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Effect of tannin and amino acid supplementation on growth, digestibility, and blood parameters in heifer calves

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Abstract. *Salsabila LN, Fitri A, Sitanggang FEM, Permana IG, Farida WR, Anzhany D, Ridwan R. 2025. Effect of tannin and amino acid supplementation on growth, digestibility, and blood parameters in heifer calves. Nusantara Bioscience 17: 194-201.* Nutritional management during the growth phase of heifer calves is crucial for optimal performance and health. Tannins and amino acids have shown potential benefits in ruminant nutrition through improved protein utilization and antimicrobial properties. This study aimed to evaluate the effect of tannins (1% DM), amino acids (0.3% DL-methionine + 0.7% L-lysine), and their mixture on heifer calves' performance and metabolic indicators. To achieve the objective, a 4 × 4 Latin square design was used with 4 calves (109.00 ± 18.17 kg of body weight), each receiving 4 treatments in 4 periods. The treatment period lasted 14 days, including 10 days for diet adaptation and 4 days for sample collection. The basal diet was a Total Mixed Ration (TMR) consisting of 20 kg/d of forage and 2 kg/d of concentrate. Treatments included: P1 (control, TMR only), P2 (TMR + 1% dry matter intake (DMI) tannin), P3 (TMR + 1% DMI amino acids), and P4 (TMR + 1% DMI tannin and 1% DMI amino acids). The results showed that individual supplementation in P2 (0.66 kg/h/d) and P3 (0.73 kg/h/d) had higher Average Daily Gain (ADG) compared to P1 (0.54 kg/h/d) and P4 (0.41 kg/h/d) ($p < 0.05$). Feed efficiency in P2 (2.65%) and P3 (3.02%) is higher than in P1 (2.49%) and P4 (1.74%) ($p < 0.05$). Although nutrient intake did not differ, Crude Protein (CP) intake in the P1 (0.32 kg/day) was lower than in the P2 (0.44 kg/day), P3 (0.39 kg/day), and P4 (0.40 kg/day) ($p < 0.05$). Plasma protein and cholesterol levels were lower in the P1 (7.07 g/dL; 94.14 mg/dL) than those (7.46-7.62 g/dL; 125.98-149.85 mg/dL, respectively) ($p < 0.05$). Overall, supplementation of 1% DMI tannin or amino acids alone improved the performance without compromising hematological profiles and blood metabolites.

Keywords: Amino acids, blood protein, calves, performance, tannin

INTRODUCTION

Efficiently managing dairy calves, especially in milk production, is crucial for the long-term success of dairy farming (van Niekerk et al. 2020). The importance of managing replacement heifers is often overlooked, especially in high mortality and morbidity rates. Mortality rates of calves can be substantially high, particularly in developing areas, with approximately 35% (Li et al. 2011). High morbidity and mortality rates cause substantial economic losses, contradicting animal welfare and food safety. Growth and mortality rates in calves have proven to be suitable indicators for health assessment. High growth rates also show proper nutrition and feeding, as insufficient nutrition will inhibit immunity, leading to disease and mortality (Tautenhahn et al. 2020). In Indonesia, the yearly rate of calf births ranges from 52-67%, while calf mortality rates range from 8 to 48%, deemed very high (Talib et al. 2003). Common health issues in calves include respiratory diseases, gastrointestinal disorders, and nutritional deficiencies, which can lead to stunted growth and increased mortality rates (McGuirk and Peek 2014).

Nutritional management is one of the primary concerns in the growth phase of the heifer calves. This is because insufficient nutrition can inhibit the growth rate, disrupt reproductive growth, and hinder the immunity of the calves (Tautenhahn et al. 2020; van Niekerk et al. 2020). The transition to solid feed has been proven effective through significant metabolic changes. For instance, calves must adapt from a carbohydrate-rich diet to one that includes more complex nutrients, which affects their blood biochemical profiles and overall health (Nagy et al. 2014; Khan et al. 2016). Around 4 to 6 weeks, ruminal fermentation and absorption will develop in calves (Mirzaei-Alamouti et al. 2025). Moreover, calves weaned early without adequate solid feed intake tend to reduce growth rates, increase susceptibility to illness, and impact overall digestive health (Bhatti et al. 2012; Benetton et al. 2019; Hao et al. 2021). The stress associated with weaning and dietary changes is inevitable. Therefore, effective management of nutrition strategies is essential to mitigate risks and promote better health outcomes for calves.

Feed supplements can enhance performance and increase livestock growth (Abitante et al. 2024). Tannins and amino acids have beneficial effects on calves' health and performance. Tannins are a heterogeneous group of polyphenols, serving as secondary metabolites in plants that are synthesized in response to biotic and abiotic stressors. These compounds also have properties such as antioxidant, antimicrobial, anthelmintic, anti-inflammatory, and antimethanogen (Fraga-Corral et al. 2021; Fitri et al. 2022;

Boukrouh et al. 2024). Previous studies explained that supplementing tannin in ruminant diets with moderate levels (0.2-0.6% dry matter (DM) in steers and 10 g/day in calves) has been associated with improved performance and reductions in both gastrointestinal parasitism and nitrogen pollution (Dell'Anno et al. 2024). Moreover, the addition 1% chestnut-tannin extract in piglets' diets and 6 g/day of tannin extract in preweaning calves' diet improved gut health through antibacterial properties and reduced incidence of diarrhea, whereas feed intake and the average daily gain were unaffected (Girard et al. 2018; Dell'Anno et al. 2024). Tannin supplementation has also been reported to improve growth rates in post-weaning calves by enhancing the colonization of beneficial microbiota that play a key role in the development and function of the gastrointestinal tract, which is subsequently associated with increased body weight and improved feed efficiency in growing ruminants (Hassan et al. 2020; Al Rharad et al. 2025). Feed processing techniques have been developed to minimize undegradable protein and increase the direct delivery of amino acids to the small intestine (Mirzaei-Alamouti et al. 2025), particularly using tannin to protect amino acids.

Essential amino acids, such as lysine and methionine, are crucial for protein synthesis and overall growth (Montout et al. 2021). Supplementing diets with lysine and methionine enhanced growth performance and immune function, reducing the incidence of illness in livestock (Montout et al. 2021). However, Silva et al. (2021) found that lysine and methionine (17 and 5.3 g/d) supplemented in the solid concentrate calves' diet detrimentally affect the animals' performance and metabolism. This might be because the amino acids are quickly degraded in the rumen, causing inefficient absorption (Schwab and Broderick 2017).

Although the potential benefits of tannin and amino acids supplementation have been investigated, a limited number of studies have investigated the combination of tannin and amino acids in post-weaned calves. Tannins can bind proteins and form tannin-amino acid complexes, reducing excessive protein degradation in the rumen and improving protein utilization. Under the acidic conditions of the abomasum, these complexes dissociate, increasing the amount of bypass protein that reaches the small intestine (Lorenz et al. 2014).

Based on the background above, this study aimed to address the knowledge gap by assessing the influence of tannin, amino acids, and their combination on performance, nutrient intake, digestibility, and blood metabolites in heifer calves. It was hypothesized that the supplementation of tannin, amino acids, and their mixture would increase average daily gain, protein digestibility, and immune profile, as shown by the blood metabolites.

MATERIALS AND METHODS

Experimental design and animals

Four Friesian Holstein post-weaned heifer calves (109.00 ± 18.17 kg of live weight, aged 6 to 7 months) were used for the experiment conducted at PT Sumber Citarasa Alam, Ciawi, Bogor, Indonesia. The animal used in this experiment was approved by the Animal Care and Use Committee of the Ethical Clearance, BRIN (Number: 004/KE.02/SK/01/2024). A Latin square design with 4 treatments and 4 replications was used. Each experimental period consisted of 10 days for diet adaptation and 4 days for data and sample collection. The sample size was determined based on data from the previous period of calves.

Treatment and feeding management

Calves were given a Total Mixed Ration (TMR) consisting of 20 kg/day of fresh forage and 2 kg/day of commercial concentrate (as fed basis). Diets were offered twice daily at 0900 and 1700, with ad libitum access to drinking water. The diets were supplemented with tannin, amino acids, and a mixture of tannin and amino acids. The experimental treatments were: P1: Control ration (TMR only), P2: TMR + 1% tannin (DM of diet), P3: TMR + 1% amino acids (0.3% DL-methionine and 0.7% L-lysine, DM of diet), P4: TMR + 1% tannin + 1% amino acids.

Tannin, amino acids, and a mixture of tannin and amino acids were added on top and then mixed into the TMR. The tannin used was a commercial extract from the Chestnut tree (Hydrolyzable tannin, Saviolife, Italy), while the amino acids were DL-methionine and L-lysine (PT Cheil Jedang, Indonesia).

Feed analysis

Representative samples of the TMR for each treatment were collected daily and composited by treatment and period. Samples were dried at 60°C for 48 hours and analyzed for dry matter (DM), organic matter (OM), ash, crude protein (CP), ether extract (EE), and crude fiber (CF) according to AOAC (1995) procedures. The nutrient composition of rations is presented in Table 1.

Growth performance

Growth performance in this study was evaluated based on average daily gain (ADG) and feed efficiency. The calves were weighed on 1 and 14 of each period (14-day intervals), before their morning feeding using a digital livestock scale. Average daily gain (ADG) was calculated using the following formula:

ADG (kg/head/day) = [final weight (kg) - initial weight (kg)] / 14 days

Table 1. Composition of the nutrient composition of the ration

Nutrient composition	P1	P2	P3	P4
Dry matter (DM, %)	21.25	20.18	24.65	22.53
Organic matter (% DM)	86.40	86.43	83.32	86.88
Ash (% DM)	13.60	13.57	16.68	13.12
Crude protein (% DM)	10.02	12.54	12.58	12.99
Ether extract (% DM)	1.13	0.83	0.84	0.87
Crude fiber (% DM)	26.12	28.91	29.05	29.04

Note: P1: control (TMR only), P2: TMR + 1% tannin (DM of diet), P3: TMR + 1% amino acids (0.3% DL-methionine and 0.7% L-lysine, DM of diet), P4: TMR + 1% tannin + 1% amino acid supplementation

While feed efficiency was calculated using the following formula:

Feed efficiency (%) = ADG (kg/head/day) / dry matter intake (kg/day)

Nutrient intake and digestibility

Offered feed and feces of individual calves were weighed and sampled every day from day 10 to 14 on each experimental period (5-day collection at the end of each period). Fecal samples were collected daily (24-hour collection) and 10% composited by treatment and period. All samples were dried in the oven at 60°C for 48 hours, ground finely, and kept until analyzed. The samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and crude fiber (CF) content using the in vitro method (AOAC 1985). Subsequently, nutrient intake was calculated based on the feed intake and nutrient composition, following the formula below:

Nutrient intake (kg/head/day) = [%nutrient in feed × feed offered (kg/day)] - [%nutrient in refusal × feed refused (kg/day)]

While the following formula calculates nutrient digestibility:

Nutrient digestibility (%) = [(nutrient intake - nutrient in fecal) / nutrient intake] × 100%

The nutrients intake and digestibility measured included the DM, OM, CP, EE, and CF.

Blood metabolites and haematological

Blood samples were collected on day 14 in an EDTA vacuum blood collection tube, which was stored in a cooler box container for a while and brought to the laboratory for analysis. Haematological analysis measured were erythrocytes, hemoglobin, hematocrit, and leukocytes. Initially, blood tests were performed using the automated hematology analyzer (NIHON KOHDEN CelltacEs Automated Hematology Analyzer, Japan), which took approximately 18 to 27 μL. Blood metabolites analysis measured in this experiment were glucose, triglyceride, blood urea nitrogen (BUN), cholesterol, and protein. The analysis was calculated based on the general procedure of KIT reagents. Samples were analyzed using a colorimetric enzymatic method with KIT reagents brand GLORY DIAGNOSTICS (Spain). After obtaining the absorbance, the blood metabolite value was calculated by dividing the sample absorbance value by the standard absorbance value.

Statistical analysis

The study used a Latin square design with 4 treatments and 4 replications. Data were analyzed using the Analysis of Variance (ANOVA) method with SAS software, followed by Duncan's Further Test. Significance was declared at $p < 0.05$ and trends toward a significant difference at $0.05 < p < 0.10$.

RESULTS AND DISCUSSION

Performance of the calves

Table 2 shows a significant difference in average daily gain (ADG) and feed efficiency ($p < 0.05$). Based on the results, supplementation of tannin (P2) and amino acid (P3) had a higher ADG and feed efficiency than the control, with the highest values found in the P3 treatment. Meanwhile, the P4 treatment had the lowest ADG and feed efficiency compared to other treatments. Tannin and amino acid supplementation did not affect the fresh daily intake, which ranged between 13.65 and 17.14 kg/head/day, and there was no significant difference between treatments.

Nutrient intake

As shown in Table 3, the treatments did not affect intake of DM, OM, EE, and CF ($p > 0.05$). However, CP intake was significantly higher in P2 (0.44 kg/d), P3 (0.39 kg/d), and P4 (0.40 kg/d) compared to the control (P1: 0.32 kg/d, $p < 0.05$).

Table 2. Performance in female Friesian Holstein dairy calves

Parameters	Treatment				SEM	*p-value*
	P1	P2	P3	P4		
Average daily gain (kg/head/day)	0.54[ab]	0.66[bc]	0.73[c]	0.41[a]	0.04	0.015
Fresh daily intake (kg/head/day)	14.86	17.14	13.65	14.68	0.49	0.086
Feed efficiency (%)	2.49[ab]	2.65[b]	3.02[b]	1.74[a]	0.17	0.036

Note: Means in the same row with different superscripts differ significantly ($p < 0.05$); Note: P1: control (TMR only), P2: TMR + 1% tannin (DM of diet), P3: TMR + 1% amino acids (0.3% DL-methionine and 0.7% L-lysine, DM of diet), P4: TMR + 1% tannin + 1% amino acid supplementation, SEM: Standard Error Mean

Table 3. Nutrient intake in female Friesian Holstein dairy calves

Parameters (kg/head/day)	P1	P2	P3	P4	SEM	*p-value*
Dry matter intake	3.16	3.51	3.37	3.31	0.09	0.584
Organic matter intake	2.73	3.04	2.80	2.87	0.08	0.559
Crude protein intake	0.32[a]	0.44[b]	0.39[b]	0.40[b]	0.01	0.021
Ether extract intake	0.04	0.03	0.03	0.03	0.00	0.079
Crude fiber intake	0.82	1.02	0.98	0.96	0.03	0.118

Note: Means in the same row with different superscripts differ significantly ($p < 0.05$); Note: P1: control (TMR only), P2: TMR + 1% tannin (DM of diet), P3: TMR + 1% amino acids (0.3% DL-methionine and 0.7% L-lysine, DM of diet), P4: TMR + 1% tannin + 1% amino acid supplementation, SEM: Standard Error Mean

Table 4. Digestibility nutrient in female Friesian Holstein dairy calves

Parameters (%)	P1	P2	P3	P4	SEM	*p-value*
Dry matter digestibility	68.91	64.90	68.55	60.25	1.86	0.262
Organic matter digestibility	72.88	70.17	72.78	64.40	1.66	0.164
Crude protein digestibility	58.79	66.70	68.76	67.06	2.13	0.216
Ether extract digestibility	66.40	66.04	72.10	58.08	2.44	0.308
Crude fiber digestibility	74.56	68.62	69.14	61.62	1.88	0.081

Note: Means in the same row with different superscripts differ significantly ($p < 0.05$); Note: P1: control (TMR only), P2: TMR + 1% tannin (DM of diet), P3: TMR + 1% amino acids (0.3% DL-methionine and 0.7% L-lysine, DM of diet), P4: TMR + 1% tannin + 1% amino acid supplementation, SEM: Standard Error Mean

Table 5. Hematology in female Friesian Holstein dairy calves

Parameters	P1	P2	P3	P4	SEM	*p-value*	Normal range*
Erythrocytes (10^6/μL)	9.74	9.32	9.72	10.65	0.40	0.521	5.0-10.0
Leukocytes (10^3/μL)	12.81	22.75	22.71	31.91	2.79	0.068	4.0-12.0
Haemoglobin (g%)	9.28	9.91	9.54	10.53	0.35	0.487	8.0-15.0
Haematocrit (%)	34.48	33.09	35.64	38.91	1.12	0.391	24.0-46.0

Note: Means in the same row with different superscripts differ significantly ($p < 0.05$); *) Normal ranges according to Latimer et al. (2011), P1: control (TMR only), P2: TMR + 1% tannin (DM of diet), P3: TMR + 1% amino acids (0.3% DL-methionine and 0.7% L-lysine, DM of diet), P4: TMR + 1% tannin + 1% amino acid supplementation, SEM: Standard Error Mean

Table 6. Blood metabolites in female Friesian Holstein dairy calves

Parameters	P1	P2	P3	P4	SEM	*p-value*	Normal range*
Glucose (mg dL^{-1})	86.10	85.40	88.51	84.70	1.34	0.749	40.0-100.0
Triglyceride (mg dL^{-1})	29.41	27.39	27.02	29.04	1.04	0.889	17.0-30.0
BUN (mg dL^{-1})	29.44	20.97	29.38	32.74	2.03	0.388	10.0-25.0
Protein (g dL^{-1})	7.07[a]	7.58[b]	7.46[b]	7.62[b]	0.07	0.012	6.0-8.0
Cholesterol (mg dL^{-1})	94.14[a]	125.98[b]	138.59[bc]	149.85[c]	5.98	0.001	80.0-170.0

Note: Means in the same row with different superscripts differ significantly ($p < 0.05$); *) Normal ranges according to Latimer et al. (2011) and Mitruka et al. (1977); BUN: blood urea nitrogen; P1: control (TMR only), P2: TMR + 1% tannin (DM of diet), P3: TMR + 1% amino acids (0.3% DL-methionine and 0.7% L-lysine, DM of diet), P4: TMR + 1% tannin + 1% amino acid supplementation; SEM: Standard Error Mean

Nutrient digestibility

Table 4 shows no significant differences in nutrient digestibility in all treatments ($p > 0.05$). However, CF digestibility was lower in P4 (61.62%) compared to other treatments ($p = 0.081$).

Hematological and blood metabolites

The treatment effect on blood hematology, including erythrocytes, leukocytes, hemoglobin, and hematocrit, is presented in Table 5. The results show no significant differences in the hematological blood ($p > 0.05$).

The effect of treatment on blood metabolites, including glucose, triglyceride, blood urea nitrogen (BUN), protein, and cholesterol, is presented in Table 6. All treatments had no significant differences in glucose, triglyceride, and BUN in all treatments ($p > 0.05$). However, blood protein levels were significantly higher in supplemented treatments (P2: 7.58 g/dL, P3: 7.46 g/dL, P4: 7.62 g/dL) compared to the control (P1: 7.07 g/dL, $p < 0.05$). Cholesterol levels were significantly elevated in all supplemented groups compared to the control ($p < 0.01$), with the highest levels observed in P4 (149.85 mg/dL), followed by P3 (138.59 mg/dL) and P2 (125.98 mg/dL), compared to P1 (94.14 mg/dL).

Discussion

Performance of the calves

Average daily gain (ADG) in this study ranged from 0.41 kg/head/day to 0.73 kg/head/day. The normal range of ADG of 6-to-7-month-old calves varied from 0.4 kg/head/day to 0.9 kg/head/day, reported by Gitau et al. (1994). The supplementation of amino acid (P3: 0.73 kg/head/day) had the highest ADG. Amino acids, particularly methionine, are crucial for the growth of all animals and basic life functions (Vázquez-Añón et al. 2006) and can increase ADG (Zhou et al. 2016). Adding amino acids lysine and methionine increased the availability of methionine and lysine in the intestine, post-ruminal segment, and the entire gastrointestinal tract (Mazinani et al. 2020), thereby contributing to increased feed efficiency and average daily gain (Zhou et al. 2016). In this study, tannin supplementation (P2: 0.66 kg/head/day) in the ration increased ADG due to improved feed efficiency. Adding tannins in ruminant feed can improve feed efficiency by increasing the amount of rumen undegradable protein (Ma et al. 2024). Tannin-protein complexes formed at near-neutral pH (3.5-7.5) help more protein avoid breaking down in the rumen and release protein at a pH less than 3.5 (Jones and Mangan 1977). Furthermore, the protein is released and digested in the small intestine (Ma et al. 2024). In previous studies, tannin supplementation in calves increased average daily gain (Soleiman and Kheiri 2018). However, supplementation with tannin-amino acids (P4) exhibited low. Supplementing tannin-amino acids in monogastrics it could decrease the absorption of essential amino acids (especially methionine) and reduce the animals' growth (Reed 1995). Moreover, tannins interfere with protein digestion by inhibiting the activity of proteases (Bhat et al. 2013; Jing et al. 2021), pectinases, amylases, cellulases, and lipases (Bhat et al. 2013), thereby decreasing nutritional digestibility in the rumen (Zhang et al. 2019a). This suggests that the rumen in young calves is still developing during the transition phase, which could make nutrient digestion less efficient than in adult ruminants (Hassan et al. 2020; Schwarzkopf et al. 2022). This could explain part of the lower ADG and feed efficiency observed in the P4.

The highest feed efficiency was found in amino acids supplementation (P3: 3.02%) and tannin supplementation (P2: 2.65%), although daily fresh intake remained relatively the same in all treatments. Based on the calculation, lower feed intake balanced with high average daily gain would lead to more feed efficiency (Krueger et al. 2010). Additionally, the presence of amino acids in the diet effectively promoted significant body weight gain, showing good feed efficiency (Niroumand et al. 2020). The increase in feed efficiency in tannin supplementation ration caused by lower protein degradation and CH_4 emissions (Orzuna-Orzuna et al. 2021). This indirectly increased the utilization of protein and energy in the calves. However, supplementation of tannin-amino acid (P4: 1.74%) reduced feed efficiency. Lower feed efficiency is indirectly caused by the formation of excessive tannins and proteins, which interfere with absorption and digestion. Incorrect levels of tannins and proteins can lead to growth suppression in livestock (Ojo 2022). Based on meta-analysis, hydrolyzable tannin (HT) has an adverse effect, causing inactivation of ruminal microorganisms, inhibiting the action of a microbial deaminase only at doses between 101 and 200 g/kg DM (Brutti et al. 2023). Still, the HT doses in this study, 0.91 g/kg, were lower and did not produce a lethal effect.

Nutrient intake

The CP intake in this study was significantly higher in the added supplementation compared to the control treatment. CP intake was linked to higher protein levels in the feed. According to Negesse et al. (2001), Katangole and Yan (2020), feeding with more protein could lead to more CP intake, and if animals eat enough, it might help with digestion, which can improve average daily gain (Etman et al. 2020). Sharma et al. (2020) also mentioned feeding more protein could help calves grow faster.

On the other hand, in P4, CP intake was higher, and ADG was lower. This was probably because of the complex formation of tannins and amino acids, which is difficult to break down. Tannins can react with many nitrogen-based compounds, including amino acids (Adamcyzk et al. 2017), and are more reactive when the amino acids have bigger molecular sizes and more amine groups (Adamcyzk et al. 2011). Lysine has two amine groups and methionine has one (Adamcyzk et al. 2017). As phenolic compounds, tannins showed the ability to bind and the high bond strength between tannin and protein (Besharati et al. 2022). In this study, different results for ADG were caused by the changes in how the supplements acted chemically. Moreover, the various levels of bond strength will modify the enzyme activity (Adamcyzk et al. 2017). Therefore, it is suggested that tannin-amino acid chemistry is crucial and requires further consideration.

Nutrient digestibility

The DM, OM, CP, EE, and CF digestibility in this study were within the normal range (Coutinho et al. 2014; Sarker et al. 2019; Geberemariyam et al. 2020; Suryani et al. 2020). Tannins can influence proteins and starch degradation (Besharati et al. 2022). Hydrolyzable tannins have more potentially adverse effects compared to condensed (Zhang et al. 2019b). However, small doses used in this study did not significantly affect the nutrient digestibility. Previous studies suggested that concentrations higher than 5% would limit nutrient digestibility (Atiku et al. 2016). Adding hydrolyzable tannin from chestnuts up to 2% did not really affect DM and OM breakdown in the rumen based on an in vitro study (Sadarman et al. 2019). Also, giving amino acids (2.0 mL/100 kg BW) might help improve how well nutrients are digested (Abdel-Raheem et al. 2022). However, in this study, adding 1% amino acids did not significantly affect how nutrients were digested, although it showed a significant effect compared to the control treatment.

There was a bit of a trend toward higher CP digestibility and lower CF digestibility when tannins, amino acids, or both were added. Tannins added to the feed have been shown to decrease rumen undegradable protein, thereby increasing nitrogen and protein to be used more (Orzuna-Orzuna et al. 2021). The lower CF digestibility was caused by the tannin-protein complex, which affected fiber digestion in the P4 group (Besharati et al. 2022). Amino acids like lysine and methionine have also been said to lower fiber digestion in calves compared to the control (Mudgal et al. 2018). However, young calves don't have a fully developed rumen, so fiber digestion is lower (Metiya et al. 2023). Since there were no significant differences in nutrient digestibility between the treatments, adding tannins, amino acids, or both did not change overall digestibility much.

Hematological and blood metabolites

Erythrocytes, hemoglobin, and hematocrit levels were within the normal range, indicating that the animals were in good health, according to Latimer et al. (2011), and the treatments in the diets did not affect the hematological profiles in this study. This response might be due to the relatively short period (14 days), which may not have been sufficient to express treatment effects fully. The added supplementation contributed to an increase in leukocytes and the adaptation process of the calves. Similarly, Ma et al. (2024) reported that adding 0.5 and 1% tannin increased leukocytes, serving as a protective mechanism and enhancing the organism's defense against potential threats (Naidenko and Mikhail 2020).

In this study, glucose levels were still in the normal range, according to Latimer et al. (2011), and blood triglyceride levels also fell within the normal range, according to Brito et al. (2021). However, BUN levels were a bit high, which is above the normal range, according to Latimer et al. (2011). This suggested the nitrogen in the feed was not being used efficiently. BUN levels are often used to check how well animals use nitrogen; nevertheless, calves with higher BUN levels could keep their body condition during cold weather (Tshuma et al. 2014). When animals fed with a lot of protein, especially the kind that breaks down in the rumen, BUN levels can increase because of the extra nitrogen in the blood (Xia et al. 2018). Adding tannins to the feed might help protect protein, allowing it to break down later in the gut, where the animal can better use it.

Protein levels in this study were within the normal range, as reported by Latimer et al. (2011). Blood protein levels are directly proportional to the amount of protein the livestock consumes. This is consistent with the findings of Schwab and Brodericket (2017), protein is converted into amino acids and will be transported through the blood. Mitruka et al. (1977) reported cholesterol levels in the normal range. Cholesterol levels increased with the added supplementation.

Furthermore, a high-protein diet can reduce total cholesterol in the blood of growing calves (Croccodrilli et al. 1970). This finding was not in line with the current study. Water-insoluble fats must bind to proteins, allowing the formation of low-density lipoproteins, which transport cholesterol from the liver to tissues (Laka et al. 2022). However, as a health marker in calves, there is a need to monitor blood cholesterol levels. Cholesterol deficiency in calves can cause diarrhea, leading to mortality (Otter and Hateley 2017).

In conclusion, the addition of individual tannins and amino acids at 1% DMI of calves' diets showed a significant effect compared to the control treatment, reduced fresh daily intake, resulting in higher average daily gain, improved feed efficiency, and increased CP utilization, without impairing calves' health. Adding the tannin-amino acids mixture produced intermediate effects, suggesting an antagonistic interaction. However, this combination shows potential for improving nutrient digestibility and protein utilization in young calves. Further studies should focus on the tannin-amino acids ratio to maximize these benefits and avoid negative interactions.

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