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PAPER

Effect of corn particle size and inclusion of organic acid in the diet on growth performance and gastrointestinal structure in young chicks

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Abstract

The effect of 3 corn particle sizes (d_{gw}: 375, 1117, and 2402 μm) combined with or without organic acids (0.3 g/kg of Galliacid S®) was investigated on broilers from day 1 to day 21; 540 1-day old Ross 708 males were raised in 36 pens (3x2 factorial design, 6 blocks each). We measured: body weight, feed intake, feed conversion ratio, liver weight, pH weight and height of empty gizzard, pH and length of intestine and caeca, height and width of ileal villi, crypt depth/gland diameter, total bacteria count. Different corn particle sizes and organic acid supplements only affected feed intake (days 14 and 21), feed conversion ratio (day 14), villus height, and crypt depth. On day 21, fine milling had negative effects on body weights compared with larger feed particle size (816 *vs* 848 and 844 g); acidic additive had a positive effect on broiler growth (859 *vs* 813 g). Length of small intestinal villi and crypt depth were affected by both particle size and organic acids (fine to coarse small intestinal villi: 1869[a], 1401[c], and 1039[d] μm in non-acidified; 1708[b], 1535[c], and 942[e] μm in acidified. Fine to coarse crypt depth: 102[ab], 98[b]; 65[c] μm in non-acidified; 106[a], 70[c], and 66[c] μm in acidified). No difference was observed in total bacteria counts of the gut in relation to the different treatments. Use of organic acids during starter phase is useful, especially when the milling process is inappropriate.

Introduction

Many Authors have reported on the effect of the form and particle size of poultry feed on performance parameters (Healy, 1992; Nir *et al.*, 1994ab; Nir *et al.*, 1995; Engberg *et al.*, 2002, Amerah *et al.*, 2007b). It is also well known that physical properties of feed, such as *proventriculus by-passing* organic acids, may influence food deterioration, gut development, pathogen proliferation and production of toxic metabolites (Ricke, 2003; Gauthier *et al.*, 2007; Grilli *et al.*, 2007). However, there is still no information available about the contemporary use of different particle sizes in the feed and the use of *proventriculus by-passing* organic acids. Given this, the aim of the present study was to investigate the effects of different corn particle sizes combined or not with *proventriculus by-passing* organic acids on growth performances and some histological traits of the gut of the young broiler chicks during the starter phase when they are establishing their ability to use energy and amino acids from feed. The presence of organic acids in the small intestine can, in fact, be guaranteed by bacterial degradation of the unabsorbed starch contained in the coarser particles that remain undigested in the upper part of the gut, or can be added directly to the feed as an organic acid blend.

Materials and methods

This study was approved by the Ethical Board of the Universities of Pisa (Italy) and every phase of animal husbandry and slaughter was carried out according to international ethical and welfare standards.

A total of 576 1-day-old Ross 708 males were randomly allocated to 6 groups and 36 pens (0.8×1.2 m) randomised in 6 blocks, 16 birds to a pen. The experimental mash (*i.e.* non-crumbled) broiler starter diets (Table 1) were formulated with the same composition but with three different corn particle sizes (fine, medium and coarse milling) and were combined or not (in a 3×2 factorial design) with a *proventriculus by-passing* organic acid blend (Galliacid S®). The mash feed form was used to increase the effects of different milling approaches since the benefits associated with crumble or pellet feed form on broiler performance are well known (Attia *et al.*, 2012; Engberg *et al.*, 2002; Bjerrum *et al.*, 2005; Huang *et al.*, 2006). The particle sizes of the different feeds were determined by a standard set of sieves (Committee on Classification of Particle Size in Feeedstuffs, 1969) and calculated as follows:

$d_{gw} = \log^{-1} [\Sigma(W_i \ \log d_i) \ \Sigma W^{-1}]$ and $S_{gw} = \log^{-1} [\Sigma W_i (\log d_i - \log d_{gw})^2 \Sigma W^{-1}]^{½}$

where
d_{gw} is geometric mean diameter of the particle sizes;
S_{gw} is geometric standard deviation of the particle sizes;
d_i is geometric diameter of particles on the i[th] sieve ($=d_i$ X $d_{i+1}]^{½}$;
W_i is the weight fraction on the i[th] sieve.

All the diets were distributed *ad libitum* and met or exceeded NRC recommendations (1994). Each pen was provided with a wood shaving floor, heated by liquid propane gas heaters, and submitted to a 24-h artificial lighting regime. Individual live weight and pen feed intake were measured weekly. At Day 22, 4 broilers from 3 pens were randomly selected and slaughtered by cervical dislocation after diazepam injection. The liver and the whole gastrointestinal structure was removed and dissected, and the following traits were measured:

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Key words: Broiler performance, Particle size, Organic acids, Gut traits.

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- liver weight: after opening the abdomen, the liver was removed and immediately weighed;
- filled and empty gizzard weight: the entire gut was removed, and the gizzard was carefully excised and immediately weighed. The gizzard was then slit longitudinally, washed to remove food particles, and re-weighed;
- gizzard height: the longitudinally slit gizzard was measured by means of a scanner-based image acquisition. Each half dissection of the gizzard was placed with its frontal surface toward the scanner (Microtek Scanmaker 4, Redondo Beach, CA, USA; 300 dots per inch, auto contrast off and no active adjustment, black felt covering the glass below the lid); the anterior and posterior sections were not used because of variability in size. The uncompressed TIFF files obtained were analysed using Image Pro Plus® 4.0 (Media Cybernetics, Silver Spring, MD, USA);
- intestine length: the distance from the gizzard to the entry of the caeca was measured by suspending from the pyloric extremity against a centimetre scale;
- caeca length: total length of right and left caecum were measured from the ileo-caecal junction against a centimetre scale;
- pH of gizzard, small intestine and caeca: contents were collected, homogenised with a little deionised water, and pH was measured using a digital pH meter;
- total bacterial count: approximately 1 g of the small intestinal and caecal contents was mixed with 9 mL of 0.85% NaCl sterile dilution (Bryant and Burkey, 1953), and homogenised for 3 min in an Omnimixer. Serial dilutions were made in saline and prepared in duplicate on pour plates or in shake tubes. The bacterial count comprehensive of evaluation of total aerobes and anaerobe populations in the small intestine and caecum, was performed using pour plates with Tryptone Glucose Extract Agar (TGEA). Anaerobic counts were established by use of either shake culture tubes containing thioglycolate medium with added agar (15 g/L) or pour plates of the same medium overlaid with 3% agar (all media were manufactured by either Difco or BBL). Incubation was at 37°C for 48 h for aerobes and for 96 h for anaerobes. All colonies that developed in the shake culture tubes or overlaid agar plates were recorded as anaerobes, although we realised that some facultative anaerobic bacteria were included. Total numbers of bacterial colonies were counted at the end of each incubation period;
- height and width of small intestinal villi and crypt depth measurements: from each animal, a 2 cm section of distal ileum next to the ileo-caecal valve was fixed overnight in 70% ethanol, routinely paraffin-embedded, sectioned at 2-3 μm and stained with haematoxylin and eosin (at least 10 fields with 8-10 villi per field and 10 coronal sections were examined for each chicken); measurements were performed using an image analysis system (Image-Pro Plus; Media Cybernetics, Silver Spring, MD, USA) on digitalised images (Nikon Optiphot-2).

Data were analysed as a randomised complete block design with 6 treatments in a factorial arrangement, and main effects (particle size and organic acids) and interactions were analysed by ANOVA using the GLM procedure (SAS, 2008). Differences between groups were tested for significance by Tukey test. Bacteria counts were log transformed before the analysis, and weight and length of the organs were analysed as absolute values, adding the live weights as a covariate in the model (relative values).

Results and discussion

Particle size

Corn textures used in the different diets are shown in Table 1. The particle sizes, categorised in coarse, medium and fine milling, were: 375±1.819 μm, 1117±1.442 μm, and 2402±1.193 μm (d_{gw}±S_{gw}).

Table 1. Ingredients, chemical composition and particle sizes of corn in the diets.

Ingredients		Calculated analysis	
Maize, g/kg	530.0		
Soybean meal solv. extr. 44, g/kg	335.0	Metabolisable energy, MJ/kg	12.88
Corn gluten, g/kg	50.0	Crude protein, g/kg	224.2
Soybean oil, g/kg	39.0	Fat, g/kg	67.8
$CaHPO_4$, g/kg	18.0	Crude fibre, g/kg	51.3
$CaCO_3$, g/kg	16.0	Ash, g/kg g/kg	72.6
Vitamin and mineral premix°, g/kg	5.0	Ca, g/kg	10.0
NaCl, g/kg	2.4 (2.1)[§]	P total, g/kg	7.6
$NaHCO_3$, g/kg	1.6	Na^+, g/kg	1.6 (1.5)
DL-methionine, g/kg	1.5	Cl^-, g/kg	1.7 (1.6)
L-lysine HCl, g/kg	1.5		
Organic acids - Galliacid S®[#], g/kg	0 (0.3)[§]		

		Sieve diameter, μ										
		2830	2000	1180	1000	710	595	425	300	250	90	Residuals
Corn particle sizes^												
Fine	d_{gw}=375; S_{gw}=1.819	0.00	0.00	0.00	7.14	10.29	15.81	16.04	13.02	21.21	15.12	1.37
Medium	d_{gw}=1117; S_{gw}=1.442	0.00	6.61	59.00	13.30	11.79	5.19	1.86	0.00	0.00	0.00	2.25
Coarse	d_{gw}=2402; S_{gw}=1.193	31.21	59.59	8.88	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.17

°Premix supplies (mg/kg diet): vitamin A (retinol), 4.5; vitamin D_3 (cholecalciferol), 0.075; vitamin E (dl-alpha-tocopherol), 30; vitamin K_3 (menadione), 3; vitamin B_1 (thiamin), 2; vitamin B_2 (riboflavin), 8; vitamin B_6 (pyridoxine), 5; Vitamin B_{12} (cyanocobalamin), 0.03; d-biotin, 0.1; vitamin PP (nicotinic acid), 40; vitamin B_5 (pantothenic acid), 15; vitamin B_9 (folic acid), 1.25; choline chloride, 600; Mn, 150; Zn, 60; Fe, 35; Co, 0.5; Cu, 10; J, 0.5; Se, 0.1; antioxidant (ethoxyquin), 2.5. [#]Organic acid composition: fumaric acid 25%, calcium formate + calcium propionate + potassium sorbate 25%, hydrogenated vegetable oil matrix 50% (microencapsulation). [§]In brackets the values for the diets containing the organic acids. ^Geometric mean and log normal geometric standard deviation according to Ensor *et al*., 1969.

Growth performances

Total mortality was 2.25% (min 2-max 3 birds per group). The particle size by organic acid interaction was not significant for body weights. The presence of the organic acids in the feed improved the broiler growth starting already from Week 1. The negative effect on body weight of the chicks fed fine-milled feed started to be evident from the Week 2, but no difference was observed between medium and coarse particles (Table 2).

Our results on the growth of the broiler confirmed the generally negative effects of the fine milling of corn (Parson *et al*., 2006). The reduced body weights observed in the chicks fed the fine-milled feed were probably due the reduced palatability of this feed when it is presented in mash form. As already reported by Portella *et al*. (1988), regarding the chicks preference for particular particle sizes, significant avoidance of finer particles was shown by the decrease in the concentration of the larger particles in the feeder over time, either using mash or crumbled diets. Feed intake, in fact, was significantly lower in the fine-milled feed group both in the medium-milled or coarse-milled groups. The significant interaction between particle size and organic acids in the last week was probably due to the reduction in the negative effect of fine milling in the group receiving feed with added organic acids and the remarkable increase in feed intake in the medium group who did not receive organic acids. This increase in feed intake affected overall performance indices (the same trend was shown by Feed Conversion Ratio, FCR). This increase (over 100 g more than that of coarse particle-fed treatment without organic acids) confirmed the results of Nir *et al.* (1994a) who obtained the best broiler performance from Days 7 to 21 of age with diets prepared from the medium texture. The reduction in the negative effects due to the inappropriate milling (too fine but also too coarse) obtained with the presence of the organic acids might be explained by a greater palatability induced by the presence of the organic acids which became evident only with apparently unimproved milled diets. The suggestions of Gauthier *et al.* (2007) and Grilli *et al.* (2007) that dietary supplemental organic acids generally improve the digestibility of proteins, Ca, P, Mg, Zn and also, as a substrate, organic acids enter the intermediary metabolism, are in line with our results regarding growth performance of chicks.

Table 2. Growth performance in relationship to the particle size of the corn and the presence of the organic acids in the diet.

		Without organic acids			With organic acids		
	n	Fine	Medium	Coarse	Fine	Medium	Coarse
Body weight, g							
Day 1	576	42	42	41	41	41	41
Day 7	572	164^{b}	167^{ab}	167^{ab}	170^{ab}	175^{a}	175^{a}
Day 14	570	402^{c}	407^{bc}	415^{abc}	413^{abc}	424^{ab}	429^{a}
Day 21	563	782^{b}	830^{a}	828^{a}	849^{a}	866^{a}	861^{a}
Feed intake, g							
Day 1-7	36	165	162	163	168	166	166
Day 7-14	36	383	376	371	396	364	381
Day 14-21	36	611^{d}	755^{a}	661^{cd}	671^{bcd}	720^{ab}	713^{abc}
Day 1-21	36	1159^{c}	1293^{a}	1195^{bc}	1235^{abc}	1250^{ab}	1261^{ab}
Feed conversion ratio							
Day 1-7	36	1.35	1.30	1.32	1.31	1.25	1.25
Day 7-14	36	1.61	1.59	1.52	1.63	1.48	1.51
Day 14-21	36	1.64^{ab}	1.85^{a}	1.62b	1.58^{b}	1.64^{ab}	1.69^{ab}
Day 1-21	36	1.58	1.67	1.54	1.55	1.52	1.55

	Particle size			Organic acids		SEM	F value of interaction
	Fine	Medium	Coarse	No	Yes		
Body weight, g							
Day 1	41	41	41	42	41	0.074	0.091
Day 7	167	171	171	166^{b}	173^{a}	0.189	0.190
Day 14	408^{b}	416^{ab}	422^{a}	408^{b}	422^{a}	0.293	0.169
Day 21	816^{b}	848^{a}	844^{a}	813^{b}	859^{a}	0.407	1.972
Feed intake, g							
Day 1-7	166	164	165	164	167	0.455	0.030
Day 7-14	389	370	376	376	380	0.757	1.232
Day 14-21	641^{c}	738^{a}	687^{b}	676^{b}	701^{a}	0.959	7.702**
Day 1-21	1197^{b}	1272^{a}	1228^{ab}	1216^{b}	1248^{a}	1.115	6.469**
Feed conversion ratio							
Day 1-7	1.33	1.27	1.28	1.32^{a}	1.27^{b}	0.042	0.229
Day 7-14	1.62	1.53	1.51	1.57	1.54	0.064	0.643
Day 14-21	1.61	1.75	1.65	1.70	1.64	0.062	3.418*
Day 1-21	1.57	1.60	1.55	1.60	1.54	0.050	2.659

^{a,b,c}Means bearing different letters differ per P<0.05; * P<0.05; ** P<0.01.

Carcass traits

Due to the fact that the differences between blocks were significant, we discarded the worst block to minimise bird use without any significant loss in sensitivity of the experiment (Festing, 1994). A random selection of 4 birds from each of the remaining 3 blocks showed the weights of the slaughtered birds were slightly heavier than the whole reared group, but the differences between these were not significant (Table 3). Coarse-milled diets produced the highest liver development in the chicks (P<0.05). No significant interaction was found between particle size and organic acids, and the organic acids did not influence liver weight. Since fatty acids are synthesised from dietary carbohydrate in liver and then transported as triglycerides in the plasma to the storage sites, the greater mass of the liver relates to the better utilisation of carbohydrates that can be obtained with the use of coarse milling. The weight of the gizzard was significantly lower in the fine milling group (3.22% *vs* 3.41% and 3.53% in medium and coarse milling). In fact, particles that are too fine are not able to sufficiently stimulate gizzard development, so that gizzard function may be reduced due to lower contraction intensities and retention times (Amerah *et al*., 2007a). The smaller size of the empty gizzard, reported also by Nir *et al*. (1994ab) and Healy (1992) in the chicks receiving the fine-milled feed, was probably due either to the faster passage of the feed in the gut or to the lower feed consumption. It is well known that particle size and form of the feed influence the development and the physiology of the avian digestive tract in chicks from Day 7 of age (Nir *et al*., 1994a; Nir *et al*., 1995; Engberg *et al*., 2002). In our experiment, the reduction in gizzard size was linked with the reduction in growth, contrary to observations made by Amerah *et al*. (2007a) who reported that a reduction in particle size may not be consistent because gizzard efficiency may vary with gizzard size according to gizzard volume. The presence of the organic acids that bypass the gizzard did not seem to have any interaction effect on the particle size and did not influence gizzard development. No interaction was observed between pH of the different intestinal tracts and intestine length, and no difference was found between the main effects of particle sizes and organic acids. However, as expected, the greater caeca development (measured as length of both tracts) observed in chicks receiving the fine milled diet without organic acids suggests that the fine ground maize may be retained longer in the digestive tract of these chicks and, consequently, there may be a longer digestion phase (Parson *et al*., 2006; Jensen *et al*., 1962; Nir *et al*., 1994b). Contrary to observations by Engberg *et al*. (2002) and Huang *et al*. (2006), we did not observe any decrease in pH due to increased production of volatile fatty acids (VFA) in the digestive tract of any broilers in relation to the physical properties of the feed. Also the presence of the organic acids did not influence the pH of the digestive tract of any of the broilers, confirming that the antibacterial mechanism for organic acid action is not still fully understood; activity may vary depending on the physiological status and physicochemical characteristics of the organism but cannot be explained by pH modulation (Ricke, 2003).

Histological and microbiological traits

Independently of the use of organic acids or different particle sizes, all the villi were elongated proportionately and were well defined; morphology was similar or better defined than that reported in the literature (Awad *et al*., 2009; Nourmohammadi and Afzali, 2013). No villi were found atrophied or with pieces scattered in the lumen of the intestine (Nir *et al*., 1994a). Either the two main effects (particle size and organic acid content) or the interaction (particle size*organic acid content) affected the villi characteristics in the small intestine. Finer particle sizes enhanced the height of villi (1869 μm without organic acids and 1708 μm with organic acids) (Table 4). The same effect was observed in villi width: finer particle size showed larger villi than every other group (P<0.05). Also crypt depths were affected by the particle size and the organic acids with the same trend. Villus-height-to-crypt-depth ratios confirmed the interactive effect (F4.873**) but showed the best value for coarse feed without acids (20.5), followed by fine, medium and coarse feeds with acids (18.6, 18.9 and 18.4, respectively); the worst were fine and medium feeds without acids (16.5 and 16.9, respectively).

In contrast to reports by other Authors, we observed greater villi height when organic acids were not used and coarser particles were used (Amerah *et al*., 2007a, 2007b). In this study, all the villi and crypt depths were well defined suggesting for all of them a good rate of nutrient absorption and a reduced rate of enterocyte cell migration from the crypt to the villus (Nourmohammadi and Afzali, 2013; Van Leeuwen *et al*., 2004). The trend of the villi height, crypt depth and their ratio might follow a parabolic curve with an optimal maximum rather than a linear curve with a slope without a maximum so that, after the optimal maximum, the further increase of a positive factor may not respond like that observed at a lower level (decreasing instead of increasing the villi height). For this reason, to be able to understand the phenomenon, when all villi are well elongated and defined, the only geometric mean diameter and geometric standard deviation might be not sufficient to describe the characteristics of the corn particles used in a feed but the papers should report the complete distribution of the particles between the different sieves. The presence of a particular fraction, in fact, could greatly influence the gastrointestinal structure:

- particles <250 (dust-like particles) negatively influence the villi height and the gastrointestinal structure causing ulcerations, haemorrhages and hardly affect performance;
- particles >1100 μm may be too large for chicks to use;
- particles >0.500 μm (medium and large) may only be useful in a feed to promote correct development of the intestine, at least till day 21 of age (Amerah *et al*., 2007a, 2007b). On the other hand, the short-chain fatty acids, which promote the proliferative activity in the crypt and villus height, are directly produced by bacterial degradation of the starch. Starch contained in the coarse particles led to longer residence time within the gizzard and small gut leading to a more muscular gizzard; however, more starch reached, undigested, the intestine. Addition of short-chain fatty acids, with optimal particle size, may be sufficient for the optimal development of the intestine, and a further supplementation can reduce bacterial activity and villus height. The ratio between villus-height-to-crypt-depth, however, confirmed the positive effect of coarse particle size and the presence of the organic acids. In fact, the villus-height:crypt-depth ratio are directly correlated with increased epithelial cell turnover, digestive capacity and better performance (Nourmohammadi and Afzali, 2013).

In our experiment, analysis of total bacteria count did not reach the minimum statistical difference; coarse milling of corn and organic acid additive did, however, show greater mean counts of total bacteria. Feed acidification with various short-chain organic acids, such as fumaric, propionic, lactic and sorbic, have been reported to increase nutrient absorption and to decrease colonisation of pathogens and production of toxic metabolites (Jørgensen *et al*., 1999; Kranker *et al*., 2001; Stege *et al*., 2001; Leontides *et al*., 2003; Huang *et al*., 2006 Mikkelsen *et al*. 2004).

Table 3. Carcass traits in relationship to the particle size of the corn and the presence of the organic acids in the diet.

		Without organic acids			With organic acids		
	n	Fine	Medium	Coarse	Fine	Medium	Coarse
Slaughtering weight, g	71	780^{c}	925^{ab}	892^{b}	920^{ab}	959^{a}	932^{ab}
Liver°, %	71	2.22^{b}	2.45^{b}	2.48^{ab}	2.16^{b}	2.20^{b}	2.51^{a}
Empty gizzard°, %	69	3.36^{ab}	3.45^{ab}	3.52^{ab}	3.08^{b}	3.37^{ab}	3.54^{a}
Gizzard pH	70	2.79	2.91	2.92	2.92	2.92	2.95
Small intestine pH	69	6.66	6.54	6.94	6.52	6.60	6.56
Caeca pH	70	7.03	7.41	7.37	7.11	7.00	7.43
Gizzard high°, cm	72	1.627	1.472	1.449	1.392	1.565	1.500
Intestine length§, mm	71	143.4	148.0	141.1	142.6	146.6	147.9
Caeca length§, mm	69	30.7^{a}	30.2^{ab}	27.5^{b}	28.9^{ab}	29.0^{ab}	28.7^{ab}

	Particle size			Organic acids		SEM	F value of interaction
	Fine	Medium	Coarse	No	Yes		
Slaughtering weight, g	850^{b}	942^{b}	912^{a}	937	866	0.995	4.255**
Liver°, %	2.2^{b}	2.3^{b}	2.5^{a}	2.3	2.4	0.199	2.903
Empty gizzard°, %	3.22^{b}	3.41^{a}	3.53^{a}	3.44	3.33	0.219	0.416
Gizzard pH	2.85	2.91	2.93	2.87	2.93	0.084	0.108
Small intestine pH	6.60	6.60	6.75	6.72	6.56	0.123	0.251
Caeca pH	7.07	7.23	7.40	7.27	7.18	0.092	1.261
Gizzard height°, cm	1.510	1.518	1.475	1.516	1.486	0.052	2.027
Intestine length§, mm	141.4	148.8	144.9	144.1	145.7	0.381	1.158
Caeca length§, mm	29.3	30.1	28.1	29.4	28.8	0.180	2.774

°Relative organ weights reconverted to percentages; SEM reported as original. §Lengths, expressed to parity of slaughter weights. [a,b,c] Means bearing different letters differ per P<0.05; *P<0.05; **P<0.01.

Table 4. Histological and microbiological traits of the gut in relation to the particle size of the corn and the presence of organic acids in the diet.

		Without organic acids			With organic acids		
	n	Fine	Medium	Coarse	Fine	Medium	Coarse
Villus height, m	211	1869^{a}	1401^{c}	1039^{d}	1708^{b}	1535^{c}	942^{e}
Villus width, m	211	178^{a}	105^{b}	101^{b}	175^{a}	106^{b}	98^{b}
Crypt depth, m	211	102^{ab}	98^{b}	65^{c}	106^{a}	70^{c}	66^{c}
Villus-height-to-crypt-depth ratio	211	16.5^{b}	16.9^{b}	20.5^{a}	18.6^{ab}	18.9^{ab}	18.4^{ab}
Total bacteria°, n	61	1.26×10^{9}	1.78×10^{9}	3.83×109	9.98×10^{9}	2.46×10^{9}	2.85×10^{9}

	Particle size			Organic acids		SEM	F value of interaction
	Fine	Medium	Coarse	No	Yes		
Villus height, μm	1788^{a}	1468^{b}	991^{c}	1436^{a}	1395^{b}	0.748	4.964**
Villus width, μm	177^{a}	105^{b}	100^{b}	128	126	0.294	0.073
Crypt depth, μm	104^{a}	84^{b}	66^{c}	88^{a}	81^{b}	0.228	9.371**
Villus-height-to-crypt-depth ratio	17.5^{b}	17.9^{ab}	19.4^{a}	17.9	18.6	0.148	4.873**
Total bacteria°, n	2.24×10^{9}	2.09×10^{9}	3.30×10^{9}	2.05×10^{9}	3.03×10^{9}	0.147	0.444

[a,b,c,d,e] Means bearing different letters differ per P<0.05. °Bacteria counts re-converted to number of colonies in the table. SEM is expressed as log values.

Conclusions

The negative effects observed on the starting growth performances suggest sub-optimal chick wellbeing. From this point of view, the acidic additive plays a positive role in the starting phase of the broiler growth but its effect can be observed mainly when an inappropriate milling process is performed. In fact, this positive effect completely disappears when the feed milling process is correctly performed and the correct particle size distribution is achieved. Probably the negative effect of too fine a milling process is less evident in commercial enterprises where pelleted/crumbled diets, which increase apparent metabolisable energy of starch, are used. In addition, the subsequent compensatory growth of the broilers can reduce the negative effects observed during the starting phase (Zubaira and Leesona, 1996). Finally, due to the possible induction of pathogen acid resistance (Patterson *et al.*, 2005), organic acids should only be used in situations in which an optimal milling process is not guaranteed.

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