IMPACT OF AN EXCLUSIVE MILK VS. MILK AND DRY FEED INTAKE TILL WEANING ON INTAKE, GROWTH, AND ON THE CAECAL BIODIVERSITY AND FIBROLYTIC ACTIVITY OF THE YOUNG RABBIT

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ABSTRACT

The effect of an immediate shift from milk to solid feed at weaning (30 d) was studied on intake, growth, caecal ecosystem and health of the young rabbit. Two groups of 11 litters were weaned at 30 d of age: PILA group, where the kits were fed exclusively with maternal milk until weaning, and then immediately fed with a pelleted feed; Control group where the kits were classically bred, with a progressive increase in feed intake before weaning from mother feeder. The adaptation of the young to the solid feed was quick, since their relative intake became similar to that of Control between 32 and 36 d (respectively 89.9 vs. 89.3 g/d/kg LW). From 30 to 49 d of age, the growth of PILA did not differ from Control (meanly 50.7 g/d). From 49 to 70 d, the intake of PILA group did not differ from Control, as well growth rate or feed conversion. The mortality (4/154) and the morbidity (3/154) were low and similar among the 2 groups. An exclusive milk intake till weaning strongly inhibited the pectinolytic and xylanasic bacterial activities at 30 d (respectively 7 and 4 times lower for PILA than for Control), but they were similar to Control at 37 and 44 d. The bacterial biodiversity in the caecum, estimated by Simpson index, did not change significantly between 30 and 44 d of age for the control group. In return, an exclusive milking reduced the caecal biodiversity at 30 d of age (PILA), but it increased rapidly and reached a level similar to control group at 37 and 44 d of age.

Key words: Milky diet, Caecal microflora, pH, Growth performance, Caecal enzymatic activities.

INTRODUCTION

The weaning is a critical period for the young rabbits, with a high sensitivity to digestive troubles. It is hypothesized that caecal flora and microbial activity play an important role (Gidenne *et al*., 2007a). In standard breeding conditions, before weaning (28-35 days), young rabbits are exclusively suckled by their mothers until 17-20 days and then they begin to consume solid food (Gidenne and Lebas, 2006). The inhibitory effect of milk on the caecal microbial activity was previously shown by Padilha *et al.* (1999), and Marounek *et al*. (2002) showed in-vitro that medium chain fatty acid, specific of rabbit milk, decreased the fermentation rate and the growth of some caecal bacteria. Furthermore, Gallois *et al*. (2007) demonstrated that maternal milk protects the young rabbit from E. coli infection. On the other hand, the intake of solid feed by the young promotes the caecal microbial activity as shown by a higher fermentative activity and lower pH in the caecum (Xiccato *et al*., 2003; Gidenne *et al*., 2002). However, the respective effect of milk vs. milk and dry feed intake on the caecal ecosystem was never studied, particularly in-vivo and respect to bacterial biodiversity and fibrolytic activity. We thus aimed to compare the effect of exclusive milk vs. milk and dry feed intake in pre-weaned rabbit on caecal microbial activities, microflora diversity, and on intake behaviour and growth performances.

MATERIALS AND METHODS

Animals and experimental design

Two groups of 11 litters from female rabbits (hybrid INRA line) were either exclusively milked (PILA) or had access to milk and to maternal pelleted feed (Control) until weaning at 30 days of age. Litters were equalised at birth to 9 pups and to 7 at 25 d. Control rabbits were caged with their mother until weaning. At 14 days of age, PILA rabbits were caged separately from their mother, and suckling was ensured by moving the does in the PILA litter cage during 5–10 min every morning (8–9:00, until weaning). At weaning, rabbits of both groups were caged collectively (one cage per litter) and fed freely a post-weaning pelleted feed (Table 1). Live weight and feed intake was measured twice a week from 30 to 42 days old, and once a week till 70 d. Morbidity was checked twice a week, while mortality was checked daily.

Table 1: Composition of the experimental diets

Ingredients (g/kg)	Does diet	Post-weaning diet
Dehydrated beet pulp	130	170
Dehydrated alfalfa meal	250	190
Wheat straw	-	60
Soya bean meal	50	90
Wheat	110	100
Barley	-	97
Extruded whole soya seeds	40	-
Sunflower meal	138	100
Wheat bran	200	177
Sugar	40	-
Sunflower oil	20	-
Vitamins and minerals	22	16
Chemical composition (g/kg raw basis)		
Moisture	88	97
Crude protein	159	171
NDF	357	363
ADF	188	193
ADL	49	43

Chemical Analyses

One kit per litter (with a weight near the average of its litter) was sacrificed after stunning at 30, 37, and 44 days of age. Caecum was removed, and weighed before and after emptying. The pH of the caecal content was taken with a glass electrode pH meter. A sample of caecal content was designed for the analysis of bacterial fibrolytic activity and was treated according to Gidenne *et al.* (2002, 2007b). Dry matter, ash, crude protein, and Van-Soest fibre analysis were conducted according to EGRAN (2001).

PCR-CE-SSCP of *16S rRNA* genes

PCR-CE-SSCP procedure was described in Michelland *et al.* (2008). Total DNA was extracted using QIAamp® DNA Stool Mini kit (QIAGEN). Genes coding for V3 region of *16SrRNA* were targeted for PCR amplification. CE-SSCP mix contained 1 µl of PCR product, 7.8 µl of desionised formamid and 0.2 µl of HD400 internal standard (Applied Biosystems). Mix were denatured at 95°C for 5 min and placed on ice before loading on ABI Prism 3100. Capillary electrophoresis was performed at 25°C for 3500 s at 15 kV and produced chromatograms where bacterial communities were spread out on 1200 scan. Using Safum software in Matlab Zemb (Zemb *et al*., 2007), chromatograms were firstly together aligned with a same reference internal standard and secondly normalized (area under bacterial community curve set to 1). Bacterial diversity was investigated by estimation of Simpson index on each CE-SSCP chromatogram.

Statistics

Data of growth and intake were subjected to analysis of variance according to the GLM procedure SAS (OnlineDoc®, release 8.01 for SunOS). For data of growth of suckled rabbits, the maternal group nested within group was declared as a random effect and was included in the model with group as main effects. After weaning, rabbit growth and feed intake were tested including group as main effect in the model. Results of caecal traits were subjected to ANOVA and Tukey's HSD post-hoc using R software with the following fixed effects: the rabbit age, the group and the interaction group X age.

RESULTS AND DISCUSSION

From kindling to 21-d old, only 8 kits died, and two litters were replaced because of milking troubles. At 15 d old, young of both groups had a similar weight (meanly 240 g, Table 2). From 25 to 70 d of age, live weights in strictly milk fed rabbit (PILA group) were significantly lower than Control (Table 2), with a maximum difference (-39%) at 30 d. Indeed, exclusive milk consumption in PILA group did not cover the nutritional needs of the young after 21 d, either qualitatively and particularly respect to the quantity of nutrients. A lower growth was even found for young exclusively milk fed rabbit with two does, compared to standard conditions (Padilha *et al.*, 1999). More generally, the growth potential of the young rabbit is not met before weaning, since a double milking and a free access to solid feed increased the growth rate (Gyarmati *et al*., 2000). Already at 21 d the solid feed intake brought nutrients for growth, since body weight of control rabbits tended to be higher compared to PILA (resp. 325 vs. 296 g, P=0.057).

Table 2: Intake and growth performances of young rabbits fed exclusively with milk (PILA) or with milk and dry feed (Control) till weaning (30 d)

	PILA	Control	RMSE	Pr > F
Body weight (g/rabbit)				
15 d	237	243	21.8	NS
25 d	370	436	36.7	<0.01
30 d (weaning)	413	683	71.1	<0.001
36 d	698	941	93.8	<0.001
49 d	1401	1620	184.5	<0.001
70 d	2452	2605	249.4	<0.01
Daily weight gain (g/d)				
15 – 25 d	13.3	19.3	2.4	<0.001
25 – 30 d	8.8	49.4	3.6	<0.001
30 – 49 d	51.8	49.6	6.1	NS
49 – 70 d	49.5	47.7	6.9	NS
30 – 70 d	50.8	48.6	4.5	0.040
Daily food intake (g/d/rabbit)				
30 – 49 d	78.8	93.8	86.3	<0.001
49 – 70 d	147.6	145.1	13.3	NS
30 – 70 d	113.2	119.7	7.6	0.073
Feed conversion ratio				
30 – 49 d	1.51	1.89	0.10	<0.001
49 – 70 d	2.98	3.06	0.26	NS
30 – 70 d	2.22	2.47	0.13	<0.001

After weaning, the intake of solid feed increased rapidly in PILA group. Two days after weaning, the feed intake of PILA groups averaged 22 g/d (±4.8) vs. 58 g/d (±5.6) for control. From 32 to 36 d of age, the intake doubled for PILA (54.2 g/d) and reached 76.3 g/d for the control. If expressed relatively to the body weight, then intake of both groups did not differ significantly (89.9 vs. 89.3 g/d/kg BW, resp. for PILA and control). Then intake of PILA rabbits continued to increased, but remained 8% lower than control for the period 36-49 d (106.3 vs. 115.3 g/d). Relative intake of PILA group was 13% higher compared to control between 36 and 42 d (105 vs. 93 g/d/kg/BW), and 25% higher between 42 and 49 d. Accordingly, after weaning, a compensatory growth took place in PILA rabbits, but was not sufficient to reach the growth of control group. Although food conversion ratio was better in PILA group than in control group, the food intake of PILA rabbit was not sufficient to allow a total compensatory growth. The health status was high during the whole experiment, since only 3 rabbits (on 154) were morbid between 30 and 49 d, and 4 rabbits died from acute diarrhoea between 49 and 70d (three in PILA and one in control group). Therefore, the abrupt change from milk to solid feed seemed not to provoke digestive troubles after weaning. As expected, the weight of the caecal wall (CW) and caecal content (CC) increased with age (P<0.001) in both groups (Table 3). The caecal wall developed similarly with age among the two groups, whereas that of caecal content differed among groups. At 37 d of age, rabbits of PILA group exhibited a significantly lighter caecal content with a higher dry matter (+5 units, P<0.05) and higher pH value than control rabbits. At 44 d of age, PILA rabbit CW weight was also lighter (P<0.05) than in control rabbits. Therefore, till weaning, the caecal development of young exclusively milked was strongly depressed compared to control, but within one week the caecum developed quickly, reaching dry matter and pH level similar to control.

Table 3: Evolution of caecal traits for young rabbits fed exclusively with milk (PILA) or with milk and dry feed (Control) till weaning (30 d)

Group	PILA			Control						age X
Age (days)	30	37	44	30	37	44	RMSE	age	group	group
Caecal wall										
(g)	7.6 d	13.2 c	17.8 b	9.7 d	15.2 c	20.1 a	1.6	<0.001	<0.001	NS
(% BW)*	1.8 a	1.9 a	1.7 ab	1.4 b	1.6 ab	1.5 b	0.2	NS	<0.001	NS
Caecal content										
(g)	11.7 d	52.9 b	72.7 a	30.0 c	58.7 b	79.0 a	9.9	<0.001	<0.001	NS
(% BW)*	2.8 c	7.5 a	6.8 a	4.4 b	6.3 a	6.1 a	1.1	<0.001	NS	<0.001
Dry matter (%)	27.4 a	22.6 b	21.6 b	22.9 b	23.1 b	21.8 b	2.0	<0.001	<0.05	<0.001
pH	6.98a	5.59 b	5.62b	5.80 b	5.70 b	-	0.25	<0.001	<0.001	<0.001

Means with different letters on the same row differ significantly (P<0.05 Tukey test)
RMSE: root mean square error; *: % of body weight, at 30d due to missing data for some sacrificed rabbits, values were reported to the mean BW of the group

Carboxymethyl cellulase activity (CMCase) was not affected by age or group, and was more than ten times lower than pectinolytic and xylanolytic activities (Figure 1), that agreed with previous studies (Gidenne *et al.*, 2002, 2007b). Significant interactions were detected among effects of group and age for pectinase and xylanase activity. For instance in control group, pectinase or xylanase activity did not change significantly with age (Figure 1). Reversely, an exclusive milk intake till weaning (PILA group) strongly inhibited the pectinolytic and xylanasic activities only at 30 d (resp. 7 and 4 times lower than control), but then within one week the microbial activity of PILA group increased greatly and reached a level similar to control animals.

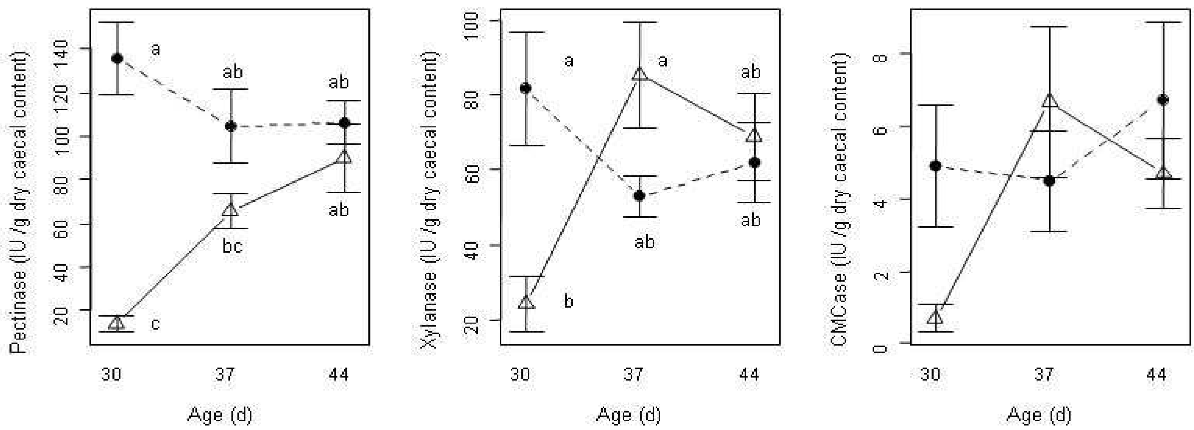

Figure 1: Caecal enzymatic activities in young rabbits fed exclusively with milk (PILA: Δ) or with milk and dry feed (Control: ●), till weaning (30 d); IU = µmol of reducing sugars released in 1 h by enzymes extracted from 1 ml of supernatants digesta

As for fibrolytic activity, significant interaction was detected among effects of group and age, for the caecal bacterial diversity estimated by Simpson index (Figure 2). In control group, the bacterial biodiversity did not changed significantly between 30 and 44 d of age, thus suggesting that biodiversity in the caecum was already achieved at weaning. In return, an exclusive milking reduced the caecal biodiversity at 30 d of age (Figure 2), but this effect was transient since the biodiversity increased rapidly for PILA group to reach a level similar to control already at 37 d old. The lower biodiversity of 30 d rabbit fed exclusively with milk would be explained by a lack of "solid" substrate from feed, e.g. for the growth of fibrolytic bacteria. However, we cannot exclude a bacteriostatic role of the milk, even in the hindgut.

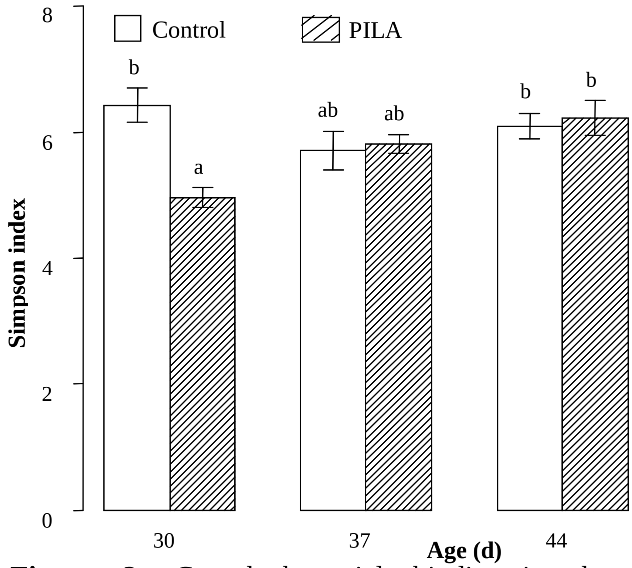

Figure 2: Caecal bacterial biodiversity by estimation of Simpson index on CE-SSCP chromatogram, in young rabbits fed exclusively with milk (PILA) or with milk and dry feed (Control), till weaning (30 d)

CONCLUSIONS

After an abrupt weaning at 30 d of age, with an immediate shift from milk to solid pelleted feed, the young rabbit was able to increase rapidly its solid feed intake, and within one week reached the control level. An exclusive milking greatly impaired the caecal development and also the bacterial activity and diversity. A sharp change in feeding behaviour at weaning seemed not to provoke further digestive troubles, suggesting a high digestive adaptability of the young rabbit. However, this must be confirmed on a higher number of animals. Under classical breeding system, with a progressive increase in solid feed intake since 18 d of age, the bacterial biodiversity as well the fibrolytic potential of the caecal ecosystem seemed already achieved between 4 and 5 weeks of age.

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