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Effects of corn silage and grass silage in ruminant rations on diurnal changes of microbial populations in the rumen of dairy cows

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ABSTRACT

Here, we examined diurnal changes in the ruminal microbial community and fermentation characteristics of dairy cows fed total mixed rations containing either corn silage (CS) or grass silage (GS) as forage. The rations, which consisted of 52% concentrate and 48% GS or CS, were offered for *ad libitum* intake over 20 days to three ruminal-fistulated lactating Jersey cows during three consecutive feeding periods. Feed intake, ruminal pH, concentrations of short chain fatty acids and ammonia in rumen liquid, as well as abundance change in the microbial populations in liquid and solid fractions, were monitored in 4-h intervals on days 18 and 20. The abundance of total bacteria and *Fibrobacter succinogenes* increased in solids in cows fed CS instead of GS, and that of protozoa increased in both solid and liquid fractions. Feeding GS favored numbers of *F. succinogenes* and *Selenomonas ruminantium* in the liquid fraction as well as the numbers of *Ruminobacter amylophilus*, *Prevotella bryantii* and ruminococci in both fractions. Minor effects of silage were detected on populations of methanogens. Despite quantitative changes in the composition of the microbial community, fermentation characteristics were less affected by forage source. These results suggest a functional adaptability of the ruminal microbiota to total mixed rations containing either GS or CS as the source of forage. Diurnal changes in microbial populations were primarily affected by feed intake and differed between species and fractions, with fewer temporal fluctuations evident in the solid than in the liquid fraction. Interactions between forage source and sampling time were of minor importance to most of the microbial species examined. Thus, diurnal changes of microbial populations and fermentative activity were less affected by the two silages.

1. Introduction

The rumen harbors a complex microbial community capable of breaking down and fermenting ingested carbohydrates to the primarily volatile fatty acids that serve as the main energy source for the host animal. The composition of the diet fed to the animal is one of the most important factors influencing the ruminal microbial community and its fermentation products; for example, changing the forage-to-concentrate ratio has been shown to alter the composition of the microbial community in the rumen, as well as dry matter (DM) intake and ruminal pH [1]. The forage source itself also has a considerable impact on the ruminal ecosystem [2,3], as forages account for more than 40% of ruminant rations.

The most important forages used for feeding dairy cows and fattening cattle in Europe and North America are corn silage (CS) and grass silage (GS). Previous studies have shown divergent effects of the two silage types on ruminal digestibility, fermentation characteristics [2,4–6] and methane production in dairy cows [4] and fattening bulls [2]. Inclusion of CS as a substitute for GS in total mixed rations increased the ruminal digestibility of organic matter in dairy cows but decreased methane production per kg of digested organic matter [4], whereas GS increased the digestibility of neutral detergent fiber (NDF) in both cows [4–6] and bulls [2] while decreasing methane production per kg of digested NDF [2,4]. In addition, changes in the production of short chain fatty acids (SCFA) were observed depending on the type of silage fed to cows [4–6]. The ruminal pH was not affected by silage in the studies of Staerfl et al. [2], Brask et al. [4] and Owens et al. [6] but

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Abrahamse et al. [5] showed a decrease of pH upon inclusion of CS. Results for feed intake were also inconsistent; fattening bulls consumed more GS than CS [2], whereas no difference [4] or an increase in feed intake was observed in dairy cows when replacing GS by CS [5,7,8]. Moreover, utilization of nitrogen by ruminants differs between CS- and GS-based rations with a higher efficiency of ruminal microbial protein synthesis being observed for CS [9]. Givens and Rulquin [9] related this observation to the abundance of readily fermentable carbohydrates in the form of starch and thus to a more synchronized supply of nutrients for microbes in case of feeding CS [9].

Most of our knowledge about effects of both silages on the ruminal microbial community stems from *in vitro* studies that revealed structural changes within the phylogenetic groups of *Firmicutes* and *Bacteroides-Prevotella* [10,11] as well as quantitative changes in different microbial populations when incubating GS instead of CS [12,13]. *In vivo* studies on the effect of CS and GS on the ruminal microbial community are rare and restricted to rumen liquids. So, Staerfl et al. [2] analyzed the ruminal microbial community in rumen liquids of fattening bulls after slaughter. The authors detected a higher abundance of total bacteria but a lower abundance of protozoa in bulls fed GS, whereas methanogens and cellulolytics were less affected by silage type. However, these results refer only to a single point of sampling and do not lead to any inference on the effect of both silages on the abundance of different microbial species in the rumen, as diurnal changes in microbial populations are well known [14]. Current understanding of the effects of CS and GS on within-day changes of microbial populations stem solely from recently published *in vitro* research [13] demonstrating that sampling time has a major effect on the results for protozoa, methanogens, *Fibrobacter succinogenes, Ruminococcus albus, Prevotella bryantii, Clostridium aminophilum* and *Ruminobacter amylophilus*.

If the two silages have differential impacts on ruminal microbiota, and consequently their fermentation products, this might also influence the host animal itself. Thus, we conducted this study to gain further insight into the changes in the rumen ecosystem when using either CS or GS as forage in rations for dairy cows, focusing on the effects of both silages on the ruminal microbial community and fermentation characteristics *in vivo* by taking into account diurnal variations in the feed intake of the host animal. We hypothesized that (1) silages would differentially affect temporal fluctuations of microbial species with different physiologies, and thus fermentation products *in vivo*; and (2) the feed intake of animals would have an effect on diurnal changes of microbial populations regardless of *ad libitum* consumption.

2. Materials and methods

2.1. Ethics statement

The cows used in this study were housed at the Agricultural Experiment Station of Hohenheim University, Meiereihof, Stuttgart (Germany), in strict accordance with German Animal Welfare legislation. All procedures regarding animal handling and treatments described in this study were approved by the Regierungspräsidium Stuttgart, Stuttgart, Germany.

2.2. Animals and diets

Three rumen-cannulated lactating Jersey cows were used in a 2×3 incomplete Latin square design to test the effect of two total mixed rations containing either CS or GS as forage source ($n = 3$). The animals were housed in a free-stall barn in a herd of six cows that were fed individually using transponders that provided access only to one trough per cow. Each feed trough was equipped with an electronic scale to measure weight before and after feeding as a means of calculating feed intake. The forage-to-concentrate ratio of the experimental rations was 48:52 based on DM, with the concentrate consisting of 19% wheat, 19% barley, 7% soybean meal, 6% molasses, and 1% mineral mix. The chemical composition of silages and total mixed rations is shown in Table 1. Urea was added to the CS-based ration to adjust the ruminal nitrogen balance, as CS contains less nitrogen than does GS. For more effective mixing, 310 ml water/kg DM was added to the GS-based ration due to the high DM content of this silage. The troughs were filled with fresh feed once daily after the morning milking, with rations available for *ad libitum* consumption, and the cows were given free access to drinking water throughout the experimental periods. Each feeding period lasted for 20 days, with the first 17 days allocated to dietary adaption; samples were then collected on days 18 and 20.

2.3. Sampling

Six samples were taken from each cow via the rumen cannula at 4 h-intervals beginning at 8:30 a.m. on days 18 and 20 of each feeding period. Solid samples were collected from five different locations (cranial, caudal, dorsal, ventral, medial) in the rumen, using disposable, arm-length polyethylene gloves. The samples were squeezed manually, with the ruminal fluid collected considered to be the liquid fraction and the remainder composing the solid fraction. Equal parts from samples of the solid and liquid fractions were pooled across the five sampling locations per sampling time within each cow and period, and were stored at −80 °C until DNA extraction. Prior to DNA extraction equal parts of samples taken on day 18 and 20 were pooled by cow and sampling time within each feeding period thus resulting in one sample of solid fraction and one sample of liquid fraction per cow, sampling time and period.

Additional rumen liquid was collected from the ventral sac of the rumen with a vacuum pump. The pH of this fluid was measured immediately (pH meter: Type CG 842, pH electrode: Blueline 14 pH, Schott Instruments, Germany), and the rumen liquid was then stored at −20 °C for determination of the ammonia and SCFA concentrations.

2.4. Chemical analysis

Individual ingredients of the total mixed rations were oven-dried and ground to pass through a 1-mm sieve to analyze the concentrations of DM (method 3.1), ether extract (method 5.2),

Table 1
Chemical composition of silages (analyzed) and total mixed rations (calculated based on analysis of single ingredients) fed to cows.

	Silages		Total mixed ration	
	CS[a]	GS[b]	CS	GS
Dry matter (DM), g/kg	383	618	634	606
Crude protein, g/kg DM	78	128	142	153
Ether extract, g/kg DM	32	37	28	32
Crude ash, g/kg DM	41	119	52	89
NDF[c], g/kg DM	413	529	272	332
ADF[d], g/kg DM	210	318	133	188
ADL[e] g/kg DM	17	26	10	14

[a] Corn silage.
[b] Grass silage.
[c] Neutral detergent fiber without residual ash after α amylase treatment.
[d] Acid detergent fiber without residual ash.
[e] Acid detergent lignin.