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Interrelations between the rumen microbiota and production, behavioral, rumen-fermentation, metabolic, and immunological attributes of dairy cows

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ABSTRACT

Different studies have shown a strong correlation between the rumen microbiome and a range of production traits (e.g., feed efficiency, milk yield and components) in dairy cows. Underlying dynamics concerning cause and effect are, however, still widely unknown and warrant further investigation. The aim of the current study was to describe possible functional interrelations and pathways using a large set of variables describing the production, the metabolic and immunological state, as well as the rumen microbiome and fermentation characteristics of dairy cows in early lactation (n = 36, 56 ± 3 d in milk). It was further hypothesized that the feed intake-associated behavior may influence the ruminal fermentation pattern, and a set of variables describing these individual animal attributes was included. Principal component analysis as well as Spearman's rank correlations were conducted including a total of 265 variables. The attained plots describe several well-known associations between metabolic, immunological, and production traits. Main drivers of variance within the data set included milk production and efficiency as well as rumen fermentation and microbiome diversity attributes, whereas behavioral, metabolic, and immunological variables did not exhibit any strong interrelations with the other variables. The previously well-documented strong correlation of production traits with distinct prokaryote groups was confirmed. This mainly included a negative correlation of operational taxonomic units ascribed to the *Prevotella* genus with milk and fat yield and feed efficiency. A central role of the animals' feed intake behavior in this context could not be affirmed. Furthermore, different methodological and interpretability aspects concerning the microbiome analysis by 16S rRNA gene sequencing, such as the discrepancy between taxonomic classification and functional communality, as well as the comparability with other studies, are discussed. We concluded that, to further investigate the driving force that causes the difference between efficient and inefficient animals, studies including more sophisticated methods to describe phenotypical traits of the host (e.g., rumen physiology, metabolic and genetic aspects) as well as the rumen microbiome (e.g., metagenome, metatranscriptome, metaproteome, and metabolome analysis) are needed.
Key words: rumen microbiota, feed efficiency, behavior, PCA

INTRODUCTION

The cow, being a ruminant, lives in a symbiotic relationship with her rumen microbiota. By ingesting feed, she delivers new substrates to the microorganisms, which in return produce valuable nutrients through fermentation and form a nutrient source themselves (Mizrahi, 2013). Early studies showed that the rumen microbial composition is very much determined by the feed composition, as well as feed intake pattern of the host (Bryant and Burkey, 1953; Warner, 1962; Mackie et al., 1978; Leedle et al., 1982). It was further described that the rumen protozoal population especially exhibits a host individuality (Kofoid and MacLennan, 1933; Eadie, 1962). With the advent of DNA fingerprinting and sequencing techniques, this finding was extended to the rumen prokaryotes (Li et al., 2009; Kong et al., 2010; Welkie et al., 2010; Jami and Mizrahi, 2012). The current understanding of rumen microbial dynamics is that the rumen microbiota consists of a core and a variable microbiota (Wu et al., 2012; Creevey et al., 2014; Henderson et al., 2015). The core microbiota is found across a wide geographical range and consists of different taxa that increase or decrease in their abundance according to the diet fed (Henderson et al., 2015). It therefore constitutes a key element in the survival strategy of ruminants by

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allowing fast and appropriate adaptation to new diets (redundancy and resilience; Weimer, 2015; Dieho et al., 2017a; Schären et al., 2017b; Solden et al., 2017). It is thought that the variable or individual microbiota is a result of interanimal variation in behavioral and genetic attributes, as well as environmental influences (Mizrahi, 2013; McCann et al., 2014a; Henderson et al., 2015; Weimer, 2015; Malmuthuge and Guan, 2017). Different studies have shown interrelations between production variables, such as feed efficiency (Guan et al., 2008; Zhou et al., 2009; Hernandez-Sanabria et al., 2010, 2012; Zhou et al., 2010; Carberry et al., 2012; Rius et al., 2012; McCann et al., 2014b; Myer et al., 2015; Jewell et al., 2015; Shabat et al., 2016; Li and Guan, 2017) and milk production and composition (Jami et al., 2014; Lima et al., 2015), and the rumen microbiota. However, the underlying dynamics concerning cause and effect still need to be elucidated (Weimer, 2015; Malmuthuge and Guan, 2017).

Therefore, the aim of the current study was to investigate the associations between the rumen microbiota and a large set of variables describing the production, as well as the metabolic and immunological state of dairy cows in early lactation, plus behavioral attributes, in an attempt to describe possible functional interrelations and pathways.

MATERIALS AND METHODS

Experimental work was conducted at the experimental station of the Institute of Animal Nutrition (Friedrich-Loeffler-Institut) in Brunswick, Germany. The experiment was carried out in accordance with the German Animal Welfare Act (http://www.gesetze-im-internet.de/tierschg/BJNR012770972.html) approved by the LAVES (Lower Saxony State Office for Consumer Protection and Food Safety, Germany).

Experimental Design

The data were collected in a trial investigating the influence of monensin and a blend of essential oils on production, rumen fermentation, and metabolic variables (Drong et al., 2016), immunology (Drong et al., 2017b), and the rumen microbiome (Schären et al., 2017a) of transition dairy cows. Sixty German Holstein cows were divided into low- (n = 15) and high-condition groups (n = 45) at the beginning of the dry period according to their BCS. The animals in the high-condition group were further divided into a control group and 2 treatment groups (either receiving a blend of essential oils or a monensin controlled release bolus, n = 15). The animals of the low-condition group were then fed a normal transition ration [80% roughage (50% maize silage, 50% grass silage) and 20% concentrate based on DM content] during the dry period; after calving they were fed a TMR with an initial concentrate feed proportion of 30%, which was increased stepwise to 50% of the daily ration within 2 wk (details in Drong et al., 2016). The animals in the high-condition group were exposed to a ketogenic ration by an oversupply with energy during the dry period (concentrate feed proportion of 60%) and a subsequently decelerated increase in concentrate feed proportion postpartum (from 30 to 50% in 3 instead of 2 wk; animal model described in Schulz et al., 2014). Production data, blood, and liver and rumen fermentation samples were collected throughout the trial at different points in time and showed that monensin increased the energy availability in the animal by increasing the ruminal propionate production, whereas the blend of essential oils failed to elicit any positive effect (Drong et al., 2016, 2017b). At d 56 postpartum (**p.p.**), oral rumen liquid samples were collected from 48 animals (n = 12) to investigate the underlying microbial alterations. The PCR-single strand conformation polymorphism and 16S rRNA gene amplicon sequencing analysis revealed alterations in the rumen microbiota due to the monensin treatment and, corresponding to the results on an animal level, no effect of the blend of essential oils was observed (Schären et al., 2017a). At that stage of lactation (d 56 p.p.), no significant difference between the control and the essential oil groups on production, metabolic, and rumen fermentation level was observed (except higher serum protein concentrations in the high-condition control group), whereas the monensin-treated animals exhibited lower serum BHB as well as higher rumen propionate concentrations (Schären et al., 2017a). Because the present study aimed to investigate the interrelations between phenotypic traits of dairy cows and their rumen microbiota at a normal physiological state, it was decided to exclude the animals of the monensin group in the current analysis. The animals in the 2 control and the essential oils group (n = 36) were considered representative for dairy cows at this stage of lactation with an average milk production of 30.7 ± 6.0 kg/d (fat % = 4.3 ± 0.7, protein % = 2.8 ± 0.2; means ± SD), DMI of 17.7 ± 2.9 kg/d, a BCS of 2.9 ± 0.4, and all measured metabolic variables in physiological ranges (e.g., serum BHB of 0.86 ± 0.36 mmol/L and fatty acids of 0.48 ± 0.29 mmol/L; Schären et al., 2017a).

Sample Collection and Analysis

All variables presented in the manuscript were assessed in samples collected at d 56 p.p., a detailed