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EFFECTS OF FEEDING STRATEGY ON THE MATURATION OF CECAL MICROBIOTA IN YOUNG RABBITS

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ABSTRACT

The objectives of this study were to evaluate the influence of the composition of feed distributed to rabbit kits before weaning on feed intake, and the composition and maturation of cecal microbiota in young rabbits. Three experimental feeds were used: feed H (10.37 MJ DE/kg and 102 g DP/kg), feed L (9.63 MJ DE/kg and 95 g DP/kg) and feed R (10.57 MJ DE/kg, 128 g DP/kg). Kits in the group RL received the feed R from 18 to 28 d and the feed L from 28 to 49 d. In the groups LL and HH, rabbit kits received feed L or H during both periods, respectively. Between 18 and 28 d, feed intake was highest (+26%) in group RL compared to the other two groups ($P<0.01$). The intra-group variability of microbiota was highest in the group LL at 28 d ($P<0.01$), but no differences were determined at 35, 43 and 49 d. Similarity of bacterial profiles between two consecutive ages was high from 18-28 d in the three groups and decreased thereafter. Variability between 43 d and 49 d was lower in the group RL compared to the group LL ($P<0.01$), group HH being intermediary. Our results suggest that composition of feed at the onset of solid feed ingestion in rabbit kits plays a role in accelerating the maturation of microbiota through a modulation of the feed intake.

Key words: Cecal microbiota, maturation, health, rabbit, feed intake onset

INTRODUCTION

Digestive disorders in young rabbits are a major problem in rabbit breeding systems, leading to reduced performances due to an increase in mortality and poor growth. Bacterial communities present in the gastrointestinal tract of rabbits, in particular in the cæcum, play an important role in gut health. It has become apparent in recent research that microbiota have a large number of physiological roles, such as hydrolysis and fermentation of nutrients, immune system regulation and barrier action against infectious agents. The composition of the feed distributed to young rabbits before weaning has a large impact on the development and maturity of the digestive physiology (Gidenne et al., 2008). Solid feed, which acts as the substrate for microorganisms, can modulate the physico-chemical conditions of the biotope, intestinal motility and digestive transit (Gidenne et al., 2008). The objectives of this study were to evaluate the influence of the composition of feed distributed to rabbit kits on feed intake, and the composition and the maturation of cecal microbiota in young rabbits.

MATERIALS AND METHODS

The experiment was designed and carried out according to the European Union recommendations on the protection of animals used for scientific purposes (2010) at the PECTOUL Experimental Unit (INRA, Toulouse, France), and was approved by the French government (n°2015100817517471).

Experimental diets, animals and measurements

Three experimental diets were used during the experiment (Table 1): feed H (high in digestible fibre and energy), feed L (high in digestible fibre, low in energy) and a feed R (low in digestible fibre, high

in energy). The R diet was formulated to meet the nutrient needs of reproductive females while diets L and H were formulated to meet the nutrient requirements of fattening rabbits while limiting nitrogen emission and preventing digestive disorders, with a similar DP/DE ratio.

Table 1: Chemical composition of experimental diets.

	Diet R	Diet L	Diet H
Chemical composition (g/kg)[a]			
Digestible energy (DE; MJ/kg)[b]	10.57	9.35	10.23
Digestible protein (DP)	128	98	107
Starch	166	72	115
Crude fat	31	26	26
Digestible fibre	199	258	240
Acid detergent fibre (ADF)	173	220	200
Neutral detergent fibre (NDF)	320	390	350
Acid detergent lignin (ADL)	56	69	58
DP/DE	12.1	10.5	10.5

[a,b]Calculated according to the tables of ingredients by Sauvant et al. (2004)[a], exception of DE by Maertens et al. (2002)[b].

A total of 537 kits from 56 litters (INRA 1077 x Hyplus) were divided into three experimental groups at birth depending on the does' weight at parturition (4254±392 g) and parity (4.7±3.5), as well as the litter size at birth (11±3 kits). The litters were equalized at 10 kits 3 days after birth (0 d) by cross-fostering or culling. After weaning (35 d), the litters were split into cages (5 kits/cage) from the same litter. The experiment took place between 18 and 49 d, and corresponds to the distribution of the experimental diets. Experimental groups differed by the diet received during 2 different periods: from 18-28 d, and from 28 d until the end of the study. Kits in the group RL received the feed R from 18-28 d, and feed L from 28-49 d. In the groups LL and HH, rabbit kits received feed L or H during both periods. Between 18 d and weaning, animals were fed *ad libitum*. After weaning, feed was restricted according to average weight of the group at weaning. During this period, feed was distributed daily. Kits were weighed at 18, 28, 35, 43 and 49 d. Mortality was recorded daily and health status was assessed at the time of weighing. A rabbit with signs of diarrhoea, bloating or abnormally low weight (average minus 2 standard deviations) was declared sick.

Cecal microbiota composition

At 18, 28, 35, 43 and 49 d, 10 rabbits from each treatment were sacrificed by electrical stunning and exsanguination. The animals sacrificed were healthy and their weight was representative of their litter before weaning or cage after weaning. Samples of cecal contents were collected and stored at -80° C. Phylogenetic analysis of cecal bacterial communities was carried out by pyrosequencing genes coding for the 16S RNA (V3-V4 region) by Miseq (Illumina). The 12,117,327 raw sequences obtained were cleaned, clustered into OTU's (operational taxonomic unit) and affiliated to taxa using the FROGS pipeline (Escudie et al, 2015).

Statistical Analysis

All statistical analyses were performed with version 3.0.3 of the software R. The variability of the bacterial community intra-age and between two consecutive ages were evaluated by Euclidean distance from the nMDS coordinates. Animals at 18 d were not included in the linear model; although assigned to an experimental group, they did not differ from an experimental point of view (feed distribution started at 18 d). The averages per group were compared using Tukey test. The percentage of mortality was compared by a Chi^2 test. Feed intake was analyzed by a linear mixed model, taking into consideration the group as a fixed effect, the doe as a random effect, and the weight at equalization as a covariable.

RESULTS AND DISCUSSION

The feed intake of kits at the onset of solid feed ingestion (18–28 d) was on average 6.8 g/d/kit, although feed intake was higher in the RL group compared to the LL and HH groups (+18% and +21%, respectively; P<0.05; Table 2). These results are similar to those found by Gidenne et al. (2007), where the kits which received the same feed as their mothers had a higher intake before weaning compared to kits receiving a diet high in fiber. During the period 28–35 d, a large variation of feed intake between litters within a group was observed (CV = 40%, 23% and 29% for the groups RL, LL, and HH, respectively) and no differences were perceived during this period (P=0.392). During the period of feed restriction (35-49 d), no feed remained in the feeder, therefore feed intake corresponded to feed offered.

Table 2: Effect of the feeding strategy on feed intake of young rabbits (g/d/kit).

Groups	RL	LL	HH	s.e.m.	P value
18 - 28	7.8[a]	6.4[b]	6.2[b]	0.11	0.032
28 - 35	19	21	16	0.51	0.392
35 - 42	70	60	65	0.23	nc
42 - 49	95	85	85	0.29	nc

When the kits had access to solid feed at 18 d, the average live weight was 304 g and no difference was observed between groups (*P*=0.205). No difference of live weight was observed at 28 or 35 d (*P*=0.573 and *P*=0.280). At 42 d, kits from the group RL were heavier than kits in the LL group (1143 vs. 1064 g, respectively; *P*<0.01), while the group HH was intermediary (1129 g). The final weight of the HH group was similar to the RL group, and both were significantly heavier than the LL group (+6.7% and + 7% for the groups HH and RL, respectively, *P*<0.001).

Kit mortality was low throughout the study, and was similar in all groups (1.8% total for all treatments). Of the mortality, 0.6% was caused by digestive problems and the cause of the remaining deaths was inconclusive.

In all samples, five bacterial phyla were found in the cecum, where 99.4% of total sequences were represented by 2 phyla (Firmicutes 83.1% and Bacteroidetes 16.3%). Due to a large variation intra-group, the ratio Firmicutes/Bacteroidetes, which has been proposed as an indicator for microbiota maturity (Combes et al., 2011), did not differ between the groups at 49 d (33.9/1 ± 2.2/1; P=0.399). Of the 49 genera detected, the relative abundance of 5 genera was found to be affected by diet: Lachnospiraceae *Marvinbryantia,* L. *Roseburia*, L. *Shuttleworthia*, Ruminococcaceae *Flavonifractor*, and Oxalobacteraceae *Oxalobacter*. The relative abundance for *Marvinbryantia* and *Flavonifractor* were highest in the HH group compared to the LL group (P<0.05), with the RL group as intermediary. Both the HH and RL groups were found to have higher relative abundances of *Shuttleworthia* compared to the LL group (*P*=0.012). *Roseburia* was found to be the highest in the RL group compared to both the LL and HH groups (+35% and +33% of the genera represented, respectively). This could be related to the higher fat content of the H diet. Mice fed a fat-rich diet had a proportion of Lachnospiraceae twice that of mice fed a low fat diet (Evans et al., 2014). The composition of the microbiota was more variable in the group LL compared to the groups RL and HH at 28 d (Figure 1A; P<0.01). The variability was found to decrease in the HH group between 43 and 49 d. No difference of intra-group variability was observed in the RL group (P>0.05), although variability was low at all ages (Figure 1A). The cecal microbiota evolves greatly with age (Figure 1B), as observed previously by Combes et al. (2011). The differences between two consecutive ages were highest between 18-28 d, which correspond to the introduction of solid feed intake. Between 28-35 d, the difference of the composition of the bacterial community was lower in the RL group than the other two groups (P<0.05), and lower in the RL group between 43-49d compared to the LL group (P<0.05). This suggests that the higher feed ingestion at the onset of solid feed intake allows for earlier digestive ecosystem stability.

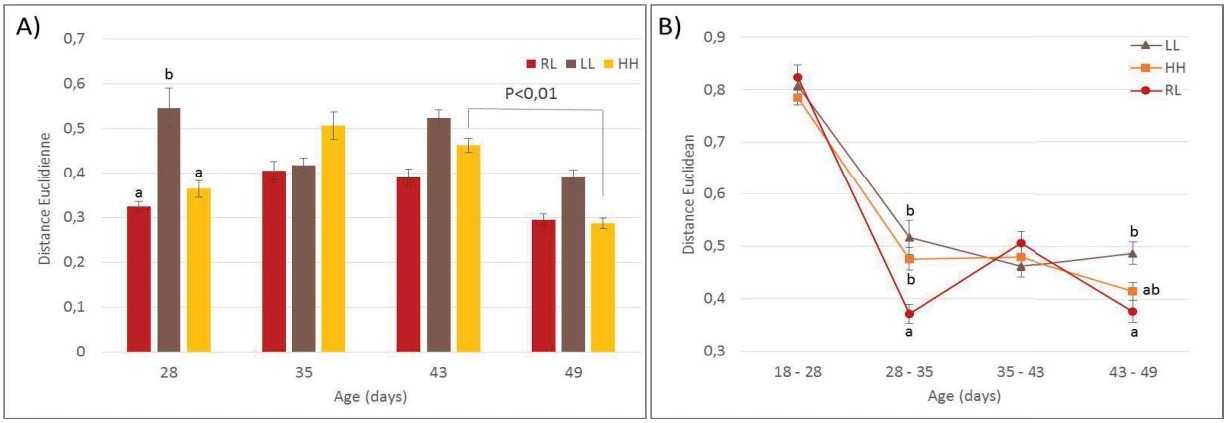

Figure 1: The intra-group variability (A) and the similarity of bacterial profiles between two consecutive ages within a group (B). The higher the value, the greater the difference of the bacterial profile is important from one age to another (P<0.05).

CONCLUSIONS

Our results suggest that composition of the feed at the onset of solid feed ingestion in rabbit kits plays a role in accelerating the maturation of microbiota through modulation of feed intake and quantity of substrate provided for bacterial growth. Further studies on kit feed preference and the roles of the bacteria present in the cæcum are needed.

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