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ORIGINAL PAPER

Effects of fermented red ginseng by-product on *in vitro* rumen fermentation and nutrient digestibility

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ABSTRACT. This study systematically evaluated the effects of fermented red ginseng by-product (FRGB) supplementation on rumen fermentation characteristics and nutrient digestibility using *in vitro* experiments. The results demonstrated significant dose- and time-dependent effects of FRGB on gas production kinetics, with the 4% supplementation level (T4) showing the most favourable performance during the mid- and late fermentation stages, reaching the theoretical maximum gas production. Although all treatments showed a characteristic pattern of increasing gas production, the T4 group maintained greater persistence, indicating optimal substrate composition and effective microbial regulation that sustained fermentation activity. Compared with the control, FRGB slightly reduced the initial gas production rate but significantly improved the overall fermentation potential. The T4 group produced the highest propionic acid concentration and the lowest acetate-to-propionic acid ratio, indicating improved energy utilisation efficiency. Nutrient digestibility analyses showed that 3% FRGB (T3) optimised organic matter digestibility and metabolizable energy, while maximising the neutral detergent fibre and acid detergent fibre degradation, indicating enhanced cellulolytic activity. These differential dosage effects showed functional complementarity: 4% FRGB mainly improved fermentation kinetics and energy release, whereas 3% FRGB enhanced nutrient digestibility and fibre degradation. These results collectively suggest that the optimal addition level of FRGB to ruminant diets is 3–4%, depending on specific production objectives. The present study provides data basis for the development of FRGB as a functional feed additive.

Introduction

As the primary digestive organ of ruminants, rumen health directly determined nutrient absorption, growth performance, and overall host health (Mizrahi et al., 2021). The complex rumen microbial community plays a central role in host nutrition by fermenting poorly degradable substrates, such as plant fibres, into volatile fatty acids, microbial proteins, and metabolic energy (Hungate, 1966; Chen et al., 2021). Therefore, maintaining microbial homeostasis and efficient fermentation is essential for optimising ruminant productivity (Russell and Rychlik, 2001). *In vitro* fermentation techniques, due to their ease of operation, low cost, and high reproducibility, are widely applied to evaluate the effects of feed ingredients and additives on rumen microbial fermentation (Beauchemin et al., 2008; Bayat et al., 2017). Measurements of gas production, volatile fatty acid concentration,

ammoniacal nitrogen level, and dry matter digestibility enable a systematic evaluation of fermentation responses, providing a theoretical basis for practical applications (Bergman, 1990). In ruminant nutrition, phytogenic compounds are widely studied and applied as feed additives due to their natural origin, safety, and growth-promoting properties (Benchaar et al., 2008). Red ginseng, a traditional Chinese medicinal herb, is rich in bioactive components, including ginsenosides, polysaccharides, and antioxidants, and has been reported to enhance immune responses, metabolism, and intestinal health. However, red ginseng processing generates substantial quantities of by-products, such as residues and fibrous roots, which are often discarded, resulting in inefficient resource utilisation. In recent years, microbial fermentation has been widely used to improve value of agricultural by-products. This process can degrade antinutritional components (such as cellulose and lignin) in red ginseng by-products (RGB), thereby improving their digestibility. It may also generate new bioactive metabolites, including small peptides and organic acids, which enhance availability (Nyonyo et al., 2014). Research has shown that RGB exert growth-promoting and antioxidant effects in monogastric animals, such as pigs and poultry (Chung et al., 2018). However, research on their application in ruminant nutrition remains limited, particularly with respect to their effects on rumen fermentation. Furthermore, most *in vitro* studies rely on single time-point measurements (e.g., 48 or 72), which may fail to capture short-term changes in microbial metabolic activity due to the dynamic rate of rumen microbial processes. Therefore, comprehensive monitoring at multiple time points (3, 6, 9, 12, 24, 36, and 48 h) can provide a more comprehensive understanding of the impact of fermented RGB on rumen microbiome. This study aimed to investigate changes in the functional components of RGB before and after fermentation, as well as to assess their effects on rumen fermentation characteristics and *in vitro* nutrient digestibility. The findings provide a foundation for the development of fermented RGB as functional feed additives in ruminant production systems.

Material and methods

Experimental design and animal feeding management

In this experiment, *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus rhamnosus* were selected to RGB to improve the bioconversion efficiency of common ginsenosides into rare ginsenosides. The two two bacterial strains were cultured and propagated to a concentration of 1×10^8 CFU/g, mixed at a 1:1 ratio, and inoculated into RGB at a 5% (w/w) inoculation rate. The mixture was anaerobically fermented at 37 °C for 5 days to obtain fermented red ginseng by-product (FRGB), which was used for subsequent experiments. The study comprised two experiments: an *in vitro* gas production assay and an *in vitro* digestibility assay. The fermentation substrate for the control group consisted of a total mixed ration (TMR, dry matter basis). FRGB was added to the control substrate at 1, 2, 3, 4, 5, and 6% of the *in vitro* fermentation substrate (corresponding to 200 mg or 1 g, dry matter basis) for groups T1–T6, respectively. Each treatment was performed in triplicate. Based on practical beef cattle production conditions, the relationship between the 30 ml *in vitro* fermentation system and the rumen volume (approximately 100–200 l) of a 500 kg beef cow (with a feed intake of approximately 2% body weight) was calculated. Three healthy Yanbian yellow cattle (body weight 500 ± 20 kg) were selected as rumen fluid donors. The rumen-fistulated animals were fed a diet consistent with the *in vitro* fermentation substrate, formulated according to the Chinese Beef Cattle Feeding Standards (NY/T 815-2004). The diet composition and nutrient levels are presented in Table 1. Animals were adapted to the diet for two weeks prior to sam-

Table 1. Composition and nutritional level of basal diet, dry matter basis)

Items	Composition
Ingredient, %	
Corn stalk	40
Corn	36
Soybean meal	9
Cottonseed meal	6
Bran	6
NaCl	0.5
$NaHCO_3$	1.3
Premix[1]	1.2
Total	100
Nutrient levels, %	
NEg[2], MJ/kg	2.07
CP	14.5
CF	16.3
ADF	21.7
NDF	36.6
EE	2.4
ash	10.8
Ca	0.23
P	0.38

CP – crude protein, CF – crude fibre, NDF – neutral detergent fibre, ADF – acid detergent fibre, EE – ether extract; [1] provided the following per kg of the diet: IU: vit. A 85000, vit. D 329000; mg: vit. E 500, Cu 350, Fe 190, Zn 900, Mn 1000, Co 15, Se 10; [2] NEg (net energy for growth) was estimated from the analyzed value of the dietary ingredients (based on Ministry of Agriculture of P.R. China (2018))

pling. Feeding was carried out twice daily (08:00 and 17:00), with *ad libitum* access to water.

Sample collection and processing

Fourteen saponin components in RGB before and after fermentation were quantitatively analysed using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS). The samples were crushed and finely ground, followed by ultrasonic extraction using 70% methanol aqueous solution as the solvent. After centrifugation and filtration, the extracts were subjected to chromatographic analysis. Separation was performed on a C18 column (100 mm × 2.1 mm, 1.8 μm; Agilent Technologies, Santa Clara, USA) using gradient elution with water and acetonitrile at a flow rate of 0.3 ml/min and a column temperature of 35 °C. Mass spectrometric detection was carried out using an electrospray ionisation source in negative ion scanning mode, operated in multiple reaction monitoring mode. The concentrations of the 14 saponins showed a linear correlation with response intensity over the range of 0.1–10 μg/ml, with correlation coefficients exceeding 0.995. The limits of quantification ranged from 0.001 to 0.010 g/kg, recoveries from spiked samples were 90.68–97.21%, and relative standard deviations were 1.93–6.33% (n = 6).

In vitro fermentation experiments were conducted according to the method of Menke and Steingas, 1988). Rumen fluid was collected from fistulated cattle before the morning feeding, pooled, and transferred to a preheated insulated container (approximately 39 °C), and immediately transported to the laboratory. The rumen fluid was filtered through gauze and mixed with a pre-prepared buffer solution at a volume ratio of 1:2. The fermentation substrates from each group were oven-dried at 65 °C for 48 h, ground, and passed through a 1-mm sieve. A sample of substrate (200 mg, dry matter basis) was combined with 30 ml of buffered rumen fluid in a 100-ml glass syringe, after which the headspace was purged with carbon dioxide to establish anaerobic conditions. The corresponding concentration of FRGBs was added to the glass syringes of the experimental group, while the control group received no supplementation. All syringes were incubated in a constant-temperature shaking water bath at 39 °C. The experiment was conducted at 3, 6, 9, 12, 24, 36, and 48 h, and pH, ammoniacal nitrogen, lactic acid, and volatile fatty acid concentrations were determined at each sampling point. The *in vitro* digestibility experiment was performed according to Menke et al. (1979). A weighed sample of the dried and ground substrate was placed in a fibre filter bag, sealed, and transferred to a 100-ml *in vitro* digestion tube preheated to 39 °C. Then, 70 ml of buffered rumen fluid was injected, and carbon dioxide was flushed to maintain anaerobic conditions. The experimental design and treatment groups were identical to those used in the *in vitro* gas production assay. Incubations were carried out for 3, 6, 9, 12, 24, 36, and 48 h, after which *in vitro* digestibility of dry matter, crude protein, neutral detergent fibre, and acid detergent fibre were determined. In the *in vitro* gas production experiment, pH was measured using a pH meter. Volatile fatty acids are analysed using gas chromatography. Ammoniacal nitrogen concentration was determined spectrophotometrically following the method of Broderick and Kang (1980), while lactate concentration was measured using a UV-visible spectrophotometer according to the method of Baker and Summerson (1941).

After completion of the *in vitro* digestion assay, the culture tubes were immediately placed in an ice-water bath to terminate fermentation. The fibre filter bags were rinsed with distilled water and dried in a forced-air oven at 105 °C for 12–24 h to a constant weight. The resulting dry matter was recorded and used to calculate *in vitro* dry matter digestibility. Crude protein content was determined using a fully automatic Kjeltec nitrogen analyser (Kjeltec 8400, Volkswagen, Denmark). NDF and ADF contents were determined in accordance with national standards (GB/T 20806-2006; NY/T 1459-2007).

Statistical data analysis

This study used SPSS 21.0 to perform one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test for multiple comparisons. Linear (L) and quadratic (Q) models were applied to compare the effects of different FRGB supplementation levels on *in vitro* rumen fermentation parameters. Differences were considered statistically significant at $P < 0.05$, while $P > 0.05$ indicated no significant effect.

Results

Changes in conventional nutritional components and ginsenoside content of RGB before and after fermentation

In this experiment, no significant differences were observed in the conventional nutritional components of RGB prior and after fermentation (Table 2). In contrast, marked changes were observed in the ginsenoside profile. As shown in Figure 1, of the common ginsenosides, the contents of Rb1 and Rb2 decreased significantly. Specifically, Rb1 declined from 3.37 to 1.95%, and Rb2 from 2.17% to 1.25%. Re and Rc also showed a decreasing trend. In contrast, all measured rare ginsenosides increased, with significant differences observed for

Table 2. Conventional nutritional components before and after RGB fermentation, dry matter basis

Items	Groups†		SEM	*P*-value
	RGB	FRGB		
EE, %	1.28 ± 0.04	1.30 ± 0.02	0.03	0.09
CF, %	15.85 ± 0.41	15.48 ± 1.13	0.21	0.81
CP, %	14.22 ± 0.45	14.53 ± 0.47	0.14	0.71
ASH, %	3.05 ± 0.08	3.72 ± 0.14	0.05	<0.01

EE – ether extract, CF – crude fibre, CP – crude protein, RGB – red ginseng by-product, FRGB – fermented red ginseng by-product, SEM – standard error of the mean; $P > 0.05$ (not statistically significant)

Rd, F2, Rh2, Rg1, Rg2, 20(S)-Rg3 and 20(R)-Rg3. Consequently, the total content of rare ginsenosides increased from 1.59 to 2.27%.

Gas production parameters

The effects of FRGB supplementation on *in vitro* rumen fermentation gas production are shown in Figure 2A. As expected, gas production increased significantly over time in all groups ($P < 0.01$). A comparison of the treatment groups demonstrated that at 3 h, the T5 group had the highest gas production, which was not significantly different from that of the T4 group but markedly higher compared to the remaining groups. At 6 h, T3 exceeded both T4 and T5, and showed the highest gas production. From 9 h onward, gas production in the T4 group stabilised and remained the highest among all groups. At all time points, the control group consistently showed lower gas production than all treatment groups ($P < 0.01$). Gas production at each time point initially increased and then decreased with incrementing FRGB supplementation, with a peak observed at a 4% inclusion level. As shown in Table 3, the theoretical maximum gas production of the T4 group was 90.52 ml, which was significantly

Table 3. Effect of FRGB on *in vitro* fermentation gas production parameters

Items	Groups							SEM	*P*-value		
	CON	T1	T2	T3	T4	T5	T6		T	L	Q
Theoretical maximum gas production, ml	63.86^{c}	82.56^{b}	88.36^{ab}	84.97^{ab}	90.52^{a}	83.89^{b}	85.98^{ab}	1.911	<0.001	<0.001	<0.001
Gas production rate, ml/h	0.039^{a}	0.030^{bc}	0.031^{bc}	0.034^{b}	0.032^{bc}	0.032^{bc}	0.029^{c}	0.001	0.001	0.001	0.254

CON – control group, T1–T6 – treatment 1–treatment 6 groups were added fermented red ginseng by-product (FRGB) accounting for 1, 2, 3, 4, 5, and 6% of the *in vitro* fermentation substrate weight (200 mg, dry matter basis) based on control group, respectively; SEM – standard error of the mean; T – treatment, L – linear, Q – quadratic; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

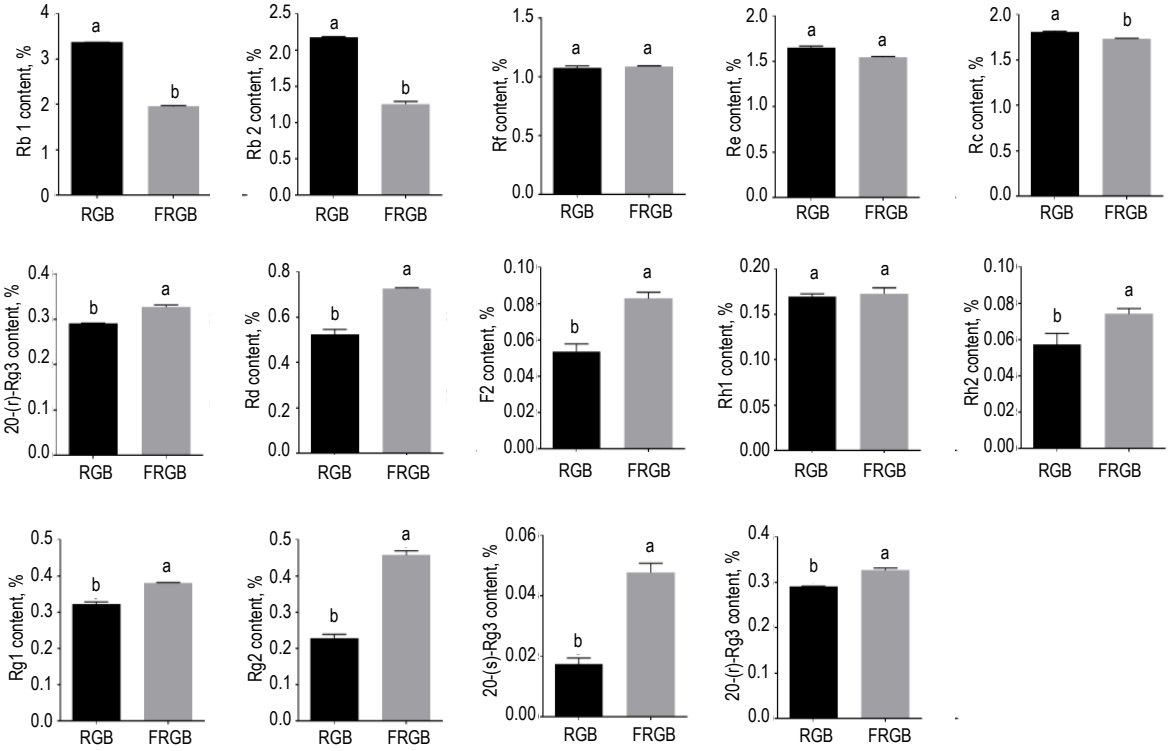

Figure 1. Changes in the content of various ginsenosides before and after fermentation. Different letters indicate significant differences ($P < 0.05$), while the same letters indicate no significant differences ($P > 0.05$).

RGB – red ginseng by-product, FRGB – fermented red ginseng by-product

higher than that of other groups. Similarly, theoretical maximum gas production followed a quadratic trend with increasing FRGB addition, also reaching its maximum at 4%. In contrast, the gas production rate was highest in the control group (0.039 ml/h), which was significantly higher than in the treatment groups.

Fermentation parameters

The effects of different FRGB addition levels on *in vitro* rumen fermentation parameters are shown in Figure 2. The pH values decreased significantly over time in all groups, with no significant differences observed in pH values after 9 h of fermentation. detected in T2. Overall, ammoniacal nitrogen levels showed a quadratic relationship, first increasing and then decreasing along with the addition of FRGB (Figure 2C). Lactic acid concentrations increased significantly over time in all groups, with differences observed across time points ($P < 0.05$) between the groups. The results concerning volatile fatty acids (VFAs) are shown in Figure 3. Acetic acid concentrations increased significantly over time in all groups ($P < 0.05$). Comparisons between groups revealed no significant differences at most time points, except for 6 h and 48 h. In general, acetic acid levels were not substantially affected by incrementing FRGB

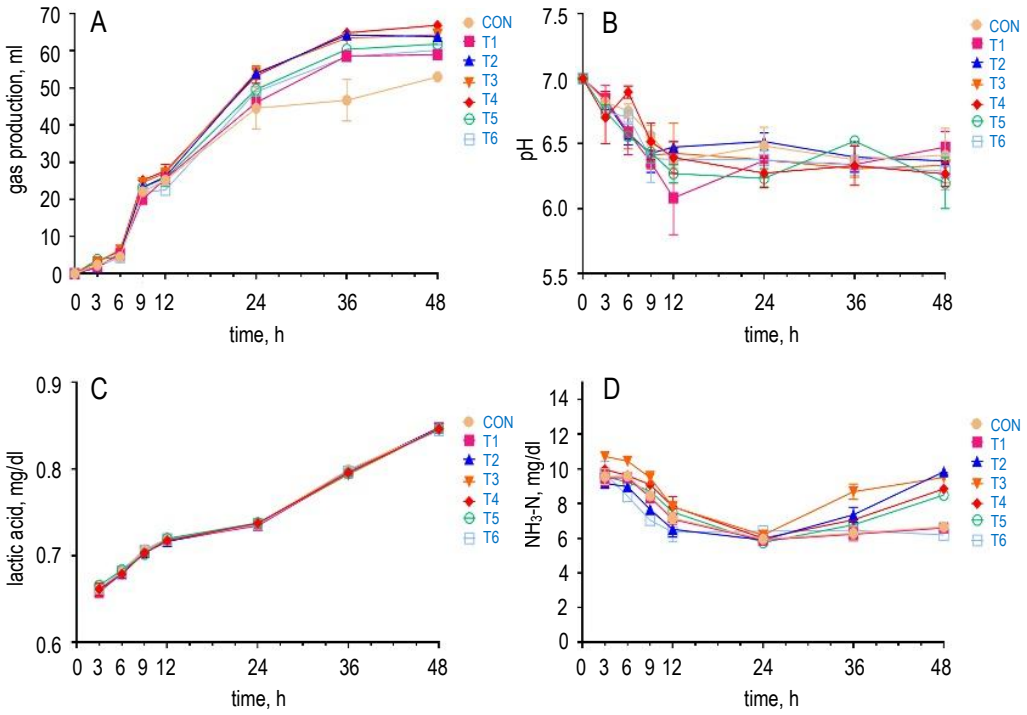

Figure 2. Gas production (A), pH (B), lactic acid (C), NH_3-N content (D) for each time period.

CON – control group, T1–T6 – treatment 1–treatment 6 groups were added fermented red ginseng by-product accounting for 1, 2, 3, 4, 5, and 6% of the *in vitro* fermentation substrate weight (200 mg, dry matter basis) based on control group, respectively; NH_3-N – ammonia nitrogen

Throughout the incubation period, pH values in all treatments remained within the normal physiological range (Figure 2B). Ammoniacal nitrogen concentrations in all groups initially decreased and then increased over time, reaching the lowest values at 24 h of fermentation, which were significantly lower than other time groups ($P < 0.001$). After 24 h, ammoniacal nitrogen levels gradually increased but remained below the baseline values. At 3 h, ammoniacal nitrogen in the T3 group was significantly higher than in the other groups ($P < 0.05$), while no significant differences were observed among the remaining groups. The highest ammoniacal nitrogen concentration at the end of fermentation was supplementation. Propionic acid concentrations also increased significantly over time in all groups ($P < 0.05$). No significant differences were observed between groups at 3 h of fermentation, while at the end of fermentation, the highest propionic acid content was recorded in T4. Similarly, butyric acid concentrations increased significantly over time ($P < 0.05$), with no significant differences observed among groups. The acetate-to-propionate ratio decreased significantly ($P < 0.05$). At 3 h, the highest ratio (3.63) was observed in the control group and the lowest (3.42) in T4. After 48 h of fermentation, no significant differences in this ratio were observed among groups (Figure 3).

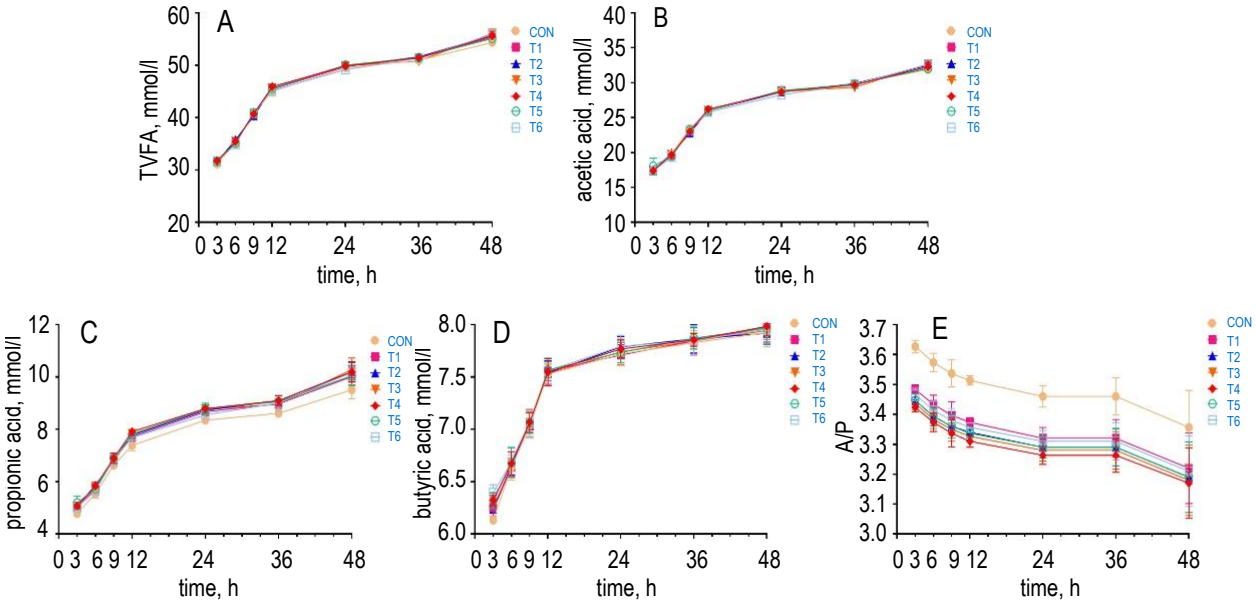

Figure 3. TVFA content (A), acetic acid content (B), propiotic acid content (C), butyric acid content (D), A/P – ratio of acetic acid content to propionic acid content (E) for each time period.

CON – control group, T1–T6 – treatment 1–treatment 6 groups were added fermented red ginseng by-product accounting for 1, 2, 3, 4, 5, and 6% of the *in vitro* fermentation substrate weight (200 mg, dry matter basis) based on control group, respectively; TVFA – total volatile fatty acid

Organic matter digestibility and metabolizable energy

The effects of FRGB supplementation on OMD and ME are shown in Table 4. OMD followed a quadratic trend, initially increasing and then decreasing, with the highest value recorded in the T3 group (72.55%). ME showed the same pattern, reaching a maximum of 10.28 MJ/kg in T3. For both parameters, all treatment groups reached significantly higher values than the control.

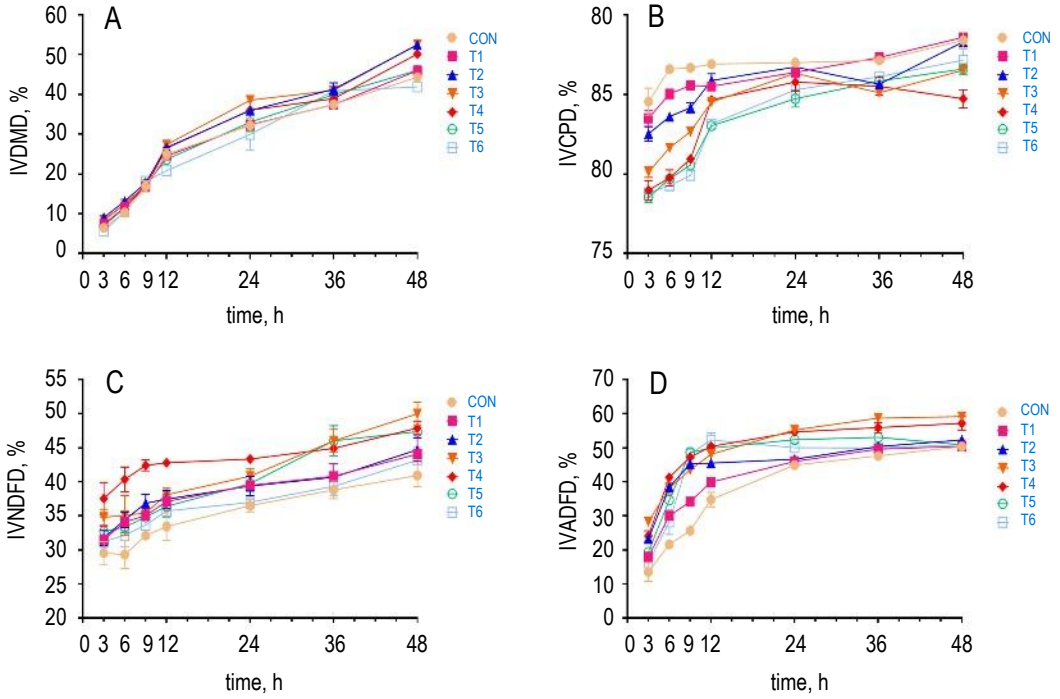

Figure 4. IVDMD (A), IVCPD (B), IVNDFD (C), IVADFD (D) for each time period.

CON – control group, T1–T6 – treatment 1–treatment 6 groups were added fermented red ginseng by-product accounting for 1%, 2%, 3%, 4%, 5%, and 6% of the in vitro fermentation substrate weight (200 mg, dry matter basis) based on control group, respectively; IVDMD – *in vitro* dry matter digestibility, IVCPD – *in vitro* crude protein digestibility, IVNDFD – *in vitro* neutral detergent fibre digestibility, IVADFD – *in vitro* acid detergent fibre digestibility

Table 4. Effect of FRGB on OMD and ME *in vitro* fermentation

Items	Groups‡							SEM	*P*-value		
	CON	T1	T2	T3	T4	T5	T6		T	L	Q
OMD, %	64.02^{d}	65.40^{cd}	72.33^{a}	72.55^{a}	71.79^{ab}	68.52^{abc}	67.98^{bcd}	0.817	0.001	0.021	<0.001
ME, MJ/kg DM	8.98^{d}	9.19^{cd}	10.25^{a}	10.28^{a}	10.17^{ab}	9.67^{abc}	9.59^{bcd}	0.125	0.001	0.021	<0.001

CON – control group, T1–T6 – treatment 1–treatment 6 groups were added FRGB accounting for 1, 2, 3, 4, 5, and 6% of the *in vitro* fermentation substrate weight (200 mg, dry matter basis) based on control group, respectively; ME – metabolizable energy, OMD – organic matter digestibility, SEM – standard error of the mean, T – treatment, L – linear, Q – quadratic; $^{a–d}$ – means within a row with different superscripts are significantly different at $P < 0.05$

In vitro digestibility

The effects of different FRGB additions levels on the *in vitro* digestibility of dietary nutrients are shown in Figure 4. The *in vitro* digestibility of dry matter increased significantly in time in all groups ($P < 0.05$), with T2 and T3 consistently reaching higher values than the other groups at all time points. Crude protein digestibility also increased significantly over time ($P < 0.05$), but comparisons among groups revealed a slight decreasing trend with rising levels of FRGB supplementation. NDF digestibility also increased significantly over time in all groups ($P < 0.05$), and responded quadratically to FRGB inclusion, reaching the highest value in T3 at 48 h (49.98%). ADF *in vitro* digestibility showed a similar pattern, increasing over time ($P < 0.05$), and reaching its highest value in T3 at 48 h (59.15%), which was significantly higher than in the other groups.

Discussion

The experimental results demonstrated that the addition of FRGB significantly affected the gas production kinetics of *in vitro* ruminal fermentation, showing clear dose-dependence and temporal dynamics. Among the groups, T4 showed the most favourable effects for many indicators, particularly during the mid-to-late fermentation stages and in theoretical maximum gas production. The latter increased significantly over time in all groups ($P < 0.01$), reflecting the continuous microbial degradation of the substrate typical of *in vitro* rumen fermentation. At 3 h, gas production was highest in the T5 group, showing no significant difference from T4 but exceeding the other groups. This suggests that, in the early fermentation stages, higher FRGB doses or its rapidly degradable components may have stimulated specific microbial populations or provided readily available carbon sources, accelerating the initiation of gas production. At 6 h, gas production in the T3 group surpassed both T4 and T5 and reached its maximum, indicating that T3 achieved the optimal level of short-term fermentative stimulation at this stage. From 9 h onwards, gas production in the T4 group consistently remained the highest. This represents one of the most significant findings, indicating that the 4% inclusion level provided the most sustained and effective enhancement in the middle and late stages of fermentation. The substrate structure, nutritional composition, and regulatory effects on the microbial community likely promoted stable fermentation activity and more efficient gas production, resulting in prolonged efficiency (Kongmun et al., 2011). Gas production in the control group was significantly lower than in all treatment groups at all time points ($P < 0.01$), clearly demonstrating that FRGB supplementation promoted rumen fermentation and increased gas production. Thus, FRGB is not a neutral additive but exerts a positive stimulatory effect (Johnson and Johnson, 1995). Importantly, the control group had the highest gas production rate (0.039 ml/h), significantly exceeding the treatment groups. This is a significant finding, and a plausible explanation is that the basal diet alone contained a relatively higher proportion of rapidly fermentable components, such as soluble carbohydrates and readily degradable protein, resulting in a faster initial gas release. Despite this higher initial rate, both the theoretical maximum and total gas production were significantly lower than in the FRGB-supplemented groups. This suggests that, although FRGB addition may have slightly reduced the initial rapid fermentation phase, it significantly enhanced the total fermentation potential and duration during the mid-to-late stages. Consequently, a higher initial gas production rate does not necessarily translate into greater cumulative production or sustained fermentation efficiency (France et al., 2000). In this context, the sustained high gas production observed in T4 is of particular relevance.

The pH values in all groups decreased significantly over time but stabilised after 9 h, indicating that the system entered a relatively stable metabolic phase. No significant differences in pH were observed between the treatment groups, and values remained within the normal ruminal range throughout the experiment. This indicates that the addition

of FRGB did not disrupt fermentation acid-base balance or adversely affect the stability of the rumen microbial environment.

All groups showed a distinct trend of initial decrease followed by an increase in NH_3-N concentration, reaching a minimum at 24 h ($P < 0.01$). The early decline may be due to rapid utilisation of nitrogen sources by microorganisms for protein synthesis, while the subsequent increase likely reflects continued protein degradation and the release of ammonia nitrogen. At 3 h, the T3 group showed significantly higher NH_3-N levels than the other groups ($P < 0.05$), suggesting that the 3% FRGB supplementation level may promote initial protein degradation. At the end of fermentation, the highest NH_3-N concentration was observed in T2, indicating lower efficiency in regulating nitrogen metabolism, leading to ammonia accumulation during the later stages. In contrast, the higher-dose groups (T4, T5) may have reduced ammonia release due to increased microbial nitrogen assimilation efficiency or by partially inhibiting deaminase activity (Itabashi et al., 1984).

Lactate levels increased significantly over time ($P < 0.05$) but did no differ between the groups, indicating that FRGB had no influence on the glycolytic pathway (Michalski et al., 2014). Acetate concentrations also increased significantly over time ($P < 0.05$), while overall differences among groups were not significant, suggesting that FRGB inclusion did not exert a targeted regulatory effect on acetate-type fermentation. At the end of fermentation, the highest propionate concentration was observed in T4, consistent with the previously identified optimal gas production duration in the this group. As propionate is the primary glucose precursor in ruminants, the elevated propionate production in T4 indicates a significant improvement in energy utilisation. Butyrate concentrations increased significantly over time ($P < 0.05$), with no significant differences between groups, indicating that the butyrate metabolic pathway was not specifically affected by FRGB supplementation. The ratio of acetic acid content to propionic acid content (A/P) decreased significantly over time in all groups ($P < 0.05$), reflecting a progressive shift towards propionate production during the later stages of fermentation. At 3 h, the control group had the highest A/P ratio, while T4 showed the lowest, demonstrating that T4 promoted propionate generation even in the early phase of fermentation. At the end of fermentation, no significant differences in the A/P ratio were observed between the groups, suggesting that microbial metabolism tended to stabilise during the later fermentation stages. The T4 group showed the most favourable combination of propionate yield, A/P ratio, and sustained fermentation activity, indicating that this inclusion level was optimal for improving overall fermentation efficiency. The lack of further improvement in the T5 group suggests possible substrate saturation or microbial metabolic limitation at higher FRGB levels. The current findings confirm that FRGB regulates rumen fermentation in a dose-dependent manner, with the T4 group demonstrating optimal balance between fermentation activity, nitrogen utilisation, and energy conversion.

Both organic matter digestibility (OMD) and and metabolizable energy (ME) showed a quadratic trend of initial increase followed by decline, reaching highest values in the T3 group. This suggests that a 3% supplementation level is optimal for nutrient utilisation efficiency, whereas exceeding this dose may trigger negative feedback mechanisms. The reductions observed in the T4 and T5 groups suggest that higher supplementation levels may impair substrate conversion efficiency, possibly due to an increased microbial metabolic load or the accumulation of inhibitory compounds. Compared with the control group, OMD and ME were significantly increased in all treatment groups, confirming that FRGB exerts a general stimulatory effect on digestion that is not limited to a single dose, and its bioactive components may act synergistically to enhance microbial enzyme activity. A 3% dose may optimally match the metabolic capacity of rumen microorganisms, maximising the activity of cellulose-degrading or starch-hydrolysing bacteria. It also provides the most appropriate carbon-to-nitrogen ratio, thereby improving the utilisation of energy and nitrogen sources. In contrast, excessive inclusion may dilute effective fermentable substrates from the basal diet by excessive addition, lowering overall digestibility (Tilley and Terry, 1963). Although the T4 group showed advantages in fermentation kinetics (gas production, propionate yield), T3 had a better conversion to nutritional end products, as reflected by higher OMD and ME values. In the T4 group, energy may have been preferentially allocated to microbial growth and maintenance, as indicated by elevated gas and VFA production rates, without a proportional increase in microbial protein synthesis. On the other hand, the T3 group could be more efficient at converting energy into absorbable nutrients (Huhtanen et al., 2015). Overall, the peak OMD and ME achieved at a 3% supplementation level (T3), confirming the strong stimulatory effect of

FRGB on ruminal organic matter digestion and energy conversion; however, efficiency declined when supplementation exceeds this optimal dose.

Dry matter digestibility increased significantly over time ($P < 0.05$), with the T2 and T3 groups consistently outperforming the other treatments throughout the experiment, indicating that a supplementation level of 2–3% optimised substrate degradation. The underlying mechanisms may involve enhancement of microbial enzyme secretion by red ginseng polysaccharides, while saponins suppress protozoa development and thereby reduce bacterial losses. Crude protein digestibility also increased significantly over time ($P < 0.05$), but the differences between the groups gradually evened out as fermentation progressed. This suggests that FRGB mainly accelerates early-stage protein hydrolysis, but its effect weakens at later phases, when slowly degradable protein fractions become dominant (Pilajun and Wanapad, 2011). Neutral detergent fibre digestibility followed a quadratic pattern, with an initial increase followed by a decline, reaching a maximum of 49.98% in the T3 group, confirming 3% as the optimal dose for fibre degradation. Acid detergent fibre digestibility was also highest in the T3 group, reaching 59.15%, indicating enhanced degradation of recalcitrant fibre components like lignin and cellulose (Van Soest et al., 1991; Priambodo et al., 2014). The highest ADF/NDF digestibility ratio in the T3 group further reflects more extensive cell wall degradation. Overall, FRGB regulates nutrient digestion through differential dosage effects. A 3% addition level represents the optimal point for fibre conversion and general digestibility improvement, while a 4% level appears to favour energy release efficiency, with the two levels forming functionally complementary effects.

In this study, VFA concentrations did not differ significantly between individual FRGB supplementation levels. This may be attributed to the fact that after substrate utilisation by rumen microorganisms, carbon is distributed among three main metabolic pathways. From an energetic perspective, VFA synthesis is one of the major carbon sinks. Additionally, carbon sources play a role in establishing favourable conditions for microbial growth and are also involved in the formation of gaseous products such as methane and carbon dioxide. Consequently, although VFA concentrations remained relatively stable with increasing FRGB inclusion, the improvements observed in digestibility parameters in the *in vitro* assays may be attributable to elevated microbial biomass production and gas generation rather than to changes in VFA synthesis. It should be noted that, although NH_3-H concentrations differed significantly during the 48-hour incubation period, *in vitro* dry matter digestibility did not show corresponding variation. This may be explained by extensive degradation of the protein fraction in treatments with higher NH_3-H, leading to increased ammonia release. At the same time, the carbohydrate fractions, particularly NDF and ADF, may have been relatively resistant to microbial degradation in these samples. As a result, despite higher protein breakdown, a significant residues of indigestible fibre limited the overall increase in dry matter digestibility. From these findings, it can be inferred that fermentation treatment makes the protein fraction of RGB more readily degradable.

Rare ginsenosides are secondary saponins produced during processing or *in vivo* metabolism of common ginsenosides, typically exhibiting stronger biological activity. In *in vitro* rumen microbial fermentation, their effects are complex and display bidirectional regulatory properties. At appropriate concentrations, rare ginsenosides can act as prebiotics, selectively stimulating the growth and metabolic activity of certain beneficial microorganisms. However, at higher concentrations, they may exert antibacterial effects, inhibiting microorganisms involved in fibre decomposition and protein degradation, thereby altering the VFA profile, including the acetate-to-propionate ratio. In the present study, whole RGB fermentation was conducted to produce FRGB, which demonstrated beneficial effects on *in vitro* rumen microbial fermentation. Nevertheless, given the structural diversity and functional complexity of rare ginsenosides, the precise mechanisms underlying these effects require further investigation.

Conclusions

The present *in vitro* study systematically evaluated the effects of fermented red ginseng by-product (FRGB) supplementation on rumen fermentation characteristics and nutrient digestibility. The results showed that FRGB significantly affects rumen fermentation kinetics and nutrient utilisation in a dose-dependent manner, with distinct optimal inclusion levels for different fermentation parameters. In terms of gas production dynamics, the 4% addition level (T4) was most effective in sustaining prolonged fermentation and achieving the highest theoretical maximum gas production. Regarding volatile fatty acid (VFA) profiles, the T4 group

showed the highest propionate concentration at the end point and the lowest acetate-to-propionate ratio during the early fermentation phase, indicating improved energy usage. Nutrient digestibility analyses showed that the T3 dose optimised organic matter digestibility (OMD) and metabolizable energy (ME). The observed dose-dependent responses revealed distinct functional advantages: 4% FRGB enhances fermentation kinetics and energy release efficiency, whereas 3% more effectively improves nutrient digestibility and fibre degradation. This functional complementarity indicates potential for targeted dietary applications. The current findings provide important insights into the application of FRGB in ruminant nutrition, suggesting an optimal inclusion range of 3–4%, depending on specific production objectives. Future research should focus on *in vivo* validation and elucidation of the precise underlying microbial regulatory mechanisms.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

Baker S.B., Summerson W.H., 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138, 535–554, https://doi.org/10.1016/S0021-9258(18)51379-X

Bayat A.R., Ventto L., Kairenius P., Stefanski T., Leskinen H., Tapio I., Negussie E., Vilkki J., Shingfield K.J., 2017. Dietary forage to concentrate ratio and sunflower oil supplement alter rumen fermentation, ruminal methane emissions and nutrient utilization in lactating cows. Transl. Anim. Sci. 1, 277–286, https://doi.org/10.2527/tas2017.0032

Beauchemin K.A., Kreuzer M., Omara F., Mcallister T.A., 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48, 21–27, https://doi.org/10.1071/EA07199

Bergman E.N, 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70, 567–590, https://doi.org/10.1152/physrev.1990.70.2.567

Benchaar C., Calsamiglia S., Chaves A.V., Fraser G.R., Colombatto D., McAllister T.A., Beauchemin K.A., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed. Sci. Tech. 145, 209–228, https://doi.org/10.1016/j.anifeedsci.2007.04.014

Broderick G.A., Kang J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63, 64–75, https://doi.org/10.3168/jds.S0022-0302(80)82888-8

Chen X., Su X., Li J., Yang Y., Wang P., Yan F., Yao J., Wu S., 2021. Real-time monitoring of ruminal microbiota reveals their roles in dairy goats during subacute ruminal acidosis. NPJ Biofilms Microbioms 7, 45, https://doi.org/10.1038/s41522-021-00215-6

Chung T.H., Kim C.M., Choi I.H., 2018. A study on growth performance of ducks fed diets with different types of sipjeondaebo-tang byproduct meal and red ginseng marc with fermented red koji and ammonia fluxes in duck litter using alum or aluminum chloride. J. Poult. Sci. 55, 112–116, https://doi.org/10.2141/jpsa.0170092

France J., Dijkstra J., Dhanoa M.S., López S., Bannink A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. Br. J. Nutr. 83, 143–150, https://doi.org/10.1017/S0007114500000180

Huhtanen P., Cabezas-Garcia E.H., Krizsan S.J., Shingfield K.J., 2015. Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. J. Dairy Sci. 98, 3182–3196, https://doi.org/10.3168/jds.2014-8215

Hungate R.E, 1966. The Rumen and Its Microbes. Academic Press. Cambridge, MA (USA)

Itabashi H., Kobayashi T., Matsumoto M., 1984. The effects rumen ciliate protozoa on energy metabolism and some constituents in rumen fluid and blood plasma of goats (in Chinese). Jpn. J. Zootech. Sci. 55, 248–256, https://doi.org/10.2508/chikusan.55.248

Johnson K.A., Johnson D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492, https://doi.org/10.2527/1995.7382483x

Kongmun P., Wanapat M., Pakdee P., Navanukraw C., Yu Z., 2011. Manipilation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. Livest. Sci. 135, 84–92, https://doi.org/10.1016/j.livsci.2010.06.131

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci. 93, 217–222, https://doi.org/10.1017/S0021859600086305

Menke K.H., Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28, 7–55

Michalski J.P., Czauderna M., Litwin W., Puzio N., Kowalczyk J., 2014. Incorporation of endogenous urea nitrogen into amino acids of milk in goats fed diets with various protein levels. J. Anim. Feed. Sci. 23, 212–216, https://doi.org/10.22358/jafs/65681/2014

Mizrahi I., Wallace R.J., Morais S., 2021. The rumen microbiome: balancing food security and environmental impacts. Nat. Rev. Microbiol. 19, 553–566, https://doi.org/10.1038/s41579-021-00543-6

Nyonyo T., Shinkai T., Mitsumori M., 2014. Improved culturability of cellulolytic rumen bacteria and phylogenetic diversity of culturable cellulolytic and xylanolytic bacteria newly isolated from the bovine rumen. FEMS Microbiol. Ecol. 88, 528–537, https://doi.org/10.1111/1574-6941.12318

Pilajun R., Wanapat M., 2011. Effect of coconut oil and mangosteen peel supplementation on ruminal fermentation, microbial population, and microbial protein synthesis in swamp buffaloes. Livest. Sci. 141, 148–154, https://doi.org/10.1016/j.livsci.2011.05.013

Priambodo T.W., 2014. Effect of medium-chain fatty acids and ration type on *in vitro* ruminal methane production. PhD Thesis. Rheinische Friedrich-Wilhelms-Universität Bonn. Bonn (Germany)

Russell J.B., Rychlik J.L., 2001. Factors that alter rumen microbial ecology. Science 292, 1119–1122, https://doi.org/10.1126/science.1058830

Tilley J.M.A., Terry R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18, 104–111, https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2