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Improving Feed Intake, Digestibility, Rumen Fermentation, and Blood Profiles in Kacang Goats through *Pueraria phaseoloides* Supplementation in Kume Grass Hay Diets

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ABSTRACT

This study was conducted to investigate the effects of supplementing *Pueraria phaseoloides* on feed intake, nutrient digestibility, rumen fermentation, and blood profiles in male Kacang goats fed Kume grass hay as a basal diet. Twelve male Kacang goats aged between 6 and 8 months with a mean body weight of 13.63 ± 1.40 kg were randomly assigned to four dietary treatments (three goats per treatment) in a 3x4 incomplete Latin square design. The treatments consist of a control group (P0) receiving grass hay and concentrate and three groups supplemented with 10% (P10), 20% (P20), and 30% (P30) *P. phaseoloides* of protein requirement on a dry matter basis. The data obtained in this study were statistically analyzed using the GLM procedure following ANOVA in SPSS Statistics for Windows, version 22. The results indicated that *P. phaseoloides* significantly ($p<0.05$) increased feed intake, with goats receiving *P. phaseoloides* consuming more dry matter compared to the control group (P0). Furthermore, nutrient digestibility improved ($p<0.05$) with *P. phaseoloides* supplementation. However, rumen fermentation characteristics, including NH_3-N concentration, VFA concentration, and ruminal pH, did not differ significantly among the dietary treatments. Additionally, there were no significant differences in blood profiles among the Kacang goats under different dietary treatments. In conclusion, supplementation of *P. phaseoloides* in male Kacang goats fed Kume grass hay as a basal diet improved feed intake and nutrient digestibility but had no significant effect on rumen fermentation or blood profiles. Therefore, *P. phaseoloides* can be used as a feed supplement for ruminants consuming low-quality grass.

Keywords: blood profiles; feed intake; kacang goats; Pueraria phaseoloides; supplementation

INTRODUCTION

Kacang goats are highly valued by smallholder farmers in the East Nusa Tenggara Province of Indonesia due to their contribution to the local economy. However, the productivity of this breed is limited by the availability and quality of feed, particularly during the dry season. Kume grass hay (*Sorghum plumosum* var. Timorense) is a commonly accessible forage source during this season, but its low nutrient content and digestibility restrict its utilization as a sole feed source for ruminant animals. Protein supplementation is crucial for increasing goat production by adding grain concentrate or forage legumes to their basic diet, which may lack essential nutrients (Patil *et al.*, 2021). However, this endeavor often proves costly and unaffordable for smallholder farmers. An economically viable and method involves utilizing alternative protein sources, such as legumes integrated into the concentrate base. This approach serves as a supplement for goats and concurrently minimizes feeding expenses within the production system. Therefore, exploring strategies to enhance the nutritional value and utilization of Kume grass hay is essential for improving the feeding efficiency and productivity of ruminants.

Pueraria phaseoloides, commonly known as tropical kudzu, is a promising leguminous forage with high nutritive value (Da Paz *et al.*, 2016) and beneficial effects on rumen fermentation. It is rich in protein, fiber, and bioactive compounds (Akingbade *et al.*, 2015), accounting for 11.85% DM, 16.35% DM, and 0.62% DM, respectively (Obua *et al.*, 2012), which contribute to enhanced rumen microbial activity, nutrient digestibility, and overall animal performance. This legume has been utilized as a protein supplement for ruminant animals because it is palatable and provides good protein (Gulizia & Downs, 2019). Supplementation of *P. phaseoloides* up to 75% on a dry matter basis in ruminants has been reported to increase dry matter intake and nutrient digestibility in Santa Ines rams when the animals were offered soybean-based concentrate or *P. phaseoloides* (Da Paz *et al.*, 2016). Indeed, the same study also confirmed that *P. phaseoloides* can be used to replace soybean meal without impairing animal performance and at the same time reducing feed costs (Da Paz *et al.*, 2016). Supplementing Kume grass hay with *P. phaseoloides* has the potential to improve feed intake, nutrient digestibility, rumen fermentation, and subsequently, the overall health and productivity of ruminant animals. However, there is limited information regarding the levels of supplementation of *P. phaseoloides* in Kacang goats fed Kume grass hay as a basal diet and its effect on feed intake, nutrient digestibility, rumen fermentation, and blood parameters.

The objective of this study was to investigate the effects of levels of *P. phaseoloides* supplementation on feed intake, nutrient digestibility, rumen fermentation, and blood parameters in Kacang goats fed Kume grass hay. We hypothesized that increasing the levels of *P. phaseoloides* supplementation would enhance these parameters, leading to the increased feed intake, improved nutrient utilization, enhanced rumen fermentation, and beneficial alterations in blood parameters.

MATERIALS AND METHODS

Experimental Site, Animals, and Management

The experiment was conducted at the dry land laboratory (-10.1761° S, 123.3793° E) of Universitas Nusa Cendana Kupang, West Timor, Indonesia. This study was approved by the Livestock Research Ethics Committee, Universitas Nusa Cendana No. 051/1. KT/KEPP/V/2020.

Twelve male Kacang goats aged between 6-8 months with a mean body weight of 13.63 ± 1.40 kg were used in this study. The animals were randomly assigned to one of the four treatments following a 3x4 incomplete Latin Square Design. Each goat has an equal chance of being assigned to each treatment within each period. The dietary treatments consist of P0 (grass hay and concentrate) supplemented with 10% (P10), 20% (P20), and 30% (P30) of *P. phaseoloides* of protein requirement. Supplementation of *P. phaseoloides* was calculated based on protein requirement from 12%-14% on a dry matter basis. The experiment lasted for four consecutive periods, with each period lasting 21 days. During this period, there were 14 days of feeding adaptation and 7 days of sample collection (including feeds, feces, urine). The natural grass hay used in this study was Kume grass (*S. plumosum var.*Timorense) harvested at the vegetative stage. The animals were housed in metabolic crates (1 x 1.5 m) within a naturally ventilated shed throughout the experiment. The animals were fed formulated diets at a 70:30 grass hay: concentrate ratio and supplemented with *P. phaseoloides* at different concentrations (0%, 10%, 20%, and 30% on a dry matter basis; Tables 1 and 2). Thirty percent of the concentrate portion consisted of corn meal, rice bran, fish meal, and mineral mixture. Feeds were given to the animals twice daily (at 8 A.M. and 5 P.M.) in approximately equal amounts to ensure *ad libitum* intakes. The amount of hay provided to each animal was calculated based on the previous day's intake plus 20% of hay (w:w). Water was available to the animals *ad libitum* at all times. Hay refusals were recorded once daily at 8 A.M. before morning feeding.

P. phaseoloides Meal Preparation

To prepare *P. phaseoloides* meal, fresh *P. phaseoloides* at the vegetative growth stage were harvested from eastern Flores between May and June. The harvested *P. phaseoloides* was sun-dried for 4-d to make hay, which was then transported to Kupang. The hay was milled using a milling machine with a 1 mm sieve to obtain *P. phaseoloides* meal which is then used for supplementation.

Samples Collection and Chemical Analysis

Feeds and refusals were collected daily throughout the study and were composited by period prior to chemical analysis. Samples of feeds, fecal, and urine were collected during the last seven days of each period. Fecal samples were collected using the total collection method. The composited feed samples were dried in the oven at 60 °C and ground to a particle size of 1 mm using a Willey mill to analyze crude protein, ash, EE, and CF following the procedures outlined in the (AOAC, 2005) guidelines. Urine samples were also collected at the same time each day as for fecal collection using the total collection method. Urine was collected from collection buckets via the tiny hole at the bottom of the collection tray. The buckets were filled with 10 % H_2SO_4 to prevent N losses during collecting. Urine

Table 1. Chemical composition of experimental diets of Kacang goat treated with different level of *Pueraria phaseoloides*

Chemical composition	*Pueraria phaseoloides*	Kume grass hay	Corn meal	Rice bran	Fishmeal	Mineral mixture
Dry matter (%)	89.90	94.20	86.52	89.27	81.95	97.11
Organic matter (% DM)	80.00	88.49	83.50	75.92	61.19	49.06
Crude protein (% DM)	18.80	7.09	7.81	11.82	58.94	0.00
Ash (% DM)	9.90	5.71	3.01	13.34	20.75	48.05
Crude fiber (% DM)	31.13	30.62	5.64	19.91	2.53	28.28
Carbohydrate (% DM)	65.54	83.24	67.94	56.89	8.64	47.50
Nitrogen-free extract (% DM)	34.40	52.62	62.29	36.98	11.17	19.22
Ether Extract (% DM)	5.65	3.15	7.74	7.21	10.89	1.55
Gross energy (MJ/Kg DM)	15.40	15.77	16.29	15.16	16.50	8.74

Note: DM= dry matter. GE (MJ/kg DM)= CP content (kg)*24.237 + crude fat content (kg)*34.116 + CHO content (kg)*17.300 (Hvelplund *et al.*, 1995).

Table 2. Feed ingredients of the basal diets offered to Kacang goats and supplemented with different levels of *Pueraria phaseoloides*

Feed ingredients (% DM)	Treatments			
	P0	P10	P20	P30
Kume grass hay	70	70	70	70
Corn meal	15	15	15	15
Rice bran	9	9	9	9
Fishmeal	5	5	5	5
Mineral mixture	1	1	1	1
Total	100	100	100	100
Calculated nutrient content, % of total DM				
Dry matter	92.02	10.10	11.00	11.89
Organic matter	84.85	92.85	10.08	10.88
Crude protein	10.14	12.02	13.90	15.78
Ash	7.17	8.16	9.15	10.14
Crude fiber	24.48	27.59	30.71	33.82
Carbohydrate	74.49	81.04	87.60	94.15
Nitrogen-free extract	50.26	53.7	57.14	60.58
Ether extract	4.58	5.14	5.71	6.27

Note: P0= control group containing grass hay and concentrate; P10= P0 supplemented with 10% of *P. phaseoloides*; P20= P0 supplemented with 20% of *P. phaseoloides*; P30= P0 supplemented with 30% of *P. phaseoloides.*

samples were then analyzed for N content (Kjeldahl method, AOAC, 2005). Gros energy of feeds was calculated using equations from Hvelplund *et al*. (1995) as follows:

GE (MJ/kg DM)= CP content (kg)*24.237 + crude fat content (kg)*34.116 + CHO content (kg)*17.300

At the end of the digestibility trial, rumen fluid samples were collected from each goat using a stomach tube. The rumen liquor samples were then filtered using four layers of cheesecloth before measurement of pH using a digital pH meter. Ammonia concentration was measured using micro diffusion plates following the method of Conway (1947). VFA was determined using the steam distillation method (Krooman *et al.*, 1967). Blood samples from each goat were collected at the end of each period 4 hours after the morning meal. The samples were drawn from the jugular vein into two vacutainer tubes, with one containing 3 mL of EDTA for determining hematological parameters and the other containing 5 mL without EDTA for the determination of metabolite parameters. The samples were immediately placed on ice and brought to the laboratory for later analysis. The blood samples were then analyzed for red blood cells (RBC) and white blood cells (WBC) were measured using a Neubauer counting chamber (hemocytometer). Packed Cell Volume (PCV) was determined using the hematocrit test and blood hemoglobin (Hb) levels were measured using a hemoglobinometer.

Variable Measurements

The determination of the dry matter intake, nutrient intake, and nutrient digestibility of the animals was conducted using the following equations:

Dry matter intake= dry matter offered - dry matter refusal

Nutrient intake= DMI x % nutrient content

Nutrient digestibility= [(Nutrient intake - Nutrient in feces) / Nutrient intake] x 100

Statistical Analysis

The data obtained in this study were statistically analyzed using the GLM procedure in SPSS Statistic for Windows, version 22, following the user's guide for ANOVA. The polynomial orthogonal test within the SPSS program was used to determine the optimum levels of *P. phaseoloides* supplementation in the test diet.

RESULTS

Nutrient Intake and Digestibility

Feed intakes of goats fed kume grass hay and supplemented with different levels of *P. phaseoloides* are presented in Table 3. Results of the present study showed that the intake of grass hay and concentrates was not affected by the increasing level of *P. phaseoloides* supplementation. However, *P. phaseoloides* was completely consumed even at the highest level of supplementation. Consequently, the total intake linearly increased ($p<0.05$) with the increasing supplementation level of *P. phaseoloides.*

In the present experiment, the intake of organic matter, crude protein, ether extract, crude fiber, carbohydrate, nitrogen free extract, and energy were improved with *P. phaseoloides* supplementation ($p<0.05$) as a result of the increase of DM intake.

The digestibility coefficient of dry matter as well as nutrients in goats consuming kume grass hay as a basal diet and supplemented with different levels of *P. phaseoloides* are presented in Table 4. There were signicant increases ($p<0.05$) in organic matter, ether extract, crude fiber, carbohydrate, nitrogen-free extract, and energy digestibility with increasing level of *P.*

phaseoloides supplementation. The highest digestibility was observed in the P20 group, indicating that 20% was the optimum level of supplementation of *P. phaseoloides*, which improved the utilization of nutrients by goats. However, no further increase was observed in this study when *P. phaseoloides* supplementation up to 30%. On the other hand, crude protein digestibility did not show significant differences among the treatment group. Although the values varied slightly the differences were not statistically significant.

Apparent digestible coefficients of various nutrients in goats supplemented with different levels of *P. phaseoloides* are presented in Table 5. Dry matter intake digestible, organic matter intake digestible, crude protein intake digestible, ether extract intake digestible, crude fiber intake digestible, carbohydrate intake digestible, and energy intake digestible significantly increased (p<0.05) with the increasing levels of *P. phaseoloides* supplementation. The highest digestible nutrient was mostly observed in the P20 group, indicating that 20% was the optimum level of supplementation of *P. phaseoloides*. However, there was no further increase observed when *P. phaseoloides* supplementation up to 30%.

Nitrogen Balance

Table 6 shows that nitrogen intake significantly improved (p<0.05) with the increasing levels of *P. phasoloides* supplementation. The highest intake was observed in the P30 group, indicating that higher supplementation levels resulted in a greater nitrogen intake. However, the daily fecal and urinary nitrogen excretion were unaffected by *P. phasoloides* supplementation. N balance was linearly increased (p<0.05) up to 20% *P. phasoloides*, but no further increase was observed at 30% *P. phasoloides* supplementation.

Rumen Fermentation

Rumen pH was slightly acidic, varying between 6.2-6.4, but no effect of *P. phaseoloides* supplementation was found on rumen pH. Similarly, the concentration of VFA and NH_3 was not affected by the increasing level of *P. phaseoloides* supplementation (Table 7).

Blood Profiles

As presented in Table 8, neither blood components (hemoglobin, erythrocyte, and leucocyte) nor the con-

Table 3. Nutrient intake of Kacang goats fed basal diet kume grass and supplemented with different levels of *Pueraria phaseoloides*

Variables	Treatments				SEM	p-value
	P0	P10	P20	P30		
DM intake (g/h/d/)						
Grass hay	200.72	195.94	197.06	194.35	8.59	0.89
Concentrate	165.93	160.43	173.64	165.77	5.86	0.18
P. phaseoloides	0.00^a	42.500^b	81.00^c	118.50^d	2.82	<0.01
Total	366.65^a	398.87^b	451.71^c	478.63^d	10.77	<0.01
Nutrient intake (g/h/d)						
Organic matter	303.98^a	335.36^b	380.60^c	404.11^d	9.26	<0.01
Crude protein	25.75^a	28.47^b	33.14^c	34.86^d	0.77	<0.01
Ether extract	17.08^a	17.68^a	20.29^b	21.27^c	0.45	<0.01
Crude fiber	88.16^a	104.97^b	123.61^c	137.42^d	3.12	<0.01
Carbohydrate	261.15^a	289.20^b	327.17^c	347.96^d	8.31	<0.01
Nitrogen-free extract	172.98^a	184.22^b	203.55^c	210.54^c	5.25	<0.01
Gross energy (MJ/h/d)	5.72^a	12.18^b	18.38^c	24.01^d	0.45	<0.01

Note: P0= control group containing grass hay and concentrate; P10= P0 supplemented with 10% of *P. phaseoloides*; P20= P0 supplemented with 20% of *P. phaseoloides*; P30= P0 supplemented with 30% of *P. phaseoloides*. Means in the same row with different superscripts differ significantly (p<0.05). SEM= standard error of mean.

Table. 4. Nutrient digestibility in Kacang goats fed basal diet kume grass hay and supplemented with different levels of *Pueraria phaseoloides*

Digestibility (%)	Treatments				SEM	p-value
	P0	P10	P20	P30		
Dry matter	76.48	77.16	80.69	81.20	1.30	<0.01
Organic matter	77.11^a	78.09^a	81.50^b	82.03^b	1.25	<0.01
Crude protein	86.13	84.97	89.12	88.62	2.27	0.22
Ether extract	85.63^{ab}	83.14^a	86.18^{ab}	89.74^b	2.11	0.03
Crude fiber	69.12^a	72.44^b	77.49^c	78.87^d	1.54	<0.01
Carbohydrate	75.63^a	77.10^a	80.43^b	80.85^b	1.36	<0.01
Nitrogen-free extract	78.93^a	79.75^{ab}	82.22^b	82.14^b	1.34	0.04
Gross energy	77.83^a	88.93^b	92.96^c	94.47^c	0.81	<0.01

Note: P0= control group containing grass hay and concentrate; P10= P0 supplemented with 10% of *P. phaseoloides*; P20= P0 supplemented with 20% of *P. phaseoloides*; P30= P0 supplemented with 30% of *P. phaseoloides*. Means in the same row with different superscripts differ significantly (p<0.05). SEM= standard error of mean.

centration of blood metabolites (urea and glucose) was affected by *P. phaseoloides* supplementation.

DISCUSSION

In this experiment, the total dry matter intake (DMI) increased as expected. This result is consistent with many experiments where DMI was increased with *P. phaseoloides* supplementation. For example, Akinlade *et al.* (2005) reported that the DMI of *Panicum maximum* increased with increasing levels of supplementation of *P. phaseoloides* in the diet of steers. Similarly, Nguyen *et al*. (2008) found an increased DMI when heifers were fed guinea grass and supplemented with *P. phaseoloides* silage and hay, reflecting the higher crude protein (CP) content in *P. phaseoloides*. Furthermore, Monteiro *et al.* (2009) also reported an increase in voluntary feed intake of sheep when quicuio-da-Amazônia (*Brachiaria humidicola*) was substituted with different levels of *P. phaseoloides*. However, Akingbade *et al.* (2015) did not find a significant increase in DMI when goats were fed concentrates varying in *P. phaseoloides* leaf meal. Similarly, Da Paz *et al.* (2016) also reported no significant differences in DMI for sheep fed elephant grass as a basal diet and supplemented with *P. phaseoloides* at different levels.

The increase in DMI is likely due to the associative effect of *P. phaseoloides* supplementation on rumen fermentation, which increases rumen emptying and stimulates intake. Positive associative effects of supplementing high-quality forages often occur in animals fed basal diets of low to medium quality (Riaz *et al.,* 2014). In such basal diets, rumen fermentation is commonly hampered by the low availability of rumen-degradable protein (nitrogen) and/or energy (Idan *et al.,* 2020). When protein-rich feeds, including forages, are supplemented to these diets, microbial population and/or activity are increased, leading to the improved ruminal feed degradation, facilitated rumen emptying, and increased intake (Lazzarini *et al.,* 2013; Detmann *et al.,* 2014; Riaz *et al.,* 2014).

Table 5. Digestible nutrient intake in Kacang goats fed basal diet kume grass hay and supplemented with different levels of *Pueraria phaseoloides* (g/kg/d)

Variables	Treatments				SEM	p-value
	P0	P10	P20	P30		
DDMI	280.59^{a}	307.52^{b}	364.13^{c}	388.38^{c}	11.99	<0.01
DOMI	234.59^{a}	261.67^{b}	309.88^{c}	331.33^{d}	10.19	<0.01
DCPI	22.16^{a}	24.24^{a}	29.56^{b}	30.99^{b}	1.02	<0.01
DEEI	14.65^{a}	14.71^{a}	17.48^{b}	19.07^{c}	0.62	<0.01
DCFI	61.02^{a}	75.95^{b}	95.65^{c}	108.28^{d}	3.45	<0.01
DCHOI	197.77^{a}	222.71^{b}	262.83^{c}	281.26^{c}	9.17	<0.01
DNFEI	136.74^{a}	146.75^{a}	167.17^{b}	172.97^{b}	5.81	<0.01
DEI	4.45^{a}	10.83^{b}	17.08^{c}	22.69^{d}	0.47	<0.01

Note: P0= control group containing grass hay and concentrate; P10= P0 supplemented with 10% of *P. phaseoloides*; P20= P0 supplemented with 20% of *P. phaseoloides*; P30= P0 supplemented with 30% of *P. phaseoloides*. Means in the same row with different superscripts differ significantly (p<0.05). SEM= standard error of mean; DDMI= digestible dry matter intake; DOMI= digestible organic matter intake; DCPI= digestible crude protein intake; DEEI= digestible ether extract intake; DCFI= digestible crude fiber intake; DCHOI= digestible carbohydrate intake; DNFEI= digestible non-fiber extract intake; DEI= digestible energy intake.

Table 6. Nitrogen (N) balance in Kacang goats fed basal diet kume grass hay and supplemented with different levels of *Pueraria phaseoloides*

Variables	Treatments				SEM	p-value
	P0	P10	P20	P30		
N intake (g/d)	4.12^{a}	4.55^{b}	5.30^{c}	5.57^{d}	0.12	<0.01
Fecal N excretion (g/d)	0.57	0.67	0.57	0.62	0.10	0.70
Urinary N excretion (g/d)	0.82^{b}	0.65^{ab}	0.54^{a}	0.58^{ab}	0.11	0.11
N balance (g/d)	2.71^{a}	3.22^{b}	4.18^{c}	4.37^{c}	0.21	<0.01

Note: P0= control group containing grass hay and concentrate; P10= P0 supplemented with 10% of *P. phaseoloides*; P20= P0 supplemented with 20% of *P. phaseoloides*; P30= P0 supplemented with 30% of *P. phaseoloides*. Means in the same row with different superscripts differ significantly (p<0.05). SEM= standard error of mean.

Table 7. Rumen fermentation of Kacang goats fed basal diet kume grass and supplemented with different levels of *Pueraria phaseoloides*

Variables	Treatments				SEM	p-value
	P0	P10	P20	P30		
NH_3-N (mg 100^{-}mL)	221.74	208.75	204.17	205.16	21.21	0.83
VFA (mM)	117.47	115.80	114.49	118.65	6.89	0.93
Ruminal pH	6.38	6.30	6.30	6.27	0.08	0.62

Note: P0= control group containing grass hay and concentrate; P10= P0 supplemented with 10% of *P. phaseoloides*; P20= P0 supplemented with 20% of *P. phaseoloides*; P30= P0 supplemented with 30% of *P. phaseoloides*. SEM= standard error of mean; NH_3-N= ammonia nitrogen; VFA= volatile fatty acids.

Table 8. Blood profiles of Kacang goats fed basal diet kume grass and supplemented with different levels of *Pueraria phaseoloides*

Variables	Treatments				SEM	p-value
	P0	P10	P20	P30		
Blood urea nitrogen concentration (mg/dL)	48.60	48.66	47.78	49.74	1.04	0.32
Blood glucose concentration (mg/dL)	89.91	90.14	90.77	91.16	1.78	0.89
Hemoglobin (g/dL)	10.77	10.68	10.93	10.84	0.18	0.60
Packed cell volume (%)	32.31	32.05	32.81	32.53	0.56	0.59
Erythrocyte (x 10^6/μL)	9.99	9.67	9.98	9.75	0.24	0.46
Leucocyte (10^3/μL)	10.10	10.76	10.38	10.43	0.28	0.15

Note: P0= control group containing grass hay and concentrate; P10= P0 supplemented with 10% of *P. phaseoloides*; P20= P0 supplemented with 20% of *P. phaseoloides*; P30= P0 supplemented with 30% of *P. phaseoloides*. SEM= standard error of mean.

Although supplementation of *P. phaseoloides* as a protein source increased both nutrient intake and digestibility in this study, there were no significant differences in rumen ammonia. This phenomenon can be attributed to the efficient utilization of ammonia, which is likely facilitated by the substantial amount of highly degradable concentrate. In this study, concentrate constituted around 30% of the basal diet. This ample concentrate supply likely provides enough energy and carbon structures for microbial synthesis, thereby limiting the absorption of ammonia from the rumen wall and resulting in stable blood urea levels. Nevertheless, the rumen ammonia (NH_3-N) concentration in all treatments was above 200 mg/L, which is considered deficient (Preston & Leng, 1987), contrary to the previously mentioned 50 mg/L threshold for deficiency. Different researchers have reported variabilities of rumen NH_3-N concentration supporting microbial activities in the rumen. For example, Satter & Slyter (1974) reported that NH_3-N concentration of 5 mg/100 mL was optimal for rumen microbial growth and fermentation. Preston & Leng (1987) reported that the optimum supply for rumen microbial growth was between 2-25 mg NH_3-N/100 mL rumen fluid. This indicates that NH_3-N concentration may not be a good indicator for assessing the sufficiency of nitrogen requirement by microbes, as described by Calsamiglia *et al*. (2010). Additionally, Satter & Roffler (1975) stated that when NH_3-N accumulates in the rumen and surpasses 5 mg NH_3-N /100 mL rumen fluid, there is no improvement with additional supplementation of either non-protein-nitrogen (NPN) or dietary true protein.

Similarly, the volatile fatty acids (VFA) concentration did not differ significantly between treatments and was above the minimum requirement for microbial growth. The mean VFA in this study ranges between 114.497-118.658 mM and is within the optimum range of 80-160 mM known to support microbial growth (Van Soest, 1994). This finding suggests that rumen degradation was optimum in the control treatment, where goats were fed grass hay only (P0). Therefore, further supplementation of *P. phaseoloides* at different levels was unable to further increase rumen VFA concentration. Additionally, ruminal pH did not differ significantly between treatments and was maintained within the optimal range of 6.2-6.3 for rumen proteolytic enzyme activity (Bach *et al*., 2005).

The nitrogen balance increased in goats supplemented with 10%, 20%, and 30% *P. phaseoloides* in the present experiment consistently exceeded that of the control group (non-supplemented group). This suggests that *P. phaseoloides* supplementation, such as body weight gain, positively impacts the animal's production. Nitrogen retention in the body is a reliable measure of weight gain in ruminant animals (Hamchara *et al.,* 2018).

The increased nitrogen retention observed with *P. phaseoloides* supplementation is likely due to the improved utilization of the digested nitrogen for productive purposes, such as protein deposition, despite the increase in nitrogen intake. In the present experiment, the percentage of nitrogen loss through urine was 50% less in *P. phaseoloides* supplemented goats compared to the control group. Several factors may contribute to this enhanced nitrogen utilization. Firstly, *P. phaseoloides* supplementation may improve amino acid absorption from the intestine rather than ammonia from the rumen (Nichols *et al*., 2022). Secondly, *P. phaseoloides* supplementation may increase the energy supply in the form of ATP, facilitating protein accretion from the absorbed amino acids (Meehan *et al*., 2021). Moreover, condensed tannin contained in *P. phaseoloides* could be another aspect that may also contributed to the absorption of amino acids (da Paz *et al.,* 2016).

The levels of metabolites in the bloodstream often reflect how nutrients are absorbed, metabolized, and utilized in animal's body. For example, blood urea concentration in blood plasma depends on protein digestion, absorption, and metabolism, while blood glucose concentration indicates the digestion, absorption, and metabolism of carbohydrates. However, there were no significant differences in the concentration of these blood metabolites in this study between the group that received *P. phaseoloides* supplementation and the control group, as shown in Table 7.

The absence of an increase in blood urea concentration in this study can be attributed to the ammonia concentration. Ammonia concentration is highly correlated to blood urea concentration (Bach *et al*., 2005; Getahun *et al*., 2019). Another factor contributing to the lower blood urea concentration is the RUP of *P. phaseoloides*. Leon *et al.* (2023) found that supplementation of *P. phaseoloides* to basal grass diet improved the concentration of RUP from 72.0-79.9 g/kg compared to 23.9-32.5 g/kg DM in the grass forage alone. In their study, Leon *et al.* (2023) found that adding *P. phaseoloides* to the basal diet improved the RUP. This increase can potentially boost RUP concentration, offering a greater supply of amino acids for absorption in the small intestine. This enhanced amino acid availability can, in turn, support higher levels of production.

It was initially expected that increasing levels of *P. phaseoloides* supplementation would lead to higher glucose concentration. When nitrogen availability limits rumen fermentation, adding protein-rich feed as a supplement typically enhances rumen fermentation (Bach *et al.,* 2005). This increased fermentation rate stimulates higher propionate concentrations (Rodríguez *et al.,* 2007) and microbial protein synthesis, promoting greater amino acid absorption (Bach *et al.,* 2005). Since propionate and amino acids are essential precursors for glucose synthesis in the liver (Forbes, 2009), it was anticipated that supplementing with *P. phaseoloides* would increase blood glucose concentration.

However, the lack of an increase in blood glucose concentration with increasing levels of *P. phaseoloides* supplementation in this experiment may be attributed to the degradable protein present in the concentrate of the basal diet (approximately 30%). This degradable protein provided sufficient amino acids for microbial synthesis, resulting in a decrease in blood glucose. Another reason for the lower blood glucose concentration could be the liver's role in regulating nutrient uptake from the digestive tract and maintaining stable levels of metabolites such as glucose and amino acids in the general circulation (Forbes, 2009).

No significant differences were observed among the dietary levels of *P. phaseoloides* supplementation ($p>0.05$) on hemoglobin, packed cell volume, erythrocyte, and leucocyte. Hemoglobin concentration indicates the amount of hemoglobin present in the blood. Packed cell volume measures the proportion of red blood cells in the total blood volume and is often used to indicate hydration status and oxygen-carrying capacity. Both hemoglobin and packed cell volume can indicate how the body adapts to challenging environmental conditions (Bezerra *et al.,* 2017). The lack of significant differences suggests that *P. phaseoloides* supplementation did not significantly impact the packed cell volume values in this study. Erythrocyte count represents the number of red blood cells per unit of blood volume. These parameters are important for oxygen transport and overall blood function, which is bound to hemoglobin (Roland *et al.,* 2014). The leucocyte count, which represents the number of white blood cells in the blood, also showed no significant differences among the dietary levels of *P. phaseoloides* supplementation. Leucocytes play a crucial role in the immune response and defence against pathogens (Roland *et al.,* 2014). The non-significant differences suggest that *P. phaseoloides* supplementation did not significantly influence blood parameters in the Kacang goats.

CONCLUSION

Supplementation of *P. phaseoloides* in male Kacang goats fed Kume grass hay as a basal diet improved feed intake and nutrient digestibility but had no significant effect on rumen fermentation or blood profiles. The optimum supplementation level in this study was 20% of protein requirements on a dry matter basis.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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