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Research Article

Effect of *Leucaena leucocephala* Protein Supplement on Nutrient Intake, Milk Yield and Quality, Hematology, Metabolites and Economy Efficiency in Etawah Crossbreed Goats

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Abstract: Legumes, such as *Leucaena leucocephala* (LL), have high protein and Rumen Degradable Protein (RUP) content, so adding legumes to the diet of dairy goats can meet the protein requirements for milk synthesis. However, the addition of LL alone is insufficient to meet the RUP requirement of dairy goats, thus requiring other protein sources. LL Protein Supplement (LLPS) is a feed ingredient containing high-quality RUP formulated with LL as the base ingredient, complemented by high-quality protein sources such as roasted soybeans, autoclaved black cumin meal, CGM, and SBM. This study investigated the effect of LLPS on dairy goat performance and productivity. This study used sixteen lactating Etawah crossbreed goats with an average milk production of 0.72 L/head/day and 49.69±7.85 kg body weight. A randomized block design consisting of four treatments and four replications was utilized. The experimental diet consisted of the farmer's ration (R0) and R0 supplemented with 5% LLPS (R1), 10% LLPS (R2), and 15% LLPS (R3). The data were statistically analyzed using ANOVA, followed by Duncan's multiple range test to conduct pairwise comparisons between the treatments. The results revealed that the LLPS significantly improves nutrient consumption, milk yield, milk component yield, milk urea, and erythrocytes counts. Additionally, LPPS supplementation demonstrated economic benefits while maintaining the overall milk quality and health status. In summary, incorporating LLPS into dairy goat diets can enhance palatability and positively impact their productivity without compromising the health status of the animals, as indicated by average hematological and blood metabolite values.

Keywords: Dairy Goat, Etawah Crossbreed, Heat Treatment, *Leucaena leucocephala*, Rumen Undegradable Protein

Introduction

Milk is a highly nutritious animal-derived food source with a pleasant taste profile, enjoyed by people of all ages. It provides excellent nutritional value, promotes health, and is easily digestible by the human digestive system. Goats are small ruminants with significant potential for development in Indonesia as a source of animal products, including meat and milk. Goat's milk offers many benefits, including being lactose intolerant-free, rich in calcium, more accessible to digest than cow's milk, containing prebiotics that support digestive health, containing selenium that boost the immune system, and containing monounsaturated and polyunsaturated fatty acids, as well as medium-chain triglycerides, which have the potential to prevent cardiovascular diseases (Anam et al., 2022).

Dairy animals have a rumen that contains various bacteria or microorganisms that degrade feed nutrients, making them available for use (Kurniawan et al., 2019). In ruminant nutrition, protein components are classified based on their ruminal degradation characteristics; specifically, the distinction is made between Rumen Undegradable Protein (RUP) and Rumen Degradable Protein (RDP). RDP is metabolized into ammonia, which rumen microbes utilize to produce microbial protein (Dunlap et al., 2000). According to the NRC (2001) guidelines, the ideal dietary protein for ruminants should maintain a 60:40 balance between RDP and RUP. However, excessive RDP can lead to increased urinary nitrogen excretion, indicating inefficient nitrogen use (McGuire et al., 2013). On the other hand, diets with higher RUP can promote livestock growth by improving amino acid availability in the small intestine, thereby supporting more effective protein metabolism (Rastgoo et al., 2020).

High-producing livestock require higher levels RUP as they need an adequate supply of amino acids for direct use in their metabolic processes. Additionally, increasing the percentage of RUP in the feed can reduce methane emissions from compounds released during the fermentation of amino acids in the rumen. Enhancing RUP involves protecting feed protein from rumen fermentation to improve amino acid efficiency and reduce nitrogen waste (Rigon et al., 2022). Current nutritional formulations for dairy cattle in Indonesia demonstrates a limited approach to nutrient requirements, predominantly focusing on meeting the necessary level of Crude Protein (CP), Total Gigestible Nutrients (TDN), and the mineralization profiles of calcium and phosphorus (Rosendo et al., 2013). The balance of RDP and RUP content in the ration must be carefully considered to ensure optimal dairy cattle productivity. Based on this, creating a product as a high RUP protein supplement can be a practical solution to meet the daily RDP and RUP requirements for animals.

The nutritive value of dairy goat ration can be improved through with concentrates or legumes. Due to the high cost of concentrates, legume supplementation may be more promising. Legumes can grow anywhere, are not affected by seasons, have high protein content ranging from 19% to 26%, and can serve as organic minerals like nitrogen, phosphorus, and potassium sources (Phelan et al., 2015). Using leguminous plants as an alternative protein source can help reduce production costs because they are easier and cheaper to cultivate than animal products or grains. Therefore, leguminous plants have great potential for use as part of ruminant concentrate feed (Ernawati et al., 2021).

Leucaena leucocephala (LL), commonly known as Lamtoro in Indonesia, is a leguminous tree species with high potential and widely used as forage for livestock in tropical regions. LL is a promising protein supplement for ruminants, characterized by its sustainable availability throughout both the rainy and dry seasons (De Angelis et al., 2021). According to the analysis conducted by the IPB Dairy Nutrition Laboratory, LL contains 28.37% Crude Protein (CP), 52.88% RDP, and 47.12% RUP. Previous research findings have reported that incorporating 25% LL into dairy rations can enhance palatability and increase milk production by 15% (Barwani et al., 2023; Leketa et al., 2019a; Susanti et al., 2022). Despite its ability to enhance palatability, the use of LL in the ratio is limited due to the presence of antinutritional compounds, such as mimosine.

In addition to using LL, LL Protein Supplement (LLPS) is formulated using feed ingredients that contain high RUP, such as autoclaved black cumin meal, roasted soybeans, Soybean Meal (SBM), and Corn Gluten Meal (CGM) (Rosmalia et al., 2021). The heating process of feed ingredients represents a nutritional intervention strategy designed to mitigate protein degradation within the rumen, consequently optimizing nutrient delivery to the duodenum (Panah et al., 2020). The heat treatment method is considered superior and does not cause excessive protection because it can still be digested by post-rumen enzymes compared to chemical methods using formaldehyde (Rosmalia et al., 2023). Based on the study conducted by Haryanto (2014), using heat-treated soybeans and CGM in dairy cattle diets potentially increases milk production and improves milk's compositional quality by elevating fat, protein, and casein concentrations. This study aims to formulate a protein supplement with a minimum content of CP and RUP of 40% and 50%, using leguminous base ingredients and protected protein feed sources. Additionally, the study aims to evaluate its effects on nutrient consumption, milk production, milk quality, blood profile, and the economic aspects of dairy goats.

Materials and Methods

Experimental Animals

The experimental study was carried out at Kampung 99 Pepohonan Farm, Depok, Indonesia, from June to July 2023. Institutional ethical clearance for animal research was obtained from the Standard Ethical Committee of IPB University, under protocol authorization (license No: 105/KEH/SKE/VIII/2023) governing animal use and welfare standards. In accordance with the methodology outlined by Rosmalia et al. (2022), sixteen multiparous (in their first to third lactations) Etawah crossbred goats with an average body weight of 49.69±7.85 kg were randomly allocated into four experimental groups based on milk production. Each group consisted of four goats and was arranged in a randomized complete block design, with blocks determined by differences in milk yields among lactating goats. The animals were maintained under intensive management protocols and housed in individual stalls. The experiment lasted a total of 49 days, including a 14-day preliminary phase dedicated to dietary adaptation and acclimatization, followed by a 35-day data collection and experimental intervention phase.

The study employed four dietary treatments: a standard farmer's ration serving as the control (R0), R0 + 5% LLPS (R1), R0 + 10% LLPS (R2), and R0 + 15% LLPS (R3). These diets were provided twice a day, at 8.00 am and 3.00 pm, prior to the milking procedures, with unlimited access to drinking water. The experimental diet formulation and nutrient composition were analytically characterized using standardized AOAC methods (AOAC, 2005), as presented in Table 1.

Environmental temperature and relative humidity values surrounding the barn were continuously monitored using a calibrated digital thermo-hygrometer (HTC-1, OneMed) during morning, afternoon, and evening. The Temperature-Humidity Index (THI) value was calculated based on the equation proposed by Mader et al. (2006), enabling precise quantification of thermal stress potential within the experimental microenvironment.

Table 1: Composition and nutritional content of experimental diet

Items	R0	R1	R2	R3
Feed ingredients (% DM)				
King grass	43.00	43.00	43.00	43.00
Tofu waste	44.00	40.14	36.28	32.42
Pollard	11.58	10.43	9.29	8.15
Protein supplement	0.00	5.00	10.00	15.00
$CaCO_3$	0.57	0.57	0.57	0.57
DCP	0.86	0.86	0.86	0.86
Nutrient content of the ration[1]				
Dry matter (%)	15.33	17.47	17.74	19.11
Ash (% DM)	9.37	9.60	9.19	10.40
Ether extract (% DM)	5.98	4.15	4.27	4.10
Crude protein (% DM)	12.68	13.69	15.29	16.41
RDP (% CP)	64.46	62.16	61.97	58.51
RUP (% CP)	35.54	37.84	38.03	41.49
Crude fibre (% DM)	24.17	22.68	22.55	21.81
NFE (% DM)	47.80	49.87	48.70	47.28
TDN[2] (% DM)	65.90	65.64	66.17	66.72

[1]Laboratory test results from the IPB Biotech Center (2023); [2]TDN calculated using the Sutardi formula (Indah et al., 2020) ; R0 = farmer's ration (control); R1 = R0 + 5% LLPS; R2 = R0 + 10% LLPS; R3 = R0 + 15% LLPS; DCP = dicalcium phosphate; DM = dry matter; CP = crude protein; RDP = rumen degradable protein; RUP = rumen undegradable protein; NFE = nitrogen-free extract; TDN = total digestible nutrient

Production of Protein Supplements

The *Leucaena leucocephala*-based Protein Supplement (LLPS) used in this research was formulated using LL harvested from the area surrounding the IPB Dramaga campus. LL was sun-dried for five days and ground before being used in the supplement mixture. Other protein feed ingredients such as Corn Gluten Meal (CGM), Soybean Meal (SBM), soybeans, and black cumin meal (*Nigella sativa*) were obtained from feed stores in the Bogor Regency. The formulation of the research supplement contained 40% CP and 52% RUP. Soybeans were subjected to a protection treatment using a roasting method with an electric coffee roaster (CAFEMASY, made in China) for 20 minutes and 200°C temperature (Rosmalia et al., 2022), while black cumin meal underwent moist heating using an autoclave at 120°C for 60 minutes (Rosmalia et al., 2023).

Data Collection

The feed offered, and refusals were collected and measured every morning during the experimental period to assess daily nutrition intake. The chemical composition of the diet was analyzed using proximate methods, including Dry Matter (DM), ash, Crude Protein (CP), Ether Extract (EE), and Crude Fibre (CF). Analysis of milk production and composition was performed following the same methods reported by Rosmalia et al. (2022). Milk samples were collected by hand milking twice a day after feeding. The milk production was measured using a measuring cup with a capacity of 1 L and then analyzed for milk composition (fat, protein, lactose, and solid non-fat content) using Milkotester Ltd. (Milk Analyzer Master Pro). At the end of the experimental period, milk samples were collected for analysis of Milk Urea Nitrogen (MUN) using a spectrophotometer in the laboratory.

On the 28^{th} day of the experimental period, blood was drawn from the jugular vein using a 3 mL syringe and collected into vacutainer tubes with Ethylenediamine tetra-acetic acid (EDTA) and kept on ice. The blood was taken four hours after the morning feeding to assess haematological (red blood cells, white blood cells, hematocrit, haemoglobin) and metabolite (glucose, triglyceride, and blood urea nitrogen). Determination of haematological parameters in whole blood of Etawah crossbreed blood was carried out according to the method of Sastradipradja et al. (1989), while the blood metabolites were analyzed using Microlab 300 based on enzymatic reactions by the KIT method and measured using a spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific). The glucose and blood triglyceride levels were measured at 500 nm, while Blood Urea Nitrogen (BUN) was measured at 578 nm (Retnani et al., 2019). Economic aspects can be calculated using the values of economic efficiency, feed efficiency, cost of feed per litre of milk, and income over feed cost (IOFC) (Linn, 2006).

Statistical Analysis

Nutrient intake, milk production, milk composition, blood haematological, and metabolite data were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS software for Windows version 25. The statistical model comprised four treatment diets as fixed effects and four different sources of rumen inoculum as random effects, defined as follows:

$$\mathrm{Y}_{ij} = \mu + \tau_i + \alpha_j + \varepsilon_{ij}$$

Where Y_{ij} represented the dependent variable (nutrient intake, milk production, milk composition, blood haematology, or metabolites), μ was the overall mean, τ_i is the fixed effect of treatment diets (i=1–4), α_j is the random effect of group based on milk production j (j=1–4), and ε_{ij} is the random residual error. Significant differences were identified using Duncan's multiple-range test at a significance level of $p<0.05$.

Results

Figure 1 presents the THI in the surrounding environment of the barn. As shown in Figure 1, the highest THI occured during the daytime, while the lowest THI occurs in the morning.

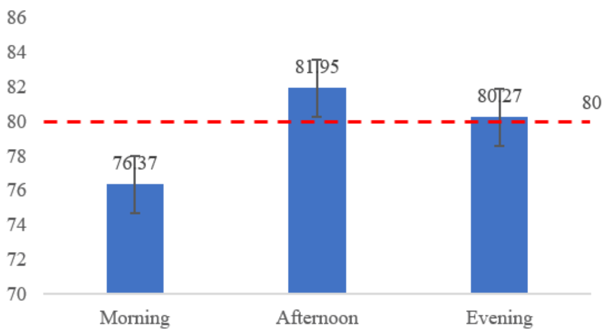

Fig. 1: Temperature humidity index around experiment barn

Table 2 shows the daily nutrient intake of Etawah crossbred goats. The higher (p<0,05) dry matter intake (DMI), organic matter intake (OMI), crude protein intake (CPI), crude fibre intake (CFI), nitrogen-free extract intake (NFEI), and true digestible nutrient intake (TDNI) were observed in goats receiving the LLPS treatments, while the EE intake was not significantly affected.. The R1 and R2 groups exhibited the highest DMI, OMI, and NFEI, while CPI and TDNI of R1, R2, and R3 were higher than R0.

Table 2: Effect of LLPS on daily feed intake

Parameters (g/day)	R0	R1	R2	R3	SEM	p-value
Dry matter	706.10^{b}	1162.59^{a}	1138.22^{a}	992.83^{ab}	70.28	0.024
Organic matter	652.00^{b}	1070.37^{a}	1055.62^{a}	903.08^{ab}	64.63	0.023
Crude protein	104.09^{b}	190.10^{a}	214.49^{a}	205.58^{a}	14.59	0.006
Ether extract	55.27	60.06	61.25	51.15	2.85	0.493
Crude fiber	147.09	214.52	206.38	167.38	12.49	0.055
NFE	345.55^{b}	605.70^{a}	573.51^{a}	479.31^{ab}	36.89	0.013
TDN	474.16^{b}	786.68^{a}	792.94^{a}	696.19^{a}	48.23	0.021

R0 = farmer's ration (control); R1 = R0 + 5% LLPS; R2 = R0 + 10% LLPS; R3 = R0 + 15% LLPS; NFE = nitrogen-free extract; TDN = total digestible nutrient; SEM = standard error of mean.

The LLPS increased (p<0.05) the daily milk production and yield of milk components (Table 3), while the milk density and the percentages of components (solid non-fat (SNF), lactose, protein, fat) showed no notable difference between treatments. The MUN concentration of goats fed R1 and R2 was higher than R3, and R3 had higher MUN than R0.

Hematological parameters of treated Etawah crossbreed goats are presented in Table 4. The table shows increased red blood cell (RBC) count in R2 and R3 treatments. All treatments did not significantly influence the White Blood Cell count (WBC), Haemoglobin (Hb), and hematocrit. The WBC differentiation count was also not affected by the treatments but by monocytes. The average concentration of metabolite parameters is presented in Table 5, indicating indicates that the treatments had no impact on blood glucose, triglyceride, and BUN.

Table 3: Effect of LLPS on milk production and quality

Parameters	R0	R1	R2	R3	SEM	p-value
Milk yield (L/day)	0.53^{b}	0.78^{ab}	0.91^{a}	0.86^{a}	0.09	0.009
Milk composition (g)						
Fat	29.63^{b}	40.61^{ab}	51.89^{a}	48.84^{a}	3.80	0.016
SNF	42.17^{b}	61.78^{ab}	74.45^{a}	73.02^{a}	5.63	0.024
Lactose	23.18^{b}	33.91^{ab}	40.64^{a}	40.10^{a}	3.09	0.024
Protein	15.39^{b}	22.56^{ab}	27.15^{a}	26.66^{a}	2.05	0.025
Milk composition (%)						
Fat	5.82	5.20	5.64	5.49	0.16	0.718
SNF	7.90	7.92	8.11	8.35	0.11	0.427
Lactose	4.34	4.34	4.43	4.58	0.06	0.462
Protein	2.88	2.89	2.96	3.05	0.04	0.411
Milk density (g/mL)	1.03	1.03	1.03	1.03	0.39	0.214
MUN (mg/dL)	8.33^{b}	13.25^{a}	12.50^{a}	11.71^{ab}	0.65	0.036

R0 = farmer's ration (control); R1 = R0 + 5% LLPS; R2 = R0 + 10% LLPS; R3 = R0 + 15% LLPS; SNF = solid non-fat; MUN = milk urea nitrogen; SEM = standard error of mean

Table 4: Effect of LLPS on haematological profiles

Parameters	R0	R1	R2	R3	SEM	p-value	Standard*
WBC (× 10^3/mm^3)	9.35	11.40	11.13	12.78	0.69	0.446	4 – 13
Lymphocytes (%)	50.50	56.19	55.35	53.28	1.23	0.188	50 – 70
Neutrophils (%)	35.46	32.77	33.81	34.79	1.06	0.738	30 – 48
Eosinophils (%)	9.57	6.32	7.09	8.06	0.60	0.267	1 – 8
Monocytes (%)	3.66^{ab}	3.83^{a}	2.87^{c}	3.01^{bc}	0.18	0.037	0 – 4
Basophils (%)	0.82	0.90	0.89	0.87	0.01	0.261	0 – 1
RBC (× 10^6/mm^3)	13.19^{b}	14.04^{b}	18.52^{a}	17.27^{a}	0.69	0.012	8 – 18
Hb (g %)	9.40	9.55	9.80	9.60	0.23	0.916	8 – 12
Hematocrit (%)	25.25	26.50	26.50	26.75	0.56	0.714	22 – 38

[*]Samira et al. (2016); R0 = farmer's ration (control); R1 = R0 + 5% LLPS; R2 = R0 + 10% LLPS; R3 = R0 + 15% LLPS; RBC = red blood cells; WBC = white blood cells; Hb = hemoglobin; SEM = standard error of mean

Table 5: Effect of LLPS on blood metabolites

Parameters (mg/dL)	R0	R1	R2	R3	SEM	p-value	Standard
Glucose	53.45	60.65	55.68	63.40	1.89	0.258	50 – 75[1]
Triglyceride	14.62	19.10	18.02	23.38	1.48	0.245	8 – 27[2]
BUN	23.28	29.08	27.78	27.10	1.46	0.643	25 – 60[3]

[1]Kaneko et al. (2008); [2]Bagnicka et al. (2015); Kohn et al. (2005); R0 = farmer's ration (control); R1 = R0 + 5% LLPS; R2 = R0 + 10% LLPS; R3 = R0 + 15% LLPS; BUN = blood urea nitrogen; SEM = standard error of mean

Table 6 presents the economic aspects measured through economic efficiency, feed efficiency, feed cost per litre of milk, and IOFC. Adding LLPS affected economic efficiency but did not impact feed efficiency, feed cost per litre of milk, or IOFC.

Table 6: Economic aspects of the treatment ration

Parameters	R0	R1	R2	R3	SEM	p-value
Feed cost (Rp/kg)	5,595	5.608	5,591	5,554	-	-
Economic efficiency	2.84^{b}	4.19^{ab}	4.91^{a}	4.68^{ab}	0.45	0.008
Feed efficiency	0.72	0.69	0.77	0.92	0.07	0.439
Feed cost per litre of milk (Rp/L)	13,574	8,829	7,203	6,808	1,330	0.152
IOFC (Rp)	11,892	16,868	20,902	20,294	2,239	0.147

R0 = farmer's ration (control); R1 = R0 + 5% LLPS; R2 = R0 + 10% LLPS; R3 = R0 + 15% LLPS; IOFC = income over feed cost; SEM = standard error of mean

Discussion

Environmental Temperature Humidity Index

Environmental factors, particularly temperature and humidity, significantly affect goat productivity by contributing to animal stress levels. Goats adapt to hot climates through changes in behaviour, physical traits, physiological processes, and genetic expression (Suyadi et al., 2021). According to Qisthon and Widodo (2015), the comfort zone temperature for goats ranges from 18°C to 30°C, and heat stress occurs when the environmental temperature exceeds 32°C (Paim et al., 2014). Meanwhile, the suitable relative humidity for goats housing in a closed system ranges from 60% to 80% (Battini et al., 2014). Heat stress in goats can result in a decline in reproductive performance.

The THI combines air temperature and humidity to assess heat stress levels. It incorporates both temperature and relative humidity to provide a comprehensive assessment (Habeeb et al., 2018). Dairy goats remain within their comfort zone at THI values below 80 (Silanikove & Koluman, 2015). If the THI value is between 80 and 85, dairy goats will experience mild stress, while moderate stress occurs at THI values of 85 to 90. Dairy goats will experience severe stress at THI values above 90. The THI values in the environment around the research barn ranged from 74.1 to 83.4, with an average of 79.5, indicating normal to mild stress conditions (Figure 1). In this study, the calculation of THI was only used to evaluate whether the goats experienced heat stress or not, and therefore, its effect on production and performance was not further investigated.

Daily Nutrient Intake

Feed consumption refers to the amount of feed intake and nutrients contained within it consumed by the animal and subsequently used for the body maintenance and production of the animals. Feed intake is related to the balance of nutrients in the digestive system. Several factors, including feed quality, palatability, nutrient availability in the feed, physical form of the feed, management, and the animals' physiological status, influence the feed intake rate (Mertens & Grant, 2020).

The results show significant differences in the DMI in Etawah crossbreed goats supplemented with LLPS in the diet (R1, R2, and R3). High DMI indicates that the LLPS can enhance palatability as the animals can accept and consume it well. This result is consistent with the result of research conducted by Ababakri et al. (2021) and Firozi et al. (2023), showing that adding protected protein sources such as formaldehyde-treated sesame meal and extruded flaxseed in goat diets can increase daily DMI. Research by Susanti et al. (2022) also states that Etawah crossbreed goats given concentrates based on legume leaves resulted in higher DMI compared to the control. Moreover, Leketa et al. (2019b) reported that substituting 25% of the diet with LL for Saanen goats led to increased DMI and CPI while reducing feed costs.

According to the data in Table 2, the addition of LLPS into the diet has an impact on the increase in DMI, OMI, CPI, NFEI, and TDNI in Etawah crossbreed goats. In contrast, treatment diets did not affect the EE intake. The average of CPI goats treated with R1, R2, and R3 is higher than the control diet (R0). This indicates that improving diet quality by adding LL-based protein supplements increases animal nutrient intake. The increase in CPI might be due to the part of the concentrate, the CP content in the diet, and different concentrate feeding levels resulting in variations in crude protein consumption (Beoang et al., 2022). Moreover, in the research done by Marhaeniyanto et al. (2023), the supplementation of 10% to 40% LL in basal diet resulted in no significantly different in DMI, OMI, CPI, CFI, EEI, and TDNI.

Milk Production and Quality

The daily milk production and components in this study are presented in Table 3. The average milk production observed in this study aligns with the findings of Cyrilla et al. (2015), who reported that tropical dairy goats produce between 770 and 1,090 mL of milk per day. Significant differences were observed among the treatments. R2 and R3 resulted in the highest milk production (0.84 L/head/day) compared to the other treatments, while treatment R1 was higher than R0. This indicates that providing LLPS in R1, R2, and R3 treatments improved the performance of Etawah crossbreed goats. Including protected protein sources in the diet increases the supply of amino acids in the small intestine, meeting the nutrient requirements for milk synthesis in the mammary glands (Borucki Castro et al., 2007). Furthermore, the increase in milk production might also be triggered by the tannin content (low to moderate levels) in LL, which reduces protein degradation in the rumen, consequently increasing the amount of absorbed amino acids in the small intestine (Mohammadabadi et al., 2023).

Factors that affect milk production are the size and weight of the animal, animal breed, age, length of lactation, udder size and shape, kidding season, feed, environmental temperature, and diseases (Getaneh et al., 2016). The relationship between protein intake and milk production shows a positive linear correlation, indicating that the higher the protein intake, the higher the milk production (Prihatiningsih et al., 2015). According to Kholif et al. (2018), the primary factors contributing to increased milk production are improved nutrient digestion, rumen fermentation, and energy intake in the diet. Previous research results state that increased milk production can occur by adding protected protein sources to dairy animals' diets (Kholif et al., 2018). Leketa et al. (2019b) reported that goats fed a Leucaena-based diet produced higher milk yields than those on a control diet, likely due to the enhanced organic matter digestibility associated with Leucanea. The observed increase in milk yield in this study may be attributed to the presence of Leucaena in LPPS. Leucaena is recognized for its high CP and mineral content. Additionally, the amino acid profile of Leucaena is comparable to that of soybean and fishmeal, making it a nearly complete feed for ruminants (Sethi & Kulkarni, 1995).

The yield of milk nutrient components showed significant differences among the treatment diets in fat, SNF, lactose, and protein. Fat, SNF, lactose, and protein production are higher in R1 to R3 compared to R0. The elevated milk nutrient components yield also aligns with the higher milk yield generated in treatments R1 to R3. The results indicate that the treatment had no effects on the percentage of fat content, SNF, lactose, and protein in Etawah crossbred goat's milk. This result is in line with the findings of the study conducted by Koushki et al. (2019), which stated that adding RUP feed sources did not affect the quality percentage of milk components such as fat, protein, lactose, and dry matter, but the MUN levels were significantly higher in goats supplemented with RUP in the form of extruded soybean. Akhtar et al. (2017) also found non-significant results in the composition of cow's milk fed with different RDP and RUP levels.

The urea concentration in milk can be applied to monitor dairy animals' nutritional status and reproduction. The MUN level in goats fed with R1, R2, and R3 were higher compared to the control. The urea concentration in milk is generally affected by various factors, with nutrition playing a major role. Key influences include the level of crude protein intake, the proportions of RDP and RUP, the energy-to-protein ratio in the diet, the availability of readily digestible carbohydrates, and the quantity of water consumed. Other factors that could affect the MUN level include the type of animal, lactation stage, season, environment, litter size, and body weight (Ljoljić et al., 2020). The higher MUN levels in treatments R1, R2, and R3 are due to the diet's higher protein content than the control. High urea levels in milk may result from an excess of dietary protein, a deficiency of degradable carbohydrates in the rumen, inadequate water intake, liver detoxification overload, or energy losses caused by poor management practices. Conversely, low urea levels in milk can stem from insufficient dietary protein, an excess of easily digestible carbohydrates, or an imbalance in energy and protein intake (Ljoljić et al., 2020).

Long-term use of LL should be monitored and limited due to its antinutritional factors, such as tannins and mimosine. A case of overexposure was reported in Sumbawa, eastern Indonesia, where farmers observed hair loss in Bali bulls consuming high levels of LL (up to 100% of the diet) over 4 to 6 months, likely due to mimosine toxicity (Panjaitan et al., 2014). However, Semenye (1990) reported that diets with less than 30% LL are generally safe for goats, but farmers should be warned about potential toxicity if the LL content exceeds 30%. Combining moderate levels of LL into the diet for a short duration (2–3 months) can reduce its toxic effects (Halliday et al., 2013). In this study, the LL in the protein supplement was approximately 40%, but the total protein supplement in the dairy goat ration was limited to 15%, resulting in only 6% LL in the overall ration. Additionally, the LL was used in dry powder form, which reduced its mimosine content. No symptoms of toxicity were observed. Consequently, the supplementation of LLPs had a positive effect on nutrient intake and milk yield in goats.

Blood Hematology and Metabolite

The feed consumed and digested by animals will be absorbed and transported by the blood to all needy body organs. Analyzing blood concentrations as an indicator of animal health needs to be conducted because blood is a bodily fluid that rapidly responds if the consumed feed is toxic (Khalifa et al., 2015). A healthy condition in Etawah crossbreed goats is characterized by a balanced profile of metabolites in their blood, which can be identified by WBC, RBC, Hb, hematocrit, blood glucose levels, triglycerides, and BUN. In this study, the experimental goats, especially those consuming LLPS, showed no clinical signs of illness, such as general depression, head pressing, mouth foaming, teeth grinding, or body twitching and jerking (Odenyo et al., 1997). The absence of these signs indicates that the LLPS level in the diet was non-toxic. Table 4 shows that LPPS had a significant effect on RBC count while maintaining WBC, Hb, and hematocrit count.

White Blood Cells (WBC) aid the body in fighting various infectious diseases as part of the immune system (Ghozali, 2018). The use of LLPS in the diet did not affect WBC count in the blood of dairy goats, and their count remains within the normal range (Table 4). This result means that all treated goats remained healthy. An elevated WBC count is typically linked to microbial infections or the presence of foreign bodies or antigens in

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the circulatory system (Babeker & Abdalbagi, 2015). The differential WBC count, including lymphocytes, neutrophils, eosinophils, and basophils, showed no statistically significant differences ($p>0.05$) among the treatments.

WBCs are classified into granulocytes (neutrophils, eosinophils, and basophils) and agranulocytes (lymphocytes and monocytes). Previous studies reported that lymphocytes play a protective role against viral infections, and high lymphocyte values indicate a weakened immune system (Alam et al., 2013; Ghozali, 2018). Neutrophils play a primary protective role against infections and antigens, and a high neutrophil count suggests an active infection, while a low count indicates a weakened or compromised immune system. Eosinophils can detoxify toxins that can cause inflammation or local inflammation. Meanwhile, monocytes are essential in combating acute infections and serve as the body's last line of defence against bloodstream infections. Basophils are responsible for allergic reactions and respond to antigens by releasing histamine, which causes inflammation. Basophils are granulocytic leukocytes with the smallest number, accounting for 0.5–1.5% of the total leukocytes.

RBCs play a role in binding oxygen and circulating it throughout the body's tissues. The RBC count in this study ranges between 12.66 – 18.52 million/mm^3, which falls within the normal range. Statistical analysis results indicate that the RBC count in treatments R2 and R3 is higher compared to R0 and R1. The production of RBC in the bone marrow is influenced, in part, by amino acids. The high protein content in the diet contributes nitrogen to rumen microbes, resulting in the production of amino acids that are absorbed by the intestinal wall and then circulated throughout the body, used in the formation of RBC. Adding a 10% LLPS to the treatment diet can increase the protein RBC uses, affecting the produced RBC count (Yanti et al., 2013).

Haemoglobin (Hb) serves the physiological function of transporting oxygen to the animal's body tissues, enabling the oxidation of consumed feed into energy, which is then utilized for various bodily functions. Also, Hb plays a role in transporting carbon dioxide from the animal's body (Etim et al., 2013). The addition of LLPs does not impact Hb levels, as their values remain within the normal range. This suggests that incorporating LLPS does not interfere with Hb levels, ensuring the proper functioning of blood metabolism, including oxygen binding in the lungs and its distribution throughout the body.

Hematocrit (HCT) represents the percentage of RBC in 100 mL of blood. It serves as an indicator of dietary toxicity. HCT levels falling below the normal range may indicate poor feed quality or the presence of anaemia in animals. Any treatments did not influence the HCT levels in this study, and the results ranged from 25.25% to 26.75%, which is within the normal range, indicating that the animals are in a healthy condition (Samira et al., 2016).

Blood glucose reflects the energy supply in animals. Glucose is needed by the body for nerve function, muscle, fat tissue, fetal growth, and milk production (Marhaeniyanto et al., 2020). The analysis revealed no significant differences in blood glucose levels among Etawah crossbreed goats across the treatments. This aligns with findings from Alkass et al. (2017) and Pormalekshahi et al. (2020), which reported that varying the balance of RDP and RUP in diets did not influence goat blood glucose levels. Similarly, El-Basiony et al. (2015) observed that adding Nigella sativa to goat diets had no effect on blood glucose levels. The blood glucose levels recorded in this study ranged normally from 50–75 mg/dL, suggesting that the treatment diets adequately met the animals' daily energy requirements. However, these levels were lower than those reported by Yupardhi et al. (2014), who reported an average blood glucose level of 64.67 mg/dL in Etawah crossbreed goats. Blood glucose levels are influenced by feed intake and digestibility, as all feed components—such as organic matter, fats, carbohydrates, and proteins—are metabolized into glucose for absorption by the body. Other factors that can affect animal blood glucose levels include physiological status, animal health, and sampling time. Blood glucose levels increase around 3–4 hours after feeding. This increase can occur because the precursor of glucose production, propionic acid in the rumen, is highest around 3 hours after feeding (Chanjula et al., 2021).

Blood triglycerides level reflect lipid metabolism and fat intake in livestock (Cakra et al., 2022). The analysis results show that adding a Leucaena protein supplement to the diet had no effect on blood triglyceride levels in Etawah crossbreed goats. These results align with the finding of Valizadeh et al. (2021), who also concluded that variations in the balance of RDP and RUP in the diet do not influence blood triglyceride levels. However, the study by Pormalekshahi et al. (2020) indicated that an increase in RUP supply, especially methionine, positively impacts VLDL synthesis, increasing blood triglycerides in young goats. According to Astuti and Laconi (2019), nutrients in the blood (glucose, triglycerides, and total protein) can be influenced by feed absorption in the intestines and the animal's physiological status. The average glucose, triglycerides, and protein absorbed into the udder and expressed as lactose, fat, and milk protein are 64%, 58%, and 76%, respectively.

The BUN concentration correlates with MUN and can indicate protein use in livestock bodies. Several factors affecting BUN and MUN levels include the energy content in the feed, protein intake, RDP and RUP ratio (Akhtar et al., 2017). Urea in the blood rapidly responds to changes in protein intake from feed, and its concentration in the blood reflects the amount of protein digested by the animals (Soares et al., 2020). BUN levels can be used as an indicator of kidney function in animals. High BUN levels in the blood may indicate kidney damage (Maxiselly et al., 2022). Additionally, BUN and MUN levels may increase when animals experience a negative energy balance (Akhtar et al., 2017).

Statistically, the BUN levels in this study are insignificant (p>0.05), but the BUN levels in goats given the R0 treatment tend to be lower than in other treatments. This BUN level is consistent with the lower average MUN (Table 3). The percentage of RDP in the feed can influence BUN levels in a linear fashion, as demonstrated by the research of Akhtar et al. (2019). Their study found that goats fed a diet containing 70% RDP had the highest BUN concentration compared to those fed diets with lower RDP levels (30–60%). Moreover, according to Marhaeniyanto et al. (2020), blood urea concentration is related to feed protein consumption. The research conducted by Wiyabot (2022) showed that substituting LL at 25–100% neither reduced nor increased BUN levels in goats.

Economic Aspects

Economic efficiency was calculated based on milk production, price, dry matter intake, and feed cost (Linn, 2006). Providing protein supplements to Etawah-crossbred goats results in higher economic efficiency values (p<0.05) compared to goats without protein supplements or the control group. This result indicates that providing protein supplements in the diet can be well-consumed and digested by the animals, leading to increased milk production and economic efficiency.

The feed efficiency values in this study did not differ among the treatments. Feed efficiency in dairy goats is affected by multiple factors, including dry matter digestibility, the forage-to-concentrate ratio, the fibre content of the feed, corrected milk fat, and the animal's body weight (Oliveira et al., 2014). The selling price of goat milk at Kampung 99 Pepohonan Farm was Rp. 35,000/liter (2.275 USD). Based on the research results, R2 and R3 produced IOFC values that tend to be higher, although not statistically different from those of the other treatments. This result indicates that providing LLPS to Etawah crossbred goats can result in more significant economic benefits.

The cost of feed per litre of milk was the expense required to produce one litre of milk in goats. The feed cost per litre of milk is calculated by dividing the feed cost during the maintenance period by the amount of milk produced. The research results show that the average feed cost per litre of milk in the control group (R0) is Rp. 13,574, while the cost of feed per litre of milk in goats given protein supplementation is Rp. 8,829 (R1), Rp. 7,203 (R2), and Rp. 6,808 (R3). Statistical analysis showed no significant difference in feed cost per litre of milk among the treatment groups. However, goats receiving protein supplements incurred lower feed costs compared to the control group (R0). This reduced cost per litre of milk is linked to higher feed efficiency, suggesting that even with relatively high and costly feed intake, milk production improved compared to the control.

Conclusion

The inclusion of legume-based protein supplements to dairy goats can enhance palatability and have a positive impact on their productivity (nutrient intake, milk production, milk nutrient components, milk urea, and economic efficiency) without disrupting the health status of the animals as indicated by average haematological and blood metabolite values. For practical application, supplementation with 10% LLPS s recommended to achieve optimal feed intake, milk production, and MUN level.

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Author's Contributions

Idat Galih Permana: Conceptualized, conceived and designed the analysis and was the project administrator, reviewing and finalizing the manuscript.

Salwa Iffat Zahidah Arif: Performed the experiments, gathered and analyzed the data, and drafted the manuscript.

Fajar Rezki Pambudi: Conducted experimental work and collected data.

Despal: Data validation, supervised and reviewed the manuscript.

Annisa Rosmalia: Data curation, reviewed and improved the content of the manuscript.

Ethics

Ethical validation for the research methodology was conducted by the Institutional Committee for Animal Care and Use at IPB University, with official documentation and ethical clearance certification

(Protocol No. 105/KEH/SKE/VIII/2023), which validates the study's adherence to scientific and animal welfare standards.

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