



## Research article

# Effect of protein intake on nutrient utilization, blood biochemical status, and reproductive performance in dry Saanen goats (*Capra hircus*)

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## Abstract

This study aims to determine the impact of protein intake on nutritional and reproduction of dry Saanen goats in malnourished conditions. This research consisted of the following two stages: [1] the condition of livestock with basal feed provided by smallholder farmers was identified and [2] the protein quality of feed was improved by adding concentrates and legumes. Fourteen dry Saanen goats, with an average body weight of  $16.62 \pm 1.46$  kg<sup>0.75</sup>, were fed using basal feed for the first four weeks, followed by supplementary high protein feeding for the subsequent two months. Several parameters, such as nutrient intake and digestibility, nitrogen balance, estrus response, blood metabolite profile, hormones, body condition score (BCS), and BW, were identified during initial data collection at the commencement of the study and after the provision of improved feed. Paired T-test analyses were employed to analyze the obtained data. Results revealed that goats during stage 1 observation were in a nutrient-deficient condition, indicated by a BCS range of only 1.1–1.3. Significant increase ( $P < 0.05$ ) in nutrient feed intake parameters, such as increased nutrition digestibility, enhanced nitrogen balance, and BCS values, were shown after feed improvement in stage 2. This increase markedly influenced blood metabolite and estrus responses but did not significantly affect hormones. Nutrient enhancement in dry Saanen goats was considered effective in improving the nutritional status and impacting blood metabolite profiles and the estrus response.

**Keywords:** Balance nitrogen, Blood metabolite, Estrus response, Hormones, Malnourished goat.

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## INTRODUCTION

Saanen goats (*Capra hircus*) from subtropical countries are widely developed in tropical countries to address animal protein requirements (De Vasconcelos et al., 2019), with most of the management handled by smallholder farmers, particularly in Indonesia. Despite the extensive engagement of smallholder farmers in the population growth of small ruminants, various factors, including nutrition, affect livestock production (Matovu and Alcicek, 2023). Low-quality agricultural byproducts, such as rice straw, are used in the preparation of feeds and feeding systems (Kotupa and Sommart, 2020; Thirawong et al., 2025). The main challenge is the relatively limited understanding and proficiency of smallholder farmers concerning the nutritional requirements of livestock. This includes comprehension of dietary requirements and various aspects of livestock production. Nutrient deficiencies in composition or quantity negatively affect nutritional status and reproductive performance.

A decline in fulfillment and reproduction could be attributed to inadequate feed provision, which affects the fulfillment of livestock requirements (Sitaresmi et al., 2023). Nutrient deficiencies lead to decreased nutritional status as well as reduced feed intake, digestibility, and reproductive performance (Widiyono et al., 2023). Malnourishment is the imbalance of nutrient intake with the nutrient requirement over a certain period (Jansman and Te Pas, 2015). Malnourishing might initiate a prolonged deficit in energy balance (NEB), subsequently resulting in the utilization of stored fat in adipose tissue as an energy source (Esposito et al., 2014). This affects animal physiology and can cause various metabolic disorders. Body condition score (BCS) has been proven to recognize disease or nutritional deficiencies (Stilwell, 2016). Precise and practical indicator of the nutritional status (Guyoti et al., 2015; Kadhem and Tahreer, 2022). The minimum BCS value of dry or non-lactating goats is 2.5 out of 5 (Ghosh et al., 2019). Additionally, the malnourishment condition causes a decrease in adenosine triphosphate (ATP) production, leading to insufficient gonadotropin-releasing hormone (GnRH) signalling from hypothalamus to the anterior pituitary gland. This subsequently decreases the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Widayati et al., 2019). Low nutrient levels are a primary factor that could reduce productivity indices, particularly in terms of reproductive parameters, potentially impairing reproductive activities (Nascimento et al., 2020).

Production and reproductive performance can be improved by enhancing feed with protein source for malnourished livestock. Feed intake and reproduction are mainly associated with protein molecules, including enzymes and hormones (Atiba et al., 2020). Protein availability through increased dry matter intake (DMI) will trigger high microbial protein synthesis, increasing nutrient intake due to enhanced microbial activity in the rumen (Law et al., 2009). The breakdown of an excess microbial protein and ruminant undegradable protein contributes to the amino acid carbon. Approximately 60% of the total metabolizable protein is composed of microbial protein. (Weston et al., 2023). The balance of rumen concentration requires degraded protein and carbohydrates to achieve an optimal ammonia concentration. The gluconeogenesis process is stimulated by optimal ammonia availability in the rumen (Law et al., 2009). This tends to be responsible for 90% of the total glucose supply in dairy cattle (Weston et al., 2023).

The fulfillment of nutrient requirements could increase follicular diameter, lengthen corpus luteum, and boost the production of steroid hormones and prostaglandins, which positively affect productive and reproductive performance (Binyameen et al., 2023). Suppression of cortisol hormone concentrations through the fulfillment of feed requirements correlates with a decrease in animal stress and an increase in the responsiveness of the hormone insulin (Widiyono et al., 2023).

Considering nutritional impacts on goat production, energy supplementation is found to affect body weight (BW) gain, organ function, activity, cell renewal, nutrient utilization (NU) (Mahgoub et al., 2000; Kustantinah et al., 2025), and

reproductive processes (Biehl et al., 2011; Kustantinah et al., 2025). The nutritional gaps in Dry-Phase Saanen goats have not been investigated at the hormonal level and through reproductive status. Therefore, this research aims to conduct a novel observation on NU, blood biochemical status, and reproductive performance among malnourished and nourished Dry-Phase Saanen goats.

## MATERIALS AND METHODS

### Ethical Approval

The research protocol involving animals was approved by the Ethics Committee for Experimental Animals of the National Research and Innovation Agency, Indonesia, under approval number 082/KE.02/SK/10/2022. The study was conducted under widely accepted international standards on the ethical treatment and utilization of animals for scientific research.

### Experimental Animals

This research used 14 dry Saanen goats ( $16.62 \pm 1.46$  kg  $BW^{0.75}$ ) and comprised two phases as follows. The first phase (farm basal feed) included an assessment of the livestock conditions in smallholder farms, while the second (feed supplementation) focused on enhancing the feed. The investigation process was conducted using an identical group of animals consistently at the Center for Livestock Development, Gadjah Mada University, from November 2022 to February 2023. Proximate analysis method included numbers 934.01 Dry Matter (DM), 942.05 Ash, 2001.11 Crude Protein (CP), 962.09 Crude Fiber (CF), and 2003.05 Ether Extract (EE) (AOAC, 2005). Hormone analyses were carried out at the Animal Nutrition and Feed Science Laboratory as well as the Animal Breeding and Reproduction Laboratory, Gadjah Mada University, Indonesia. Simultaneously, the examination of blood metabolite samples was performed at the Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University.

### Management

A metabolic barn equipped with feeding and drinking facilities was used to house the Saanen goats in a dry state. The first research phase was conducted during the fourth week to assess the conditions of Saanen goats kept on smallholder farms. During this time, commercial concentrates were provided at 08:00 AM, and Napier grass (*Pennisetum purpureum*) was given once daily at 03:00 PM. In Phase 1, forage and concentrate were provided on an as-fed basis at 1.0 kg/day and 1.2 kg/day, respectively. Phase 2, which was conducted over two months, aimed to enhance livestock welfare. In this period, a self-mixed concentrate and Napier grass (*Pennisetum purpureum*) were supplied *ad libitum*, with each measuring 1.0 kg/day provided at 08:00 AM and 03:00 PM, respectively, while the forage-to-concentrate ratio was balanced at 50:50. Leguminous *Leucaena leucocephala* and *Gliricidia maculata* were provided once daily at 12:00 PM, with each measuring 1 kg/day, and water was provided *ad libitum* during both phases. In Phase 2, the feed formulation was adjusted to have higher levels of DM, CP, EE, nitrogen-free extract (NFE), and total digestible nutrient (TDN), as well as a lower CF composition compared to Phase 1. Comprehensive details regarding the formulation and chemical composition of the feed ingredients are presented in Tables 1 and 2, respectively.

**Table 1** Ingredients ratios and chemical compositions of experimental diets phase 1 and phase 2 (%DM basis) for dry Saanen goat.

Item	DM (%)	Chemical Composition (% DM)						Percentage (%)	
		OM	CF	EE	CP	NFE <sup>1</sup>	TDN <sup>2</sup>	Phase 1	Phase 2
Ingredients									
Copra	91.26	94.36	17.30	1.83	13.87	61.36	75.54	-	12
Soybean hulls	83.00	81.47	38.45	3.75	13.00	26.27	41.98	-	12
White Pollard	88.50	94.76	14.82	4.35	18.50	57.09	76.57	-	23
Soybean meal	93.20	98.80	3.13	1.02	47.00	47.65	89.61	-	25
Molasses	15.81	92.25	-	-	5.65	-	27.69	-	7.5
Corn gluten feed	87.80	95.40	9.10	1.46	21.60	63.24	82.19	-	3.5
Palm kernel meal	91.20	94.92	19.80	9.20	16.17	49.22	71.47	-	10
DDGS	91.16	96.02	12.17	1.19	15.64	66.49	80.67	-	7
Count (%DM)									100
Concentrate selfmixing	88.07	79.51	13.96	5.12	20.80	39.62	57.42	-	55
Concentrate commercial	89.87	88.82	16.50	5.25	15.13	41.64	53.08	79	-
<i>Pennisetum purpureum</i>	15.17	43.44	49.97	1.46	5.07	26.93	51.30	21	10
<i>Gliricidia Maculate</i>	22.15	91.75	43.68	3.71	13.60	30.74	61.73	-	17
<i>Leucaena Leucocephala</i>	25.59	92.32	39.93	3.15	13.29	35.94	59.89	-	18
Total (%DM)								100	100
Ration									
Phase 1	35.00	83.30	28.18	3.97	15.83	35.33	56.91	-	-
Phase 2	37.49	84.25	27.32	4.15	16.60	36.18	57.87	-	-

DM: Dry Matter; OM: Organic Matter; CF: Crude Fiber; CP: Crude Protein; EE: Extract Ether; NFE: Nitrogen-free Extract; TDN: Total Digestible Nutrient; DDGS: Distillers Dried Grains with Solubles

<sup>1</sup> NFE = 100 - (% CP + % CF + % EE + % ash)

<sup>2</sup> TDN =  $-133,726 - (0,254 \times CF\%) + (19,593 \times EE\%) + (2,784 \times NFE\%) + (2,315 \times CP\%) + (0,028 \times CF\%^2) - (0,341 \times EE\%^2) - (0,008 \times CF\% \times NFE\%) - (0,215 \times EE\% \times NFE\%) - (0,193 \times EE\% \times CF\%) + (0,004 \times EE\%^2 \times CP\%)$  (Hartadi et al., 2005)

**Table 2** Effect of feed improvement on the nutrient intake and digestibility in dry Saanen goats.

Variable	1 Phase	2 Phase	P Value
GE (Mcal/Kg DM)	4,342	4,341	
Ratio P/GE	1:26.69	1:20.36	
Nutrient intake (g/kg BW <sup>0.75</sup> )			
DM	68.34 ± 5.21	76.34 ± 10.37	0.010
EE	3.61 ± 0.41	3.94 ± 0.41	0.009
CF	18.95 ± 1.41	15.10 ± 2.64	0.000
CP	9.52 ± 1.04	14.89 ± 1.89	0.000
NFE	28.89 ± 2.44	29.26 ± 3.79	0.702
TDN	36.59 ± 2.84	44.71 ± 5.99	0.000
Digestibility (%)			
DM	59.93 ± 7.93	71.48 ± 4.17	0.00
EE	92.80 ± 3.25	91.75 ± 2.04	0.38
CF	36.53 ± 12.44	35.53 ± 10.97	0.84
CP	78.76 ± 5.85	82.55 ± 3.20	0.05
NFE	70.55 ± 6.17	89.99 ± 3.90	0.00
TDN	61.68 ± 6.87	68.04 ± 3.24	0.00

Significant value p<0.05; GE: Gross Energy; DM: Dry Matter; OM: Organic Matter; CF: Crude Fiber; CP: Crude Protein; EE: Extract Ether; NFE: Nitrogen-free Extract; TDN: Total Digestible Nutrient

## Feed Samples

The in vivo method was employed to obtain representative samples of feed, which were extracted from the comprehensive sample and data collection performed in two distinct research phases, particularly phases 1 and 2. Proximate analysis was conducted on the feed samples to determine the chemical composition of feed, based on the method (AOAC, 2005).

## Feed intake, digestibility determination, and Protein-to-Energy Ratio

Observations of feed intake and nutrient digestibility were conducted daily throughout the total collection period during phases 1 and 2. Data were collected from each goat using the total collection method to determine nutrient digestibility, feed samples, refusal feed, and feces. The collection of feed, feces, urine, and blood data in Phase 1 was conducted over 15 days. Phase 2 was carried out over two months, including the feed adaptation for 14 days, treatment phase for 31 days, and a second total collection (feed, feces, urine, and blood) conducted during the final 15 days. Nutrient measured for intake and digestibility included DM, organic matter (OM), CP, CF, EE, NFE, and TDN. Meanwhile, the feed intake was calculated by subtracting the remaining feed per livestock from the amount of feed given. The assessment of nutrient digestibility was determined by comparing the quantity of feed intake (in DM) with the total feces output (in DM), as well as the digestible nutrient (Sallam et al., 2023). In calculating protein-to-energy (P/GE) ratio, the crude protein analysis of nutrient was performed (Brown and Lassiter, 1962). The energy values used were those estimated through gross energy regression equations (Son and Kim, 2013). The digestibility (%), digested nutrient (g/d/BW<sup>0.75</sup>), and regression equations for the gross energy of nutrient were measured by using the following formulas:

$$\text{Nutrient Digestibility (\%)} = \frac{\text{Nutrient Intake (g/d)} - \text{Nutrient output (g/d)}}{\text{Nutrient intake (g/d)}} \times 100 \dots\dots\dots(1)$$

$$\text{Digested Nutrient (g/d/BW}^{0.75}\text{)} = \frac{\{\text{Nutrient Intake (nutrients)(g/d)} - \text{Nutrient output (g/d)}\}}{\text{Metabolic Body Weight (g/d/BW}^{0.75}\text{)}} \times 100 \dots\dots\dots(2)$$

$$\text{Gross Energy (Kcal/Kg DM)} = 4,341 + (11 \times \text{CP\% DM}) + (54 \times \text{EE\% DM}) - (24 \times \text{Ash\% DM}) \dots\dots\dots(3)$$

## Nitrogen balance determination

Nitrogen balance was determined based on the total nitrogen in the feed intake compared to the total N content in feces and urine (Sinz et al., 2008). The total nitrogen intake was determined by multiplying the feed's nitrogen content by the feed intake. The amount of nitrogen in feces and urine was calculated by multiplying the nitrogen content in the feces and urine by the total daily volume of feces and urine, thereby determining the retained nitrogen value. Nitrogen balance was calculated using the following formula (Decandia et al., 2000):

$$\text{Nitrogen Balance (\%)} = \frac{\{\text{N Ingested} - (\text{N Feces} + \text{N Urine})\}}{\text{N Ingested}} \times 100 \dots\dots\dots(4)$$

## Blood Collection and Analysis of Biochemical Substances

Samples of blood (5 cc) were taken from the jugular vein and transferred into EDTA tubes (OneMed, Indonesia) for the analysis of blood biochemistry at the conclusion of phases 1 and 2. The serum samples underwent centrifugation at 3000 rpm for 15 minutes to isolate them, after which they were stored at a

temperature of  $-20^{\circ}\text{C}$  until the metabolite parameter analysis (Widayati et al., 2019). The metabolite parameters that were assessed included the following: the total protein (g/dl), albumin (g/dl), glucose (mg/dl), BUN (mg/dl), creatinine level (mg/dl), phosphorus (mg/dl), calcium (mg/dl), total bilirubin (mg/dl), cholesterol (mg/dl), triglycerides (mg/dl), high-density lipoprotein (HDL) (mg/dl), and low-density lipoprotein (LDL) (mg/dl). These parameters were evaluated using a blood hematology analyzer. Insulin and cortisol hormones were determined using the ELISA Insulin kit (Bioenzy: BZ-22086187-EB) and the ELISA Cortisol kit (Bioenzy: BZ-22084580-CPEB), respectively. The reading was performed with an ELISA reader (Thermo-Fisher, Canada), following the protocol procedures outlined in the provided kit (Sitaresmi et al., 2023).

## Body Weight, Body Condition Score, and Estrus Response Determination

Weight of an animal (BW) and BCS evaluations were conducted routinely every week in the morning before feeding during the first and second phase (Bell et al., 2018). The spinous, transverse, and pin bones were considered in the assessment of BCS throughout both research phases, employing a BCS scale ranging from 1 to 5 (Villaquiran et al., 2017). The estrus response was evaluated by observing the keratinization process in vaginal epithelial cells using vaginal smear method. Vaginal epithelial cells were collected every 2 days for 12 days during the first and second phases of the study using cotton bud swabs. The gathered vaginal epithelium was applied to a slide and allowed to air-dry. Giemsa stain was then applied to the prepared slide, which was subsequently examined under a microscope at 40x magnification (Widayati et al., 2018).

## Statistical Analysis

An analysis of statistics was conducted to compare the differences between the initial and subsequent stages of this research. A single-factor methodology was employed, utilizing the paired T-test. Statistical analysis was carried out utilizing IBM Statistical Product and Service Solutions software version 25 (Zumbo et al., 2022). Correlation analysis visualization was determined using R studio software.

# RESULTS

## Effect of protein intake and nutrient digestibility

The improved feed in phase 2, with a lower P/GE ratio compared to phase 1, showed that nutrient intake of DM, EE, CF, CP, and TDN was significantly affected ( $P<0.05$ ). During phase 1, nutrient intake signified that the livestock were malnourished, as evidenced by the low body condition score (BCS) of dry Saanen goats (Table 4). In phase 2, CF nutrient intake significantly decreased (Table 2) due to feeding of self-mixed concentrate, *Gliricidia maculata*, *Leucaena leucocephala*, and *Pennisetum purpureum*. The positive results from phase 2 of nutrient intake had a similar effect on nutrient digestibility in dry Saanen goats (Table 2). The increase in nutrient digestibility for DM, CP, NFE, and TDN during phase 2 was found to be significant ( $P<0.05$ ).

## Effect of protein intake in the feed on nitrogen balance

Significant positive values ( $P<0.05$ ) in the second phase of improvement for dry Saanen goat feed were observed in the increase N intake, N feces, N urine, and N absorbed input, N balance, and N absorbed output (Table 3). Feeding in stage 2 with a high TP composition resulted in an increase in N intake by animals, which was followed by positive correlation with increased N urine and N feces excretion.

**Table 3** Effect of feed improvement on the N balance in dry Saanen goats.

Variable	1 Phase	2 Phase	P Value
	-----g of N/BW <sup>0.75</sup> -----		
N intake	1.29 ± 0.12	2.66 ± 0.40	0.000
N Excretion			
N feces	0.31 ± 0.79	0.46 ± 0.10	0.001
N urine	0.15 ± 0.09	0.45 ± 0.18	0.000
N balance			
Absorbed N	0.97 ± 0.11	2.19 ± 0.35	0.000
N balance	0.82 ± 0.20	1.74 ± 0.44	0.002
N output	-----% of N intake-----		
Absorbed	65.47 ± 6.70	64.80 ± 8.90	0.813
Retained	86.51 ± 5.32	78.59 ± 9.74	0.016

Significant value p&lt;0.05

### Effect protein intake on the blood metabolite profiles, hormonal concentrations, estrus response, body weight, and BCS

Research conducted on dry Saanen goats that were malnourished revealed low BCS (Table 4). After the improved feeding, dry Saanen goats showed significant (P<0.05) differences in blood biochemical profile, including total protein, BUN, phosphorus, cholesterol, HDL, and LDL. A significant decreased in serum lipids during the second phase indicated that changes in body metabolism occurred in the malnourished animals. In the second phase, enhancing the feed had a notable positive impact (P<0.05) on elevating body weight and BCS, which was correlated with an increase in estrus response in dry Saanen goats (Table 4).

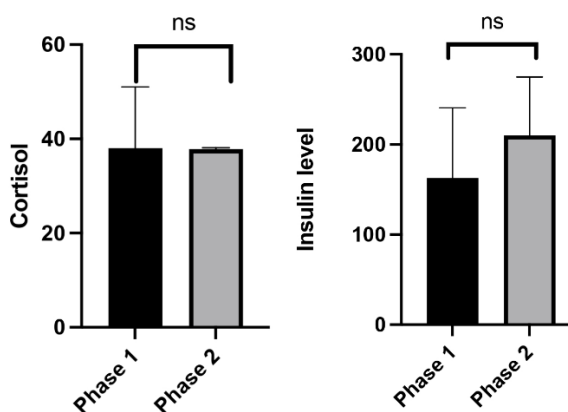
**Table 4** Effect of feed improvement on blood metabolite profiles, hormone levels, estrus response, body weight, and body condition score of dry Saanen goats.

Variable	1 Phase	2 Phase	P Value	Normal range	Reference
Blood metabolite					
TP (g/dl)	7.45 ± 0.56	6.94 ± 0.43	0.005	5.70–7.68	(Sarmin et al., 2021)
ALB (g/dl)	3.61 ± 0.25	3.53 ± 0.53	0.200	2.64–3.05	(Sarmin et al., 2021)
Glucose (mg/dl)	54.40 ± 6.80	59.45 ± 12.49	0.202	36–62	(Sarmin et al., 2021)
BUN (mg/dl)	11.50 ± 1.60	31.45 ± 4.12	0.000	28–32	(Pouillet et al., 2019)
Creatinine (mg/dl)	0.78 ± 0.16	0.87 ± 0.16	0.154	0.51–1.10	(Sarmin et al., 2021)
Phosphorus (mg/dl)	1.78 ± 0.28	6.13 ± 1.01	0.000	5.10–9.40	(Sarmin et al., 2021)
TB (mg/dl)	0.32 ± 0.04	0.34 ± 0.49	0.428	0.2–0.3	(Widiyono et al., 2023)
Cholesterol (mg/dl)	122.78 ± 19.52	79.09 ± 11.08	0.000	68–162	(Widiyono et al., 2013)
TGL (mg/dl)	22.25 ± 4.18	23.71 ± 6.59	0.431	16–160	(Shittu et al., 2016)
HDL (mg/dl)	63.61 ± 9.00	23.17 ± 3.72	0.000	29–74	(Sarmin et al., 2021)
LDL (mg/dl)	91.62 ± 14.85	56.02 ± 8.74	0.000	17–59	(Sarmin et al., 2021)
Ca (mg/dl)	10.36 ± 2.32	9.72 ± 0.20	0.275	2.22–2.24	(Sarmin et al., 2021)
Estrus response	381.00 ± 153.56	531.07 ± 118.61	0.004	ND	
BW	36.31 ± 4.89	42.48 ± 5.02	0.000	ND	
BCS	1.1 ± 0.17	2.55 ± 0.49	0.000	2.5–3.0	(Ghosh et al., 2019).

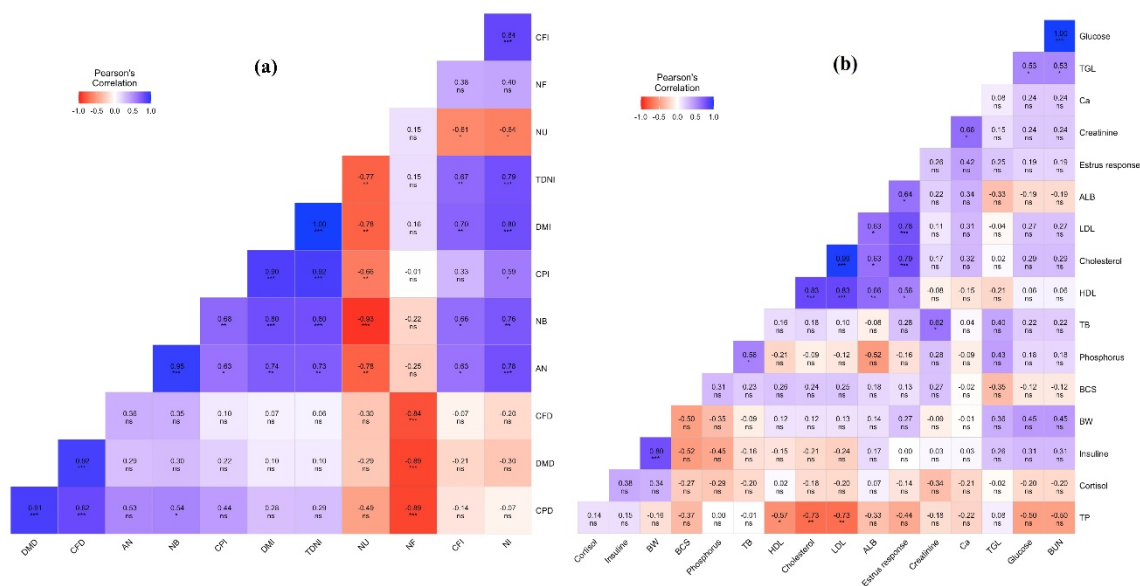
ALB: albumin; BCS: body condition score; BUN: blood urea nitrogen; BW: body weight; Ca: calcium; HDL: high-density-lipoprotein; LDL: low-density-lipoprotein; ND: no data available; TB: total bilirubin; TGL: triglyceride; Significant value p<0.05.

## Correlation between nutrient status and reproductive performance of dry Saanen goats in phase 1

The correlation between nutritional status and reproductive performance in phase 1 of malnourished dry Saanen goats is presented in Figure 2. Research showed that nitrogen balance (NB), DMI, crude protein intake (CPI), total digestible nutrient intake (TDNI), and nitrogen intake (NI) had a significant effect ( $P < 0.01$ ). NI simultaneously showed a significant positive correlation ( $P < 0.001$ ) with increases in DMI, crude fiber intake (CFI), TDNI, and absorbed nitrogen (AN). Furthermore, a significant positive relationship ( $P < 0.001$ ) was identified in dry matter digestibility (DMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD). DMI, CPI, and TDNI were positively related, while the correlation of NU with DMI, CPI, TDNI, NB, and AN was significantly negative ( $P < 0.01$ ). Fecal nitrogen (NF) showed a significant negative correlation ( $P < 0.001$ ) with DMD, CPD, and CFD. In the blood biochemical profiles (Figure 2), Creatinine and phosphorus concentrations had a significant positive correlation ( $P < 0.05$ ) with TB. Glucose and blood urea nitrogen (BUN) showed a significant positive correlation ( $P < 0.01$ ) with triglyceride concentration, while insulin concentration had a positive correlation ( $P < 0.001$ ) with BW. Cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), albumin (ALB), and estrus response showed a positive correlation relationship, and total protein (TP) had a negative correlation ( $P < 0.05$ ) with HDL, LDL, and cholesterol.



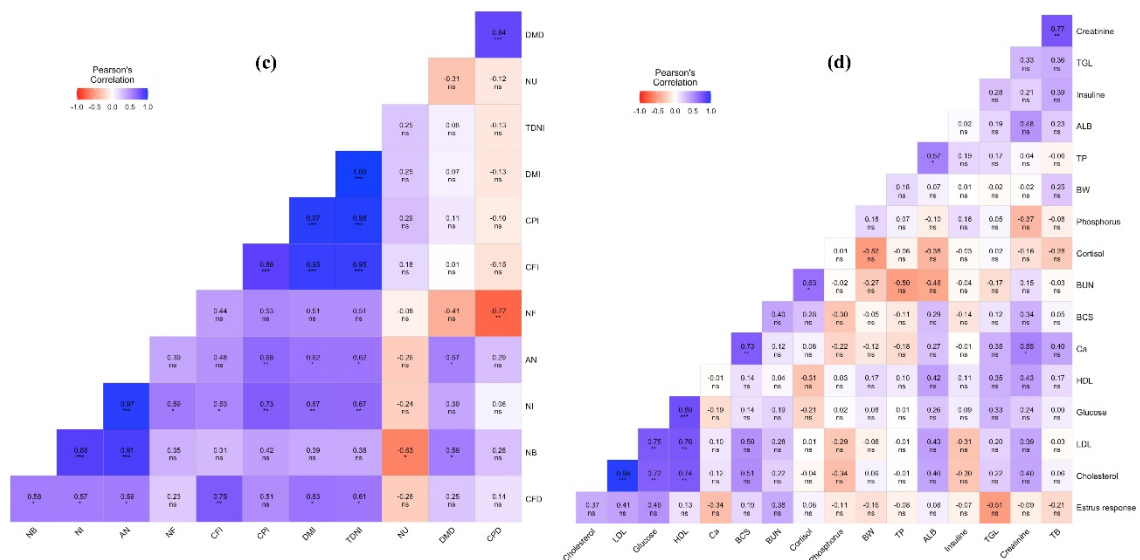
**Figure 1** Insulin and cortisol levels in Saanen dry goat before (phase 1) and after (phase 2) feed improvement.



**Figure 2** The heatmap correlation phase 1 on dry Saanen goat (a) nutrient status (b) reproductive performance. ns:  $p > 0.05$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; DMI: dry matter intake; CFI: crude fiber intake; CPI: crude protein intake; TDNI: total digestible nutrient intake; DMD: dry matter digestible; CFD: crude fiber digestible; CPD: crude protein digestible; NI: nitrogen intake; NF: nitrogen feces; NE: nitrogen urine; AN: absorbed nitrogen; NB: nitrogen balance; BUN: blood urea nitrogen; TGL: triglyceride; Ca: calcium; ALB: albumin; LDL: low-density-lipoprotein; HDL: high-density-lipoprotein; TB: total bilirubin; BCS: body condition score; BW: body weight.

## Correlation between nutrient status and reproductive performance of dry Saanen goats in phase 2

The correlation between nutritional status and reproductive performance in phase 2 of protein intake improvement is presented in Figure 3. The results showed a positive relationship ( $P < 0.001$ ) with NI, but AN had a negative association ( $P < 0.05$ ) with NU. NI showed a significant positive correlation ( $P < 0.01$ ) with DMI, CPI, and TDNI, while NF had a significant negative correlation ( $P < 0.001$ ) with CPD. Additionally, CFD had a significant positive correlation ( $P < 0.01$ ) with the increase in CFI. The blood biochemical profile (Figure 3) showed a significant positive correlation ( $P < 0.01$ ) between creatinine concentration and TB, as well as a positive correlation ( $P < 0.05$ ) between BUN concentration and cortisol. The concentration of calcium (Ca) showed a significant positive effect ( $P < 0.05$ ) on both creatinine concentration and BCS. There was a positive correlation ( $P < 0.01$ ) in the blood biochemical profile for glucose, cholesterol, HDL, and LDL.



**Figure 3** The heatmap correlation phase 2 on dry Saanen goat (c) nutrient status (d) reproductive performance. ns:  $p > 0.05$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; DMI: dry matter intake; CFI: crude fiber intake; CPI: crude protein intake; TDNI: total digestible nutrient intake; DMD: dry matter digestible; CFD: crude fiber digestible; CPD: crude protein digestible; NI: nitrogen intake; NF: nitrogen feces; NE: nitrogen urine; AN: absorbed nitrogen; NB: nitrogen balance; BUN: blood urea nitrogen; TGL: triglyceride; Ca: calcium; ALB: albumin; LDL: low-density-lipoprotein; HDL: high-density-lipoprotein; TB: total bilirubin; BCS: body condition score; BW: body weight.

## DISCUSSION

The standard nutrient requirements for dry Saanen goats measuring  $16.62 \pm 1.46 \text{ BW}^{0.75}$  are 55.35, 2.40, and 29.59 g/kg  $\text{BW}^{0.75}$  of DM, CP, and TDN, respectively (NRC, 2006). The results showed that nutrient intake of dry Saanen goats in phase 1 met these requirements in terms of DM, CP, and TDN. However, a discrepancy was observed between the fulfilment of nutrient requirements and the visual condition of BCS in the livestock (Table 4). The discrepancy could be attributed to the adaptation of Saanen goats to tropical climates.

Saanen goats, originally bred in sub-tropical regions with high milk production genetics (Nicory et al., 2023). Changes in climatic conditions experienced by animals in the long term will have negative consequences for decreased performance and body condition (Favreau-Peigné et al., 2024). The feed provided by farmers was insufficient to meet the requirements of the livestock, as evidenced by low BCS observed in dry Saanen goats (Table 3). A BCS below the normal range suggested that energy mobilization from body fat is occurring to meet the needs of livestock. The ideal BCS for goats is ranged from 2.5 to 3.0 on a scale of five (Ghosh et al., 2019).

The increase in nutrient intake in dry Saanen goats was attributed to the feed improvement implemented in the second phase. This enhancement allowed for ad-libitum feeding, enabling the ewes to consume and utilize the maximum amount of feed according to their needs. Consequently, DMI increased compared to restricted feeding, aligning with the first phase data (Martins et al., 2019). The provision of adequate energy and protein level is crucial for the proliferation of rumen microbes (Kustantinah, 2021). Previous studies have identified Bacteroides and Proteobacteria-I as the predominant microorganisms in diets with high energy and protein content (Cui et al., 2019; Kustantinah, 2021). Thus, administering low levels of energy and protein may lead to a decline in rumen performance and function. Conversely, the current research indicates that providing a higher DM of feed could stimulate microbial growth and the rumen's synthesis of volatile fatty

acids, which shows that the rumen bacteria may break down components of feed (Xiaokang et al., 2020).

The improved quantity and quality of feed supplied were responsible for the second phase's increase in digestibility and nutritional intake, in contrast to the first phase, where lower feed DM led to reduced intake and efficiency. The impact of feed levels on digestibility may not exhibit uniformity and is influenced by the chemical composition of the feed, the interrelationships between feed components, and overall feed digestibility (Huhtanen et al., 2009). Research indicates that an increase in CFI concentration could make other nutrients harder to digestibility. (Pontes et al., 2020). A crucial component of plant cell walls, lignin is an amorphous polymer of phenolic chemicals that binds plant fibers to give mechanical support. Due to its structure, lignin is indigestible by animals in the digestive tract (Oladosu et al., 2016).

The elevated intake and digestion of CP noted in the second phase of the study enhance NI. This was evidenced by positive correlation of NI with increased DMI, CPI, TDNI. The elevation in AN by animals could be attributed to the proportional improvement in CPD. However, the increase in NI might also lead to greater N excretion through urine and feces (Oladosu et al., 2016). Indicated that feeding in tropical regions with a high CF could increase undigested NF (Bohnert et al., 2011). This hypothesis aligns with the data shown in Table 3, where CFI and digestibility exhibited nonsignificant outcomes.

The enhancement in NB during phase 2 was attributed to the increased nutrient digestibility in the animals, leading to augmented nutrient excretion through urine, but NB values remained positive. This process occurred without necessitating energy mobilization from body reserves, thereby ensuring an adequate protein supply for rumen microorganisms. The primary factors contributing to the high nitrogen retention included the decline in intra-rumen nitrogen recycling and the rise in microbial nitrogen flow to the duodenum (Zhang et al., 2019). NB offered a comprehensive conceptual understanding of protein requirements for maintenance. Meanwhile, measurements over several days during metabolic experiments showed susceptibility to influences under diverse conditions during specific periods, affecting nutrient metabolism (Almeida et al., 2015). This observation corresponded with the condition of Saanen goats in phase 1, which had positive retention values and a low BCS (Table 4).

Malnutrition in dry Saanen goats in phase 1 may adversely affect metabolism and body condition. However, serum protein, glucose, and minerals remained in a normal range, suggesting that the livestock maintained body homeostasis even under malnourished conditions. The increase in BUN levels was associated with the augmented intake of CP by the livestock. The differences in blood metabolite concentrations despite similar nutrition intake were due to the enhancement of nutrient mobilization (Widayati et al., 2024). The serum urea concentration reflects dietary protein intake, but it may elevate during periods of NEB when muscle tissue is catabolized to support metabolic requirements (Roche et al., 2013). A previous research including Kacang goats during underfeeding and refeeding periods found sufficient rumen microbial nitrogen to sustain liver function (Widiyono et al., 2023). Increased blood urea and reduced fertility rates have also been connected to high-protein diets in postpartum cows (Roche et al., 2000; Puppel et al., 2016). In phase 1, BUN levels were significantly lower than the normal standard, suggesting insufficient protein content in the feed. This phenomenon probably occurred because protein in the feed was inadequate or poorly digested and metabolized by goats. During phase 2, the liver metabolized blood protein from the feed more effectively, maintaining BUN levels within normal limits, which could be efficiently used by the body (Torres-Cavazos et al., 2023).

The significant decrease in cholesterol, HDL, and LDL concentrations observed in this research might be associated with the administration of legumes, *Leucaena Leucocephala*, and *Gliricidia Maculata* during phase 2 of feed improvement compared with the basal feed in phase 1. These legumes tended to

inhibit the activity of pancreatic lipase enzymes (Liu and Xu., 2015). Flavonoids, which serve as cofactors for cholesterol esterase enzymes, have been recognized for the ability to reduce total cholesterol levels. Moreover, flavonoids can induce bile excretion by activating cytochrome P-450 enzymes, facilitating the interaction with various components in bile to decrease cholesterol levels in the body (Ballanntyne et al., 2019). The grains included in the concentrates during the second phase contain omega-3 nutrient, which exerts an inhibitory effect on total blood cholesterol by reducing blood lipid content and disrupting the synthesis of cholesterol and triglycerides. Omega-3 fatty acids act as enzymes that hinder fat biosynthesis in the liver (Teama and El-Tarabany, 2016).

Despite the significant reduction in cholesterol, HDL, and LDL concentrations, the enhancement of estrus response in the second phase still occurred. This increase in the estrus response was largely based on improving the general nutrition of goats, leading to a rise in BCS and BW. The observed improvement in BCS value introduced a superior estrus response or reproductive performance compared to low BCS goats (Sitaresmi et al., 2020). The ideal BCS size showed that goats had sufficient nutrient or energy for daily maintenance and directly provided the main response to the hypothalamus–pituitary axis (HPGx) to resecret reproductive hormones, such as GnRH, and reinstate the reproductive process of livestock (Maurya et al., 2010; Sitaresmi et al., 2020). Sufficient protein and other nutrient contents in the feed contributed to the increased estrus response in dry Saanen goats, which provided a substantial resource for the synthesis of reproductive hormones and the development of ovarian follicles (Wu et al., 2014).

A positive correlation with the increased nutrient intake could be attributed to the minimal rise in insulin levels. This correlation is presented in Figure 1 and Table 4, which show similar concentration values of insulin and glucose, despite experiencing a slight increase. Cortisol can stimulate appetite, which is essential for addressing low BW (Sitaresmi et al., 2020). Plasma insulin concentrations decreased during feed restriction, suggesting the correlation between insulin and the extent of feed consumption (Poulet et al., 2019). Nutrient deficiency led to an increase in cortisol levels during the maintenance period before feed improvement and a concentration reduction of these levels when feeding was conducted ad libitum. Moreover, a slight decrease in cortisol was observed with improved feeding in phase 2 (Ekpe et al., 2000; Widiyono et al., 2023). The stress condition in the livestock was signified by the concentration of cortisol hormone in both phases 1 and 2. The hypothalamic–pituitary–adrenal axis stimulates the adrenal cortex when the body passes through stress to release cortisol hormones (Sheriff et al., 2011).

## CONCLUSIONS

In conclusion, enhancement of the feed for dry Saanen goats led to a significant improvement in the nutritional status, as shown by increased nutrient intake, augmented nutrient digestibility, and improved NB. This positively affected reproductive parameters, including estrus response, BCS, and BW, along with various biochemical markers such as BUN, phosphorus, cholesterol, HDL, and LDL levels.

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## AUTHORS' CONTRIBUTIONS

**Yananto aryo wicaksono:** Writing – original draft (lead); conceptualization (equal); data curation (equal); formal analysis (lead); writing – review and editing (lead); methodology (equal).

**Kustantinah:** Conceptualization (equal); methodology (equal); data curation (equal); funding acquisition (equal); investigation (equal); supervision (equal).

**Diah Tri Widayati:** Conceptualization (equal); data curation (equal); methodology (equal); investigation (equal); supervision (equal).

**Pradita Iustitia Sitaresmi:** data curation (equal); investigation (equal); supervision (equal); methodology (equal); funding acquisition (equal).

**Herdis:** validation (supporting); supervision (supporting).

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## CONFLICT OF INTEREST

There was no conflict of interest.

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