

Variation among Dairy Cows in Rumen Liquid Fermentation Characteristics

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ABSTRACT: Volatile fatty acids are the main energy product from rumen fermentation. This study investigated the individuality of VFA concentrations in samples of rumen fluid obtained from 10 Holstein cows using an esophageal probe to take samples repeatedly over a 7 week period. Systematic changes were seen for propionic acid after onset of lactation, but acetic and butyric acid was not affected. Repeatability was generally low, but highest for propionic acid. VFA traits should be used as supplementary information in metagenomic studies.

Keywords:
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Introduction

The digestive efficiency of the rumen of each individual cow depends on the microbiota contained in its rumen. Different types of symbiotic anaerobic microorganisms, including Bacteria, Archaea, ciliated protozoa, and yeast, inhabit the rumen, and play important roles in affecting the host's performance (Hungate, 1984). In fact, a number of small experiments have demonstrated that the microbiota has a strong host dependency, so that following a complete exchange of flora between animals they revert to the pre-exchange state over few days (Weimer et al., 2010). During development from calf to adult cow, the microbiota changes in distinct patterns (Li et al., 2012) as well as fermentation is affected by fibrous diets (Zebeli et al., 2012). More importantly, host genome was found to have influence on ruminal bacterial structure, indicating an association between the structure and diversity of rumen bacteria and cattle production efficiency (Guan et al., 2008). All these facts imply that methanogen structure may also be associated with host (cow) genetic variation. New methods to characterize rumen microbiota, metagenomics, have evolved over recent years and offer insights and high capacity at the same time, but generate large volumes of data. Tools for handling such data (bioinformatics) are developed concurrently, but need to be dedicated to handle data from ruminants to be of use for agricultural purposes. Then they offer better opportunities for investigating host-microbe interactions (Benson, 2010) and their joint effects on methane emission, production and efficiency traits.

However, before embarking on large scale metagenomic experiments some basics need to be investigated in dairy cows. These include the huge changes at the onset of lactation and the stability of the metabolome

over consecutive weeks. To the cow, the most important products of the fermentation processes are the volatile fatty acids, as they are the key energy source. We used the VFA's as pilot traits for investigating systematic changes and repeatability in cows from before parturition and into early lactation.

Materials and Methods

Animals and design. Ten Holstein heifers were used for the experiment. They were housed at the Danish Cattle Research Centre (KFC-Foulum), and fed a total mixed ration ad libitum. Following calving cows were milked in an automatic milking system (VMS, DeLaval, Tumba, Sweden), with free cow traffic. During milking visits cows were offered restricted amounts of concentrates (max. 3 kg / d).

Rumen liquid sampling and assaying for VFA. Rumen liquid was sampled from all 10 heifers / cows on each of 7 occasions, with one week intervals, making a total of 70 samples. Sampling was done by an esophageal rumen probe (Flora rumen scoop; Geishauser et al. (2012)) "swallowed" by the cow. Each sample contained 40 mL of rumen liquid. The samples were immediately chilled on crushed ice and take to the lab for further processing and storage. Aliquots of the samples were assayed for a range of VFA's by gas-chromatography. For this study we focus on Acetic, Propionic and Butyric acid, although a larger array of VFA's were actually included in the total profile. The ratio between acetic and propionic acid is an often used variable in nutrition studies as an indicator of the dietary methane mitigation potential (more P less methane), thus also reported here (AP_ratio).

Statistical analyses. Concentrations of VFA's were analyzed using a linear mixed model. The model included "stage of lactation" as a fixed factor, and test_days were another fixed factor. Animals were considered random. Data was analyzed using HPMIXED in SAS (SAS 9.3, SAS Institute Inc.). The consistency of VFA concentrations were estimated repeatability calculated from the variance components as an intra-class correlation. Standard errors of repeatability were calculated from the standard errors of variance component estimates and their asymptotic correlation.

Results and Discussion

VFA changes at onset of lactation. Figure 1 shows that changes were generally small over the first weeks of lactation, indicating a level of stability in the cows. This may be an effect of the cows eating the same diet during all days, although in increasing amounts. However, for propionic acid concentrations increased from 16 to 23 mmol/kg when going from dry-cow status to day 15 in lactation. Propionic acid is a product of starch fermentation, and the change could be an effect of concentrates eating during milking sessions. The changes in the AP_ratio were clearer than the changes for each of the variables.

Linear mixed model estimates for repeatability.

The estimated repeatability was rather low for all 3 VFA's: Acetic acid $t = 0.03$; Propionic acid $t = 0.18$; $t = 0.03$. These estimates are across important metabolic changes at the onset of lactation. For propionic acid, the repeatability increased substantially if only data after onset of lactation were used. The AP_ratio had slightly higher repeatability $t = 0.25$ and is thereby more useful than the individual variables.

Robinson et al. (2010) showed repeatabilities in the magnitude of 0.20 for VFA profiles in 708 sheep and found phenotypic correlations between Acetic acid, propionic acid, butyric acid and 1-hour methane measurements of 0.15-0.20. The authors concluded that it was unlikely that VFA profiles could be used to predict methane emission from sheep, even though the rumen samples was taken directly after the sheep left the chamber where methane emission was measured. The low repeatabilities found in the present study also suggest that there is limited room to select for high and/or low content of VFA in dairy cattle.

The VFA profiles are much more dependent on feeding time than the microbial profiles. In this study samples were taken within a 3 hour window on the same weekday in all weeks, and simultaneously the feeding of the cows were also done in limited windows. However the present study have not included information on feeding time in the data, and it has therefore not been possible to estimate this effect. In a dairy cattle study it has been shown that there is a very high repeatability between samples taken in different layers of the rumen content (Ross et al. (2012)). This indicates that samples can be taken randomly in the rumen. It also indicates that there is room for additive genetic variation in rumen microbial content and thereby opportunities to select for or against specific microbes such as Archaea that are methanogenic. In the data available in this study such information is not available. The methods used in the present study however are applicable to large scale phenotyping (Lassen et al. (2013)). That study showed that rumen microbial (bacterial) profiles can be clustered and that these clusters had significant effect on methane production and milk production in a study of 50 Holstein first lactation cows.

Conclusion

These results indicate that the VFA concentrations in rumen are not strongly characteristic of the cow at least over shorter periods of time including the onset of lactation. Results of this type should be included in metagenomic profiling studies.

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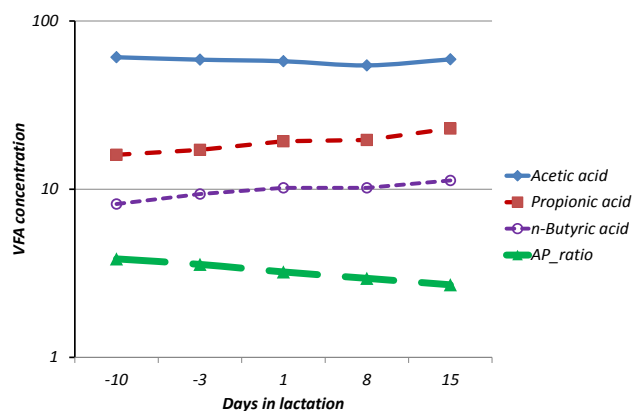


Figure 1. Concentrations (mmol/kg wet sample) of VFA in rumen fluid before and following parturition, in 10 Holstein cows.