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Article

The effects of probiotics administration on the milk production, milk components and fecal bacteria microbiota of dairy cows

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ABSTRACT

Probiotics administration can improve host health. This study aims to determine the effects of probiotics (*Lactobacillus casei* Zhang and *Lactobacillus plantarum* P-8) administration on milk production, milk functional components, milk composition, and fecal microbiota of dairy cows. Variations in the fecal bacteria microbiota between treatments were assessed based on 16S rRNA profiles determined by PacBio single molecule real-time sequencing technology. The probiotics supplementation significantly increased the milk production and the contents of milk immunoglobulin G (IgG), lactoferrin (LTF), lysozyme (LYS) and lactoperoxidase (LP), while the somatic cell counts (SCC) significantly decreased ($P < 0.01$). However, no significant difference was found in the milk fat, protein and lactose contents ($P > 0.05$). Although the probiotics supplementation did not change the fecal bacteria richness and diversity, significantly more rumen fermentative bacteria (*Bacteroides*, *Roseburia*, *Ruminococcus*, *Clostridium*, *Coprococcus* and *Dorea*) and beneficial bacteria (*Faecalibacterium prausnitzii*) were found in the probiotics treatment group. Meanwhile, some opportunistic pathogens e.g. *Bacillus cereus*, *Cronobacter sakazakii* and *Alkaliphilus oremlandii*, were suppressed. Additionally, we found some correlations between the milk production, milk components and fecal bacteria. To sum up, our study demonstrated the beneficial effects of probiotics application in improving the quality and quantity of cow milk production.

1. Introduction

Dairy cows are ruminant animals. The nutrition acquisition of this animal group is characterized by a microbe-rather than host-based feed degradation [1]. The gastrointestinal tract of ruminant animals harbours a wide diversity of strictly anaerobic bacteria, ciliate protozoa, anaerobic fungi, and archaea, which are responsible for degradation and fermentation of 70–75% of the dietary compounds for providing energy. The cellulose, hemicellulose and lignin are hydrolyzed and converted into short-chain fatty acids that are easily absorbed by the host. Meanwhile, these microbes also help eliminate the toxins produced by the host metabolic processes [2]. Because of the crucial role of the dairy cow gut microbiota in nutrition and energy acquisition, there is no doubt it should be regarded as a target for subsequent improvement of cow health, milk yield and quality.

Probiotics is defined as 'live microorganism which when administered in adequate amounts confers a health benefit on the host' [3]. They can regulate the balance of gut microbes, promote the growth and development of animals, and improve the host resistance to diseases [4]. Since the traditional probiotic bacteria comprise a significant proportion of the cow rumen microbes, it is not surprising that many previous studies have investigated the influence of feeding probiotics to dairy cow; and so far, probiotics supplementation has been proven to change the rumen bacteria fermentation pattern, improve the feed utilization rate, the milk yield and component profiles, and increase the dry matter intake [5,6]. Moreover, Sun et al. and Qiao et al. found that *Bacillus subtilis* improves the milk yield and rumen fermentation of dairy cows [7,8]. In addition, Sun et al. reported that the supplementation of *Bacillus subtilis natto* could increased the serum immunoglobulin (Ig) G and interferon (IFN)-gamma levels in calves [9]. *Saccharomyces cerevisiae* can modulate the fermentation of ruminal microbes and stimulate bacterial lactate uptake and cellulose digestion in *in vitro* experiments [10]. However, most of the published works have focused on the effects of *Bacillus subtilis* and/or

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Saccharomyces on dairy cow health; and the effects of lactic acid bacteria (LAB) on the ruminal gut micobiota and milk yield and quality of dairy cattle have not been adequately addressed.

Recently, the emergence of high-throughput sequencing techniques has deepened our knowledge and understanding in the areas of microbial community and ecology. Particularly, the Pacific Biosciences (PacBio) single molecule, real-time sequencing technology (SMRT) is a powerful platform that is advantageous over other technology in producing long sequence reads and comprehensive microbiota profiles based on full-length 16S rRNA amplicons [11,12].

The objective of the present study was to assess the effects of supplementing a probiotic mix (two different LAB, *Lactobacillus casei* Zhang and *Lactobacillus plantarum* P-8) on the milk yield, milk composition, and fecal microbiota of dairy cow. We also aimed to profile the probiotics-driven changes in the dairy cow ruminal microbiota at phylogenic metagenomic level by using the SMRT technology.

2. Materials and methods

2.1. Animals

The study was performed in a commercial dairy farm near Zhangjiakou city, northern Hebei Province, between 12 December 2015 and 12 January 2016. All procedures involving animals were approved and conducted according to the standards of the Institute of Animal Science, Inner Mongolia Agricultural University. Twenty lactating primiparous Chinese Holstein dairy cows (60 days postpartum) were selected and divided into two (control and treatment) groups. The milk yield of lactation was similar at the start of the experiment. To ensure all animals share the same housing environment, all animals were kept in a single shed, having free access to separate open-air paddocks. All cows were fed the same basal diet as a total mixed ration.

2.2. Probiotics supplementation

Probiotics supplementation was given to the treatment group 60 days after parturition continuously for 30 days. The control group received the basal diet with no probiotics supplement throughout. Each treated animal received 50 g/day probiotics (containing 1.3×10^9 CFU/g of a mixture probiotics supplementation) mixed with the basal diet. The live probiotics used in this study were *Lactobacillus casei* Zhang and *Lactobacillus plantarum* P-8 (the proportion of each strain is 1:1) provided by Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University in China. The beneficial effects of *Lactobacillus casei* Zhang and *Lactobacillus plantarum* P-8 to humans have been shown previously [13,14]. The yeast strain has been proven to improve milk yield and ruminal bacterial diversity in cattle [15].

2.3. Milk sampling and analyses

Cows were milked twice daily in their tie stalls at 9:00 am and 9:00 pm; and the milk yield was recorded electronically. Milk samples (approximately 50 mL) from individual cows were collected on the first day of the trial (day 0, before feed administration), and at day 15 and day 30 from two milking. The two samples milked on the same day were combined at a ratio of 1:1 (volume:volume) to ensure a fair representation of the milk quality of the specific sample day. Samples were stored at 4 °C until analysis. The fat, protein and lactose contents were determined by the MilkoTMScan (MilkoScan Type FT120, Foss Electric, Hillerød, Denmark). The somatic cell counts (SCC) were determined using the Fossomatics 5000 (Foss Analytical A/S; Foss Electric, Hillerød, Denmark). The sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine the milk immunoglobulin G (IgG), lactoferrin (LTF), lysozyme (LYS) and lactoperoxidase (LP) levels.

2.4. Fecal sample collection and DNA extraction

The fecal samples of twenty cows were obtained at day 0 (before the supplementation) and at day 30 (post probiotics supplementation) and stored at −80 °C until analysis. The genomic DNA extraction of fecal samples was performed using a QIAGEN DNA Stool Mini-Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions [16]. The quality of the extracted genomic DNA was checked by agarose gel electrophoresis and spectrophotometric analysis (optical density at 260 nm/280 nm ratio). All extracted DNA were stored at −20 °C until further experiment.

2.5. Single-molecule real-time sequencing analysis of fecal microbiota

Bacterial 16S rRNA gene sequences of all genomic DNA samples were sequenced, and raw data processing was carried out according to the previous describe [17]. Alpha and beta diversity were calculated on the basis of the de novo taxonomic tree constructed by the representative chimera-checked OTU set using FastTree [18]. The Shannon-Wiener, Simpson's diversity, Chao1 and rarefaction estimators were performed for evaluating the sequence depth and biodiversity richness. The weighted and unweighted principal coordinate analysis (PCoA) based on the UniFrac distances [19] derived from the phylogenetic tree were applied to assess the microbiota structure of different samples. The sequence data reported in this study have been deposited in the MG-RAST database (Accession No. 4733612.3, 4733614.3 to 4733652.3).

2.6. Statistical analyses

All experimental data were analyzed with the R software (version 3.1.3). Statistical significant differences were tested based on Mann-Whitney Test in a pairwise manner. P-values below 0.05 were considered statistical significant. To adjust for falsely rejected null hypotheses, the Benjamin–Hochberg method controls the False Discovery Rate (FDR) were calculated by comparing the proportions of fecal bacteria at each phylogenetic level separately [20]. The graphic presentations were generated by Graph Pad Prism 6. The correlation between fecal bacteria and milk production, SCC and other measured parameters were represented by the Spearman rank correlation coefficient and visualized by heatmap in R using the "pheatmap" package.

3. Results

3.1. Milk composition, milk production and SCC

The results of milk analyses are summarized in Table 1. The probiotics intervention showed an increasing trend in milk at day 15 ($P = 0.052$) post probiotics application. At day 30, the increment became significant ($P < 0.01$), while the milk production of the control group remained stable throughout the experiment. The probiotic treatment also significantly lowered the SCC in the treatment group at day 15 and day 30 ($P < 0.01$, Table 1). No significant difference was observed in the proportions of milk protein, fat and lactose at any time points after probiotics supplementation.

Table 1
Effects of probiotics supplementation on cow milk yield and composition.

	Day 0			Day 15			Day 30		
	Treated group	Control group	*P*-value	Treated group	Control group	*P*-value	Treated group	Control group	*P*-value
Milk yield (kg)	23.79 ± 4.11	23.75 ± 3.99	N.S.	27.82 ± 3.19	25.28 ± 2.87	0.052	33.43 ± 4.54[a]	24.35 ± 3.70	<0.01
Fat (%)	4.01 ± 0.11	3.79 ± 0.09	N.S.	3.34 ± 0.21	3.42 ± 0.12	N.S.	3.48 ± 0.23	3.56 ± 0.17	N.S.
Protein (%)	3.26 ± 0.17	3.27 ± 0.23	N.S.	3.31 ± 0.14	3.42 ± 0.20	N.S.	3.40 ± 0.21	3.45 ± 0.13	N.S.
Lactose (%)	5.18 ± 0.11	5.25 ± 0.07	N.S.	5.15 ± 0.13	5.11 ± 0.12	N.S.	5.20 ± 0.10	5.22 ± 0.06	N.S.
SCC ($\times 10^4$ cell/mL)	8.27 ± 0.25	8.40 ± 0.37	N.S.	5.00 ± 0.20[a]	8.16 ± 0.34	<0.01	3.96 ± 0.54[a]	8.21 ± 0.21	<0.01

Note: The superscript letters represent significant differences of the same parameter between the same groups at different time points. N.S. represents no significant.

3.2. Milk IgG, LTF, LYS and LP contents

The effects of probiotics supplementation on the contents of some milk functional components, IgG, LTF, LYS and LP, are presented in Fig. 1. The milk IgG (Fig. 1a) and LP (Fig. 1d) content significantly increased at day 15 and day 30 compared to day 0 before the probiotics treatment. Compared to the control group, the increases in these two parameters were also significant. The levels of LTF (at day 15) (Fig. 1b) and LYS (at both day 15 and day 30) (Fig. 1c) were significantly higher in the probiotics compared to the control group.

3.3. Sequencing coverage and bacterial diversity

The SMRT sequencing of the full length 16S rRNA gene generated 171,632 reads from 40 samples, with an average of 4290 reads for each sample. The total number of unique and classifiable representative OTU sequence for bacteria was 93,042 (average = 180 OTUs per sample, range = 48–304, SD = 59, Table S1, online). The Shannon-Wiener diversity curves showed that the sequence depth here obtained was adequate for all samples, although further sequencing might identify new phylotypes based on the rarefaction curves (Fig. S1, online).

Results from the diversity indexes interestingly reflected that the bacterial richness and diversity of the control group significantly decreased at the end of the experiment ($P < 0.01$). In contrast, the treatment group maintained similar levels of diversity indexes after probiotics treatment ($P < 0.01$) (Fig. 2).

3.4. Principal coordinate analysis

The PCoA analysis based on the weighted and unweighted UniFrac distances (Fig. 3) revealed apparent bacterial structural differences between before and after the probiotics intervention. Symbols representing the treatment groups before and after probiotics administration were largely separated on both PCoA score plots with only minor overlap. Symbols representing the bacterial community of control cows were also distributed away from the probiotics-treated group.

3.5. Changes in microbial composition after probiotics administration

Collectively, 12 bacterial phyla, 143 genera, and 284 species were identified in the fecal samples of dairy cows. At phylum level, the Firmicutes, Bacteroidetes and Proteobacteria were the three predominant phyla, representing 83.52%, 12.07% and 4.16% of all sequences, respectively (Fig. S2, online). After adjustment for falsely rejected null hypotheses, there was no statistically significant difference in the relative proportions between treated and control group before and after treatment. However, based on the unadjusted P values, the differences in relative abundances of the three major phyla were obvious. Though the abundance of the Firmicutes phylum increased in both the control and the treatment groups, the increase in the control group was more apparent. An opposite trend was observed in the phylum Bacteroidetes. The abundance of the phylum Proteobacteria also increased in both groups at day 30 (Fig. 4).

The distribution of the 16S sequences were assigned to the genus level was analyzed and represented in Fig. 5 (only genera with $\geq$0.1% of the total sequences are displayed). Large individual variations are observed. Some genera apparently increased after probiotics treatment, including *Bacillus*, *Paenibacillus*, *Enterococcus*, *Cronobacter* and *Alkaliphilus*, while other genera were suppressed (including *Bacteroides*, *Faecalibacterium*, *Lactobacillus*, *Roseburia*, *Ruminococcus*, *Clostridium Coprococcus* and *Dorea*). However, it is worth noting that statistically significant differences were only detected in the fecal microbiota structure of the control but not the treatment group at day 0 and day 30 (Table S2, online).

Together from all samples, 37 species had a relative abundance of >0.1%. Significantly more *Bacillus cereus*, *Cronobacter sakazakii* and *Alkaliphilus oremlandii* were found at day 30 compared to day 0 for both the control and treatment groups. In contrast, some species of the *Bacteroides* genus (e.g. *Bacteroides plebeius*, *Bacteroides dorei* and *Bacteroides uniformis*) significantly decreased in the control but not treatment group at day 30 (versus day 0). The abundances of these lineages were significantly higher in the treatment than the control group. A similar trend was observed in the abundance of *Ruminococcus gnavus*, *Ruminococcus bromii*, *Roseburia hominis*, *Faecalibacterium prausnitzii* and *Lactobacillus rogosae* (Table S3, online).

3.6. Correlation between fecal bacteria, milk yield, SCC, and other milk components

Some of the changes in the bacterial abundances were correlated with the milk yield, SCC, functional milk components as determined by Spearman's rank correlation analysis (Fig. 6). The milk concentrations of LYS and IgG were significantly and negatively associated with the abundance of *Bacillus cereus*. The milk yield was significantly and negatively correlated with *Bacillus cereus*, *Paenibacillus barcinonensis* and *Paenibacillus odorifer*. The concentration of LP was positively correlated with some species belonging to the genera *Bacteroides*, *Ruminococcus*, and *Roseburia*, while negatively correlated with members of the *Bacillus*, *Brevibacillus* and *Paenibacillus* genera. Opposite correlation trends were observed for SCC (Fig. 6).

4. Discussions

This study investigated the effects of supplementing a probiotic mix (*Lactobacillus casei* Zhang and *Lactobacillus plantarum* P-8) on milk production, milk composition, several functional components and fecal microbial communities of dairy cow. Many studies have demonstrated that certain microorganisms can exert beneficial effects to the host and thus boost the production performance of dairy cows [21–23]. Furthermore, administering probiotics can help maintain the balance of gut microbiota and even improve

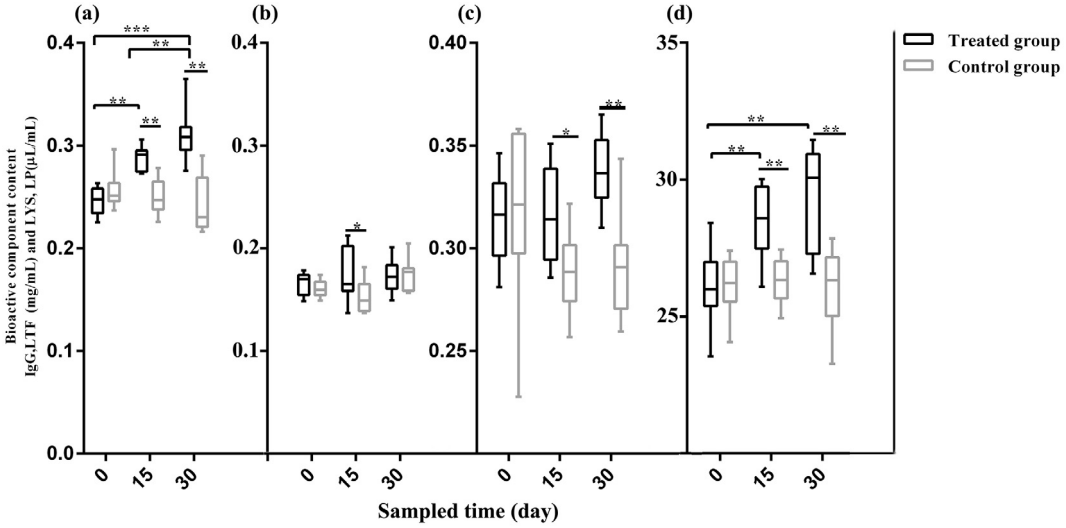

Fig. 1. Concentrations of milk functional components (a: immunoglobulin G, b: lactoferrin, c: lysozyme and d: lactoperoxidase) in the control and probiotics treatment groups ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

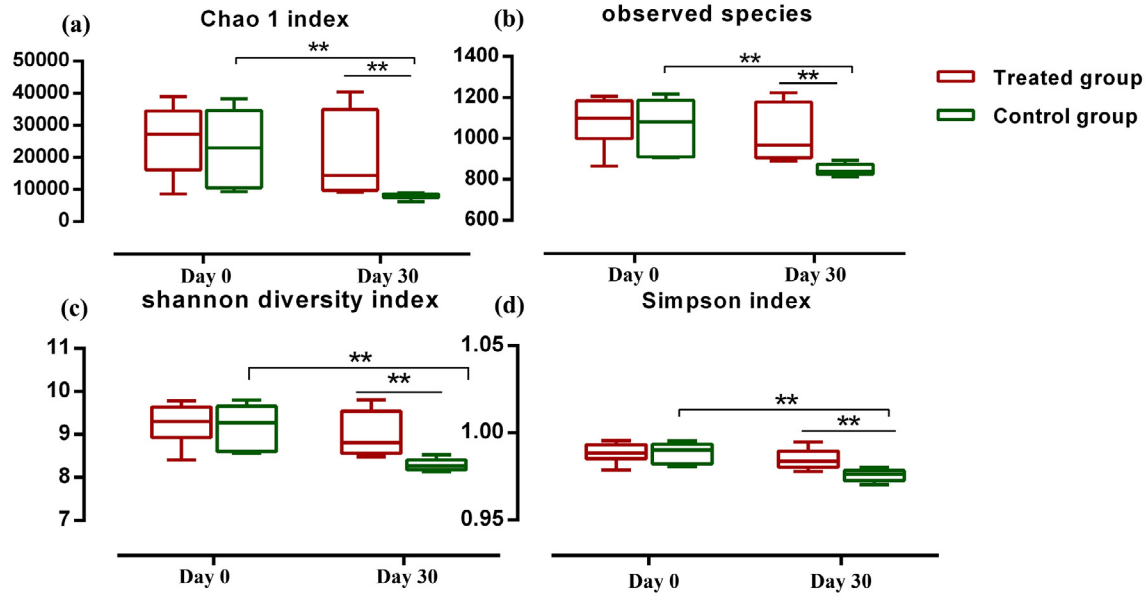

Fig. 2. Changes in the alpha-diversity of fecal microbiota of dairy cow subject to probiotics treatment ($^{**}P < 0.01$).

the ruminal bacterial composition [1,15]. However, many of these studies applied the species *Bacillus subtilis* and *Saccharomyces cerevisiae* as the probiotics strain, and the beneficial effects of LAB have not been adequately investigated.

Our work indicates that the probiotic mix application could increase the milk yield while suppress the SCC. The major milk composition, including protein, fat, and lactose contents, remained similar before and after probiotics treatment. The beneficial effects are likely related to an improved ruminal microbiota subject to probiotics treatment. For instance, microbial–derived amino acids can constitute up to two-thirds of the amino acids absorbed from the ruminant small intestine, some ruminal bacteria have been characterized as potent proteolytic and/or ammonia hyper-producing bacteria (*Clostridial* species) [24]. The SCC is a main indicator of mammary gland inflammation and mastitis, which are directly related to milk quality. The majority of somatic cells are leukocytes that traffic to the mammary gland and related tissues due to the localized inflammation. The SCC values were lower in treated cows than in control cows, suggesting mastitis in treated herd was low, it was perhaps unlikely that there was an opportunity to decrease it further. The reduction of SCC subject to probiotics administration suggests that such treatment can reduce mammary gland inflammation in dairy cow and subsequently increase in milk yield as shown also by our results.

Although we show that probiotics supplementation can improve udder inflammation and milk yield but not the milk composition, some of the currently published reported show inconsistent findings. For example, some studies showed that probiotic administration to dairy cows increased the milk production and simultaneously improved the milk fat, protein and lactose yield, accompanied by a decrease in milk SCC [21,9]. Two other studies found no significant changes in the milk fat and protein, lactose subject to microbial supplementation [22,7]. Possibly, different factors contribute to the discrepancies. Since the experiments were

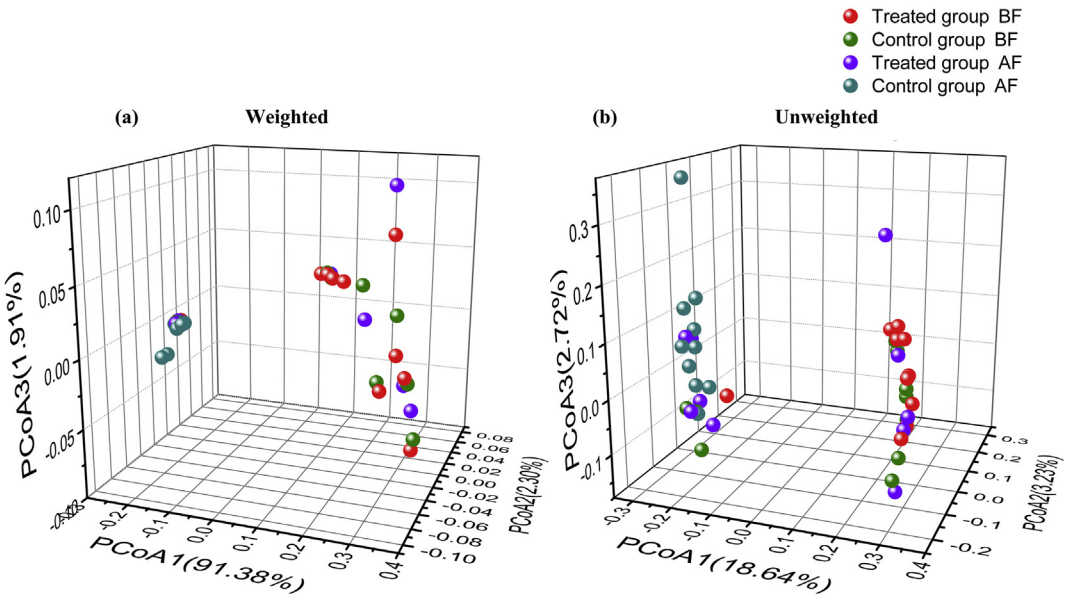

Fig. 3. Weighted (a) and unweighted (b) UniFrac principal coordinate analysis of the bacterial communities before (BF) and after (AF) probiotics administration.

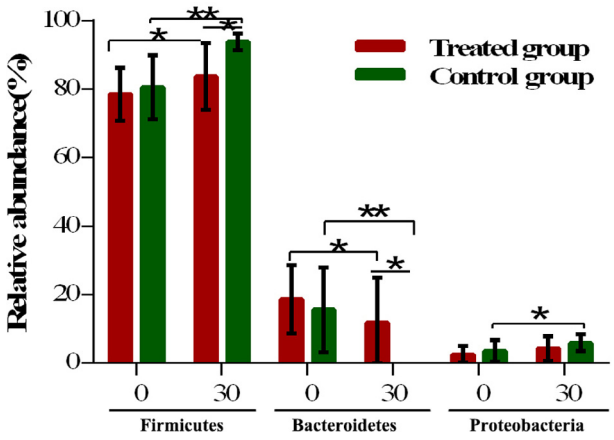

Fig. 4. Changes in fecal microbiota composition at phylum level subject to probiotics treatment.

performed by different laboratories and under different experimental conditions. A number of factors relating to the experiments were different, including probiotic mix preparation, the host-specific factors like age, physiological stage, health, feeding regime of the subject animals. Moreover, it is very likely that any beneficial effects seen are probiotic strain-specific.

We also monitored the changes of several milk functional components, including IgG, LP, LTF, and LYS. The milk IgG is originated from blood; the molecules are transported across the mammary alveolar cells mediated by an active receptor mechanism. Most likely, the probiotics treatment enhanced the plasma IgG level (as reported also by Sun [9]), and hence more of these molecules are transported to the udder area. Moreover, the species *Lactobacillus casei* significantly enhanced the IgG level in dairy cows, although such increase was only seen by a combination with babesiosis vaccine [25].

The other measured functional components, LTF, LYS and LP, are synthesized by the epithelial cells of the mammary gland before being transported to the mammary secretory cells. Lactoferrin can release the antimicrobial peptide, lactoferricin, subject to proteolytic cleavage, and function to suppress other microbes. Lactoperoxidase catalyses the oxidation of thiocyanate (SCN^-) in the presence of H_2O_2 to produce an antimicrobial intermediate, while some *Lactobacillus* can produce H_2O_2 [26]. Although we found correlations between these functional factors and some of the identified fecal microbes after probiotics supplementation, whether there is any causal relationship between them and the mechanisms behind remain unclear.

Our data showed that the probiotics treatment did not induce changes in the bacterial microbiota richness and diversity, which was consistent with another published study [27], while significant decreases in the diversity indexes were seen in control cows at day 30. Such results illustrate that the probiotics application can maintain the bacterial community richness and a high number of different species. On the PCoA score plot, symbols representing the control group at day 30 formed a distinct cluster, suggesting that the fecal microbiota of this subgroup differed from that of other samples. Possible, the loss of microbial richness and diversity was due to the high grain and low roughage feed given to the cows, leading to the change of rumen microbial population [28]. In contrast, the probiotics supplements contain a rich array of carbohydrate hydrolyzing genes/enzymes that aid in maximizing the feed utilization and support the microbial diversity.

Firmicutes is the most prevalent phylum in our dataset, particularly the control compared to the treatment group at day 30, as in other published results [29–31]. Bacteroidetes is also often found to be another dominant phylum in the rumen of dairy cows [32,33]; the abundance of this phylum was very low at day 30 (compared to that at day 0) in the control cows. The reason for the apparent decrease is unclear. Possibly, the high grain low roughage diets fed to the cow during the experiments reduced the proportion of Bacteroidetes [34]. The samples of the treatment group (at day 30) had an enriched level of Bacteroidetes while reduced abundances of *Firmicutes* and Proteobacteria. Normally, the proportion of Proteobacteria is less than 4% of the total ruminal community [35]; and an increased Proteobacteria population may cause subacute ruminal acidosis [30]. Such result indicated that the probiotics might contribute to the balance of the rumen microorganism at phylum level.

We found the probiotics used in this study stabilized the gut microbiota and reduced the risk of pathogen colonization. Although the probiotics treatment did not result in any significant

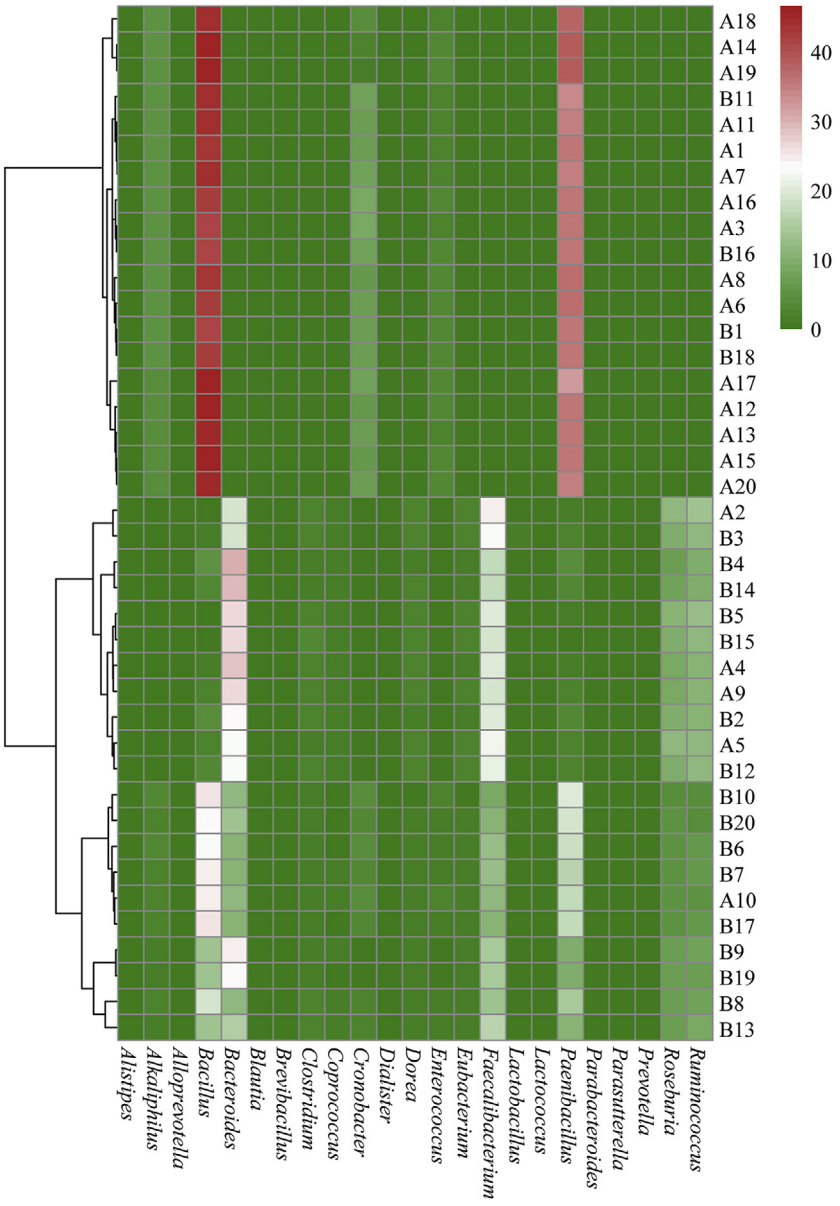

Fig. 5. Heatmap showing changes in major bacterial genera subject to probiotics administration. *Note*: Subject codes are shown at the right; 'B' and 'A' indicate before and after probiotics administration, respectively.

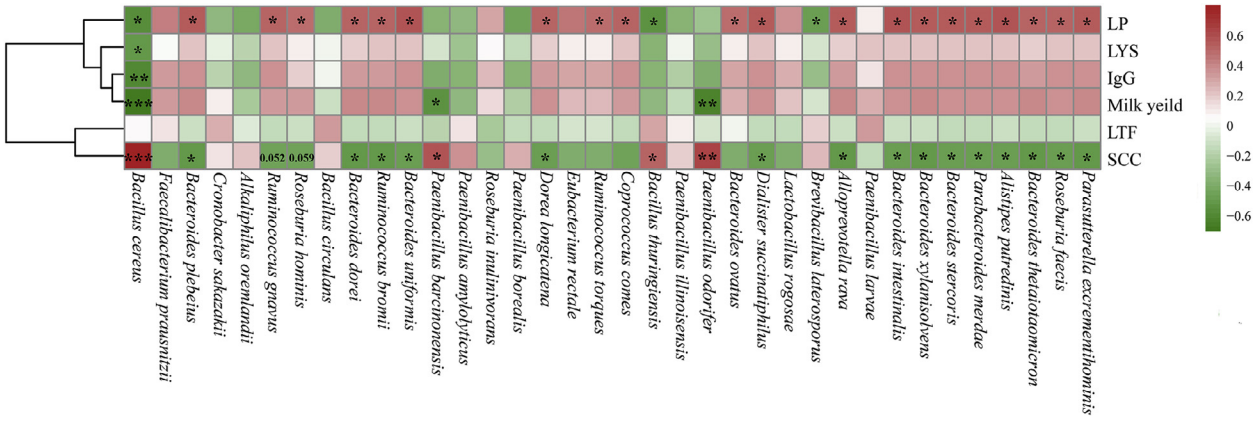

Fig. 6. Heatmap showing correlations between significantly altered fecal bacteria species between milk production capacity and various milk components ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

change in the fecal microbiota composition at genus and species level, the difference between the treatment and control groups at day 30 was striking. *Bacillus*, *Enterococcus*, *Cronobacter* and *Alkaliphilus* in the control groups increased, while some rumen digestion bacteria such as *Bacteroides*, *Roseburia*, *Ruminococcus*, *Clostridium*, *Coprococcus* and *Dorea* decreased. It was shown that cattle dysbiosis, characterized by low abundances of *Clostridium* and *Ruminococcus* and high contents of *Bacillus* and *Enterococcus*, led to malabsorption, diarrhea, and weight loss [36]. Significantly more *Bacillus cereus* was found in the control compared to the treated group, which is considered as a common contaminant in milk [37] and a predisposing factor for developing clinical mastitis [38]. The bacterium maybe temporally exists in the intestinal tract mammals, or, alternatively acting as an opportunistic pathogen involved in local and systemic infection [39]. Moreover, we identified some other opportunistic pathogens e.g. *Cronobacter sakazakii*, *Alkaliphilus oremlandii* [40,41] which were significantly less in the treatment than the control group at day 30.

On the other hand, one important function of the ruminal microbes is to degrade cellulose, hemicelluloses starch, fiber and peptides [42]. The probiotics treatment group had a higher proportion of the rumen fermentative bacteria, such as *Bacteroides uniformis*, *Ruminococcus gnavus* and *Roseburia hominis*, which may enhance the ruminal fermentation capacity. The enrichment of cellulolytic bacteria by probiotics treatment has previously been reported [43]. In addition, the abundance of another interesting bacterium, *Faecalibacterium prausnitzii*, was elevated in the treatment group. This bacterium enhances weight gain and reduces incidences of diarrhea [44]. These microbiota compositional changes upon probiotics supplementation steer the ruminal microbiota to a balance and healthy state that potentially improves fermentation and lowers the risk of diseases of lactating cows [45].

We also found that some correlations between the probiotics-driven variation of several fecal microbes and the investigated parameters (including milk yield, SCC and milk functional components), suggesting that the gut bacteria may be associated with the changes in these parameters after probiotics treatment. It is well known that the gut microbiota play an important role in the health of cows, if the gut is infected, dairy cows will often experience poor digestion and reduced absorption of nutrients and these digestive problems often precipitate into diarrhea. *Lactobacillus* have been linked to increased resistance to infection and diarrheal disease and stimulation of immune system activity, possibly due to the chemical composition and structure of their cell wall components. Indeed, some *Lactobacillus* species form substances that are antagonistic to other organisms, such as organic acids and bacteriocins, hence our probiotics improved milk parameters via maintaining the stability of ruminal bacteria [46]. In addition, some members of *Bacteroides*, *Roseburia*, *Ruminococcus* and other rumen digestion bacteria can hydrolyze the feed and promote nutrition absorption [42], which could have led to the increased of the milk functional components and resulted in the positive correlation. A previous paper has confirmed the improvement of immunity in cows is positively correlated with IgG concentration [47]. These bacteria meanwhile decreased the SCC and caused the negative correlations. The SCC is an important marker of mammary gland inflammation, the performance and health state of dairy cow are linked to characteristic changes in the rumen microbiota [48,49]. Also, the ability of live probiotics supplementation in improving milk yield and weight gain in cattle is accompanied with a shift in the major bacterial groups within the communities [1]. On the contrary, the increased abundance of *Bacillus cereus* might have increased the SCC and decreased the milk production and functional components [39]. *Bacillus cereus* is a causative agent in gangrenous mastitis [43]. Thus, the probiotics supplementation used in this experiment can modulate the ruminal microbiota composition and structure, steering toward a more stable and healthier status.

Overall this study demonstrated that our probiotics preparation was able to enhance the milk production and the functional substances meanwhile reduce the SCC. The fecal bacteria microbiota was significantly different from the control group without receiving probiotics. The treatment group was characterized with more rumen digestion bacteria, including members of *Bacteroides*, *Roseburia*, *Ruminococcus*, *Clostridium*, *Coprococcus* and *Dorea*, while less opportunistic pathogens *Bacillus cereus*, *Cronobacter sakazakii* and *Alkaliphilus oremlandii*.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scib.2017.04.019.

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