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Effect of Dietary Rubber Leaves and Probiotics "*Bacillus pumilus St.* L1" on Blood Profile, Milk Yield and Milk Quality in Goats

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ABSTRACT

Background: This study aims to evaluate the potential of rubber leaves (RL) as an alternative feed ingredient supplemented with *Bacillus pumilus St.* L1 probiotics in diets on the hemogram profile and milk production and quality of etawah crossbred (EC) goats.

Methods: This study used twelve lactating EC goats. The treatment diets were T0: 60% native grass (NG) + 40% concentrate (CON). T1: 45% NG + 15% RL + 40 CON + 1% probiotics. T2: 30% NG + 30% RL + 40% CON + 1% probiotics. T3: 15% NG + 45% RL + 40 CON + 1% probiotics. The experiment was conducted in a randomized block design (RBD). Probiotics *Bacillus pumilus St.* L1 (2.8×10^{17} cfu/ml) supplement as 1% based on the dry matter. The parameters observed were dry matter intake (DMI), milk production, milk quality, feed efficiency and blood profile (erythrocytes and Hematocrit. Analysed variance was applied and the differences between treatments were measured using Duncan's multiple.

Result: Including RL and probiotics in the diets showed a significant ($P<0.05$) improvement in DMI, milk production, milk quality and blood profile. The research results showed an increase in DMI with an average of 597.92 - 890.84 g/head/day, milk production 265.49-360.24 g/head/day and feed efficiency 37.19 - 39.87%. Normal blood profile (erythrocytes 8.300 - 9.210 $\times 10_6$/µL, Hematocrit 26.500 - 34.000%, Hemoglobin 8.133 -9.500 g/dL; Leukocytes 10.758 - 13.308 10_3/mm^3). The use of 45% RL and probiotics in the diet was significantly higher ($P<0.05$) than other treatments.

Key words: Blood profile, EC goats, Probiotics, Rubber leaves.

INTRODUCTION

Goat milk is very beneficial for human health because the milk nutrients contain fatty acids (FA) (Nudda *et al.,* 2020). Goat's milk is useful for treating intestinal inflammation (Yadav *et al.,* 2016). One of the goat breeds that produces milk is the Etawa Crossbred (EC) goat. EC goat milk contains dry matter 13.63-14.54%, fat 3.74-4.19%, protein 3.62-3.80%, lactose 2. 71-3.73% and SNF 8.31-8.84% (Raguati, 2016). Dairy goat milk contains fat (2.51-2.72%), protein (3.15-3.67%) and lactose (4.54-4.75%) (Costa *et al.,* 2021). The health status of goats is reflected in their blood haemogram profile. One of the determining indicators that a goat is infected with a disease can be seen in the hematological and biochemical parameters of its serum (Singh *et al*., 2014). Healthy livestock and high productivity can be achieved with a nutritious and balanced diet. Feed is essential for blood metabolism because it needs protein, vitamins and minerals to form blood cells.

Smallholder farmers usually provide only native grass for the livestock. Field grass has an average crude protein (CP) of 6.18%, DM of 91.64% and ash of 15.87% (Faji *et al.,* 2021). The CP content of nine field grass species in Indonesia is 3.48-7.60% (Wahyono *et al.,* 2019). Alternative feed such as rubber leaves (RL) can replace field grass as a fiber source for livestock. Rubber leaves can be used as forage for goats and their availability throughout the year. The dry matter content of RL is 36.93%, organic matter 95.39%, crude fat 6.95%, crude fiber 30.50% crude protein of 17.56%-21.82% and undegradable protein (UDP) 40.88% (Raguati, 2016; Wigati *et al.,* 2016). RL contains high protein, which increases blood protein and blood viscosity. Several factors, such as management, feed quality and blood nutrient content in the body, can influence animal physiological conditions.

To enhance the efficiency of using rubber leaves in animal diets, it is important to incorporate probiotics derived from *Bacillus pumilus* St. L1. *Bacillus pumilus* St. L1 is

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classified as a probiotic (Raguati *et al.,* 2015). Probiotics can increase feed consumption and reduce pathogenic bacteria in the rumen and small intestine, leading to improved nutrient metabolism. It also supply ruminal N-ammonia, promote overall health and milk production (Khattab *et al.,* 2020; Zhang *et al.,* 2020; Sahoo *et al.,* 2020).

This study aims to evaluate the potential of rubber leaves as an alternative feed ingredient *Bacillus pumilus St.* L1 probiotics in diets on the hemogram, milk production and quality in EC goats.

MATERIALS AND METHODS

Twelve EC goats with an average body weight of 30 kg were divided in to four groups. The feed consists of forages consisting of field grass and rubber leaves (60%) and concentrate (40%). The concentrate consists of 38% rice bran, 28% refined corn, 27% soybean meal, 5% coconut meal, 1% salt and 1% mineral mix. The nutritional content (%) of feed is shown in Table 1. The probiotic used was *Bacillus pumilus* St. L1 developed in rice bran as much as 1% of the total feed with the number of bacteria: 2.8 × 1017 CFU/ml (Raguati *et al.,* 2015).

Blood sampling

Blood samples were taken from the jugular vein using venoject vacutainer containing EDTA and analyzed for blood profile in the laboratory.

Milk production and quality

100 ml of milk samples were taken twice a day at the morning and evening during the last week of the study and stored in the refrigerator at 5°C before quality analysis in the laboratory.

This study used a randomized block design (RBD) with four treatments and three groups (milk production): T0: 60% native grass + 40% concentrate. T1: (45% native grass + 15% rubber leaves) + 40% concentrate + 1% probiotics. T2: (30% native grass + 30% rubber leaves) + 40% concentrate + 1% probiotics. T3: (15% native grass + 45% rubber leaves) + 40% concentrate + 1% probiotics. The parameters measured were dry matter intake, milk production, feed efficiency and milk quality (specific gravity, protein, fat, total solids, solid Non-Fat and lactose). Blood profile analyses include erythrocytes, leukocytes, hematocrit value and hemoglobin. Data obtained from the experiment were analyzed using analysis of variance (ANOVA). Comparisons among treatments were performed Duncan's multiple range test using SPSS statistical software version 17.0.

RESULTS AND DISCUSSION

Dry matter intake

The average DMI of EC goats was significantly increased ($P<0.05$) by the use of rubber leaves supplemented with probiotics in the diet (Table 2). The DMI ranged from 597.92-890.83 g/head/day, which was higher than that obtained by Raguati (2016). The high DMI obtained in this study was influenced by the quality, palatability and energy content of the diet with a higher use of rubber leaves. Wigati *et al.* (2015) reported that rubber leaves contain up to 21.82% protein and 40.88% non-degradable protein. Meanwhile,

Table 1: Nutrient content of treatment feed.

Nutrient content	Treatments			
	T0	T1	T2	T3
DM (%)	53.516	54.887	56.258	57.629
CP (%)	13.991	15.400	16.808	18.217
Fat (%)	5.690	5.256	4.821	4.386
CF (%)	21.810	21.325	20.841	20.356
Ash (%)	9.286	9.151	9.016	8.881
NFE	36.284	30.197	24.110	18.023

Note: DM: Dry matter. CP: Crude protein. CF: Crude fiber. NFE: Nitrogen free extract. T0: 60% native grass (NG) + 0% rubber leaves (RL) + 40% concentrate (CON) + 0% probiotic; T1: 45% NG + 15% RL + 40 CON + 1% probiotics; T2: 30% NG + 30% RL + 40% CON + 1% probiotics; T3: 15% NG + 45% RL + 40 CON + 1% probiotics.

Table 2: Effect of dietary rubber leaves and the probiotics "*Bacillus Pumilus* ST. I1" on dry matter intake, milk production and feed efficiency of EC goats.

Parameter	Treatment				SEM	p-value
	T0	T1	T2	T3		
Intake DM. gr/head/day	597.92^{c}	739.96^{b}	831.84^{a}	890.84^{a}	22.12	<0.001
Milk yield. g/head/day	265.49^{b}	283.67^{ab}	318.74^{a}	360.24^{a}	23.63	0.039
Feed efficiency. %	38.64	37.17	37.67	39.87	4.12	0.102

Note: $^{a.b.c.d}$Different superscript letters in each row indicate significant differences at $p<0.05$.

Khatab *et al.* (2020) reported that supplementation of probiotics in diets increased DMI and milk production and reduced the effect of heat stress.

Feed efficiency

The treatment had no significant effect (P>0.05) on the feed efficiency of EC goats. The average feed efficiency in this study was 37.19-39.87% (Fig 1), however, T3 treatment showed a feed efficiency value of 45%, which tended to be higher than that of the other treatments. The high feed efficiency value in the T3 treatment was due to the role of probiotics, which influence milk production. Arowolo and He (2018) and Bain *et al.* (2022) stated that the use of probiotics effectively increases the digestive process, milk production and milk fat and improves animal health by preventing pathogenic microbes.

Erythrocytes

The results indicated that the treatment significantly affected the total blood erythrocyte levels in EC goats (P<0.05) (Table 3). A higher proportion of rubber leaves supplemented with the probiotic *Bacillus pumilus* St. L1 in the diet resulted in an increased production of erythrocytes. The crude protein (CP) content of the feed, which included rubber leaves ranged from 15.40% to 18.22%. This CP is essential for synthesizing microbial proteins in the rumen, which then flow to the small intestine, where they are converted into amino acids that act as precursors for erythrocyte formation, known as erythropoiesis. The mean erythrocyte count ranged from 8.30 to 9.21 million/mm^3, indicating good health condition of goats. Normal erythrocyte levels for goats range from 8 to 18 million/mm^3 (Weiss and Waldrop, 2010). These normal levels of erythrocytes suggest that the transport of oxygen and necessary substances in the body is stable. Cases of anemia in cattle occur due to oxidative damage to red blood cells (Kumar *et al.,* 2018).

Hematocrit/ packed cell volume (PCV)

The treatment had a significant effect on hematocrit levels (P<0.01). The hematocrit levels observed in the T0 treatment were lower than those in T1, T2 and T3 (Table 3). For EC goats fed rubber leaves supplemented with probiotics, the mean hematocrit levels ranged from 26.50% to 34.00%. Including rubber leaves in the diet can enhance hematocrit values because the protein in the feed is effectively utilized by EC goats. The feed protein is utilized optimally in livestock due to the protective effects of tannins found in rubber leaves (Raguati *et al.,* 2022).

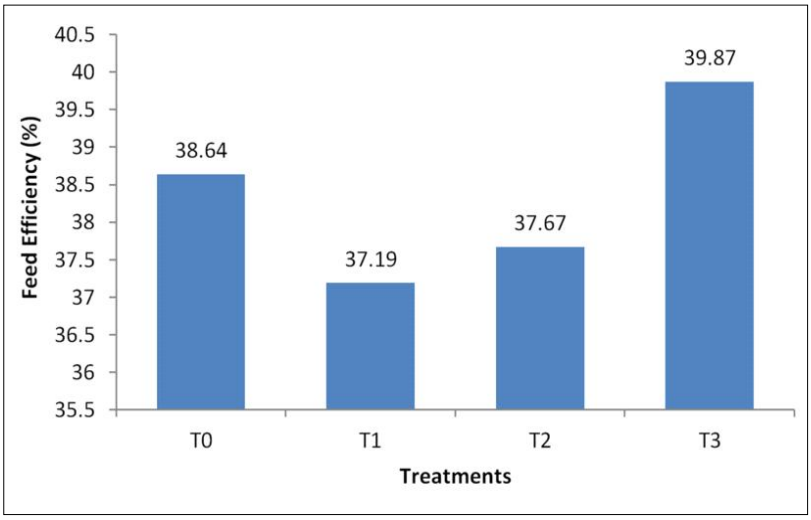


Fig 1: Effect of dietary rubber leaves and the probiotics "*Bacillus pumilus* ST. L1" on feed"efficiency of EC goats.

Table 3: Effect of dietary rubber leaves and the probiotics "*Bacillus pumilus* ST. L1" on blood profile of EC goats.

Parameter	Treatment				SEM	p-value	NV/Rf.*
	T0	T1	T2	T3			
Erythrocytes (×10^6/µL)	8.300^D	8.677^C	8.913^B	9.210^A	0.074	0.0001	8-18
Hematocrit (%)	26.500^B	28.333^B	29.333^B	34.000^A	0.961	0.0014	22-38
Hemoglobin (g/dL)	8.133^B	8.333^B	9.100^A	9.500^A	0.244	0.0014	8-12
Leukocytes (10^3/mm^3)	13.308^A	12.017^{AB}	11.600^B	10.758^B	0.473	0.0033	4-13**

Note: A. B. C. D Different superscript letters in each row indicate significant differences at p<0.01.

*NV/RL= Normal value/Reference Limit (Feldman *et al.,* 2000; Weiss and Wadrop, 2010).

** = Normal value/Reference Limit (Mudawamah and Sumartono, 2020); EC= Etawah crossbred.

Table 4: Effect of dietary rubber leaves and the probiotics "*Bacillus pumilus* ST. l1" on milk quality of CE goats.

Parameter	Treatment				SEM	p-value
	T0	T1	T2	T3		
Specific gravity	1.0281	1.0302	1.0293	1.0286	0.009	0.052
pH	5.49	5.38	5.34	5.32	0.184	0.540
Protein (%)	3.28[b]	4.17[a]	4.29[a]	4.31a	0.021	0.009
Fat (%)	3.19[c]	4.56[a]	3.76[b]	4.12b	0.228	0.009
Laktosa (%)	2.49[b]	3.96[a]	4.03[a]	4.09a	0.483	0.044
SNF (%)	5.53[b]	8.81[a]	8.97[a]	9.05a	1.071	0. 456
Total solid (%)	8.72[b]	13.70[a]	12.79a	13.98a	2.137	0.134

Note: [a.b.c.d]Different superscript letters in each row indicate significant differences at p<0.05. SNF: solid non-fat.

Hematocrit levels observed were within the normal range, indicating that the goats were in good health of goats. The hematocrit value ranged from 16.6% to 19.0%, which is higher than the findings reported by Hamzaoui *et al.* (2021). Additionally, the hematocrit levels in livestock are closely related to the levels of erythrocytes and hemoglobin (Laka *et al.,* 2019). An increase in hematocrit levels typically occurs when there is an increase in blood cell counts or a decrease in blood plasma volume.

Hemoglobin

The blood hemoglobin levels of EC goats were significantly affected by the treatment (P<0.01). The hemoglobin in the T0 treatment was lower than in T1, T2 and T3 (Table 3). Normal hemoglobin levels indicate that the goats are in good health due to a high-protein intake from rubber leaves. The average hemoglobin level of EC goats ranged from 8.13 to 9.50 g/dl, while normal hemoglobin levels in goats ranged from 8 - 12 g/dl (Weiss and Waldrop, 2010). An increase in blood erythrocyte levels leads to higher hemoglobin, which ensures that the goats blood is sufficiently oxygenated. The elevated hemoglobin is attributed to the protein content in rubber leaves and the action of tannins, which help protect feed protein, making vital nutrients readily available for livestock.

Leukocytes

The treatment had a significant effect on the leukocyte levels of EC goats (P<0.01). Feeding rubber leaves and probiotics resulted in an average decrease of 19.16% in leukocyte levels. In this study, the total leukocyte count ranged from 10.75-13.30 $\times$ 10^3/mm^3, which falls within the normal range of 5.1-13.3 $\times$ 10^3/µL (Mudawamah and Sumartono, 2020) Fernandez *et al.* (2021) reported leukocyte counts ranging from 11.6 to 12.11 thousand/mm^3 with a basal treatment using sorghum-pea butterfly silage.

The mean leukocyte count in the T0 treatment was notably high at 13.30 thousand/mm^3, indicating that the goat was unhealthy. Rubber leaves, which are rich in tannins, have an immunomodulatory effect and are bioactive against inflammation in EC goats, as evidenced by normal leukocyte levels. The addition of rubber leaves along with probiotics (T1, T2, T3) helps normalize leukocyte formation. A normal total leukocyte count reflects the livestock's ability to defend itself. Probiotic supplementation can enhance nutrient consumption and positively impact the blood metabolism of goats (Han *et al.,* 2019).

Milk yield and quality

Supplying goats with rubber leaves supplemented with probiotics significantly increased both the yield and quality of milk (P<0.05) (Table 2 and 4). The feed protein level in treatment T3 (CP 18.217%) was higher than in the other treatments. Treatment T3, which consisted of 45% rubber leaves and probiotics, produced the highest milk output. Increased DMI correlated with greater milk production. Milk production relies on quality feed and effective management (Zucali *et al.,* 2020). The tannins found in rubber leaves can protect degraded protein in the rumen, improving metabolism in the udder glands. This enhanced metabolism contributes to increased milk production and quality. Additionally, introducing varying doses of probiotics into the diets of the experimental groups of pregnant goats resulted in a significant increase in average daily milk yield and milk quality (Olchowy *et al.,* 2019; Funk *et al.,* 2021).

The average protein content in goat's milk treated with rubber leaves and supplemented with *Bacillus pumilus* St. L1 ranged from 4.17% to 4.29%. The quality of the milk produced in this study is considered premium, meeting the following characteristics: protein greater than 3.7% (Thai Agriculture, 2008) and above the fresh milk standard of greater than 2.7% (SNI, 2011). The administration of probiotic-supplemented rubber leaves significantly increased (P<0.05) the fat, total solids and lactose content in the milk of EC goats. The average milk content was found to be as follows: fat 3.76% to 4.56%, solid non-fat (SNF) 8.81% to 9.05%, total solids 12.79% to 13.98% and lactose 3.96% to 4.09% (Table 4). For comparison, the milk quality of dairy goats in Brazil showed moisture content between 89.54% and 90.02%, fat content between 2.51% and 2.72%, protein content between 3.15% and 3.67% and lactose between 4.54% and 4.75% (Costa *et al.,* 2021). These results were higher than those obtained from feeding pineapple peel powder with probiotics, which yielded an average total milk solids content of 12.38% to 12.58% (Raguati, 2016). Additionally, the SNF content in this study according to previous research, ranges from 12% to 18% and 7.08% to 8.21% (Marwah *et al.,* 2010; Arief *et al.,* 2020).

CONCLUSION

Providing rubber leaves up to 45% and supplemented with probiotics (*Bacillus pumilus* St. L1) increased milk yield and quality and improved the hemogram of goats.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from using this content.

Informed consent

The Committee of Experimental Animal Care approved all animal procedures for experiments and the University of Animal Care Committee approved handling techniques.

Conflict of interest

The authors declare that they have no conflicts of interest or competing interest.

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