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Nitrogen Retention Value in Local Sheep Fed with Dwarf Elephant Grass (*Pennisetum purpureum cv. Mott*) and Indigofera (*Indigofera zollingeriana*)

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ABSTRACT. This study aimed to determine the nitrogen retention in local sheep fed dwarf elephant grass (*Pennisetum purpureum cv. Mott*) and indigofera (*Indigofera zollingeriana*). A completely randomized design (CRD) was used, consisting of four treatments with four replications each. The dietary treatments included: T= 100% dwarf elephant grass, T= 95% dwarf elephant grass + 5% indigofera, T2= 90% dwarf elephant grass + 10% indigofera, and T= 85% dwarf elephant grass + 15% indigofera. The parameters measured were dry matter intake (DMI), dry matter digestibility (DMD), nitrogen (N) intake, N retention, and average daily gain (ADG). The results showed that DMI, DMD, N intake, N retention, and ADG were significantly ($P<0.05$) influenced by the dietary treatments. Based on these findings, the combination of 95% dwarf elephant grass and 5% indigofera was the most effective in improving DMI, DMD, N intake, N retention, and ADG in local sheep.

Keywords: Average daily gain, dwarf elephant grass, indigofera, nitrogen retention, sheep

INTRODUCTION

Sheep are one of the most commonly raised small ruminants among farmers in Indonesia. They possess several advantages over other ruminants, including adaptability, rapid breeding, high prolificacy (ability to give birth to multiple offspring), and seasonal polyestrous reproductive cycles (Hasan and Sitepu, 2017). One of the most prevalent local sheep breeds is the Thin-Tailed Sheep (DET), which is known for its tolerance to a wide range of feed types and its ability to adapt well to suboptimal environmental conditions (Najmuddin and Nasich, 2019). However, a major challenge faced by local farmers is the high cost of feed, leading them to rely on low-nutrient forage without supplementing with concentrates. This practice can negatively impact livestock productivity due to inadequate nutrient availability. Therefore, this study aims to provide an alternative feeding strategy that enhances livestock productivity by offering cost-effective, high-quality forage.

One of the commonly used forages for sheep feed is dwarf elephant grass (*Pennisetum purpureum cv. Mott*), which has a higher leaf-to-stem ratio than other forages, making it a favorable feed option (Sirait, 2018). Additionally, dwarf elephant grass is easy to cultivate and widely available. Besides grasses, another potential forage source for sheep is indigofera (*Indigofera zollingeriana*), a leguminous plant with a crude protein content of 23.01% (Setiyaningrum et al., 2018). Indigofera is highly tolerant to dry conditions, making it adaptable to various land typologies (Tresia and Saenab, 2020). However, its use in animal feed requires careful consideration due to the presence of antinutritional factors, such as tannins, which can form protein-tannin complexes that reduce feed digestibility (Wijaya et al., 2016).

Combining indigofera with dwarf elephant grass is expected to enhance the crude protein content of the diet. However, excessive inclusion of indigofera may negatively impact protein degradation by rumen microbes due to its tannin content, which can reach up to 7% (Antari et al., 2022). An optimal combination of these forages is essential to meet the target dietary protein requirement of 12%, which is the minimum protein level needed for sheep fattening. This protein level can be achieved with a diet comprising 90% dwarf elephant grass and 10% indigofera.

MATERIALS AND METHODS

Research Time and Location

This study was conducted from August to October 2021 at the Digestion Cage, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang. The analysis of dry matter, ash, crude fiber, and crude fat was carried out in August 2021 at the Nutrition and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University. Nitrogen analysis was conducted in October 2021 at the Laboratory of Beef and Dairy Livestock Production, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang.

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Research Materials

The experimental animals used in this study were 16 Thin-Tailed Sheep (DET), aged approximately six months, with an average body weight of 16.03±1.54 kg (coefficient of variation= 9.61%). The sheep were housed individually in stage cages measuring 1.50×1.25 meters, with a total of 16 cages. Each cage was equipped with a feed and water bucket, a feces container made of paranet, and a urine collection container made of plastic. The tools used for animal maintenance included a chopper machine, shovels, a hanging scale with a maximum capacity of 50 kg and an accuracy of 10 g, plastic bags, broomsticks, buckets, hoses, label paper, stationery, and a smartphone camera. The feed materials used in the study consisted of dwarf elephant grass (*Pennisetum purpureum cv. Mott*) and indigofera (*Indigofera zollingeriana*), with their respective nutrient compositions presented in Table 1. Other materials used included urine and fecal samples, 0.1 N H_2SO_4, $CuSO_4$ and K_2SO_4 catalysts, concentrated H_2SO_4, distilled water, 45% NaOH, 4% H_3BO_3, and indicators such as Methyl Red (MR) and Bromocresol Green (BCG). For nitrogen content analysis, the study utilized a grinder, sieve, analytical balance, and a complete set of Kjeldahl method apparatus.

Table 1. Nutrient content of Dwarf elephant grass and Indigofera

Feed Ingredients	DM [a)]	CP [b)]	CF [a)]	CF [a)]	Ash [a)]	NNFE [c)]	TDN [c)]
	--- % ---	---------- 100% DM ----------					
Dwarf elephant grass	31.70	11.12	30.18	4.11	13.04	41.56	58.60
Indigofera	24.28	23.08	25.06	1.70	18.20	31.97	57.23

Note: [a)]Nutrition and Feed Science Laboratory Analysis Results, 2021; [b)]Laboratory Analysis Results of Beef and Dairy Livestock Production, 2021; [c)]Calculation Results Based on Hartadi *et al.* (1980); DM= dry matter, CP= crude protein, CF= crude fiber, EE, extract ether, NNFE= non nitrogen free extract, TDN= total digestible nutrients

Research Design

This study employed a completely randomized design (CRD) with four treatments and four replicates. The feed treatments applied were as follows, and the nutrient composition of the treatment feed is presented in Table 2.

T0 = 100% Dwarf elephant grass
T1 = 95% Dwarf elephant grass + 5% indigofera
T2 = 90% Dwarf elephant grass + 10% indigofera
T3 = 85% Dwarf elephant grass + 15% indigofera

Table 2. Nutrient content of feed treatment

Nutrient components	T0	T1	T2	T3
	-------- % --------			
DM	31.70	31.33	30.96	30.59
	-------- 100% DM --------			
CP	11.12	11.71	12.31	12.91
CF	30.18	29.93	29.67	29.41
EE	4.11	3.99	3.87	3.75
Ash	13.04	13.29	13.55	13.81
NNFE	41.56	41.08	40.60	40.12
TDN	58.60	58.53	58.46	58.39
Tannins[*)]	0.31	0.36	0.40	0.48

Note: Calculation results based on Table 1, *) Laboratory analysis results

Research Method

This study consisted of five stages: preparation, preliminary, adaptation, treatment, and data collection. The preparation stage involved assembling the necessary tools and materials for the study. The preliminary stage lasted for 20 days, during which the animals were fed the control diet (T0) ad libitum to determine their daily feed consumption. This data was used as a reference for feed allocation during the adaptation stage. The adaptation stage lasted for 10 days, during which the animals were gradually introduced to the treatment diets to familiarize them with the new feed. The treatment stage was conducted for 14 days, during which the animals received the assigned treatment diets according to predetermined levels. Feeding was carried out three times daily at 07:30, 13:30, and 19:30 WIB. The data collection stage was conducted over 10 days using the total collection method. Daily feed intake was recorded, and urine and feces were collected for measurement and weighing. To prevent nitrogen volatilization, 10% H_2SO_4 was sprayed on the feces and urine. Fecal samples were collected at 10% of the total dry matter (DM) of feces, while urine samples were taken at 5% of the total urine volume. These samples were then pooled by replicate within each treatment group and analyzed for nitrogen content using the Kjeldahl method. The parameters observed included dry matter intake (DMI), dry matter digestibility (DMD), nitrogen (N) intake, nitrogen retention, and average daily gain (ADG).

$$\text{DMI} = [\text{Intake(g)} \times \%\ \text{DM Intake}] - [\text{Remaining (g)} \times \%\ \text{DM remaining}]$$

$$\text{DMD}\ (\%) = \frac{\text{Consumed DM - Fecal DM}}{\text{DM consumed}} \text{x}\ 100\%$$

Fecal N and urinary N were calculated using the formula:

Fecal N (g) = Fecal DM × % Fecal N

Urine N (g) = Urine volume (ml) × % Urine N

The formula for calculating nitrogen retention is as follows:

N retention (g) = N consumption (g) - (Fecal N (g) + Urine N (g) (McDonald et al., 2002)

$$\text{ADG (kg/head/day)} = \frac{\text{Final weigth (kg) - Initial weight (kg)}}{\text{Length of maintenance (day")}}$$

Data Analysis

The data on dry matter intake (DMI), dry matter digestibility (DMD), nitrogen (N) intake, nitrogen retention, and body weight gain were analyzed using analysis of variance (ANOVA). If a significant treatment effect was detected, Duncan's multiple range test was conducted to determine differences between treatment means. Data analysis was performed using SPSS Version 24.

RESULTS AND DISCUSSION

The data on average dry matter intake (DMI), dry matter digestibility (DMD), nitrogen (N) intake, nitrogen retention, and average daily gain (ADG) in local sheep fed with dwarf elephant grass and Indigofera are presented in Table 3.

Table 3. Average DMI, DMD, N consumption, N retained and ADG

Parameters	Treatment			
	T0	T1	T2	T3
DMI (g/head/day)	$632.88^{b}\pm46.41$	$775.81^{a}\pm50.10$	$639.32^{b}\pm79.28$	$633.31^{b}\pm50.73$
DMD (%)	$78.68^{a}\pm1.76$	$76.15^{ab}\pm2.08$	$74.17^{b}\pm4.09$	$72.04^{b}\pm1.68$
N consumption (g/head/day)	$11.26^{b}\pm0.83$	$14.54^{a}\pm0.94$	$12.59^{b}\pm1.56$	$13.07^{ab}\pm1.05$
N retained (g/head/day)	$5.91^{b}\pm0.84$	$8.30^{a}\pm0.79$	$6.98^{ab}\pm1.37$	$6.85^{ab}\pm1.02$
ADG (g/head/day)	$33.75^{c}\pm1.04$	$50.31^{a}\pm4.45$	$42.64^{b}\pm4.57$	$38.23^{bc}\pm3.42$

Note: Different superscripts in the same row indicate significant differences (P<0.05)

Dry Matter Intake (DMI)

The results of the statistical analysis showed that dry matter intake (DMI) was significantly (P<0.05) influenced by the combined feeding treatment of dwarf elephant grass and Indigofera. The observed DMI values were higher than the average DMI of local sheep reported by Muktiani et al. (2013), who stated that sheep fed grass, concentrate, and silage forage waste had an average DMI ranging from 514 to 627 g/head/day. The high DMI in this study was attributed to the nutrient availability in the treatment feeds and the fiber requirement as an energy source. This finding aligns with Sutardi (1981), as cited by Purwanto et al. (2014), who stated that feeds with high fiber content tend to reduce consumption, whereas feeds with lower fiber content promote higher DMI, as livestock attempt to increase feed intake to meet their energy requirements.

DMI in T1 was significantly different (P<0.05) from T0, T2, and T3, indicating that the inclusion of Indigofera at a 5% level effectively increased DM consumption. This increase was likely due to the lower crude fiber (CF) content in T1 compared to T0, and the tannin content of 0.31% in Indigofera at this level was not sufficient to negatively affect DM digestibility. Aluko (2012) stated that tannins are polyphenolic compounds that can inhibit the digestive system of livestock. The DMI values in T2 and T3, which were not significantly different (P>0.05) from T0, suggest that the tannin content in Indigofera at the 10% (0.40%) and 15% (0.48%) levels affected rumen microbial activity, leading to a reduction in DMI despite the lower CF content in these treatments compared to T1. This finding is consistent with Yudianto et al. (2021), who reported that rations supplemented with 0.50% tannin protection reduced DM intake.

The lower DMI in T2 and T3 was also likely due to the palatability of Indigofera. The tannin content of 0.70% in Indigofera at the 10% level and 1.05% at the 15% level may have negatively influenced feed consumption due to the bitter and astringent properties of tannins, which create an undesirable taste for livestock. This aligns with Suryaningsih (2019), who noted that tannins impart a bitter taste, affecting feed intake. The bitterness becomes apparent during mastication, as described by Jayanegara et al. (2009), who explained that the mastication process in ruminants releases tannins, which then bind to salivary proteins, reducing palatability and ultimately decreasing feed consumption.

Dry Matter Digestibility (DMD)

The results of the statistical analysis showed that dry matter digestibility (DMD) was significantly (P<0.05) influenced by the combined feeding treatment of dwarf elephant grass and

Indigofera. The DMD values obtained in this study were relatively high compared to the average DMD of dwarf elephant grass reported by Sirait (2018), which was 63.98%. The results also indicated that the DMD in the T0 treatment was not significantly different (P>0.05) from T1, but it was significantly different (P<0.05) from T2 and T3. DM digestibility is closely related to the nutrient composition of the treatment feed. A decrease in crude fiber (CF) content alongside an increasing proportion of Indigofera would typically be expected to increase DMD, as feeds with lower CF content generally have higher digestibility.

However, contrary to expectations, the highest DMD was observed in the T0 treatment, while the lowest was in T3. The decrease in DMD, despite the lower CF content, is likely attributed to the presence of tannins, which negatively impact digestibility. This finding aligns with Prasetyo et al. (2019), who stated that lower CF content generally leads to higher digestibility. However, Hidayat, Tanuwiria, and Hidayat (2019) highlighted those tannins can reduce feed digestibility. This is evident in T3, which had the lowest CF but the highest tannin content, resulting in the lowest DMD. Conversely, T0, with the highest CF and the lowest tannin content, had the highest DMD value. These results suggest that increasing levels of Indigofera in the feed led to a decline in digestibility due to the inhibitory effect of tannins on rumen microbial activity. This is consistent with Rahman et al. (2020), who noted that tannins can reduce the degradative ability of rumen microbes by forming complexes that interfere with digestion, ultimately decreasing digestibility values.

Nitrogen Intake (N)

The results of the statistical analysis showed that nitrogen (N) consumption was significantly (P<0.05) influenced by the combined feeding treatment of dwarf elephant grass and Indigofera. The average N consumption in this study ranged from 11.26 to 14.54 g/head/day, which falls within the optimal category, as the N requirements of the sheep were met. The crude protein (CP) requirement for sheep with an average body weight of 16.03 kg and an average daily gain (ADG) of 23.75 g/day, based on the Kearl formula (1982), is 43.74 g/day, meaning the minimum N requirement is 7 g/day. These results are consistent with the findings of Yulistiani et al. (2011), who reported that sheep fed with elephant grass and concentrate had an average N consumption of 13 g/head/day. The N consumption in T1 was not significantly different (P>0.05) from T2 and T3, but it was significantly different from T0. This indicates that the inclusion of Indigofera at all three treatment levels effectively increased N consumption, as Indigofera contributes a substantial amount of N to the feed composition.

N consumption is influenced by dry matter intake (DMI) and the N content percentage in the feed, as N is a fundamental component of the protein content in organic matter (OM) within DM. This aligns with the findings of Puastuti et al. (2017), who stated that N consumption is strongly correlated with DMI. Although the percentage of N in the T1 treatment feed was lower than in T2 and T3, the higher DMI in T1 led to an increased N intake. As shown in Table 3, T1, which had the highest DMI (775.81 g/head/day), also exhibited the highest N consumption (14.54 g/head/day). This was followed by T3 and T2, while the lowest values were observed in T0, which had a DMI of 632.88 g/head/day and an N consumption of 11.26 g/head/day. These findings are consistent with Albuquerque et al. (2020), who stated that an increase in total N consumption is directly proportional to the amount of DM consumed.

Nitrogen Retention

The results of the statistical analysis showed that nitrogen (N) retention was significantly (P<0.05) influenced by the combined feeding treatment of dwarf elephant grass and Indigofera. The values obtained in this study did not differ substantially from the average N retention in local sheep. Hanun et al. (2018) reported that sheep fed with elephant grass and concentrate, containing a crude protein content of around 12.97%, had an average N retention value of 8.82 g/head/day. As the level of Indigofera feeding increased, the crude fiber (CF) content also increased. However, the N retention value showed a decrease, likely due to the inability of post-rumen protease enzymes to hydrolyze proteins, due to the presence of free tannins in the digestive tract. As shown in Table 3, T1 had the highest N retention value, compared to T2 and T3. This is consistent with the findings of Simanihuruk et al. (2006), who observed a decrease in nitrogen retention as the level of passion fruit peel (containing tannins) in the diet of goats increased.

The results also indicated that the N retention in T2 and T3 did not significantly differ (P>0.05) from T0. This can likely be attributed to the presence of 0.70% tannins in 10% Indigofera and 1.05% tannins in 15% Indigofera, which inhibited protein digestion. Hidayah (2016) stated that tannin compounds form complex bonds with proteins,

linking the phenol groups from tannins with the carboxyl groups from proteins. These strong bonds reduce the degradation ability of rumen microorganisms in digesting proteins. In the rumen, with a pH between 4-7, condensed tannins can form stable complexes with proteins. Mayangsari et al. (2013) also noted that this pH range in the rumen allows for stable tannin-protein binding. Proteins that remain undigested in the rumen will pass to the abomasum, where the bond between tannin and protein is released due to the low pH. Jayanegara and Sofyan (2008) explained that the protein-tannin complex can be broken down in the abomasum, allowing protein to be digested in the post-rumen. However, this process can be hindered by free tannins in the intestine, which may bind to post-rumen protease enzymes and decrease their ability to hydrolyze feed. Tanuwiria and Hidayat (2019) suggested that free tannins can form bonds with protease enzymes, thus reducing enzyme efficiency and impacting digestion.

Moreover, the increasing level of Indigofera feeding resulted in a decreased DM digestibility (DMD), which in turn led to a decrease in N retention. Table 3 illustrates that T1, which had the highest DMD, also had the highest N retention, followed by T2, T3, and T0. Notably, T0 had the highest DMD, but its N retention was the lowest. This is likely due to the relatively lower crude protein content in T0 compared to the other treatments, despite the absence of tannins.

Average Daily Gain (ADG)

The results of statistical analysis showed that average daily gain (ADG) was significantly ($P<0.05$) influenced by feeding a combination of dwarf elephant grass and Indigofera. The average ADG value obtained in this study is considered to be in the low category when compared to the average ADG of local sheep. Muktiani et al. (2013) stated that local sheep with a DM intake of 541 g/day were able to achieve an ADG of 96 g/day. In contrast, the results of this study did not differ substantially from those of Metkono et al. (2011), where local sheep fed with field grass and tofu pulp, with an average DM intake of 583-774 g/head/day, showed ADG values ranging from 36-73 g/head/day.

The ADG value of local sheep in the T1 treatment was significantly higher ($P<0.05$) than those in T0, T2, and T3 treatments, indicating that the inclusion of Indigofera at the 5% level was effective in increasing ADG. The percentage of nitrogen (N) content in feed treatments T2 and T3 was higher than in T1. However, the T1 treatment demonstrated higher N consumption, likely due to its higher DM intake compared to the other treatments. High DM intake leads to greater N consumption, as nitrogen is a key component of protein, which is present in DM. Higher N consumption results in increased protein retention, which is crucial for tissue growth. Li et al. (2019) stated that N retention represents the portion of absorbed nitrogen used in new tissue formation. High N retention is associated with increased ADG, as it reflects greater protein digestibility and deposition, which in turn promotes the formation of new tissue. This is consistent with Nggajung et al. (2021), who argued that high N retention values indicate high protein deposition, resulting in the formation of more new tissue. As shown in Table 4, T1, with the highest DM intake, also exhibited the highest N consumption, N retention, and ADG, while T0, with the lowest DM intake, had the lowest values for N consumption, N retention, and ADG.

The feed conversion ratio obtained in this study ranged from 12.91 - 19.97, which is higher than the average feed conversion for sheep. Supratman et al. (2016) stated that the feed conversion value for sheep typically ranges from 7.63 - 9.72. Feed efficiency in this study ranged from 5.01 - 7.74%, placing it in the very low category, as it is below the standard value for feed efficiency. Wijaya et al. (2018) indicated that the feed efficiency value for sheep typically ranges from 7.62%-14.16%.

CONCLUSION

Feeding a combination of 95% dwarf elephant grass and 5% Indigofera can serve as an effective forage feed that enhances dry matter intake, nitrogen consumption, nitrogen retention, and daily body weight gain.

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