Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effects of mineral-vitamin supplementation on growth performance, serum parameters, fecal microbiota and SCFAs in growing male Ashan wapiti (Cervus canadensis sibiricus)

Jiali Wei
Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences
Huitao Liu
Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences
Xingyao Hua
Shenghua Red Deer Breeding and Training Base
Guiwu Wang
Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences
Kaiying Wang
wangkaiying@caas.cn
Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences

Research Article

Keywords: Mineral-vitamin supplementation, Wapiti (Cervus canadensis sibiricus), Fecal microbiota, SCFAs, Immune status

Posted Date: November 6th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11239873/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

• Background

• Commercial feeding systems for farmed wapiti (*Cervus canadensis sibiricus*) generally adopt cattle-based mineral-vitamin formulas, which fail to satisfy the species-specific nutritional requirements of wapiti. This study aimed to investigated the effects of 2% mineral-vitamin supplementation on growth performance, serum parameters, fecal microbiota, and short-chain fatty acids (SCFAs) profiles in one-year-old male wapiti (n = 6/group) over 45 days.

• Results

• The supplementation significantly increased final body weight by 3.38% ($P < 0.05$), and elevated serum GH, IGF-1, immunoglobulin (IgA, IgG, IgM), T-AOC, and ALB levels ($P < 0.05$). The dietary intervention reshaped the fecal microbiota community structure, and increasing the relative abundance of *unclassified_Bifidobacteriaceae*, *Turicibacter*, *Romboutsia*, and *Oscillospiraceae UCG-005*. Consequently, fecal butyrate concentration increased dramatically by 658.94% (2,227.00 μg/mL), with its proportion elevated by 140.09% ($P < 0.05$). Spearman analysis demonstrated that these enriched keystone taxa were positively associated with butyrate accumulation and serum parameters, indicating a coordinated microbiota-butyrate-immunity-growth regulatory pattern in growing wapiti.

• Conclusions

• Dietary mineral-vitamin supplementation effectively improves the growth and physiological health of growing male wapiti, which is potentially mediated by integrated modulation of gut microbiota and butyrate metabolism. This study provides a feasible nutritional strategy for developing species-specific feed formulas and supports the sustainable feeding of farmed wapiti.

Background

Ashan wapiti (*Cervus canadensis sibiricus*) is a distinctive local deer breed native to northern Altay Mountains, Xinjiang Uygur Autonomous Region, China [1]. Its high-value velvet antler makes it an important farmed cervid in northwest China [2]. Ashan wapiti exhibits strong adaptability and good tolerance to low-quality roughages, which favors local deer farming. At present, no unified feeding standards or dedicated dietary additives are available for farmed wapiti. In practical production, additives formulated for beef cattle are widely used, and they cannot fully satisfy the unique nutritional and developmental requirements of wapiti, thereby greatly restricting the growth performance and breeding benefits of farmed wapiti [3–6].

Juvenile growth stage is a critical developmental window that determines lifelong growth and production performance in ruminant animals [7, 8]. Specifically, the growing stage (from weaning to 12 months of age) is critical for pedicle development and the establishment of antler production potential in deer [9]. Body growth, nutritional reserve and intestinal microbial homeostasis during this stage exert long-term effects on subsequent antler performance [10, 11]. To date, most studies on wapiti microbiota have focused on rumen functions in mature individuals [12, 13]. Nevertheless, juvenile wapiti have an incompletely developed rumen, and their hindgut microbiota plays a vital regulatory role in growth and development. Although dietary intervention can remarkably reshape microbial community structure [14–17], few studies have focused on the effects of mineral and vitamin supplementation on the growth performance and health status of growing wapiti. Mineral elements (Zn, Cu, Mn, Co) and vitamins are essential cofactors and growth regulators for microbial fermentation, and can effectively modulate intestinal microbiota structure and short-chain fatty acids (SCFAs) synthesis [18, 19]. SCFAs supply 70–80% of the energy requirements for host growth and metabolism [20]. Among them, butyrate is closely associated with gastrointestinal development, immune homeostasis, nutrient absorption, and antler growth in cervids [21–23]. Recent studies have reported that dietary supplementation with 25-hydroxyvitamin D could reshape rumen bacterial composition and enrich butyrate-producing bacteria for better antler growth [24]. Similarly, vitamin A supplementation triggered gut microbiota remodeling in sika deer, elevating the abundance of *Oscillospiraceae UCG-005* and enhancing the production of lipid mediators linked to antler development [25].

Previous studies regarding wapiti gut microbiota are predominantly descriptive, focusing on microbial variations among different genders [26], wild and captive populations [27], various deer species [28, 29], and simple supplementary feeding regimes [30], whereas systematic nutritional regulation trials remain scarce for wapiti. Moreover, most nutritional-intervention feeding trials of cervids have been performed in sika deer[10, 24]. Few investigations have explored responses of growing wapiti to compound mineral-vitamin supplementation at the hind-gut microbial level. Given these research gaps together with practical constraints in wapiti farming, we hypothesized that dietary mineral-vitamin supplementation would alter hindgut microbial composition and SCFA metabolism, and further benefit growth and immune performance in growing male wapiti. Therefore, this 45-day feeding trial was conducted to investigate the effects of 2% mineral-vitamin supplementation on the growth performance, serum biochemical and immune indices, fecal microbiota community, and SCFA profiles of growing male wapiti. These findings will support the development of species-specific dietary supplements for farmed Ashan wapiti.

Methods

Animals and experimental designs

All animal experimental procedures were approved by the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences and performed according to the guidelines for animal experiments of the National Institute of Animal Health (No. ISAPSAEC-2023-037SD).

The Ashan wapiti (*Cervus canadensis sibiricus*) used in this experiment were provided by the Shenghua wapiti breeding and training base in Changji City, Xinjiang Uygur Autonomous Region. A total of 12 healthy one-year-old male Ashan wapiti were randomly selected, all with similar body weights (109.21 ± 13.92 kg). The animals were randomly divided into two groups ($n = 6$). The animals were housed in an enclosed breeding system with a stocking density of approximately 5–10 m² per animal. Throughout the experimental period, the animals had ad libitum access to water and feed, and the pens were regularly cleaned to maintain hygiene. Group A (control) was fed the basal diet, while Group B (treatment) received the basal diet supplemented with 2% compound mineral-vitamin supplementation. The composition of the basal diet is shown in Table 1. A 7-day adaptation period was allowed for the animals to acclimatize to the experimental diets and to determine *ad libitum* feed intake. During the 45-day experimental period, feed was provided twice daily at 07:30 and 15:30, and orts were recorded to calculate daily feed intake. Daily feed intake was calculated as the difference between feed offered and orts, recorded on a group basis.

Table 1
Ingredients and nutrient composition of the basal diets (%, as-fed basis)

Items	**Basal-diet** [1]
Ingredients (%)	
Corn	63.61
Soybean meal	12.00
Cottonseed meal	11.69
Wheat bran	8.00
NaCl	1.20
Limestone	1.00
Premix[2]	2.5
Addictives[3]	0.00
Total (%)	100.00
Nutrient composition	
ME[4](MJ/kg)	11.43
CP[4] (%)	17.07
Ca[4] (%)	1.24
P[4] (%)	0.70
EE[4] (%)	1.93
CF[4] (%)	4.44
Lysine[4] (%)	0.70

[1].Group A served as the basal-diet control, and group B was the treatment group (basal diet supplemented with 2% feed additive).[2].The premix provides each kilogram of the diet with: MgO, 0.076 g; ZnSO4.H2O, 0.036 g; MnSO4.H2O, 0.043 g; FeSO4.H2O, 0.053 g; NaSeO3, 0.031 g; vitamin A, 2484 IU; vitamin D, 3496.8 IU; vitamin E, 0.828 IU; vitamin K, 0.23 mg; vitamin B1, 0.092 mg; vitamin B2, 0.69 mg; vitamin B12, 0.00138 mg; folic acid, 0.023 mg; nicotinic acid, 1.62 mg; calcium pantothenate, 1.15 mg; CaHPO4, 5.17 g; CaCO3, 4.57g.[3].The feed additives provide the following amounts per kilogram of diet: calcium: 7.355g, phosphorus: 4.051g, copper: 28.865mg, manganese: 166.95mg, zinc: 189.75mg, iron: 142.5mg, magnesium: 39.75mg, selenium: 0.235mg, cobalt: 0.475mg, vitamin A: 3750 IU, vitamin D3: 375 IU, vitamin E: 50 IU.[4] ME, metabolic energy; CP, crude protein; EE, ether extract; CF, crude fiber. ME was a calculated value, while the others were measured values. The proportion of each basic raw material in the experimental group = the proportion of the corresponding raw material in the control group × 0.98.

Items	Basal-diet [1]
Methionine[4] (%)	0.17

[1].Group A served as the basal-diet control, and group B was the treatment group (basal diet supplemented with 2% feed additive).[2].The premix provides each kilogram of the diet with: MgO, 0.076 g; ZnSO4.H2O, 0.036 g; MnSO4.H2O, 0.043 g; FeSO4.H2O, 0.053 g; NaSeO3, 0.031 g; vitamin A, 2484 IU; vitamin D, 3496.8 IU; vitamin E, 0.828 IU; vitamin K, 0.23 mg; vitamin B1, 0.092 mg; vitamin B2, 0.69 mg; vitamin B12, 0.00138 mg; folic acid, 0.023 mg; nicotinic acid, 1.62 mg; calcium pantothenate, 1.15 mg; CaHPO4, 5.17 g; CaCO3, 4.57g.[3].The feed additives provide the following amounts per kilogram of diet: calcium: 7.355g, phosphorus: 4.051g, copper: 28.865mg, manganese: 166.95mg, zinc: 189.75mg, iron: 142.5mg, magnesium: 39.75mg, selenium: 0.235mg, cobalt: 0.475mg, vitamin A: 3750 IU, vitamin D3: 375 IU, vitamin E: 50 IU.[4] ME, metabolic energy; CP, crude protein; EE, ether extract; CF, crude fiber. ME was a calculated value, while the others were measured values. The proportion of each basic raw material in the experimental group = the proportion of the corresponding raw material in the control group × 0.98.

Growth performance

Body weights were measured on day 1 (initial) and day 45 (final) before morning feeding, following a 12-h overnight fast. Daily feed intake was recorded on a group basis throughout the experimental period. Average daily feed intake (ADFI, g/day per animal) was calculated as total feed intake divided by the number of experimental days and the number of animals per group. Average daily gain (ADG, g/day) was calculated as (final body weight - initial body weight) divided by the number of experimental days.

Sample collection

On the last day of the experiment, following a 12 h overnight fast, animals were anesthetized with xylazine hydrochloride (1 mL/animal, intramuscular). Blood samples were collected via jugular venipuncture into clot activator tubes, followed by centrifugation at 3500× g for 15 min at 4°C to isolate serum for immune indices and growth hormone levels. Fresh feces were collected by rectal retrieval under gentle manual restraint using single-use lubricated obstetrical sleeves and sterile gloves; approximately 5–10 g was collected into pre-labeled sterile tubes and immediately stored in liquid nitrogen for 16S rRNA sequences and UHPLC-MS/MS analysis. Anesthesia was reversed with phenazoxine hydrochloride (2 mL/animal, intramuscular).

Serum immune parameters and growth hormone analysis by ELISA

The concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), growth hormone (GH), insulin-like growth factor-1 (IGF-1), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), total antioxidant capacity (T-AOC), total protein (TP), albumin (ALB), and alkaline phosphatase (ALP) in serum were measured using cervid-specific ELISA kits (JX-50391A1, JX-8912A1, JX-8914A1, JX-8994A1, JX-77304A1, JX-W-002, JX-W-195, JX-W-001, JX-W-

006-96, JX-W-508, JX-W-107, and JX-W-152-96, respectively; Yancheng Junxing Biotechnology Co., Ltd, Yancheng, Jiangsu, China) according to the manufacturer's instructions.

DNA extraction and 16S rRNA gene sequencing of fecal microbiota

The microbial DNA was extracted from fecal sample using the EZNA soil kit (Omega Bio-Tek, USA) according to the manufacturer's protocol. The DNA concentration and purity were determined using a NanoDrop™ 2000 instrument, and the integrity was verified by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using the primer pair 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The purified amplicons were used to construct paired-end (PE) 2 × 250 bp sequencing libraries. Libraries were quantified using a Qubit™ 4.0 fluorometer and the KAPA Library Quantification Kit (Roche, Basel, Switzerland), and sequenced on the NovaSeq™ 6000 platform (Illumina, San Diego, CA, USA). The row reads were deposited into NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA1543715).

Short-chain fatty acid analysis by UHPLC-MS/MS

Fecal samples were ground in liquid nitrogen, extracted with 80% methanol, and centrifuged at 12,000 × g at 4°C for 10 min. A 50 μL aliquot of the supernatant was derivatized at 40°C for 40 min, diluted, spiked with isotope-labeled internal standards, and subjected to UHPLC-MS/MS analysis. Chromatographic separation was performed on a Waters ACQUITY BEH C18 column (2.1 × 100 mm, 1.7 μm; column temperature 40°C) using a Vanquish™ Flex UHPLC system coupled to a TSQ Altis™ triple-quadrupole mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Mobile phase A was 10 mM ammonium acetate in water and B was acetonitrile - isopropanol (1:1, v/v) delivered at 0.30 mL min^{-1} with a gradient of 25–100% B over 12 min. The MS was operated in negative MRM mode with a spray voltage of − 4.5 kV, source temperature 550°C, curtain gas 35 psi, auxiliary gas 50 psi and collision gas 55 psi. Nine SCFA standards (acetate, propionate, butyrate, isobutyrate, 2-methylbutyrate, valerate, isovalerate, 4-methylvalerate, hexanoate) and their isotope analogues (ZZ Standard, Shanghai, China) were prepared as mixed calibrators and stored at − 20°C.

Bioinformatics and statistical analysis

Raw paired-end reads were demultiplexed by sample using barcode information, and adapter and primer sequences were removed. Forward and reverse reads were merged using FLASH, followed by quality filtering to obtain high-quality sequences. Chimeric sequences were removed using DADA2 de-novo algorithm, yielding non-chimeric reads for downstream analysis. The classify-sklearn algorithm of QIIME2 was used to annotate each ASV with a pre-trained Naive Bayes classifier. The annotation database was: Silva 138.2. The QIIME2 software was used to calculate the chao1, shannon, dominance and pielou_e indices. Relative abundance of the top 10 phyla and genera was visualized as stacked bar charts generated using [ggplot2 in R / in-house Perl scripts]. Beta diversity was assessed by principal

coordinate analysis (PCoA) based on bray-curtis dissimilarity matrices using the ade4 and ggplot2 packages in R (version 4.3.1). Differential abundance analysis was performed using Linear discriminant analysis effect size (LEfSe) with an LDA score threshold of 4.0 to identify biomarker taxa significantly associated with each group. Spearman correlation analysis was performed on all samples from the control and treatment groups to analyze correlations among fecal microbiota, SCFAs, serum parameters, and growth performance. Significant correlations were visualized as a network diagram using Cytoscape (version 3.10.0).

All data were analyzed at the individual-animal level using independent samples t-tests to compare means between the two groups. Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $P < 0.05$. Data visualization (including box plots, stacked bar charts, and bar charts) was performed using the same software.

Results

Dietary mineral-vitamin supplementation improved growth performance in growing wapiti

As shown in Table 2, relative to group A, group B showed a 3.38% increase in final body weight ($P < 0.05$), a 4.81% decrease in average daily feed intake ($P = 0.707$), and a 35% increase in average daily gain ($P = 0.403$). These results suggest that the 2% mineral-vitamin supplementation promoted body weight, potentially reflecting improved feed utilization efficiency.

Table 2
Effects of feed additives on growth performance of growing cervus wapiti

Items	**A**[1]	**B**[1]	***P*-value**
Initial body weight (kg)	115.45 ± 12.14	113.41 ± 17.80	0.338
Final body weight (kg)	133.35 ± 15.14	138.53 ± 18.14	0.0448
Average daily feed intake (kg/d)	3.12 ± 0.16	2.97 ± 0.40	0.707
Average daily gain (kg/d)	0.40 ± 0.22	0.56 ± 0.37	0.403

[1].Group A fed as the basal-diet control, and group B was the treatment group (basal diet supplemented with 2% feed additive). Data are presented as mean ± standard deviation.

Table 2
Effects of feed additives on growth performance of growing male wapiti

Items	A[1]	B[1]	*P*-value
Initial body weight (kg)	115.45 ± 12.14	113.41 ± 17.80	0.338
Final body weight (kg)	133.35 ± 15.14	138.53 ± 18.14	0.0448
Average daily feed intake (kg/d)	3.12 ± 0.16	2.97 ± 0.40	0.707
Average daily gain (kg/d)	0.40 ± 0.22	0.56 ± 0.37	0.403

[1]Group A fed as the basal-diet control, and group B was the treatment group (basal diet supplemented with 2% feed additive). Data are presented as mean ± standard deviation.

Dietary mineral-vitamin supplementation modulated serum parameters in growing wapiti

After the 45-day feeding trial, compared to group A, serum GH and IGF-1 concentrations were elevated by 17.85% and 21.28%, respectively (Fig. 1A and B) ($P < 0.05$). Similarly, IgA, IgG, and IgM levels in the group B increased by 25.46%, 29.94%, and 34.76%, respectively (Fig. 1C, D, E) ($P < 0.05$). The SOD in group B decreased by 12.63%, T-AOC increased by 16.65%, and ALB increased by 4.86% (Fig. 1F, I, K) ($P < 0.05$), while others showed no significant changes (Fig. 1G, H, J, L) ($P > 0.05$). These results indicate that 2% mineral-vitamin supplementation increased serum GH and IGF-1 concentrations, enhanced humoral immunity, and modified the serum antioxidant profile.

Effects of dietary mineral-vitamin supplementation on fecal microbial α-diversity and community structure

Compared with group A, group B showed no significant differences in fecal microbial (Chao1), diversity (Shannon), or evenness (Pielou's E) (Fig. 2A, B, D) ($P > 0.05$). The dominance index showed a numerical increase (Fig. 2C) ($P > 0.05$). Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity revealed distinct clustering and clear separation between the two groups along PC1 (36.74%) and PC2 (14.94%) (Fig. 3A), indicating that the 2% mineral-vitamin supplementation restructured the fecal microbiota of growing wapiti.

Dietary mineral-vitamin supplementation promoted enrichment of SCFAs-producing bacteria

At the phylum level, the fecal microbiota of growing wapiti was dominated by Firmicutes, Methanobacteriota, Actinomycetota and Proteobacteria (Fig. 3B). The genus-level taxonomic composition was shown in Fig. 3C. The LEfSe analysis revealed that *unclassified_Bifidobacteriaceae* and three SCFAs-producing genera (*Turicibacter*, *Romboutsia*, and Oscillospiraceae *UCG-005*) were significantly enriched in group B (Fig. 3D, E). Compared with group A, the relative abundance of Clostridia

increased by 138.46% (Fig. 3F), while *Turicibacter* and *Romboutsia* increased by 937.97% and 157.73%, respectively (Fig. 3G) ($P < 0.05$). These results indicate that the 2% mineral-vitamin supplementation reshaped the fecal microbiota structure by selectively enriching SCFA-producing bacteria.

Dietary mineral-vitamin supplementation increased fecal SCFA concentrations

Compared with group A, group B showed markedly elevated concentrations of acetate (3,337.04 μg/mL, + 170.42%), butyrate (2,227.00 μg/mL, + 658.94%), 2-methylbutyrate (43.61 μg/mL, + 84.51%), valerate (115.30 μg/mL, + 88.15%), and isovalerate (55.82 μg/mL, + 82.12%) ($P < 0.05$) (Fig. 4A, B). Notably, butyrate proportion increased by 140.09% ($P < 0.05$), whereas isobutyrate and valerate proportions decreased by 63.94% and 44.65%, respectively ($P < 0.05$) (Fig. 4C, D). These metabolic shifts coincided with the enrichment of *Turicibacter*, *Romboutsia*, and *Oscillospiraceae UCG-005*, suggesting microbiota-mediated modulation of hindgut fermentative metabolism.

Correlation network between fecal microbiota, SCFAs, serum parameters and growth performance of growing wapiti

Figure 5 presents the Spearman correlation network integrating fecal microbiota, SCFAs, serum parameters and growth performance indices of growing wapiti ($P < 0.05$). Network topology analysis identified four keystone taxa with distinct structural roles. *Turicibacter* and *unclassified_Bifidobacteriaceae* served as the global hub and structural bridge, respectively, while *Romboutsia* and Oscillospiraceae *UCG-005* acted as local module cores with the highest clustering coefficient. Turicibacter, Oscillospiraceae UCG-005 and *Romboutsia* formed a tightly clustered sub-module ($R > 0.8$), which was positively correlated with SCFAs, IgG and T-AOC. Notably, acetate, propionate and butyrate exhibited the strongest correlations with Oscillospiraceae *UCG-005* ($R = 0.865$, 0.795 and 0.845, respectively). In contrast, *Peribacillus* constituted a negative association hub, establishing 13 negative correlations with multiple indices ($R < -0.5$), the strongest negative associations were detected for butyrate ($R = -0.944$) and valerate ($R = -0.938$). T-AOC was a core node within the serum-related network, positively linked to keystone microbiota, while GH was positively correlated with ADFI ($R > 0.6$).

Discussion

Most deer-nutrition feeding trials focus on sika deer, and dedicated feed additives for wapiti remain unavailable. The present study investigated the effects of 2% compound mineral-vitamin supplementation on growth performance, serum parameters, fecal microbial community, and SCFA metabolism in growing Ashan wapiti. These results demonstrated that the supplementation effectively improved body weight gain, accompanied by increased serum GH and IGF-1 concentrations, enhanced humoral immunity, and modified serum antioxidant profile. Meanwhile, it restructured hindgut microbial

composition, enriched SCFA-producing functional taxa, and increased both the concentration and proportion of butyrate, ultimately benefiting the health of growing male Ashan wapiti.

Growth performance is an intuitive indicator for assessing the developmental status of juvenile ruminants. At present, commercial wapiti farming largely relies on feeding standards and additives originally developed for beef cattle. Such practices cannot satisfy the species-specific nutritional demands of wapiti and may restrict animal growth as well as overall farming productivity. This study demonstrated that dietary mineral-vitamin supplementation (Mn 166.95 mg/kg, Se 0.235 mg/kg, Vit D_3 375 IU/kg, Vit E 50 IU/kg, etc.) significantly increased the final body weight of growing wapiti (Table 2). Although average daily gain showed a considerable numerical rise (35%) without statistical significance and average daily feed intake was not altered, these observations imply that the supplementation might enhance feed utilization efficiency rather than elevating voluntary feed intake. This result highlights that tailored mineral-vitamin nutrition represents an effective strategy to unlock the growth potential of farmed wapiti, which has practical implications for commercial wapiti production.

Serum physiological indices reflect the endocrine, immune, antioxidant, and hepatic metabolic status of the host. In this study, dietary mineral-vitamin supplementation significantly increased serum GH, IGF-1, elevated serum IgA, IgG, and IgM, and modified the serum antioxidant profile including decreased SOD but increased T-AOC and ALB (Fig. 1). Si et al. (2022) reported that dietary 25-hydroxyvitamin D supplementation promotes antler growth in sika deer partly by increasing circulating IGF-1 concentrations [24]. The concurrent rise of GH and IGF-1 observed in our wapiti suggests that endocrine changes might contribute to host growth. Furthermore, Spearman correlation analysis revealed a positive correlation between GH and ADFI (Fig. 5), suggesting that increased feed intake may provide nutritional signals to activate the GH-IGF-1 endocrine axis. For humoral immunity, several previous ruminant feeding studies have demonstrated beneficial effects of trace-element supplementation. Kachuee et al. reported that dietary Se supplementation increased serum IgG concentration and improved immunity in goats [31]. Tian et al. found that Se-anthocyanin supplementation elevated SOD and IgA levels and altered hindgut bacterial composition together with butyrate proportion in goats [32]. Klein et al. observed increased serum IgA after Zn-Se supplementation in dairy cows [33]. With regard to antioxidant responses, Bao et al. demonstrated that Mn improved growth performance of sika deer by acting as a co-factor for antioxidant-related enzymes, while combined Se and VE benefited growth and IgG levels via antioxidant-dependent pathways[34, 35]. In our study, SOD activity was reduced while T-AOC was increased, indicating reshaping of the systemic antioxidant profile instead of uniform up-regulation of all antioxidant molecules. Serum ALB is synthesized by hepatocytes and rapidly released into the bloodstream, and its concentration directly reflects hepatic protein biosynthetic capacity [36]. Zhang et al. reported that higher serum ALB levels were associated with improved nutritional status and metabolic efficiency in postpartal dairy cows [37]. The increase of ALB in the supplemented group suggests an improvement in hepatic protein metabolism under improved nutritional conditions. Collectively, the mineral-vitamin supplementation may improve final body weight in growing male Ashan wapiti through coordinated improvements in endocrine status, humoral immune defense, systemic antioxidant profile, and hepatic protein anabolism.

In this study, the 45-day with dietary mineral-vitamin supplementation had no significant effect on the α-diversity (Chao1, Shannon, and Pielou's E) in growing wapiti, although the microbial dominance index showed a numerical increase (Fig. 2). Consistent with our findings, previous ruminant nutritional intervention studies reported unchanged fecal microbial richness and evenness under short-term non-antibiotic additive treatments, including active dry yeast supplementation in forest musk deer and D-ribose supplementation in Hu sheep [38, 39]. However, PCoA analysis based on Bray-Curtis dissimilarity revealed clear separation of microbial community structure between the two groups (Fig. 3), demonstrating that mineral-vitamin supplementation reshaped the hindgut microbial composition rather than altering microbial diversity.

LEfSe analysis further identified significant enrichment of three core SCFA-producing genera, including *Turicibacter*, *Romboutsia*, and *Oscillospiraceae_UCG-005*, as well as unclassified Bifidobacteriaceae in the supplementation group (Fig. 4). These functional microorganisms are closely involved in host energy metabolism and intestinal fermentation. *Turicibacter*, participates in lipid metabolism and bile acid regulation, contributing to improved feed energy utilization[40, 41]; *Romboutsia* is a typical butyrate-producing bacterium responsible for intestinal carbohydrate fermentation [42]; *and Oscillospiraceae_UCG-005* is a key cellulolytic taxon positively correlated with fatty acid metabolism in ruminants [43, 44]. It enriched in feces of wild wapiti compared to captive individuals [27], and its abundance can be upregulated by vitamin and amino acid supplementation, accompanied by elevated SCFA concentrations [25, 45]. In addition, vitamin D3 supplementation has been reported to increase the abundance of Oscillospira in calves [46], which is consistent with the microbial variation observed in the present study. Si et al. further confirmed that vitamin A-enriched *Oscillospiraceae UCG-005* participates in regulating host lipid metabolism and anti-inflammatory responses in sika deer [25]. The *unclassified Bifidobacteriaceae*, another enriched taxon in this study, can directly degrade carbohydrates and promote intestinal SCFA accumulation, thereby facilitating host growth performance [47]. Selenium supplementation, as an essential antioxidant trace element, can reshape intestinal microbial community structure and optimize microecological homeostasis [48]. Collectively, compound mineral-vitamin supplementation selectively enriches multiple beneficial fermentation bacteria, laying a critical microbial foundation for improved hindgut metabolic function in growing male Ashan wapiti.

SCFAs are key metabolites produced by microbial fiber fermentation, and changes in their concentrations are valuable for evaluating fiber digestion [49, 50]. In this study, butyrate concentration increased by 658.94% and its relative proportion increased by 140.09%, whereas the proportions of proteolytic fermentation markers (isobutyrate and valerate) decreased by 63.94% and 44.65%, respectively. The drastic increase in butyrate relative to acetate (+ 170.42%), together with the reduction of branched-chain fatty acids, clearly indicates a metabolic transition from amino acid-driven putrefactive fermentation toward carbohydrate-based saccharolytic fermentation[51]. This metabolic shift reflects improved dietary protein utilization efficiency and reduced hindgut protein catabolism[52], and we propose that the butyrate-to-BCFA ratio may serve as a valuable index for evaluating hindgut metabolic health in wapiti nutritional studies. The co-enrichment of *Turicibacter*, *Romboutsia*, and *Oscillospiraceae UCG-005* constitutes a butyrogenic guild specialized in butyrate synthesis via the

butyryl-CoA: acetate CoA-transferase pathway [53]. As the primary energy source for colonocytes, butyrate contributes up to 70% of colonic oxidative metabolism, promotes intestinal epithelial proliferation, and maintains mucosal barrier integrity [49, 54–58]. The substantial butyrate accumulation observed herein facilitates intestinal nutrient absorption capacity, which provides a reasonable explanation for the significant elevation in final body weight (+ 3.38%, $P < 0.05$) in the supplemented wapiti. Furthermore, trace minerals including Mn, Cu, and Zn have also been verified to enhance fiber-degrading enzyme activity and improve ruminal fiber digestibility and SCFA production in ruminants [59–62]. Collectively, mineral-vitamin supplementation contributes to the optimization of hindgut microbial composition and fermentation profile.

Co-occurrence network analysis further confirmed the microbe-metabolite-host interactive relationship shaped by mineral-vitamin intervention. Co-occurrence network analysis further verified the functional synergism of these enriched taxa. *Turicibacter*, *Romboutsia*, and *Oscillospiraceae UCG-005* formed a tightly clustered functional submodule, which was positively correlated with SCFA accumulation, serum IgG, and T-AOC (Fig. 5). In contrast, *Peribacillus* acted as a negative hub taxon, exhibiting extremely strong negative correlations with butyrate (R = − 0.944) and valerate (R = − 0.938) (Fig. 5). As the most potent histone deacetylase (HDAC) inhibitor among SCFAs, butyrate can upregulate B cell immunoglobulin synthesis and antioxidant gene expression, which mechanistically explains the synchronous increases in serum IgA, IgG, IgM, and T-AOC in the present study [63, 64]. In addition, butyrate stimulates intestinal enteroendocrine L cells to secrete GLP-1, which modulates the GH/IGF-1 endocrine axis and elevates circulating GH and IGF-1 concentrations [65]. Combined with the positive correlation between GH and ADFI, these findings demonstrate a complete regulatory cascade: compound mineral-vitamin supplementation selectively enriches beneficial butyrate-producing microbiota, optimizes saccharolytic fermentation and butyrate accumulation, and further coordinates endocrine functions, host immune, and antioxidant, ultimately improving the growth performance and health status of growing male Ashan wapiti.

Conclusion

This study demonstrated that dietary mineral-vitamin supplementation effectively improves growth performance, enhances serum immune and antioxidant capacity, and optimizes hindgut microecology and metabolic profiles in growing male Ashan wapiti. The nutritional intervention enriched key butyrate-producing microbial taxa and markedly elevated fecal butyrate concentration, which collectively contributed to the improved host physiological health and growth status. These findings reveal that gut microbiota and butyrate metabolism serve as crucial microbial metabolic foundations mediating the beneficial effects of mineral-vitamin nutrition on wapiti growth. Furthermore, the present results provide valuable experimental evidence for the development of stage-specific mineral-vitamin feeding strategies and support sustainable nutritional regulation systems for farmed wapiti.

Abbreviations

SCFAs short-chain fatty acids

ADFI average daily feed intake

ADG average daily gain

IgA immunoglobulin A

IgG immunoglobulin G

IgM immunoglobulin M

GH growth hormone

IGF-1 insulin-like growth factor-1

SOD superoxide dismutase

GSH-Px glutathione peroxidase

MDA malondialdehyde

T-AOC total antioxidant capacity

TP total protein

ALB albumin

ALP alkaline phosphatase

Declarations

Ethics approval and consent to participate

Informed consent was obtained from the owner of the Shenghua Wapiti Breeding and Domestication Base to permit the use of the wapiti in this study. All animal experimental procedures were approved by the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences and performed according to the guidelines for animal experiments of the National Institute of Animal Health (No. ISAPSAEC-2023-037SD).

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest.

Funding

This study was supported by Innovation Project of the Chinese Academy of Agricultural Sciences, and National Nanfan Research Institute of Chinese Academy of Agricultural Sciences “Nanfan Special Project” (Project No.: YBXM2554).

Author Contribution

JW: formal analysis, software, data curation, writing-original draft, and writing-review and editing. HL: writing-review and editing. XH: data curation. GW: funding acquisition. KW: conceptualization, methodology, supervision, project administration, and writing-review and editing.

Acknowledgement

Thanks to Shenghua Red Deer Breeding and Training Base for supporting this experiment.

Data Availability

The original contributions presented in the study are publicly available. Microbiota data are deposited in NCBI (Accession Number: PRJNA1543715) and are available at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1543715

References

1. Hu P, Shao Y, Xu J, Wang T, Li Y, Liu H, Rong M, Su W, Chen B, Cui S, et al. Genome-wide study on genetic diversity and phylogeny of five species in the genus Cervus. BMC Genomics. 2019;20(1):384.
2. Wu F, Li H, Jin L, Li X, Ma Y, You J, Li S, Xu Y. Deer antler base as a traditional Chinese medicine: a review of its traditional uses, chemistry and pharmacology. J Ethnopharmacol. 2012;145(2):403–15.
3. Huang J, Sheng Y, Jia X, Qian W, Li Z. Comparative Analysis of Microbial-Short-Chain Fatty Acids-Epithelial Transport Axis in the Rumen Ecosystem Between Tarim Wapiti (Cervus elaphus yarkandensis) and Karakul Sheep (Ovis aries). Microorganisms 2025, 13(5).
4. Víquez-R L, Henrich M, Riegel V, Bader M, Wilhelm K, Heurich M, Sommer S. A taste of wilderness: supplementary feeding of red deer (Cervus elaphus) increases individual bacterial microbiota diversity but lowers abundance of important gut symbionts. Anim Microbiome. 2024;6(1):1–14.

5. Guo J, Li Z, Liu X, Jin Y, Sun Y, Yuan Z, Zhang W, Wang J, Zhang M. Response of the gut microbiota to changes in the nutritional status of red deer during winter. Sci Rep.
6. Shin HT, Hudson RJ, Gao XH, Suttie JM. Nutritional Requirements and Management Strategies for Farmed Deer - Review. Asian-Australasian J Anim Sci 2000(4):561–73.
7. Zanton GI, Heinrichs AJ. Meta-analysis to assess effect of prepubertal average daily gain of Holstein heifers on first-lactation production. J Dairy Sci. 2005;88(11):3860–7.
8. Gelsinger SL, Heinrichs AJ, Jones CM. A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. J Dairy Sci. 2016;99(8):6206–14.
9. Bao K, Wang X, Wang K, Li G, Liu H. Energy and Protein Requirements for the Maintenance of Growing Male Sika Deer (Cervus nippon). Front Vet Sci. 2021;8:745426.
10. Gao Z, Tian J, Zhang Q, Sun H, Jiang Q, Zhang T. Effects of Dietary Protein and Fat Levels on Growth Performance, Nutrient Digestibility, Serum Indexes, and Rectal Fecal Microbiota of Sika Deer (Cervus nippon) Fawns in Early Wintering Period. Anim (Basel) 2025, 15(7).
11. Mu R, Li S, Zhang Y, Li Y, Zhu Y, Zhao F, Si H, Li Z. Microbiota and Metabolite Profiles in the Feces of Juvenile Sika Deer (Cervus nippon) from Birth to Weaning. Anim (Basel) 2024, 14(3).
12. Qian WX, Li ZP, Ao WP, Zhao GY, Li GY, Wu JP. Bacterial community composition and fermentation in the rumen of Xinjiang brown cattle (Bos taurus), Tarim red deer (Cervus elaphus yarkandensis), and Karakul sheep (Ovis aries). Can J Microbiol. 2017;63(5):375–83.
13. Glendinning L, Genç B, Wallace RJ, Watson M. Metagenomic analysis of the cow, sheep, reindeer and red deer rumen. Sci Rep 2021, 11(1).
14. Gnat S, Dziedzic R, Nowakiewicz A, Zieba P, Troscianczyk A, Majer-Dziedzic B, Ziólkowska G, Beeger S, Wójcik M. Effect of balanced supplementary feeding in winter on qualitative and quantitative changes in the population of microbes colonizing the rumen of red deer. *MEDYCYNA WETERYNARYJNA-VETERINARY MEDICINE-SCIENCE AND PRACTICE* 2018, 74(2):119–24.
15. Zheng P, Gao WZ, Cong SB, Leng L, Wang T, Shi L. High-Energy Supplemental Feeding Shifts Gut Microbiota Composition and Function in Red Deer (Cervus elaphus). *ANIMALS* 2024, 14(10).
16. Viquez -RL, Henrich M, Riegel V, Bader M, Wilhelm K, Heurich M, Sommer S. A taste of wilderness: supplementary feeding of red deer (Cervus elaphus) increases individual bacterial microbiota diversity but lowers abundance of important gut symbionts. Anim MICROBIOME 2024, 6(1).
17. Qian WX, Ao WP, Hui XH, Wu JP. Lower dietary concentrate level increases bacterial diversity in the rumen of Cervus elaphus yarkandensis. Can J Microbiol. 2018;64(7):501–9.
18. Guimaraes O, Jalali S, Wagner JJ, Spears JW, Engle TE. Trace mineral source impacts rumen trace mineral metabolism and fiber digestion in steers fed a medium-quality grass hay diet. J Anim Sci 2021.
19. Graziella JO, Loh HY, Muco C, Heldt JS, Spears JW, Engle TE. The Effects of Trace Mineral Source, Diet Type, and Monensin and Tylosin on Rumen Fermentation Characteristics in Beef Cattle. Biol Trace Elem Res. 2026;204(2):969–75.

20. Xue Y, Lin L, Hu F, Zhu W, Mao S. Disruption of ruminal homeostasis by malnutrition involved in systemic ruminal microbiota-host interactions in a pregnant sheep model. Microbiome. 2020;8(1):138.
21. Zhao X, Zhang Y, Rahman A, Chen M, Li N, Wu T, Qi Y, Zheng N, Zhao S, Wang J. Rumen microbiota succession throughout the perinatal period and its association with postpartum production traits in dairy cows: A review. Anim Nutr. 2024;18(000):10.
22. Li S, Wang S, Zhu Y, Mu R, Wang T, Zhen Y, Si H, Du R, Li Z. In vitro dynamics of rumen microbiota and fermentation profiles with Antler growth of Sika deer. Microbiol Spectr 2025.
23. Roberto B. Canani, Margherita, Di, Costanzo, Ludovica, Leone, Monica, Pedata: Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol 2011.
24. Si H, Li S, Nan W, Sang J, Xu C, Li Z. Integrated Transcriptome and Microbiota Reveal the Regulatory Effect of 25-Hydroxyvitamin D Supplementation in Antler Growth of Sika Deer. *Animals (Basel)* 2022, 12(24).
25. Si H, Li S, Liu H, Duan X, Deng R, Zhu Y, Zhang Y, Chen S, Wang S, Ma C et al. Vitamin A Supplementation Induces AMFK Production to Promote Cartilage Proliferation and Antler Growth in Sika Deer. *Animals (Basel)* 2025, 15(19).
26. Sun Y, Yu Y, Guo J, Zhong L, Zhang M. Alterations in Fecal Microbiota Linked to Environment and Sex in Red Deer (Cervus elaphus). Anim (Basel) 2023, 13(5).
27. Zhou Y, Wu Y, Ma C, Ruan X, Cha M, Zhou Y, Li T, Sun W, Liu H. Comparative Profiling of the Fecal Bacteriome, Mycobiome, and Protist Community in Wild Versus Captive (Cervus canadensis). Anim (Basel) 2025, 16(1).
28. Yan J, Wu X, Wang X, Shang Y, Zhang H. Uncovering the Fecal Bacterial Communities of Sympatric Sika Deer (Cervus nippon) and Wapiti (Cervus canadensis). Anim (Basel) 2022, 12(18).
29. Zhao C, Yang Y, Zhao P, Bai L. Comparative analysis of the fecal microbiota in Père David's deer and five other captive deer species. Front Microbiol. 2025;16:1547348.
30. Zheng P, Gao W, Cong S, Leng L, Wang T, Shi L. High-Energy Supplemental Feeding Shifts Gut Microbiota Composition and Function in Red Deer (Cervus elaphus). Anim (Basel) 2024, 14(10).
31. Kachuee R, Moeini M, Souri M. Effects of organic and inorganic selenium supplementation during late pregnancy on colostrum and serum Se status, performance and passive immunity in Merghoz goats. Anim Prod Sci. 2014;54(8):1016–22.
32. Tian X, Li J, Wang X, Lu Q, Fang Y, Zhong R. Effects of selenium and anthocyanin on apparent digestibility, blood parameters, rumen fermentation, and microbiota compositions of goat doe. Sci Rep. 2025;15(1):38207.
33. Klein GS, Leal KW, Rodrigues CA, Draszevski TMR, Brunetto ALR, Vitt MG, Klein MS, Cauduro VH, Flores EMM, da Silva GB et al. Organic Zinc and Selenium Supplementation of Late Lactation Dairy Cows: Effects on Milk and Serum Minerals Bioavailability, Animal Health and Milk Quality. *ANIMALS* 2025, 15(4).

34. Bao K, Wang K, Wang X, Zhang T, Liu H, Li G. Effects of dietary manganese supplementation on nutrient digestibility and production performance in male sika deer (Cervus Nippon). Anim Sci J. 2016;88(3):463–7.
35. Bao K, Wang X, Wang K, Yang Y, Li G. Effects of Dietary Supplementation with Selenium and Vitamin E on Growth Performance, Nutrient Apparent Digestibility and Blood Parameters in Female Sika Deer (Cervus nippon). Biol Trace Elem Res. 2019;195(2):454–60.
36. Raggio G, Lobley GE, Berthiaume R, Pellerin D, Allard G, Dubreuil P, Lapierre H. Effect of protein supply on hepatic synthesis of plasma and constitutive proteins in lactating dairy cows. J Dairy Sci. 2007;90(1):352–9.
37. Zhang SY, Xu YM, Chen TY, Gao D, Wang JJ, Zhuang YM, Jiang W, Hou GB, Liu S, Li SL et al. Effects of Postpartal Relative Body Weight Change on Production Performance, Serum Biomarkers, and Fecal Microbiota in Multiparous Holstein Cows. *ANIMALS* 2025, 15(9).
38. Ying Z, Yang Q, Xie S, Cai M, Fan W, Gao H, Feng X, Wu Y. Active dry yeast enhances immunity through modulation of gut microbiota and serum metabolic processes in captive forest musk deer (Moschus berezovskii). BMC Vet Res. 2025;21(1):262.
39. Qiu Q, Li L, Pan K, Ouyang K, Qu M, Liang H. Dietary D-Ribose Supplementation in Sheep: Implications on Rumen, Fecal Microbiota, and Metabolic Function. Microorganisms 2025, 13(11).
40. Lynch JB, Gonzalez EL, Choy K, Faull KF, Jewell T, Arellano A, Liang J, Yu KB, Paramo J, Hsiao EY. Gut microbiota Turicibacter strains differentially modify bile acids and host lipids. Nat Commun. 2023;14(1):3669.
41. Zheng P, Ding P, Gao W-Z, Chen X, Shi L. The Jarman-Bell principle revisited: Gut microbiota facilitate body size-dependent nutritional strategies in herbivores. Curr Res Microb Sci. 2025;9:100508.
42. Qin R, Wang J, Chao C, Yu J, Copeland L, Wang S, Wang S. RS5 Produced More Butyric Acid through Regulating the Microbial Community of Human Gut Microbiota. J Agric Food Chem. 2021;69(10):3209–18.
43. Wu Z-L, Yang X, Zhang J, Wang W, Liu D, Hou B, Bai T, Zhang R, Zhang Y, Liu H, et al. Effects of forage type on the rumen microbiota, growth performance, carcass traits, and meat quality in fattening goats. Front Vet Sci. 2023;10:1147685.
44. Gao Q, Sun G, Duan J, Luo C, Yangji C, Zhong R, Chen L, Zhu Y, Wangdui B, Zhang H. Alterations in gut microbiota improve SCFA production and fiber utilization in Tibetan pigs fed alfalfa diet. Front Microbiol. 2022;13:969524.
45. Yang B, Zhu Y, Li Y, Gao Y, Si H, Li Z. Effects of rumen-protected lysine on antler growth performance, fecal bacterial community, and blood gene expression in sika deer. Front Vet Sci. 2025;12:1583605.
46. Vigors S, Flores-Villalva S, Meade KG. The impact of vitamin D3 supplementation on the faecal and oral microbiome of dairy calves indoors or at pasture. Sci Rep. 2023;13(1):9111.
47. Zhuang Y, Liu S, Gao D, Xu Y, Jiang W, Chen T, Xiao J, Wang J, Hou G, Li S, et al. The Bifidobacterium-dominated fecal microbiome in dairy calves shapes the characteristic growth phenotype of host. NPJ Biofilms Microbiomes. 2024;10(1):59.

48. Callejón-Leblic B, Selma-Royo M, Collado MC, Gómez-Ariza JL, Abril N, García-Barrera T. Untargeted Gut Metabolomics to Delve the Interplay between Selenium Supplementation and Gut Microbiota. J Proteome Res. 2021;21(3):758–67.
49. Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev. 1990;70(2):567–90.
50. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 2017;8(2):172–84.
51. Gilbert MS, Noortje I, Kies AK, Van MSWC. Protein fermentation in the gut; implications for intestinal dysfunction in humans, pigs and poultry. AJP Gastrointest Liver Physiol. 2018;315(2):G159–70.
52. Zhang L, Piao X. Different dietary protein sources influence growth performance, antioxidant capacity, immunity, fecal microbiota and metabolites in weaned piglets. Anim Nutr. 2022;8:71–81.
53. Vital M, Howe C, Tiedje M. Revealing the Bacterial Butyrate Synthesis Pathways by Analyzing (Meta) genomic Data. Mbio. 2014;5(2):1–11.
54. Mukhopadhya I, Louis P. Gut microbiota-derived short-chain fatty acids and their role in human health and disease. Nat Rev Microbiol. 2025;23(10):635–51.
55. Hays KE, Pfaffinger JM, Ryznar R. The interplay between gut microbiota, short-chain fatty acids, and implications for host health and disease. Gut Microbes. 2024;16(1):2393270.
56. Burlakova KP, Dimitrov KK. Sodium Butyrate in Pig Nutrition: Applications and Benefits. Agriculture; Basel 2026, 16(1).
57. Yu C, Liu S, Chen L, Shen J, Niu Y, Wang T, Zhang W, Fu L. Effect of exercise and butyrate supplementation on microbiota composition and lipid metabolism. J Endocrinol. 2019;243(2):125–35.
58. Brahe LK, Astrup A, Larsen LH. Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases? Obes Rev. 2013;14(12):950–9.
59. Kišidayová S, Pristaš P, Zimovčáková M, Blanár Wencelová M, Homol'ová L, Mihaliková K, Čobanová K, Grešáková Ľ, Váradyová Z. The effects of high dose of two manganese supplements (organic and inorganic) on the rumen microbial ecosystem. PLoS ONE. 2018;13(1):e0191158.
60. Guimaraes O, Wagner JJ, Spears JW, Brandao VLN, Engle TE. Trace mineral source influences digestion, ruminal fermentation, and ruminal copper, zinc, and manganese distribution in steers fed a diet suitable for lactating dairy cows. Animal. 2022;16(4):100500.
61. Guimaraes O, Jalali S, Wagner JJ, Spears JW, Engle TE. Trace mineral source impacts rumen trace mineral metabolism and fiber digestion in steers fed a medium-quality grass hay diet. J Anim Sci 2021, 99(9).
62. Loh HY, Guimaraes O, Thorndyke MP, Jalali S, Spears JW, Heldt JS, Engle TE. Trace Mineral Source Impacts Volatile Fatty Acid Profile and Rumen Trace Mineral Solubility in Feedlot Steers. Anim (Basel) 2025, 15(9).

63. Wachamo S, Gaultier A. The emerging role of microbiota derived SCFAs in neurodegenerative disorders. *Brain, Behavior, & Immunity - Health*.
64. Thomas SP, Denu JM. Short-chain fatty acids activate acetyltransferase p300. eLife Sciences Publications Limited; 2021.
65. Siddiqui MT, Cresci GAM. The Immunomodulatory Functions of Butyrate. Dove; 2021.

Figures

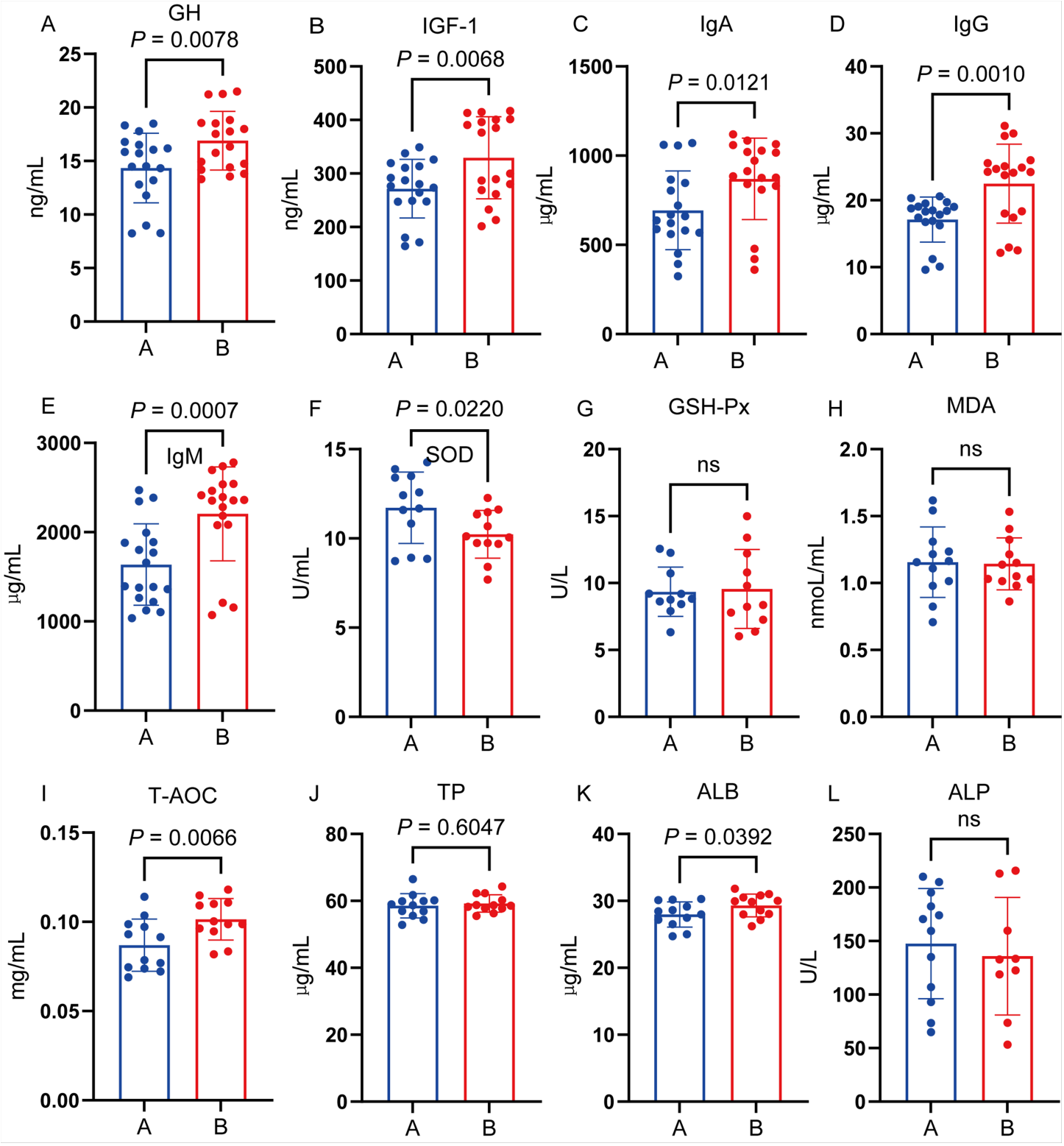


Figure 1

The influence of dietary mineral-vitamin supplementation on the content of immune factors and growth factors in the serum of growing Ashan wapiti. (A) Growth Hormone (GH). (B) Insulin-like Growth Factor-1 (IGF-1). (C) Immunoglobulin A (IgA). (D) Immunoglobulin G (IgG). (E) Immune globulin M (IgM). (F) Superoxide dismutase (SOD). (G) Glutathione peroxidase (GSH-Px). (H) Malondialdehyde (MDA). (I) Total

antioxidant capacity (T-AOC). (J) Total protein (TP). (K) Albumin (ALB). (L) Alkaline phosphatase (ALP). Group A: Control diet; Group B: Basic diet + 2% mineral-vitamin supplementation.

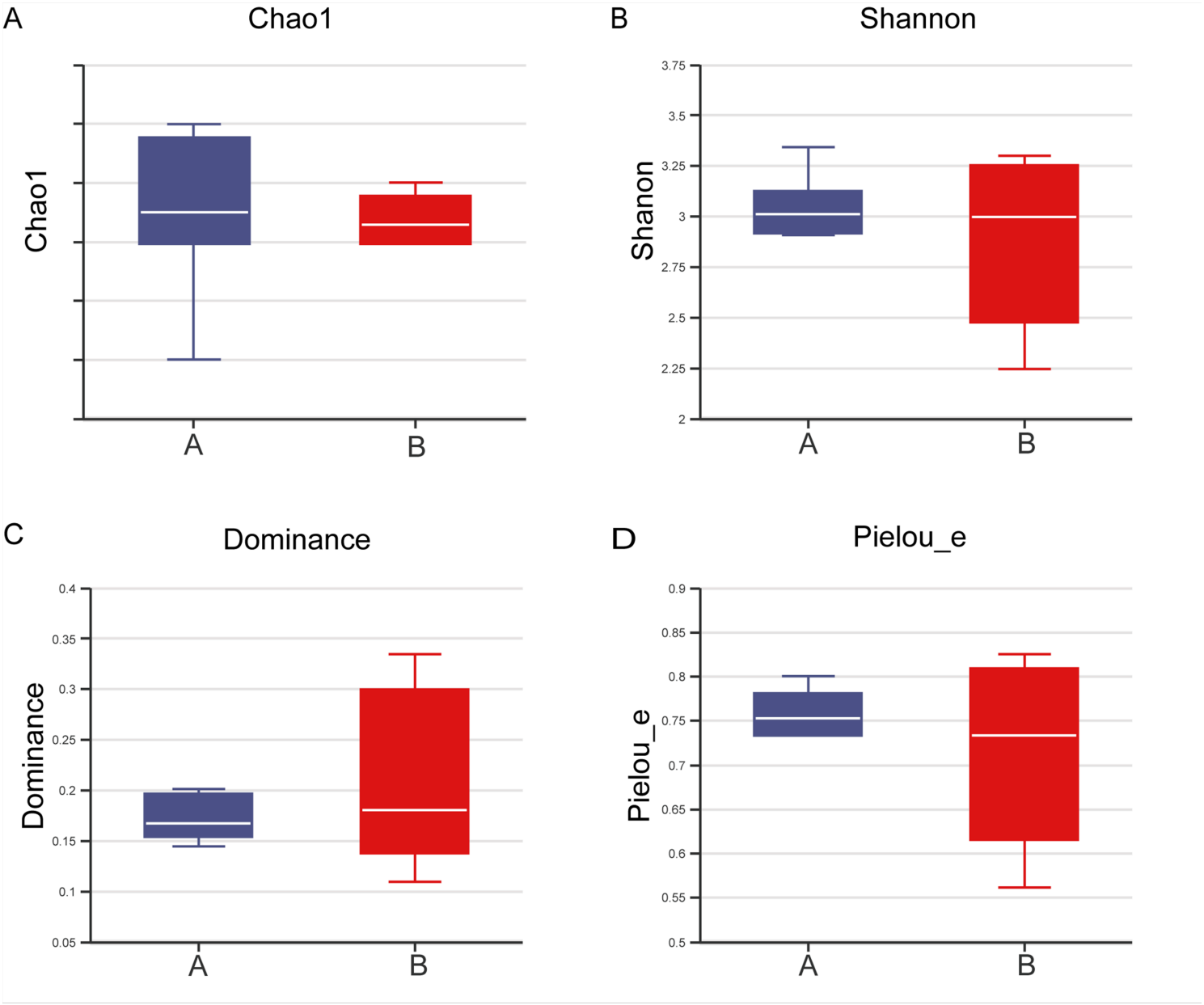


Figure 2

The effect of dietary mineral-vitamin supplementation on the microbial α-diversity of growing Ashan wapiti feces. (A) Chao1 index. (B) Shannon index. (C) Dominance index. (D) Pielou_e index. Group A: Basic diet; Group B: Basic diet + 2% mineral-vitamin supplementation.

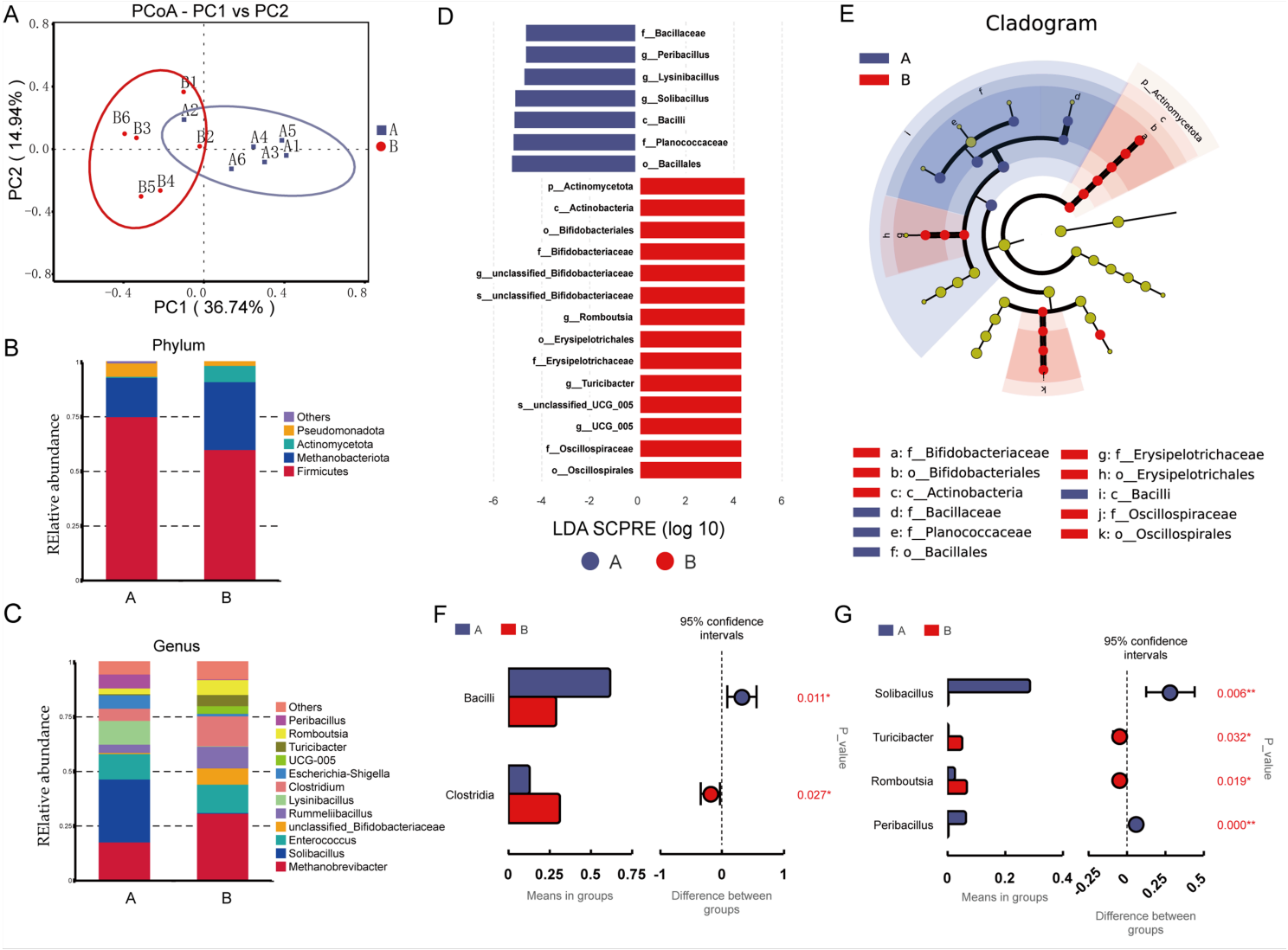


Figure 3

The influence of dietary mineral-vitamin supplementation on the microbial composition of growing Ashan wapiti feces. (A) Principal Component Analysis (PCA). (B) The composition of the fecal microbial community at the phylum level. (C) The composition of the fecal microbial community at the genus level. (D) LEfSe analysis, LDA value distribution bar chart. (E) LEfSe analysis, evolutionary branch diagram. (F) Statistical test for overall level differences. (G) The T-test is a type of statistical test for comparing differences in levels. Group A: Control diet; Group B: Basic diet + 2% mineral-vitamin supplementation.

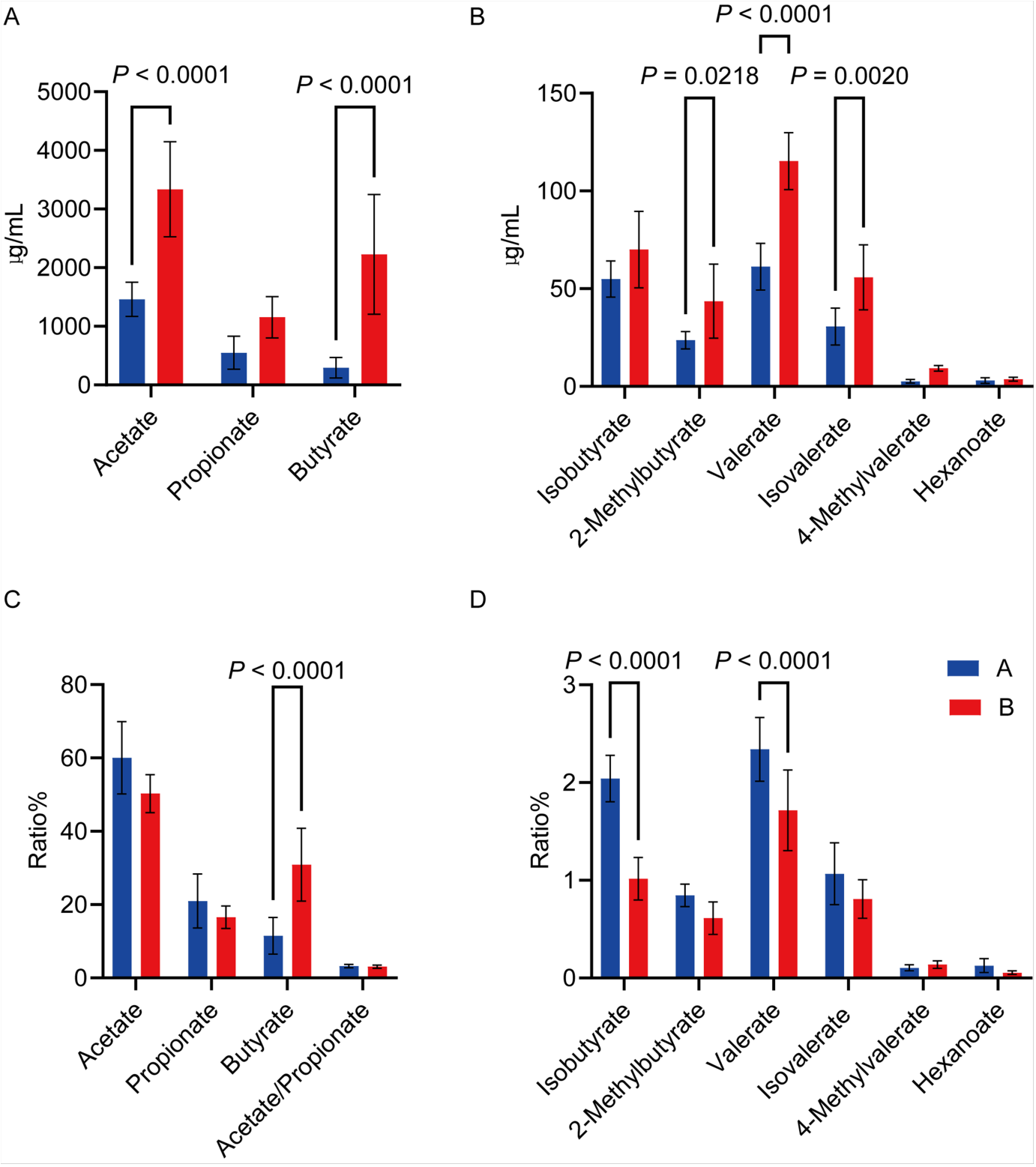


Figure 4

The influence of dietary mineral-vitamin supplementation on the content of short-chain fatty acids in the feces of growing Ashan wapiti. Group A: Control diet; Group B: Basic diet + 2% mineral-vitamin supplementation.

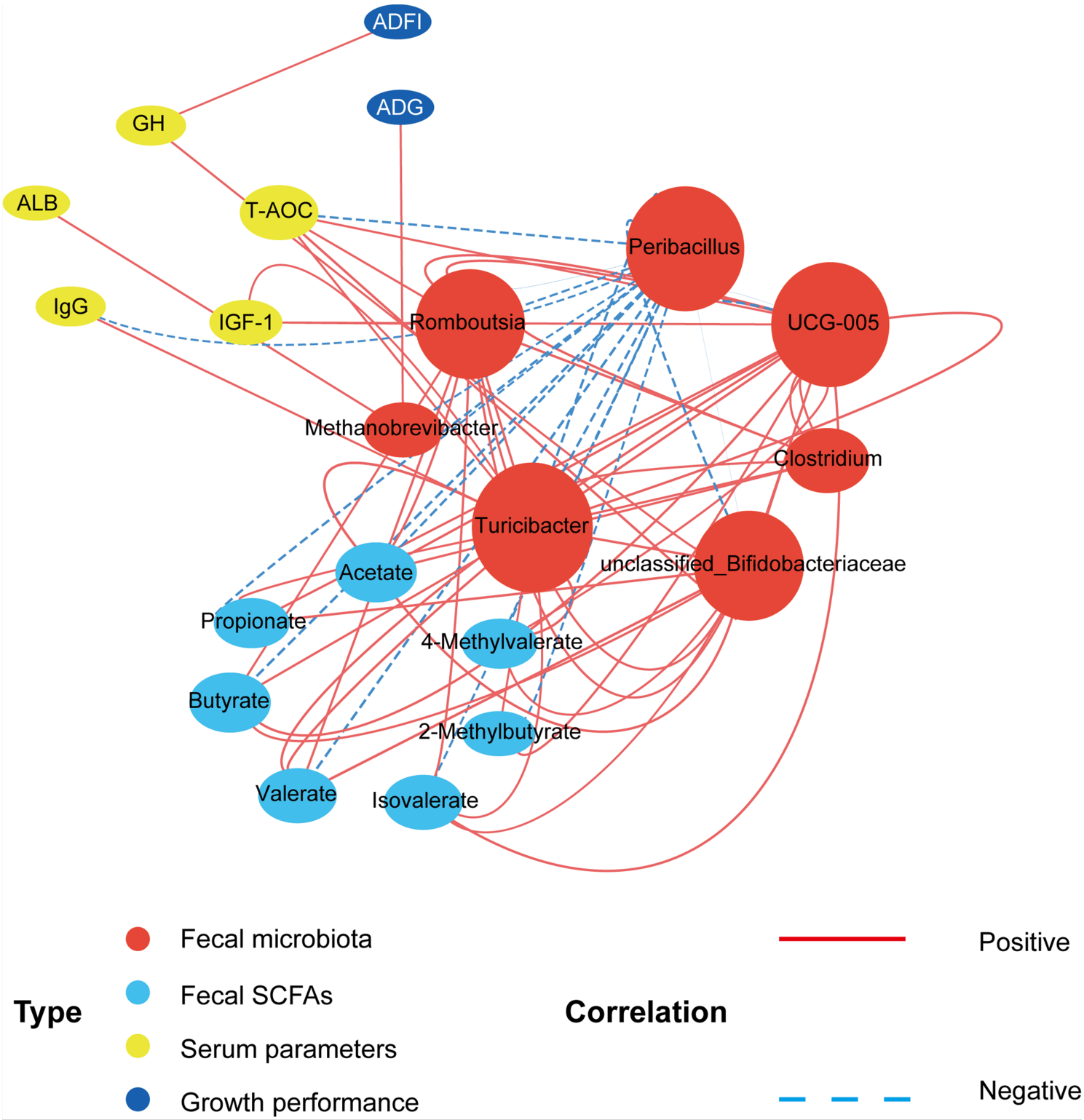


Figure 5

Spearman correlation network analysis between fecal microbiota, SCFAs, serum parameters, and growth performance in growing Ashan wapiti. $P < 0.05$; Spearman correlation coefficient (R) > 0.5.