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DIETARY EFFECT OF SHORT-CHAIN ORGANIC ACIDS ON GROWTH PERFORMANCE AND DEVELOPMENT OF INTESTINAL LYMPHOID TISSUES IN FATTENING RESTRICTED RABBITS

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Abstract: This work aimed to test the effect of a dietary inclusion of formic and citric acids on growth performance, mortality, jejunal histology and development of intestinal lymphoid tissues in growing non-medicated rabbits. To this end, a control diet (diet C; 35.9 and 34.7% NDF and 18.9 and 19.1% CP, on DM basis, in the 28-55 d and 56-77 d periods, respectively) was compared with the same diet supplemented with a blend of microencapsulated formic and citric acids (diet A: diet C enriched with 0.4% and 0.2% in the 28-55 d and 56-77 d periods, respectively). Sixty rabbits weaned at 28 d were assigned to each diet. At 56 and 77 d, 5 rabbits per diet and age were slaughtered to assess caecal traits, jejunal histology and follicular development in the caudal ileal Peyer's patch and the appendix. No dietary effects were observed on growth performance in the 28-55 d period. In the 56-77 d period, average daily gain of rabbits fed diet A was greater than that of control rabbits (48.0 *vs.* 43.9 g; P=0.019). Mortality rate was not affected by the diet (11.8% on average). Caecal pH, volatile fatty acids and ammonia concentration, and appendix size were not affected by diet. Caecal pH was lower at 77 than at 56 d (6.02 *vs* 6.19; P=0.016). The concentrations of ammonia (P=0.003) and volatile fatty acids (P<0.001) in the caecal contents increased, respectively, from 9.62 and 70.5 to 14.2 and 81.9 mmol/L when rabbits reached 77 d of age. At 77 d the appendix was heavier (4.30 *vs.* 3.21 g/kg BW; P<0.001), longer (13.3 *vs.* 10.4 cm; P<0.001) and wider (1.74 *vs.* 1.45 cm; P=0.006) than at 56 d. At 56 d, villi of control rabbits were shorter than those of rabbits fed diet A and than those of rabbits aged 77 d, irrespective of the dietary treatment (662 *vs.* 807 µm; P<0.001). In the Peyer's patch, the average follicle area was greater at 77 than at 56 d of age (118 *vs.* 88.4 x 10^3 µm^2; P<0.001) and was also greater in rabbits fed diet C than in those fed diet A (109 *vs.* 97.5 x 10^3 µm^2; P=0.049). In the appendix, no differences in the average follicle area were found at 56 d of age (115×10^3 µm^2), whereas at 77 d the area increase was higher for rabbits fed diet C than for those fed diet A (95.5 *vs.* 50.8%; P<0.001). In conclusion, including formic and citric acids in growing rabbit diets improved weight gain in the 56-77 period, had a trophic effect on the jejunal mucosa at 56 d and controlled the hypertrophy of gut-associated lymphoid tissues.

Key Words: rabbits, growth performance, gut histology, health status, lymphoid tissue, organic acids.

INTRODUCTION

According to Boucher and Leplat (2005) and Rosell *et al.* (2009), digestive diseases are currently the main cause of morbidity in growing rabbits, resulting in dramatic mortality rate increases in fattening rabbitries (Rosell, 2003; Licois, 2004). In recent years, the occurrence of digestive disorders was prevented by the inclusion of antibiotics in the feed. However, the European ban

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on antibiotics as growth promoters together with the price and legal restrictions on the authorized products represent forceful reasons that justify the search for viable alternatives.

Organic acids are among the prospective replacements for antibiotics in rabbit feeding (Falcão-e-Cunha *et al.*, 2007). First chosen as feed preservatives for their antifungal activity (Chichester and Tanner, 1972), organic acids have been regularly used in the feed industry for decades and their numerous benefits are now admitted. Beyond the antifungal property of organic acids, some other benefits such as their antibacterial activity against anaerobic opportunistic pathogens (Cherrington *et al.*, 1990; Skrivanova *et al.*, 2006) and the reduced mortality due to digestive diseases (Hollister *et al.*, 1990) can be cited. Recent investigations have pointed out further benefits of organic acids that would stem from a direct stimulation of gastrointestinal mucosa growth (Dibner and Buttin, 2002; Cardinali *et al.*, 2007). However, the response of gut-associated lymphoid tissue to the inclusion of organic acids in growing rabbit diets is yet to be determined. Since the appendix and Peyer's patches serve a major role in the synthesis of antibodies against intraluminal antigens (Page Faulk *et al.*, 1970; Dasso *et al.*, 2000), the reduction of acid-intolerant bacteria counts by feeding organic acids could help control the incidence of subclinical infections and consequently the hypertrophy of the appendix and the Peyer's patches.

The current work itself is in fact the second part of a two-sided study whose first part (Romero *et al.*, 2010b) was based on a health-challenge study in which the dietary inclusion of formic and citric acids had a similar effect to that of bacitracin in controlling the mortality rate and improving the gut health of rabbits experimentally infected with enteropathogenic *Escherichia coli* O103 and *Clostridium perfringens*. The first part of this two-sided study focused on the post-weaning period (28-55 d) and the present work now centres on evaluating the effect of feeding a non-medicated diet including a blend of formic and citric acids on growth performance, mortality rate, gut histology and follicular development of intestinal lymphoid tissues in the final stage of the fattening period of growing rabbits reared under field conditions.

MATERIALS AND METHODS

Animals, dietary treatments and experimental procedures

A total of 120 New Zealand×Californian mixed-sex rabbits (15 litters) were weaned at 28 d of age and immediately split into 2 homogeneous groups submitted to the following dietary treatments. Sixty rabbits were fed a non-medicated standard diet meeting their nutrient requirements (control diet, diet C). Two control diets were formulated according to the age of the rabbits: post-weaning diet for the 28-55 d period and finishing diet for the 56-77 d period. An experimental diet (acids diet, diet A) consisting of the control diet supplemented with a blend of microencapsulated formic and citric acids (0.4% in the post-weaning period (28-55 d) and 0.2% in the finishing period (56-77 d)) was assigned to another 60 rabbits. All diets were pelleted. The control diet formulation and the average chemical composition of the 2 experimental diets are reported in Table 1.

All rabbits were housed individually in flat-deck cages measuring 600×250×330 mm (with a daily photoperiod of 16 h light starting at 07:30) and the quantity of feed daily allocated to each animal is shown in Figure 1. The feeding programme was adjusted according to previous voluntary feed intake records obtained at the experimental facilities where this study was conducted. Since there was not a control group of rabbits fed *ad libitum* for the whole length of

Table 1: Ingredients of the control diet and average chemical composition of the 2 experimental diets[1] for each period.

Ingredients, %		Nutrients, % DM	
Post-weaning period (28–55 d of age)			
Lucerne hay	39.0	Gross energy, MJ/kg DM	17.9
Soybean meal	13.0	Dry matter	87.9
Barley	9.00	Ash	9.98
Wheat bran	35.0	Crude protein	18.9
Maize oil	1.00	Ether extract	4.16
Calcium carbonate	1.00	Starch	15.7
Sodium chloride	0.50	Sugars[3]	4.60
Monocalcium phosphate	0.50	Neutral detergent fibre[4]	35.9
Mineral and vitamin premix[2]	1.00	Acid detergent fibre[4]	19.8
		Acid detergent lignin	4.78
		Lysine[3]	0.997
		Sulphur amino acids[3]	0.645
		Threonine[3]	0.796
		Digestible energy[5], MJ/kg DM	11.1
Finishing period (56–77 d of age)			
Lucerne hay	35.5	Gross energy, MJ/kg DM	18.7
Soybean meal	13.8	Dry matter	86.6
Barley	5.00	Ash	9.20
Maize	6.20	Crude protein	19.1
Wheat bran	35.0	Ether extract	5.46
Maize oil	2.00	Starch	17.9
Sodium chloride	0.50	Sugars[3]	4.66
Monocalcium phosphate	1.00	Neutral detergent fibre[4]	34.7
Mineral and vitamin premix[2]	1.00	Acid detergent fibre[4]	18.7
		Acid detergent lignin	4.55
		Lysine[3]	1.02
		Sulphur amino acids[3]	0.662
		Threonine[3]	0.797
		Digestible energy[5], MJ/kg DM	11.8

[1] Two experimental diets at each period. Diet C: non-medicated control diet; Diet A: control diet with a blend of formic and citric acids (0.4% in the post-weaning period -wheat bran was included at 34.6%- and 0.2% in the finishing period -wheat bran was included at 34.8%-). [2] Added per kg of diet: vitamin A 11000 IU; vitamin D_3 2000 IU; vitamin B_1 2.5 mg; vitamin B_2 4 mg; vitamin B_6 1.25 mg; vitamin B_{12} 0.01 mg; vitamin E 25 mg; biotin 0.06 mg; vitamin K 2.5 mg; niacin 15 mg; folic acid 0.30 mg; D-pantothenic acid 10 mg; choline 600 mg; Mn 60 mg (MnO); Cu 3 mg ($CuSO_4$); Fe 50 mg ($FeSO_4$); Zn 15 mg (ZnO); I 0.5 mg (KI); Co 0.5 mg ($CoSO_4$); lysine 50 mg; methionine 40 mg. [3] Values calculated from FEDNA (2003). [4] Samples for sequentially analysed NDF were assayed with a heat stable amylase and expressed exclusive of residual ash. [5] Value estimated according to de Blas *et al.* (1992): digestibility of gross energy = 0.814±0.0293 (P<0.001)–(0.0012±0.0001)×ADF (P<0.001)+(0.00024±0.00011)×CP (P=0.040); R^2=0.879; rsd=0.020.

the current study, feed distribution was limited on the basis on those records and was intended to be approximately 80% of the average voluntary intake of growing rabbits from 28 to 56 d of age. Thereafter, rabbits were no longer restricted, although they were still given a pre-weighed quantity of feed (at 08:00). Water was supplied *ad libitum* and no medication was provided in it.

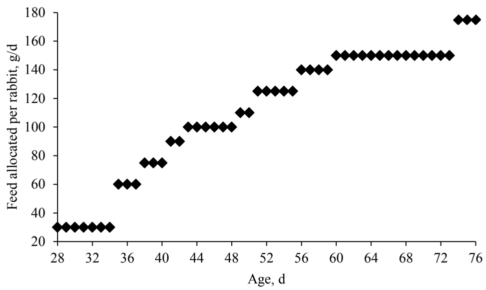

Figure 1: Feeding programme for all groups during the whole study.

Rabbits were weighed at weaning and then weekly until the end of the study (77 d of age). Mortality was recorded daily throughout the whole fattening period (28 to 77 d). On days 56 and 77 of age, 5 rabbits per diet were slaughtered (at 19:00 at both ages) by intravenous overdose of Tanax (Hoechst, Frankfurt, Germany). Rabbits slaughtered at 56 d of age still had feed available at 19:00. At both ages, from all 20 animals, the volatile fatty acid (VFA) concentration, pH, ammonia (Verdouw *et al.*, 1978) and dry matter (DM) were evaluated on the supernatant of caecal contents. Caecal concentration of VFA was determined with a CP-3800 Varian gas chromatograph unit following the procedures described by Xiccato *et al.* (2003). Furthermore, the appendix was excised and its weight, length and diameter were measured. Moreover, the caudal ileal Peyer's patch was removed and a 6 cm sample not bearing Peyer's patches was excised from the middle part of the jejunum to determine mucosal histology. The appendix, Peyer's patch and jejunal sample were placed in a 10% buffered neutral formaldehyde solution (pH 7.2).

The animals were cared for according to the International Guiding Principles for Biomedical Research Involving Animals (Council of International Organisation of Medical Science, 1986). The current experimental protocol was planned according to guidelines from the animal committee of the University of Perugia.

Histological methods

All samples were gradually dehydrated with increasing concentrations of ethanol (50 to 100%). These dehydrated samples were then embedded in paraffin, sectioned (6 µm) and stained with haematoxylin and eosin (Armed Forces Institute of Pathology, 1968).

For each rabbit, 5 cross sections of the jejunal sample, a medial cross section of the caudal ileal Peyer's patch and 3 cross sections of the appendix (proximal, medial and distal) were viewed at 40× magnification under a light microscope (Olympus BX40, Olympus Optical Co., 20097, Hamburg, Germany). Images were digitally captured for later assessment of their morphologies using computer-assisted image analysis (The ImageJ v 1.26. Wayne Rasband, National Institutes of Health, Bethesda, MD 20892, USA). For each rabbit, villus heights were measured in the jejunal sample (30 vertically oriented villi per animal) according to the procedure described by Hampson (1986). The area of 4 complete lymphoid follicles and the area of the whole follicular region were measured in the caudal ileal Peyer's patch of each animal. In the appendix cross sections, all follicles were counted and the area of ten complete follicles was determined. A follicle with entire dome and germinal centre was considered complete.

Analytical methods

Chemical analyses of each experimental diet were conducted in triplicate. AOAC procedures (2000) were used to determine DM by the oven-drying method (930.15), total ash by muffle furnace (942.05), Dumas N (968.06) using a LECO equipment (model FP-528, Leco Corporation, St. Joseph, MI, USA) and starch (996.11). NDF content was determined using the Mertens method (2002), whereas the ADF and ADL analyses were done according to the official AOAC method (973.18) (2000). All fibre analyses were done sequentially without sodium sulphite and corrected by ash content of ADL residue. Both NDF and ADF were determined using a filter bag system (Ankom Technology, New York, NY). Ether extract was analysed by Soxhlet fat analysis (method 4.B) after 3 N HCl acid hydrolysis (Spanish Royal Decree 2257/1994; Spanish Government, 1995). Gross energy was determined using an isoperibol bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL, USA) and digestible energy was estimated according to de Blas *et al.* (1992).

Statistical analysis

All data were analysed for normal distribution using the UNIVARIATE procedure and for homogeneity of variances through the Levene's Test using the SAS GLM procedure HOVTEST option (1990). Growth performance data were analysed as a completely randomised design with the diet as the main source of variation by using the SAS GLM procedure (1990). Litter was used as a block effect and weaning weight as a linear covariate. The experimental unit was the rabbit. Caecal and mucosa morphology traits were also analysed as a completely randomised design with the GLM procedure of SAS but in these cases, the diet, the age and their interaction were the explanatory variables.

Fattening mortality results were analysed using generalised linear models (McCullagh and Nelder, 1989) with the GENMOD procedure of SAS (1990). A binomial distribution was used for mortality data and the link function was the logit transformation, $\ln [\mu/(1-\mu)]$, where μ was the mean value. The dietary effect was included as an explanatory variable. All data are presented as least-squares means. All the means were compared using a protected t-test, and differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The dietary inclusion of formic and citric acids did not affect growth performance in the post-weaning period (28-55 d of age; Table 2), but in the finishing period (56-77 d), average daily gain of rabbits fed diet A was greater than that of control rabbits (48.0 *vs.* 43.9 g; P=0.019). Moreover, when considering the whole fattening period (28-77 d of age), feeding diet A also led to higher but not significant values for average daily gain of rabbits (33.7 *vs.* 32.1 g; P=0.065). Increases in body weight gain resulting from the addition of short-chain organic acids (formic, sorbic, tartaric and lactic acids) have been reported in broilers (Vogt *et al.*, 1982; Versteegh and Jongbloed, 1999) and swine (Galfi and Bokori, 1990; Partanen, 2001). However, it should be noted that the effects of organic acids on animal performance often lack consistency and reproducibility (Dibner and Buttin, 2002). Thus, studies failing to report benefits of organic acids in animal performance are also numerous (Falcão-e-Cunha *et al.*, 2007).

Mortality rate was not affected by the diet in any of the periods studied (11.8% on average; Table 2). Most animals (>93%) died of digestive diseases displaying symptoms such as low body weight, bloated abdomen and diarrhoea. The mean value of global mortality remained at a moderate level in this study, especially taking into account that rabbits were not medicated. This low average mortality percentage in the whole fattening period could be a consequence of the feed restriction, as some authors (Boisot *et al.*, 2003; Gidenne *et al.*, 2009) found that the health status of restricted rabbits was improved compared with rabbits fed *ad libitum*. In fact, Romero

Table 2: Effect of the dietary inclusion of organic acids on growth performance.

	Diets[1]			
	C	A	SEM[2]	*P*-value
Post-weaning period (28-55 d of age)				
ADG[3], g	23.3	23.0	0.47	0.607
FCR[3]	3.54	3.59	0.097	0.716
Live weight at 56 d, g	1299	1265	15.9	0.138
Mortality rate[4], %	3.6 (2/55)	9.1 (5/55)	-	0.234
Finishing period (56-77 d of age)				
ADG[3], g	43.9	48.0	1.17	0.019
FCR[3]	3.52	3.37	0.13	0.427
Live weight at 77 d, g	2215	2270	28.9	0.201
Mortality rate[4], %	5.4 (3/55)	5.4 (3/55)	-	1.000
Whole fattening period (28-77 d of age)				
ADG[3], g	32.1	33.7	0.54	0.065
FCR[3]	3.48	3.36	0.070	0.276
Mortality rate[4], %	9.1 (5/55)	14.5 (8/55)	-	0.374

[1] Two experimental diets in each period: Diet C: non-medicated control diet; Diet A: control diet with a blend of formic and citric acids [0.4% in the post-weaning period (wheat bran was included at 34.6%) and 0.2% in the finishing period (wheat bran was included at 34.8%)].[2] SEM: Standard error of the mean, n=60 per diet in 28-55 d period and n=55 in 56-77 and 28-77 d period (as at 56 d, 5 healthy rabbits of each dietary group were slaughtered). n=55 per diet for mortality. [3] ADG: Average daily gain. FCR: Feed conversion rate. [4] In brackets: No. of dead/No. of animals.

et al. (2010a) reduced the average mortality rate of restricted rabbits down to 6.3% by applying a 20% feed restriction.

Previous studies (Hollister *et al.*, 1990; Romero *et al.*, 2010b) reported a sharp reduction in the mortality rate due to diarrhoeal syndromes when rabbits were fed diets including short-chain organic acids, whilst in the present work the dietary inclusion of formic and citric acids had no effect on mortality. However, it should be noted that in these studies the mortality rate of rabbits fed the control diet was much higher than that of the control rabbits in the present work (38.9 *vs.* 9.1%, respectively). In fact, it was observed that feeding organic acids is an efficient strategy, drastically reducing the mortality percentage in a context of high incidence of digestive diseases or after an experimental infection with harmful pathogenic bacteria (Cardinali *et al.*, 2007), but it may be of no help when sanitary conditions of the farm are adequate. Dibner and Buttin (2002) pointed out that the action of short-chain organic acids is dependent, among other factors, on the environment in which the study is conducted. Thus, the dietary use of organic acids seems much more pertinent in places where good hygienic and sanitary conditions are not guaranteed.

As shown in Table 3, the caecal concentration of VFA increased from 56 to 77 d (70.5 *vs.* 81.9 mmol/L; $P<0.001$) but was not affected by the diet. The proportions of acetic and propionic acids were greater at 77 than at 56 d (78.8 *vs.* 75.8%; $P<0.001$, and 7.73 *vs.* 6.81%; $P=0.001$, respectively) whereas the proportion of butyric acid was lower (8.85 *vs.* 12.0%; $P<0.001$). Neither were caecal acidity or ammonia concentration influenced by the diet, although they were lower at 56 than at 77 d (6.19 *vs.* 6.02; $P=0.016$, and 9.62 *vs.* 14.2 mmol/L; $P=0.003$). The decrease in caecal pH and the increase in caecal VFA concentration are fully in keeping with the results found by Gidenne and Fortun-Lamothe (2002). These authors reported that caecal pH continuously decreases from the second to the seventh week of age of growing rabbits, while the caecal concentration of VFA increases steadily. It must be pointed out that these results might be

Table 3: Effect of the dietary inclusion of organic acids and age on caecal traits.

Diets[1]	C		A			*P*-value		
Age, d	56	77	56	77	SEM[2]	Diet	Age	Diet×Age
Caecal VFA[3] concentration, mmol/L	69.5	81.0	71.4	82.8	2.54	0.463	<0.001	0.984
Acetic acid, %	75.8	78.4	75.8	79.2	0.49	0.447	<0.001	0.424
Propionic acid, %	6.64	7.66	6.98	7.80	0.24	0.329	0.001	0.681
Butyric acid, %	12.4	8.96	11.6	8.74	0.30	0.101	<0.001	0.330
Valeric acid, %	4.76	4.64	4.60	4.36	0.19	0.272	0.370	0.760
Caecal pH	6.19	6.00	6.19	6.05	0.061	0.676	0.016	0.629
Caecal ammonia concentration, mmol/L	8.84	14.7	10.4	13.7	1.321	0.858	0.003	0.327
Dry matter of the caecal contents, %	18.5	21.3	20.9	22.4	0.822	0.045	0.017	0.479
Appendix								
Weight, g	4.36	9.84	4.25	9.65	0.392	0.704	<0.001	0.924
Weight, g/kg BW	3.22	4.39	3.20	4.21	0.214	0.644	<0.001	0.711
Length, cm	10.5	12.7	10.4	13.90	0.443	0.240	<0.001	0.180
Diameter, cm	1.42	1.74	1.48	1.74	0.091	0.744	0.006	0.744

[1] Two experimental diets at each period. Diet C: non-medicated control diet; Diet A: control diet with a blend of formic and citric acids [0.4% in the post-weaning period (wheat bran was included at 34.6%) and 0.2% in the finishing period (wheat bran was included at 34.8%)].[2] SEM: Standard error of the mean n=5 per diet and age. [3] Volatile fatty acids.

affected not only by age but also by the feeding regime (restricted until 55 d and then *ad libitum*), although according to the results from Gidenne and Feugier (2009) the influence of feeding regime might be lesser than that of age. The DM percentage of the caecal contents was greater at 77 than at 56 d (21.8 *vs.* 19.7%; P=0.017). In addition, feeding diet A to the rabbits led to a higher DM percentage than that of rabbits fed diet C (21.6 *vs.* 19.9%; P=0.045). Even if more liquid caecal contents are often associated to morbid animals (Dewrée *et al.*, 2007; Romero *et al.*, 2009), differences obtained in this work for the DM content did not bear any relation to mortality rate and so they do not reflect the health status of animals. Moreover, it should be highlighted that the values of the present work could perfectly correspond to healthy growing rabbits (Carabaño and Piquer, 1998).

No differences in the dimensions of the appendix due to dietary effect were reported (Table 3). Nevertheless, the appendix was heavier (4.30 *vs.* 3.21 g/kg BW; P<0.001), longer (13.3 *vs.* 10.4 cm; P<0.001) and wider (1.74 *vs.* 1.45 cm; P=0.006) at 77 than at 56 d. In this scope, Dasso *et al.* (2000) carried out a study of the growth of the rabbit appendix and stated that most lymphoid tissue development took place during the first 12 wk of life.

The interaction between diet and age was revealed to be significant (P<0.001) for the villus height (Table 4). At 56 d of age, villi of control rabbits measured 662 µm whereas those of rabbits fed diet A were 817 µm high. Moreover, the latter height was not different from the average villus height obtained at 77 d for both dietary treatments (802 µm). These differences observed at 56 d show that, at younger stages, the dietary inclusion of short-chain organic acids has a positive effect on the jejunal mucosa morphology. This deduction seems to be confirmed by the results of Romero *et al.* (2010b) who found at the same age (56 d) that jejunal villi of rabbits receiving a diet enriched with formic and citric acids were much longer than measures taken in control rabbits (595 *vs.* 790 µm; P<0.001). Dibner and Buttin (2002) pointed out that one of the benefits of the organic acids is the direct stimulation of gastrointestinal cell proliferation. Indeed, Frankel *et al.* (1994) observed that infusions of short-chain organic acids increased jejunal villus height in rats, suggesting thereby that these acids might exert a trophic effect on the small intestine mucosa. Moreover, the results of Cardinali *et al.* (2007) confirmed that a mixture of

Table 4: Effect of the dietary inclusion of organic acids and age on jejunal histology and development of intestinal lymphoid tissues.

Diets[1]	C		A			*P*-value		
Age, d	56	77	56	77	SEM[2]	Diet	Age	Diet×Age
Villus height, µm	662[a]	806[b]	817[b]	798[b]	28.0	< 0.001	<0.001	<0.001
Caudal ileal Peyer's patch								
Follicle area, ×10³ µm²	95.9	122	81.0	114	10.7	0.049	<0.001	0.573
Whole patch area, ×10³ µm²	1468	1620	1798	1376	322	0.904	0.707	0.428
Appendix								
Follicle area, ×10³ µm²	112[a]	219[c]	118[a]	178[b]	17.5	0.002	<0.001	<0.001
Number of follicles	39.8	38.0	38.8	40.8	1.44	0.541	0.945	0.206

[1] Two experimental diets at each period. Diet C: non-medicated control diet; Diet A: control diet with a blend of formic and citric acids [0.4% in the post-weaning period (wheat bran was included at 34.6%) and 0.2% in the finishing period (wheat bran was included at 34.8%)]. [2] SEM: Standard error of the mean, n=5 per diet and age. [a,b,c] Within a row, means without a common superscript differ ($P < 0.05$).

microencapsulated formic and citric acids can lengthen villi in young weaned rabbits. In a similar way to antibiotics, organic acids have an antimicrobial activity against enteropathogenic bacteria that damage jejunal mucosa morphology (Cherrington *et al.*, 1990; Skrivanova *et al.*, 2006). The trophic effect of short-chain organic acids on jejunal villi may benefit the young rabbit by increasing the epithelial cellular mass and thereby enlarging the absorptive surface area (Sakata, 1987). The transmission of the stimuli of the acids to the mucosa of the digestive tract via a systemic mediatory mechanism (Blottière *et al.*, 2003) and the involvement of the autonomic nervous system (Sakata, 1987), the stimulation of the mucosal blood flow (Dobson, 1984) and the use of organic acids as an energetic source by epithelial cells (Roediger, 1980) are some possible explanations for this enhancement of intestinal villi length.

In the caudal ileal Peyer's patch, the average follicle area was larger at 77 than at 56 d of age (118 *vs.* 88.4 ×10³ µm²; $P<0.001$) and was greater in rabbits fed diet C than in those fed diet A (109 *vs.* 97.5 ×10³ µm²; $P=0.049$). On the contrary, the area of the whole patch was not influenced either by the diet or the age, being on average 1565×10³ µm². In the present work, the average follicle area in the caudal ileal Peyer's patch at 56 d was 18.4% greater in rabbits fed diet C than in those fed diet A, whereas in the work of Romero *et al.* (2010b) these differences reached 49.0% at the same age. The greater hypertrophy of a lymphoid structure in the latter study in which rabbits were experimentally infected with *E. coli* and *C. perfringens* suggests that the follicle area is more influenced by antigenic stimuli than aging. As gut-associated lymphoid tissues, the development of Peyer's patches in young rabbits is dependent upon antigenic stimulation (Dasso and Howell, 1997), so the greater areas found in the previous study (Romero *et al.*, 2010b) may reflect an immune response. In the current study, this response was especially decreased in rabbits fed diet A, probably because the ileal counts of pathogenic and opportunistic bacteria had been reduced by the antimicrobial activity of organic acids (Skrivanova *et al.*, 2006). Moreover, Skrivanova and Marounek (2007) specified that citric acid was able to reduce *in vitro* the counts of viable enteropathogenic *E. coli* cells in a pH range very close to usual pH values in the small intestine.

In the appendix, an interaction between the diet and the age was detected (*P*<0.001) on the average follicle area. Thus, no differences were found between dietary treatments at 56 d of age (115×10^3 μm^2) whereas, at 77 d, the area increase of rabbits fed diet C was higher than that of rabbits fed diet A (95.5 *vs.* 50.8%). Since the appendix is the largest lymphoid organ in rabbits and makes an important contribution to the antibody-forming potential of the gut-associated lymphoid tissue (Dasso *et al.*, 2000), this increase in area is also probably related to an antigenic stimulation. In fact, Dasso and Howell (1997) showed that the appendix was greatly responsible for the levels of IgA, IgM and IgG found in the serum. Finally, the number of follicles in the appendix remained unaffected by the treatments being on average 39.3. As reported by Romero *et al.* (2010b), the number of follicles in the appendix was not affected by the diet or the age. Therefore, it could be discussed whether this trait is genetically determined.

CONCLUSIONS

On balance, the results of the present work bring out that the inclusion of formic and citric acids in growing rabbit diets improved weight gain in the 56-77 period, had a positive effect on the jejunal mucosa at 56 d and controlled the hypertrophy of gut-associated lymphoid tissues.

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