

# Effects of dietary organic acid supplementation on the intestinal mucosa in broilers

S. KUM<sup>1\*</sup>, U. EREN<sup>1</sup>, A.G. ONOL<sup>2</sup>, M. SANDIKCI<sup>1</sup>

<sup>1</sup>Department of Histology-Embryology, Faculty of Veterinary Medicine, Adnan Menderes University, 09 013 Isikli-Aydin, TURKEY.

<sup>2</sup>Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Adnan Menderes University, 09 013 Isikli-Aydin, TURKEY.

\*Corresponding author: skum@adu.edu.tr

## SUMMARY

The aim of this study was to investigate some histological intestinal modifications induced by a dietary organic acid supplementation in broilers. Forty eight male Ross 308, one day old broilers were randomly allotted in 2 equal control and assay groups: whereas control birds were not supplemented, organic acids were added to the ration of the assay birds and the half of each group were euthanized at 21 days then at 42 days for semi-quantitative histological assessment of the small intestine mucosa (duodenum, jejunum and ileum) throughout Crossman's triple stain and periodic acid Schiff (PAS) stain. It was observed that organic acid supplementation markedly increased the intestinal absorption area by promoting villus growth in height and in width at 21 and 42 days. In parallel, the goblet cell counts were also significantly higher in treated group whatever the intestinal segment. Moreover, the induction of these histological modifications appeared early (before the 21<sup>st</sup> day), transient in duodenum and jejunum but prolonged (after the 21<sup>st</sup> day) in the ileum part since the magnification rates calculated for ileum villi in supplemented birds for the 21-42 days period were significantly higher than in controls. These results demonstrate a positive effect of organic acids on intestinal mucosa.

**Keywords:** Organic acid, broiler, histology, small intestine, intestinal villus.

## RÉSUMÉ

**Effets d'une supplémentation alimentaire en acides organiques sur la muqueuse intestinale chez les poulets de chair**

L'objectif de cette étude était de rechercher d'éventuelles modifications histologiques de l'intestin induites par une supplémentation alimentaire en acides organiques chez le poulet de chair. Au total, 48 poussins Ross 308 mâles de 1 jour ont été aléatoirement répartis en 2 groupes égaux, soit un groupe contrôle et un groupe essai: alors que les contrôles n'ont pas été supplémentés, les acides organiques ont été ajoutés à la ration dans le groupe essai, puis la moitié de chaque groupe a été sacrifiée à J21 et l'autre moitié à J42 afin de réaliser une étude histologique semi-quantitative de la muqueuse intestinale (duodénum, jéjunum et iléum) après triple coloration de Crossman et coloration à l'acide périodique de Schiff (PAS). La supplémentation en acides organiques a fortement augmenté la surface d'absorption intestinale en favorisant la croissance des villosités en hauteur et en largeur à J21 et à J42. En parallèle, le nombre des cellules à mucus était aussi significativement plus élevé chez les traités quelque soit le segment intestinal. En outre, l'induction de ces modifications histologiques est apparue précoce (avant le 21<sup>ème</sup> jour), transitoire dans le duodénum et le jéjunum mais plus durable dans l'iléum, étant donné que les taux d'agrandissement calculés pour les villosités iléales du 21<sup>ème</sup> au 42<sup>ème</sup> jour ont été significativement plus élevés chez les poulets supplémentés que chez les contrôles. Ces résultats démontrent un effet positif des acides organiques sur la muqueuse intestinale.

**Mots clés :** Acides organiques, poulet de chair, histologie, intestin grêle, villosité intestinale.

## Introduction

Organic acids have been used for a long time as food additives and preservatives for preventing food deterioration and extending the shelf life of perishable food ingredients. Specific organic acids have also been used to control microbial contamination and dissemination of food born pathogens in preharvest and postharvest food production and processing [21]. Organic acids commonly referred to as acidifiers or acidifying agents, have shown favourable effects when used as additives in diets for weanling pigs [14]. Like antibiotics, short-chain organic acids also exhibit a specific antimicrobial activity, but unlike antibiotics, their antimicrobial activity is pH dependent. Dietary supplementation with organic acids is associated with reductions of bacteria, especially of species which are acid-intolerant, such as *E. coli*, *Salmonella* and *Campylobacter* [8]. Use of organic acids has clearly and significantly been beneficial in weanling piglets and their interest in poultry performance, particularly in broilers [7, 9, 11, 12], is currently under evaluation. It has been shown that the fumaric acid [18, 22, 26, 31] and sorbic acid [31, 32]

significantly improved food efficiency and weight gain of broiler chickens whereas addition of 0.5 or 1.0% calcium formate into diet had no significant effect on weight gains or feed utilisation [18] and even at the dosage of 1.5% significantly reduced body weights of both males and females at 21 and 42 days [18]. However, IZAT *et al.* [15] have shown that the live bird performance was not adversely affected by supplementation with formic acid (1%) or calcium formate (1.45%). HAZIMOGLU [13] stated that dietary organic acid supplementation had no positive impact on broiler live weight, food consumption, food efficiency and carcass characteristics.

Nevertheless, putative benefits of a dietary organic acid supplementation would be associated with the increase of intestinal nutrient assimilation but there are only few studies on the effects of organic acids on the gut histology in broilers [1, 17, 19, 20, 33]. Consequently, the aim of the present study is to evaluate the effects of a dietary supplementation with various organic acids on growth performance and on the intestinal histology in broilers.

## Materials and Methods

### ANIMALS AND EXPERIMENTAL DESIGN

Forty eight male (Ross 308) one day old broilers were used in this study. Birds were kept in a storey cage system under conventional conditions: lighting was continuous and the room temperature was  $33 \pm 2^\circ\text{C}$  until the birds were 7 days old and thereafter it was gradually reduced on alternate days until  $23 \pm 2^\circ\text{C}$ . Food and water were provided *ad libitum*.

Birds were randomly divided into 2 equal groups (24 animals per group): the control group received a basal diet containing 23% crude protein, 3000 kcal/kg metabolizable energy and 1% calcium whereas during the experimental period, the basal diet for birds of the assay group was supplemented with calcium butyrate, calcium propionate, calcium lactate, calcium formiate and a pure essential oil (Biacid Salt, Provimi B.V., Veerlaan 17-23-3072 AN, Rotterdam, The Netherlands) that contains a mixture of organic acids by replacing 0.15% corn (Table I). Half of the birds in each group were slaughtered by decapitation on day 21 and the remaining animals on day 42. All studies with animals were

	Controls	Assays
<b>Ingredients (% of the ration)</b>		
Corn	52.53	54.74
Soybean meal	39.64	39.64
Vegetable oil	3.96	3.96
Limestone	1.34	1.34
Dicalcium Phosphate	1.68	1.68
Sodium chloride	0.25	0.25
Vitamin premix <sup>1</sup>	0.25	0.25
Mineral premix <sup>2</sup>	0.20	0.20
DL-Methionine	0.15	0.15
<b>Mixed organic acids<sup>3</sup></b>	<b>0.00</b>	<b>0.79</b>
<b>Chemical analysis</b>		
Crude proteins (%)	22.05	22.98
ME (kcal/kg)	3 000	2 973
Ca (%)	1.00	1.00
P (%)	0.46	0.46
DL-Methionine (%)	0.49	0.49
Lysine (%)	1.22	1.22
Linoleic acid (%)	1.15	1.15

<sup>1</sup>including per 2.5 kg of food: Vitamin A: 15 000 000 U, Vitamin D<sub>3</sub>: 2 000 000 U, Vitamin E: 100 000 mg, Vitamin K<sub>3</sub>:5 000 mg, Vitamin B<sub>1</sub>: 3 000 mg, Vitamin B<sub>2</sub>: 6 000 mg, niacin: 40 000, calcium-D-pantotenate:12 000 mg, Vitamin B<sub>6</sub>: 8 000 mg, Vitamin B<sub>12</sub>: 30 mg, biotine: 150 mg, Vitamin C: 80 000 mg, etoiquin 125 000 mg; <sup>2</sup>including per 2.0 kg of food: Mg:100 000 mg, Fe:60 000 mg, Zn: 60 000 mg, Cu: 5 000 mg, Iodine: 2 000 mg, Se:150 mg, Choline chloride: 40 000 mg; <sup>3</sup>Mixed organic acid including per kg of food: Calcium butyrate: 25 000 mg, Calcium lactate: 20 000 mg, Calcium formiate:10 000 mg, Citric acid: 210 000 mg, Essential oil: 55 000 mg, Sepiolite: 680 000 mg; ME: Metabolisable Energy.

TABLE I: Composition (ingredients and chemical analysis) of the ration distributed to broilers supplemented with organic acids (assays) or not (controls).

approved by University of Adnan Menderes Institutional Animal Ethics Committee. Tissue samples (duodenum, jejunum and ileum) were immediately collected and fixed in 10% neutral buffered formalin.

### HISTOLOGICAL ANALYSIS

The sampled tissues were processed according to a standard alcohol dehydration-xylene sequence and embedded in paraffin. Transversal serial sections (5  $\mu\text{m}$ ) were cut at 50  $\mu\text{m}$  intervals from the tissues [23, 27]. The sections were stained with the Crossman's triple stain for general histological examination and with the periodic acid Schiff (PAS) for demonstration of goblet cells inside the villi. In each of the six sections taken serially from sampled tissues, the villus height and width were determined by examining randomly 5 villi and the determination of goblet cells was performed by counting the number of goblet cells in the central 100  $\mu\text{m}$  portion of the 5 villi [2, 3, 30] using an image analysis program (Leica Q win standard). Later, the mean of 30 values obtained for each animal was calculated.

### STATISTICAL ANALYSIS

To determine whether or not organic acid salt has an effect on the goblet cell count, villi height and width in the intestinal mucosa, independent t test (SPSS 11.5) was used to compare the control and treatment groups. Difference was considered as significant when *P* value was less than 0.05.

## Results

The intestinal villus dimensions (width, height and area) and the goblet cell counts according to the small intestinal parts in controls and in broilers dietary supplemented with organic acids are summarized in Table II. The villus width was significantly increased in supplemented birds compared to not supplemented controls since the 21st day in duodenum and jejunum ( $P < 0.05$  and  $P < 0.001$ , respectively) and in all parts of the small intestine on day 42 ( $P < 0.01$  to  $0.001$ ). Additionally, villi, whatever their localization, were markedly greater in treated birds than in controls on days 21 (figures 1, 2, 3) and 42 ( $P < 0.001$ ) (figure 4) and consequently, the villus areas from all intestinal parts were significantly enhanced ( $P < 0.05$  to  $0.001$ ) when chickens were supplemented with organic acids for 21 days or for 42 days. Nevertheless, the magnification rates of villus width, height and area were similar between the 21<sup>st</sup> and the 42<sup>nd</sup> days in controls and treated birds except for ileum villi. In ileum, villi won 13.3% in width, 8.5% in height and 23.0% in area in treated birds whereas they have poorly grown in controls (0% to 1.3%) (figures 3 and 4).

As far as the goblet cell frequency in small intestine was concerned, the number of this cell type was significantly increased in supplemented chickens compared to controls ( $P < 0.05$  to  $0.001$ ) except in jejunum of 42 day old birds (Table II).

Histological parameters		Day 21			Day 42		
		Control	Assay	P	Control	Assay	P
Villus width (µm)	Duodenum	130.17 ± 1.21	142.80 ± 1.32	< 0.001	153.16 ± 1.20	168.23 ± 0.98	< 0.001
	Jejunum	151.78 ± 1.45	153.53 ± 1.27	NS	151.80 ± 0.89	156.54 ± 1.21	< 0.01
	Ileum	139.03 ± 0.86	142.14 ± 1.19	< 0.05	133.41 ± 0.66	161.11 ± 1.07	< 0.001
Villus height (µm)	Duodenum	1559.29 ± 6.33	1730.30 ± 7.66	< 0.001	1718.69 ± 8.38	1930.66 ± 11.71	< 0.001
	Jejunum	864.37 ± 6.08	901.01 ± 5.24	< 0.001	1130.88 ± 4.34	1149.32 ± 4.32	< 0.01
	Ileum	546.09 ± 2.61	628.12 ± 4.01	< 0.001	553.04 ± 2.12	681.52 ± 3.42	< 0.001
Villus area (x10 <sup>3</sup> µm <sup>2</sup> )	Duodenum	202.97 ± 2.31	247.09 ± 2.40	< 0.001	263.23 ± 2.47	324.79 ± 2.73	< 0.001
	Jejunum	131.19 ± 1.54	138.33 ± 1.69	< 0.05	171.67 ± 1.31	179.91 ± 1.54	< 0.01
	Ileum	75.92 ± 0.61	89.28 ± 0.95	< 0.001	73.78 ± 0.44	109.80 ± 0.95	< 0.001
Goblet cells (number/100 µm)	Duodenum	7.32 ± 0.06	8.66 ± 0.06	< 0.001	9.38 ± 0.06	9.83 ± 0.05	< 0.001
	Jejunum	9.43 ± 0.07	9.99 ± 0.06	< 0.001	11.81 ± 0.05	11.90 ± 0.05	NS
	Ileum	12.39 ± 0.06	12.60 ± 0.06	< 0.05	14.24 ± 0.07	15.42 ± 0.06	< 0.001

NS: not significant

TABLE II: Intestinal villus size (width, height and area) and goblet cell counts according to the small intestinal parts in control (not supplemented) broilers and in broilers dietary supplemented with organic acids for 21 days and for 42 days. Results (obtained from 12 x 30 measures) are expressed as mean ± standard deviation.

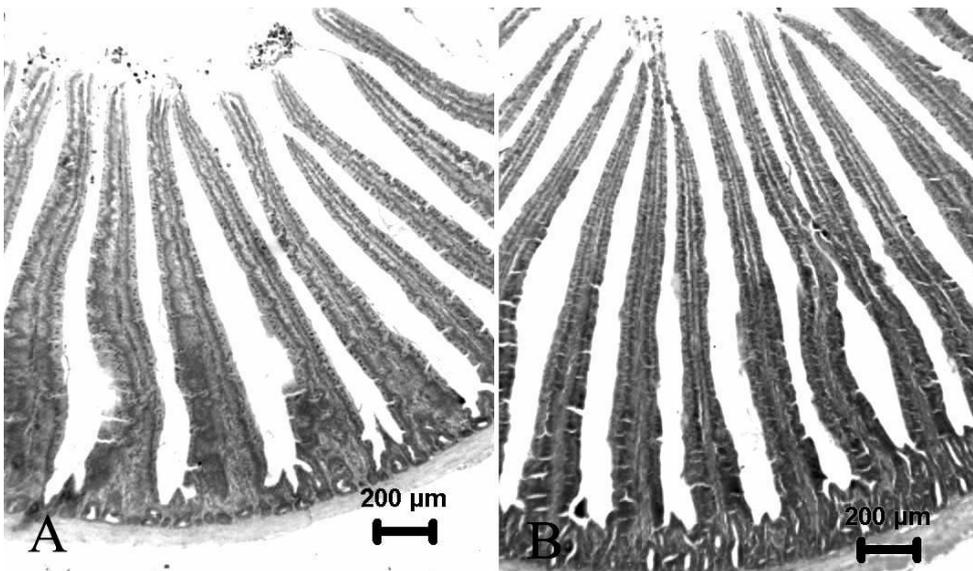


FIGURE 1: Villus height in duodenum from a not supplemented control (A) and a broiler dietary supplemented with organic acids for 21 days (B). PAS, Bar: 200 µm.

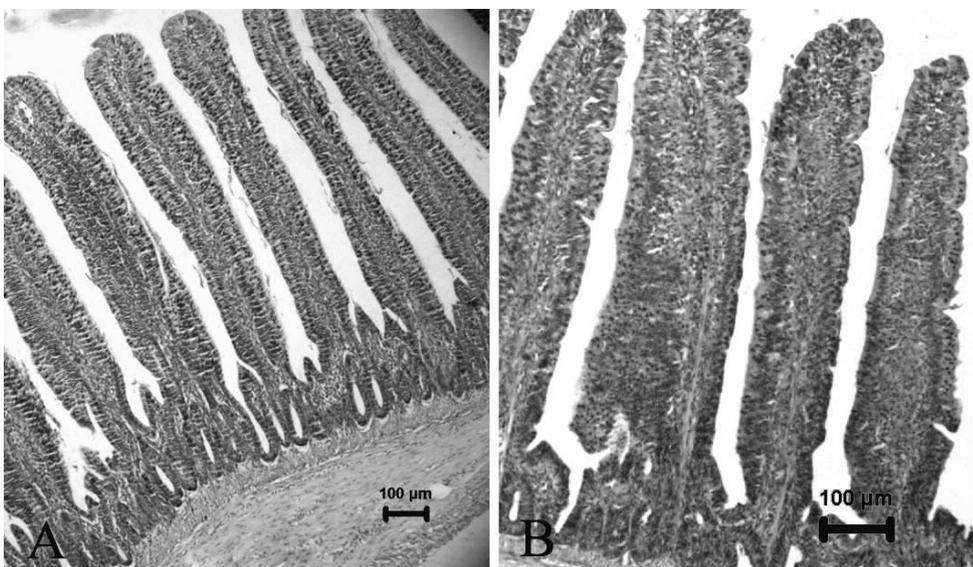


FIGURE 2: Villus height in jejunum from a not supplemented control (A) and a broiler dietary supplemented with organic acids for 21 days (B). PAS, Bar: 100 µm.

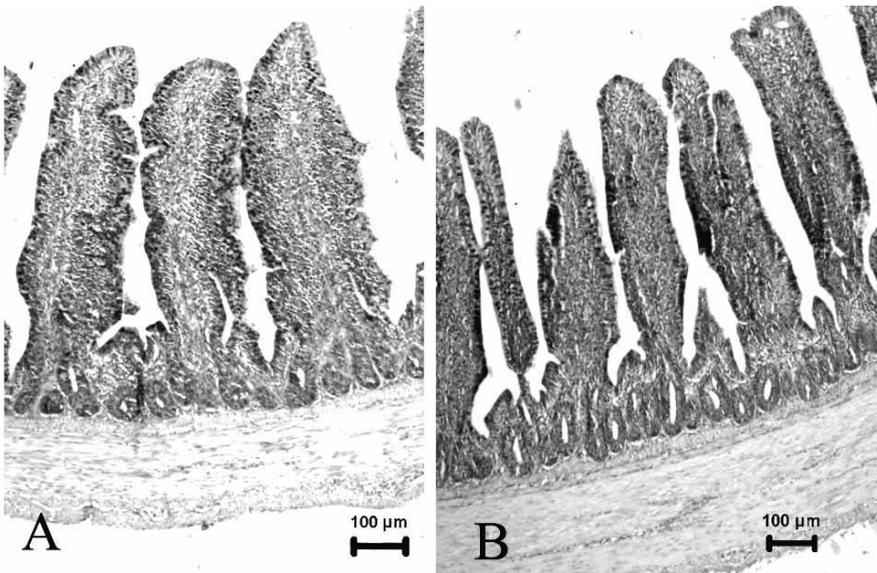


FIGURE 3: Villus height in ileum from a not supplemented control (A) and a broiler dietary supplemented with organic acids for 21 days (B). PAS, Bar: 100 µm.

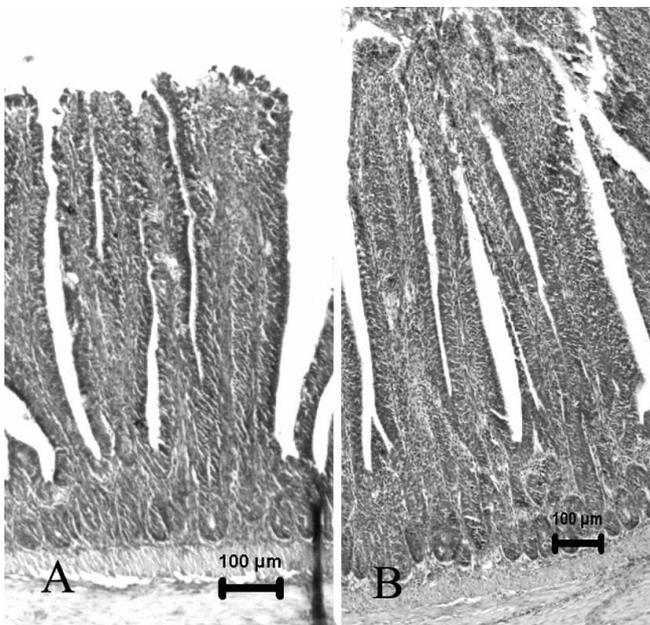


FIGURE 4: Villus height in ileum from a not supplemented control (A) and a broiler dietary supplemented with organic acids for 42 days (B). PAS, Bar: 100 µm.

However, the percentages of increase of goblet cell counts between the 21<sup>st</sup> and the 42 days appeared to be smaller in treated birds than in controls in duodenum and jejunum (13.5% and 19.1% in assay group vs. 28.1% and 25.2% in control group) while this parameter in ileum was markedly higher in treated broilers (22.4% vs. 14.9% in controls).

## Discussion

UNI *et al.* [28] have studied changes in the structure and function of the duodenum, jejunum and ileum in chickens from hatch to 14 days of age: the development of all parts of the small intestine was rapid from hatch to the 2<sup>nd</sup> day and thereafter, the increase rate has differed according to the

considered segment of the small intestine. Whereas the villus volume reached a plateau on day 7 in duodenum, it continued to increase in jejunum and ileum. Besides, FERRER *et al.* [10] reported that microvillus length remained constant in duodenum and jejunum during the development. By contrast, BOZKURT and SANDIKCI [5] reported that the height of villi from all small intestine segments generally increased from hatch to 6 weeks in chickens, whereas it was shortened on 8 week old chickens. They have also observed that the villus width gradually increased in duodenum and jejunum. In the present study, whereas the villus size remained constant in ileum, the surface of villi from duodenum and jejunum markedly increased by 29.7% and 30.9% respectively between the 3<sup>rd</sup> and the 6<sup>th</sup> week of age in controls mainly because of the height gain while the width gain was notably reduced in jejunum.

Several factors such as fasting and diet characteristics were known to affect the villus growth in broilers: SHAMOTO and YAMAUCHI [25] observed a decrease of the villus height in birds fasted for 24-72hours and LANGAR *et al.* [16] reported that this reduction was associated with decline in the nutrient absorption in poultry receiving diets included 1.00 to 1.75% uric acid. APTEKMANN *et al.* [1] observed that the height of villi from the jejunum and ileum was significantly higher in quails receiving 3.0% calcium diets than in birds fed with 3.5% calcium diets. TARACHAI and YAMAUCHI [27] found that the villus height was dramatically reduced in the duodenum from 142 day old Leghorn chickens starved for 5 days but they also demonstrated that this histological parameter was restored when birds were fed for 2 days after a 3 day fasting period. These results suggest that the dietary effects on villus morphology were rapid and easily reversible within a short term. YAMAUCHI *et al.* [34] indicated that villi were larger in broilers fed with high protein and low energy diets compared to broilers fed with low protein and high energy diets. Moreover, in their study, they observed a proportional increase of the villus height to the age in all small intestine parts and of the villus width only in the duodenum.

SANDIKCI *et al.* [24] have reported that environmental conditions like heat stress could significantly modified intestinal

histological parameters; they observed significant reduction of the villus height in the duodenum, jejunum and ileum from quails exposed to heat stress and significant variations of the goblet cell counts particularly in the ileum. In addition, treatment with yeast and with bacitracin zinc also induced a significant decrease of the villus height in jejunum and in jejunum and ileum respectively whereas the goblet cell count increased [24]. Probiotics are used as food additives instead of antibiotics. Without any stress condition, the villus size (height and width) was not significantly affected by dietary probiotic supplementation in chickens [3], humans and rats [6]. PELICANO *et al.* [20] reported higher ileum villus height in chickens fed with diets enriched with organic acid salts than in those fed with diets depleted in mannose oligosaccharide. Whereas OWENS *et al.* [17] did not evidence significant difference in ileum histology according to the organic acid dietary supplementation or not, PAUL *et al.* [19] observed that some organic acid salts (ammonium formate and calcium propionate at the dose of 3 mg/kg) significantly improved the intestinal villus height. XIA *et al.* [33] also found that the most organic acidifiers added to diet induced a significant increase of the intestinal villus height in chickens, but they also reported that modifications of the villus height in ileum were different according to the utilization of probiotics (viable microbial additives in the host organism) or prebiotics (non digestible food ingredients stimulating growth and activity of beneficial bacteria in the digestive system). In agreement with these previous reports, the dietary supplementation with organic acids in the present study has significantly increased the height of villi from all segments of the small intestine in both 21 day old and 42 day old broilers. Nevertheless, the histological modifications induced by the dietary organic acid supplementation should be early (before the 21<sup>st</sup> day) but transient in the duodenum and jejunum since the magnification rates of the villus size between the 21<sup>st</sup> and the 42<sup>nd</sup> days of age in these 2 intestinal segments were closely related between controls and treated broilers whereas they appeared to be more lasting in the ileum in which the magnification rate of the villus size was markedly higher in supplemented birds. Discrepancies between the different studies would be related not only to the amount of organic acid salts into diets but also to the bird age (and the growth capacities of the small intestine) at the beginning of the supplementation.

Mucin glycoproteins, synthesized and secreted by goblet cells distributed along the villi [29], play a key role in the intestinal epithelium function. According to BOZKURT and SANDIKCI [5], the goblet cell count increases according to the bird age. BRADLEY *et al.* [3] reported a decrease of the goblet cell density in the ileum from hens reared under normal conditions and supplemented with *S. cerevisiae* while BAUM *et al.* [2] noted no difference in the goblet cell numbers in the intestines from swine supplemented with probiotics during the suckling period or from controls. On the contrary, BREVES *et al.* [4] reported an increase of the number of goblet cells in the small intestine of swine supplemented with probiotic during growth. In this study, organic acid salts caused moderate to marked increases of the goblet cell counts ( $P < 0.05$  to  $< 0.001$ ) compared to the not supplemented controls according to the part of the small intestine and to the supplementation duration (or to the bird age). As for the villus size increase,

the effects of organic acids on goblet cell counts appeared to be early installed (before the 21<sup>st</sup> day), transient in the duodenum and jejunum and more prolonged in the ileum.

As a conclusion, the dietary organic acid supplementation exhibits some benefits on intestine histology by early promoting the villus growth and the cell differentiation into goblet cells, these effects being long-lasting in ileum. Further studies are required for investigating the organic acids mechanisms on intestine mucosa.

## References

1. - APTEKMANN K.P., ARTONI S.M.B., STEFANINI M.A., ORSI M.A.: Morphometric analysis of the intestine of domestic quails (*Coturnix coturnix japonica*) treated with different levels of dietary calcium. *Anat. Histol. Embryol.*, 2001, **30**, 277-280.
2. - BAUM B., LIEBLER-TENORIO E.M., ENB M.L., POHLENZ J.F., BREVES G.: *Saccharomyces boulardii* and *Bacillus cereus* var. *toyoi* influence the morphology and the mucins of the intestine of pigs. *Z Gastroenterol.*, 2002, **40**, 277-284.
3. - BRADLEY G.L., SAVAGE T.F., TIMM K.I.: The effects of supplementing diets with *Saccharomyces cerevisiae* var. *boulardii* on male poult performance and ileal morphology. *Poult. Sci.*, 1994, **73**, 1766-1770.
4. - BREVES G., SZENTKUTI L., SCHRODER B.: Effects of oligosaccharides on functional parameters of the intestinal tract of growing pig. *Dtsch. Tierarztl. Wochenschr.*, 2001, **108**, 246-248.
5. - BOZKURT M., SANDIKCI M.: Farklı yaşlardaki civcivlerin barsak villus boyu ve çapı ile kadeh hücresi ve mitotik hücre sayılarındaki değişimler. *YYU Vet. Fak. Derg.*, 2009, **20**, 5-9.
6. - BUT J., BERNASKONI P., VAN CRAYNEST M., MALDAGUE P., DE MEYER R.: Response of human and rat small intestinal mucosa to oral administration of *Saccharomyces boulardii*. *Ped. Res.*, 1986, **20**, 192-196.
7. - BROWN D.R., SOUTHERN L.L.: Effects of citric and ascorbic acids on performance and intestinal pH of chicks. *Poult. Sci.*, 1985, **64**, 1399-1401.
8. - DIBNER J.J., BUTTIN P.: Use of organic acids as a study the impact of gut microflora on nutrition and metabolism. *J. Appl. Poult. Res.*, 2002, **11**, 453-463.
9. - EIDELSBURGER U., KIRCHGESSNER M.: Effect of organic acids and salts in the feed on fattening performance of broilers. *Arch. Geflügelkunde*, 1994, **58**, 268-277.
10. - FERRER R., PLANAS J.M., MORETO M.: Cell apical surface area in enterocytes from chicken small and large intestine during development. *Poult. Sci.*, 1995, **74**, 1995-2002.
11. - GARCIA R.G., ARIKI J., MORAES V.M.B., KRONKA S.N., BORGES S.A., MURATA L.S., CAMPOS V.A.: Isolated or combined action of organic acids and growth promoter in broiler rations. *Rev. Bras. Cienc. Av.*, 2000, **2**, 149-154.
12. - GUERRERO R., HOYOS G.: Direct fed microbials and acidifiers for poultry. In: *Biotechnology in the Feed Industry*, Alltech Technical Publications (Ed. Lyons T.P.), Nicholasville, Kentucky, 1991, pp.: 407-411.
13. - HAZIMOĞLU S.: Etlik civciv rasyonlarına organik asid katkısının performans ile ileum pH'sı ve barsak mikroflorası üzerine etkisi. Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü, 2004, Yüksek Lisans Tezi Aydın, Türkiye.
14. - HOLDEN P., EWAN R., JURGENS M., STAHLY T., ZIMMERMAN D.: Life cycle swine nutrition. Iowa State University, 2000, [http://www.ipic.iastate.edu/LCSN/LCSN\\_metric.pdf](http://www.ipic.iastate.edu/LCSN/LCSN_metric.pdf).
15. - IZAT A.L., ADAMS M.H., CABEL M.C., COLBERG M., REIBER M.A., SKINNER J.T., WALDROUP P.W.: Effects of formic acid or calcium formate in feed on performance and microbiological characteristics of broilers. *Poult. Sci.*, 1990, **69**, 1876-1882.
16. - LANGAR P.N., VIRK R.S., GILL S.P.S.: Effect of poultry dropping uric acid on nutrient utilization by broiler chick. *Ind. J. Anim. Sci.*, 1993, **63**, 339-343.

17. - OWENS B., TUCKER L., COLLINS M.A., Mc CRACKEN K.J.: Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. *Br. Poult. Sci.*, 2008, **49**, 202-212.
18. - PATTEN J.D., WALDROUP P.W.: Use of organic acids in broiler diets. *Poult. Sci.*, 1988, **67**, 1178-1182.
19. - PAUL S.K., HALDER G., MONDAL M.K., SAMANTA G.: Effect of organic acid salt on the performance and gut health of broiler chicken. *J. Poult. Sci.*, 2007, **44**, 389-395.
20. - PELICANO E.R.L., SOUZA P.A., SOUZA H.B.A., FIGUEIREDO D.F., BOIAGOM M., CARVALHO S.R., BORDON V.F.: Intestinal mucosa development in broiler chicken fed natural growth promoters. *Braz. J. Poult. Sci.*, 2005, **4**, 221-229.
21. - RICKE S.C.: Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poult. Sci.*, 2003, **82**, 632-639.
22. - RUNHO R.C., SAKOMURA N.K., BANZATTO S.D., JUNQUEIRA O.M., STRINGHINI J.H.: Use of organic acid (fumaric acid) in broiler rations. *J. Braz. Soc. Anim. Sci.*, 1997, **26**, 1183-1191.
23. - SAMANYA M., YAMAUCHI K.: Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. natto. *Comp. Biochem. Physiol. Part A.*, 2002, **133**, 95-104.
24. - SANDIKCI M., EREN U., ONOL A.G., KUM S.: The effect of heat stress and the use of *Saccharomyces cerevisiae* or (and) bacitracin zinc against heat stress on the intestinal mucosa in quails. *Rev. Med. Vet.*, 2004, **155**, 552-556.
25. - SHAMOTO K., YAMAUCHI K.: Recovery responses of chick intestinal villus morphology to different refeeding procedures. *Poult. Sci.*, 2000, **79**, 718-723.
26. - SKINNER J.T., IZAT A.L., WALDROUP P.W.: Fumaric acid enhances performance of broilers chickens. *Poult. Sci.*, 1991, **70**, 1444-1447.
27. - TARACHAI P., YAMAUCHI K.: Effects of luminal nutrient absorption, intraluminal physical stimulation, and intravenous parenteral alimentation on the recovery responses of duodenal villus morphology following feed withdrawal in chickens. *Poult. Sci.*, 2000, **79**, 1578-1585.
28. - UNI Z., GANOT S., SKLAN D.: Posthatch development of mucosal function in the broiler small intestine. *Poult. Sci.*, 1998, **77**, 75-82.
29. - UNI Z., SMIRNOV A., SKLAN D.: Pre- and posthatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. *Poult. Sci.*, 2003, **82**, 320-327.
30. - VAREDI M., GREELEY G.H., HERNDON D.N., ENGLANDER E.W.: A thermal injury-induced circulating factor(s) compromises intestinal cell morphology, proliferation and migration. *Am. J. Physiol.*, 1999, **277**, 175-182.
31. - VOGTH H., MATTHES S., HARNISCH S.: The effect of organic acids in the rations on the performances of broilers and laying hens. *Arch. Geflugelkunde.*, 1981, **45**, 221-232.
32. - VOGTH H., MATTHES S., HARNISCH S.: The effect of organic acids on the performances of broilers. *Arch. Geflugelkunde.*, 1982, **46**, 223-227.
33. - XIA M.S., HU C.H., ZU Z.R.: Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. *Poult. Sci.*, 2004, **83**, 1868-1875.
34. - YAMAUCHI K., YAMAMOTO K., ISSHIKI Y.: Development of the intestinal villi associated with the increased epithelial cell mitosis in chickens. *Anim. Sci. Technol.*, 1993, **4**, 340-350.