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INFLUENCE OF AGE AT WEANING AND NUTRITIVE VALUE OF WEANING DIET ON GROWTH PERFORMANCE AND CAECAL TRAITS IN RABBITS

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Abstract: To investigate the relationship between dietary nutrient concentration and weaning age on growth performance and caecal characteristics of rabbits, a trial was carried out on 64 litters (eight rabbits/litter) comparing two weaning ages (25 *vs*. 34 d) and two diets (HC: high concentrated and LC: low concentrated diet) offered to the kits from 18 to 34 d of age. At 34 d of age 54 animals per group were caged individually and all were fed the LC diet until 45 d of age. All animals were then fed a standard fattening diet (37% neutral detergent fiber, 3.4% ether extract and 18.0% crude protein on dry matter basis) until slaughter at 80 d. The digestibility trial was performed from 56 to 60 d of age on another group of 16 rabbits (8 per diet). During the experimental period (18-80 d of age), feed intake and animal weights were recorded. Caecal volatile fatty acids were measured at 45 and 80 d of age, while microbiological analysis was performed at 25 and 34 d on healthy suckling rabbits. Digestibility of dry matter, protein, neutral detergent fiber, fat and energy was higher in rabbits fed the HC diet (by 7.7, 7.4, 13.1, 26.7, 10.6%, respectively; $P<0.001$) than in those fed the LC diet. Growth rate and feed conversion ratio (FCR) from 18 to 34 d improved by 7.7 and 9.5% in rabbits fed HC in comparison to the LC diet, respectively ($P\leq0.061$). Diet offered around weaning did not influence growth rate or FCR from 34 to 80 d of age. Rabbits weaned earlier increased feed intake (by 67.8%) from 25 to 34 d, but reduced growth rate (by 26.8%) compared to animals weaned at 34 d ($P<0.001$). Early weaning reduced live weight during the entire fattening period ($P\leq0.018$), as well as the FCR (by 4%; $P<0.001$). Caecal pH was lower at 45 d of age in rabbits weaned at 25 d than in those weaned at 34 d (5.53 *vs*. 5.83; $P=0.019$). Treatments did not affect total caecal VFA concentration, but HC diet decreased caecal acetic concentration and increased butyric acid level compared to the LC diet at 45 d ($P\leq0.001$). HC diet tended to reduce caecal counts of *Enterobacteriaceae* ($P=0.093$), while it did not affect facultative anaerobic bacteria, *E. Coli* and *Costridium* spp. The LC diet increased at 25 d the number of *Clostridium perfringens* in comparison with the HC group at the same age and in comparison with animals fed both two diets at 34 d ($P<0.001$). The values of *E. Coli* and *Enterobacteriaceae* increased ($P\leq0.008$) from 25 to 34 d of age, whereas that of *Clostridium* spp. decreased. Weaning at 25 d increased mortality from 18 to 34 d compared with rabbits weaned at 34 d (7.02 *vs*. 2.46%, $P=0.017$). However, during the fattening period (34-80 d) rabbits weaned earlier showed lower mortality (7.41 *vs*. 17.6%; $P=0.024$).

Key Words: rabbit, nutritive value, weaning age, caecal fermentation, caecal microbiota population.

INTRODUCTION

The rabbit *caecum* is characterized by the presence of a rich microbial community, which plays an important role in feed digestion through fermentation of non-absorbed nutrients and it is essential for maintaining intestinal health by preventing colonization by pathogenic bacteria strains (Carabaño *et al*.,

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2006). The composition of intestinal flora depends on the age of the animal and may change according to dietary composition, weaning age, and level of intake (Carabaño *et al*., 2006; Gidenne *et al*., 2008).

At weaning, when the kit has to replace milk with solid feed, changes in the microbiota population and the incomplete development of enzymatic and immune systems may cause various digestive disorders (Gidenne, 1997) that can lead to high mortality after weaning. Composition of the diet is very important during the weaning period because of the major predisposition to digestive disorders at this time (Piattoni *et al*., 1999). This is because diet supplies nutrients to the microbiota and influences intestinal motility and rate of passage of digesta (Gidenne *et al*., 2008). In particular, fibre level intake plays a major role in the occurrence of digestive disorder. Several authors report that a low fibre diet is often related to higher retention time and this is associated with intestinal troubles (de Blas *et al*., 1999; Gidenne and Garcia, 2006). Moreover, dietary fibre deficiency (neutral detergent fiber; NDF <30%) decreases the resistance of growing rabbits to intestinal disease, when entheropaty was experimentally induced (Gidenne and Licois, 2005).

Because of the high lipase activity of kits, the use of a high fat diet during the weaning period, as a partial substitution of starch, could be interesting; but there is a lack of information about interactions between the fat level of the diet and caecal microbial activity. Fekete *et al*. (1990) and Fernández *et al*. (1994) found a positive effect of fat addition on fibre digestibility, while Falcao-E-Cunha *et al*. (2004) reported that the nature of fibre plays a greater role than fat inclusion on caecal microbial activity.

On commercial rabbit farms, dams and kits eat solid feed from the same feeder and diet is often inadequate to cover the antagonistic nutritional requirements of lactating females and litters. Early weaning, therefore, could enable better cover for the nutritional requirements of kits, promote their digestive development (Xiccato *et al*., 2003a), and favour the establishment of a balanced caecal ecosystem (Gidenne and Fortun-Lamothe, 2002).

The aim of this study was to investigate the relationship between dietary nutritive value and weaning age on growth performance, caecal characteristics, and the microbiota population of rabbits.

MATERIALS AND METHODS

Housing

The trial was performed at the experimental rabbit farm of the Department of Animal Science using litters from multiparous females of the Martini hybrid strain. At birth the litters were equalised to eight kits without considering the sex of animals. Rabbits were kept under a 16/8 h light/dark cycle in a building with a constant temperature between of 16-20°C. Dams and their kits were bred in the same cages, in which the feed was offered to the litters from 18 d of age onwards using experimental feeders able to separate litter and dam intake. For the entire experimental period, all rabbit does were fed the same commercial diet *ad libitum*.

Diets and growing trial

Between 18 and 34 d of age, 64 litters were assigned at random to one of the two experimental weaning diets: HC, high concentrated and LC, low concentrated diet. The diets were offered *ad libitum* (32 litters/diet). The HC diet contained higher levels of fat and protein, and lower NDF and starch levels than the LC treatment (Table 1). In both diets, starch was maintained low to reduce the risk of digestive disorders and the starch/acid detergent fiber (ADF) ratio was similar to the *optimum* value reported in the literature (Gidenne and Fortun-Lamothe, 2002). Diets were designed to contain the same digestible protein/energy

Table 1: Ingredients and chemical composition of experimental diets.

	High concentrated	Low concentrated
Ingredients (%)		
Dehydrated alfalfa meal, 18%	24.0	32.0
Wheat bran middlings	22.0	-
Wheat bran	-	26.0
Soybean meal, 44% CP	6.00	
Sunflower meal, 28% CP	19.0	14.0
Sugar beet pulp	19.0	14.0
Barley	5.00	10.0
Soybean oil	3.00	-
Cane molasses	-	2.00
Minerals & vitamins	2.00	2.00
Chemical composition (% of dry matter)	-	-
Crude protein (CP)	16.9	15.6
Ether extract	6.19	3.09
Ash	9.52	10.3
Neutral detergent fiber (NDF)	33.3	36.3
Acid detergent fiber (ADF)	18.8	20.9
Acid detergent lignine (ADL)	4.88	5.15
Crude fibre	16.3	18.3
Starch	8.71	11.3
NFC[1]	34.1	34.7
Starch/ADF ratio	0.46	0.54
Lysine[2]	0.89	0.91
Methionine + Cysteine[2]	0.70	0.62
Threonine[2]	0.76	0.68

[1]Non-fibrous carbohydrate=100–(ash+crude protein+ether extract+NDF). [2]Values calculated according to Maertens *et al*. (2002).

ratio consistent with the present recommendations (De Blas and Mateos, 1998; Carabaño *et al*., 2009). The diets contained coccidiostat (Robenidine, 66 ppm/kg) as medication, but no antibiotics.

Half of the rabbits of each group were weaned at 25 or 34 d of age (16 litters/diet and weaning age). Rabbit does of the litters weaned earlier were moved after 25 d of lactation to other cages. To improve animal welfare by increasing social activities, the litter remained together in the same cage until 34 d of age. At 34 d of age, 54 animals per group, representative in terms of average weight, were caged individually for fattening. All animals were fed the LC diet *ad libitum* until 45 d of age. A standard fattening diet without antibiotics (characterized by 18% crude protein, 3.4% ether extract, 18% starch, 37% NDF and 11 MJ digestible energy (DE)/kg dry matter) was then given to the rabbits until slaughter at 80 d of age. During the weaning period, between 18 and 34 d of age, solid feed intake and the weight of litters were controlled every 3 d, while from 34 d of age until slaughter the live weight of each rabbit was recorded every week and feed intake was determined twice weekly. Rabbits were clinically monitored every day and all animals that died during the trial were submitted to necropsy and microbiology examinations to investigate the possible development of pathogenetic intestinal microbiota population.

Faecal digestibility trial

The apparent faecal digestibility of HC and LC diets were determined *in vivo* on another group of 16 rabbits from 56 to 60 d of age (eight rabbits/diet, weaned at 34 d, and with live weight of 1686 g ± 62.4 at 56 d), following the procedure standardized by the European Group of Rabbit Nutrition (Perez *et al*., 1995). From 34 to 48 d, all rabbits were fed a commercial diet. They were then fed the experimental diets for 7 d (adaptation period). To record feed intake and collect faeces, animals were allocated to single metabolic cages and fed *ad libitum* the two experimental diets. Faeces samples, collected during the experimental period for digestibility, were stored at −20°C until chemical analysis.

Caecal trial

At 45 and 80 d of age, 5 rabbits per group, representative in terms of average weight and apparently healthy, were weighed and then slaughtered (by cervical dislocation according to the Report of the American Veterinary Medical Association Panel on Euthanasia) to measure *caecum* weight and caecal content composition. Animals were slaughtered from 2 pm to 6 pm in the afternoon. The pH was measured using a pH-meter equipped with a specific electrode, while 10 g caecal content was diluted with HPO_3 and maintained at −20°C for volatile fatty acid analysis.

Microbial population trial

To study the effect of age and diet composition on the microbiota population, 5 healthy suckling rabbits per diet were sacrificed at 25 and 34 d of age (live weight of 416 g ± 14 and 807 g ± 29, respectively). Qualitative and quantitative microbiological analysis was carried out in accordance with the method employed by Grilli *et al*. (1992, modified). In particular, the following were assessed: the facultative anaerobic bacteria (using tryptic glucose yeast agar medium and hatching at 37°C for 24-48 h); *Enterobacteriacae* and coliforms (on violet red bile glucose medium added with methylumbelliferyl β-D-glucuronide, hatching at 37°C for 24 h); *Escherichia coli* (incubating at 37°C for 24 h on EC-X–gluc agar); and *Staphylococcus aureus* (determined on Baird-Parker agar medium with egg yolk tellurite emulsion and hatching at 37°C for 48 h).

The study on clostridia was made in strictly anaerobic conditions using anaerobic jars (Oxoid, Cambridge, UK) on pasteurized caecal samples at 80°C for 10 min. The samples were cultivated on a tryptose sulphite cycloserine (TSC) agar base supplied by egg yolk emulsion and incubated at 37°C for 24-48 h.

The colony counts of the caecal bacteria were expressed in $\log_{10}$ colony forming units (CFU) related to 1 g of a sample.

Chemical analysis

Diets and faeces were analyzed following the recommendations of the Association of Official Analytical Chemists (AOAC, 2000). Dry matter (DM; 930.15), ash (942.05), crude protein (CP; 984.01), crude fibre (CF; 978.10), ether extract after acid-hydrolysis (EE; 954.02), and starch concentration were measured enzymatically (996.11). NDF, ADF, and acid detergent lignin (ADL) were determined using the F57 filter bag system (ANKOM Technology, New York). In particular, NDF was assessed according to the procedure of Mertens (2002), while ADF and ADL were determined according to Van Soest *et al*. (1991). Gross energy was measured according to EGRAN (2001) using an adiabatic calorimeter (IKA C 4000). Volatile fatty acids were determined according to Osl (1988). Each sample was centrifuged for 10 minutes at 9000 *g* and the concentration of volatile fatty acids (VFA) was determined on the supernatant by using gas chromatography (Varian Chrompack gas-chromatograph Model CP-3800) with a fused silica capillary column (0.25 mm Ø.30 m long). The temperature was programmed from 40 to 200°C and helium was used as the carrier gas at 1.0 mL/min.

Statistical analysis

The litter was considered as the experimental unit from 18 d to weaning, whereas from weaning onwards the experimental unit was the rabbit. Data recorded during the growth performance and caecal trial was analyzed by ANOVA using the general linear model procedure of SAS (Statistical Systems Institute Inc., Cary, NC, USA), including diet composition and age at weaning as main effects in the model. Statistical analysis revealed no significant interaction between diet and age at weaning, and therefore the discussion of the results concerns only these two main factors. Results of the digestibility trial were analysed considering only the diet composition in the model. Data from the microbiota trial was analysed as a factorial structure. The model included diet composition, age of suckling rabbits, and their interaction as the main effects. Mortality data were analysed using the SAS CATMOD procedure.

RESULTS AND DISCUSSION

In the HC diet, the apparent faecal digestibility of DM, CP, NDF, EE and gross energy (Table 2) was higher than in the LC diet (by 7.7, 7.4, 13.1, 26.7, and 10.6 units of percentage, respectively; $P<0.001$). A high level of lipids improved the digestibility of the diet, especially that of fat, as reported by Fernández *et al*. (1994) and Falcao-E-Cunha *et al*. (2000). This improvement, higher than that reported by the above mentioned authors, could be related to a different composition of the diets. In particular, the low digestibility coefficients found for CP and NDF digestibility found in the LC diet were due to protein

Table 2: Feed intake, apparent faecal digestibility and nutritive value of experimental diets in rabbits from 55 to 60 d of age.

		HC[1]	LC[2]	RSD[3]	*P*-value
Number of animals		8	8		
Feed intake	g DM/d	125	137	7.64	0.007
Apparent faecal digestibility, %					
Dry matter (DM)		61.8	57.4	1.15	<0.001
Organic matter		61.8	57.0	1.12	<0.001
Crude protein		72.7	67.7	1.98	<0.001
Neutral detergent fibre		33.7	29.8	1.84	<0.001
Acid detergent fibre		22.7	19.6	3.55	0.092
Crude fibre		24.0	18.3	2.83	<0.001
Ether extract		83.9	66.2	2.62	<0.001
Starch		93.9	93.5	5.60	0.88
Energy		61.5	55.6	1.09	<0.001
Nutritive value					
Digestible Energy (DE)	MJ/kg DM	11.5	10.1	0.20	<0.001
Digestible Protein (DP)	g/kg DM	123	106	3.15	<0.001
DP/DE	g/MJ	10.7	10.5	0.23	0.15

[1] HC: High concentrated diet, [2] LC: Low concentrated diet, [3]RSD: Residual standard deviation.

Table 3: Effect of weaning age and composition of weaning diet on growth performance and mortality during the weaning period (18 to 34 d of age).

			Weaning age (d)			Weaning diet			
			25	34	*P*-value	HC[1]	LC[2]	*P*-value	RSD[3]
Number of litters			16	16		16	16		
Rabbit weight at 18 d		g	290	289	0.89	287	292	0.38	21.2
Daily weight gain	18-25	g/rabbit	18.3	19.1	0.40	19.4	18.0	0.16	3.61
	25-34	g/rabbit	32.2	44.0	<0.001	39.4	36.4	0.015	5.22
	18-34	g/rabbit	26.1	33.1	<0.001	30.7	28.5	0.041	3.95
Daily feed intake	18-25	g/rabbit	3.15	3.13	0.95	3.09	3.19	0.79	1.38
	25-34	g/rabbit	50.0	29.8	<0.001	38.3	41.5	0.093	6.44
	18-34	g/rabbit	29.6	18.6	<0.001	22.9	25.3	0.096	4.25
Solid DE intake	18-25	kJ/d/rabbit	34.2	33.0	0.79	35.5	31.7	0.37	15.1
	25-34	kJ/d/rabbit	538	322	<0.001	440	419	0.31	70.1
	18-34	kJ/d/rabbit	320	199	<0.001	263	255	0.61	47.0
Feed conversion ratio[4]	18-34	g/g	1.05	0.55	<0.001	0.76	0.84	0.061	0.13
Mortality	18-34	%	7.02	2.46	0.017	4.77	4.71	0.97	-

[1] HC: High concentrated diet, [2]LC: Low concentrated diet, [3]RSD: Residual standard deviation, [4]Data determined without accounting milk intake.

origin (soybean and sunflower meals) and different levels of wheat bran, alfalfa meal, and sugar beet pulp in these diets. DE concentration differed between the diets (11.5 and 10.1 MJ/kg DM in HC and LC, respectively; *P*<0.001) mainly due to the inclusion of vegetable fat, but also due to the different chemical composition.

Between 18 and 34 d (Table 3), the feed conversion ratio tended to be lower (*P*=0.061) in animals fed the HC diet. Assuming there is no differences in milk intake, this can be explained because rabbits of the HC group showed a higher weight gain (30.7 *vs*. 28.5 g/d; *P*=0.041) and have a lower mean feed intake value (22.9 *vs*. 25.3 g/d; *P*=0.096, respectively) in comparison with the rabbits given LC treatment. In addition, the HC diet showed a higher digestible nutrient concentration than the LC diet. However, daily weight gain and feed intake of animals were not affected by the weaning diets between 18 and 25 d of age. Instead, between 25 and 34 d of age the daily weight gain was higher in animals fed the HC diet in comparison with those fed the LC diet (39.8 *vs*. 36.4 g/d; *P*=0.015, respectively). In contrast with Gidenne *et al*. (2004a), fat inclusion in the diet could reduce feed intake (*P*=0.096) from 18 to 34 d of age. This means that in our trial, rabbits were able to regulate their feed consumption according to the energetic level of the diet and maintain a constant solid DE intake (on average 259 kJ/d/rabbit).

The mortality rate during the weaning period (Table 3) remained low over the complete period, taking into account that the diets did not contain antibiotic substances. The administration of a specific weaning diet did not affect the health of rabbits from 18 to 34 (4.74 %, on average, Table 3) and from 34 to 80 d of age (12.5 %, on average, Table 4).

During the entire fattening period (34-80 d), in which all rabbits ate the same diet, the experimental diets had no effect on feed intake, daily weight gain, and feed conversion rate (FCR) (Table 4). Consequently, the final body weight of rabbits was similar in both groups (2690 g, on average).

Table 4: Effect of weaning age and composition of weaning diet (fed from 18 to 34 d of age) on growth performance and mortality during the growing period (34 to 80 d of age).

			Weaning age			Weaning diet			
			25	34	*P*-value	HC[1]	LC[2]	*P*-value	RSD[3]
Number of animals			54	54		54	54		
Feed intake (LC diet)	34-45	g/d	110	109	0.75	110	109	0.76	20.4
(Common diet)	45-80	g/d	135	141	0.13	138	138	0.76	15.3
	34-80	g/d	129	133	0.21	131	131	0.90	14.2
Live weight at	34 d	g	729	818	<0.001	790	742	0.28	74.3
	80 d	g	2654	2725	0.018	2704	2675	0.33	201
Weight gain	34-45	g/d	44.6	42.2	0.18	43.1	43.6	0.79	12.2
	45-80	g/d	41.0	41.0	1.00	41.1	41.4	0.27	4.78
	34-80	g/d	41.8	41.4	0.52	41.6	42.0	0.24	4.17
Feed conversion ratio	34-80	g/g	3.09	3.22	<0.001	3.16	3.13	0.18	0.22
Mortality	34-45	%	3.70	2.78	0.70	3.70	2.78	0.70	
	34-80	%	7.41	17.6	0.024	10.2	14.8	0.30	

[1] HC: High concentrated diet, [2] LC: Low concentrated diet, [3]RSD: Residual standard deviation

The type of diet that the rabbits had been eating around weaning did not influence the empty weight of *caecum*, its content, the caecal pH, and total VFA concentration at 45 and 80 d of age (Table 5). Instead, the molar proportion of caecal VFA of those rabbits that had been fed the HC diet around weaning showed a lower concentration of acetic acid (84.2 *vs*. 87.4%; *P*=0.001) accompanied by a higher proportion of butyric acid (12.3 *vs*. 9.46%; *P*<0.001) than those given LC diet. Similar results were found by Falcao-E-Cunha *et al*. (2000) in rabbits fed a diet characterized by a higher fat level. According to Gidenne *et al*. (2004b) the different proportion of VFA found at 45 d of age in rabbits that had been fed the HC diet underlined a change in caecal microbial activity, probably due to the lower level of poorly digested fibre (such as lignin and cellulose) and the higher concentration of fat present in this diet. Anyway, it must be pointed out that at 45 d animals from the LC group had not changed their diet, whereas HC rabbits changed diet at 34 d of age. However, at slaughter, no residual effects were observed from the experimental treatments in total volatile fatty acid concentration and in molar proportion.

Between 25 and 34 d of age (Table 3), the feed and DE intake was higher in rabbits weaned at 25 d (50.0 *vs*. 29.8 g/d and 538 *vs*. 322 kJ/d/rabbit, respectively *P*<0.001) compared to those weaned at 34 d. This is due to compensation for the interruption of milk intake and to cover rabbits' nutritional requirements, as reported by Scapinello *et al*. (1999) and Debray *et al*. (2002). This higher level of intake, however, was insufficient to ensure a similar weight at 34 d of age (Table 4) because the daily weight gain was lower in early weaned rabbits (32.2 *vs*. 44.0 g/d; *P*<0.001). The same results were reported by Xiccato *et al*. (2003a) and Gidenne and Fortun-Lamothe (2004), who registered higher body weight and lower feed consumption in animals weaned later.

On the contrary, after 34 d of age feed intake was similar in both weaning groups (Table 4). At slaughter, the live weight of rabbits weaned at 25 d remained lower than those weaned at 34 d (2654 *vs*. 2725 g; *P*=0.018) because the daily weight gain from 34 to 80 d was not different between both groups. This result is in contrast with some authors who determined a compensatory growth during post-weaning period (De Blas *et al*., 1981; Xiccato *et al*., 2003a; Gidenne and Fortun-Lamothe, 2004), but in accordance with Feugier *et al*. (2005) and Xiccato *et al*. (2003b) who found higher live weight at slaughter as weaning age

Table 5: Effect of weaning age and composition of weaning diet (fed from 18 to 34 d of age) on caecal characteristics of rabbits at 45 (all fed LC diet) and 80 d of age (all fed on standard commercial diet).

		Weaning age (d)			Weaning diet			
		25	34	*P*-value	HC[1]	LC[2]	*P*-value	RSD[3]
Number of animals		10	10		10	10		
Caecal characteristics at 45 d of age								
Empty *caecum* weight	%BW[4]	1.83	1.80	0.73	1.81	1.81	0.98	0.21
Caecal content weight	%BW	6.13	5.48	0.11	5.99	5.61	0.34	0.86
pH		5.53	5.83	0.019	5.63	5.73	0.40	0.26
Total VFA[5]	mmol/L	83.9	79.6	0.58	80.1	83.4	0.68	15.7
Molar proportions of VFA								
Acetic acid	mol/100mol	86.2	85.3	0.25	84.2	87.4	0.001	1.57
Propionic acid (C3)	mol/100mol	3.33	3.30	0.95	3.45	3.18	0.48	0.75
Butyric acid (C4)	mol/100mol	10.4	11.4	0.13	12.3	9.46	<0.001	1.19
C3/C4		0.32	0.30	0.46	0.28	0.34	0.15	0.07
Caecal characteristics at 80 d of age								
Empty *caecum* weight	%BW	1.54	1.55	0.80	1.60	1.48	0.14	0.16
Caecal content weight	%BW	3.92	3.81	0.62	3.66	4.06	0.073	0.61
pH		6.14	6.20	0.25	6.17	6.18	0.84	0.16
Total VFA	mmol/L	62.5	68.6	0.28	62.7	68.4	0.31	13.7
Molar proportions of VFA								
Acetic acid	mol/100mol	86.8	87.5	0.49	88.3	88.7	0.79	3.15
Propionic acid	mol/100mol	5.89	5.05	0.27	5.70	5.30	0.54	1.82
Butyric acid	mol/100mol	7.41	7.55	0.86	5.90	6.00	0.90	1.96
C3/C4		0.79	0.67	0.12	0.97	0.88	0.35	0.32

[1] HC: High concentrated diet, [2] LC: Low concentrated diet, [3]RSD: Residual standard deviation, [4] BW: body weight, [5] VFA: volatile fatty acids.

increased. However, FCR from 34 to 80 d was lower for rabbits weaned at 25 d in comparison with those weaned later, even if the daily weight gain and the feed intake were not statistically different between the two experimental groups.

Between 18 and 34 d of age, the mortality observed at 25 d for weaned animals resulted higher than in rabbits weaned at 34 d (7.02 *vs*. 2.46%; $P=0.017$, respectively). This is similar to that observed by Feugier *et al*. (2005). However, during the fattening period (Table 4) the mortality rate of animals weaned at 25 d was lower than those weaned later (7.41 *vs*. 17.6%; $P=0.024$), and this is in agreement with observations by Garrido *et al*. (2009). Necropsy carried out on dead rabbits showed the mortality was related to a specific event of epizootic rabbit enteropathy characterized by a higher incidence of caecal paresis which occurred mainly in groups weaned later and fed the LC diet (data not shown).

The empty weight of *caecum* and its content in animals slaughtered at 45 and 80 d of age (Table 5) were not affected by the weaning age and these values were similar to those reported by García *et al*. (2000) and Gallois *et al*. (2005) on growing rabbits. In accordance with Xiccato *et al*. (2003a) early weaning stimulated the solid feed intake that determined at 45 d a reduction of caecal pH value (by 5.15%;

Table 6: Effect of age and diet composition on caecal microflora population (log_{10}CFU[1]/g) in suckling rabbits.

	Age		Diet			*P*-value		
	25	34	HC[1]	LC[2]	RSD	Age	Diet	Age×Diet
Number of animals	10	10	10	10				
Facultative anaerobic bacteria	5.62	5.86	5.94	5.54	0.88	0.44	0.19	0.55
E. coli	2.76	4.10	3.53	3.33	1.39	0.008	0.68	0.51
Clostridium spp.	3.73	2.16	3.13	2.76	1.09	<0.001	0.33	0.49
Clostridium perfringens	3.56	2.00	2.37	3.19	0.85	<0.001	0.008	0.008
Enterobacteriacae	3.67	5.08	3.96	4.80	1.42	0.007	0.093	0.44

[1] CFU: colony-forming unitl, [2]HC: High concentrated diet, [3] LC: Low concentrated diet, [4] RSD: Residual standard deviation

P=0.019). This is an indirect symptom of a higher caecal fermentative activity, compared to that found in animals weaned at 34 d. Moreover, lower caecal pH could be related to the lower values of mortality found during the fattening period in animals weaned at 25 d (Piattoni *et al*., 1999; Xiccato *et al*., 2003a). At 45 and 80 d the concentration of total VFA and the values of VFA molar proportion were not influenced by the age of weaning and were in accordance with those reported in the literature (Gidenne *et al*., 2000).

The study on the microbiota population of the *caecum* content took into account the effect of diet composition and the age of suckling rabbits (Table 6). The effect of weaning age on caecal microbiota was not evaluated. The amount of facultative anaerobic bacteria detected in our study was higher than that found by other authors (Gouet and Fonty, 1979; Kovacs *et al*., 2004, 2008). *Enterobacteriacae* counts tended to be lower in the HC group when compared with the LC group (*P*=0.093), with no effect for diet on facultative anaerobic bacteria, *E. Coli* and *Costridium* spp.

The interaction between diet composition and the age of rabbits detected in our study for *Clostridium perfringens* (*P*=0.008) suggest that the use of an LC diet in the first phase of weaning could increase the number of the *Clostridium perfringens* at 25 d (2.74 *vs*. 4.39 log_{10}CFU/g; *P*<0.001, in HC and LC groups, respectively). The counts of *Clostridium perfringens* found at 25 d in the LC group, moreover, resulted higher than that found in animals fed the two diets at 34 d (2.00 and 2.00 log_{10}CFU/g; *P*<0.001, in the LC and HC groups at 34 d). However, these results should be confirmed due to the relatively small number of rabbits used.

The increased values of *E. Coli* (*P*=0.008) at 34 d when compared to 25 d of age were as reported by Bonai *et al*. (2008). In fact, according to Padilha *et al*. (1996), the concentration of coliforms seems to depend more on the age of animals than on dietary factors. Enterobacteria increased from 25 to 34 d of age (3.67 *vs*. 5.08 log_{10} CFU/g; *P*=0.008,) in accordance with Carabaño *et al*. (2006) and Gidenne *et al*. (2007). Its counts reached high values when solid feed intake became significant, even if our trial data found that at 25 d of age it was lower than that reported by Gouet and Fonty (1979) (3.67 *vs*. 7.00 log_{10}CFU/g on average, respectively). The *Clostridium* spp. count showed the opposite trend to that of coliforms, and at 25 d of age the counts were higher (*P*<0.001) than those recorded in animals slaughtered at 34 d. The concentration of *Staphylococcus aureus* resulted undetectable at 25 and 34 d of age, as expected in healthy rabbits during the weaning period.

CONCLUSIONS

In our study weaning at 25 d impaired the live weight of rabbits during the entire experimental period and increased mortality rate up to 34 d. However, the resulting fattening period showed lower mortality rates and this could be related to higher caecal acidity. In the first phase of rearing, the use of a hight nutritive diet resulted in a greater weight gain for animals between 25 and 34 d of age and higher live weights at 45 d.

More studies should be performed to understand the relationship between diet composition and age at weaning, commensal microbiota maturation, and the health of rabbits, taking also into account the ethological and economic aspects.

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