

*Proceedings of the World Congress on Genetics Applied to Livestock Production, 897*  
**Fecal Archaeol Concentration and Rumen Microbial Community Structure  
in Lactating Dairy Cows**

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## Summary

The study provides an initial insight into the informative value of fecal archaeol, a potential biomarker for methanogenesis in cattle, with regard to the abundance of the ruminal population structures. Analysis of rumen fluid samples with 16s rRNA amplicon sequencing revealed differences in abundance of rumen archaeal and bacterial community for lactating cows grouped according to their fecal archaeol concentration (low, medium or high). The results demonstrate that the abundance of the rumen methanogen and bacterial microbiota can vary widely between different archaeol phenotypes. Different methanogen genotypes may be associated with different hydrogen-producing organisms and protozoa might play an important role in this context in addition to the microbiota we have studied. Therefore, further studies on the interactions of different microbiota are necessary to obtain greater knowledge about their role for methanogenesis.

*Keywords: fecal archaeol, microbial community, dairy cattle, rumen microbiota*

## Introduction

Selection for lower methane contribution and an increased production efficiency would improve the environmental footprint of the livestock sector. Therefore, ruminal methane production is emerging as a potential new trait in cattle (Negussie *et al.*, 2017). Heritability estimates for methane production on different scales ranged from 0.19 to 0.29 (Hayes *et al.*, 2016). Knapp *et al.* (2015) suggested a reduction potential of enteric methane emissions of 9 to 19 % due to animal breeding and management strategies. A reliable and simple method to quantify individual methane production is required to identify low- and high-emitting animals on farm. To overcome challenges of individual direct methane measurements, the use of a suitable proxy, such as fecal archaeol concentration, was examined in previous studies. Archaeol is a cell membrane lipid from methanogens. The archaeol concentration in feces is indicative for the level of enteric methane production (Gill *et al.*, 2011; McCartney *et al.*, 2013). However, the explanatory power of this correlation is still to be explored and might differ between different methanogenic species. The use of fecal archaeol concentration as a proxy for methane emission requires a better understanding of factors causing individual variation, and thus, knowledge about the microbial community structure of the hosts is needed in more detail. The aim of this study was to investigate the relationship between fecal archaeol and the ruminal microbiota in Holstein Friesian cattle. The hypothesis was that cows with diverse fecal archaeol concentrations differ in individual archaeal and bacterial community compositions. Therefore, fecal and ruminal samples of 40 cows fed the same diet were analyzed.

## Material and Methods

The study was conducted at the dairy research farm Karkendamm of the Institute of Animal Breeding and Husbandry of Kiel University. Cows received an ad libitum mixed ration based on grass (25±2 %) and maize (36±5 %) silage, daily feed intake was recorded, and fixed amounts of concentrates were provided at concentrate feeders. 40 Holstein Friesian cows, entering first (n = 11), second (n = 20) or third (n = 9) lactation, were recorded at day 150 in milk (±3). The cows were milked twice a day at 0500 and 1600 h. Fecal and ruminal samples were collected between 0900 to 1000 h. Feces were obtained from rectum by grabbing, then dried and grounded, and archaeol analysis was performed as described by Görs *et al.* (2016). Rumen fluid samples were obtained by suction. A tube with a bolus in form of a coarse strainer connected to a manual vacuum pump was inserted orally. To avoid salivary contamination samples (50 ml) were collected after discarding the first approximately 30 ml rumen fluid. Samples were subsequently stored on crushed ice and brought to the lab. DNA was isolated from rumen fluid with NucleoSpin kit for soil (Machery Nagel, Germany). Structure of rumen archaeal and bacterial community was characterized by sequencing 16S rRNA genes with Illumina MiSeq, Nextera XT V2. To this aim, we targeted the V5 and V6 region for archaea and the V1 and V2 region for bacteria.

Statistical analysis was performed using SAS (9.4, SAS Institute, 2013). Animals were grouped in low (lArch, < 25 µg/g fecal dry matter (fDM), n = 7), medium (mArch, 25 ≤ x < 35 µg/g fDM, n = 24) and high (hArch, ≥ 35 µg/g fDM, n = 9) fecal archaeol concentration (fArch). Individual methane emission was estimated with the formula: methane production (L/d) = 330.78 + 4.93 × archaeol, based on a respiration chamber experiment using the same feeding regime that was conducted earlier by Sandberg *et al.* (2015). Correlation coefficients for microbes and archaeol groups were estimated by the CORR procedure. The following model and the procedure GLM were used to analyze differences between archaeol groups ( $y_i$ , n = 40):  $y_i = \text{Group}_i + e_{ij}$ , where the term is the fixed effect of the group (i = 3) and  $e_{ij}$  is the residual error. Principal coordinate analysis (PCoA) based on bray-curtis dissimilarity matrix was performed in order to identify clustering patterns among samples using the PAST Software (3.16, Hammer *et al.*, 2006). Differences were declared significant at  $P < 0.05$ .

## Results and Discussion

The fArch ranged between 14.5 and 49.9 µg/g fDM. Animals belonging to the group hArch had highest feed intake and body weight on average and accordingly highest estimated methane production (Table 1).

Table 1. Descriptive statistics for recorded and estimated traits of cattle grouped in to low (< 25 µg/g fDM), medium (25 ≤ x < 35 µg/g fDM) or high (≥ 35 µg/g fDM) fecal archaeol concentration.

Trait	Low (n = 7)		Medium (n = 24)		High (n = 9)	
	Mean	SD	Mean	SD	Mean	SD
Dry matter intake, kg	24.5	3.47	23.4	3.65	26.8	5.38
Milk yield, kg	38.9	6.02	39.3	5.48	35.2	8.34
Body weight, kg	629	74.9	647	78.0	651	71.5
Fecal archaeol, µg/g fDM	19.8	3.32	30.8	2.96	39.6	4.04
Estimated methane <sup>1</sup> , L/d	428	16.4	482	14.6	526	19.9

<sup>1</sup>Based on fecal archaeol concentration (Sandberg *et al.*, 2015).

A total of 2.278.128 sequences of the domain archaea were obtained from rumen fluid

samples. Main phyla Euryarchaeota (99.9 %) and minor phyla Crenarchaeota (0.01 %) represented the archaeal population. The Euryarchaeota was dominated by the class *Methanobacteria* (71.5 %), followed by *Thermoplasmatales* (9.5 %). At genus level, *Methanobrevibacter* was most abundant in all archaeol groups, but relative abundance was 13.7 percent points higher in the lArch compared to the hArch (Figure 1a). On the contrary, *VadinCA11* had 15.4 percent points lower abundance in the lArch than in the hArch.

Domain of bacteria, with a total of 2,266,275 sequences, were represented by 27 different phyla, while 23 phyla were found across all samples. The bacterial community was dominated by *Bacteroidetes* (42.5 %), followed by *Firmicutes* (29.3 %) and *Proteobacteria* (13.6 %). On class level, abundance of *Bacteroidia* dominated in all archaeol groups. Figure 1b indicates that there are main differences between archaeol groups. The second most abundant class in lArch and mArch was *Gammaproteobacteria* with 20.4 % and 28.9 % respectively, whereas in hArch the class *Clostridia* with 14.4 % was more present.

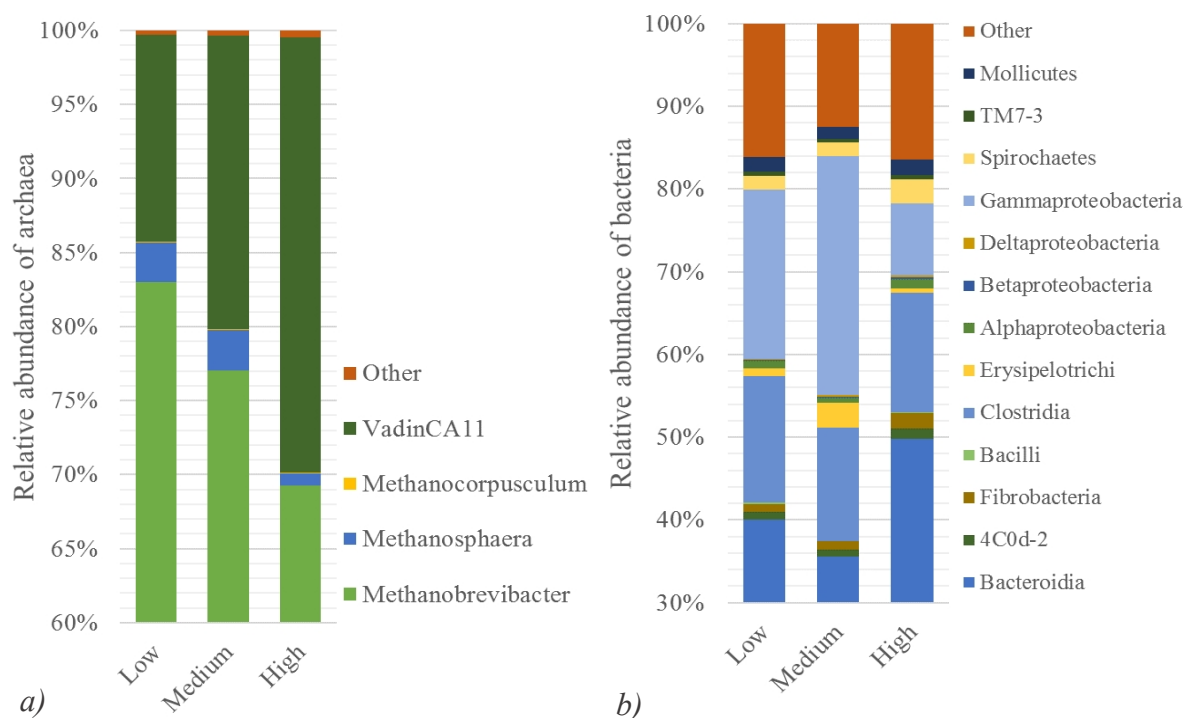
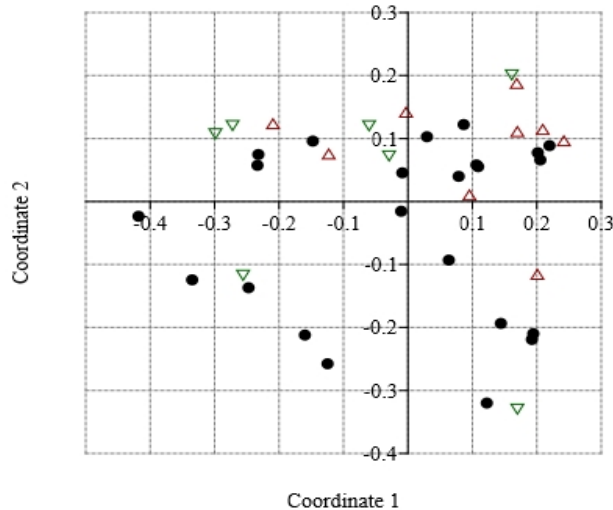


Figure 1a and b. Relative abundance of archaea at genus level and bacteria at class level in rumen fluid of cattle grouped in low, medium or high fecal archaeol concentration at 150 ( $\pm 3$ ) days in milk. Genera or classes with lower abundance than 0.05 % were subsumed as others. The y-axis shows in a) that > 60 % of archaea sequences were *Methanobrevibacter* and in b) that > 30 % of bacteria sequences were *Bacteroidia* for all groups.

A positive significant correlation could be observed between hArch and abundance of *Methanosphaera* ( $r = 0.95$ ,  $P < 0.001$ ). Furthermore, lArch and *Bacilli* showed a significant negative correlation ( $r = -0.78$ ,  $P = 0.03$ ). The PCoA in figure 2 displayed the distance between samples, but the point cloud showed no clear separated clusters of archaeol groups. Pairwise comparison between groups revealed only for hArch and mArch significant differences in bacterial composition ( $P < 0.01$ ). No significant relationships could be observed for different archaeol groups and the total abundance of archaea, bacteria or the ratio of archaea:bacteria. It has to be assumed that the abundance of the ruminal archaea and bacteria population is not remarkable for fArch. Considering the findings of Ng *et al.* (2016) that essential symbiotic contacts between protozoa and archaea exist, it can be concluded, that

the abundance of protozoa, which were no object of the present study, may obtain a greater understanding of the variations in fArch and should be considered in further studies in this area.



*Figure 2.*  
Principal coordinate analysis (PCoA) showing the relationship of rumen fluid samples based on abundances of archaea and bacteria. Colors represent different groups: **green** = low (< 25 µg/g fDM, n = 7); **black** = medium (25 ≤ x < 35 µg/g fDM, n = 24); **red** = high (≥ 35 µg/g fDM, n = 9) fecal archaeol group. Coordinate 1 described 30.6 % and Coordinate 2 17.8 % of the variance.

## Conclusion

Investigation of rumen microbial population revealed some significant differences for animals diverse in fArch. Differences in archaeal and bacterial community composition might indicate host genetic differences, and as consequence, abundance and characteristic of rumen microbial genes might be useful as selection criterions to mitigate methane.

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