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THE PHYSICAL FORM OF CORN INFLUENCES THE RUMEN BACTERIAL BIODIVERSITY– PRELIMINARY RESULTS

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Preliminary communication

SUMMARY

The aim of this study was to investigate the rumen bacteria in terms of genetic biodiversity and variation due to different physical form of corn in cow diet. A total of twenty dry cows were fed for 3 months with the same diet, only differed for corn physical form, ten received corn grains, while the other ones received corn flour. To investigate the biodiversity of the bacterial 16S rRNA gene clone library analysis has been conducted and then the sequencing has been carried out using Ion Torrent PGM™ System. Bacterial population was tested using R statistical software. The Kruskal-Wallis one-way analysis of variance (Kruskal-Wallis, 1952) confirmed that the bacterial populations were different when the animals were fed grain compared with flour corn. Both the OUT's abundance (Operational Taxonomic Unit) and the biodiversity indexes presented a significant difference among the two sample groups, underlining the large changes that take place even with small diet modifications in ruminal environment. There is still the need to deepen how exactly the diet changes the rumen phylogenetic structure and the consequences on bacteria's activity.

Key-words: rumen bacterial biodiversity, diet composition, physical form, OTUs.

INTRODUCTION

Dietary component and variation cause shifts in rumen bacterial ecology playing a role in animal health and productivity. Because of this complexity further investigations are required. For several years the rumen has been studied for its role in nutrient digestion and to manipulate its microbial ecosystem to increase animal performance and efficiency. Microbial population is not stable and changes to ruminal environmental characteristics and diet (Biavati and Mattarelli, 1991; Tajima et al., 2000). Feeding large proportions of starch to ruminants increase rumen microbial activity and the animal productivity but on the other hand, can negatively affect the rumen environment and its functionality, the fibre digestibility, and the animal health (Theurer, 1985).

The use of grain instead of cereal flour in ruminant nutrition can affect the site of starch utilization leading to a shift from the rumen to the intestine with positive effect on efficiency of energy utilization and on rumen environment. For this reason there is a great interest to investigate the impact of the physical form of cereals on the rumen microbial population diversity. New technologies of DNA sequencing ("ultra-high throughput" Ion Torrent Personal Genome Machine, PGM) allow the simultaneous analysis of huge amounts of sequences at very low cost, improving accuracy in quantification, enabling the identification even of minor species.

The PGM™ System has been used by Patel et al (2014) to describe rumen microbiome of Indian cattle (Kankrej breed) under different dietary treatments, where cattle were gradually adapted to a high-forage diets. The study revealed significant differences between all the diet treatments. The aim of this study was to investigate

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the rumen bacteria in terms of genetic biodiversity and its variation feeding finishing dry cows with corn grain and flour.

MATERIAL AND METHODS

A total of twenty dry cows of three different breeds (Holstein, Brown Swiss, and Simmental) reared in L. Toniolo experimental farm (Legnaro, PD, Italy) were fed for 3 months a finishing diet composed of corn silage 44.6%, corn 34.2%, sunflower 8.3%, straw 5.8%, sugar beet pulp 3.7%, others additives 3.4% of DM (chemical composition: starch 38.8%, NDF (Neutral Detergent Fiber) 37.4%, crude protein 10.9%). The cows were divided in 2 homogeneous groups fed the same diet that differed only for the corn physical form, ten received corn grains, while the other ones received corn flour. Rumen fluid samples were collected from each one using rumen probe and stored at -80°C. Subsequently, the DNA has been obtained using a method based on guanidine hydrochloride buffer and common DNA extraction columns (Yaffe et al., 2012) and then purified with silica and DNAse (Rohland and Hofreiter, 2007). After the isolation the bacterial 16S rRNA gene clone library analyse has been conducted and then the sequencing of V1-V2 region has been carried out using Ion Torrent PGM™ System. After sequencing, data were combined and sample identification numbers assigned to multiplexed reads using the MOTHUR software environment (De La Fuente et al. 2014). Data were denoised, low quality sequences, pyrosequencing errors and chimeras were removed, then sequences were clustered into OTU's at 97% identity using the pipeline available from http://www.brmicrobiome.org/#!16s-profiling-ion-torrent/cpdg (Pylro et al., 2014). OTU's containing fewer than 5 reads were excluded due to the likelihood of them being a sequencing artifact. Samples were normalised by randomly resampling to the lowest number of sequences per sample using Daisychopper (De La Fuente et al., 2014). The OTUs' study was made using R software. A principal component analysis (PCA), and subsequently, a k-mean cluster analysis, were performed to test the whole dataset without any prior information. The cluster analysis has shown that the cow breed did not affect the separation of samples in different groups so this factor has been removed from analysis. After this preliminary step the Kruskal-Wallis one-way analysis of variance (Kruskal-Wallis, 1952) has been applied to verify the difference between the two diets treatments. The number of sequences of each normalised sample was 14,289 sequences/sample and the number of sequences per each OTU was log transformed. Three indexes had been used to study the bacterial biodiversity. The Simpson's Index was computed as $D = \sum(n / N)2$, the Shannon's diversity index as $H = - \Sigma_{j=1} p_j \ln p_j$ and Richness as mean of the number of OTUs of each sample.

RESULTS AND DISCUSSION

The 16S rRNA gene clone library analyses and the sequencing of V1-V2 region allowed obtaining 4.108 operational taxonomic units (OTUs) as sum of all samples analysed. As shown in Figure 13622 and 3089 different OUTs have been globally identified for grain and flour groups, respectively. The log transformation of the sequence number evidenced a different distribution of OTUs abundances among diet treatments. In almost all the OTUs, the grain group had higher abundance of sequence, compared to flour group, with the exception of only 7 OTUs where flour groups showed a much higher abundance.

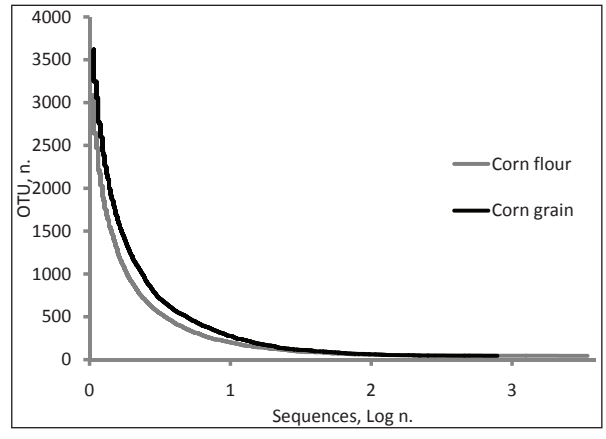

Figure 1. OTUs and Sequences graphical distribution for both groups (Flour and Grain corn).

The k-means cluster analysis graphically and clearly explained the differences among the groups and allowed two clusters identification. As shown in Figure 2, the two ellipses divided the twenty animals in two clusters that clearly identify the diet treatments even if there is an intersection area where some samples were not assigned and there is also an attribution error in corn flour ellipse. The Kruskal test, used to statistically analyse the biodiversity indexes, identified an outlier within the corn grain group, that behaviours differently from the other and was excluded from the statistical analysis. As reported in Table 1, the diversity indexes were significantly different for flour and grain groups.

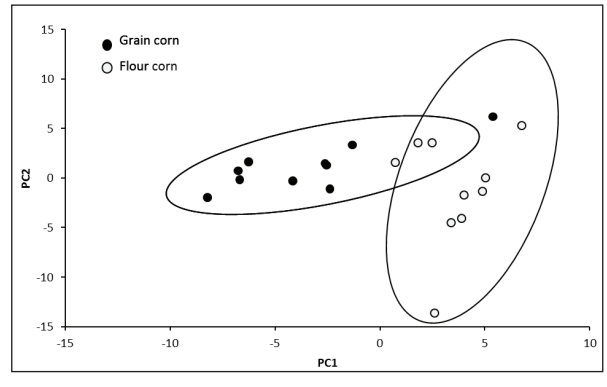

Figure 2. OTUs Cluster Analysis regarding principal component 1 (PC1) and principal component 2 (PC2).

The Shannon index increases as both the richness and the evenness of the community increase, and evidenced that animal fed corn grain instead of corn flour were characterized for a more biodiverse rumen microbial population. The richness index suggests that the grain group has a much higher diversity in term of OTUs number compared to the flour (1692 vs. 1261 OUTs, respectively). Finally, also the Simpson index that measure the microbial dominance, suggests also a slightly higher evenness of microbial population of grain group compared to the flour group.

Table 1. X^2, mean of corn flour group (flour) and corn grain group (grain) and P-value of Simpson, Shannon and Richness diversity indexes

		Corn treatment		
Biodiversity Index	X^2	Flour	Grain	*P-value*
Shannon	5.85	4.29	4.9	0.015
Richness	7.4	1261	1692	0.006
Simpson	3.72	0.91	0.89	0.053

To our knowledge, there are no studies that investigate the specific effect of diet physical form on rumen bacterial dynamics. However, some authors, who have worked on different levels of forages and concentrates, reported important variations in the rumen bacterial biodiversity. In particular, Fernando et al. (2010) found out a reduction of biodiversity increasing the proportion of concentrates in the diet. This result suggests that diet manipulation has an important role on rumen bacterial selection. In the present study these sensible effects of diet treatments on rumen diversity can be related to the different fermentative properties of corn fed to the animal as whole grains or after milling. The reduction of grain particle size is commonly associated to an increase of rumen fermentation rate and to a reduction of starch passage rate (Theurer, 1985). The rumen degradation of starch stimulate the microbial activity and the production of high proportions of VFA. However, at the sometime, the increase of starch fermentation in the rumen is commonly related to a reduction of cellulolytic bacteria activity and fibre digestion (Russell, 2002). Indeed, when the rate of VFA production overcome the buffering and absorption capacity of rumen, their accumulation lead to fluctuation of rumen pH and may have a selective effect on microbial population (Tajima et al., 2001). The reduction of bacteria biodiversity can impair the fermentative activity of the rumen microbial consortium (Wang and McAllister, 2002). Indeed, rumen bacteria adhere and colonize feed particles in the rumen, however, not all bacteria are equipped with a complete array of digestive enzymes. Co-culture of different microbial species demonstrated the importance of cross-feeding among bacterial species in attaining greatest bacterial growth rates and complete digestion of feed (Huntington, 1997).

CONCLUSION

The present study confirmed the significant difference between rumen bacterial populations in cows fed corn with different physical form within the same diet. Thus underlines the extreme dynamism of the bacteria and the susceptibility to even small changes in diet composition. There is a different rumen environmental equilibrium for the two theses that proved the variation in terms of bacterial diversity. The technology applied in this research does not allow to investigate the rumen bacterial activity. Anyway, this work represents a preliminary study that requires further investigations to understand the relation between the physical form of diet and the phylogenetic structure of the rumen population.

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