The Impact of Bypass Protein Supplementation and Neem (*Azadirachta indica A. Juss*) Induced Defaunation on Napier *(Pennisetum purpureum Schumach*) Grass-Based Diets

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Abstract: This study investigated the effects of ruminal defaunation and bypass protein supplementation on dry matter intake (DMI), weight gain, and feed digestibility in sheep. The experimental results indicated that the combination of ruminal defaunation and bypass protein supplementation, achieved through the administration of liquefied soybean meal (LSBM), resulted in statistically significant increases in DMI and weight gain compared to control animals. While bypass protein supplementation alone did not elicit a statistically significant increase in DMI in animals possessing an intact ruminal microbiome, it did enhance weight gain and overall feed digestibility, particularly in animals subjected to defaunation using Neem Tree Liquid Extract (NTLE) and Sodium laurel sulfate (SLS). Mechanisms, including enhanced bacterial fiber digestion, increased dietary nitrogen availability, and improved nutrient delivery to the small intestine, explain the observed improvements in DMI and weight gain in defaunated, LSBM-supplemented animals. Furthermore, ruminal defaunation alone also led to increased DMI and digestibility, thereby suggesting that the elimination of protozoa enhances the efficiency of bacterial digestive processes. These findings indicate that the combined strategy of ruminal defaunation and bypass protein supplementation is a promising approach for optimizing nutrient utilization and enhancing production efficiency in ovine livestock, contingent upon the economic viability of supplementation.

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I. INTRODUCTION

Small ruminant production, with a particular emphasis on ovine farming, represents a significant element of the socioeconomic and food security framework for numerous communities globally (Derbib, 2021; Kyei-Gyamfi & Kyei-Athur, 2025). These animals demonstrate considerable adaptability across a range of environmental conditions and contribute substantially to sustainable agricultural systems through the efficient bioconversion of low-quality forages into economically valuable products (Oyedeji, 2024; Wróbel et al., 2025). However, nutritional limitations frequently constrain sheep productivity, especially in areas where dietary regimens are predominantly based on low-quality roughages.

A common challenge in ovine nutrition involves the frequent reliance on forages such as Pennisetum purpureum (Napier grass). Despite its high biomass yield, P. purpureum exhibits inherent nutritional deficiencies, including a low protein concentration and a high fiber content, which limit its efficient utilization by ruminant livestock (Islam, 2024; Botero-Londoño, 2021). Addressing these limitations is therefore crucial for optimizing ovine performance and enhancing the economic sustainability of agricultural practices reliant on sheep production (Aouina, 2025).

To address these nutritional constraints, researchers have investigated various strategies, including bypass protein supplementation and manipulation of rumen microbial ecology through defaunation (Cherdthong, A., 2025; Lavelle

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et al., 2007). Bypass protein, designed to resist degradation in the rumen, facilitates the direct delivery of essential amino acids to the small intestine (Lata & Mondal, 2021; Singh, 2019; Summers, 2012). Defaunation, involving the selective reduction or elimination of protozoa within the rumen, aims to enhance nutrient utilization by mitigating the adverse effects of protozoa on bacterial protein synthesis and overall nutrient bioavailability (Holtshausen & Beauchemin, 2010).

The potential application of Azadirachta indica (neem), a tropical tree species recognized for its inherent anthelmintic and antimicrobial properties, warrants particular attention as a naturally derived defaunating agent (Valleser & Caraca, 2024). Valleser and Caraca (2024) specifically propose the increased utilization of A. indica as a defaunating agent within ovine husbandry practices. Through the selective inhibition or reduction of protozoal populations within the rumen, A. indica-induced defaunation may potentiate bacterial protein synthesis, augment the flux of microbial protein to the small intestine, and thereby enhance the overall efficiency of nutrient utilization in ovine livestock.

This article presents a comprehensive analysis of the effects of bypass protein supplementation and neem-induced defaunation, both independently and in combination, on the productivity of sheep maintained on dietary regimens based on P. purpureum (Campbell, et al.,2021). . It provides a critical review of current knowledge regarding the nutritional limitations of P. purpureum, the underlying principles and mechanisms governing bypass protein supplementation and defaunation, and the potential benefits of these strategies for enhancing ovine performance (Te Pas, 2021; Thakur et al., 2024). By addressing these objectives, this review aims to inform the development of sustainable feeding strategies that optimize ovine productivity and improve economic outcomes for smallholder farmers.

II. MATERIALS AND METHODS

A. Preparation of the Bypass Protein Supplement

Soybean oil meal, used as a source of bypass protein, was obtained from the Visayas State University (VSU) Pilot Feed Mill and subsequently reduced to a fine particulate consistency through grinding. The resultant material was then subjected to hydration by immersion in water for 12 hours, using a ratio of 1 part soybean oil meal to 3 parts water. Incorporating 40 grams of commercially available corn starch (designated as "zoy") into the solution maintained a homogenous suspension of the soybean meal particles during administration (Fig. 8). The prepared supplement was administered to the animals at a rate equivalent to 0.75% of their total body weight on a dry matter basis, delivered either through voluntary consumption from a feeding receptacle or, in cases of incomplete ingestion, via drenching.

B. Preparation and Feeding of Experimental Animals

Before the commencement of the experimental period, animals were subjected to deworming and administered an intramuscular injection of Vitamin ADE. Researchers fed the sheep a basal diet of *Pennisetum purpureum* (Napier grass) throughout the study, providing it daily at a rate of approximately 3% of their body weight on a dry matter basis. The growth measurement phase extended for 35 days, with feeding intervals occurring at 0700, 1200, and 1600 hours daily. The facility consistently provided ad libitum access to potable water. The researchers recorded the live weights of the experimental animals every week and maintained pen hygiene through daily cleaning protocols.

C. Dietary Treatments and Experimental Set-up

The experimental protocol consisted of five distinct treatment groups, designed to evaluate the individual and combined effects of ruminal defaunation and bypass protein supplementation on ovine subjects. Group A, serving as the control, consisted of animals with an intact ruminal microbiome and no supplemental protein. Group B comprised animals with an intact ruminal microbiome receiving bypass protein supplementation. Researchers chemically induced defaunation in Groups C and D by administering neem tree leaf extract; Group C received no supplemental bypass protein, while researchers supplemented Group D with bypass protein. Group E consisted of animals subjected to ruminal defaunation via sodium lauryl sulfate, in conjunction with bypass protein supplementation. Twenty (20) sheep were weighed and randomly assigned to the aforementioned treatment groups, with individual confinement in metabolism cages. The experimental design employed a randomized complete block design, utilizing sex-period combinations as the basis for blocking.

D. Administration of Bypass Protein Supplement

Post-defaunation, from day 15 to day 45 of the experimental period, animals received a bypass protein supplement in the form of liquefied soybean meal (LSBM), administered daily at 0700 hours via bucket feeding (Fig. 9). The researchers used LSBM, hypothesizing that its liquid consistency would stimulate closure of the reticular groove, facilitating direct transit to the omasum through the reticulo-omasal orifice (Owens & Basalan, 2016) and effectively circumventing ruminal fermentation. Subsequently, the researchers expected the supplement to traverse the sulcus omasi within the omasum (Reddy & Hyder, 2023) and enter the abomasum, where enzymatic hydrolysis would yield amino acids for absorption in the small intestine (Moughan, 2018; Good, 2018). The research team kept meticulous daily records of feed offered and refused for each animal.

E. Apparent Digestibility Trial

The apparent digestibility coefficient rigorously quantifies the proportion of a feedstuff presumed absorbed by an animal, based on the difference between intake and fecal excretion. Convention dictates expressing this coefficient as a percentage of feed dry matter. To ensure a comprehensive assessment of both the foliar and stem components of the basal roughage diet, the digestibility trial was implemented following the 45-day feeding period, with feed offered at 90% of ad libitum intake levels. The digestibility trial methodology followed the established procedures outlined by Bestil and Espina (1992) and Bestil (2009). The research team collected data for five consecutive days immediately after the feeding trial. The research team quantified daily feed intake and fecal output before the morning feeding regimen.

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F. The Process of Dry Matter Determination in a Convection Oven.

The process involved weighing feed and fecal samples collected over five days with a triple-beam balance. Subsequently, the Department of Animal Science Nutrition Laboratory determined dry matter (DM) content by drying the samples in a convection oven at 100°F for 24 hours.

G. Data Gathered

➢ *Cumulative Biweekly Voluntary Dry Matter Intake (CVDMI).*

CVDMI = Σ(TFGi – TFRi)

Where: Σ = summation

TFGi = total feed given at the ith period of measurement

TFRi = total feed refused at the ith period of measurement

➢ *Cumulative Biweekly Weight Gain (CWG)*

CWG = Σ(BWi – BWo)

Where: Σ = summation

BWi = body weight at the ith period of measurement

BWo = initial body weight

➢ *Dry Matter Digestibility*

DMI of Napier = VFI of Napier x %DM of Napier

DMI of LSBM = VFI of LSBM x & DM of SBM

DM Excreted = Fecal output x % DM of feces

Total DM Intake – DM Excreted

DM Digestibility = X 100

Total DM Intake

➢ *Statistical Analysis of Data*

The data acquired from this study were subjected to statistical analysis using a two-way analysis of variance (ANOVA), appropriate for the randomized complete block design implemented. Detecting significant treatment effects led researchers to compare treatment means using Tukey's Honestly Significant Difference (HSD) test in post-hoc analyses. We performed all statistical computations with the Statistical Package for the Social Sciences (SPSS), version 12.

III. RESULTS AND DISCUSSION

➢ *Cumulative Voluntary Dry Matter Intake*

Analysis of cumulative voluntary dry matter intake (DMI) in ovine subjects revealed statistically significant treatment effects attributable to both ruminal defaunation and bypass protein supplementation (Table 1, Figure 1). Specifically, animals in Treatments D and E, which were subjected to ruminal defaunation and received bypass protein supplementation, exhibited the highest DMI values, considering both the consumption of *Pennisetum purpureum* (Napier grass) alone and total DMI (i.e., Pennisetum purpureum plus liquefied soybean meal). Furthermore, the data indicated that bypass protein supplementation, delivered via liquefied soybean meal, did not elicit a statistically significant increase in the DMI of *Pennisetum purpureum* in animals with an intact ruminal microbiome.

Table 1 Cumulative Dry Matter Intake (kg) in Sheep Fed Napier Grass as Affected by Defaunation and Bypass Protein Supplementation.

Treatment		2 Weeks		4 Weeks	
		Napier	Total	Napier	Total
Undefaunated					
Without supplement	A	7.17^{c}	7.171^{c}	11.23^{b}	11.23c
With supplement	B	7.24^{c}	9.72^{b}	12.80^{b}	18.11bc
Defaunated (with NTLE)					
Without supplement	C	7.46^{bc}	7.46^{c}	11.49^{b}	11.49c
With supplement		11.71^{a}	14.19^{a}	23.31^{a}	28.94a
Defaunted (with SLS)					
With Supplement	D	11.44^{a}	13.87^{a}	23.31^{a}	29.42^{a}
p-value		0.004	0.004	0.003	0.004

Means with a Column of Similar Letter Superscript are Not Significantly Different.

The findings about voluntary dry matter intake (DMI) in ovine subjects indicated that bypass protein supplementation facilitated an increase in the voluntary consumption of dry matter from *Pennisetum purpureum* (Napier grass), demonstrating a 'supplementing effect' rather than a 'substitution effect.' This observed increase in DMI may be

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attributable to the influence of liquefied soybean meal (LSBM) on the parotid salivary glands, potentially stimulating the production of salivary buffers (Cappai et al., 2021; Dall'Aglio et al., 2020), or to the provision of an augmented quantity of protein, which is inherently deficient in Pennisetum purpureum, thereby enhancing the voluntary intake of the basal diet. The increased voluntary DMI observed in ruminally defaunated animals, relative to those with an intact ruminal microbiome, corroborates previous findings of Valleser & Caraca 2024) (Tables 2 and 3) demonstrating that a reduction in protozoal populations, coupled with a concomitant increase in bacterial populations, leads to enhanced intake and digestibility (Zhang et al., 2022). Therefore, bypass protein supplementation provides the most significant benefits to voluntary DMI in ruminally defaunated sheep.

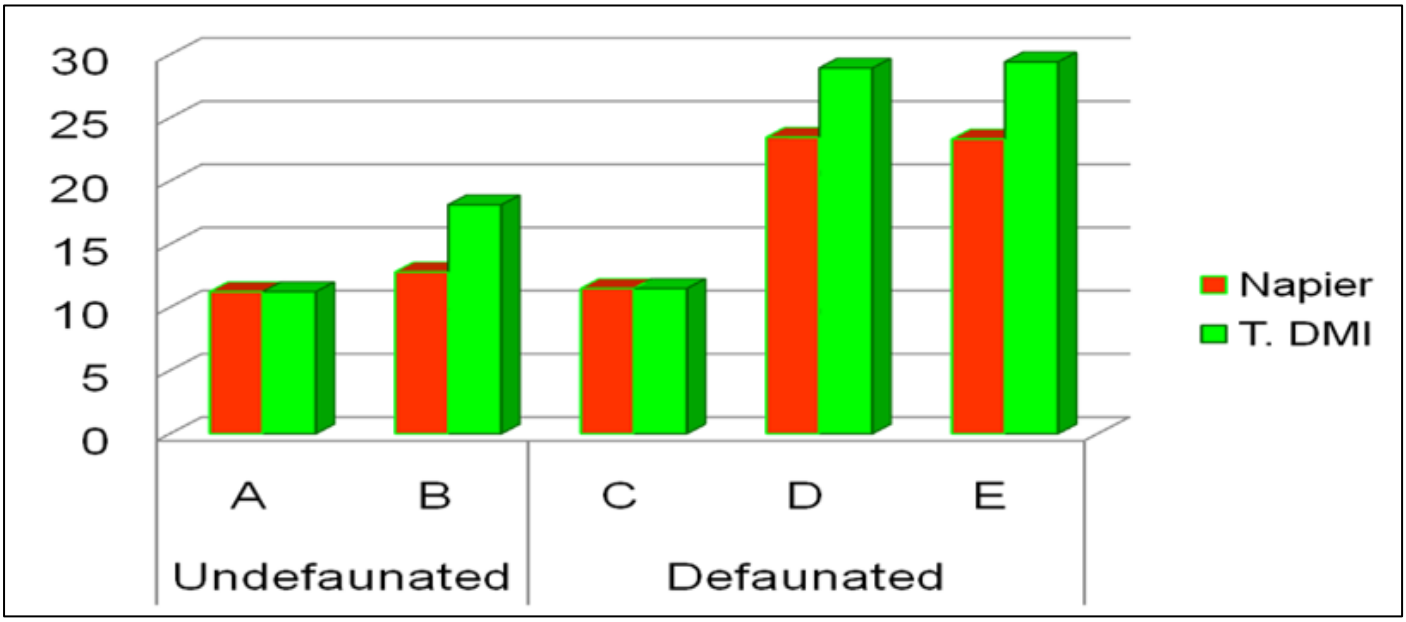

Fig 1 Cumulative DM Intake of Undefaunated and Defaunated Sheep Fed Napier Grass with or without Bypass Protein Supplement.

➢ *Cumulative Weight Gain*

The data presented in Table 2 demonstrate statistically significant differences in weight gain among the experimental treatments. Ovine subjects receiving liquefied soybean meal (LSBM) supplementation exhibited a statistically significant ($P > 0.0054$) increase in weight gain compared to those not receiving supplementation. Furthermore, ruminally defaunated animals demonstrated superior weight gain performance relative to their non-defaunated counterparts.

Table 2 Cumulative Weight Gain (kg) in Sheep Fed Napier Grass as Affected by Defaunation and Bypass Protein Supplementation

Treatment		2 Weeks	4 Weeks
Undefaunated			
Without supplement	A	0.65^{c}	0.20^{b}
With supplement	B	0.70^{c}	$0.55b^{c}$
Defaunated (with NTLE)			
Without supplement	C	1.05^{c}	1.05^{c}
With supplement	D	2.15^{a}	4.43^{a}
Defaunted (with SLS)			
With Supplement	E	2.35^{a}	4.55^{a}
p-value		0.007	0.004

Means with a Column of Similar Letter Superscript are Not Significantly Different.

The observed weight gain outcomes exhibited a positive correlation with dry matter intake, wherein animal performance was significantly influenced by both ruminal defaunation and bypass protein supplementation, as illustrated in Figure 18. The enhanced digestion of *Pennisetum purpureum* (Napier grass) by ruminal bacteria augmented the availability of both carbohydrate moieties and dietary nitrogen within the rumen, which, according to Singh

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et al. (2020), constitutes an essential substrate for the bacterial synthesis of microbial protein. Furthermore, the administration of liquefied soybean meal (LSBM) facilitated the circumvention of ruminal degradation, thereby providing an adequate nutrient supply within the small intestine (Thakur et al., 2024). Because LSBM supplementation (Treatments D and E) augmented intestinal nutrient supply and elevated amino acid concentrations, ruminally defaunated animals experienced a 95% increase in body tissue development. Conversely, a limiting supply of rumen-degradable nitrogen enhanced microbial protein synthesis, influencing a 76% increase in weight gain observed in defaunated animals receiving NTLE (Treatment C). The elimination of protozoa is associated with a more efficient overall digestive process (Zhang et al., 2022).

Fig 2 Cumulative Weight Gain of Undefaunated or Defaunated Sheep Fed Napier Grass with or without Bypass Protein Supplement.

➢ *Apparent Digestibility of Dry Matter*

Feed digestibility rigorously quantifies the fraction of ingested material that is presumed to be absorbed by an animal, based on the difference between intake and fecal excretion. Researchers typically express this as a percentage of dry matter, which they call the digestibility coefficient. The digestibility coefficients for the treatment diets, as presented in Table 3 and Figure 3, exhibited statistically significant differences ($p > 0.000$). The results indicated that defaunated animals achieved superior digestion compared to undefaunated animals. Furthermore, defaunated animals receiving liquefied soybean meal (bypass protein supplement) demonstrated statistically improved diet digestibility compared to those without bypass protein supplementation.

Table 3 The Apparent Digestibility of Dry Matter (%) of Undefaunated or Defaunated Sheep Fed Napier Grass with or without Bypass Protein Supplement.

Treatment		DM Intake			DM Excreted (Kg)	DM Digestibility (%)
		Napier	LSBM	Total		
Undefaunated						
Without supplement	A	3.28	0.00	3.28	1.66	49.45^{c}
With supplement	B	2.58	0.88	3.46	1.37	60.53b^{c}
Defaunated (with NTLE)						
Without supplement	C	3.07	0.00	3.07	0.92	69.97^{b}
With supplement	D	4.82	1.01	5.82	0.45	94.26^{a}
Defaunted (with SLS)						

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With Supplement	E	4.95	0.95	5.90	0.50	91.94[a]
p-value						0.000

Means with a Column of Similar Letter Superscript are Not Significantly Different.

Ruminally defaunated ovine subjects receiving bypass protein supplementation (Treatments D and E) exhibited the highest dry matter (DM) digestibility coefficients, a result that was statistically significantly different (P > 0.000) compared to those observed in Treatments A, B, and C. This finding suggests that the efficiency of ruminal bacteria in digesting both fiber and other feedstuffs is significantly enhanced in the absence of ruminal protozoa (Zhang et al., 2022). Consequently, bypass protein supplementation remains a judicious nutritional strategy for ruminally defaunated animals when elevated production levels warrant the incremental cost associated with the supplement.

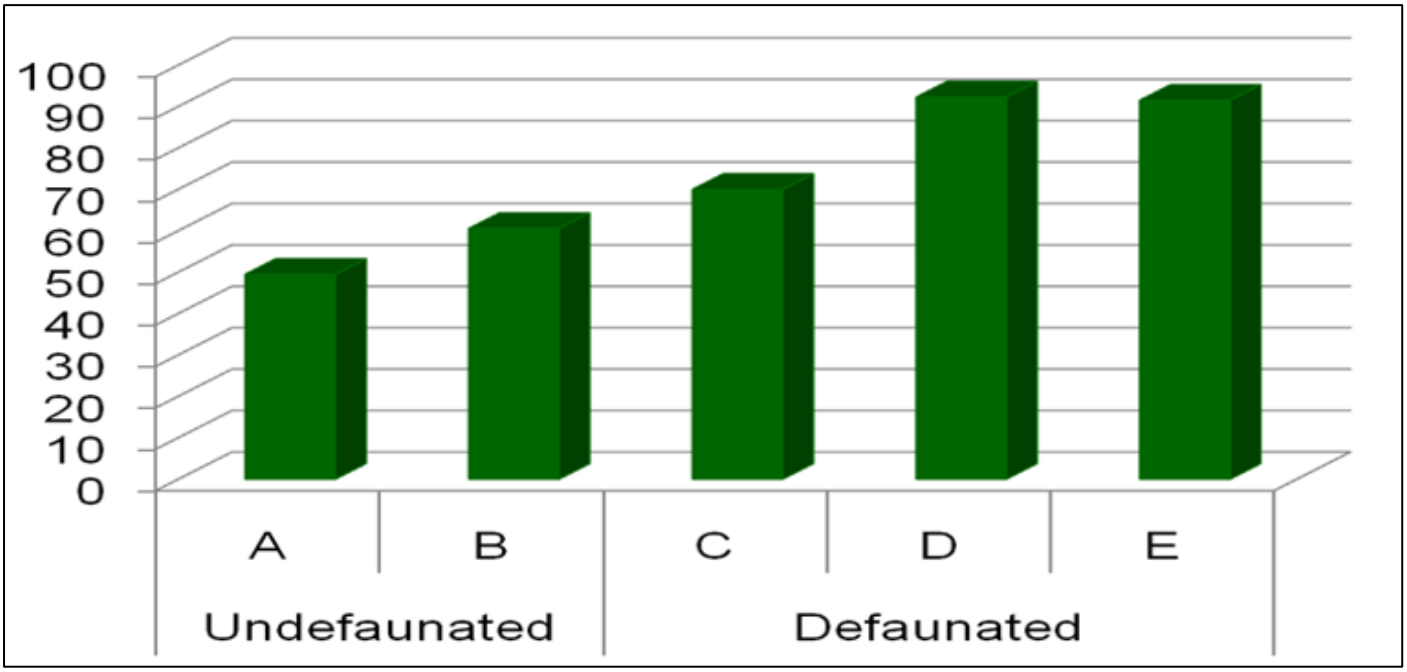

Fig 3 Apparent Digestibility (%) of Undefaunated and Defaunated Sheep Fed Napier Grass with or without Bypass Protein Supplement.

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