Report: Deliverable 2.2.1 Keeping cow and calf together – impacts on gut microbiota development (INRAE)

Authors: Martin, B.[1]., Nicolao, A.[1], Pomies, D. [1], Mathieu, A.[1], Koczura, M.[1], Bouchon, M.[2], Constant, I.[1], Popova M.

[1] Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

[2] Université Clermont Auvergne, INRAE, UE Herbipole, F-15203 Marcenat, France

1. Research question

This deliverable is directly linked to the work done in Task 2.1 related to animal performances obtained in the "Trial 3-Volame 3". The main results obtained in this task are presented in "D2.1 Keeping cow and calf together – impacts on animal performances, behaviour, welfare and farm economy (INRAE, SLU)".

Briefly, at INRAE experimental farm 'Herbipole', we tested two different suckling strategies on animal performance and behaviour. A 14-cow 'Classic' rearing system (C group) was compared during 14 weeks to two suckling systems. In the C group, calves were separated from dams immediately after birth and fed with an automatic milk feeder until weaning. In the 'Dam' group (D), dam-calf contact was allowed from birth to weaning, between morning and evening milking. In the 'Mixed' group (M), calves were kept with dams until three weeks (as in D group) before being separated and reared as in C group. All calves were weaned at about 11 weeks. On average, over 14 weeks, D and M cows produced 25.1% less milk at parlour than C cows; milk fat content was 3.6 g/kg lower in D group compared to C and M, and milk protein content was intermediate between C and M. After 11 weeks, D-calves weighed 20.5 kg more than C and M calves. Cows and calves both vocalised for one week after separation or after weaning. All calf vocalisations were at a maximum during the first four days. Cows' vocalisations were less notable when calves were removed after three weeks compared to 11. The frequency of health disorders was not significantly different between groups of cows and calves. In conclusion, a three-week suckling period seems better for farmers' income and cows' distress, but it induces stress for calves at both separation and weaning, without benefit on growth.

Additional sampling and analyses were done in order to get insights into the development of gut microbiota of calves depending on the rearing method, including or not contacts with the dam. We hypothesised that the gut microbiota of calves could develop differentially if the calves are suckling or not thanks to direct or indirect transfer of microorganisms from adults to the calves. In case different gut microbiota development would be evidenced, it could help understand the possible implication of gut microbiota in calves' health and therefore support the reduction of antibiotics use. The initial plan was to analyse the cow teat skin and milk's microbiota and calves' faeces before and after weaning. By the end, as it was possible to sample rumen fluid directly in calves, we decided to replace the analyses on milk and teat skin by direct analyses of rumen fluid.

2. Material and Methods

Calves rumen fluid samples were taken at weeks 3, 10, 13 and 20 of age (+/- 3 days) before the morning meal, only in female calves. 50 ml of rumen contents were collected through a stomach tube and a hand vacuum pump, and they were immediately frozen by immersing in a liquid nitrogen bottle/tank and stored at -80°C until extraction.

- For volatile fatty acids (VFA) analysis, 0.8 ml of liquid phase was put in 2 ml Eppendorf tubes containing 0.5 ml deproteinizing solution (4 g crotonic acid + 20g metaphosphoric acid in 5N HCl. qsp 1L). It was kept for at least 2h at 4°C.
- For Protozoa analysis, 1 ml of liquid phase was mixed with 1 ml of a mixture prepared with 1 L of 35% formaldehyde, 0.9 L distilled water, 0.6 g methylgreen, 8.0 g NaCl. The resulting mix was taken and stocked in the dark at room/ambient temperature.
- For Microbial ecosystem analysis, the remaining liquid was centrifuged for 10 min at 14,000 × g and frozen immediately in liquid nitrogen. It was stored at -80°C until nucleic acid extraction.

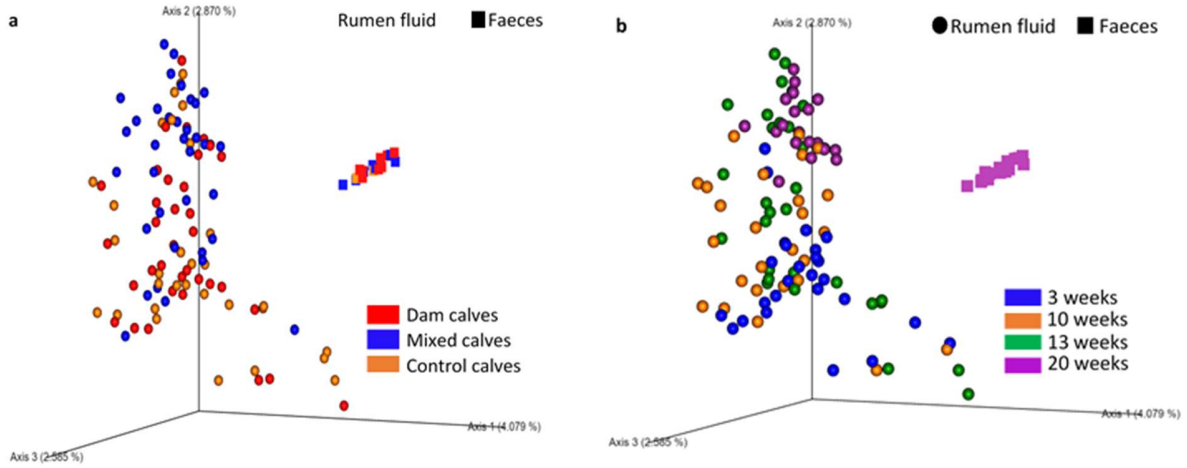
The genomic DNA was extracted following a protocol routinely applied in the laboratory (Popova et al, 2019). DNA was sent to an external provider/service for PCR amplification using universal primers targeting the 16S rRNA gene. Sequencing data were analyzed by the QIIME2 default pipeline.

Calves faeces samples were taken simultaneously as the rumen fluid samples by rectal search/ inspection (only in female calves). The samples were placed in sterile Falcon tubes and stored at min -20°C until further analysis.

3. Results

Results related to rumen VFA and Protozoa are not yet available. Preliminary analyses of results related to microbial ecosystems revealed a clear separation between faecal and ruminal samples reflecting two distinct microbial communities (Figure 1). However, the group/rearing system did not significantly affect the structure of the ruminal microbiota (Figure 1a). On the other hand, as expected, the age of the animal was a major factor influencing the installation of the microbiota: well-distinct communities were observed at 3 and 20 weeks of age, whereas 10 and 13 weeks of age were intermediate (Figure 2b). This can be explained by the feeding changes, as the 20 weeks-old calves are completely weaned.

Figure 1. Multivariate analysis of ruminal and fecal microbiota structure in female calves, expressed by group/rearing system (Figure 1a) or by week of age (Figure 1b).



4. Conclusions:

These preliminary results and data treatments do not confirm our hypothesis that the development of the gut microbiota in calves is changing according to the CCC contacts. Nevertheless, some results are still missing, and further in deep data treatments will be pursued to check specific microbial communities.

References:

Popova, M., Guyader, J., Silberberg, M., Seradj, A. R., Saro, C., Bernard, A., … Morgavi, D. P. (2019). Changes in the Rumen Microbiota of Cows in Response to Dietary Supplementation with Nitrate, Linseed, and Saponin Alone or in Combination. *Applied and Environmental Microbiology*, *85*(4). https://doi.org/10.1128/AEM.02657-18

Acknowledgment: The authors acknowledge the financial support provided by transnational funding bodies, being partners of the H2020 ERA-net project - CORE Organic Cofund - and the cofund from the European Commission, under the project ProYoungStock "Promoting young stock and cow health and welfare by natural feeding systems".

The authors thank the staff of the INRAE farm at Marcenat (UE Herbipôle) for animal care. This research was supported by French government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25). A. Nicolao received a doctoral fellowship from the Fondazione Cariparo at University of Padova (Italy).