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Protected Protein Supplement Based on Rumen Undegradable Protein to Enhanced Productivity of Etawah Crossbred Dairy Goats

Research Article

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ABSTRACT

High-lactating dairy goats require a substantial amount of protein. Protected protein supplements could provide a significant quantity of rumen undegradable protein (RUP), ensuring an adequate protein supply for high-lactating dairy goats. This study aimed to evaluate the impact of protected protein supplements on the productivity of Etawah crossbred dairy goats. The study involved 16 Etawah crossbred lactating goats with an average milk production of 0.94 ± 0.38 L head^{-1} day^{-1} and an average body weight of 46.80 ± 7.50 kg. A randomized block design of four treatments and four replications was used. The treatment ration were: R0= a ration with 0% protected protein supplement content as control, R1= R0 + 5% protected protein supplement, R2= R0 + 10% protected protein supplement, and R3= R0 + 15% protected protein supplement. Data was analyzed using ANOVA and continued to the Duncan test. The results showed that the R1, R2, and R3 treatments significantly ($P<0.05$) influenced the increase in feed intake, milk production, milk component production, milk urea nitrogen, and blood urea nitrogen. However, no significant effect was observed on milk quality, milk density, blood hematology, blood glucose, triglycerides, or economic factors. It can be concluded that adding a 5% protected protein supplement improved the performance of Etawah crossbred dairy goats without compromising milk quality and animal health.

KEY WORDS dairy goat, Etawah crossbred, heat treatment, protected protein, rumen undegradable protein.

INTRODUCTION

Goats are an important source of food that provides meat and milk. Based on the data from BPS (2023), the goat population in Indonesia continues to increase every year, reaching 18.56 million heads in 2022. The Etawah crossbred dairy goat (a crossbreed between the local Kacang goat and the Etawah goat) is a popular breed among farmers in Indonesia (Sudrajat *et al*. 2021). This has led to the Etawah crossbred goat being a significant contributor to goat milk production in Indonesia.

Goat milk has better nutritional value than cow's milk because it is easily digestible and has higher nutrient content such as protein, fat, and vitamins (Kumar and Sharma, 2016; Al Mazroea *et al.* 2018). Consequently, there has been a consistent rise in the demand for goat milk in Indonesia, fueled by a growing awareness of its health benefits (Mahendra *et al.* 2023). The increasing enthusiasm for goat milk underscores the need to improve dairy goat performance in Indonesia to meet domestic milk requirements.

Dairy goats require high-quality nutrients to achieve optimal productivity (Salo, 2018). One essential nutrient is

protein. The protein requirements for dairy goats are divided into two types of protein fractions: rumen degradable protein (RDP) and rumen undegradable protein (RUP) (Schwab and Broderick, 2017). Rumen microorganisms need ammonia from RDP, while RUP is essential for livestock. Degradation of feed in the rumen transforms RDP into nitrogen that can be utilized by rumen microbes to produce high-quality microbial protein synthesis (Manoukian *et al.* 2021). Excessive RDP in the feed can increase ammonia production in the rumen, which will eventually be absorbed and converted in the liver for excretion through urine (Savari *et al.* 2018). Meanwhile, RUP remains unhydrolyzed when entering the rumen, allowing digestion in the small intestine (Manoukian *et al.* 2021). According to the NRC (2001), the minimum ratio of RDP to RUP in dairy rations is 60:40. However, in the ration of dairy goats in Indonesia, this ratio has not been considered. Meanwhile, local feed ingredients used in the diet have high biological value and RDP content (Rosmalia *et al.* 2021). This results in dairy goat diets in Indonesia having high RDP content. This indicates the need for RUP supplementation to achieve the ideal RDP and RUP ratio.

Supplementing protected protein as RUP in the ration of dairy goats is a way to enhance the amount and the quality of milk production (Thapa *et al.* 2019). The feed ingredients for these protected supplements require feed processing techniques to improve the RUP value (Seifdavati and Taghizadeh, 2012). One of the methods involves the use of heating techniques. The heating technique can alter the chemical structure and protein fractions, reducing protein degradation in the rumen and providing nutrients for livestock (Doiron *et al.* 2009). The heating technique did not reduce the total amino acid content in the feed and decreased digestibility in the small intestine. Still, it lowered the degradation level of feed in the rumen (Karlsson *et al.* 2012). Previous research results have shown that local feed treated with moist heat techniques using an autoclave at a temperature of 120 ˚C for 60 minutes can reduce the RDP level in the feed (Rosmalia *et al.* 2024). In an *in vitro* study on diets supplemented with protected soybeans, it was observed that there was a decrease in rumen ammonia levels (indicating a reduction in RDP levels). Still, it did not affect its *in vitro* digestibility (Pambudi *et al.* 2023). This demonstrates that heating techniques can protect the protein in feed ingredients, making them suitable protein supplements.

Protected feed ingredients in feed supplements can enhance protein utilization for dairy livestock. Therefore, this research aims to evaluate the impact of protected protein supplements on the productivity of Etawah crossbred dairy goats. The benefits of this research are to optimize the productivity of dairy goats through the availability of feed nutrients, especially RUP so that the direct utilization of protein for the animal body increases. Additionally, the formation of a protein supplement product with high protein content as an RUP supply is established, allowing the dairy goat ration in Indonesia to achieve an ideal ratio of RDP and RUP.

MATERIALS AND METHODS

All the experimental procedures applied to livestock in this study were approved by the Animal Ethics Committee School of Veterinary Medicine and Biomedical Sciences, IPB University, with the number 115/KEH/SKE/IX/2023.

Experimental diet

This study used four treatments: R0 (control)= a ration with 0% protected protein supplement, R1= R0 + 5% protected protein supplement, R2= R0 + 10% protected protein supplement, R3= R0 + 15% protected protein supplement. Feedstuff consisted of king grass, pollard, tofu waste, and protected protein supplement. Dicalcium phosphate (DCP) and calcium carbonate ($CaCO_3$) were used as mineral sources.

The protein supplement comprised roasted soybeans, autoclaved black cumin seed meal, corn gluten meal, and soybean meal. The protein supplement contained crude protein at 45.34% (RDP 47.50% and RUP 52.50%). Protein protection was carried out using two different methods: the autoclaving method to protect the black cumin seed meal and the roasting method to protect soybeans. The autoclaving method refers to Doiron *et al.* (2009), where 500 g of black cumin seed meal was placed in a hollow aluminum container. Subsequently, the material was placed in an autoclave to be heated at 120 ˚C for 60 minutes. The roasting method for protein protection was performed using a coffee roaster (Cafemasy Sonifer SF-3561, China). A total of 500 g of soybeans was placed in the roasting apparatus and roasted at 200 ˚C for 20 minutes. Table 1 shows the composition of the treatment rations and their nutritional components.

Livestock selection and environmental condition

The study was conducted in Kampung 99 Pepohonan, Meruyung, Depok, West Java, Indonesia. The experimental animals were sixteen multiparous (1^{st} and 2^{nd} lactation stages) Etawah crossbred dairy goats, aged 2–3 years, with an average body weight of 46.80 ± 7.50 kg and average milk production of 0.94 ± 0.38 L $head^{-1}$ day^{-1}. The animals were kept in individual pens (1.4 m×1 m) for 35 days and had a feed adaptation period of 14 days. Each pen was equipped with a feeding trough and a water trough. Feeds were provided separately for forage and concentrate, with *ad libitum* water.

Table 1 The composition and nutrient content of the dairy goat ration

Feed (dry matter, %)	Treatments[1]			
	R0	R1	R2	R3
King grass	43.00	43.00	43.00	43.00
Tofu waste	44.00	40.14	36.28	32.42
Pollard	11.58	10.42	9.29	8.15
DCP	0.57	0.57	0.57	0.57
$CaCO_3$	0.86	0.86	0.86	0.86
Protein supplement	0.00	5.00	10.00	15.00
The nutritional content of the ration[1]				
DM (%)	14.92	17.38	18.26	18.92
Ash (% DM)	9.66	8.85	9.09	8.95
Ether extract (% DM)	4.36	4.08	4.67	5.10
Crude protein (% DM)	12.18	13.00	14.58	18.73
RDP[2] (% CP)	64.83	62.26	60.15	58.70
RUP[2] (% CP)	35.17	37.74	39.85	41.63
Crude fiber (% DM)	21.82	20.93	21.53	20.75
NFE (% DM)	51.98	53.13	50.13	46.47
TDN[2] (% DM)	68.73	69.95	69.32	69.93

[1] Proximate analysis conducted at the Biotechnology Laboratory, IPB University.
[2] TDN= 2.79 + 1.17 CP + 1.74 EE – 0.295 CF + 0.81 NFE, (Indah *et al*. 2020).
R0: Control ration; R1: R0 + 5% protected protein supplement; R2: R0 + 10% protected protein supplement and R3: R0 + 15% protected protein supplement.
DM: dry matter; DCP: dicalcium phosphate; RDP: rumen degradable protein; RUP: rumen undegradable protein; TDN: total digestible nutrient and NFE: nitrogen-free extract.

The temperature and relative humidity (RH) inside the pens were observed using a Thermo-Hygrometer (HTC-1, OneMed, Indonesia) and recorded daily at 07:00 a.m., 12:00 p.m., and 04:00 p.m. The temperature-humidity Index (THI) was measured using the following formula (Mader *et al*. 2006):

THI= (0.8×temperature) + [(RH) × (temperature×14.4)] + 46.4

The average THI values inside the pens were 76.58 (morning), 82.14 (midday), and 80.54 (afternoon). According to Silanikove and Koluman (2015), dairy goats are in normal condition when THI is below 80, begin to experience heat stress at THI 80–85, experience severe heat stress at THI 85–90, and can face mortality at THI greater than 90. Based on this reference, the dairy goats in the study were comfortable in the morning and began to experience heat stress during midday and in the afternoon.

Feed intake and analysis of economic aspects

The feed intake was determined daily by weighing the offered and rejected feed amounts. Subsequently, this feed intake was used to calculate nutrient intake, including dry matter intake, crude protein intake, ether extract intake, crude fiber intake, nitrogen-free extract intake, and total digestible nutrient intake. The economic aspects measured in this study include economic efficiency, feed efficiency, ration cost per liter of milk, and income over feed cost (IOFC).

Economic efficiency= Milk price (Rp) × Milk production (L) / Feed cost (Rp)

Feed efficiency= Milk production (L) / Dry matter intake (kg)

Feed cost per liter of milk (Rp)= Feed cost (Rp) / Milk production (L)

IOFC= Milk price - Feed cost (Rp)

Milk production and quality

Milk collection was conducted twice daily, at 9:00 a.m. and 3:30 p.m. The milk production was recorded daily during the experiment period.

The milk quality parameters, including fat, protein, lactose, and solid non-fat (SNF), were analyzed using the LACTOSCAN SLP: ultrasonic milk analyzer (Milkotronic Ltd., Bulgaria). The milk urea levels were analyzed in two stages: first, sample preparation based on the method by Broderick and Reynal (2009); second, reading the milk urea levels using a spectrophotometer (Genesys 10S UV-Vis, ThermoScientific, USA) based on the method by Astuti *et al*. (2011).

Hematology and blood metabolite analysis

Blood was collected from the jugular vein in 3 mL using a syringe. The blood collection area was sterilized before the collection process with a cotton swab soaked in 70% alcohol.

The blood sample was placed in a vacutainer tube containing EDTA as an anticoagulant, then stored in a cool box and transported to the laboratory for analysis of blood profiles (hematocrit, erythrocyte count, leukocyte count, leukocyte differentiation, and hemoglobin) (Sastradipradja *et al*. 1989) and blood metabolites (glucose, triglycerides, and blood urea nitrogen) using enzymatic colorimetric techniques with a spectrophotometer (Genesys 10S UV-Vis, ThermoScientific, USA) (Astuti *et al*. 2011).

Statistical analysis

The experimental design used in this study was a randomized block design consisting of four treatments (0%, 5%, 10%, and 15% protected protein supplement) and four groups based on milk production. The data was analyzed using the analysis of variance (ANOVA) method using SPSS IBM Vers. 20 (SPSS, 2011). The significant differences ($P<0.05$) between each treatment were further tested with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The feed intake of the Etawah crossbred is presented in Table 2. The study showed a significant difference in the feed intake of dairy goats. The addition of protected protein supplements increases the consumption of dry matter intake. The dry matter intake in this study ranged from 806.35-1111.44 g head^{-1} day^{-1}. Furthermore, there was a significant increase in organic matter due to the addition of protected protein supplements in the ration. The organic matter intake in this study ranged from 738.57-1033.04 g head^{-1} day^{-1}. The increase in dry and organic matter intake was also aligned with the elevation of all nutrient intake, such as crude protein (109.89-250.61 g head^{-1} day^{-1}), crude fiber (147.22-182.93 g head^{-1} day^{-1}), ether extract (42.09-69.24 g head^{-1} day^{-1}), nitrogen-free extract (439.36-595.57 g head^{-1} day^{-1}), and total digestible nutrients (594.25-849.95 g head^{-1} day^{-1}). The results of milk production and quality for dairy goats in the study are shown in Table 3. The treatment significantly influenced the increase in milk production. The average milk production in goats fed R1, R2, and R3 (0.81–0.95 L head^{-1} day^{-1}) rations were higher than in goats fed the control ration (0.56 L head^{-1} day^{-1}).

The treatment rations did not have a significant effect on milk density. The range of milk density values in this study ranged from 1.026 to 1.027 g mL^{-1}. The treatment rations did not significantly affect the fat, SNF, lactose, protein, and milk density. Milk fat content in this study ranged from 5.09-5.63%, SNF ranged from 8.05-8.32%, milk lactose ranged from 4.43-4.57%, and milk protein ranged from 3.00-3.04%. Regarding milk component parameters, significant results were observed in fat, SNF, lactose, and protein production.

Milk fat production ranged from 27.82-53.50 g, SNF production ranged from 47.38-80.76 g, milk lactose production ranged from 26.05-44.40 g, and milk protein production ranged from 17.31-29.48 g.

The treatment significantly influenced the increase in milk urea levels. Milk urea levels increased with the increasing level of protected protein supplements in the ration. The milk urea levels in this study ranged from 7.10–14.53 mg dL^{-1}.

The number of erythrocytes, leukocytes, hemoglobin levels, and hematocrit can be seen in Table 4. The treatment did not significantly affect blood hematology. The erythrocytes in this study ranged from 13.46-15.02 10^6 mm^{-3}, leukocytes ranged from 7.68-12.63 10^3 mm^{-3}, hemoglobin level ranged from 10.10-11.05 g%, and hematocrit ranged from 25.50-29.00%. According to normal standards defined by Feldman *et al*. (2002), the number of erythrocytes, leukocytes, hemoglobin levels, and hematocrit of the dairy goats in this study falls within the normal range.

The leukocyte differentiation results during this study can be seen in Table 5. The treatment had no significant effect on changes in lymphocyte (48.03-54.11%), monocyte (2.90-4.36%), and basophil (0.80-0.88%) values, but it had a significant increase in the percentage of neutrophil and eosinophil. The percentage of neutrophil and eosinophil values in this study ranged from 31.60 to 40.05% and 6.60 to 9.18% of the total leukocytes. This range falls within the normal range (Feldman *et al*. 2002).

The metabolic status of the dairy goats, including glucose, triglycerides, and blood urea nitrogen, is presented in Table 6. The treatment rations did not significantly affect blood glucose and triglyceride levels. This study's blood glucose value range was 53.78-68.53 mg dL^{-1}. These values fall within the normal range according to the reference values provided by Christian and Pugh (2012). The range of triglyceride values in this study was 15.73-22.15 mg dL^{-1}. This range falls within the normal standard for goats (Bagnicka *et al*. 2014). The treatment significantly affected the increase in blood urea nitrogen (BUN) levels. The range of blood urea levels in this study ranged from 21.75 to 34.48 mg dL^{-1}. This range falls within the normal range (Kohn *et al*. 2005).

The economic aspects were measured through economic efficiency, feed efficiency, the price of feed per liter of milk, and income over feed cost (IOFC), as seen in Table 7. The treatment did not significantly affect economic efficiency (3.91-4.61), feed efficiency (0.65-0.95), feed cost per liter of milk (Rp 7109-10168 L^{-1} milk), and IOFC (Rp 20162-32018 L^{-1} milk kg^{-1} ration).

Our findings showed that adding protected protein supplements to the ration of dairy goats can increase dry matter intake.

Table 2 Feed intake of Etawah crossbred dairy goat

Parameter (g head^{-1} day^{-1})	Treatments			
	R0	R1	R2	R3
Dry matter intake	806.35±108.03^{b}	1042.39±86.35^{a}	1111.44±108.21^{a}	1010.21±215.65^{a}
Organic matter intake	738.57±125.10^{b}	972.29±78.75^{a}	1033.04±101.37^{a}	943.41±203.99^{a}
Crude protein intake	109.89±16.53^{c}	160.52±11.30^{b}	198.43±21.16^{b}	250.61±60.28^{a}
Ether extract intake	42.09±5.86^{c}	53.13±3.56^{b}	66.92±7.32^{a}	69.24±16.73^{a}
Crude fiber intake	147.22±31.59^{b}	163.07±18.04^{ab}	182.93±17.87^{a}	148.01±24.96^{b}
Nitrogen-free extract intake	439.36±71.47^{b}	475.56±45.80^{b}	584.76±57.97^{a}	595.57±102.19^{a}
Total digestible nutrient intake	594.25±94.06^{b}	805.94±60.60^{a}	849.95±86.11^{a}	790.53±178.00^{a}

R0: control ration; R1: R0 + 5% protected protein supplement; R2: R0 + 10% protected protein supplement and R3: R0 + 15% protected protein supplement.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effect of treatment on the production and quality of milk from Etawah crossbred goats

Parameter	Treatments			
	R0	R1	R2	R3
Milk production (L head^{-1} day^{-1})	0.56±0.43^{b}	0.81±0.24^{a}	0.87±0.29^{a}	0.95±0.34^{a}
Milk density (g mL^{-1})	1.027±1.61	1.026±2.00	1.027±2.00	1.027±0.54
Milk urea nitrogen (mg dL^{-1})	7.10±0.91^{c}	10.70±1.40^{b}	11.96±1.79^{ab}	14.53±2.22^{a}
Milk quality (%)				
Fat	5.09±0.65	5.63±0.50	5.39±0.37	5.52±0.25
Solid non fat (SNF)	8.24±0.44	8.05±0.46	8.31±0.38	8.32±0.19
Lactose	4.53±0.24	4.43±0.25	4.57±0.21	4.57±0.10
Protein	3.01±0.16	3.00±0.16	3.03±0.14	3.04±0.07
Production of milk component (g)				
Fat	27.82±19.36^{b}	47.11±15.45^{a}	47.83±15.70^{a}	53.50±19.09^{a}
SNF	47.38±37.80^{b}	66.59±18.19^{ab}	74.87±25.62^{a}	80.76±28.95^{a}
Lactose	26.05±20.78^{b}	36.62±10.00^{ab}	41.16±14.08^{a}	44.40±15.91^{a}
Protein	17.31±13.81^{b}	24.33±6.57^{ab}	27.31±9.35^{a}	29.48±10.57^{a}

R0: control ration; R1: R0 + 5% protected protein supplement; R2: R0 + 10% protected protein supplement and R3: R0 + 15% protected protein supplement.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 4 Effect of treatment on the blood hematology of Etawah crossbred dairy goats

Parameter	Treatments				Standard*
	R0	R1	R2	R3	
Erythrocyte (10^{6} mm^{-3})	15.02±1.72	14.11±2.83	13.46±2.49	14.38±3.17	8–18
Leukocyte (10^{3} mm^{-3})	7.68±1.65	10.15±3.01	10.83±1.18	12.63±5.50	4–13
Hemoglobin (g %)	10.10±1.64	10.25±0.30	11.05±0.67	10.10±0.64	8–12
Hematocrit (%)	26.25±3.19	25.50±0.50	29.00±1.87	26.75±1.30	22–38

R0: control ration; R1: R0 + 5% protected protein supplement; R2: R0 + 10% protected protein supplement and R3: R0 + 15% protected protein supplement.
* Feldman *et al*. (2002).

Table 5 Effect of treatment on the differentiation of leucocyte of Etawah crossbred dairy goats

Parameter	Treatments				Standard*
	R0	R1	R2	R3	
Lymphocyte (%)	52.62±1.40	53.27±2.83	54.11±3.41	48.03±4.37	50–70
Neutrophil (%)	33.99±4.31^{b}	36.17±4.12^{ab}	31.60±1.53^{b}	40.05±5.11^{a}	30–48
Eosinophil (%)	9.18±2.26^{a}	6.60±1.06^{b}	9.10±1.49^{a}	8.19±1.49^{ab}	1–8
Monocyte (%)	3.41±1.01	3.08±0.47	4.36±1.71	2.90±0.28	0–4
Basophil (%)	0.80±0.03	0.88±0.03	0.84±0.06	0.84±0.07	0–1

R0: control ration; R1: R0 + 5% protected protein supplement; R2: R0 + 10% protected protein supplement and R3: R0 + 15% protected protein supplement.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* Feldman *et al*. (2002).

Table 6 Effect of treatment on the blood metabolites of Etawah crossbred dairy goats

Parameter	Treatments				Standard*
	R0	R1	R2	R3	
Glucose (mg dL^{-1})	53.78±1.21	68.53±2.45	56.23±2.95	55.45±3.78	50–75†
Triglyceride (mg dL^{-1})	22.15±7.36	16.95±2.86	20.38±2.99	15.73±2.92	8–27‡
BUN (mg dL^{-1})	21.75±4.76^{b}	23.13±1.38^{b}	27.70±5.42^{ab}	34.48±7.88^{a}	25–38£

R0: control ration; R1: R0 + 5% protected protein supplement; R2: R0 + 10% protected protein supplement and R3: R0 + 15% protected protein supplement.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
† Christian and Pugh (2012); ‡ Bagnicka *et al*. (2014) and £ Kohn *et al*. (2005).

Table 7 Economic analysis of the treatment for Etawah crossbred dairy goats

Parameter	Treatments			
	R0	R1	R2	R3
Dry matter (g $head^{-1}$ day^{-1})	806.35±108.03^{b}	1042.39±86.35^{a}	1111.44±108.21^{a}	1010.21±215.65^{a}
Milk production (L $head^{-1}$ day^{-1})	0.56±0.43^{b}	0.81±0.24^{a}	0.87±0.29^{a}	0.95±0.34^{a}
Feed cost (Rp kg^{-1})	5279	5580	5890	6160
Economic efficiency	3.91±2.50	4.34±1.21	4.48±1.54	4.61±1.60
Feed efficiency	0.65±0.37	0.78±0.24	0.79±0.26	0.95±0.31
Feed cost per liter of milk (Rp L^{-1} milk)	10168±6030	7463±2260	7729±3.916	7109± 2230
Income over feed cost (Rp L^{-1} milk kg^{-1} ration)	20162±12264	27131±6874	29289±8428	32018±9723

R0: control ration; R1: R0 + 5% protected protein supplement; R2: R0 + 10% protected protein supplement and R3: R0 + 15% protected protein supplement.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The increase in dry matter intake is due to the high palatability of the feed resulting from the addition of protein supplements to the ration. Feed palatability is influenced by the aroma, taste, texture, and anti-nutrients contained in the feed (Naveen *et al*. 2022; Phesatchaa *et al*. 2022). Protecting feed protein using heating methods causes a strong aroma, especially in black cumin seed meal. In addition, the heating method can reduce the level of anti-nutrients in the feed (Carvalho *et al*. 2013). Based on the research findings of Martins *et al*. (2019), dry matter consumption is higher in rations containing feed treated by a heat process. This is also supported by the research results of Chesini *et al*. (2023), indicating that the increase in RUP values in rations by adding heated soybean meal can enhance dry matter consumption and milk production.

The increase in nutrient intake aligns with the rise in dry matter intake. Increasing the dry matter intake led to increased feed nutrient intake, positively correlating to livestock performance (Astuti *et al*. 2020). The intake of crude protein (CP) in rations with protein supplements was higher compared to the control ration. This suggests that protected protein supplements can enhance the ration's quality by increasing nutrient content. Novianti *et al*. (2021) also reported that high CP content in the ration increased the CP intake. The total digestible nutrients (TDN) intake in supplemented rations surpasses that in the control ration. TDN represents the total energy derived from the digestion of organic matter consumed by livestock (Nakano *et al*. 2018). The utilization of TDN as an energy source and protein availability significantly impacted microbial protein synthesis, making nutrients more accessible to the livestock's body (Suryani *et al*. 2020).

The average crude protein (CP) and TDN intake in this study ranged from 109.89 to 250.61 g per head and 577.17 to 849.95 g per head, respectively. According to the NRC (1981), the average nutrient requirements for dairy goats were 663.32 g TDN and 103.44 g CP. Therefore, in the control treatment, dairy goats experienced a deficiency of nutrients because of the farm's ration, specifically in the form of TDN. Meanwhile, in the treatment with protein supplements, the dairy goats in the study received sufficient nutrients.

This indicates that the addition of protected protein supplements can enhance the ration's nutrient content and fulfill the livestock's requirements. This will support the increase in livestock productivity.

This increase in milk production was in line with the increase in dry matter consumption. According to Craig *et al*. (2022), the higher the dry matter consumption, the higher the milk production due to the increased nitrogen and energy efficiency. In addition, rations with protected protein supplements, such as roasted soybeans (Junior *et al*. 2017), have a higher RUP value, making the protein more available and allowing the livestock to synthesize more milk. According to Thapa *et al*. (2019), the high milk production in the rations with bypass protein supplementation was due to the increased supply of amino acids that can be absorbed in the small intestine.

Variations in the composition of milk impact its physical characteristics, such as milk density. In this study, the milk density showed results that were not significantly different between treatments. According to Zain (2013) and Ratya *et al*. (2017), Etawah crossbred goats have milk density in the range of 1.025 to 1.029 g mL^{-1}. Etawah crossbred goats

have milk density in the range of 1.025 to 1.029 g mL^{-1} (Zain, 2013; Ratya *et al.* 2017). Milk density positively correlates with milk SNF content (Montero-Prado *et al.* 2021). Factors influencing milk density include lactation period, animal age, and the feed consumed (Eckles *et al.* 1980; Pramono *et al.* 2023).

The quality of milk is influenced by chemical parameters, such as milk fat, protein, lactose, and SNF, which play a crucial role in determining milk's nutritional value, taste, and overall composition (Fusco *et al.* 2020). There were no significant impacts on milk fat, SNF, lactose, and protein due to the treatment rations. However, according to Craig *et al.* (2022), the more milk is produced, the less fat and protein it contains. The range of milk fat values in this study ranged from 5.09 to 5.63%. According to the Thai Agriculture Standard (TAS) 6006-2008 (TAS, 2008), goat milk with more than 4% fat falls into the premium milk category.

The production of milk nutrient components in treatments R1, R2, and R3 was higher than in the control treatment (R0). These results align with the increased milk production in treatments R1, R2, and R3. The increase in protein and lactose production is due to the addition of protected protein supplements as a source of RUP, making the absorbed amino acids more available. These findings are consistent with the research by Elsaadawy *et al.* (2022), which showed that supplementation of protected amino acids methionine and lysine can increase milk protein and lactose production.

Nichols *et al.* (2016) also demonstrated that milk protein and lactose production increased in rations with complete essential amino acid mix. Amino acids are essential components as gluconeogenic precursors (Tetrick and Odle, 2020). Amino acid supplementation affects the mammary glands' whole-body glucose practice and glucose metabolism (Lemosquet *et al.* 2009). The availability of additional amino acids to synthesize glucose contributes to the release of additional lactose and protein in the milk (Osorio *et al.* 2016). The increase in SNF levels is due to the lactose and protein components in the milk. Lactose plays a vital role in regulating the quantity of milk by prompting the movement of water into the mammary secretory vesicles from the cytoplasm of mammary epithelial cells, thus ensuring the maintenance of osmolality (Chen *et al.* 2016). This indicates that the more lactose production is generated, the more milk volume will increase.

The increase in milk fat production resulted from the availability of amino acids, methionine, and lysine (Elsaadawy *et al.* 2022; Melendez *et al.* 2023). These amino acids play a role in milk fat synthesis through increased *de novo* synthesis of short and medium-chain fatty acids or increased synthesis of chylomicrons and very-low-density lipoprotein (NRC, 2001). In treatments R1, R2, and R3, there was an increase in the RUP value in the ration due to the addition of protected protein supplements that can supply amino acids for absorption in the small intestine. RUP can provide high-quality amino acids, such as lysine and methionine, for the animal's body (Owens *et al.* 2014). The increase in amino acids absorbed in the small intestine increases available precursors for milk fat synthesis in goats fed with protected protein supplements.

Milk urea reflected the condition of protein deficiency or excess in the ration and indicated nitrogen efficiency in livestock (Roy *et al.* 2011). Furthermore, milk urea could also be used to evaluate the carbohydrate and nitrogen source balance in the rumen (Aguilar *et al.* 2012). Milk urea levels increased with the increasing level of protected protein supplements in the ration. The highest milk urea concentration was in R3, and the lowest was in R0. The milk urea concentration in goats ranged from 10.40 to 59.70 mg dL^{-1} (Čobanović *et al.* 2019). Meanwhile, the milk urea concentration in goats on a balanced ration to achieve maximum nitrogen efficiency ranged from 28 to 32 mg dL^{-1} (Brun-Bellut *et al.* 1991). Low milk urea concentrations occur due to low protein in the ration, easily digestible carbohydrates, and an imbalance between energy and protein (Biswajit *et al.* 2011). On the other hand, high milk urea concentrations result from excess protein in the ration, a lack of easily digestible carbohydrates, and excess undegraded protein in the rumen (Jonker *et al.* 1998). However, the high MUN levels in rations with RUP supply can be attributed to increased amino acid catabolism for gluconeogenesis or portal-drained viscera (PDV) oxidation (Larsen *et al.* 2014). Milk urea concentration positively correlates with blood urea parameters (Broderick and Clayton, 1997).

Hematology of blood served as an indicator to determine the health status of livestock (Madan *et al.* 2016). Hematocrit values below the normal standard indicated that the animals might be experiencing anemia and a deficiency of nutrients in their diet, while increased hematocrit values might be due to dehydration (Mudatsir *et al.* 2021). According to Mudatsir *et al.* (2021), the hemoglobin values for Etawah crossbred dairy goats ranged from 8.00 to 9.93 g%. However, in this study, the hemoglobin values ranged from 10.10 to 11.05 g %, indicating that the dairy goat produced more hemoglobin. Hemoglobin functions to bind oxygen, so an increase in hemoglobin values within the normal range allows the blood to bind more oxygen and efficiently remove carbon dioxide, leading to improved cell function (Cunningham, 2002). The blood hematology results for the dairy goats fed the treatment diets fell within the normal range, according to Feldman *et al.* (2002), indicating that the animals are healthy.

White blood cell differentiation plays a vital role in the defense mechanisms of the animals against foreign invaders

or infections. Lymphocytes have a crucial function in the immune system, participating in diverse immunological activities such as the production of immunoglobulins and the modulation of immune defense (Chaplin, 2010). Neutrophils are the primary mediators of the body's defense system and respond rapidly to bacterial and fungal pathogen attacks in response to infection, inflammation, and stress before humoral complexes, and lymphocyte cells provide immunity to these infections (Davis *et al*. 2008; Malech *et al*. 2014). Eosinophils are associated with allergic inflammation and are considered the primary effector cells against parasitic infections and allergens (Park *et al*. 2021). The granules of neutrophils and eosinophils contain antimicrobial proteins (AMP) that function as the body's defense mechanism to eliminate bacteria, fungi, parasites, and viruses (Gigon *et al*. 2021). Monocytes are the most prominent white blood cells that can migrate from the bloodstream into tissues and transform into macrophages, allowing them to ingest dead cells and microorganisms that enter the body (Shi and Pamer, 2011). Basophils are the least common type of white blood cell but mediate allergic reactions in an individual (Zhang *et al*. 2021).

Increased neutrophil and eosinophil levels indicate that the animals experienced an infection or an attack by foreign bodies within the body. Heat stress can also increase the levels of neutrophils and eosinophils in the body. According to previous research, neutrophil and eosinophil values increased in dairy cattle due to heat stress (Morar *et al*. 2018; Park *et al*. 2021). Heat stress can lead to physiological changes in animals, including metabolic disorders, and make animals more susceptible to diseases (Ju *et al*. 2014). This suggests that animals experiencing heat stress are unhealthy and vulnerable to disease, with increased neutrophil and eosinophil values indicating an ongoing immune response to the infection (Park *et al*. 2021).

The treatment rations did not significantly affect blood glucose, but this study's blood glucose values were within the normal range based on the reference values from Christian and Pugh (2012). Although there was no significant impact on blood glucose levels in this study, it was evident that the group receiving protected protein supplements experienced increased blood glucose levels. This was attributed to the availability of carbohydrates that did not undergo degradation in the rumen due to the heating technique, causing the carbohydrates to undergo hydrolysis by enzymes and transform into glucose (Perdana *et al*. 2020; Mustafa, 2021). Blood glucose concentration was regulated based on dietary patterns and hormones but might also be influenced by age, gender, breed, and the environment (Sakha *et al*. 2008; Mohammed *et al*. 2016). Furthermore, elevated blood glucose levels might also result from stress and the administration of medications, such as steroids (Amer *et al*. 1989). When animals experience stress, the central nervous system becomes active, triggering the release of glucose. This leads to increased blood glucose levels due to glycogenolysis, which is associated with elevated catecholamine and cortisol hormones under the control of the parasympathetic nervous system in response to stress (Anton *et al*. 2016).

The concentration of triglycerides in blood reflects lipid metabolism in the animal's body (Zhang *et al*. 2011). The range of triglyceride values in this study was 15.73-22.15 mg dL^{-1}. In other studies, the triglyceride values for PE goats ranged from 9.5 to 16.25 mg dL^{-1} (Perdana *et al*. 2020). The triglyceride values in this study were higher, indicating that the feed consumed by the PE goats in this study contained good nutrients. It implies that the energy requirement is fulfilled, so the triglycerides in the blood will be stored in the liver and adipose tissues (Novianti *et al*. 2021).

The increase in blood triglyceride levels might be influenced by the fat and carbohydrate content in the ration (Faza *et al*. 2017). The fat content in the ration used in this study has nearly the same value, resulting in no significant difference in triglyceride levels among each treatment.

The treatment rations significantly affected the increase in blood urea nitrogen (BUN) levels. In this study, the higher the protein supplements added to the rations, the higher the milk urea and blood urea levels. The higher the concentration of RUP in the rations, the higher the blood urea concentration would be (Lee *et al*. 2020; Ababakri *et al*. 2021). Higher blood urea content was aligned with higher milk urea value and milk production (Souza *et al*. 2021). According to Fachiroh *et al*. (2012), the average blood urea levels in Etawah crossbred dairy goats given protected protein supplements at the levels 4% and 8%, with a crude protein content in the ration of 12%, ranging from 31.06 to 33.30 mg dL^{-1}. Meanwhile, in this study, higher levels of crude protein in the ration and protected protein supplement resulted in lower blood urea levels. This indicates that the addition of protected protein supplements in this study could supply RUP, resulting in a lower accumulation of urea levels in the blood.

The higher the supplement level added to the ration, the higher the feed cost per liter of milk. This is proportional to the increase in protein in the ration. Protected protein supplements have a high protein content, the most expensive nutrient, thus increasing the feed cost.

Protected protein supplements in the ration did not significantly affect the economic aspects, but as the supplement level increased, the economic aspects also improved. This indicated that the addition of protected protein supplements might enhance economic aspects. Economic efficiency was calculated based on milk production, feed

prices, and animal feed consumption (Linn, 2006). Treatment R3 resulted in the highest economic efficiency compared to other rations. This was attributed to the enhanced milk production observed in dairy goats under treatment R3, which increased profitability.

The treatment rations did not significantly affect the feed efficiency. This was because the feed consumption in the R0 treatment was lower than in the other treatments. Therefore, despite the increase in milk production in the goats treated with protected protein supplements, the feed efficiency values between treatments were relatively similar. The selling price of goat milk received by Kampung 99 Pepohonan farm is Rp 30000 per liter. The research results show that rations with the addition of protected protein supplements resulted in a higher income over feed cost (IOFC) than the control ration (R0). This indicated that adding the protected supplements might increase profits.

CONCLUSION

In conclusion, adding a 5% protected protein supplement to the ration increases milk production without decreasing quality and affects blood hematology, indicating no disruption to livestock health and economic impact. We propose conducting *in vivo* testing for optimal recommendations, entailing a comprehensive examination of digestibility factors and nitrogen efficiency. This approach aims to gain a nuanced understanding of the environmental impact resulting from the addition of protein supplements.

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