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The physiological dissimilarities of Holstein dairy cows with different milk yields

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Abstract

Background: Even if breed, parity, dietary and environmental management are same, dairy cows still have notable differences in milk yield that may be underpinned by physiologic differences.

Objectives: This study aimed to investigate the physiological dissimilarities of dairy cows with different milk yields.

Methods: Thirty cows were sorted into high milk-yielding cows (group H: 58.93±2.31 kg/day), moderate milk-yielding cows (group M: 44.99±0.54 kg/day), and low milk-yielding cows (group L: 24.99±6.83 kg/day) according to milk yield. Blood was collected and serum parameters were assessed. Rumen fluid was collected for the evaluation of rumen fermentation parameters (RFPs) and bacterial community composition (BCC).

Results: Serum prolactin, growth hormone, glutathione peroxidase, immunoglobulin A and non-esterified fatty acid had a significantly positive correlation with milk yield ($p < 0.05$), whereas serum glucagon and total antioxidant capacity had a significantly negative correlation with milk yield ($p < 0.05$). The concentration of valeric acid and the ratio of acetic acid to propionic acid in the rumen fluid in group H was significantly lower than that in group L ($p < 0.05$). The concentration of acetic acid and butyric acid in group H was significantly lower than that in groups M and L ($p < 0.05$). The relative abundances of *Ruminococcaceae_NK4A214*_group,

Jianan Dong and Yongjun Liu contributed equally to this manuscript.

Prevotella_1, *Rikenellaceae_RC9_gut_group*, *Christensenellaceae_R-7_group*, *Muribaculaceae*, and *Ruminococcus_2* were negatively correlated with milk yield, whereas the relative abundance of *Succinivibrionaceae_UCG-001*, *Lachnospiraceae_NK3A20_group*, *Shuttleworthia* and *Dialister* were positively correlated with milk yield ($p < 0.05$).

Conclusions: This study indicates that dairy cows with different milk yields have clear divergence in serum indicators, RFPs, BCC and rumen microbial metabolism.

KEYWORDS

bacterial community composition, Holstein dairy cows, milk yield, physiological dissimilarities, rumen fermentation parameters, serum indicator

1 | INTRODUCTION

Milk, one of the most natural foods, is rich in protein, minerals and vitamins. The milk yield in cattle has almost doubled in many countries over the last 30 years (Keyserlingk et al., 2013), but this increased milk yield is still unable to meet the current demand. Many factors affect the milk yield of dairy cows, including breed (Manzi et al., 2020), parity (Hackmann & Firkins, 2015), environment (Oba et al., 2015) and dietary management (Pirondini et al., 2015). Furthermore, even if the above-mentioned factors are the same, notable differences occur in milk yields among dairy cows (Linden et al., 2009), due to individual differences in energy metabolism and health status. A series of changes take place in the metabolism of dairy cows during lactation. The beginning of lactation in dairy cows is marked by the preferential transport of nutrients to mammary tissues (Gross et al., 2017). Nutrient partitioning towards the mammary gland is under genetic control, but many individuals cannot successfully adapt to the processes associated with the preferential metabolism of the mammary tissues in the early stage of lactation (Van Knegsel et al., 2014). This may be because of the negative energy balance (NEB). Gross et al. (2011) showed that NEB can significantly reduce milk yield. They also demonstrated that the NEB of high-yield dairy cows was greater. In addition, stress and decreased immunity may also lead to a decline in the milk yield of dairy cows. Moreover, heat stress was more detrimental to cows with high milk yields than those in that have low milk yields (Staples & Thatcher, 2011). Therefore, physiological metabolism and health are closely related to the performance of dairy cows. Additionally, the rumen microbiome can directly, or indirectly, affect milk production (Collier et al., 1984; Mcguire et al., 1995). Indugu et al. (2017) found that the relative abundance of *Succinivibrionaceae* in high-yielding cows was significantly higher than their abundance in low-yielding cows. The study of the rumen microbiome is important to evaluate ruminant physiology and its effects on lactation performance in dairy cows.

Therefore, in this study, the serum hormone concentrations, oxidative stress status, immunoglobulin levels, rumen fermentation parameters (RFPs) and bacterial community composition (BCC) of Holstein dairy cows managed under identical conditions were analysed in relation to milk yield to determine the impact of these physiological parameters on milk yield.

2 | MATERIALS AND METHODS

2.1 | Animals, diets and experimental design

Thirty healthy Holstein dairy cows, in similar production stages (parities: 3.00 ± 1.22; days in milk: 67.72 ± 10.54) and feed the same total mixed ration (Table 1), were selected from a commercial dairy farm (dairy cows > 2000; Yinchuan, China). According to their milk yield, the cows were divided into three groups with 10 animals in each group. The groups comprised high milk-yielding cows (group H: 58.93±2.31 kg/day), moderate milk-yielding cows (group M: 44.99±0.54 kg/day) and low milk-yielding cows (group L: 24.99±6.83 kg/day). All cows were maintained under the same management practices and had free access to clean water.

2.2 | Collection of milk, serum and rumen fluid samples

The automatic milking system was used to collect milk and record the milk yield. Milk yields were recorded for 7 consecutive days. On the seventh day, milk composition was assessed by collecting milk samples from each experimental cow in the morning (0500 h), afternoon (1300 h) and evening (2100 h). This was achieved by using the diverter of the milking system. The milk samples of each cow were combined in the ratio of 4:3:3 corresponding to the morning, afternoon, and evening in a 50 ml centrifuge tube. Milk composition was determined using an automatic milk composition analyser (Milko Scan FT120, FOSS, 82 Denmark).

Vacuum blood collection tubes were used to collect blood samples (10 ml/head) from the tail vein. The samples were centrifuged at 3000 × *g* and 4°C for 15 min, and the supernatants were sub-packed into 1.5 ml centrifuge tubes and stored at −80°C for subsequent analysis of serum indicators. The time between blood collection and freezing was less than 30 min. The rumen fluid was collected before the morning feeding using an oral stomach tube, which comprised a probe, spiral tube, rubber tube, and syringe (overall length of oral stomach tube: 2 m; length of probe head: 15 cm; syringe: 60 ml; Anscitech Co. Ltd., Wuhan, China). The oral stomach tube was carefully inserted into the rumen

TABLE 1 Ingredients and chemical composition of the experimental diets (% of dry matter)

Item	Kg/head/day	Chemical composition	%
Corn silage	26.00	Crude protein	17.15
Alfalfa silage	2.00	Ether extract	5.18
Alfalfa hay	2.50	Ash	7.84
Corn	2.40	ME, Mcal/kg	2.74
Steam-flaked corn	4.12	NEL, Mcal/kg	1.77
Soybean meal	2.64	Neutral detergent fibre	29.48
Cotton meal	0.96	Acid detergent fibre	16.85
DDGS	1.20	Non-fibrous carbohydrate	43.11
Cotton seed	0.50	Sugar	5.78
Beet pulp	0.80	Starch	26.78
Fresh beer lees	6.00		
Molasses	1.20		
Fat powder	0.15		
Yeast culture	0.05		
0.5% Premix[1]	0.50		
Water	0.50		

[1]Premix: MgO, 0.076 g; $ZnSO_4.H_2O$, 0.036 g; $MnSO_4.H_2O$, 0.043 g; $FeSO_4.H_2O$, 0.053 g; $NaSeO_3$, 0.031 g; vitamin A, 7.27 KIU/kg; vitamin D, 2.08 KIU/kg; vitamin E, 62.32 IU/kg; vitamin K, 0.23 mg; vitamin B_1, 0.092 mg; vitamin B_2, 0.69 mg; vitamin B_{12}, 0.00138 mg; folic acid, 0.023 mg; nicotinic acid, 1.62 mg; calcium pantothenate, 1.15 mg; $CaHPO_4$, 5.17 g; $CaCO_3$, 4.57 g.

of the dairy cows from the mouth. The first two oral stomach tubes of collected fluid were discarded to avoid saliva contamination. The third tube of rumen fluid was aliquoted into 10 ml cryogenic vials and placed into a liquid nitrogen tank for cryopreservation within 5 min. These samples were stored at −80°C until further analysis.

2.3 | Measurement of serum indicators and RFPs

The serum indicators comprised the concentrations of hormones (prolactin, PRL; growth hormone, GH; insulin, INS; glucagon, GC; γ- aminobutyric acid, GABA; cortisol, COR; triiodothyronine, T3; tetraiodothyronine, T4), immunoglobulin (immunoglobulin A, IgA; immunoglobulin M, IgM; immunoglobulin G, IgG), oxidative stress indicators (reactive oxygen species, ROS; malondialdehyde, MDA; glutathione peroxidase, GSH-Px; superoxide dismutase, SOD; total antioxidant capacity), β-hydroxybutyric acid (BHBA), and non-esterified fatty acid (NEFA). The serum indicators were individually determined using the specific ELISA (Enzyme-linked immunosorbent assay) kits for the indicators listed above, according to the manufacturer's instructions (MLbio, Shanghai, China). The absorbance value was measured using a microplate reader (450 nm). The RFPs, including pH, ammonia nitrogen (NH_3-N) concentration, and total volatile fatty acids (TVFAs), were measured in the following manner. A Sanxin MP523-04 pH meter (Shanghai Sanxin Instrumentation, Inc., Shanghai, China) was used to determine the ruminal pH, while a Shimadzu UV-1201 spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure the NH_3-N concentration, as described by Chaney and Marbach (1962). TVFAs were analysed using an Agilent 7890B Gas Chromatograph (Agilent Technologies, Santa Clara, California, USA; Isac et al., 1994; Table S1).

2.4 | DNA extraction and 16S rRNA gene sequencing analysis

The BCC of the rumen fluid was analysed by Shanghai Parsons Biotech Co., Ltd. The forward primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primers 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3–V4 region of the 16S rRNA gene. The resulting DNA fragments were pair-end sequenced on an Illumina MiSeq platform. After cutting primer fragments, splicing, quality filtering, removing repetitive sequences, and removing chimeras, the high-quality sequences were clustered at 97% sequence identity using Vsearch (v2.13.4_linux_x86_64; Rognes et al., 2016). Thereafter, the high-quality sequences were used for taxonomic classification based on the Silva database (version 123). QIIME 2 2019.4 was used to process sequencing data microbiome bioinformatics, as described by Bolyen et al. (2019). The Chao1 (Chao, 1984), Good's coverage (Good, 1953), and Shannon (Shannon, 1948) indexes were estimated using the diversity plug-in. Principal coordinate analysis (PCoA) was performed to reveal differences in the bacterial communities across the three treatments based on unweighted Unifrac, weighted Unifrac, and the Bray–Curtis dissimilarity matrix. A PhyloTree of bacterial phyla and genera was generated using the R program ggtree package. PICRUSt analysis was used to predict functional profiles of the rumen's bacterial communities. Thereafter, the predicted genes were summarised according to their MetaCyc pathways.

2.5 | Statistical analysis

IBM SPSS software version 22.0 (IBM, Armonk, New York, USA) was used to analyse the data using one-way ANOVA for the hormone concentrations, oxidative stress status, immunoglobulin levels, RFPs, BCC, and the rumen microbial metabolism pathways among cows with varying milk yields. A heatmap of Spearman's correlations between serum indicators, RFPs, and milk yield was generated using the R software. The heatmap showing significant differences among high, moderate, and low yield milk were generated using the R software. $p < 0.05$ represented a significant difference.

3 | RESULTS

3.1 | Milk composition among dairy cows with different milk yields

The milk compositions are shown in Table 2. The milk fat and milk protein increased with the increase of milk yield ($p < 0.05$), and the milk

TABLE 2 Mean (7 days of collection) milk yield (kg/day) and composition (%) of the dairy cows for 67.72 (±10.54) days in milk and classified based on milk yield as high, moderate, and low milk yield

	Treatments[1]		
Item	H	M	L
Yield, kg/day			
Milk	58.93 ± 2.31^{c}	44.99 ± 0.54^{b}	24.99 ± 6.83^{a}
Milk fat	2.17 ± 0.51^{c}	1.65 ± 0.34^{b}	0.94 ± 0.24^{a}
Milk protein	1.90 ± 0.15^{c}	1.54 ± 0.09^{b}	0.87 ± 0.25^{a}
Milk composition, %			
Protein	3.23 ± 0.21^{a}	3.41 ± 0.21^{ab}	3.46 ± 0.26^{b}
Fat	3.66 ± 0.76^{a}	3.67 ± 0.78^{a}	3.81 ± 0.45^{a}
Milk urea nitrogen, mg/dl	15.01 ± 1.07^{a}	14.69 ± 1.07^{a}	15.42 ± 0.63^{a}

[1]H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group.
^{a,b,c}Values with different superscripts in the same row indicate a significant difference ($p < 0.05$).

TABLE 3 Concentrations of serum hormones of dairy cows with different milk yields

	Treatments[1]		
Item	H	M	L
Prolactin (PRL, mIU/L)	856.68 ± 51.66^{b}	727.15 ± 32.56^{a}	718.66 ± 50.82^{a}
Growth hormone (GH, ng/ml)	17.44 ± 1.57^{b}	16.85 ± 1.30^{ab}	15.68 ± 1.68^{a}
Insulin (INS, mIU/L)	40.78 ± 2.78^{a}	40.09 ± 2.28^{a}	39.74 ± 2.24^{a}
Glucagon (GC, pg/ml)	385.71 ± 22.60^{a}	425.27 ± 20.67^{b}	437.62 ± 16.09^{b}
γ- aminobutyric acid (GABA, mol/L)	23.99 ± 1.37^{a}	23.43 ± 1.13^{a}	22.89 ± 1.25^{a}
Cortisol (COR, pg/ml)	68.11 ± 5.07^{a}	70.07 ± 4.24^{a}	70.48 ± 5.33^{a}
Triiodothyronine (T3, nmol/L)	8.18 ± 0.92^{a}	7.47 ± 0.54^{a}	7.69 ± 0.72^{a}
Tetraiodothyronine (T4, nmol/L)	297.45 ± 20.89^{a}	280.10 ± 22.73^{a}	300.00 ± 15.62^{a}

[1]H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group.
^{a,b}Values with different superscripts in the same row indicate a significant difference ($p < 0.05$).

protein level in the L group was significantly higher than that in the H group ($p < 0.05$).

3.2 | Concentrations of serum indicators among dairy cows with different milk yields

The hormone concentrations within the serum are presented in Table 3. The results showed that the concentration of PRL in the H group was significantly higher than that in groups M and L ($p < 0.05$). The concentration of GH in the H group was significantly higher than that in the L group ($p < 0.05$). However, the concentration of GC in the H group was significantly lower than that in the M and L groups ($p < 0.05$).

The data for the serum oxidative stress status are presented in Table 4. The serum concentration of ROS tended to decrease with increasing milk yield. The serum concentrations of GSH-Px in dairy cows in the H and M groups were significantly higher than that in the L group ($p < 0.05$).

The serum levels of immunoglobulin, BHBA, and NEFA are presented in Table 5. The serum concentration of BHBA in dairy cows increased with increased milk yield ($p < 0.05$). The concentrations of NEFAs and IgA in groups H and M were significantly higher than those in group L ($p < 0.05$). The serum concentration of IgG in the H group was lower than that in groups M ($p = 0.09$) and L ($p = 0.23$). Meanwhile, there was no significant difference in IgM levels among groups.

Spearman's correlation analysis revealed that milk yield was positively correlated with PRL and GH ($p < 0.05$; $0.48 < R < 0.56$), but it was negatively correlated with GC ($p < 0.05$; $R = -0.61$; Figure 1a). There was a significant positive correlation between milk yield, and NEFA and IgA ($p < 0.05$; $0.37 < R < 0.56$; Figure 1b). Moreover, milk yield was positively correlated with GSH-Px ($p < 0.05$; $R = 0.64$) and negatively correlated with total antioxidant capacity (T-AOC) ($p < 0.05$, $R = -0.38$; Figure 1c).

3.3 | RFPs of dairy cows with different milk yields

The RFPs among dairy cows with different milk yields are presented in Table 6. The concentrations of acetic acid, butyric acid, and isovaleric acid in the H group were lower than those in the M and L groups ($p < 0.05$). The concentration of propionic acid in the M group was higher than that in the L group ($p < 0.05$), and the ratio of acetic acid to

TABLE 4 Serum oxidation and antioxidation indicators of dairy cows with different milk yields

Item	Treatments[1]		
	H	M	L
Reactive oxygen species (ROS, IU/ml)	604.61 ± 45.46[a]	604.10 ± 44.80[a]	603.38 ± 31.69[a]
Malondialdehyde (MDA, nmol/L)	13.95 ± 1.10[a]	14.37 ± 1.10[a]	14.09 ± 1.23[a]
Glutathione peroxidase (GSH-Px, ng/ml)	6138.53 ± 355.21[b]	6240.34 ± 314.59[b]	5259.24 ± 293.71[a]
Superoxide dismutase (SOD, ng/ml)	12.24 ± 0.36[a]	11.70 ± 0.47[a]	11.82 ± 0.56[a]
Total antioxidant capacity (T-AOC, U/ml)	55.00 ± 2.42[a]	56.37 ± 3.13[a]	56.84 ± 2.7[a]

[1]H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group.
[a,b]Values with different superscripts in the same row indicate a significant difference ($p < 0.05$).

TABLE 5 Serum immunoglobulin levels, BHBA and NEFA of dairy cows with different milk yields

Item	Treatments[1]		
	H	M	L
β-hydroxybutyric acid (BHBA, μmol/L)	540.31 ± 37.41[b]	522.77 ± 29.50[ab]	506.59 ± 28.55[a]
Non-esterified fatty acid (NEFA, μmol/L)	2172.03 ± 107.17[b]	2127.96 ± 107.65[b]	1984.58 ± 124.09[a]
Immunoglobulin levels			
Immunoglobulin A (IgA, mg/ml)	5.20 ± 0.15[b]	5.14 ± 0.15[b]	4.89 ± 0.15[a]
Immunoglobulin M (IgM, mg/ml)	2.53 ± 0.09[a]	2.47 ± 0.13[a]	2.49 ± 0.07[a]
Immunoglobulin G (IgG, mg/ml)	8.54 ± 0.43[a]	9.10 ± 0.51[a]	9.06 ± 0.74[a]

[1]H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group.
[a,b]Values with different superscripts in the same row indicate a significant difference ($p < 0.05$).

TABLE 6 Rumen fermentation parameters in dairy cows with different milk yields

Item	Treatments[1]		
	H	M	L
pH	5.67 ± 0.21[a]	5.52 ± 0.29[a]	5.70 ± 0.26[a]
Acetic acid (mmol/L)	60.82 ± 3.44[a]	67.50 ± 2.75[b]	68.62 ± 4.06[b]
Propionic acid (mmol/L)	27.13 ± 4.64[ab]	29.18 ± 6.79[b]	23.06 ± 2.90[a]
Butyric acid (mmol/L)	10.28 ± 1.26[a]	12.57 ± 0.84[b]	12.43 ± 1.12[b]
Valeric acid (mmol/L)	0.90 ± 0.10[a]	1.12 ± 0.20[ab]	1.29 ± 0.24[b]
Isovaleric acid (mmol/L)	1.57 ± 0.12[a]	1.80 ± 0.21[a]	1.73 ± 0.36[a]
Acetic acid/propionic acid	2.28 ± 0.23[a]	2.33 ± 0.38[ab]	2.76 ± 0.36[b]
Total volatile fatty acids (TVFAs, mmol/L)	100.69 ± 6.7[a]	112.17 ± 8.85[a]	107.13 ± 14.12[a]
Ammonia nitrogen (NH_3-N, mg/100 ml)	7.56 ± 1.46[a]	7.47 ± 0.84[a]	8.63 ± 0.98[a]

[1]H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group.
[a,b]Values with different superscripts in the same row indicate a significant difference ($p < 0.05$).

propionic acid decreased with the increase in milk yield ($p < 0.05$). Milk yield was negatively correlated with propionic acid, butyric acid, valeric acid, isovaleric acid, and NH_3-N ($p < 0.05$; $0.48 < R < 0.75$; Figure 1d).

3.4 | BCC in dairy cows with different milk yields

We obtained 749,287 high-quality reads and an average of 49,952±7564 reads per sample via 16S rRNA sequencing. Based on a 97% sequence similarity, 3636, 3816, and 2971 amplicon sequence variants were obtained for the H group (mean = 1748), M group (mean = 1734), and L group (mean = 1486), respectively. Taxonomic analysis showed that the sequences belonged to 15 bacterial phyla and 155 bacterial genera. After the flattening depth became greater than 2500, rarefaction curves trended to a plateau, and the sequencing coverage became saturated (Figure 2). The Goods_coverage index value increased with increased milk yield (Figure 3; Goods_coverage: $p = 0.075$), whereas the diversity index value decreased with increased

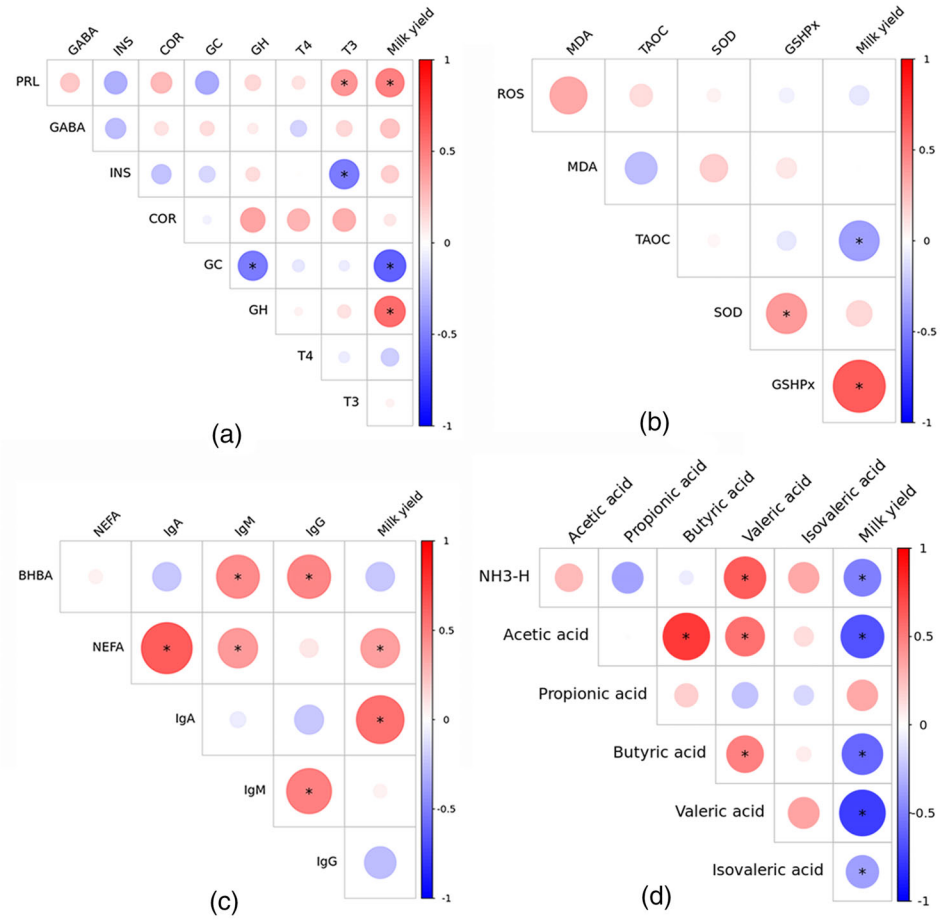

FIGURE 1 Spearman correlations between serum indicators and yield milk. (a) Spearman correlations within concentrations of hormones and yield milk. (b) Spearman correlations within oxidative status and yield milk. (c) Spearman correlations within immune, NAFA, BHBA and yield milk. (d) Spearman correlations within rumen fermentation parameters and yield milk. PRL, prolactin; GABA, γ- aminobutyric acid; INS, insulin; COR, cortisol; GC, glucagon; GH, growth hormone; T3, triiodothyronine; T4, tetraiodothyronine; ROS, reactive oxygen species; MDA, malondialdehyde; TAOC, total antioxidant capacity; SOD, superoxide dismutase; GSHPx, glutathione peroxidase; BHBA, β-hydroxybutyric acid; NEFA, non-esterified fatty acid; IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G; NH3-H, ammonia nitrogen

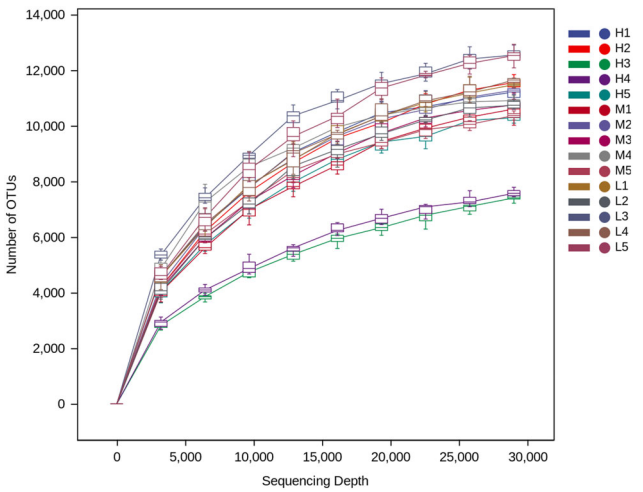

FIGURE 2 The rarefaction curve of OTU from rumen bacterial 16S rRNA gene at 97% sequence similarity. H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group

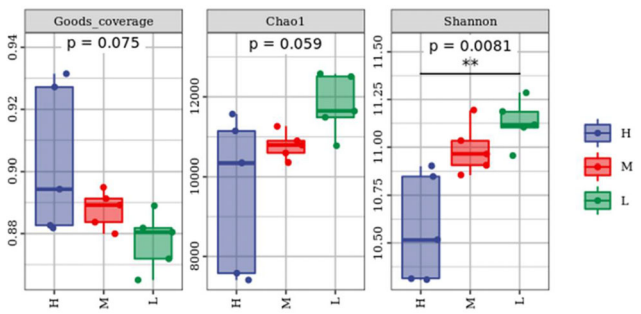

FIGURE 3 Microbial diversity and richness indices among dairy cows with different milk yields. H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group

milk yield (Figure 3; Shannon: $p < 0.01$). Moreover, the richness index value in group H was lower than that in the other groups (Figure 3; Chao 1: $p = 0.059$).

The BCC of different milk yield groups was distinctly illustrated by a PCoA plot. The unweighted UniFrac distance (Figure 4a, ANOSIM:

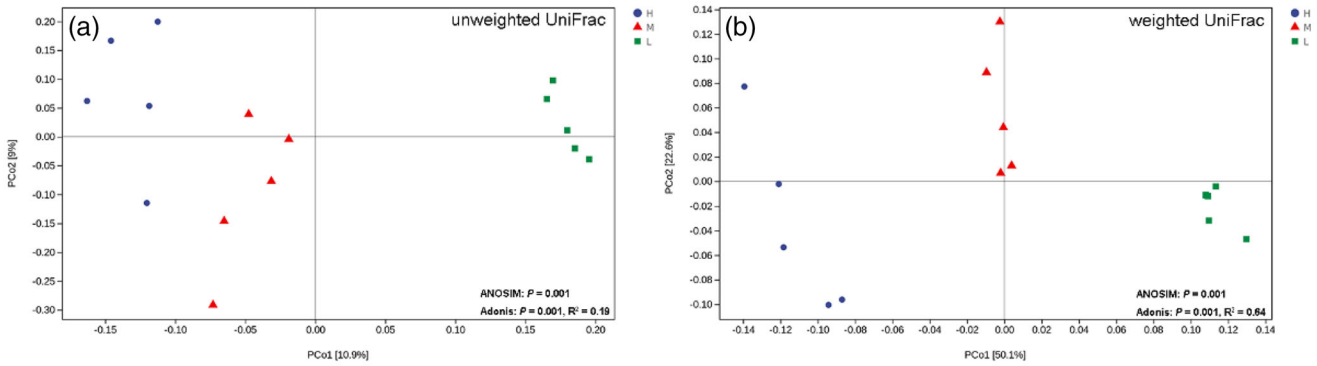

FIGURE 4 Principal coordinates analysis (PCoA) revealing bacterial genera in dairy cows with different milk yields based on unweighted UniFrac distance (a) and weighted UniFrac distance (b). H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group

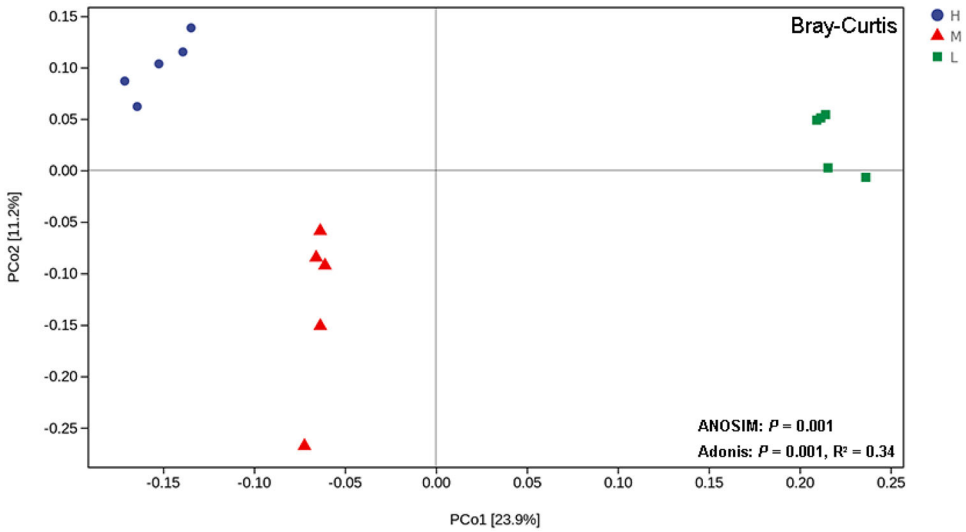

FIGURE 5 Principal coordinates analysis (PCoA) revealing bacterial genera in dairy cows with different milk yields based on Bray-Curtis dissimilarity matrix. H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group

$p = 0.001$; Adonis: $p = 0.001$, $R^2 = 0.19$), weighted UniFrac distance (Figure 4b, ANOSIM: $p = 0.001$; Adonis: $p = 0.001$, $R^2 = 0.64$), and Bray–Curtis index (Figure 5, ANOSIM: $p = 0.001$; Adonis: $p = 0.001$, $R^2 = 0.34$) all revealed clear segregation and dissimilarities among the groups. Furthermore, the BCC at the genus level was compared (Table 7 and Figure 6). The relative abundance of *Prevotella_1* and *Muribaculaceae* in the group H was significantly lower than that in groups M and L ($p < 0.05$). The relative abundances of *Ruminococcaceae _NK4A214_group*, *Rikenellaceae _RC9_gut _group*, *Christensenellaceae_R-7_group*, *Ruminococcus_2*, and *Prevotellaceae_UCG-003* in groups H and M were significantly lower than those in group L ($p < 0.05$). The relative abundance of *Succiniclasticum* in the H group was significantly lower than that in the M group ($p < 0.05$). Furthermore, the relative abundance of *Ruminococcaceae_UCG-014* in the H group was significantly higher than that in the M and L groups ($p < 0.05$). The relative abundance of *Prevotella_7* in the M group was significantly higher than that in the H and L groups ($p < 0.05$). The relative abundance of *Dialister*, *Succinivibrionaceae_UCG-001*, *Lachnospiraceae_NK3A20_ group*, and *Shuttleworthia* declined with the reduction in milk yield ($p < 0.05$). The relative abundance of *Ruminococcaceae_UCG-005* and *F082* in the M group was significantly lower than that in the L group ($p < 0.05$).

3.5 | Potential functions of rumen bacteria

Functionality of rumen bacterial found in the rumen was determined using MetaCyc. These functions were mainly enriched in Biosynthesis, Degradation, Generation, Glycan, Macromolecule, and Metabolism in the first level pathways (Figure 7a). These results show that the relative abundance of Biosynthesis, Degradation, Generation and Metabolism in the H group were significantly higher than those in the M and L groups ($p < 0.05$). Furthermore, the relative abundance in

TABLE 7 Bacterial-community composition in dairy cows with different milk yields (%)

Phylum	Genus	Treatments[1]		
		H	M	L
Bacteroidetes		50.56 ± 2.96^{a}	56.21 ± 2.31^{b}	54.24 ± 1.39^{b}
	Prevotella_1	28.35 ± 2.65^{a}	32.15 ± 2.73^{b}	33.61 ± 1.22^{b}
	Prevotella_7	7.92 ± 0.71^{b}	9.24 ± 0.51^{c}	3.02 ± 0.10^{a}
	Rikenellaceae_RC9_gut_group	2.77 ± 0.39^{a}	2.98 ± 0.24^{a}	4.51 ± 0.28^{b}
	Muribaculaceae	2.40 ± 0.20^{a}	2.74 ± 0.32^{b}	2.93 ± 0.17^{b}
	Prevotellaceae_UCG-001	1.34 ± 0.16^{a}	1.43 ± 0.07^{a}	1.49 ± 0.11^{a}
	Prevotellaceae_UCG-003	0.82 ± 0.13^{a}	0.77 ± 0.11^{a}	1.01 ± 0.10^{b}
Firmicutes		39.02 ± 2.57^{a}	39.25 ± 1.45^{a}	41.75 ± 1.70^{a}
	Ruminococcaceae_NK4A214_group	6.99 ± 0.57^{a}	7.54 ± 0.37^{a}	10.02 ± 0.77^{b}
	Succiniclasticum	7.43 ± 0.73^{a}	8.43 ± 0.45^{b}	7.76 ± 0.64^{ab}
	Ruminococcaceae_UCG-014	4.47 ± 0.48^{b}	3.88 ± 0.42^{a}	3.47 ± 0.18^{a}
	Christensenellaceae_R-7_group	2.16 ± 0.28^{a}	2.41 ± 0.08^{a}	3.64 ± 0.17^{b}
	Ruminococcus_2	1.62 ± 0.34^{a}	1.71 ± 0.17^{a}	2.13 ± 0.06^{b}
	Lachnospiraceae_NK3A20_group	1.47 ± 0.08^{c}	1.24 ± 0.21^{b}	0.92 ± 0.08^{a}
	Shuttleworthia	1.12 ± 0.10^{c}	0.92 ± 0.07^{b}	0.49 ± 0.03^{a}
	Dialister	1.13 ± 0.26^{c}	0.75 ± 0.07^{b}	0.27 ± 0.02^{a}
	Butyrivibrio_2	0.76 ± 0.12^{a}	0.78 ± 0.04^{a}	0.71 ± 0.06^{a}
	Ruminococcaceae_UCG-005	0.76 ± 0.12^{ab}	0.70 ± 0.07^{a}	0.86 ± 0.08^{b}
	F082	1.95 ± 0.33^{ab}	1.85 ± 0.31^{a}	2.29 ± 0.20^{b}
Proteobacteria		5.46 ± 0.47^{c}	1.52 ± 0.17^{b}	0.98 ± 0.14^{a}
	Succinivibrionaceae_UCG-001	5.11 ± 0.52^{c}	1.23 ± 0.11^{b}	0.76 ± 0.11^{a}
Spirochaetes		0.80 ± 0.25^{a}	0.69 ± 0.21^{a}	0.77 ± 0.12^{a}
	Treponema_2	0.80 ± 0.25^{a}	0.69 ± 0.21^{a}	0.76 ± 0.12^{a}

[1]H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group.
^{a,b,c}Values with different superscripts in the same row indicate a significant difference ($p < 0.05$).

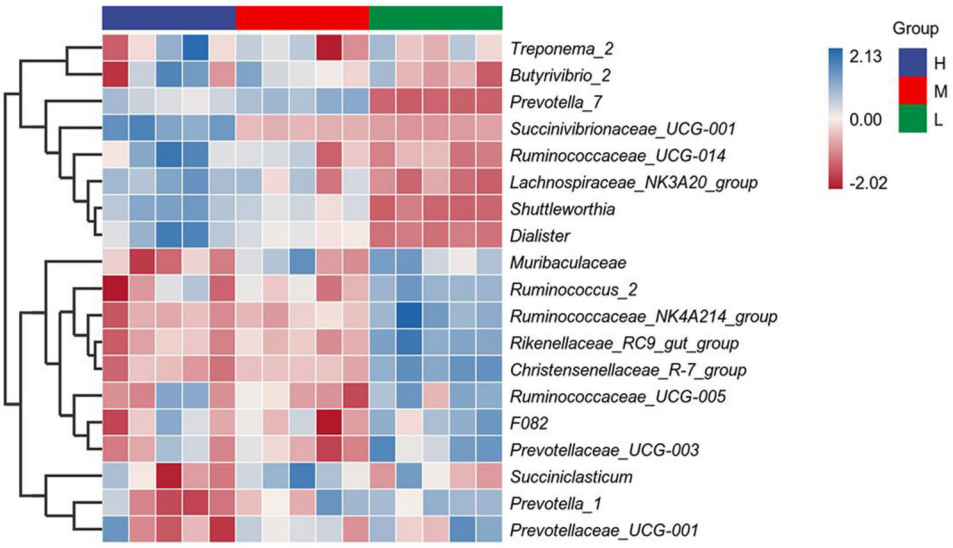

FIGURE 6 Significant differences of bacterial genera among dairy cows with different milk yields. H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group

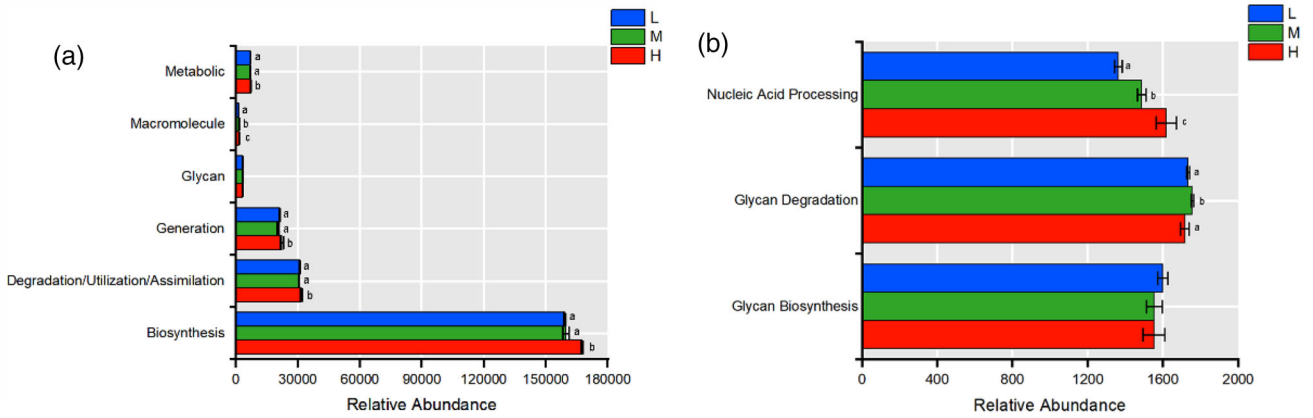

FIGURE 7 Differential MetaCyc functions among dairy cows with different milk yields. (a) First level pathway. (b) Comparison of Glycan and Macromolecule in second level pathway. H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group

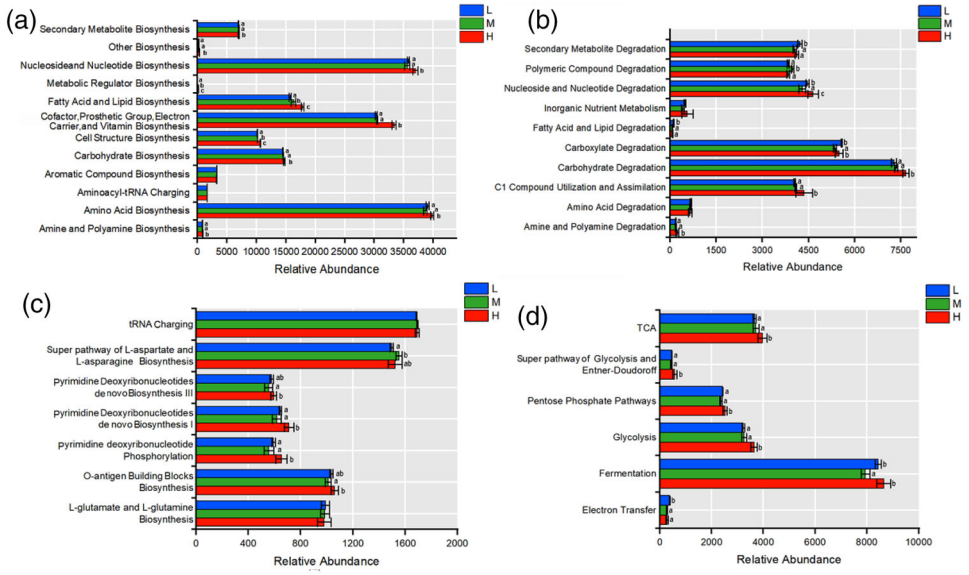

FIGURE 8 Differential MetaCyc functions in the second pathway among dairy cows with different milk yields. (a) Biosynthesis pathway. (b) Degradation pathway. (c) Metabolic pathway. (d) Generation pathway. H: high milk yield cows, H group; M: moderate milk yield cows, M group; L: low milk yield cows, L group

Macromolecule increased with increased milk yield ($p < 0.05$). For the second-level pathways, 2-s pathways belonged to Glycan and 1-s pathway belonged to Macromolecule (Figure 7b). A total of 12-s pathways belonged to Biosynthesis (Figure 8a), 10-s pathways belonged to Degradation (Figure 8b), 7-s pathways belonged to Metabolism (Figure 8c), and 6-s pathways belonged to Generation (Figure 8d). In the Biosynthesis pathway, Fatty Acid and Lipid Biosynthesis, as well as other pathways in the H group, were significantly higher than those in the L group; the exceptions to this were Aminoacyl tRNA Charging and Aromatic Compound Biosynthesis. In the Generation of Precursor Metabolite and Energy pathway, the Electron Transfer pathway in the H and M groups was significantly lower than that in the L group ($p < 0.05$). The Glycolysis, Pentose Phosphate Pathways, tricarboxylic acid cycle (TCA), and the Superpathway of glycolysis and Entner–Doudoroff in the H group were significantly higher than those in the L group ($p < 0.05$).

4 | DISCUSSION

It is well known that genetics, parity, diets and environmental management have an impact on the milk yield and milk composition of dairy cows. In addition, heat stress can significantly reduce milk yield, with the milk yield of primiparous cows being more easily affected by heat stress (Chen et al., 2022). Even cows fed the same diet under the same management presented wide variations in milk production. A study by

Xue et al. (2019) showed that the relative abundance of bacteria in the rumen had a potential impact on milk production and milk protein in dairy cows. Another study confirmed that individual physiological and metabolic differences contributed to the variations in milk yield and protein (Wu et al., 2018). This study attempted to explain the differences in the milk yield of dairy cows fed the same diets from the perspectives of the cows' self hormone secretion, humoral immune response, oxidative stress status, rumen fermentation and microbial composition.

4.1 | Serum indicators

Hormone regulators such as GH, PRL, INS and GC are related to the growth axis, glucose metabolism and lipid metabolism. They play important roles in the distribution of nutrients to the mammary tissue (Gross & Bruckmaier, 2019). The process is coordinated by a very complex interplay of hormones, and their receptors, throughout lactation (Marett et al., 2019). This suggests that the concentrations of PRL and GH in the H group were significantly higher than that in the L group in our study. PRL secreted by the anterior pituitary gland's eosinophils can promote the development and growth of the mammary tissue (Ibrahim et al., 2008), stimulate and maintain lactation and stimulate the production of luteinising hormone receptors in follicles (Groner, 2002). The specific function of GH is to support milk synthesis by coordinating catabolism in the body to increase milk yield. Davis et al. (2020) reported that the milk yield of cows injected with GH was significantly higher than that of the control group. The PRL and GH play a key role in the regulation of lactation. The more GH and PRL secreted by the individual, the greater the amount of milk produced. INS works to promote anabolism, and increase the absorption of glucose and fatty acids in peripheral tissues (non-mammary glands) for storage (Marett et al., 2019). In contrast to INS, GC promotes catabolism (Madsbad, 2013). Glucose homeostasis is regulated by the reciprocal function of INS and GC. Thus, we speculated that the high concentration of GC in cows with low milk yield inhibited INS' absorption of glucose (Zarrin et al., 2015), which resulted in a milk yield reduction.

Oxidative stress is the process of mutual transformation between oxidation and antioxidation in the body. There are two kinds of antioxidant systems in the body, one is the enzyme antioxidant system, and the other is a non-enzyme antioxidant system. The enzyme antioxidant system includes superoxide dismutase (SOD), catalase (CAT), and GSH-Px (Sordillo et al., 2009). In this study, only GSH-Px was significantly higher in groups H and M than in group L. A study also showed that when milk yield was improved, the level of GSH-Px in blood would increase (Paraskevakis, 2015). During lactation, the metabolic process of dairy cows is prone to disorder. Our results suggest that metabolic abnormalities resulting in low milk yield in some cows may be partly due to imbalances between oxidant and antioxidant systems. ROS is produced in the process of body metabolism. When ROS is produced too much, it will cause oxidative stress leading to metabolic disorder. Despite the ROS values being comparable amongst all groups of cows, low-yielding cows in our study may have been under grater oxidative stress resulting in a higher demand of antioxidant enzymes like GSH-Px to maintain ROS levels (Kurata et al., 1993).

Whether the immune function is normal or not is directly related to body health. Kaufman et al. (2017) reported that there was a positive correlation between milk yield and cow health. IgA, IgM and IgG levels can reflect one aspect of immune function of the body (Singh et al., 2014). IgA can induce immune cells and pro-inflammatory responses (Breedveld & van Egmond, 2019), which can resist some inflammation and diseases caused by bacteria, fungi, and viruses, so as to maintain the health of dairy cows. The current experiment showed that the concentrations of IgA in the H and M groups were significantly higher than that in the L group. A previous study indicated that feeding an immunomodulatory supplement from dry-off until 150 days in milk improved the milk yield and reduced the incidence of diseases (Casarotto et al., 2020). Thus, we speculate that dairy cows with higher milk yields have superior IgA response that may let them combat some disease more effectively and thus support great milk production. In addition, in this experiment, the concentration of IgG in the H group was lower than that in the M and L groups, respectively. This may be due to cows with high milk yields having more blood flowing through their mammary tissues. This brings IgG in the blood to the mammary tissues and then transfers it to the milk secreted by the mammary tissue (Herr et al., 2011). The concentration of IgG in milk would be need to confirm this but was not assessed in this study.

BHBA in the blood of cows is mainly used to diagnose cow ketosis (Suthar et al., 2013). Concentrations of 1.2 mmol/L (Drift et al., 2012) or 1.4 mmol/L (Denis-Robichaud et al., 2014) were adopted as thresholds for subclinical ketosis. In this study, the concentration of BHBA in the serum of all dairy cows in this experiment was less than 1.2 mmol/L; thus, there was no risk of ketosis. In addition, the concentrations of NEFA and BHBA are commonly used as indicators to determine energy metabolism and the extent of fat mobilisation (Shin et al., 2015). In the current study, the concentrations of BHBA and NEFA in group H were substantially greater than those in group L. One study showed that a high concentration of BHBA would increase the daily milk yield by 5−11% (Benedet et al., 2019), which was consistent with our findings. The liver is responsible for the metabolism of NEFA, which can be completely oxidised to ATP and excreted from the liver in the form of lipoprotein, or partially oxidised to ketones such as BHBA (Reynolds et al., 2003; Soosten et al., 2011). After dehydrogenation and activation, BHBA can enter the TCA cycle, which is the main metabolic pathway in the body. Therefore, the high concentrations of BHBA and NEFA in the H group may promote the metabolism of the TCA cycle, so as to produce more ATP and provide sufficient energy for dairy cows to synthesise and secrete more milk.

4.2 | RFPs and BCC

The digestion of bacteria, such as anaerobic bacteria and protozoa, plays an important role in ruminant nutrition. Denek et al. (2016) showed that frozen rumen fluid had significantly reduced numbers of protozoa and anaerobic bacteria compared with fresh rumen fluid.

Thus, fresh rumen fluid should be used for in vitro experiments. Rumen bacterial fermentation can meet the nearly 70% of the energy (Blum et al., 1983) and 60–85% of the protein (Ruoff et al., 2017) required by dairy cows. Isoacids (IA) are growth factors produced during rumen fermentation, especially for cellulolytic bacteria (Allison et al., 1962; Dehority et al., 1967). IAs mainly include isobutyric, isovaleric, 2-methyl butyric (branched-chain VFA) and valerate (a straight-chain acid). A study showed that supplementation of IA (17 g/day of isobutyrate, 17 g/day of 2-methyl butyrate, 12 g/day of isovalerate, and 14 g/day of valerate) increased milk fat yield by increasing milk fat content but not milk volume (Copelin et al., 2021). The propionate produced by ruminal fermentation is the main glycogenesis precursor; it is converted into glucose (Mccarthy et al., 2015). Water absorption in the mammary gland is influenced by the synthesis of lactose, and lactose determines milk osmolarity (Martins et al., 2019). Dairy cows fed propionate tended to have higher lactose levels (Martins et al., 2019), which improves milk yield. This theory was confirmed by the current study that the concentration of propionic acid in H and M groups were higher than that in L group in this study. In contrast, the study by Xue et al. (2019) showed that the high-yield and high-protein group had higher contents of various VFAs than those of the low-yield and low-protein group. However, in this study, the acetic acid, butyric acid, and TVFAs in the rumen of high milk-yielding and moderate milk-yielding dairy cows were significantly lower than those of low milk-yielding dairy cows. This could be due to ruminal VFA concentrations being the result of microbial production and host absorption. The leftover concentrations of VFAs in the rumen were assessed in this study, not the production or absorption concentrations. Thus, the high milk-yielding dairy cows may have a greater ability to absorb and utilise VFAs than the low milk-yielding cows. Combining the results of serum hormones and antioxidative stress status indicators, it appears that the high milk-yielding dairy cows may have a more active metabolism. Nevertheless, the absorption of VFAs requires further study.

Rumen fermentation is essential for the growth and production performance of dairy cows. Rumen bacterial composition and the metabolic functions of these bacteria are important for improving dairy cow production performance. The rumen bacterial composition of dairy cows varies with the breed (Bergman, 1990), parity (Hackmann & Firkins, 2015), diet (Cersosimo et al., 2016), milk yield, milk composition (Indugu et al., 2017) and physiological conditions of the cows (Jewell et al., 2015). These studies showed that the phyla Bacteroides and Firmicutes dominate the rumen bacteria. In this experiment, Bacteroidetes and Firmicutes accounted for 50.56–54.24% and 36.45–43.45% of the rumen bacteria, respectively, which is consistent with what was seen in the above-mentioned reports. The proportion of Bacteroides observed in this study was much higher than that of the studies reported above, but it was similar to previous findings (Pitta et al., 2014). The differences between these reports may have resulted from the differences in the diet composition, sampling time (Sanjay et al., 2015), and methods used to analyse rumen bacterial diversity. The relative abundance of Prevotellaceae_UCG-001 in the H and M groups were significantly higher than that in the L group. This is consistent with the results of Indugu et al. (2017) who selected dairy cows for primiparity and multiparity in two different pastures. They found that the relative abundance of Succinivibrionaceae in the different pastures, and the different parities of the high-yield group, was significantly greater than of the low-yield group. Succinivibrionaceae can reduce the production of methane and increase the production of propionic acid, thereby providing more energy for tissue metabolism (Liu et al., 2013; Pope et al., 2011). Moreover, propionic acid is metabolised to glucose in the liver and, because glucose is the precursor of lactose, this increases the milk yield (Soest, 1982). In the present experiment, the concentration of propionic acid in the H group was higher than that in the L group, which supports this possibility. In addition, the relative abundances of Christensenellaceae in the H and M groups were significantly lower than the abundance in the L group. However, the relative abundances of *Butyrivibrio_2* and *Treponema_2* were not significantly different among the three groups in this study. Cunha et al. (2017) reported that *Christensenellaceae*, *Butyrivibrio* and *Treponema* were negatively correlated with CH_4 emissions, and positively correlated with daily dry matter intake and daily organic matter intake. They concluded that reducing CH_4 emissions could reduce energy loss so that more energy could be used for lactation and increasing the milk yield. They also inferred that these bacteria may increase dry matter intake because their members include known fibre-decomposing bacteria. Fibre is mainly responsible for the expansion and filling of the rumen. One hypothesis is that the increase in the number of fibre-decomposing bacteria could increase the fibre degradation rate and reduce the satiety of cows, which would lead to increased feed intake. The cows would then ingest and absorb more energy, which could lead to improved production performance. However, this is contrary to what was observed in our results. One possible reason for this is that the referenced studies were not based on animals fed on the same diet. Another possible reason is that this experiment was analysed at the genus level, but *Christensenellaceae_R-7_group*, *Butyrivibrio_2* and *Treponema_2* are only members of *Christensenellaceae*, *Butyrivibrio* and *Treponema*, thus, their relative abundance may not represent the entire genus.

5 | CONCLUSIONS

Under the same management and feeding conditions, dairy cows presented great differences in milk yield. These differences may be attributed to the physiological dissimilarities among dairy cows. The higher the milk yield of dairy cows, the higher the hormone levels measured in serum that promote milk yield, and the more balanced the state of oxidative stress. Moreover, the rumen microbial metabolism in cows with high milk yields was more vigorous than that in cows with moderate and low milk yields. The results of this study suggest that improving the metabolic health and the diversity of the rumen bacterial community of dairy cows might stimulate their lactation potential. In the future, the liver, breast and other tissues could be collected for combined multi-omics analysis to reveal the mechanisms behind them.

AUTHOR CONTRIBUTIONS

Jianan Dong: Data curation; Formal analysis; Investigation; Software; Writing - original draft. **Yongjun Liu:** Data curation; Formal analysis; Investigation; Software; Writing - original draft. **Songze Li:** Project administration; Resources; Software. **Zhe Sun:** Funding acquisition; Methodology; Resources. **Xue Chen:** Resources. **Duojia Wang:** Resources. **Guixin Qin:** Resources; Writing - review & editing. **Xuefeng Zhang:** Resources; Software. **Natnael Demelash Aschalew:** Resources; Writing - review & editing. **Tao Wang:** Conceptualization; Funding acquisition; Resources; Supervision; Writing - review & editing. **Yuguo Zhen:** Conceptualization; Funding acquisition; Resources; Supervision; Writing - review & editing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

Animals were managed according to the guidelines for the care and use of experimental animals of Jilin Agricultural University (JLAUACUC-2020-015).

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