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Evolution of bacterial diversity in the rumen in dairy calves from birth to weaning using a high throughput 454 GS FLX pyrosequencing

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Introduction The rumen in adult cow is an important site of fermentations, carried out by a microbial ecosystem that predominantly consists of bacteria. At birth, the calf rumen is sterile and milk is digested in the abomasum and the intestine. The rumen becomes functional later, with solid food intake. In lambs, Fonty *et al.* 1987 showed that the colonization of the rumen by bacteria begins during the first days of life and is sequential and Bryant *et al.* 1958 showed the same pattern from the first week of age in the calves. A lack of information persists on the establishment of the bacterial population during the very first days of life of calves, and most previous studies have focused on cultivable bacteria. The aim of this study was to define, using molecular microbiology, the early implantation of the ruminal bacterial population and its evolution until weaning.

Materials and methods The experiment was carried out on 6 male Prim 'Holstein calves from birth (day 1) to 3 months of age (day 83), before weaning. The calves were separated from their mother at delivery and reared in individual pens. Body weight (BW) of the calves averaged 39.4 ± 1.7 kg and 120.5 ± 16.8 kg at birth and 83 days of age, respectively. A standard feeding program for unweaned calves, using a milk replacer, hay and a pelleted concentrate, was applied. The rumen samples were taken through a stomach tube every day until the 10th day after birth, then every 3 to 5 days to 83 days of age. Characterization of bacteria was achieved using V3 V4 hypervariable regions of the 16S RNA gene by pyrosequencing (454) on 132 samples. After cleaning and alignment, taxonomic assignment were performed using the open-source software Mothur V.1.12.3 (www.mothur.org) and a bacteria database based Silva 96 (1194263 reads). Number of and relative abundance of taxa according to phylum, class and order were analysed using a repeated-measures analysis of variance with a model that included the effects of age, calf and their interaction as fixed effects.

Results The compilation of all samples (*e.g.* 6 calves and 22 sampling days) showed 430 different taxa. The number of taxa evolved with age ($P < 0.001$). Between 2 and 12 days after birth, the number of taxa increased from 185 to 229 (Figure 1). Between 15 and 43 days of age, the number of taxa decreased from 205 to 144 taxa. After one month of age, the number of taxa was stabilized at an average value of 155 taxa. On the 132 samples, there were 10 different phyla, the major being: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacter (10, 15, 39 and 31%, respectively). Regarding classes, there were 17 different classes, the major being Actinobacteria, Bacteroidia, Bacilli, Clostridia, Betaproteobacteria and Gammaproteobacteria (11, 10, 9, 27, 9 and 15%, respectively). The importance of some classes changed between 2 and 83 days: Bacilli (from 16 to 8%) and Gammaproteobacteria decreased from 16 to 8% and from 25 to 12%, respectively, whereas Clostridia increased from 17 to 31%. Forty one orders were identified, the major being Actinobacteridae, Bacteroidales, Lactobacillales, Clostridiales, Burkholderiales, Enterobacteriales, Pasteurallales and Pseudomonadales (7, 11, 7, 28, 5, 3, 4 and 4%, respectively). Three orders were observed to change between 2 and 83 days: Lactobacillales and Enterobacteriales decreased from 13 to 5% and from 9 to 1%, respectively and Clostridiales increased from 16 to 31% (Figure 2) and showed a strong evolution during the first week.

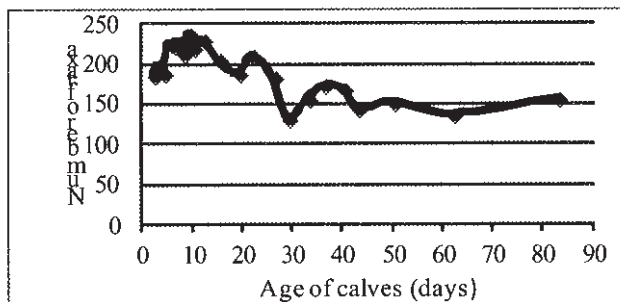


Figure 1 Evolution in the number of taxa according to the age of calves from birth to 83 days

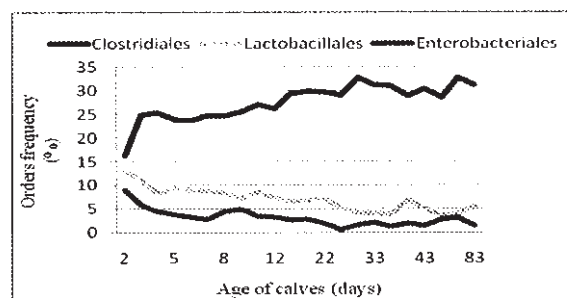


Figure 2 Evolution of 3 dominant orders frequency to the age of calves

Conclusions The use of 454 pyrosequencing showed an evolution of taxonomic diversity with the age of calves. This could be explained by successive steps of bacterial establishment in the rumen of calves from birth to weaning. The first one, between 2 and 30 to 40 days after birth would correspond to the establishment of pioneer species originating from the external environment. During the 2nd step the diversity reached stability, which could relate to an equilibrium between the microbiota and solid food arriving in the rumen.

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