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Original Research Article

# Ruminal bacterial community is associated with the variations of total milk solid content in Holstein lactating cows



Kaizhen Liu <sup>a, b</sup>, Yangdong Zhang <sup>a, b, \*</sup>, Guoxin Huang <sup>a, b</sup>, Nan Zheng <sup>a, b</sup>, Shengguo Zhao <sup>a, b</sup>, Jiaqi Wang <sup>a, b, \*</sup>

<sup>a</sup> State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China

<sup>b</sup> Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China

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## ABSTRACT

Total milk solid (TMS) content directly reflects the quality of milk. Rumen bacteria ferment dietary components, the process of which generates the precursors for the synthesis of milk solid, therefore, the variation in rumen bacterial community could be associated with milk solid in dairy cows. In this study, 45 healthy mid-lactation Holstein dairy cows with the similar body weight, lactation stage, and milk yield were initially used for the selection of 10 cows with high TMS (HS) and 10 cows with low TMS (LS). All those animals were under the same feeding management, and the individual milk yield was recorded for 14 consecutive days before milk and rumen fluid were sampled. Rumen fluid was used to determine bacterial community by 16S rRNA gene sequencing technique. The HS cows had significantly greater feed intake and milk TMS, fat, protein content than LS cows ( $P < 0.05$ ). Among the volatile fatty acids (VFA), propionic acid and valeric acid concentrations were significantly greater in HS cows than those in LS cows ( $P < 0.05$ ). There was no significant difference in the concentrations of acetate, butyrate, isobutyrate, valerate, and the total VFA ( $P > 0.05$ ), nor was the acetate-to-propionate ratio, pH value, ammonia nitrogen and microbial crude protein concentrations ( $P > 0.05$ ). Significant differences in the relative abundances of some bacterial genera were found between HS and LS cows. Spearman's rank correlation analysis revealed that TMS content was correlated positively with the abundances of *Ruminococcaceae* UCG-014, *Ruminococcaceae* NK4A214 group, *Prevotellaceae* UCG-001, *Butyrivibrio* 2, *Prevotellaceae* UCG-003, *Candidatus Saccharimonas*, *Ruminococcus* 2, *Lachnospiraceae* XPB1014 group, probable genus 10, *Eubacterium ventriosum* group, but negatively correlated with *Pyramidobacte*. In addition, *Ruminococcaceae* UCG-014, *Ruminococcus* 2, *Ruminococcaceae* UCG001, probable genus 10 and *Eubacterium ventriosum* group might boost the total VFA production in the rumen. In conclusion, the dry matter intake of dairy cows and some special bacteria in rumen were significantly associated with TMS content, which suggests the potential function of rumen bacteria contributing to TMS content in dairy cows.

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\* Corresponding authors.

E-mail addresses: [zhangyangdong@caas.cn](mailto:zhangyangdong@caas.cn) (Y. Zhang), [jiaqiawang@vip.163.com](mailto:jiaqiawang@vip.163.com) (J. Wang).

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## 1. Introduction

Total milk solid (TMS) is the sum of milk fat, milk protein, lactose, and minerals and a direct index to evaluate milk quality. It is also the pricing index in milk trade in some countries. The precursors for TMS include volatile fatty acids (VFA), long-chain fatty acids, amino acids (AA), peptides, glucose, glycerin etc., which are primarily produced in the rumen by bacterial degradation of dietary components (Hurtaud et al. 1998; Bauman and Griinari, 2003; Chen et al. 2011). Therefore, there is a relationship between the

precursor production in the rumen and the uptake by the mammary gland. The nutritional value of milk would be unbalanced if regulating one of the ingredients in milk, such as milk protein and milk fat (Jenkins and McGuire, 2006; Nichols et al., 2018). Thus, increasing TMS content is the effective measure to improve milk quality and production efficiency.

A number of factors have impact on TMS content, such as diet, environment, and animal's genetics (Dechow et al., 2007; Kmicikewycz and Heinrichs, 2015). An appropriate proportion of high-quality roughage in total mixed ration (TMR) is the key to ensure TMS content and milk fat (Kmicikewycz and Heinrichs, 2015). Seasons have an influence on TMS, and TMS content is lower in summer but higher in winter (Bernabucci et al., 2015). Dechow et al. (2007) reported that TMS content of cross dairy cows (Holstein × Brown Swiss) was significantly higher than that in Holstein cows. Within a breed, there were considerable individual variations in the feed efficiency, milk yield, and milk protein yield when cows were fed the same diet (Shabat et al., 2016; Xue et al., 2019). Understanding the mechanisms that determine these individual variations is important to increase TMS and milk quality. It has been found that these individual variations are related to the key ruminal bacterial species and rumen fermentation profiles, which influence the precursors for the synthesis of milk protein, fat, and lactose (Shabat et al., 2016; Schären et al., 2018; Xue et al., 2019). For example, the abundance of *Megasphaera elsdenii* was significantly higher in cows with high feed efficiency than cows with low feed efficiency, and the abundance of *Streptococcus*, unclassified *Enterobacteriaceae*, *Ruminobacter*, *Treponema*, and unclassified *Bacteroidaceae* were all higher in cows with a lower milk yield (Shabat et al., 2016; Zhao et al., 2019), indicating that the ruminal bacterial profile plays a key role in the conversion of feed to milk.

This study aimed to explore the relationships between the rumen bacterial profile and TMS content. We selected 2 groups of cows with high or low TMS content to measure their rumen bacterial community by using 16S rRNA gene sequencing and the real-time qPCR techniques, and analyzed the relationships between the microbial profile and TMS content.

## 2. Materials and methods

The use of the animals and the experimental procedures were approved by the Animal Care Committee of Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China) in accordance with the guidelines for animal experimental welfare and ethical inspection in China (No: IAS 2019 - 28).

### 2.1. Animals, grouping, and management

This trial was carried out in Mengde Dairy Station, Tianjin, China. There were over 1,500 Holstein lactation dairy cows in the farm, and their individual milk yield were recorded daily. All dairy cows were fed in the same cowshed with the same TMR and free access to water. On the basis of the productive records, 45 healthy lactating cows were selected with the similar body weight, parity (parity =  $1.30 \pm 0.74$ ), day in milk ( $153 \pm 16$  d) and daily milk yield ( $34 \pm 6$  kg). Milk samples were taken from those cows (as detailed below) to measure milk protein, milk fat, lactose, and TMS content using MilkoScan FT120 (Foss, Hillerød, Denmark). The TMS content varied from 10.4% to 14.1% in those animals. Ten cows with the highest or lowest TMS content respectively were further selected by the mean and standard deviation (SD) based on the method of previously research (Nkrumah et al., 2006; Ramos and Kerley, 2013). The SD values above and below the mean were used to group cows into high TMS (HS, TMS > mean +  $0.7 \times$  SD) phenotype

and low TMS (LS, TMS < mean -  $0.7 \times$  SD) phenotype. The aim of this study was to explore the relationships between the ruminal bacteria and TMS in cows at a similar level of milk production. A great variation in milk yield could have a confound effect on milk solid. To minimize other potential effect besides to TMS between 2 groups, some cows in each group with extremely high or low milk yield were removed (Appendix Table 1). In addition, the cows that were failed to obtain rumen fluid samples were also removed. Finally, there were 6 cows ( $n = 6$ ) in both groups with a similar milk yield.

All the selected 45 cows were maintained at the same farming condition and fed the same TMR containing 36% forage and 64% concentration (dry matter basis) for 14 d continuous stable feeding. The TMR consisted of corn silage, alfalfa hay, alfalfa silage, corn, soybean meal, orange peel granules, expanded soybean, distillers dried grains with soluble and wheat bran. Cows were kept in individually tethered stalls in a barn and fed 3 times a day at 07:00, 13:00, and 18:00. Fresh drinking water was available all times. The feed offered and the refusal of cows were recorded daily to calculate the daily feed intake of individual animals. Cows were milked 3 times a day at 06:30, 12:30, and 17:30 using an automatic milking system, and the daily milk yield were recorded by the automatic milking system.

### 2.2. Sampling

Milk and rumen fluid were sampled at the end of the 14-d continuous stable feeding. After each milking, milk samples (50 mL each) were collected and stored at 4 °C. The samples collected in the morning, afternoon, and evening were then mixed at a ratio of 4:3:3, and subsampled as a daily sample referenced a previously article by Wu et al. (2018). The aliquots of the samples were used to analyze milk components (see below).

Rumen fluid samples (about 200 mL each) from each cow were collected after morning milking before feeding. Sample was collected using an oral stomach tube (Shen et al. 2012). The pH value was immediately measured using a pH meter (PHS-100 portable acidity meter, Tianqi Mdt InfoTech Ltd., Shanghai, China). Then, samples were filtered through a 4-layer cheesecloth, and aliquoted into 6 sterilized tube (5 mL), then stored at -80 °C until the further analysis.

### 2.3. Measurement of rumen fermentation parameters

The rumen liquid sample was thawed on ice, then centrifuged at  $12,000 \times g$  at 4 °C for 10 min. The supernatant was harvested for the determination of VFA, ammonia nitrogen (NH<sub>3</sub>-N), and microbial crude protein (MCP) concentrations. A colorimetric method was used to detect NH<sub>3</sub>-N and MCP as described by Wang et al. (2019). The VFA concentration was measured using a liquid chromatographic method (Agilent 7890A-7000B, Agilent, Beijing, China).

### 2.4. Acquisition of DNA and construction of library

The genomic DNA in rumen microbiota in each rumen fluid sample was extracted with a cetyltrimethylammonium bromide (CTAB) reagent. The purity of extracted DNA was checked using 1% agarose gel, and the DNA concentration was determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA).

The purified DNA was used as the template, and 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GGACTACHVGG GTWCTAAT-3') were the primers. The V3 to V4 of bacterial 16S rRNA gene was amplified using an ABI GeneAmp 9700 PCR thermocycler (ABI, CA, USA) (Parada et al., 2016). The PCR protocol was

94 °C for 2 min, followed by 30 cycles at 98 °C for 10 s and 62 °C for 30 s. The PCR reaction solution consisted of 10 × KOD Buffer 5 μL, 2 mmol/L dNTP 5 μL, 25 mmol/L MgSO<sub>4</sub> 3 μL, 10 μmol/L primer 1.5 μL respectively, KOD Polymerase (TOYOBO, Japan) 1 μL, and the template DNA 100 ng. Each PCR reaction was performed in triplicate. Once the PCR reaction was completed, the amplified products were extracted using 2% agarose gel, further purified through the Axy-Prep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Finally, the purified products were quantified using a Quantus Fluorometer (Promega, USA), and pooled in equimolar, then paired-end sequenced (2 × 300 bp) at Illumina HiSeq 2500 PE250 platform (Illumina, San Diego, USA) under the standard protocols by GENE DENOVO. (Guangzhou, China). The raw reads of 16S rRNA gene sequencing were deposited into the NCBI Sequence Read Archive database (Accession Number: PRJNA722820).

All raw reads analysis and the quality control were referenced to the methods as reported by Huang et al. (2020). UPARSE (7.1 version, <http://drive5.com/uparse/>) was used to cluster the operational taxonomic unit (OTU) with 97% similarity cutoff (Liu et al., 2017), then identified, and removed the chimeric sequences. The longest read was deemed as a representative sequence of the taxonomy for each OTU. Further, those representative sequences were identified against to the Silva SSU128 database using RDP Classifier.

### 2.5. Real-time qPCR

The copy numbers of these bacterial species that were related with fiber, protein, or starch degradation as described by Satoshi and Yasuo, (2001) and Stevenson and Weimer, (2007) were determined by real-time qPCR in a BioRad CFX96 (Hercules, CA, USA). The same DNA samples in 16S rRNA gene sequencing were used for real-time qPCR. Primers were selected from the published papers (Table 1). The real-time qPCR was performed with SYBR Premix Ex Taq II assay kit (TaKaRa Bio Inc., Shiga, Japan) and calculated with the standard curve method. The standard curve for each bacterial species was generated using the method as described by Stubner, 2002 with plasmid DNA that contained the cloned marker loci. All standard curves met the requirements for further analysis ( $R^2 > 0.98$ , 90% > E > 120%).

### 2.6. Statistical analysis

The comparison of the differences in the milk component content, rumen fermentation parameters, and bacterial abundances between HS and LS groups were performed using the *t*-test procedure of SAS (Version 9.4, USA). Spearman correlation analysis

was used to analyze the relationship of the bacterial abundance with the milk component content, ruminal NH<sub>3</sub>-N, MCP, and VFA concentrations. The results were presented as the mean and standard error of means (SEM). Significant and extremely significant differences were declared at  $P < 0.05$  and  $P < 0.01$ , respectively.

## 3. Results

### 3.1. Feed intake and milk composition

As shown in Table 2, the dry matter intake (DMI) differed between HS and LS cows ( $P < 0.05$ ), and the DMI in LS cows was 11.0% lower than that in HS. Moreover, crude protein (CP) intake, neutral detergent fiber (NDF) intake and acid detergent fiber (ADF) intake were all significantly higher in HS group than that in LS group ( $P = 0.012$ ,  $P = 0.011$  and  $P = 0.010$ , respectively). The milk yield was similar between 2 groups ( $P = 0.932$ ). The TMS (both the content [%] and yield (kg);  $P < 0.001$ ,  $P = 0.001$ ), milk fat (%), milk protein (%), and the fat-to-protein ratio ( $P = 0.001$ ) were all significantly greater in HS cows than those in LS group. Meanwhile, there was no significant difference in lactose (%) and non-fat milk solid (%) between 2 groups ( $P > 0.05$ ).

When expressing milk fat, protein, and lactose as the percentages to TMS, HS cows had significantly greater milk fat ( $P < 0.001$ ),

**Table 2**  
Milk composition of lactating cows selected for high or low milk solid.

Item	HS	LS	SEM	P-value
DMI, kg/d	23.89	21.30	0.558	0.011
CP intake, kg/d	2.49	2.25	0.051	0.012
NDF intake, kg/d	13.78	12.28	0.322	0.011
ADF intake, kg/d	3.87	3.41	0.100	0.010
Milk yield, kg/d	33.66	33.55	0.639	0.932
TMS yield, kg/d	4.38	3.81	0.118	0.007
Moisture, %	86.99	88.62	0.271	0.001
Milk fat, %	4.41	2.77	0.269	0.000
Milk protein, %	3.34	2.99	0.066	0.002
Milk fat-to-protein ratio	1.32	0.93	0.072	0.001
Lactose, %	4.84	4.98	0.044	0.112
TMS, %	13.01	11.38	0.272	0.000
Non-fat milk solid, %	8.60	8.61	0.077	0.976
Percentage to TMS, %rowhead				
Milk fat	33.89	24.23	1.639	0.000
Milk protein	25.66	26.32	0.367	0.398
Lactose	37.21	43.86	1.064	0.000
Non-fat milk solid	56.11	75.77	1.639	0.000

HS = high total milk solid group; LS = low total milk solid group; SEM = standard error of the mean; DMI = dry matter intake; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; TMS = total milk solid.

**Table 1**  
Primers used for real-time qPCR.

Bacteria	Sequence (5' to 3')	Annealing temperature, °C	Size, bp	References
<i>Butyrivibrio</i>	F: GTGCCAGCMGCCGCGG R: TGCCGGCACYGACTCCCTATG	65	371	Stiverson et al. (2011)
<i>Fibrobacter succinogenes</i>	F: GGATGGGATGAGCTTGC R: GCCTGCCCTGAACTATC	53	446	Satoshi and Yasuo (2001)
<i>Ruminococcus albus</i>	F: GTTTTAGGATTGTAACCTCTGTCTT R: CCTAATATCTACGCAITTCACCGC	58	170	Li et al. (2008)
<i>Ruminococcus genus</i>	F: GAGTGAAGTAGAGGTAAGCGGAATTC R: GCCGTACTCCCCAGGTGG	60	75	Petri et al. (2013)
<i>Prevotella bryantii</i>	F: ACTGCAGCGCGAACTGTGAGA R: ACCTTACGGTGGCAGTGTCTC	57	421	Bekele et al. (2010)
<i>Prevotella genus</i>	F: GGTTCTGAGAGGAAGGTCCCC R: TCCTGCACGCTACTTGGCTG	61	121	Stevenson and Weimer (2007)

but lower lactose ( $P < 0.001$ ), and similar percentage of milk protein ( $P > 0.05$ ) compared with those in LS cows (Table 2).

### 3.2. Rumen fermentation parameters

NH<sub>3</sub>-N, MCP, pH and VFA concentration in rumen fluid were shown in Table 3. There was no significant difference in pH, NH<sub>3</sub>-N, and MCP concentrations between 2 groups ( $P > 0.05$ ). Among the VFA concentrations, propionic acid and valeric acid concentrations were significantly greater in HS cows than those in LS group ( $P < 0.05$ ). Otherwise, there was no significant difference in the concentrations of acetate, butyrate, isobutyrate, valerate, and the total VFA ( $P > 0.05$ ), nor was the acetate-to-propionate ratio ( $P > 0.05$ ). There was no significant difference in the molar percentage of any of these VFA ( $P > 0.05$ ).

### 3.3. Composition of rumen bacteria

Through the 16S rRNA gene sequencing analysis, we acquired 1,221,314 high-quality sequences, with the average of  $101,776 \pm 4,697$  sequences per sample which belonged to  $1,608 \pm 127$  OTU (Appendix Fig. 1). A total of 1,302 OTU were commonly shared by HS and LS cows (Appendix Fig. 2). Rarefaction curve showed that samples in this experiment covered the majority of rumen bacteria (Fig. 1A). The richness index values of bacteria (Sobs, Simpson and Shannon) were all significantly greater in HS cows than those in LS cows ( $P < 0.05$ ; Fig. 1B, C, and D). The similarity between and within groups were exhibited in a PCoA plot with unweighted UniFrac distance metrics (Fig. 2). The plot showed a clear differentiation in bacteria between 2 groups, and PC1 explained 34.5% of the variation and PC2 explained 24.1% of the variation.

A total of 26 phyla were identified in the rumen fluid by the taxonomic analysis, and only 0.15% bacterial phylum in HS cows and 0.09% in LS cows were unclassified. As shown in Fig. 3, Bacteroidetes and Firmicutes were 2 dominant phyla both in HS and LS cows, and the relative abundances of these 2 phyla accounted for approximately 93.0% (Bacteroidetes 53.0% and Firmicutes 40.0%). At genus level, 227 genera were identified, *Prevotella* 1 and *Succiniclaticum* were the dominant genera (Appendix Table 2). The relative abundance of *Prevotella* 1 (belonging to Bacteroidetes) was

**Table 3**

Rumen fermentation parameters in lactating cows selected for high or low milk solid.

Item	HS	LS	SEM	P-value
pH	6.84	6.79	0.094	0.772
NH <sub>3</sub> -N, mg/dL	9.74	10.11	0.784	0.829
MCP, mg/mL	0.31	0.28	0.015	0.532
VFA concentration, mmol/Lrowhead				
Acetic acid	10.91	8.70	0.637	0.081
Propionic acid	4.40	3.10	0.335	0.044
Isobutyric acid	0.72	0.50	0.057	0.054
Butyric acid	2.75	1.99	0.266	0.164
Isovaleric acid	0.41	0.28	0.071	0.386
Valerate acid	0.38	0.29	0.022	0.045
Acetate-to-propionate ratio	2.55	2.79	0.102	0.261
Total VFA, mmol/L	19.58	14.87	1.294	0.065
Molar proportion, %rowhead				
Acetic acid	56.36	58.31	0.966	0.335
Propionic acid	22.25	21.20	0.580	0.389
Isobutyric acid	3.63	3.45	0.165	0.623
Butyric acid	13.78	13.21	0.686	0.698
Isovaleric acid	2.02	1.81	0.282	0.726
Valerate acid	2.00	2.02	0.097	0.790

HS = high total milk solid group; LS = low total milk solid group; SEM = standard error of the mean; NH<sub>3</sub>-N = ammonia nitrogen; MCP = microbial crude protein; VFA = volatile fatty acid.

25.1% and 16.8% in HS and LS cows respectively; the relative abundance of *Succiniclaticum* (belonging to Firmicutes) was 12.4% and 25.5% in HS and LS cows respectively. The average relative abundances of *Ruminococcaceae* NK4A214 group, *Ruminococcaceae* UCG-014 and *Rikenellaceae* RC9 gut group were 2.20%, 2.41%, and 3.20% respectively.

### 3.4. Comparison of rumen bacteria between HS and LS cows

At genus level, there were a total of 22 bacterial genera, the abundance of which differed significantly between HS and LS cows, and all these genera were assigned to Bacteroidetes and Firmicutes phyla. The relative abundance of *Succiniclaticum* that belongs to Firmicutes was 2.06-folds higher in LS cows than that in HS group. However, *Ruminococcaceae* UCG-014, *Ruminococcaceae* NK4A214 group, *Ruminococcus* 1, *Ruminococcus* 2, *Saccharofermentans* and *Ruminococcaceae* UCG-001 in *Ruminococcaceae*, *Prevotellaceae* UCG-001 and *Prevotellaceae* UCG-003 in *Prevotellaceae*, *Butyrivibrio* 2, *Eubacterium ruminantium* group, *Lachnospiraceae* XPB1014 group, *Lachnospiraceae* AC2044 group, *Pseudobutyrvibrio*, probable genus 10, and *Eubacterium ventriosum* group in *Lachnospiraceae* were all significantly greater in HS cows than LS cows (Fig. 4, Appendix Table 2). The real-time qPCR results showed the copy number of *Prevotella bryantii* was significantly greater in LS than those in HS group. There was no significant difference in the other bacterial genera between HS and LS cows (Fig. 5).

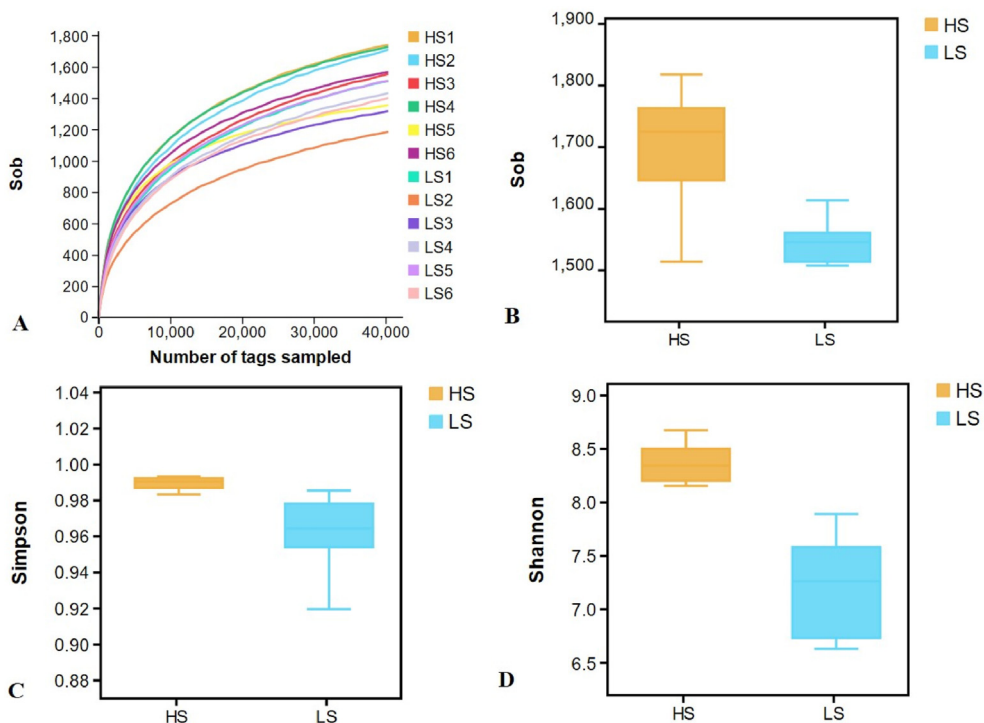
### 3.5. Correlation of rumen bacterial differentiation with milk composition and fermentation parameters

The Spearman's rank correlation analysis was used to explore the correlations between milk composition (%), ruminal fermentation parameters and the relative abundances of those bacterial genera that differed significantly between HS and LS cows. The results were shown in Fig. 6. The TMS content was positively correlated with the abundances of *Ruminococcaceae* UCG-014, *Ruminococcaceae* NK4A214 group, *Prevotellaceae* UCG-001, *Butyrivibrio* 2, *Prevotellaceae* UCG-003, *Candidatus Saccharimonas*, *Ruminococcus* 2, *Lachnospiraceae* XPB1014 group, probable genus 10 and *Eubacterium ventriosum* group, yet *Pyramidobacter* had negative correlations with TMS content. The relative abundance of these bacterial genera was negatively correlated with milk lactose concentration, but positively correlated with milk protein and fat contents. In contrast, the abundances of *Succiniclaticum* and *Veillonellaceae* UCG-001 correlated negatively with milk protein concentration, and *Pyramidobacter* correlated negatively with milk protein concentration and TMS content.

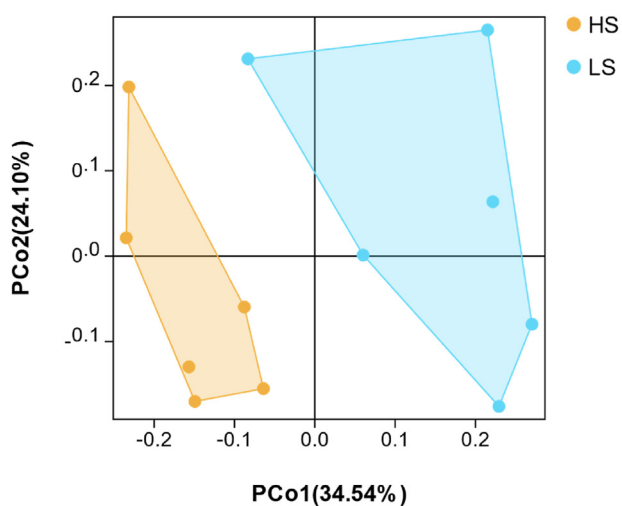
As for the rumen fermentation parameters, *Ruminococcaceae* NK4A214 group abundance correlated negatively with NH<sub>3</sub>-N concentration. *Ruminococcaceae* UCG-014, *Candidatus Saccharimonas*, *Anaeroplasma*, and *Eubacterium ventriosum* group negatively correlated with the acetate-to-propionate ratio. *Ruminococcaceae* UCG-014, *Ruminococcus* 2, *Ruminococcaceae* UCG-001, and probable genus 10 correlated positively with acetate, propionate, isobutyrate and total VFA concentrations.

## 4. Discussion

In this study, a large individual variation in TMS content was noted in the selected 45 Holstein cows, and the content ranged from 10.4% to 14.1% (Appendix Table 1) when the cows were fed the same diet. This result was not reported in the previous research, but the individual variations in feed efficiency, utilization efficiency of nitrogen, and milk protein yield had been reported (Shabat et al., 2016; Wang et al., 2019; Wu et al., 2018). The selected HS cows



**Fig. 1.** Alpha diversity analysis of rumen bacteria in lactating cows of HS and LS. (A) Number of tags sampled; (B) Sob index; (C) Simpson index; (D) Shannon indexes. HS = high total milk solid group; LS = low total milk solid group.



**Fig. 2.** Principal coordinate analysis (PCoA). The unweighted UniFrac distance metrics analysis between the rumen bacteria in lactating cows of HS or LS. HS = high total milk solid group; LS = low total milk solid group.

had greater TMS than LS cows, which attributed to the greater content of milk fat and milk protein in the present study. However, on the basis of TMS, there was more milk fat, but less lactose, and no change in milk protein in TMS in HS cows when compared with those in LS cows. Our results were consistent with a previous study by Nichols et al. (2018). We also noted that HS cows had greater feed intake, 10.8% higher than that in LS cows. When the milk yield was similar between 2 groups, the increase of the feed intake was coincident with the change in TMS content and the TMS yield (increased by 12.5% and 13.0% respectively). Moreover, the CP, NDF,

and ADF intakes were all found higher in HS than that in LS group. The results suggest that TMS content seems apparently to be associated with feed and the nutrient intakes in those lactating cows. Mostly current research showed that the DMI of different diets was associated with TMS (Jenkins and McGuire, 2006; Nichols et al., 2018), the results of this present study warrant a further study.

The TMS content was mainly affected by the interaction between feed intake and rumen fermentation (Schären et al., 2018; Pitta et al., 2018). In the present study, we found the individual VFA and total VFA concentrations were relatively greater in HS cows compared with LS cows, although some were not significant statistically. The higher TMS in HS group was likely due to the differences in the VFA concentrations which were attributed to the differentiation of the fermentation rate caused by the change in DMI (including the CP, NDF, and ADF intakes). VFA are the major precursors for TMS, so the greater VFA concentration could explain the greater content of milk fat in HS cows. In addition, there was no significant change in the VFA profile, the molar percentages of individual VFA, which suggested that the rumen fermentation pattern did not change. The similarity of pH and NH<sub>3</sub>-N indicated the stable acid-base environment of the cow fed the same composite TMR. The similarity of MCP between 2 groups explained the reason why there was no difference in the milk protein to TMS ratio in both groups.

The roles of rumen bacteria in feed and nitrogen utilization efficiency were reported recently (Shabat et al., 2016; Wang et al., 2019). Some studies have found that rumen bacteria were tightly related to the milk components (Zou et al., 2019; Zeng et al., 2019). In this study, the results of 16s rRNA gene sequencing showed that there were differences in the rumen bacterial richness and diversity (Sobs, Simpson and Shannon indices) between HS and LS cows. It had been reported that the rumen bacteria richness was negatively related to milk fat in cows (Xue et al., 2018), and a lower bacteria

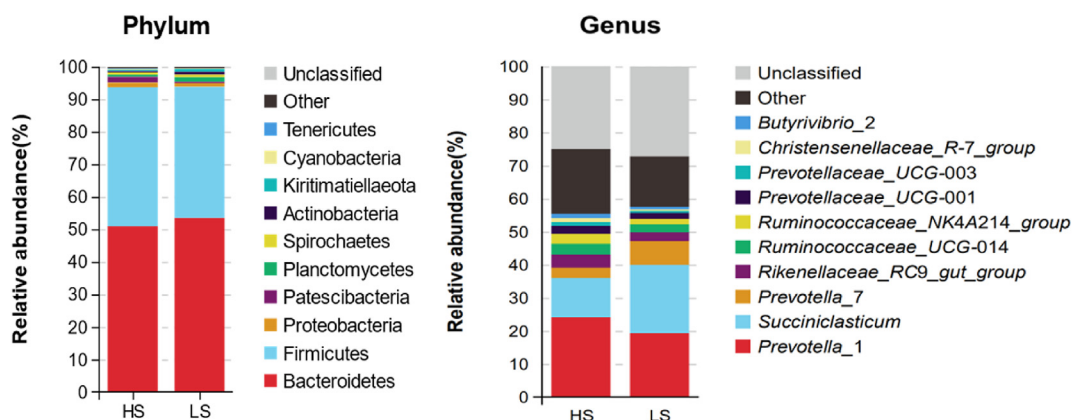


Fig. 3. Relative abundances of rumen bacteria at phylum and genus levels in lactating cows of HS and LS. HS = high total milk solid group; LS = low total milk solid group.

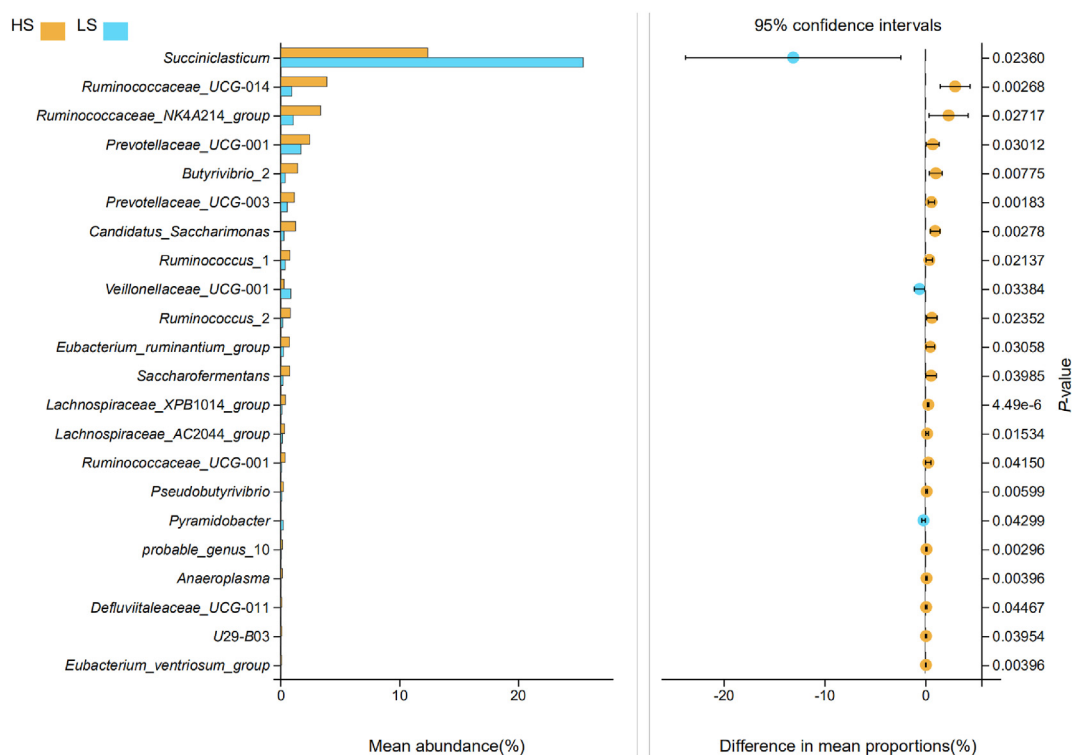


Fig. 4. Comparison of rumen bacteria at genus level in lactating cows of HS and LS. HS = high total milk solid group; LS = low total milk solid group.

richness was found in cows with a higher protein yield when compared with cows with a lower protein yield (Xue et al., 2019). As for bacterial diversity, the alpha index was not associated with milk yield (Tong et al., 2018). We assumed the greater the bacterial diversity, the more fermentation products can be produced, such as VFA, AA and glucose, which might be beneficial to milk production.

The present study also showed that the variation in TMS was associated with the changes in some bacterial genera in the rumen of cows. The higher abundance of predominant genus *Succiniclasicum* in LS cows than HS cows indicated that *Succiniclasicum* could decrease TMS by affecting milk protein content. Possibly, *Succiniclasicum* restrains the nitrogen metabolism and absorption of the host. A similar result was reported in our previous research in goats (Wang et al., 2019). *Succinivibrio dextrinsolvens* was related to the feed protein degradation (Wang et al., 2017). *Selenomonas* that is at the same order (Selenomonadales) as

*Succiniclasicum*, was a proteolytic bacterium (Zhou et al., 2019). On the contrary, Xue et al. (2019) showed that *Succinimonas* (Proteobacteria phylum), which has the same function as *Succiniclasicum* in the rumen, was positively associated with milk protein content.

A previous study showed *Succiniclasicum* could promote the use of fiber by converting succinate produced by rumen fiber decomposition to propionate which is the major precursor to gluconeogenesis in ruminants (Wang et al., 2019). A greater propionate concentration in the rumen fluid in HS cows was noted, which was not correlated with the lactose concentration in the present study. Possibly, the effect of *Succiniclasicum* might be compromised by the great complexity of interactions among different bacteria species. The specific role of *Succiniclasicum* on protein degradation and nitrogen metabolism in the rumen needs to be further explored.

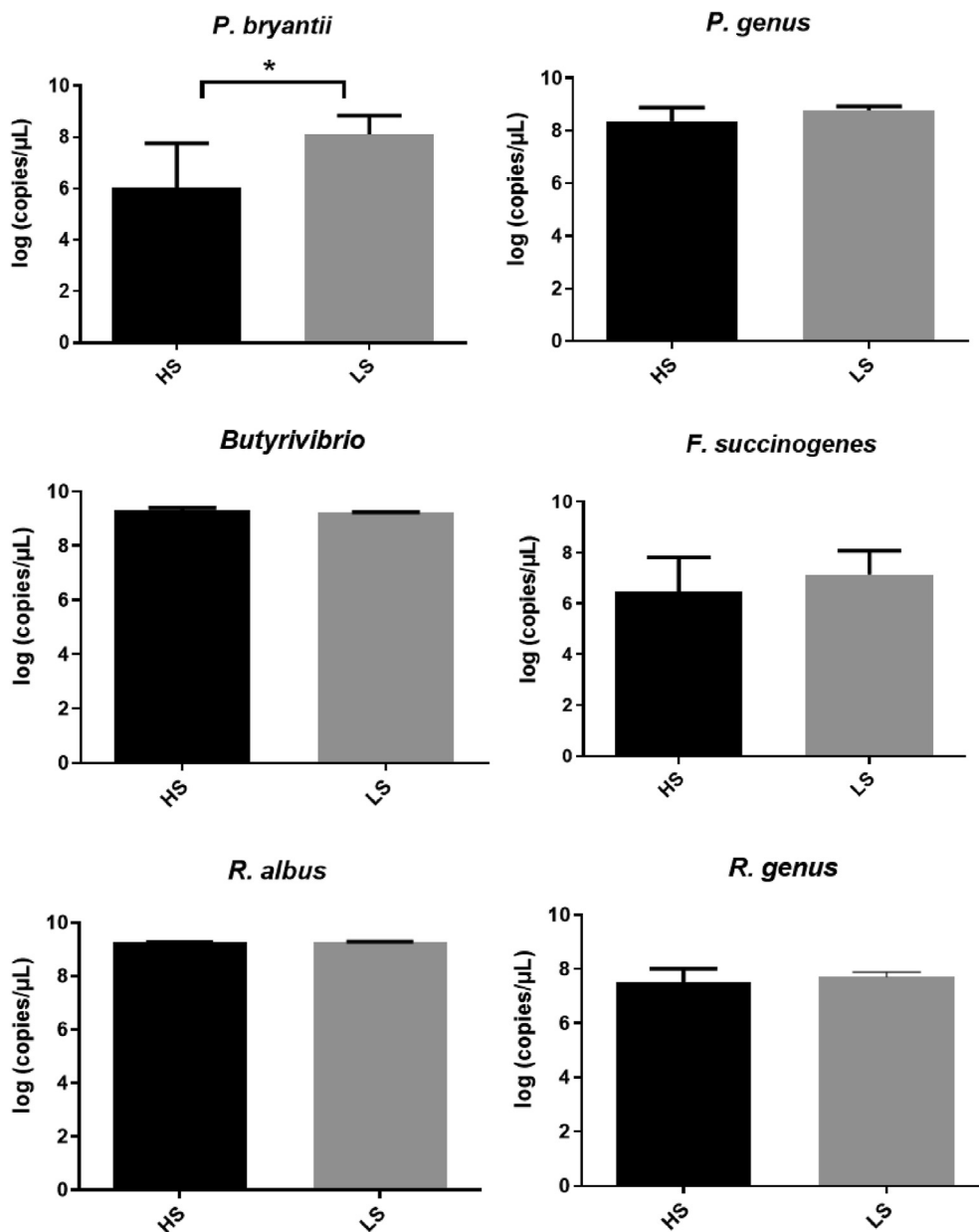
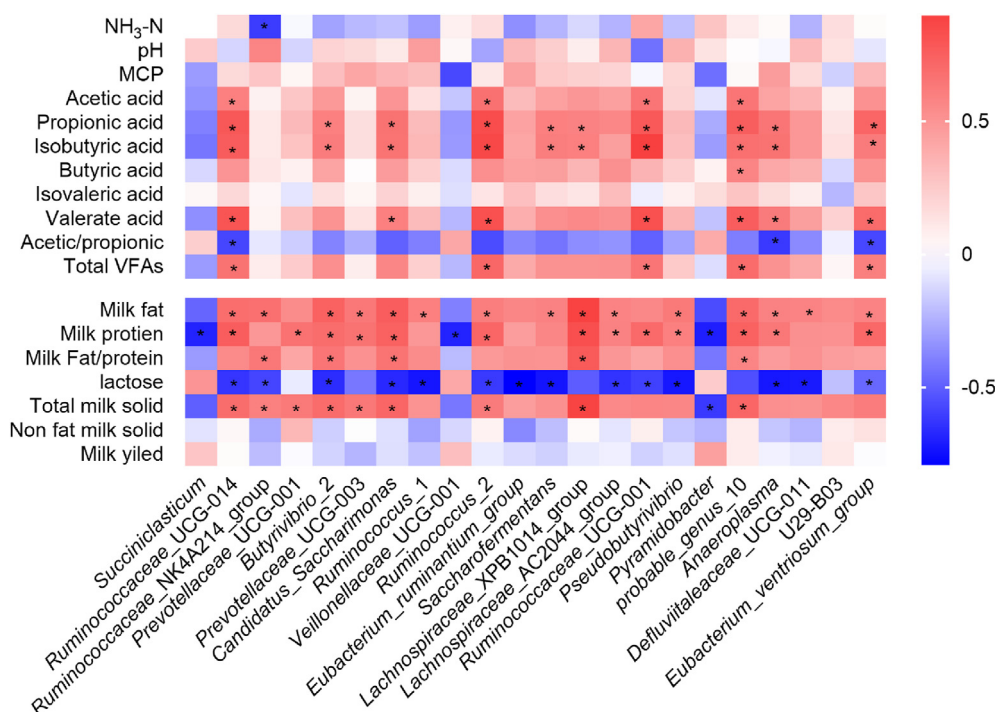


Fig. 5. The copy numbers of rumen bacteria by real-time qPCR in lactating cows of HS and LS. \* indicates the correlation is statistically significant ( $P < 0.05$ ). HS = high total milk solid group; LS = low total milk solid group.

In most cases, the closer genetic distance in animals, the more similar functions of ruminal bacteria display. The present study showed the positive correlations between the *Prevotellaceae* UCG-003, *Prevotellaceae* UCG-001 abundances and the TMS content. Xue et al., (2020) showed that *Prevotella* genus contributed to the metabolism of glutathione, phenylalanine, starch, sucrose, and galactose, and was involved in AA and carbohydrate metabolism by ruminal microbes. Moreover, *Prevotella* appeared to be a major bacterium that can reduce nitrogen loss through effectively utilizing forages to produce succinate and acetate as the end-product of glucose catabolism (Liu et al., 2019). That might partly explain the reason why the VFA and TMS in HS group compared with LS cows were greater in the present study.

Lachnospiraceae and Ruminococcaceae are deemed to be highly specialized in degradation of complex plant materials to VFA in the rumen (Biddle et al., 2013). Lachnospiraceae plays a predominant role

in biohydrogenation in the rumen, especially *Butyrivibrio* species has been identified as the most important biohydrogenation bacterium (Huws et al., 2011; Dewanckele et al., 2018; Zhang et al., 2019). However, our research showed an interesting result that the abundances of *Butyrivibrio* 2, *Eubacterium ventriosum* group *Lachnospiraceae* XPB1014 group and probable genus 10 in Lachnospiraceae positively correlated with the TMS content, milk protein and milk fat. In addition, these bacteria had positive correlations with the propionate and isobutyrate concentrations, which explained the greater proportion of milk protein, fat and TMS in HS cows. We also found in this study that HS cows harbored the greater abundance of Ruminococcaceae in the rumen, such as *Ruminococcaceae* UCG-014, *Ruminococcaceae* NK4A214 group, *Ruminococcus* 1, *Ruminococcus* 2, *Saccharofermentans*, and *Ruminococcaceae* UCG-001, within which *Ruminococcaceae* NK4A214 group, *Ruminococcaceae* UCG-014, and *Ruminococcus* 2 correlated positively with the TMS content. Acetate is



**Fig. 6.** The correlations between the differential bacteria in HS and LS cows and the milk composition and rumen fermentation parameters. Blue color indicates negative correlations, and red color represents positive correlations. Color darkness stands for the value of correlation coefficients, the darker the color, the greater the coefficient. \* indicates the correlation is statistically significant ( $P < 0.05$ ). HS = high total milk solid group; LS = low total milk solid group.  $\text{NH}_3\text{-N}$  = ammonia nitrogen; MCP = microbial crude protein; VFA = volatile fatty acid.

the main substrate produced in the rumen for the milk fat synthesis, and an energy source for the microbial protein synthesis (Xue et al., 2020). The present study showed that probable genus 10, *Ruminococcaceae* UCG-001, *Ruminococcus* 2, and *Ruminococcaceae* UCG-014 boosted milk protein, fat and TMS, which might be due to the positive correlation with the acetate concentration. Thus, we deemed that these bacteria genera from *Lachnospiraceae* and *Ruminococcaceae* might be dominant bacteria linked to TMS (Biddle et al., 2013).

### 5. Conclusion

TMS was greater with the higher DMI, and milk fat was the most changeable component in milk. Propionic acid and valeric acid concentrations were also greater in the high TMS group cows. Moreover, the significant correlations between some rumen bacterial genera and TMS content were found in this study. The positive roles of *Ruminococcaceae* UCG-014, *Ruminococcaceae* NK4A214 group, *Prevotellaceae* UCG-001, *Butyrivibrio* 2, *Prevotellaceae* UCG-003, *Candidatus Saccharimonas*, *Ruminococcus* 2, *Lachnospiraceae* XPB1014 group, probable genus 10, and *Eubacterium ventriosum* group, and the negative roles of *Succiniclasticum* in TMS content were noted. And, *Ruminococcaceae* UCG-014, *Ruminococcus* 2, *Ruminococcaceae* UCG001, probable genus 10 and *Eubacterium ventriosum* group might boost the total VFA production in the rumen of lactating cows. In conclusion, the DMI of dairy cows and some special bacteria in the rumen were significantly varied with TMS content, which suggests the potential function of rumen bacteria contributing to TMS content of dairy cows.

### Author contributions

**Kaizhen Liu:** Investigation, Data curation, Writing - original draft. **Yangdong Zhang:** Conceptualization, Writing - review. **Guoxin Huang:** Methodology, Validation. **Shengguo Zhao:**

Conceptualization, Project administration. **Nan Zheng:** Investigation, Writing - review & editing. **Jiaqi Wang:** Supervision, Conceptualization, Writing - review & editing.

### Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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### Appendix

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aninu.2021.12.005>.

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