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Impact of Long-term Soybean Meal Flushing during the Mating Period on Estrus Expression and Reproductive Physiology of Saanen Dairy Goats in Tropical Conditions

Dio Fico Felsidan Diatmono[1], Fransisca Gani Padmawati[1], Pradita Iustitia Sitaresmi[2], Budi Prasetyo Widyobroto[3], Sungkono[1], Andrian Syah[1], Herdis[2], Satoshi Ohkura[4] and Diah Tri Widayati[1*]

[1]Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia; [2]Research Center for Animal Husbandry, Cibinong Science Center, National Research and Innovation Agency (BRIN), Bogor, Indonesia; [3]Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia; [4]Department of Animal Sciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

[*]*Corresponding author:* widayati@ugm.ac.id

Abstract

Feed protein intake is crucial for successful reproduction of small ruminants, including dairy goats. However, while high protein intake is proven to increase productivity, the negative impact on reproductive efficiency is well documented. This study aimed to evaluate the effectiveness and physiological effects of a high-protein flushing diet on reproductive performance in tropical Saanen goats. Forty-two multiparous does with body condition scores (BCS) of 2.73±0.29 were assigned to a control group with no supplementation (T0; n = 14), 5% soybean meal (SBM) group (T1), or 15% SBM group (T2) on a dry matter (DM) basis. Supplementation was administered for 14 days pre- and 14 days post-artificial insemination (AI). Estrus was synchronized using CIDR and PGF2α, followed by AI. Blood metabolite profiles, plasma antioxidant status, reproductive hormone levels, estrus expression, and maternal performance were measured. Data were analyzed using two-way repeated measures analysis of variance (ANOVA), followed by Tukey's B post-hoc test. Results showed differences ($p < 0.05$) in blood metabolites in T2, with increased urea and blood urea nitrogen (BUN), whereas cholesterol was higher in T0 than in diet-flushing groups. Group T1 showed superior antioxidant profiles to T0, with elevated glutathione peroxidase-1 (GPX1) and reduced malondialdehyde (MDA) levels. Reproductive hormones, including follicle-stimulating hormone (FSH) and estrogen, increased significantly ($p < 0.05$) during estrus in the diet-flushing groups. These groups exhibited earlier estrus onset, shorter estrus duration, more pronounced estrus signs, and higher cervical opening scores than the T0 group. Supplementation with 5% SBM improved reproductive performance by enhancing metabolic and hormonal profiles without inducing systemic strain.

Keywords: antioxidant biomarker; blood biochemical profile; caprine reproduction; dietary protein supplementation; nutritional flushing

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INTRODUCTION

The global dairy goat industry is rapidly expanding. In addition to providing wholesome and nutritious milk-based products, dairy goats provide sustainable livelihoods, particularly in resource-limited areas, and enable smallholders to accumulate assets. Saanen goats are one of the

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most popular goat breeds in Southeast Asia, especially in Indonesia (Sitaresmi et al., 2020; Widayati et al., 2025). The composition of goat milk, which contains beneficial nutrients such as medicines, anti-inflammatory agents, and immunomodulatory properties, and its low allergenicity compared with cow's milk, has driven increased demand for goat milk (Widayati et al., 2025).

Saanen goats originate from subtropical environments; thus, their reproductive and productive performance often declines when reared in tropical regions due to challenges in physiological adaptation (Sejian et al., 2021; Adjassin et al., 2022). This adaptation process impacts physiological stress, disrupts hormonal mechanisms, and affects the energy allocation of reproductive processes (Sejian et al., 2021; Riaz et al., 2023). This situation is intensified by farmers' poor understanding of the need for a balanced diet to support reproductive efficiency in dairy goats (Sitaresmi et al., 2020).

In smallholder dairy goat production systems in Indonesia, providing an adequate supply of soluble feed protein is essential to optimize herd productivity. This practice successfully enhances rumen microbial protein synthesis, improves dry matter (DM) digestibility, and increases both average daily gain (ADG) and milk yield (Nuswantara et al., 2024; Abdelsattar et al., 2025). However, smallholders frequently provide excessive levels of soluble protein due to a lack of precise ration formulation. This dietary surplus exceeds the goats' physiological threshold, leading to elevated blood urea nitrogen (BUN) levels that alter the uterine microenvironment and severely disrupt reproductive hormonal pathways (Sitaresmi et al., 2023; Diatmono et al., 2025). Consequently, this excessive protein intake triggers a cascade of reproductive inefficiencies, including sub-estrus, elevated service-per-conception (S/C) rates, and prolonged kidding intervals. Because a timely pregnancy is biologically required to re-initiate peak lactation, these reproductive delays inadvertently force goats into extended, low-yielding late-lactation phases (Riaz et al., 2023). Protein over-supplementation paradoxically culminates in a sharp decline in cumulative milk yield and severe economic losses due to inflated feed costs, repetitive breeding expenses, and a reduced kid crop (Adjassin et al., 2022; Diatmono et al., 2025).

Reproductive challenges arising from adaptation and nutrient deficiencies in Saanen goats can be mitigated through balanced protein supplementation, particularly during critical periods such as the estrous phase. Dietary flushing of protein-rich feed during crucial reproductive periods can address these issues by supporting cellular regeneration, enzyme formation, and reproductive hormone synthesis, thereby enhancing mating success and fertilization (Fazel and Kia, 2014; Sitaresmi et al., 2020). Compared with energy-only flushing, protein flushing provides superior reproductive outcomes by delivering essential amino acids that are critical for folliculogenesis and embryo viability (Fazel and Kia, 2014). Furthermore, protein- and nutrient-rich flushing during the mating period significantly improves the body condition score (BCS) and maternal fertility of does (Sitaresmi et al., 2023). In this context, soybean meal (SBM) serves as an ideal protein source because of its high crude protein (CP) and energy contents, balanced amino acid profile, and favorable ruminal degradability, which collectively enhance nitrogen utilization and reproductive efficiency (Banaszkiewicz, 2011; Abdelsattar et al., 2025; Kim et al., 2025).

In addition to its superior nutritional content, SBM offers additional advantages, including high digestibility, abundance, relatively stable quality, and non-competition with human food needs (Banaszkiewicz, 2011; Oviedo-Rondón et al., 2024). SBM also contains phytoestrogen compounds, which are non-steroidal substances that exhibit estrogenic effects on the central nervous system and can bind to estrogen receptors, thereby inducing estrus and stimulating reproductive tract development in does at adequate levels (Wyse et al., 2022). A high-protein diet generally enhances the antioxidant capacity of ruminants, particularly in rumen tissue, by increasing the activity of key antioxidant enzymes. A previous study reported that a high-protein intake in sheep significantly increased superoxide dismutase (SOD) production in blood plasma (Wu et al., 2025a).

Incorporating targeted nutritional strategies within an estrus synchronization context offers a promising approach to mitigating reproductive bottlenecks. The administration of strategic, time-restricted high-protein diet flushing in conjunction with hormonal synchronization protocols, such as controlled internal drug release (CIDR) and prostaglandin F2α (PGF2α), is expected to stabilize reproductive hormone fluctuations and induce synchronous estrus symptoms (Arya et al., 2023; Wittayarat et al.,

2025). High-protein flushing using SBM can safely optimize the physiological status of does when applied within a specific timeframe, specifically spanning 14 days pre- to 14 days post-mating (Wyse et al., 2022; Wu et al., 2025a), thereby supporting early pregnancy, preventing embryonic loss, and promoting placental development (Herring et al., 2018; Sitaresmi et al., 2023) without triggering the chronic toxic effects of protein surplus.

However, a critical research gap remains: while general dietary manipulation and standard synchronization protocols are well documented, the optimal dosage of duration-specific SBM supplementation in an estrus synchronization context has not been established for temperate Saanen goats reared in challenging tropical environments. Therefore, this study aimed to evaluate the efficacy of two distinct dietary dosages of a 28-day targeted high-protein flushing regimen applied within a CIDR-PGF2α synchronization framework to improve reproductive performance in Saanen does, while ensuring it does not compromise systemic physiological functions, as evaluated through blood metabolites, plasma antioxidant activity, reproductive hormone profiles, and estrus expression.

MATERIALS AND METHOD

Ethics approval and environmental conditions

The entire data collection procedure, including animal maintenance, estrus observation, and blood sampling, was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (No. 3/EC-FKH/int./2024). All animals were housed individually in untethered pens at the Center for Livestock Development to facilitate free movement and minimize environmental stress. Each pen (0.8 m × 1.7 m) was equipped with a feed and water trough to prevent competition among the animals. The does were confirmed to be free of reproductive diseases through a veterinary examination before and throughout the experimental period. The study was conducted under tropical environmental conditions (110°23’11.0” E, 7°46’07.7” S), with climate data provided by the Meteorology, Climatology, and Geophysics Agency (BMKG, 2024), as illustrated in Figure 1.

Animals used and experimental design

Forty-two multiparous Saanen does (parity > 2), with a mean BCS of 2.73±0.29, and an average body weight (BW) of 42.32±4.81 kg, were used in this study. The selected does were non-lactating, exhibited a non-seasonal estrous reproductive cyclicity, and were fully acclimated to the constant photoperiod of a tropical region (Sleman Regency, Special Region of Yogyakarta, Indonesia). Using a completely randomized design (CRD), the animals were assigned to one of three high-protein flushing diet treatment groups (n = 14 per group), evenly balanced based on baseline BCS and BW. Breeding management, estrus synchronization, artificial insemination (AI) technique, husbandry practices, and environmental conditions were uniform across the groups. Blood samples were prepared, and vaginal epithelial cells were observed at the Laboratory of Animal Physiology and Reproduction. Blood biochemical profiles were analyzed at the Laboratory of Tropical Animal Research Center, Faculty of Animal Science, Universitas Gadjah Mada. Figure 2 shows the comprehensive experimental timeline and procedures used in this study.

Feeding and BCS assessment

Feed was administered twice daily at 08:00 AM and 02:00 PM in a total mixed ration (TMR). The basal diet consisted of commercial concentrates and forage in a ratio of 30:70, comprising Napier grass (*Pennisetum purpureum*) and dried Indigofera (*Indigofera zollingeriana*), and was provided according to the National Research Council (NRC, 2007). Water was provided *ad libitum*. Basal feeding was conducted for 2 weeks before treatment as a feed adaptation period. Subsequently, SBM (Sari Rosa Asih Co., Indonesia) supplementation was provided at levels of T0 (basal feed or control), T1 (T0+5% SBM), and T2 (T0+15% SBM) of the total DM requirements.

Supplementation levels of 5% and 15% were designed to evaluate the effects of a contrasting SBM range. The 5% level was selected to observe responses at a lower inclusion rate, whereas the 15% level was used to test the upper threshold for animal safety without inducing toxic effects. Supplementation was administered during a 28-day diet-flushing period, including 14 days pre-AI and 14 days post-AI. Feed ingredients were analyzed via proximate analysis according to the Association of Official Agricultural Chemists (AOAC, 2005) to determine nutrient content and provide the basis for feed formulation. Feed intake was monitored by subtracting the residual feed from the total daily feed offered. Table 1 shows

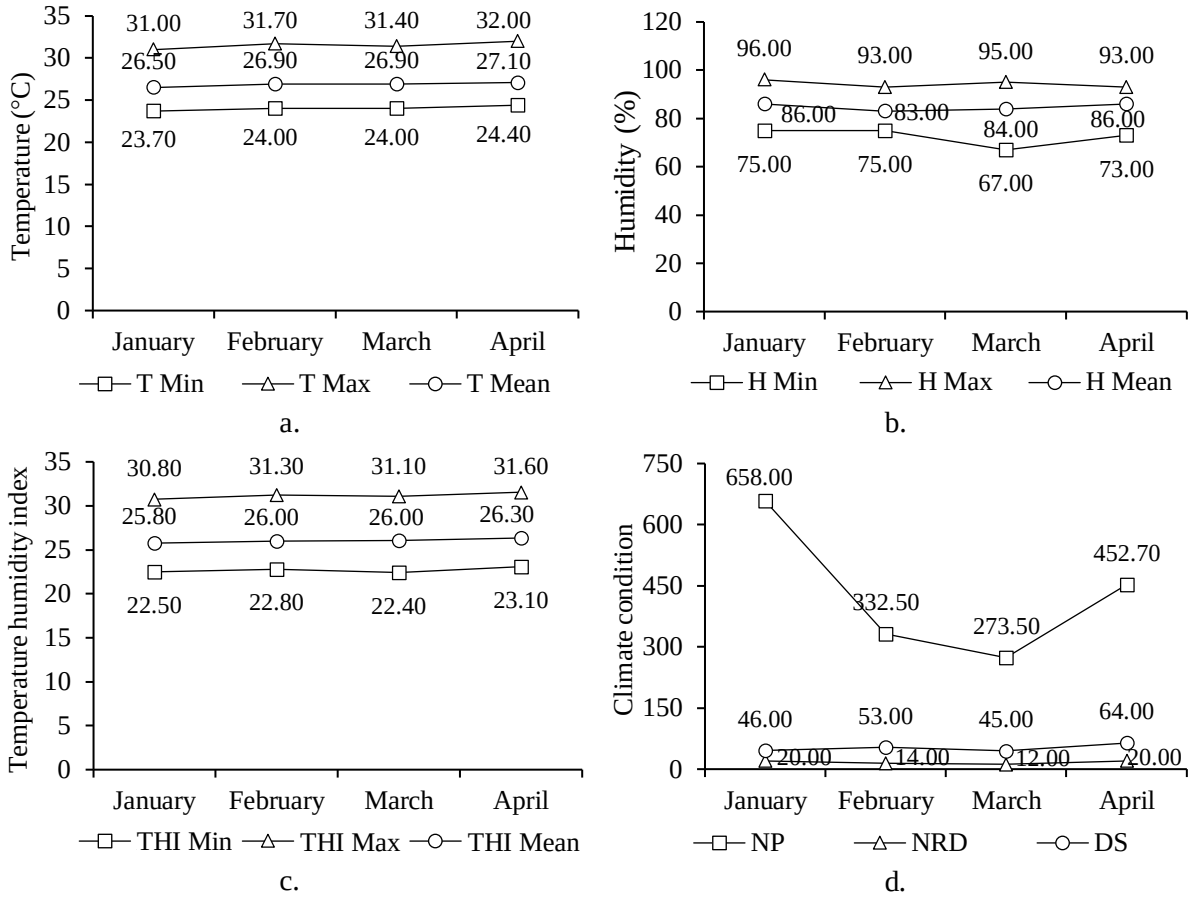

Figure 1. Environmental data for Sleman Regency from January to April 2024, (a) temperature, (b)humidity, (c) temperature humidity index, and (d) climate condition, obtained from the Meteorology, Climatology, and Geophysics Agency

Note: Max. = maximum monthly average; Mean = monthly average; Min. = minimum monthly average; NP = number of precipitation (mm per month); NRD = number of rainy days (days); DS = duration of sunshine (hours)

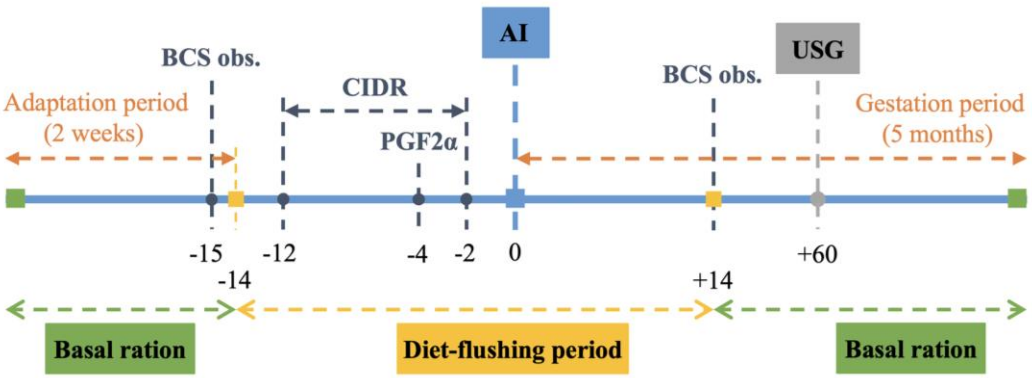

Figure 2. Timeline and procedures of the experiment, with numbers denoting day timepoints

Note: AI = artificial insemination; BCS obs. = body condition score observation (before and after diet-flushing period); CIDR = controlled internal drug release; PGF2α = prostaglandin F2α; USG = ultrasonography; (-) = days pre-AI; (+) = days post-AI

the feed nutrient composition at each treatment level.

The BCS of the does was visually evaluated on a scale of 1 to 5, with increments of 0.5, according to the method described by Luridiana et al. (2025). The BCS was assessed by five observers to ensure accuracy, and their scores were averaged to determine the final BCS for each doe. These observations were performed at the end of the basal feeding period (day -15) and immediately

following the post-flushing diet period (day +14) to evaluate alterations in BCS values.

Estrus synchronization protocol

The Saanen does were subjected to estrus synchronization using a combination of CIDR with 330 mg progesterone (EAZI-BREED™, Zoetis, Australia) and an intramuscular injection of 1.5 ml PGF2α per animal containing dinoprost 5 mg ml^{-1} (Lutalyse™, Zoetis, Belgium). The CIDR device was implanted intravaginally for 10 days, initiated 2 days after the high-protein flushing diet (day -12). On day 8 of CIDR insertion (day -4), an intramuscular injection of PGF2α was administered. This timing was selected based on the anticipated decline in circulating exogenous progesterone levels that typically occurs beyond 7 days of CIDR implantation (Arya et al., 2023). The CIDR implants were subsequently removed 2 days post-PGF2α injection (day -2) to ensure complete regression of the corpus luteum (CL) and to induce a synchronized hormonal surge (Arya et al., 2023; Wittayarat et al., 2025). Following CIDR removal, the estrus response was strictly monitored.

Estrus characteristics and behavioral observation

The estrus response and its associated characteristics were monitored for 4 consecutive days following CIDR removal (from day -2 to day +1). The estrus response and intensity were evaluated using a standardized scoring system based on specific estrus characteristics, including vulvar reddening, vulvar swelling, and vaginal mucus secretion. The intensity of these characteristics was scored on a scale from 1 to 3, representing none, medium, and high intensity, respectively (Magistrama et al., 2024; Diatmono et al., 2025). Estrus onset was determined via periodic visual observations conducted every 4 hours from the time of CIDR implant removal until the first appearance of estrus symptoms, with an average intensity score of > 1.5. Conversely, estrus duration was calculated as the time interval from the onset of estrus until the cessation of symptoms, defined by an average intensity score dropping below < 1.5 (Salleh et al., 2021).

Behavioral alterations and estrus symptoms were recorded immediately before AI on day 0. Behavioral changes associated with estrus, specifically bleating and tail wagging, were assessed in the absence of a male teaser. These behavioral observations were performed by monitoring each doe's activity intensity for 20 minutes (Salleh et al., 2021). Supplementary estrus symptoms, including vaginal mucus pH and vaginal temperature, were observed using the method previously described by Magistrama et al. (2024). The pH of the vaginal mucus was determined by applying a pH indicator strip directly to the vaginal mucus, and the resulting color change was compared against a standard indicator chart (MQuant®, Germany). The vaginal temperature was measured by inserting a digital thermometer (OMRON, China) into the vagina until an audible alert indicated that the temperature reading had stabilized.

Vaginal epithelial cell cytology was evaluated using the vaginal smear technique with 3% Giemsa staining (Merck, Germany), as developed by Magistrama et al. (2024). Cytological evaluation was performed by quantifying the population of superficial cells to identify the estrus phase. Cervical dilation was assessed by visualizing the cervical opening using a camera-equipped speculum. Cervical scores ranged from 1 to 3, representing a narrow cervical opening without cervical mucus, a moderate opening with minimal mucus, and a wide opening accompanied by copious mucus (Kanthawat et al., 2024).

AI procedure and post-thaw semen sampling

AI was performed two days following CIDR implant removal (day 0) using a single dose via the cervical insemination technique. The semen

Table 1. Nutrient composition across dietary treatment groups

Nutrient contents (%)	Treatment group		
	T0	T1	T2
Dry matter	61.09	62.35	64.54
Ash	12.30	12.26	12.20
Crude protein	13.51	14.93	17.40
Ether extract	2.17	2.17	2.18
Crude fiber	22.10	21.37	20.09
Nitrogen-free extract	45.52	44.98	44.05
Total digestible nutrient	59.28	60.00	61.25

Note: T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

straws originated from a single production batch of a superior Saanen buck, provided by the Ungaran Artificial Insemination Center (BIB Ungaran), Central Java. Each straw had a volume of 0.25 ml and contained approximately 30×10^6 spermatozoa. Prior to AI, a quality analysis was conducted on a sample of 10 post-thaw frozen semen straws as replicates, evaluating motility, viability, and abnormalities.

This assessment was performed to ensure that semen complied with the Indonesian National Standard (SNI, 2023) criteria. The post-thaw analysis protocols were adapted from Erlindra et al. (2026). Straws were thawed in a water bath (Memmert, Germany) at 37 °C for 30 seconds, and eosin staining (Merck, Germany) was used to assess viability and total abnormality. The analysis revealed that the used semen had a motility of 48.57±5.56%, viability of 87.71±1.77%, and abnormalities of 10.00±1.25%. The mathematical equations used to calculate these post-thaw spermatozoa quality parameters are expressed in Equations 1 and 2.

Blood sample collection and preparation

Blood samples were collected on days -15, -2, -1, 0 (AI), +1, and +14. Blood metabolite and plasma antioxidant biomarker profiling was performed on samples from days -15, 0, and +14 to evaluate the impact of dietary treatments at each specific observation period. Reproductive hormones were analyzed in samples from day -2 to +1 to assess hormonal fluctuations occurring 2 days before, on the day of, and 1 day following AI. Sampling was performed 2 hours before the morning feeding. Approximately 6 ml of blood was drawn from the jugular vein of each animal using a needle (BD Vacutainer®, Indonesia) and a holder (OneMed, Indonesia) connected to an ethylenediaminetetraacetic acid (EDTA) tube (Vaculab®, Sweden), and transported to the laboratory within 2 hours. The samples were centrifuged at 3,000 rpm for 15 minutes to separate the plasma. The plasma was aliquoted into several microtubes (±0.5 ml) and stored at -20 °C until further analysis (Sitaresmi et al., 2020; Diatmono et al., 2024).

Blood metabolite profiling

Blood metabolite profiles were determined spectrophotometrically using a semi-automated chemistry analyzer (Microlab 300; Vital Scientific, Netherlands) and commercial clinical chemistry reagent kits (Dumolab, Austria), according to the manufacturer's protocols. Each analysis used blank and standard solutions as controls to ensure the validity of the results. Total protein, glucose, and cholesterol levels were measured at a wavelength of 546 nm using the biuret, glucose oxidase-peroxidase aminoantipyrine (GOD-PAP), and cholesterol oxidase-peroxidase aminoantipyrine (CHOD-PAP) methods, respectively. Urea and BUN were assessed using the urease-glutamate dehydrogenase (urease-GLDH) method at a wavelength of 365 nm (Diatmono et al., 2025). The mathematical equations used to calculate each blood metabolite parameter are expressed in Equations 3, 4, 5, 6, and 7.

Plasma antioxidant and hormonal parameters

Antioxidant profiles and reproductive hormone levels were analyzed using enzyme-linked immunosorbent assay (ELISA) according

$$\text{Sperm viability (\%)} = \frac{\text{Number of live spermatozoa}}{\text{Total number of spermatozoa counted}} \times 100\% \quad (1)$$

$$\text{Sperm abnormalities (\%)} = \frac{\text{Number of abnormal spermatozoa}}{\text{Total number of spermatozoa counted}} \times 100\% \quad (2)$$

$$\text{Total protein concentration (g dl}^{-1}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 5 \text{ g dl}^{-1} \quad (3)$$

$$\text{Glucose concentration (mg dl}^{-1}) = \frac{A \text{ sample}}{A \text{ standard}} \times 100 \text{ mg dl}^{-1} \quad (4)$$

$$\text{Cholesterol concentration (mg dl}^{-1}) = \frac{A \text{ sample}}{A \text{ standard}} \times 200 \text{ mg dl}^{-1} \quad (5)$$

$$\text{Urea concentration (mg dl}^{-1}) = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 50 \text{ mg dl}^{-1} \quad (6)$$

$$\text{BUN concentration (mg dl}^{-1}) = \text{Urea} \times 0.467 \text{ mg dl}^{-1} \quad (7)$$

Where, A is the absorbance and ΔA is the change in absorbance (A1–A2 of the sample or standard).

$$\text{Pregnancy rate (\%)} = \frac{\text{Number of pregnant does}}{\text{Number of inseminated does}} \times 100\% \quad (8)$$

$$\text{Average number of fetuses} = \frac{\text{Total number of fetuses detected}}{\text{Total number of pregnant does}} \quad (9)$$

to the manufacturer's instructions (ELK Biotechnology, USA). Each assay included a blank control and 7 standards evaluated in duplicate (occupying 16 wells), while the remaining wells were used for the samples. The concentrations of SOD, glutathione peroxidase-1 (GPX1), and malondialdehyde (MDA) were determined using ELK9537, ELK9427, and ELK10930 kits, respectively. Follicle-stimulating hormone (FSH) and estrogen levels were measured using ELK7741 and ELK0296 kits, respectively. All assays were performed using a microplate reader photometer (Multiskan SKY-S1119700DP, Thermo Scientific, Germany) at a wavelength of 450 nm (Sitaresmi et al., 2020; Wu et al., 2025a). The optical density (OD) was interpolated using GraphPad Prism software version 9 (GraphPad Software, USA) to determine the final concentrations.

Determination of the pregnancy rate (PR) and number of fetuses

The PR and the average number of fetuses were evaluated 60 days post-AI using real-time transrectal ultrasonography (USG; Dramiński iScan 2, Dramiński S.A., Poland) equipped with a 7 MHz probe. Transrectal USG was selected to accommodate the does' hair density and skin thickness, thereby providing clearer and more accurate images. The parameters for each treatment group were determined according to Equations 8 and 9 (Wittayarat et al., 2025).

Statistical analysis of data

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 23 (IBM Corp., Armonk, NY, USA), with statistical significance set at $p < 0.05$. Data on blood metabolites, plasma antioxidant status, and BCS were analyzed using a two-way repeated-measures analysis of variance (ANOVA), with the diet-flushing treatment groups and observation periods as the main factors. Reproductive hormones, estrous characteristics, and feed intake data were analyzed using one-way ANOVA, with the dietary treatment group as the fixed factor, regardless of the period. Significant differences among means were further analyzed using Tukey's B post-hoc multiple comparisons test. Furthermore, the PR among treatment groups was analyzed using Fisher's exact test. Continuous data are presented as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

This study demonstrates that long-term high-protein flushing, spanning 28 days, is a highly effective nutritional strategy for optimizing the reproductive potential of Saanen does in tropical environments. A systemic improvement in physiological status was observed through the provision of a higher plane of nutrition during the mating period, as evidenced by elevated blood metabolites and superior antioxidant capacity, which directly translated into a more robust reproductive hormone profile. These physiological gains underpin the observed improvements in estrus intensity, ultimately leading to higher estrus expression and reproductive performance. These results suggest that the metabolic optimization provided by high-protein flushing is sufficient to overcome the typical reproductive challenges associated with tropical heat stress in this breed.

Feed intake, nutrient consumption, and BCS changes

Dietary flushing with high-protein feed during the flushing period significantly influenced total feed consumption and overall nutrient intake (Table 2). There were significant differences ($p < 0.05$) in the total feed and nutrient intake between the diet-flushing groups (T1 and T2) and the control group (T0) before and after AI (28 days). Flushing with a high-protein diet increased feed intake, as supported by data from the adaptation period (Table 2), which showed no significant differences among the treatment groups ($p > 0.05$). Furthermore, the high-protein flushing regimen using SBM substantially improved the BCS of Saanen does (Table 3). The initial BCS at the beginning of the study did not differ significantly across all treatment groups ($p > 0.05$). However, BCS in the T1 and T2 groups differed significantly from the adaptation period after the dietary flushing period ($p < 0.05$), whereas no significant differences were observed in the T0 group.

Diet-flushing groups consistently ate more than the control group. Their higher nutrient

Table 2. Average daily feed and nutrient intake of Saanen during the adaptation and diet-flushing periods

Observation period	Total consumption (g)	Treatment group			SEM	*p*-Value
		T0	T1	T2		
Adaptation	Feed intake	2,836.16[a]	2,871.96[a]	2,868.30[a]	39.72	0.925
	DM	1,732.72[a]	1,754.59[a]	1,752.36[a]	24.26	
	Ash	213.19[a]	215.88[a]	215.61[a]	2.98	
	CP	234.14[a]	237.09[a]	236.79[a]	3.27	
	EE	37.52[a]	37.99[a]	37.94[a]	0.52	
	CF	382.98[a]	387.81[a]	387.32[a]	5.36	
	NFE	788.65[a]	798.61[a]	797.59[a]	11.04	
	TDN	1,027.23[a]	1,040.19[a]	1,038.86[a]	14.38	
Diet-flushing	Feed intake	2,641.10[a]	2,909.59[b]	3,066.43[b]	36.43	< 0.001
	DM	1,613.55[a]	1,814.21[b]	1,979.14[c]	25.37	
	Ash	198.53[a]	222.51[b]	241.39[c]	3.05	
	CP	218.04[a]	270.92[b]	344.44[c]	6.43	
	EE	34.94[a]	39.38[b]	43.15[c]	0.55	
	CF	356.64[a]	387.65[b]	397.60[b]	4.53	
	NFE	734.41[a]	816.03[b]	871.78[c]	10.66	
	TDN	956.57[a]	1,088.57[b]	1,212.25[c]	16.34	

Note: [a,b,c] = different superscripts within the same row indicate a significant difference ($p < 0.05$). DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber; NFE = nitrogen-free extract; TDN = total digestible nutrients; SEM = standard error of the mean. T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

Table 3. Influence of diet-flushing on BCS changes in the Saanen

BCS observation period	Treatment group			SEM	*p*-Treatment (T)	*p*-Period (P)	*p*-Interaction (T × P)
	T0	T1	T2				
Before (day -15)	2.73[a,x]	2.71[a,x]	2.75[a,x]	0.08	0.074	< 0.001	0.091
After (day +14)	2.78[a,x]	3.03[a,y]	3.10[a,y]	0.67			

Note: [a] = identical superscripts within the same row indicate non-significant differences ($p > 0.05$); [x,y] = different superscripts within the same column indicate a significant difference ($p < 0.05$). *p*-Interaction = probability value for the interaction effect between dietary treatment and observation period; *p*-Period = probability value for the main effect of the observation period; *p*-Treatment = probability value for the main effect of the dietary flushing treatment; SEM = standard error of the mean; T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

intake matched the high-protein flushing diets that were designed to exceed basic needs. This diet-flushing regimen is closely associated with higher energy and CP consumption (Mohsan et al., 2019; Tahuk et al., 2024). The significant increase in TDN intake during the diet-flushing period across the treatment groups directly reflects the actual amount of energy across the treatment groups during this period (Nuswantara et al., 2024; Abdelsattar et al., 2025). Furthermore, Oviedo-Rondón et al. (2024) indicated that SBM possesses an obvious apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) of 2,856 and 2,588 kcal kg^{-1}, respectively. The increase in dietary nutrient components, such as protein, was attributed to the high protein and amino acid content in SBM (Banaszkiewicz, 2011; Oviedo-Rondón et al., 2024; Kim et al., 2025), as shown in Tables 1 and 2.

These findings are consistent with previous studies indicating that elevated energy and protein intake improves productivity, specifically average ADG, in cattle and dairy goats (Nuswantara et al., 2024; Tahuk et al., 2024; Kim et al., 2025). Furthermore, ADG was positively correlated with BCS improvements (Table 3), supporting the findings of a previous study by Mohsan et al. (2019). Ultimately, optimal BCS was reported to support overall health, reproductive performance, and productivity in dairy goats (Sitaresmi et al., 2020; Luridiana et al., 2025).

Blood metabolite profiles of Saanen does fed with high-protein flushing diets

Diet-flushing significantly affected several blood metabolite parameters ($p < 0.05$), including

total protein, urea, BUN, and cholesterol levels, but had no significant effect on blood glucose (Table 4 and Figure 3). The overall means indicated that the T1 and T2 groups exhibited higher levels ($p < 0.05$) of total protein than the T0 group (Figure 3a). Urea and BUN levels in the T2 group were consistently higher than in the other groups (Figures 3b and 3c). In contrast, the average blood cholesterol levels in the T0 group were significantly higher than those in the T1 and T2 groups (Figure 3e).

The significant increase in total protein levels observed in groups T1 and T2 (Table 4 and Figure 3a) was highly dependent on dietary composition, feed quality, and amino acid absorption efficiency across the intestinal wall (Fazel and Kia, 2014). Proteins are recognized as essential cellular components involved in cell formation, enzymatic activity, structural maintenance, and signaling pathways (Hong et al., 2024). Protein involvement is critical for oocyte and embryo development, implantation, and placental formation during the reproductive phases from estrus to early pregnancy, which require cell proliferation and differentiation (Diatmono et al., 2024; Hong et al., 2024). Furthermore, protein deficiency was associated with amino acid shortages critical for gonadotropin and gonadal hormone synthesis, leading to hormonal imbalances and impaired ovarian function, which manifested as sub-estrus, weak estrus, repeat breeding, and early embryonic death (Diatmono et al., 2024; Widayati et al., 2024).

The elevated total protein levels were associated with increased total urea and BUN levels in the T2 group (Figures 3b and 3c). Higher BUN levels usually indicate either excessive protein intake or inefficient protein utilization, whereas lower levels suggest adequate protein

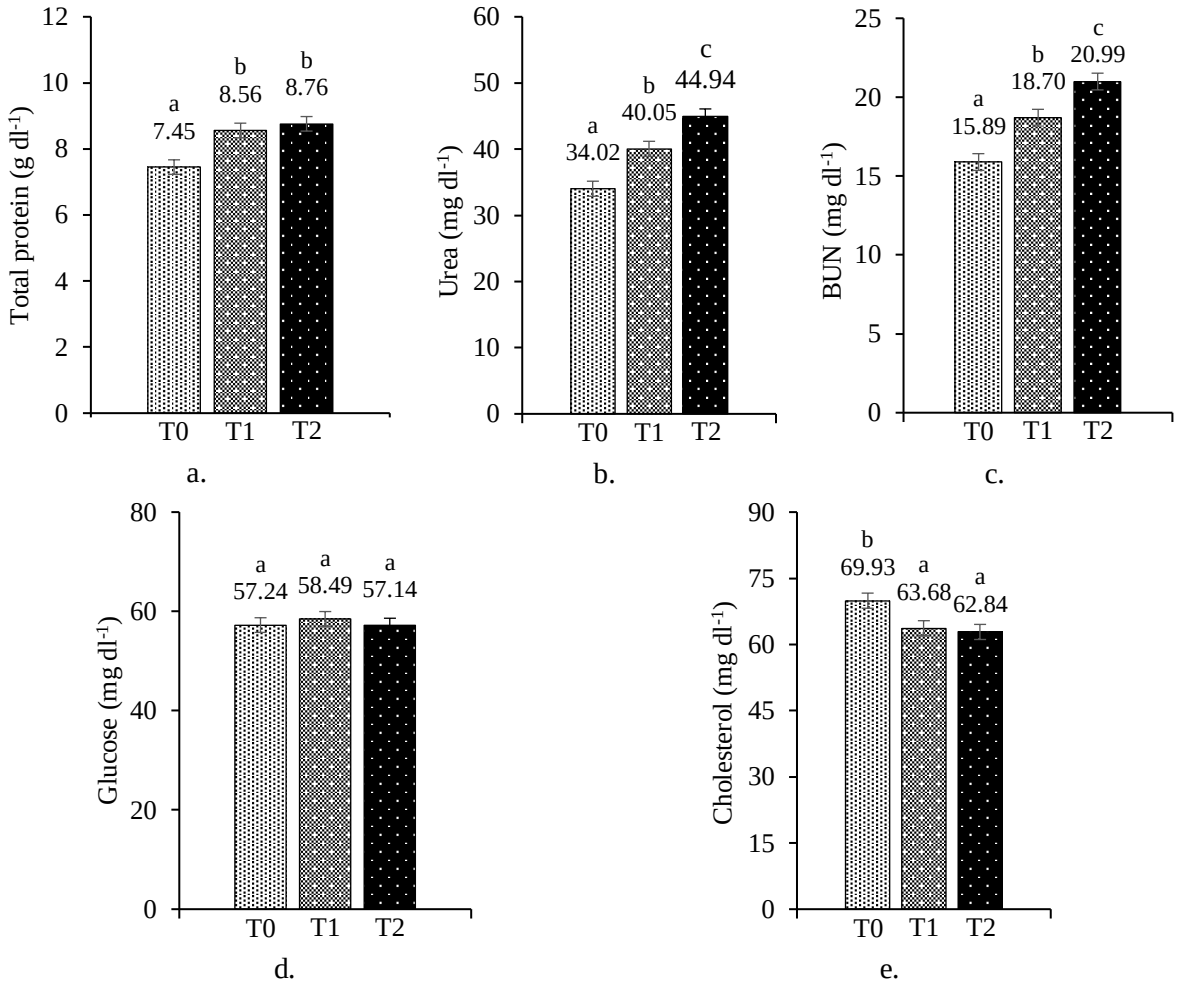

Figure 3. Mean blood metabolite concentrations of Saanen does across tratment groups, (a) total protein, (b) urea, (c) BUN (blood urea nitrogen), (d) glucose, and (e) cholesterol

Note: Values are presented as mean ± SEM of all observation periods. [a,b] = different superscripts within the same graph indicate a significant difference ($p < 0.05$). BUN = blood urea nitrogen; T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

Table 4. Changes in blood metabolite concentrations of Saanen does during different observation periods

Blood metabolite profile	Observation period	Treatment group			SEM	*p*-Treatment (T)	*p*-Period (P)	*p*-Interaction (T × P)
		T0	T1	T2				
Total protein (g dl^{-1})	Adaptation (day -15)	$7.23^{a,x}$	$7.48^{a,x}$	$7.11^{a,x}$	0.39	< 0.001	< 0.001	0.001
	AI (day 0)	$7.76^{a,x}$	$8.56^{ab,xy}$	$9.01^{b,y}$	0.26			
	Post-AI (day +14)	$7.37^{a,x}$	$9.66^{b,y}$	$10.17^{b,z}$	0.40			
Urea (mg dl^{-1})	Adaptation (day -15)	$33.89^{a,x}$	$35.42^{a,x}$	$34.43^{a,x}$	1.92	< 0.001	< 0.001	0.007
	AI (day 0)	$33.18^{a,x}$	$38.70^{a,xy}$	$48.45^{b,y}$	2.42			
	Post-AI (day +14)	$34.99^{a,x}$	$46.04^{b,y}$	$51.95^{b,y}$	2.47			
Blood urea nitrogen (mg dl^{-1})	Adaptation (day -15)	$15.82^{a,x}$	$16.54^{a,x}$	$16.08^{a,x}$	0.90	< 0.001	< 0.001	0.007
	AI (day 0)	$15.49^{a,x}$	$18.07^{a,xy}$	$22.62^{b,y}$	1.13			
	Post-AI (day +14)	$16.34^{a,x}$	$21.50^{b,y}$	$24.26^{b,y}$	1.15			
Glucose (mg dl^{-1})	Adaptation (day -15)	$55.98^{a,x}$	$56.07^{a,x}$	$54.68^{a,x}$	2.61	0.773	0.114	0.596
	AI (day 0)	$54.04^{a,x}$	$59.52^{a,x}$	$55.21^{a,x}$	2.69			
	Post-AI (day +14)	$61.70^{a,x}$	$59.89^{a,x}$	$61.54^{a,x}$	2.07			
Cholesterol	Adaptation (day -15)	$46.06^{a,x}$	$47.68^{a,x}$	$45.14^{a,x}$	2.36	0.011	< 0.001	0.015
	AI (day 0)	$83.65^{b,y}$	$70.91^{a,y}$	$73.24^{a,y}$	2.65			
	Post-AI (day +14)	$80.11^{b,y}$	$72.45^{a,y}$	$70.14^{a,y}$	2.02			

Note: ^{a,b} = different superscripts within the same row indicate a significant difference ($p < 0.05$); ^{x,y,z} = different superscripts within the same column indicate a significant difference ($p < 0.05$). *p*-Interaction = probability value for the interaction effect between dietary treatment and observation period; *p*-Period = probability value for the main effect of the observation period; *p*-Treatment = probability value for the main effect of the dietary flushing treatment; SEM = standard error of the mean; T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

consumption or reduced nitrogen metabolism (Sitaresmi et al., 2023; Widayati et al., 2024). BUN originates from ruminal feed degradation and protein metabolism, in which amino acids produced by fermentation are used for microbial protein synthesis, generating ammonia (NH_3) as a byproduct (Arias-Islas et al., 2020). Non-optimal BUN concentrations were linked to adverse reproductive outcomes, as excessive BUN increased urea concentrations in follicular and uterine fluids, altered estrogen and progesterone levels, and caused embryo loss (Sitaresmi et al., 2023; Diatmono et al., 2024; Widayati et al., 2024). Furthermore, elevated BUN levels were associated with decreased uterine pH, impaired embryo implantation, reduced fertility and pregnancy rates, and increased incidence of repeat breeding (Herring et al., 2018; Hudaya et al., 2020).

High-protein feed treatments did not significantly alter glucose levels (Table 4 and Figure 3d), as the body employs complex metabolic regulation to ensure glucose homeostasis, which is regulated by insulin and glucagon (Abbas et al., 2020; Widayati et al., 2024). Glucose absorption in ruminants tends to be lower than that in monogastric animals, as the primary energy source for ruminants is volatile fatty acid (VFA) produced from rumen fermentation (Sitaresmi et al., 2023; Diatmono et al., 2024). Furthermore, variations in glucose levels are more significantly influenced by physiological states such as pregnancy, lactation, and heat stress (Abbas et al., 2020; Hudaya et al., 2020). Glucose is the primary source of cell growth, milk production, and immune activation (Abbas et al., 2020). It was also implicated in ovarian function, supporting granulosa cell (GC) activity, steroidogenic and gonadotropin hormone synthesis, and secretion (Sitaresmi et al., 2020; Widayati et al., 2024).

The significantly higher blood cholesterol levels in the T0 group compared to T1 and T2 (Figure 3e) are associated with the high-protein flushing treatment. High-protein diets have been reported to enhance metabolic efficiency in dairy goats (Sitaresmi et al., 2023) and to reduce low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels (Meena et al., 2019). Blood cholesterol, derived from cholesterologenesis, is the metabolic conversion of dietary fats into fatty acids and subsequently acetyl-coenzyme A, which serves as a crucial precursor for steroidogenesis (Meena et al., 2019; Arias-Islas et al., 2020; Brann et al., 2022). The steroid hormones estrogen and progesterone were synthesized from cholesterol via pregnenolone, formed through enzymatic cleavage of the cholesterol molecule's side chain catalyzed by cytochrome P450 (CYP450) enzyme (Brann et al., 2022; Widayati et al., 2024). Cholesterol's role as a hormonal precursor is critical for the manifestation of estrus, ovulation, and uterine preparation for pregnancy (Diatmono et al., 2024; Diatmono et al., 2025), as well as for post-estrus progesterone production necessary for the uterine environment, embryonic development, and maintaining pregnancy (Arias-Islas et al., 2020; Widayati et al., 2024).

Plasma antioxidant activity of Saanen does

High-protein flushing significantly influenced antioxidant activity parameters (Table 5 and Figure 4), including GPX1 and MDA concentrations ($p < 0.05$), but had no significant effect ($p > 0.05$) on SOD concentrations (Figure 4a). The T1 and T2 groups exhibited significantly higher ($p < 0.05$) concentrations of GPX1 enzymes than the T0 group (Figure 4b). Furthermore, the T1 group had lower MDA concentrations than the T0 group (Figure 4c). In contrast, no significant differences in MDA concentrations were observed in the T2 group compared with either the T0 or T1 groups. The elevated antioxidant activity observed in the T1 group indicates that high-protein flushing with 5% SBM effectively enhances the redox defense system of Saanen does during the peri-conception period, in accordance with a previous study by Çetin et al. (2021).

Increased levels of antioxidant enzymes, such as SOD and GPX1, are essential as a primary defense against oxidative stress (OS), as reported in previous studies by Çetin et al. (2021) and Wu et al. (2025a). As shown in Table 5, SOD levels increased in the T1 and T2 groups during the flushing period; however, no significant difference was observed in the overall mean across all periods (Figure 4a). SOD serves as the first line of defense by catalyzing the conversion of harmful superoxide radicals into oxygen (O_2) and hydrogen peroxide (H_2O_2), consistent with Sharma et al. (2011) and Wu et al. (2025b). Furthermore, SBM is characterized by high concentrations of methionine and cysteine, which serve as essential precursors for glutathione (GSH) synthesis (Krishnan and Jez, 2018). The availability of these precursors subsequently drives GPX1 activity (Figure 4b), which plays a crucial role in reducing H_2O_2 to water (H_2O)

Table 5. Plasma antioxidant status of Saanen does during different observation periods

Blood metabolite profile	Observation period	Treatment group			SEM	*p*-Treatment (T)	*p*-Period (P)	*p*-Interaction (T × P)
		T0	T1	T2				
SOD (U ml^{-1})	Adaptation (day -15)	1.80^{a,x}	1.44^{a,x}	1.61^{a,x}	0.21	0.068	0.022	0.001
	AI (day 0)	1.51^{a,x}	2.13^{ab,xy}	2.65^{b,y}	0.26			
	Post-AI (day +14)	1.33^{a,x}	2.41^{b,y}	1.83^{b,x}	0.21			
GPX1 (ng ml^{-1})	Adaptation (day -15)	5.66^{a,x}	6.75^{a,x}	6.13^{a,x}	0.44	0.001	0.019	0.741
	AI (day 0)	5.94^{a,x}	7.23^{a,x}	6.75^{a,x}	0.49			
	Post-AI (day +14)	6.08^{a,x}	8.41^{b,x}	7.55^{ab,x}	0.53			
MDA (ng ml^{-1})	Adaptation (day -15)	145.51^{a,x}	137.86^{a,y}	141.34^{a,y}	9.07	0.013	< 0.001	0.248
	AI (day 0)	133.53^{b,x}	97.41^{a,x}	102.63^{a,x}	7.46			
	Post-AI (day +14)	144.36^{b,x}	109.91^{a,x}	127.01^{ab,xy}	9.45			

Note: ^{a,b} = different superscripts within the same row indicate a significant difference ($p < 0.05$). ^{x,y} = different superscripts within the same column indicate a significant difference ($p < 0.05$). SOD = superoxide dismutase; GPX1 = glutathione peroxidase-1; MDA = malondialdehyde. *p*-Interaction = probability value for the interaction effect between dietary treatment and observation period; *p*-Period = probability value for the main effect of the observation period; *p*-Treatment = probability value for the main effect of the dietary flushing treatment; SEM = standard error of the mean; T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

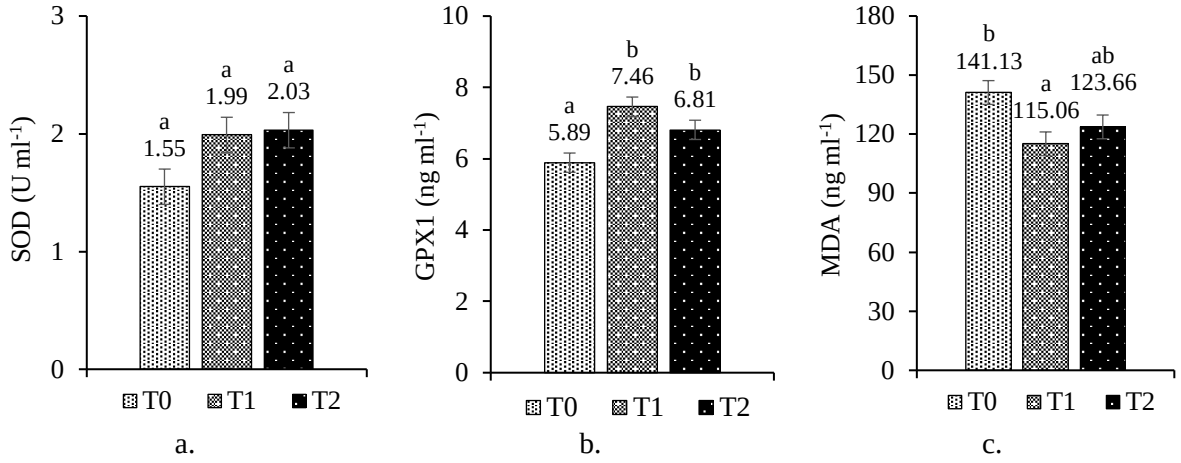

Figure 4. Mean plasma antioxidant profiles of Saanen does across treatment groups, (a) SOD (superoxide dismutase), (b) GPX1 (glutathione peroxidase-1), and (c) MDA (malondialdehyde)

Note: Values are presented as mean±SEM of all observation periods. [a,b] = different superscripts within the same graph indicate a significant difference ($p < 0.05$). T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

to prevent cellular damage (Wu et al., 2025a; Wu et al., 2025b).

Supplementation with SBM provides high concentrations of essential amino acids and isoflavone compounds, which are precursors for enzymatic protein biosynthesis and trigger endogenous antioxidant activity (Banaszkiewicz, 2011; Krishnan and Jez, 2018). Based on a previous study by Oviedo-Rondón et al. (2024), SBM exhibits an amino acid profile (expressed as a percentage of CP) consisting of 6.32% lysine, 1.35% methionine, 1.42% cysteine, 4.01% threonine, 1.44% tryptophan, 4.98% valine, 4.87% isoleucine, 7.79% leucine, 5.11% phenylalanine, 2.54% histidine, and 4.41% arginine.

Interestingly, increasing the SBM level in the T2 group did not result in higher plasma antioxidant values compared with the T1 group. On the day of AI, MDA levels in the T2 group were lower than those in the T0 group and comparable to those in the T1 group. However, by 14 days post-AI, MDA levels in the T2 group tended to increase (Table 5). This finding indicates an optimal threshold for long-term high-protein flushing, suggesting that excessive protein consumption may trigger metabolic imbalances, including elevated urea and BUN levels that subsequently suppress antioxidant enzyme efficiency (Sharma et al., 2011; Tsiplakou et al., 2018; Sitaresmi et al., 2023). Consistent with this increased enzymatic activity, the levels of MDA, as the primary marker of lipid peroxidation, reached their lowest point in the T1 group, whereas the highest concentrations were observed in the T0 group.

A previous study demonstrated that optimal nutrient intake supports cell plasma membrane integrity against the damaging effects of OS, particularly during periods of high metabolic activity such as the estrous cycle and pregnancy (Çetin et al., 2021). The elevated MDA levels in the T2 group (Table 5 and Figure 4c) suggest that excessive feed consumption significantly reduces antioxidant capacity, thereby increasing OS (Colakoglu et al., 2017; Tsiplakou et al., 2018). This mechanism is likely driven by enhanced nitrogen catabolism, which produces surplus NH_3 within the rumen and tissues (Celi and Gabai, 2015). This finding is consistent with the high urea and BUN concentrations observed in the T2 group on the day of AI (Table 4). Excessive NH_3 accumulation impairs cell membrane integrity via the OS pathway in ruminants (Celi and Gabai, 2015; Tsiplakou et al., 2018). Furthermore, a high oxidant load coupled with diminished antioxidant activity leads to poor reproductive performance, which is characterized by an increased S/C ratio and prolonged calving-to-conception intervals (Colakoglu et al., 2017).

Concentrations of circulating reproductive hormones during estrous

High-protein flushing modulated the levels of reproductive hormones (Figure 5). Significant differences ($p < 0.05$) were observed in plasma FSH levels between the pre-AI period (days -2 and -1) and the day of AI (day 0). Specifically, FSH levels in the T1 and T2 groups differed

significantly from those in the T0 group ($p < 0.05$). Additionally, plasma estrogen levels on the day of AI (day 0) were significantly higher ($p < 0.05$) in the T1 and T2 groups than in the T0 group. However, both FSH and estrogen levels subsequently decreased, showing no significant differences among the treatment groups by day +1 post-AI.

Increased FSH levels in the T1 and T2 groups on the day of AI (Figure 5a) were attributed to the positive effects of diet-flushing (Meza-Herrera et al., 2008). Sufficient protein levels are essential for ovarian follicular development, which encompasses the growth and maturation of oocyte-containing structures, due to its protein-dependent nature (Meza-Herrera et al., 2008; Arrebola et al., 2022; Sitaresmi et al., 2023). FSH concentrations increased during follicular wave emergence, peaked during estrus, and then declined post-ovulation (Arrebola et al., 2022). FSH stimulated GC proliferation and estrogen synthesis, which influenced estrus behaviors and luteinizing hormone (LH) surges for dominant follicle selection and ovulation (Khan et al., 2023). Optimal FSH levels are essential for reproductive success, with deficiencies causing smaller follicles, fewer ovulated oocytes, fertilization disruption, and reduced fertility (Arrebola et al., 2022; Khan et al., 2023).

Estrogen levels peaked highest in the T1 and T2 groups on the day of AI (Figure 5b), likely due to optimal cholesterol throughout the supplementation period, facilitated by the aromatase enzyme CYP450 (Brann et al., 2022). Estrogen, particularly estradiol (E2), was critical for estrus manifestations such as vulvar swelling, vaginal temperature increase, vaginal mucus secretion, epithelial cell changes, and behavioral signs (Magistrama et al., 2024). Nutrient adequacy influences estrogen levels, which are also modulated by blood cholesterol and glucose through the gonadotropin-releasing hormone

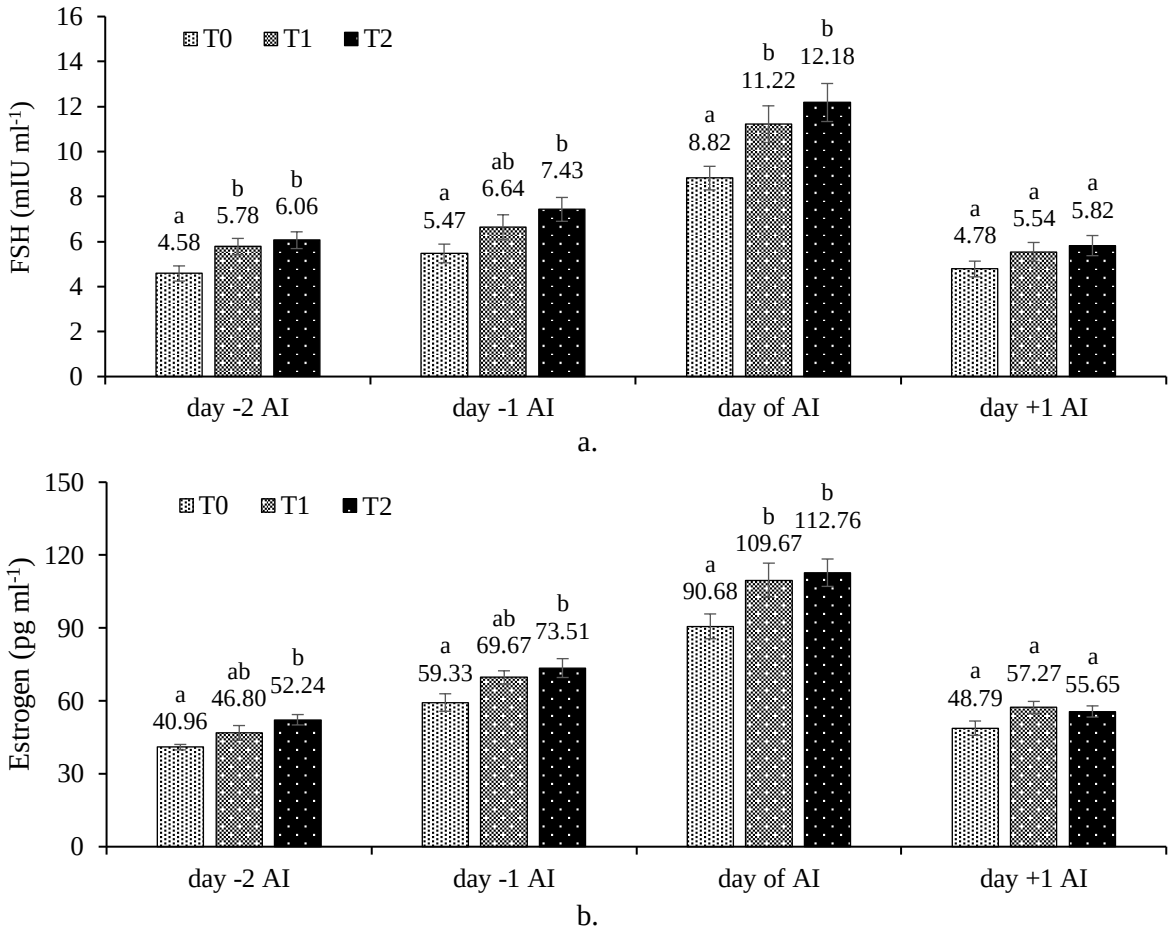

Figure 5. Reproductive hormone concentrations in Saanen does during the estrous period across treatment groups, (a) FSH (follicle-stimulating hormone) and (b) estrogen

Note: Values are presented as mean±SEM. [a,b] = different superscripts within each graph indicate significant differences ($p < 0.05$). T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

(GnRH), FSH, and LH pathways, which stimulate follicle development and estrogen production (Arrebola et al., 2022; Khan et al., 2023; Perry et al., 2023). Estrogen prepares the endometrium for embryo implantation by promoting endometrial proliferation and progesterone receptor expression during the pre-ovulation phase (Perry et al., 2023).

Estrus expression and PR of the Saanen does

All Saanen does that underwent estrus synchronization exhibited signs of estrus (100% incidence), although with varying intensities across groups (Table 6). Both the T1 and T2 groups showed significant differences ($p < 0.05$) in several estrus responses and characteristics, including estrus onset and duration, reddening and swelling of the vulva, vaginal mucus production, and cervical opening scores, when compared to the T0 group. Several estrus characteristics showed no significant differences among treatment groups ($p > 0.05$), including bleating, tail wagging, vaginal mucus pH, vaginal temperature, and superficial cell count. Furthermore, real-time transrectal USG at 60 days post-AI revealed non-significant differences in PR among the groups ($p > 0.05$), with the T1 group reaching the highest PR and the T0 group showing the lowest (Figure 6). Additionally, high-protein flushing had no significant effect ($p > 0.05$) on the number of fetuses (Table 6).

Elevated FSH and estrogen levels (Figure 5) were associated with several estrus characteristics of Saanen does (Table 6), which can be attributed to the diet-flushing intervention. Significant differences in estrus onset time and estrus duration were observed ($p < 0.05$), likely resulting from the enhanced nutrient supply (Widayati et al., 2024; Diatmono et al., 2025). Protein-sufficient feed affects complex hormonal mechanisms, thereby affecting the estrous phase (Fazel and Kia, 2014; Wyse et al., 2022). The timing and duration of estrus were considered critical for determining optimal AI timing, which supported improved insemination success rates (Arya et al., 2023; Magistrama et al., 2024). Higher estrogen levels in the T1 and T2 groups (Figure 5b) corresponded with increased vulvar color scores, vaginal mucus production, and cervical opening scores (Kanthawat et al., 2024; Diatmono et al., 2025).

Vulvar color changes (Table 6) were interpreted as manifestations of elevated circulating estrogen and FSH levels (Salleh et al., 2021; Widayati et al., 2025). The increase in follicular-phase estrogen was correlated with enhanced vaginal mucus secretion, which was attributed to the development of mucus-producing epithelial layers (Magistrama et al., 2024; Diatmono et al., 2025). In this study, estrogen concentrations were significantly elevated compared with the T0 group (Figure 5b). This increase in reproductive hormones correlates with a more pronounced and frequent expression of estrus in female goats, including vaginal vasodilation, increased blood flow in the reproductive tract, vaginal mucus production, and decreased electrical resistance of vaginal mucus, facilitating cervical canal softening and opening (Table 6) to promote sperm transport to the fertilization site (Kanthawat et al., 2024).

Table 6. Estrus expression and reproductive performance scores of Saanen does fed a high-protein flushing diet on the day of AI

Estrus expression and reproductive performance	Treatment group			SEM	*p*-Value
	T0	T1	T2		
Estrus onset (hours)	9.38^{b}	7.93^{a}	7.73^{a}	0.23	0.004
Estrus duration (hours)	48.22^{a}	53.12^{b}	55.00^{b}	4.75	< 0.001
Bleating	2.35^{a}	2.78^{a}	2.71^{a}	0.08	0.076
Tail wagging	2.42^{a}	2.75^{a}	2.78^{a}	0.08	0.161
Reddening of vulva	2.07^{a}	2.64^{b}	2.71^{b}	0.10	0.013
Swelling of the vulva	2.00^{a}	2.57^{b}	2.55^{b}	0.10	0.026
Mucus secretion	2.14^{a}	2.64^{b}	2.85^{b}	0.08	0.002
Vaginal mucus pH	8.42^{a}	8.50^{a}	8.57^{a}	0.07	0.732
Vaginal temperature (°C)	39.07^{a}	39.13^{a}	38.90^{a}	0.04	0.129
Superficial cells (%)	68.53^{a}	66.78^{a}	67.17^{a}	0.75	0.620
Cervical dilation scoring	2.28^{a}	2.82^{b}	2.85^{b}	0.08	0.008
Number of fetuses (kid)	1.50^{a}	1.62^{a}	1.60^{a}	0.13	0.959

Note: ^{a,b} = different superscripts within the same row indicate a significant difference ($p < 0.05$). SEM = standard error of the mean; T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

Several estrus parameters (Table 6) showed no significant differences among treatment groups, which can be attributed to the estrus synchronization protocol and adequate baseline nutritional intake across all groups, as shown in Table 2 (Salleh et al., 2021; Arya et al., 2023; Sitaresmi et al., 2023). These factors likely maintained circulating estrogen secretion within a uniform physiological range across the treatment cohorts (Figure 5b). Consequently, typical estrous behaviors, specifically bleating and tail wagging, were initiated before mating. In natural mating, this behavior serves as a proceptive cue to bucks before copulation (Salleh et al., 2021). Furthermore, vaginal temperature and mucus pH values did not differ significantly, further confirming that sampling occurred at the identical stage of the estrous cycle across all does (Diatmono et al., 2024; Magistrama et al., 2024). This physiological equilibrium is mirrored by the superficial vaginal epithelial cell population, which remained consistent across treatments (Table 6), as a direct result of the uniform estrogen concentration within the physiological range (Brann et al., 2022; Diatmono et al., 2025).

Based on the results (Figure 6), the PR was higher in the diet-flushing groups than in the control group, although this difference was not statistically significant. Additionally, the results presented in Table 6 show that the number of fetuses also exhibited no significant differences. Despite the lack of statistical difference ($p > 0.05$), maternal nutritional status significantly influences PR. Specifically, optimal protein intake during early pregnancy enhances embryo survival, growth, and development. This finding aligns with several previous studies, including those by Herring et al. (2018) and Sitaresmi et al. (2023), which linked amino acid availability to protein breakdown (Fazel and Kia, 2014; Leese et al., 2021; Wyse et al., 2022). Specific amino acids were required for implantation, placental development, angiogenesis, and nutrient transport (Leese et al., 2021).

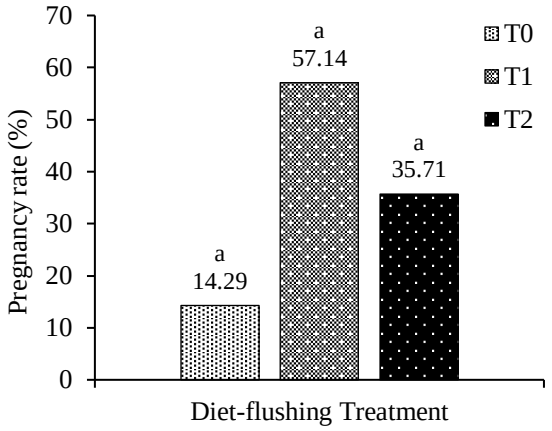

Figure 6. Pregnancy rate (PR) in Saanen does across all treatment groups (values represent the percentage of pregnant does)

Note: [a] = identical superscripts within each graph indicate non-significant differences ($p > 0.05$). T0 = basal feed (control); T1 = basal feed+5% SBM; T2 = basal feed+15% SBM

The higher PR in the T1 group than in the T0 and T2 groups may be attributed to amino acid imbalances caused by excessive protein in the T2 group and insufficient protein in the T0 group (Herring et al., 2018; Sitaresmi et al., 2023). The elevated urea and BUN levels observed in the T2 group (Table 4, Figure 3b, and Figure 3c) are suspected to be implicated in implantation failure, as high BUN can reduce uterine pH and subsequently lead to early embryonic death (Sitaresmi et al., 2023; Diatmono et al., 2024; Widayati et al., 2024).

Limitations of the study and future directions

A limitation of this study involves the selection of contrasting SBM supplementation levels, which was intentionally designed based on NRC (2007). The 5% SBM level (T1) met the lower threshold for CP supplementation recommendations, whereas the 15% level (T2) served as an upper threshold to evaluate reproductive performance under a high-protein surplus. Consequently, an intermediate 10% level was excluded because this deliberate contrast was deemed sufficient to map the nutritional response curve against the baseline. Furthermore, the overall sample size (n = 42 does; 14 animals per cohort) may have limited the statistical power required to resolve minor variations in PR and the number of fetuses. Future studies should expand the animal cohort size to strengthen the statistical robustness of maternal reproductive performance, such as conception rates and pregnancy retention. Additionally, exploring rumen digestibility and VFA profiles is recommended to clarify how this 28-day high-protein flushing modulates ruminal fermentation mechanisms. Testing intermediate protein levels (e.g., 10% SBM) would further help define the inflection point of the nutritional response curve in tropical Saanen does.

CONCLUSIONS

Long-term nutritional flushing with SBM effectively enhances estrus expression and reproductive performance in tropical Saanen does by increasing feed and nutrient intake, thereby improving blood metabolite profiles, plasma antioxidant status, reproductive hormone profiles, and estrus expression. High-protein flushing at an optimal level (5%) during the mating period facilitates superior estrus characteristics and precise timing for AI without compromising the does' physiological status. However, excessive protein supplementation elevates urea and BUN levels and may compromise antioxidant defense mechanisms during long-term administration, as evidenced by reduced GPX1 levels and increased MDA concentrations. These findings underscore the importance of balanced protein management in improving reproductive efficiency while maintaining the physiological health of Saanen dairy goats kept in tropical conditions.

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