Effects of dietary various supplementations on the mucin- and serotonin- releasing cell numbers in small intestine of quails

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SUMMARY

The present study aimed to evaluate immunohistochemical changes of mucin- and serotonin-releasing cells in the small intestine induced by various dietary supplementations in quails. A total of 300 one day old quails were randomly divided into 5 equal groups according to the 5 weeks long supplementation: whereas birds of the group 1 were fed with basal diet, the others were supplemented with prebiotics/probiotics (1 g/kg food, group 2), with organic acids (4 g/kg food, group 3), with both prebiotics/probiotics and organic acids (same dosages, group 4) or with antibiotic (active form of avilamycin, 10 mg/kg food, group 5). Weight growth and food intake were not modified among groups. Density of goblet cells were markedly increased mainly in ileum in all supplemented groups except for the group 5 and in birds co-treated with prebiotics/probiotics and organic acids, the effect was maximal and extended to duodenum and jejunum whereas in antibiotic treated quails the goblet cells were dramatically depleted. In parallel, the number of intestinal serotonin positive (closed and opened types) cells has significantly declined in all supplemented birds: cell depletion was highest in antibiotic supplemented birds and lowest in those supplemented with only organic acids. Additionally, the villus height / crypt depth ratio was also diminished in ileum from birds receiving antibiotic, prebiotics/probiotics alone or combined to organic acids. These results show that antibiotic and other dietary additives alter differently intestinal morphology and especially density of mucin- and serotonin-releasing cells.

Keywords: Quail, dietary supplementation, prebiotic, probiotic, organic acid, antibiotic, small intestine, goblet cell, serotonin cell.

RÉSUMÉ

Effets de différentes supplémentations alimentaires sur le nombre de cellules produisant des mucines ou de la sérotonine dans l'intestin grêle des cailles

Cette étude a eu pour objectif d'évaluer les modifications histologiques de l'intestin grêle et notamment les variations des populations de cellules produisant des mucines ou de la sérotonine chez des cailles soumises à différents types de supplémentation alimentaire. Au total, 300 cailles âgées de 1 jour ont été aléatoirement réparties en 5 groupes égaux en fonction de la supplémentation réalisée pendant 5 semaines: alors que les oiseaux du groupe 1 ont reçu l'aliment de base, les autres ont été supplémentés avec un mélange de probiotiques/probiotiques (1g/kg d'aliment, groupe 2), un mélange d'acides organiques (4 g/kg d'aliment, groupe 3) ou par les 2 à la fois (mêmes dosages, groupe 4) ou encore par un antibiotique (forme active de l'avilamycin, 10 mg/kg d'aliment, groupe 5). La croissance pondérale et l'ingéré alimentaire n'ont pas varié en fonction des groupes. La densité des cellules caliciformes a été fortement augmentée principalement dans l'iléum pour tous les groupes supplémentés à l'exception du groupe 5 et chez les oiseaux cotraités par les probiotiques, probiotiques et acides organiques, cet effet a été maximal et étendu au duodénum et au jéjunum alors que les cellules caliciformes ont considérablement disparu chez les cailles supplémentées par l'antibiotique. En parallèle, le nombre de cellules à sérotonine (de types ouvert et fermé) a significativement diminué chez tous les oiseaux supplémentés : ces pertes cellulaires ont été les plus élevées chez les oiseaux supplémentés par l'antibiotique et au contraire les plus faibles chez ceux supplémentés seulement par les acides organiques. De plus, le rapport hauteur des villosités / profondeur des cryptes a aussi été diminué dans l'iléum des cailles recevant l'antibiotique ou le mélange prébiotiques/probiotiques seul ou associé aux acides organiques. Ces résultats montrent que l'antibiotique et les autres additifs alimentaires agissent de façon différente sur la morphologie intestinale et notamment sur les densités des cellules caliciformes et des cellules produisant la sérotonine.

Mots clés : Caille, supplémentation alimentaire, prébiotique, probiotique, acide organique, antibiotique, intestin grêle, cellules caliciformes, cellules à sérotonine.

Introduction

Antibiotics as grow healthy have mostly been used for decades in poultry and mammalian animal production to develop farm performance and in the control of small and large intestinal pathogen microorganisms [3]. However, there is increasing concern about the risk of developing antibiotic resistance in livestock animals. The uses of antibiotics in animal feed have been prohibited in the European Union since 2006 [32]. Recently, the use of natural alternatives including probiotics, prebiotics and organic acidifier compounds instead of antibiotics has been increasing in order to improve the beneficial microbial population of the intestinal and digestive functions in poultry [3, 11, 21, 22, 32].

Probiotics are natural antibiotics that produce organic acids such as lactic acid, acetic acid and hydrogen peroxidase. These biotechnological products are useful for arrangement of intestinal flora such as *Lactobacillus* spp., *Streptococcus* spp. *Bacillus* spp., and some fungus and yeast species [26,

35]. Moreover, those are inhibiting reproduction of harmful bacteria by increasing mucin releases and acidity of the intestine [36]. Beneficial microorganisms are used by farmer for enhancing nutrient utilisation, promotion of enzyme reaction, reduction of ammonia, increase of resistance to colonisation [14, 25], fermentation of non-degradable dietary fibres and intraluminal mucoproteins [24], expression of mucin, and modulation of intestinal morphology [22]. The activities of probiotics are enhanced by the addition of prebiotics. Prebiotics are non-digestible oligo and polysaccharides such as gluco-, galacto-, fructo-, xylo-, and soybean-oligosaccharides, and lactulose, oligofructose and polydectrol [22, 34]. The prebiotics exhibited beneficial effects of growth activity or limited number of microorganisms in gut [22]. Some researches reported that the use of prebiotics might act as substrates for beneficial bacteria such as *Lactobacillus bifidus* in the intestinal microbial system, subsequently reducing pathogen invasion such with *Salmonella enteritidis* and *E. coli* [3, 27, 34, 45]. Furthermore, the prebiotics have been used for preventing colon cancer, reducing cholesterol, increasing enzyme activity and promoting the active Fe, Zn, Mg and Ca absorption by the intestinal mucosa [13].

Goblet cells secreting mucin are glandular simple columnar epithelial cells that are present throughout the small and large intestinal mucosa. The mucins are rich in polysaccharides and are destructed by highly specialised members of the gut microflora [19]. The continuous production of mucus and its oligosaccharide-rich structure constitute a favourable area for the development of bacteria [34]. More importantly, mucin layer is the first line of defence of the intestinal mucosa against invading pathogens or their associated toxins [19]. Additionally, mucins competitively bind to lectin receptors of pathogen microorganisms and play important role in transport of nutrients and lubrication of the intestinal tract [16]. Some probiotics such as *bifido* and *lactobacilli* species may reduce the secretion of mucus and the risk of diarrhoea in the gut [12]. On the other hand, constipation and diarrhoea have been found associated with deregulation of secretion of several intestinal peptides. Serotonin is one of these peptides, which approximately 95% are produced by enterochromaffin cells (EC) in the intestinal mucosa [15, 41], serotonergic neurons in the myenteric plexus and mast cells in connective tissue. The EC located primarily at base of crypts in gastrointestinal tract are involved in serotonin (5HT) synthesis, storage and release in the body [15, 20]. It is generally admitted that serotonin functions are to control crypt epithelial secretion and cell proliferation in gut [20], to regulate appetite, to stimulate gut muscle contractions, sleep and memory [39].

The objective of this study was to evaluate the relationship between mucin-, serotonin-releasing cells and dietary probiotic, prebiotic, formic acid, propionic acid and antibiotic supplementation.

Material and Methods

ANIMALS AND EXPERIMENTAL DESIGN

A total of 300 one day old Japanese quail chicks were obtained from a commercial hatchery and randomly allotted into 5 experimental groups with 4 replicates of 15 birds per replicate following weighing. Birds in each replicate were placed into cage having 40 x 35 cm^2 floor area and 25 cm in height for a 5 weeks long experimental period. Each pen used litter and was equipped with a nipple drinker line (3 nipples per pen) and a feeder (1.5 kg capacity). Nutrient compositions of the diets for quails were based on the National Research Council recommendations [31] (Table I). Food and water were given *ad libitum* to quail chicks. Temperature was maintained at 32ºC for the first 5 days and then gradually reduced according to normal management practices until a temperature of 22ºC was achieved, and during the entire 5-week study period continuous lighting was applied to quails.

The experimental design consisted of 5 dietary treatments: birds from the group 1 received a control diet without supplementation whereas in the other groups, chicks received dietary supplementation for 5 weeks with probiotic-prebiotic combination (a combination of *Enterococcus faecium* as probiotic strain, oligosaccharides as prebiotic, phytogenic substances and cell wall fragments: *Biomin®IMBO*) at 1 g/kg of food (group 2) or organic acid combination (a combination of formic acid and propionic acid based on an inorganic phyllo-silicate carrier: *Biotronic*) at 4 g/kg of food (group 3), or both probiotic-prebiotic and organic acid combinations at the same

Ingredients	**%**	**Calculated analysis[3]**	
Corn	53.08	Metabolisable energy (kcal/kg)	2929
Soybean meal	39.13	Dry matter (%)	92.00
Full-fat soybean	5.00	Crude protein (%)	24.01
$CaCO_3$	1.07	Crude fibre (%)	3.01
Dicalcium phosphate	0.93	Ether extract (%)	3.67
Salt	0.30	Crude ash (%)	6.20
Methionine	0.14		
Vitamin premix[1]	0.25		
Mineral premix[2]	0.10		

[1]Vitamin premix contained per kg: 15 000 000 IU Retinol, 2 500 000 IU Cholecalciferol, 80 000 mg Tocopherol, 5000 mg Menadion, 300 mg Thiamine, 600 mg Riboflavin, 5000 mg Pyridoxine, 30 mg Cobalamin, 50 000 mg Ascorbic acid, 50 000 mg Nicotinamide, 12 000 mg Panthotenic acid, 1500 mg Folic acid, 100 mg D-biotin; [2]Mineral premix contained per kg: 160 000 mg Mn, 120 000 mg Fe, 120 000 mg Zn, 10 000 mg Cu, 400 mg Co, 2000 mg I, 300 mg Se; [3]ÇAKIR et al., 2008 [11].

TABLE I: Chemical composition of experimental diets given to quails for 5 weeks.

levels given above (*Biomin®IMBO+ Biotronic*) (group 4) or with an antibiotic (active form of avilamycin) at 10 mg/kg food (group 5).

Slaughter processes were informed in our previous study [11]. For this purpose, feed was removed from each pen 12 hours prior to processing. Then, 10 quails for each group were randomly selected and weighed individually before slaughter for histochemical and immunohistochemical analysis of small intestine.

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL EXAMINATIONS

Small intestine tissues were collected after randomly slaughtering quails and, then a 1-cm segment of the midpoint of the duodenum, jejunum and ileum were removed and fixed in 10% buffered formalin for 72 hours. Each segment was then embedded in paraffin, and transversal two serial sections (5 µm) were cut at 50 µm intervals from the tissues. The sections were stained with the Alcian blue (AB), periodic acid/Schiff's (PAS) or combined AB plus periodic acid / Schiff's (AB + PAS) for histochemical demonstration of goblet cells in the villi and crypts.

For immunohistochemical evaluation of serotonin-releasing cells, 5 µm thick two serial sections prepared from the blocks were stained by streptavidin-biotin-peroxidase staining method. In this study, primary antibody polyclonal 5HT anti human serotonin (dilution: 1/150, M0758: Dako), biotinylated secondary antibody (Universal LSAB Kit-K0690, Dako) were used and binding sites of antibody were visualized using 3,3'-Diamino Benzidine (DAB, Sigma) as enzyme substrate. The binding of antibodies were evaluated by high-power microscopic examination. From the two serial sections per small intestine portion, the numbers of goblet and serotonin cells were determined by counts in the 1000 µm portion of the villi and/or crypts by means of an image analysis program (Kameram SLR, 1.6.1.0, Mikro Sistem Ltd. Sti.).

STATISTICAL ANALYSIS

All values were expressed as mean ± standard deviation. The numbers of goblet and serotonin cells were analysed using a one-way analysis of variance (ANOVA). Significant differences among treatment means were determined using Duncan's. Differences with a $P < 0.05$ were considered significant.

Results

In this study, changes in food consumption and body weight were recorded once a week. These results showed that dietary treatments with probiotic-prebiotic combination, organic acid combination, both probiotic-prebiotic and organic acid combinations and antibiotic did not cause any statistically significant effect on body weight gain and food intake.

On the other hand, as reported in Table II, some significant differences in histological characteristics of small intestines (goblet and serotonin cell counts and villi/crypts ratio) were recorded according to the various dietary treatments.

In controls, the goblet cells were predominantly located in the ileum segment. Dietary supplementations with probiotic-prebiotic combination (group 2) or combination of organic acids (group 3) or with both probiotics-prebiotics and organic acids (group 4) have not only preserved the preferential ileum localisation of goblet cells but also have markedly increased their numbers compared to controls ($P < 0.05$). Furthermore, this positive effect on the goblet cell numeration was maximal in the group 4 compared to the other groups ($P < 0.05$) and extended to the 3 parts of small intestine (duodenum, jejunum and ileum). A significant increase in the goblet cell count located in jejunum compared to the control group ($P < 0.05$) was also recorded for the group 3. By contrast when birds received a dietary antibiotic supplementation (group 5), the number of goblet cells in duodenum and ileum were significantly lowered compared to the controls ($P < 0.05$). The goblet cells located on the villi and crypts generally presented a purple colour with PAS + AB stain, indicating the presence of both acidic and neutral mucins (mixed mucin) while a magenta colour alone showed neutral mucins (PAS positive mucins). Only PAS + AB positive goblet cells were observed in crypt epithelium (figure 1A) whereas few PAS positive goblet cells scattered among PAS + AB positive goblet cells were also seen in villi (figures 1A and 1B). However, no differences in the goblet cells numbers in crypt were observed between the experimental groups and the control group.

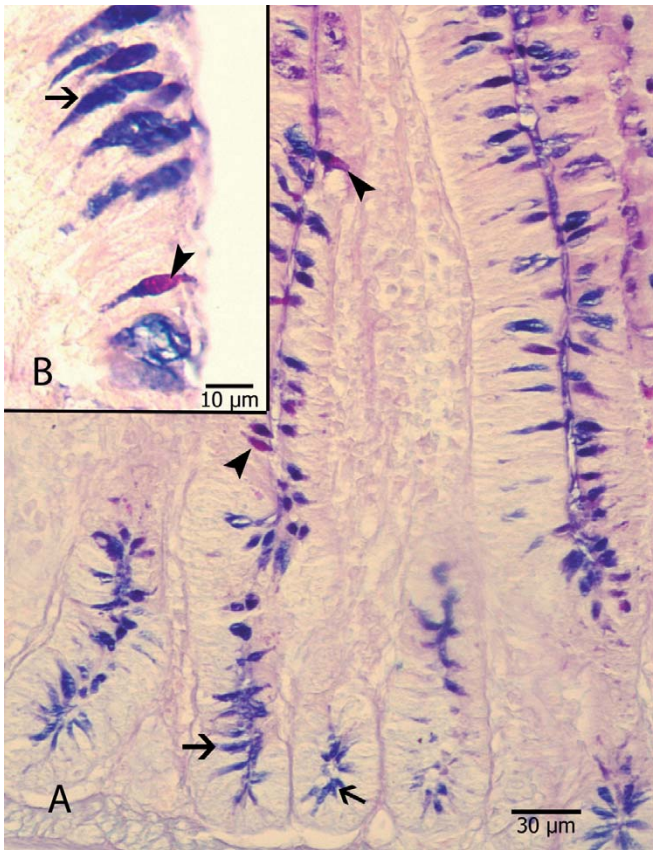
By immunohistochemistry, it was observed uniform distribution of serotonin positive (brown colour) cells in the epithelia of villi and crypts throughout duodenum, jejunum and ileum in all experimental and control groups. Two predominant serotonin-positive cell populations were determined and consisted in closed- (figure 2) and opened-type cells (figure 3). The closed-type cells were small, triangular or round shaped and serotonin positive cytoplasmic granules were localised in the basal region in contact with vessels. These epithelial cells were more commonly found in crypts, particularly in the basal region of Lieberkuhn glands, than in villi. The opened-type cells exhibited a columnar or elongated shape and the apical cytoplasmic processes were in contact with lumen. The superficial villus epithelium considerably contained more opened-type cells than closed-type cells. Compared to the control group, the numbers of closed- and opened-types serotonin cells were significantly depressed in all segments of the small intestine in all experimental groups ($P < 0.05$). The highest decreases in serotonin positive cells were recorded in the group 5 (supplemented with antibiotic) compared to the other groups ($P < 0.05$) while in groups 4 (supplemented with both probiotic-prebiotic and organic acid combinations) and 2 (supplemented only with probiotic-prebiotic combination) this effect was less marked, specifically in jejunum and for the opened-type cell in ileum in the group 2. The decrease in serotonin positive cell counts was globally reduced in the group 3 (supplemented only with organic acid combination) (Table II).

Whereas the epithelial height was not significantly affected by any dietary supplementation, the villus/crypt ratio was markedly decreased in the jejunum and ileum from antibiotic treated quails and also in ileum from quails supplemented

Histological parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Goblet cell counts (number /1000µm)					
Duodenum	38.0 ± 3.2^{b}	41.2 ± 5.0^{b}	40.9± 4.7^{b}	60.3 ± 4.8^{c}	28.5 ± 2.5^{a}
Jejunum	46.1 ± 1.2^{a}	44.7 ± 2.8^{a}	52.1 ± 3.5^{b}	74.1 ± 5.7^{c}	45.6 ± 2.3^{a}
Ileum	73.7 ± 4.6^{b}	90.5 ± 3.7^{c}	86.2 ± 4.3^{c}	104.0 ± 8.5^{d}	46.9 ± 5.4^{a}
Closed-type serotonin cell counts (number /1000µm)					
Duodenum	90.0 ± 2.7^{a}	16.0 ± 2.5^{c}	27.0 ± 3.0^{b}	15.1 ± 1.9^{c}	5.1 ± 0.3^{d}
Jejunum	80.7 ± 4.7^{a}	54.0 ± 4.5^{b}	45.3 ± 5.4^{b}	21.0 ± 2.0^{c}	12.0 ± 2.7^{d}
Ileum	103.3 ± 3.5^{a}	17.0 ± 2.3^{c}	30.6 ± 3.7^{b}	16.0 ± 2.2^{c}	15.0 ± 1.9^{c}
Opened-type serotonin cell counts (number /1000µm)					
Duodenum	34.8 ± 0.7^{a}	10.0 ± 3.9^{c}	24.0 ± 1.4^{b}	12.1 ± 2.3^{c}	15.2 ± 2.6^{c}
Jejunum	40.0 ± 2.3^{a}	21.0 ± 1.6^{b}	20.0 ± 3.1^{b}	18.0 ± 2.8^{b}	9.0 ± 2.6^{c}
Ileum	48.5 ± 2.0^{a}	24.0 ± 3.1^{b}	20.4 ± 2.0^{b}	5.0 ± 1.3^{c}	8.5 ± 2.4^{c}
Villus height / Crypt depth (µm/µm)					
Duodenum	10.0 ± 1.0	9.2 ± 2.4	10.8 ± 1.7	9.1 ± 1.1	8.1 ± 2.3
Jejunum	9.7 ± 1.4^{a}	10.3 ± 1.6^{a}	9.0 ± 2.4^{a}	9.7 ± 1.3^{a}	6.4 ± 0.5^{b}
Ileum	12.3 ± 0.7^{a}	7.9 ± 1.3^{b}	9.5 ± 1.0^{a}	6.6 ± 1.2^{b}	6.4 ± 0.8^{a}
Epithelial crypt height (µm)					
Duodenum	26.0 ± 3.9	33.0 ± 3.2	35.0 ± 2.5	28.0 ± 3.0	28.0 ± 1.9
Jejunum	28.0 ± 2.7	33.0 ± 3.4	29.0 ± 2.5	31.0 ± 2.3	28.0 ± 1.4
Ileum	29.0 ± 1.0	30.0 ± 1.5	27.0 ± 1.8	28.0 ± 1.4	27.0 ± 2.1

Group 1 control feeding with basal diet, group 2 supplemented with probiotic-prebiotic combination (a combination of Enterococcus faecium as probiotic strain, oligosaccharides as prebiotic, phytogenic substances and cell wall fragments: Biomin®IMBO) at 1 g/kg of food, group 3 supplemented with organic acid combination (a combination of formic and propionic acid based on an inorganic phyllo-silicate carrier: Biotronic) at 4 g/kg of food, group 4 supplemented with both probiotic-prebiotic and organic acid combinations at the same levels given above (Biomin®IMBO+ Biotronic) and group 5 supplemented with an antibiotic, active form of avilamycin, at 10 mg/kg food. Data are means in the 1000 µm portion of the villi and/or crypts of ten birds of each group. Different superscripts ^{a,b,c,d} in the same row indicate significant differences (P < 0.05 or more) according to the dietary treatment groups.

TABLE II: Small intestine characteristics (Goblet and serotonin cell counts, villus height/crypt depth ratio and epithelial crypt height) according to the dietary treatment groups Results are expressed as mean ± standard deviation.



with probiotic-prebiotic combination alone (group 2) or in association with organic acids (group 4) compared to controls (*P < 0.05*) (Table II).

Discussion

The intestinal tract is a crossroad between invading pathogens or their associated toxins and nutrient absorption in host. As the defence and absorption activities vary according to the mucosa integrity and to the nature of the microbial flora, the use of natural alternatives including probiotics, prebiotics and organic acidifier compounds instead of antibiotics [3, 11, 22, 32] is actually privileged. However, no data about the effects of probiotics, prebiotics, organic acids and antibiotics on both mucin- and serotonin-releasing cells was available in literature.

Delay or regression in production of enterocytes in crypts can cause growth retardation but decrease in the epithelium

FIGURE 1: Histochemical staining of mucin- releasing goblet cells located in the villi and crypt epithelium. PAS + AB positive (purple colour) cells containing both acidic and neutral mucins (arrows); and PAS positive (magenta colour) cells containing neutral mucins (arrowheads). Double staining with Periodic acid Schiff (PAS)-Alcian Blue pH 2.5 (AB). **A.** bar: 30 µm; **B.** bar: 10 µm.

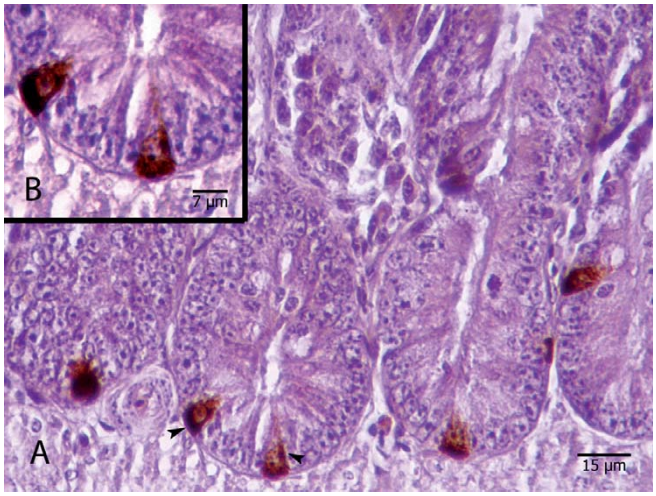


FIGURE 2: Closed-type serotonin positive cells (arrows) in crypt epithelium. Streptavidin-biotin-peroxidase staining method. **A.** bar: 15 µm; **B.** bar: 7 µm.

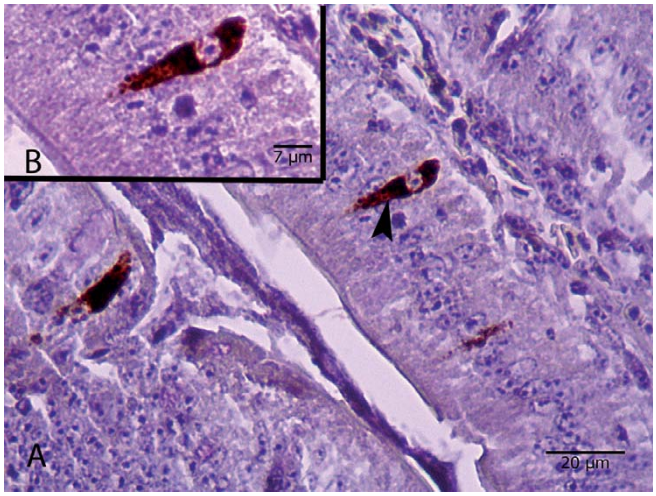


FIGURE 3: Opened-type serotonin positive cells (arrows) in villus epithelium. Streptavidin-biotin-peroxidase staining method. **A.** bar: 20 µm; **B.** bar: 7 µm.

thickness in crypts and villi can increase nutrient absorption, and facilitate absorption process in the intestinal tract [33, 44]. The digestive capacity of the small intestine can be determined according to calculated villus height/crypt depth ratio (V/C). Increments in this ratio are directly correlated with enhancement of absorption and digestion [30]. Reported values of the V/C ratio are found to be 13.48, 8.44 and 5.87 in duodenum, jejunum and ileum, respectively, in turkey hens [36] and 6.5 in the ileum of the chicken fed with maize [28]. In the present study, the reported V/C ratios are compatible with literature. According to GUNAL *et al*. [21], V/C ratios in jejunum and ileum were significantly greater in probiotic-supplemented broilers than in controls. However, it was noted here a significant decrease in the V/C ratio in ileum from quails supplemented with probiotic-prebiotic combination alone or in association with organic acids. In addition, V/C ratio was remarkably decreased in the jejunum and ileum from antibiotic treated quails, leading to a potential reduction in mucosal absorption and digestion as suggested by MONTAGNE *et al.* [30], NOY and PINCHASOV [33] and YASON *et al*. [44].

The goblet cells are specialised cells residing in the villus and crypt epithelium and are responsible for synthesis and secretion of mucin. The mucin production is required for the formation of mucus layers covering epithelium that plays important roles in transport of nutrients and bowel lubrication [16]. Mucins also constitute the first host defence against invading pathogens or their associated toxins [16], and they represent a source of nutrition for anaerobic beneficial bacteria [29]. The number of goblet cells shows significant variations along the intestinal tract segments: they were preferentially located in ileum and the mucus layer thickness and efficiency are dependent from the mucosa integrity and the activity of the microbial flora [34]. SANDIKCI *et al*. [38] have already reported the goblet cell counts in quails feeding with basal diet according to intestinal localisation (3.64, 5.73 and 7.31 per 100 µm of duodenum, jejunum and ileum, respectively). The goblet cell counts were 11.4 in jejunum and 21.2 in ileum per 100 µm in 18 days old chickens [40], and 11.9, 15.34 and 19.96 in duodenum, jejunum and ileum respectively in 56 days old chickens [7]. Findings obtained in the current study are in accordance with values reported by SANDIKCI *et al*. [38].

Probiotics can be administered as pharmaceutical forms assimilated to medicines and functional foods that have various actions such as promoting the gut barrier function, reducing permeability and fermentation of non-degradable dietary fibres and intraluminal mucoproteins, reducing colonic mucorrhoea and hydrorrhoea, regulating the action of mastocytes and inhibiting pathogen binding [24]. The probiotics increase proliferation of epithelial cells in the intestinal mucosa by increasing the amounts of short chain fatty acids [23]. According to some investigations, the density of goblet cell decreased in ileum of *Saccharomyces cerevisiae*-fed [5] and throughout the small intestine of Fermacto-fed chickens [32]. It was demonstrated in several studies that the goblet cell number and a greater amount of mucus covering the intestines were increased in probiotic-fed broilers compared to control birds [10, 17, 21, 36]. On the contrary, some researchers reported no difference in goblet cell number in animals fed with *Bacillus cereus, Saccharomyces boulardii* [5, 9] and *Saccharomyces cerevisiae* [38]. Moreover, increased goblet cell count is related to the prebiotic effect of oligosaccharides and mannanoligosaccharides in broilers, turkeys [2, 42], barley-fed birds [43] and pigs [6], but show a decrease in antibiotic treated broilers [3]. One study reported that the goblet cell counts increased in the duodenum and jejunum segments of treated birds with bacitracin zinc and in the jejunum and ileum of treated birds with *Saccharomyces cerevisiae* + bacitracin zinc [38]. According to our findings, the prebiotics, probiotics and organic acids significantly increased goblet cell counts into the villus epithelium, and these results are in agreement with previous reports in broilers and turkeys [2, 42]. Especially, the goblet cell numbers in group 4 (treated both with prebiotics, probiotics and organic acids) were significantly increased in all intestinal segments compared to the other groups. In this study, organic acid combination may probably promote the effects of probiotic-prebiotic combination. However, some discrepancies with previous works may be related to the bacteria type in diet and dosages of probiotic.

The serotonin (5-HT) releasing cells as a significant member of the entero-endocrine system are scattered in the epithelia of villi and crypts of the gastrointestinal tract. It has been recognized that intestinal endocrine cells consist of closed- and opened-type cells: the opened-type endocrine cells that are in contact with the villus and glandular lumen, and the closed-type endocrine cells, which are in contact with vessels in *lamina propria* [15]. The closed-type cells are functionally modulated by neuronal stimulation or hormones, whereas opened-type cells are well-known to be functionally coordinated by luminal pH and nutrients [41]. The present study demonstrates that serotonin releasing cells exist as both closed-type and opened-type cells in the small intestine and suggests also that each segments of the small intestinal has a different physiological and histological structure [15, 41]. In the present study, the closed-type was around 2 times more frequent than the opened-type in all regions of small intestine in controls but the presence of the opened-type gradually increases from duodenum to ileum.

The serotonin cells are involved in local regulation of electrolyte secretion and water in the gastrointestinal tract [15, 20]. These cells are inhibited by the serotonin re-uptake transporter (SERT) that mediates serotonin uptake into neurons and/or enterocytes [8, 20]. The lack of SERT and stimulation of serotonin cells lead to diarrhoea by increased water and colonic motility [8, 15, 18]. However, the lower serotonin immunoreactivity is associated to oxidative stress and inflammatory cytokines that involve a mechanism responsible for goblet cell hyperplasia [4]. The probiotics and prebiotics may play different roles in various diseases associated with diarrhoea or constipation, and would act as antidepressant and serotonergic compounds by increasing the levels of tryptophan [1, 37]. In this study, the counts of serotonin closed-type and opened-type cells have significantly decreased in all intestinal segments in quails supplemented with prebiotics / probiotics and/or organic acids and with antibiotics compared to the controls. The decline in serotonin cells was maximal with antibiotic supplementation and minimal when only organic acids were added to diets. Therefore, the decrease in the serotonin positive cells might be related to mobility decrease and/or to increase in SERT.

As a conclusion, the villus height / crypt depth ratio and the mucin- and serotonin- releasing cell counts were determined by histochemical and immunohistochemical methods in the small intestine of quails treated with basal diet, prebiotic-probiotic combination, organic acid combinations, both probiotic-prebiotic and organic acid combinations and antibiotic. It was mainly observed that whereas all supplementation procedures have lead to decrease the numeration of serotonin positive cells (opened and closed types) in all intestinal segments, dietary supplementation with combination of prebiotics, probiotics and organic acids has dramatically enhanced the presence of goblet cells contrary to the antibiotic supplementation and to simple addition of prebiotics/probiotics or organic acids into diets at a lesser extend. Further studies are required to investigate the regulation of mucin- and serotonin- releasing cell proliferation in small intestine and particularly to explore the serotonin effects on goblet cell density.

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References

1. - RAGON G., GRAHAM D.B., BORUM M., DOMAN D.B.: Probiotic therapy for irritable bowel syndrome. *Gastroenterol. Hepatol*., 2010, **6**, 140-144.
2. - BAURHOO B., PHILLIP L., RUIZ-FERIA C.A.: Effects of purified lignin and manan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poult. Sci*., 2007, **86**, 1070-1078.
3. - BAURHOO B., GOLDFLUS F., ZHAO X.: Purified cell wall of *Saccharomyces cerevisiae* increases protection against intestinal pathogens in broiler chickens. *Int. J. Poult. Sci.*, 2009, **8**, 133-137.
4. - BERZINA N., MARKOVS J., ISAJEVS S., APSITE M., SMIRNOVA G.: Cadmium-induced enteropathy in domestic cocks: a biochemical and histological study after subchronic exposure. *Basic Clin. Pharmacol. Toxicol*., 2007, **101**, 29-34.
5. - BRADLEY G.L., SAVAGE T.F., TIMM K.I.: The effects of suplementing diets with *Saccharomyces cerevisiae* var. boulardii on male poult performance and ileal morphology. *Poult. Sci*., 1994, **73**, 1766-1770.
6. - BREVES G., SZENTKUTI L., SCHROEDER B.: Effects of oligosaccharides on functional parameters of the intestinal tract of growing pigs. *Dtsch. Tieraerztl Wochenschr*., 2001, **108**, 246-248.
7. - BOZKURT M., SANDIKÇI M.: Farklı yaşlardaki civcivlerin barsak villus boyu ve çapı ile kadeh hücresi ve mitotik hücre sayılarındaki değişimler (in Turkish). *Y.Y.U. Vet. Fak. Derg*., 2009, **20**, 5-9.
8. - BUENO L., DE PONTI F., FRIED M., KULLAK-UBLICK G.A., KWIATEK M.A., POHL D., QUIGLEY E.M.M., TACK J., TALLEY N.J.: Serotonergic and non-serotonergic targets in the pharmacotherapy of visceral hypersensitivity. *Neurogastroenterol. Motil*., 2007, **19**, 89-119.
9. - BUTS 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*. *Pediatr. Res*., 1986, **20**, 192-196.
10. - CABALLERO-FRANCO C., KELLER K., DE SIMONE C., CHADEE K.: The Vsl#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *Am. J. Physiol. Gastroinest. Liver Physiol*., 2007, **292**, G315-G322.
11. - ÇAKIR S., MIDILLI M., EROL H., ŞIMŞEK N., ÇINAR M., ALTINTAS A., ALP H., ALTINTAS L., CENGIZ Ö., ANTALYALI A.: Use of combined probiotic-prebiotic, organic acid and avilamycin in diets of japanese quails. *Rev. Méd. Vét*., 2008, **159**, 565-569.
12. - CAMILLERI M.: Is there a role for probiotics in irritable bowel syndrome? *Dig. Liver Dis*., 2006, **38**, 266-269.
13. - CANIBE N., ENGBERG R.M., JENSEN B.B.: An overview of the effect of organic acids on gut flora and gut health. *J. Anim. Sci*., 2001, **79**, 2123-2133.
14. - CAVAZZONI V., ADAMI A., CASTROVILLI C.: Performance of broiler chickens supplemented with Bacillus coagulans as probiotic. *Br. Poult. Sci.*, 1998, **39**, 526-529.
15. - CETIN Y., KUHN M., KULAKSIZ H., ADERMANN K., BARGSTEN G., GRUBE D., FORSSMANN W.: Enterochromaffin cells of the digestive system, Cellular source of guanylin, a guanylate cyclase-activating peptide. *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 2935-2939.
16. - CHADEE K., PETRI Jr.W.A., INNES D.J., RAVDIN J.I.: Rat and human colonic mucins bind to and inhibit adherence lectin of Entamoeba histolytica. *J. Clin. Invest.*, 1987, **80**, 1245-1254.
17. - CHICHLOWSKI M., CROOM W.J., EDENS F.W., Mc BRIDE B.W., QIU R., CHIANG C.C., DANIEL L.R., HAVENSTEIN G.B., KOCI M.D.: Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, primalac, and salinomycin. *Poult. Sci*., 2007, **86**, 1121-1132.

18. - COLEMAN S., FOLEY S., DUNLOP S.P., WHEATCROFT J., BLACKSHAW E., PERKINS A.C., SINGH G., MARSDEN C.A., HOLMES G.K., SPILLER R.C.: Abnormalities of serotonin metabolism and their relation to symptoms in untreated celiac disease. *Clin. Gastroenterol. Hepatol*., 2006, **4**, 874-881.

19. - DEPLANCKE B., GASKINS H.R.: Microbial modulation of innate defense, goblet cells and the intestinal mucus layer. *Am. J. Clin. Nutr*., 2001, **73**, 1131S-1141S.

20. - GERSHON M.D., TACK J.: The serotonin signaling system, from basic understanding to drug development for functional GI disorders. *Gastroenterology*, 2007, **132**, 397-414.

21. - GUNAL M., YAYLI G., KAYE O., KARAHAN N., SULAK O.: The effect of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *Int. J. Poult. Sci*., 2006, **5**, 149-155.

22. - HAJATI H., REZAEI M.: The application of prebiotics in poultry production. *Int. J. Poult. Sci.*, 2010, **9**, 298-304.

23. - ICHIKAWA H., KUROIWA T., INAGAKI A., SHINEHA R., NISHIHIRA T., SATOMI S., SAKATA T.: Probiotic bacteria stimulate gut epithelial cell proliferation in rat. *Dig. Dis. Sci*., 1999, **44**, 2119-2123.

24. - JIMÉNEZ M.B.: Treatment of irritable bowel syndrome with probiotics. An etiopathogenic approach at last? *Rev. Esp. Enferm. Dig. (Madrid)*, 2009, **101**, 553-564.

25. - JIN L.Z., HO Y.V., ABDULLAH N., JALALUDIN S.: Digestive bacterial enzyme activities in broilers fed diets suplemented with Lactobacillus cultures. *Poult. Sci*., 2000, **79**, 886-891.

26. - KARADEMIR G., KARADEMIR B.: Yem Katkı Maddesi Olarak Kullanılan Biyoteknolojik Ürünler (in Turkish). *Lalahan Hay. Araşt. Enst. Derg*., 2003, **43**, 61-74.

27. - KIM S.H., PARK S.Y., YU D.J., LEE S.L., RYU K.S., LEE D.G.: Effects of feeding *Aspergillus oryzae* ferments on performance, intestinal microbiota, blood serum components and environmental factors in broiler. *Korean J. Poult. Sci*., 2003, **30**, 151-159.

28. - LANGHOUT D.J., SCHUTTE J.B., VAN LEEUWEN P., WIEBENGA J., TAMMINGA S.: Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. *Br. Poult. Sci*., 1999, **40**, 340-347.

29. - LEIPER K., CAMPBELL B.J., JENKINSON M.D., MILTON J., YU L., DEMOCRATIS J., RHODES J.M.: Interaction between bacterial peptides, neutrophils and goblet cells, a possible mechanism for neutrophil recruitment and goblet cell depletion in colitis. *Clin. Sci*., 2001, **101**, 395-402.

30. - MONTAGNE L., PLUSKE J.R., HAMPSON D.J.: A review of interactions between dietary fiber and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Technol.*, 2003, **108**, 95-117.

31. - NATIONAL RESEARCH COUNCIL: Nutrients requirements of poultry, 9th revision, National Academy Press, 1994, pp.: 1-157.

32. - NAVIDSHAD B., ADIBMORADI M., PIRSARAEI Z.A.: Effects of dietary supplementation of *aspergillus* originated prebiotic Fermacto, on performance and small intestinal morphology of broiler chickens. *Italian J. Anim. Sci.*, 2010, **9**, 55-60.

33. - NOY Y., PINCHASOV Y.: Effect of a single posthatch incubation of nutrients on subsequent performance of broiler chicks and turkey poults. *Poult. Sci*., 1993, **72**, 1861-1866.

34. - OUWEHAND A.C., DERRIEN M., DE VOS W., TIIHONEN K., RAUTONEN N.: Prebiotics and other microbial substrates for gut functionality. *Curr. Opin. Biotechnol*., 2005, **16**, 212-217.

35. - PETEK M.D.D., SÖNMEZ G., YILDIZ H., BASPINAR H.: Effects of different management factors on broiler performance and incidence of tibial dyschondroplasia. *Br. Poult. Sci.*, 2005, **46**, 16-21.

36. - RAHIMI S., GRIMES J.L., FLETCHER O., OVIEDO E., SHELDON B.W.: Effect of a direct-fed microbial Primalac, on structure and ultrastructure of small intestine in turkey poults. *Poult. Sci*., 2009, **88**, 491-503.

37. - RODRIGUES K.L., CARVALHO J.C.T., SCHNEEDORF J.M.: Anti-inflammatory properties of kefir and its polysaccharide extract. *Inflammopharmacology*, 2005, **13**, 485-492.

38. - 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.Méd. Vét*., 2004, **155**, 552-556.

39. - SIKANDER A., RANA S.V., PRASAD K.K.: Role of serotonin in gastrointestinal motility and irritable bowel syndrome. *Clin. Chim. Acta*, 2009, **403**, 47-55.

40. - SMITS C.H.M., TE MAARSSEN C.A.A., MAUWEN J.M.V.M., KONINKX J.F.J.G., BEYNEN A.C.: The antinutritive effect of a carboxymethylcellulose with high viscosity on lipid digestibility in broiler chickens is not associated with mucosal damage. *J. Anim. Physiol. Anim. Nutr.*, 2000, **83**, 239-245.

41. - SOLCIA E., RINDI G., BUFFA R., FIOCCA R., CAPELLA C.: Gastric endocrine cells, types, function and growth. *Regul. Pept*., 2000, **93**, 31-35.

42. - SOLIS DE LOS SANTOS F., DONOGHUE A.M., FARNELL M.B., FARNELL G.R., HUFF G.R., HUFF W.E., DONOGHUE D.J.: Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune). *Poult. Sci*., 2007, **86**, 921-930.

43. - VIVEROS A., BRENES A., PIZZARO M., CASTANO M.: Effect of enzyme supplementation of a diet based on barley, an autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Anim. Feed Sci. Technol.*, 1994, **48**, 237-251.

44. - YASON C.V., SUMMERS B.A., SCHAT K.A.: Pathogenesis of rotavirus infection in various age groups of chickens and turkeys, pathology. *Am. J. Vet. Res*., 1987, **48**, 927-938.

45. - YUSRIZAL T., CHEN C.: Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length. *Int. J. Poult. Sci*., 2003, **2**, 214-219.