Science Bulletin 62 (2017) 767–774

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Science Bulletin
www.scibull.com

Article

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

Haiyan Xu [a,1], Weiqiang Huang [a,1], Qiangchuan Hou [a], Lai-yu Kwok [a], Zhihong Sun [a], Huimin Ma [a], Feiyan Zhao [a], Yuan-Kun Lee [b], Heping Zhang [a,*]

[a] Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University, Huhhot, Inner Mongolia 010018, China
[b] Department of Microbiology, National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 20 February 2017
Received in revised form 29 March 2017
Accepted 29 March 2017
Available online 21 April 2017

Keywords:
Milk production
Milk components
Fecal bacteria
Probiotics
Dairy cow
PacBio single molecule real-time sequencing technology

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

* Corresponding author.
E-mail address: hepingdd@vip.sina.com (H. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scib.2017.04.019
2095-9273/

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.