EFFECT OF DIETARY ACIDIFICATION ON GROWTH PERFORMANCE AND CAECAL CHARACTERISTICS IN RABBITS

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ABSTRACT

The present work aimed to evaluate whether the addition of organic acids to feed could improve growth performance and caecal characteristics, and modify the equilibrium of caecal microbiota population in growing rabbits. For this purpose, 216 rabbits, weaned at 30 d of age, were divided into four experimental groups. Control group (C) was given the basal diet without any additive, while Antibiotic group (A) rabbits were fed the same diet with antibiotics until 55 d of age. The other two experimental groups were T1 (containing a commercial organic acid product characterized by a blend of formic acid, lactic acid and essential oil from rosemary, thyme and cinnamon) and T2 (included only formic and lactic acid). At 30, 55 and 84 d of age the caecum weight, caecal content characteristics in terms of pH, ammonia and volatile fatty acids (VFA), and caecal microbiota composition were determined in 10 rabbits per group. Between 55 and 84 d of age the daily weight gain of T1 and T2 groups were higher (34.0 and 34.5 g/d; $P<0.05$) than those found in C and A groups (31.2 and 31.2 g/d); in the same period, animals fed T1 and T2 diets showed also a significantly lower feed conversion rate ($P<0.01$). Before 55 days, T2 group presented significantly lower feed conversion ratio than those of the other groups, suggesting a positive effect of acidification during the weaning period, even if this effect was not significant in animals fed the diet T1. Rabbits given T2 diet had a significantly higher VFA concentration and a lower pH in the caecum in comparison with the other experimental groups, probably due to a higher fermentative activity; also VFA molar proportions were significantly affected by the treatments, with a lower acetate concentration in T2 and A groups, perhaps associated with changes occurred in composition of commensal intestinal microflora, not determined in our study. The data of microbial analysis at 55 days of age showed significantly lower aerobic and facultative anaerobic bacteria counts of C group, while the other bacteriological traits were similar among treatments. The mortality of animals fed the diet supplemented with essential oils (T1) was significantly lower than that of rabbits given T2 diet, including only formic and lactic acid, but not different from that of C and A treatments. In conclusion, in our trial formic acid, lactic acid and essential oils inclusion to the diet stimulated weight gain and increased feed conversion rate in the second phase of fattening, without reducing mortality when enteric disease occurred.

Key words: Dietary acidification, Formic acid, Lactic acid, Growth performance, Caecal characteristics, Microflora, Rabbit.

INTRODUCTION

Rabbits are sensitive to multifactorial digestive disorders, which can cause high mortality and morbidity rate, and seem to be related to microbial dysbiosis in the caecum. During the first growing period, in fact, changes in feeding behaviour and in housing conditions together with an incomplete maturation of the digestive immune system could promote the development of potentially pathogenic microflora, which could cause digestive troubles and lower zootechnical performance. In intensive rabbit farms, antibiotics are often added to feed or water from weaning till 8 weeks of age, in order to prevent enteric diseases and reduce high economic losses. Criticism on the antibiotics use in animal nutrition (antibiotic resistance and interest in human health and animal welfare) has stimulated the study of non-antibiotic substances, which might have similar effects on the intestinal rabbit health.

Among the approaches proposed to this purpose, the use of organic acids appears interesting, even if scientific data concerning their effect on microflora population, mucosal immunity and growth performance are few and often contradictory in rabbits (Falcao *et al.*, 2007). Also the mode of action of these products on caecal microflora is not completely understood, although it is demonstrated that organic acids play a direct action on the bacterial cell integrity (Maertens *et al.*, 2006). In pigs and poultry, several authors found a beneficial effect on health status and growth performance when using organic acids (Partanen and Mroz, 1999; Dibner and Buttin, 2002) In rabbits, Skřivanová and Marounek (2002) reported a lower post-weaning mortality without affecting performance traits when using caprylic acid, while Carraro *et al*. (2005) and Scapinello *et al*. (2001) did not reach the same results testing sodium butyrate and fumaric acid, respectively. Also plant extracts have been proposed among alternatives of antibiotics in animal production. Although in rabbits there are no data available in literature, Losa and Kohler (2001) reported a remarkable reduction of *Clostridium perfringens* in the intestine of chickens fed a diet supplemented with a commercial preparation of essential oils.

The aim of this study was to investigate the effect of formic acid, lactic acid and essential oils inclusion to the diet on growth performance, health status, caecal characteristics and microflora population in growing rabbits.

MATERIALS AND METHODS

Animals and experimental design

At weaning (30 days of age) 216 rabbits were allocated to individual commercial cages and were randomly assigned to four treatment groups characterized by the same pelleted basal diet (prepared from the same batch of raw materials and containing 66 ppm of the anticoccidial Robenidine) and by different commercial additives. In particular, the animals of the Control Group (C) received the basal diet without any additive, whereas the rabbits of the Antibiotic Group (A) were fed until 55 days of age the same diet with antibiotics (colistin 180 ppm, avilamycin 60 ppm and tiamulin 40 ppm). The other two experimental groups (T1 and T2) received the basal diet supplemented with a commercial organic acid product: treatment T1 contained a blend of formic acid, lactic acid and essential oil originating from rosemary, thyme and cinnamon (4 g/kg), while T2 contained only formic and lactic acid (5 g/kg).

Body weight of each rabbit was recorded every week from weaning to the slaughter (84 days of age), while feed consumption was determined twice a week during the experimental period. At 30, 55 and 84 days of age, 10 rabbits per group, representative in terms of average weight and in good health, were slaughtered to measure caecum weight, caecal content composition in terms of pH, ammonia and volatile fatty acids (VFA), and to analyse the caecal microbiota population.

Chemical and bacteriological analyses

Chemical analyses of diets were performed according to AOAC standard methods (AOAC, 2000); neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined according to the sequential procedure of Van Soest *et al*. (1991). In the caecal content of sacrificed animals, VFA were measured by gas chromatography according to Osl (1988), and ammonia was analysed using an autoevaluation distillation unit.

Quantitative and qualitative bacteriological analyses were performed on the same caecal content to evaluate the major bacterial species known in rabbit. In particular, aerobic and facultative anaerobic bacteria counts on Tryptic Glucose Yeast agar (PCA), *Enterobacteriaceae* and coliforms on Violet Red Bile Glucose agar (VRBG), *Escherichia coli* on Chromogenic Coliform Agar (CCA), *Lactobacillus* spp. on De Man-Rogosa-Sharpe agar (MRS), *Clostridium* spp., *Bacteroides* spp. and *Bacillus* spp. on PEA agar were determined according to Grilli *et al*. (1992, modified).

RESULTS AND DISCUSSION

The chemical composition of the basal diet (CP: 18.9%; EE: 4.40%; NDF: 36.3%; ADF: 23.9%; ADL: 5.25%; starch: 11.5%), as expected, was similar between all experimental groups because the diets were prepared from the same batch of raw materials.

The data reported in Table 1 show that the inclusion of organic acids did not affect the body weight and the daily feed intake of the animals during the whole experimental period. Between 30 to 55 days of age, the daily weight gain was very high and not statistically different in all experimental groups. In the second fattening period (56 to 84 days of age), instead, the daily weight gains of the T1 and the T2 groups supplemented with organic acids were significantly higher (P<0.05) than those found in C and A groups, and the feed conversion rates (FCR), calculated on live animals, were lower in animals fed T1 and T2 treatments. These results are in accordance with those reported by Partanen and Mroz (1999) and Mroz (2005) in growing pigs given a diet supplemented with formic acid.

Table 1: Growth performance and mortality rate from weaning to 84 days of age

			C	A	T1	T2	SE
Number of observations			54	54	54	54	
Live weight at	30^{th} day	(g)	832	831	846	831	8.21
	55^{th} day	(g)	2078	2097	2073	2099	30.4
	84^{th} day	(g)	2952	2972	3025	3074	45.5
Daily weight gain	30^{th}-55^{th}	(g/d)	48.1	48.7	47.4	48.6	1.04
	56^{th}-84^{th}	(g/d)	31.2^{b}	31.2^{b}	34.0^{a}	34.5^{a}	1.10
Daily feed intake	30^{th}-55^{th}	(g/d)	137	139	134	133	2.10
	56^{th}-84^{th}	(g/d)	170	164	168	168	3.07
Feed conversion rate	30^{th}-55^{th}		2.89^{a}	2.85^{a}	2.82^{a}	2.72^{b}	0.04
	56^{th}-84^{th}		5.55^{A}	5.38^{A}	5.05^{B}	4.92^{B}	0.13
Mortality	30^{th}-84^{th}	(%)	13.0	3.70^{B}	7.55^{B}	22.2^{A}	4.34

Values with different letters on the same row differ significantly (^{A,B}: P<0.01; ^{a,b}: P<0.05)

The good value of FCR found in T2 group before 55 days of age suggested a positive effect of acidification during the weaning period, even if a high mortality rate has been registered in this group as consequence of an acute enteric syndrome, happened even in the Control group. The inclusion of essential oils in the group T1 resulted in a lower mortality in comparison with the group T2 (7.55 vs. 22.2% ; P<0.05, respectively). This effect is difficult to explain, because the information about these extracts is limited, even if the mechanism of action seems to be related to the antibacterial activity of these products.

The caecal characteristics and the microbial population observed in the rabbits slaughtered at 55 days of age are reported in Table 2. The values of caecal pH and total volatile fatty acids were significantly influenced by the treatment; in particular, animals fed diet T2 showed VFA concentration significantly higher than those fed the other diets. The increase in total VFA and the consequent decrease in caecal pH suggest that fermentation activity was higher in the T2 group. Also VFA molar proportions were statistically affected by the treatments, with lower acetate concentration in T2 and A groups, and without the change of the C_3/C_4 ratio. The different level of each VFA could be related to changes occurred in composition of commensal intestinal microflora, not determined in our microbiological analysis.

Table 2: Caecal characteristics and microflora population at 55 days of age

		C	A	T1	T2	SE
Empty caecum weight	(% BW)	1.62 ^{a}	1.43 ^{b}	1.55	1.49 ^{b}	0.04
Caecal content weight	(% BW)	5.05	4.68	5.27	4.99	0.17
pH		6.03 ^{a}	6.06 ^{a}	6.01 ^{a}	5.82 ^{b}	0.05
Total VFA	(mmol/l)	61.2 ^{b}	66.6 ^{b}	60.2 ^{b}	73.4 ^{a}	2.78
Acetic acid	(mol/100 mol VFA)	90.1 ^{a}	87.8 ^{b}	89.8 ^{a}	87.3 ^{b}	0.84
Propionic acid	(mol/100 mol VFA)	0.58	1.33	0.99	1.93	0.43
Butyric acid	(mol/100 mol VFA)	8.93	10.3	8.85	10.3	0.69
Valeric acid	(mol/100 mol VFA)	0.43	0.57	0.38	0.49	0.09
C_3/C_4		0.06	0.13	0.11	0.20	0.05
NH_3	(mmol/l)	12.1	12.4	11.0	12.3	1.52
Aerobic and facultative anaerobic bacteria	(log UFC/g)	5.02 ^{BB}	6.47 ^{AA}	6.76 ^{AA}	6.80 ^{AA}	0.26
E. coli	(log UFC/g)	3.34	3.62	5.04	6.50	1.19
Clostridium spp.	(log UFC/g)	5.22	3.98	4.70	3.04	0.86
Bacteroides spp.	(log UFC/g)	3.45	4.25	4.66	4.95	1.24
Lactobacillus spp.	(log UFC/g)	2.58	2.75	1.42	3.29	0.82
Bacillus spp.	(log UFC/g)	5.89	5.61	6.34	6.54	0.74

Values with different letters on the same row differ significantly (^{AA,BB}: $P<0.001$; ^{a,b}: $P<0.05$)

At 55 days of age, data concerning bacteriological analysis showed high variability which obscured differences among the experimental groups; only the total aerobic/facultative anaerobic bacteria count was statistically lower in the group C than in all the other groups. *Bacteroides* spp. population was not significantly improved by the acidifiers used in our study and, according to data determined *in vitro* by Skřivanová and Marounek (2007), formic acid added to T1 and T2 treatments did not prevent coliform and *E. coli* growth. Concerning *Clostridium* spp., the concentration was lower in T2 (3.04 log CFU/g caecal content) than in the other groups (5.22, 3.98 and 4.70 log CFU/g caecal content for group C, A and T1, respectively), although this difference failed to be significant. In the literature, data on caecal microflora of rabbit are scarce (Gouet and Fonty, 1979; Comi and Cantoni, 1984; Bennegadi *et al*., 2003). In our study the total number of aerobically cultivable bacteria was higher than that found by Gouet and Fonty (1979), but lower than that reported by Comi and Cantoni (1984). Among the anaerobic microflora, counts of *Bacteroides* spp. were very low and *Clostridium* spp. was higher except in T2 group. *Bacillus* spp. was the prevalent spore-forming strain in all considered groups.

CONCLUSIONS

In our study formic acid, lactic acid and essential oils inclusion to the diet stimulated weight gain, increasing also feed conversion rate in the second phase of fattening, without reducing mortality when enteric disease occurred. The high variability of caecal microflora found in this trial and the effect of acidification on the caecal characteristics in terms of pH and total VFA suggest further studies to explain the mode of action of these products and the relationships between health status, caecal characteristics and microbiota population of the rabbit intestine.

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