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Original Article

Effect of Turmeric (Curcuma Longa) on Duodenal Structure in Broiler Chickens

■Author(s)

Bondar A[I] https://orcid.org/0000-0003-3898-8993
Slencu BG[II] https://orcid.org/0000-0002-4144-4383
Popovici I[I] https://orcid.org/0009-0004-2378-0262
Solcan C[I] https://orcid.org/0000-0002-0323-0296

[I] University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" from Iași - Department Preclinics, Faculty of Veterinary Medicine - Aleea M. Sadoveanu nr. 8 M , Iasi 700489 - Romania.
[II] "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy - Pharmaceutical Sciences I - Str. Universitatii nr. 16 , Iasi 700115 - Romania.

■Mail Address

Corresponding author e-mail address
Slencu Bogdan Gabriel
"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, Department Pharmaceutical Sciences I Str. Universitatii nr. 16, Iasi 700115, Romania.
Phone: +40.232.301.623
Email: bogdan.slencu@umfiasi.ro

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ABSTRACT

Supplementation of feed with turmeric (*Curcuma longa*) has been shown to be beneficial in poultry farming. The present study aimed at evaluating the effect of turmeric on the duodenal structure of broiler chickens during the first 42 days of life. A control and three treatment groups (turmeric powder added to feed at the following doses: E1 - 5; E2 - 10; E3 - 20 g/kg feed) were constituted (n = 8). Addition of turmeric powder in the feed resulted in an increase in intestinal villi height and a decrease in crypts depth in case of groups E1 and E2, while the villus height to crypt depth ratio generally did not differ significantly from the control. Turmeric also influenced the histological structure of the duodenum, as well as the presence of IL-6 and TNFα, as evidenced through staining. Addition of 0.5 and 1 % turmeric powder in the feed had evident results on body weight gain.

INTRODUCTION

Poultry farming has undergone a paradigm shift in regard to structure and operation, from a simple backyard activity to a major commercial activity. Sustained poultry farming requires use of medicines, including antibiotics.

Phytogenic feed additives are available alternatives for replacing antibiotics and include prebiotics, probiotics, enzymes, organic acids and essential oils (Wang & Peng, 2008; Saeed *et al.*, 2017; 2018a; 2018b; 2019; 2020; 2022). Turmeric (*Curcuma longa*) is an herbaceous plant of Asian origin, related to ginger and cultivated in India, southern China, Taiwan, Japan, Indonesia and Africa.

Curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which are used therapeutically for their anticancer, antibiotic and anti-inflammatory effects, are extracted from its root (Kotha & Luthria, 2019).

According to the available reports, besides the fact that turmeric has significant antioxidant actions, it can be concluded that it could also be a genuine source of proteins and carbohydrates, while its beneficial properties lead to improved growth and production performance in birds fed turmeric as a feed supplement (Hafez *et al.*, 2022). In addition, it has been reported that it could suppress inflammation by reducing TLR4, MyD88 and NFκB levels (Yoo *et al.*, 2018).

In poultry, mRNA expression of IL-1β, IL-6, IL-12, IL-18 and tumor necrosis factor superfamily member 15 was enhanced in macrophages of chickens treated *in vitro* with organic turmeric extract (Lee *et al.*, 2010).

Microarray hybridization results demonstrated that gene expression of myeloperoxidase, differentiation group 28 and lactotransferrin, which are associated with inflammatory response in chickens, was

down-regulated after treatment with organic turmeric extract (Gautam & Jachak, 2009).

Antioxidant activity offers great hope as a solution for heat stress in poultry, by increasing mitochondrial Mn-SOD activity and gene expression of thioredoxin-2 and peroxiredoxin-3 (Zhou *et al.*, 2015).

Curcumin, the most important active compound found in turmeric, can also decrease mitochondrial malondialdehyde levels (Zhang *et al.*, 2014), increase mitochondrial glutathione levels (Park & Chun, 2017) and increase glutathione peroxidase (GSH-Px), glutathione S-transferase (GSST) and superoxide dismutase (SOD) activities (Zia *et al.,* 2021). An increase in total antioxidant capacity in broilers leads to a reduction in oxidative stress and also in inflammation, with improved digestibility and therefore growth performance (Gowda *et al.*, 2009).

Thus, it can be said that turmeric powder can be safely and economically used as a natural supplement in broiler chicken diets, due to the presence of bioactive substances responsible for the beneficial effects on the health of the chickens and on the quality of meat.

The intestinal epithelium is a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen, which can cause disturbances in the normal microflora and intestinal epithelium. Thus, the permeability of this natural barrier can be altered, facilitating the invasion of pathogens and harmful substances, altering the metabolism and the ability to digest and absorb nutrients. All these factors lead to the development of chronic inflammatory processes in the intestinal mucosa. Eventually, reduction of villi and decrease in digestive activity and absorption capacity of enterocytes occur (Oliveira *et al.*, 1999).

The mucosa of the duodenum undergoes an important physiological stress in order to neutralize the acidity of the content coming from the stomach. In a previous experiment (Bondar *et al.*, 2022), which assessed the influence of *Spirulina platensis* extract (ZooBioR2) on the morphology of intestinal segments (duodenum, jejunum, ileum, cecum), it was found that the duodenum reacted most significantly.

The aim of this study is to assess the effect of turmeric (*Curcuma longa*) on health, and specially on the duodenal structure in broiler chickens during the first 42 days of life.

MATERIAL AND METHODS

One day after hatching, 32 male chickens were randomly divided into 4 treatment groups (n = 8; one replicate per group). Initial body weight did not differ between groups (average 49 ± 0.7 g/chick). Each group was reared for 42 days in cages on the ground (one collective cage for each group). Each bird from a particular group was individually marked for identification purposes. Throughout the experiment, conventional breeding and management procedures were applied in accordance with current legislation. The experimental protocol was evaluated and approved by the Ethics Committee of the Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" University of Life Sciences.

Turmeric powder (brand: HerbalSana; country of origin: India) used in the experiment had the following nutritional value, according to the statement on the label: total fats – 9.7 g, of which 1.8 g were saturated fatty acids; carbohydrates – 44.4 g, of which 3.2 g were sugars; dietary fiber – 22.7 g; proteins – 9.7 g; salt – 0.07 g / 100 g turmeric powder. Curcumin and total phenolic content were determined from the turmeric powder. Curcumin content was 1.13 ± 0.06 g / 100 g turmeric powder, as determined spectrophotometrically (Chauhan *et al.,* 1999). The total phenolic content was 2.703 ± 0.110 g gallic acid equivalents (GAE) / 100 g turmeric powder, as determined according to a previously described method (Luca *et al.*, 2022).

Birds in the control group (C) were fed with a commercial diet based on corn and soybean: starter (1-14 days), grower (15- 28 days), finisher (29 - 42 days), according to NRC (1994) guidelines. Birds in group E1 were offered the same diet but supplemented with 5 g turmeric powder/kg. At the same time, birds in group E2 were fed a diet with a total addition of 10 g turmeric powder/kg. Birds in group E3 were fed a diet with a total addition of 20 g turmeric powder/kg.

All birds had access to food and water for consumption, *ad libitum*.

The feed was designed to meet the requirements of broilers at all stages of growth as recommended in the nutritional management guide for the hybrid studied (COBB 500). Three separate mixed feed diets were developed, specific to each period of chick rearing, namely starter, grower and finisher feed. Turmeric powder was thoroughly mixed with the free-flowing forage. All reagents used were of analytical grade.

After the birds were sacrificed, the intestines were removed. The duodenum samples were fixed in Bouin solution for 24 hours, dehydrated in a series of baths of increasing concentrations of ethanol, clarified in xylene and then included in paraffin. Two slices of 5 µm were selected for each chicken, stained with

hematoxylin and eosin (HE) and examined with an Olympus CX41 microscope. For each field, 3 villi were measured (Kawalilak *et al.*, 2010).

For immunohistochemistry anti-IL-6 (manufacturer: Abbexa; code: abx177189), anti-TNFIP8 (supplier: Antibodies-online GmbH; code: ABIN2707009) and anti-Pax-7 (supplier: Antibodies-online GmbH; code: ABIN 27434880) antibodies were used.

For immunohistochemical staining, the slides were dewaxed with xylene, hydrated using ethanol and exposed to microwaves for 10 minutes at 95˚C in 10 mmol citrate buffer (pH 6), then cooled for 20 minutes, then washed twice in phosphate buffer saline (PBS) for 5 minutes. Further on, the slices were treated with 3% hydrogen peroxide and rinsed with PBS, then incubated overnight at 4˚C in a humid atmosphere with primary antibodies, at dilutions of 1:100 for anti-TNFIP8, anti-IL-6 and 1:500 for antiPax-7. In the next day, the slides were washed 3 times in PBS for 5 min, after which were incubated with the secondary antibodies. Goat anti-rabbit IgG secondary antibody was used to reveal TNFIP8 and IL-6, whereas goat anti-mouse IgG secondary antibody was used for Pax-7. The slides were developed in 3,3'-diaminobenzidine (DAB) and then finally counter-stained with hematoxylin, prior to their microscopic examination. The incubation temperatures and concentrations for the antibodies were set according to the indications stated by the manufacturers in the datasheets of the products.

Birds were periodically weighted and weight gain was calculated.

Results for the morphometric analysis and weight gain were expressed as mean ± standard deviation. One-way ANOVA was employed, followed by Tukey's post-hoc test, in order to assess data variance between groups. The level of significance was set as 0.05.

Regression analysis was performed in order to assess the relationship between the turmeric dose in feed as factor (independent variable) and parameters concerning duodenal histology as response (dependent variables). Linear, quadratic (second degree polynomial) and cubic (third degree polynomial) models were tested and the regression equations, coefficients of determination (R^2) and the standard errors of the estimates were calculated.

IBM SPSS 20 and MS Excel 2007 were used for statistical analysis.

RESULTS AND DISCUSSION

The availability and nature of nutrients within the gut led to intestinal histological changes. The slides with tissue samples from the duodenum were subjected to morphometric analysis and the overall microscopic structure was typical for each section analyzed. Specific to the duodenum is the presence of leaf-like villi and longitudinal, straight crypts. Longer villi are generally the result of cell mitosis, which occurs in crypts, an area where stem cells divide in order to allow for renewal of the villi, a larger crypt area indicating more intense cell production (Samanya & Yamauchi, 2002). The presence of the meander-type structure may be due to the fact that nutrient uptake is more efficient when the villi are arranged in this way, as opposed to the case in which the villi are parallel.

In the current study, the submucosa and muscularis externa had a typical, normal structure (Figure 1).

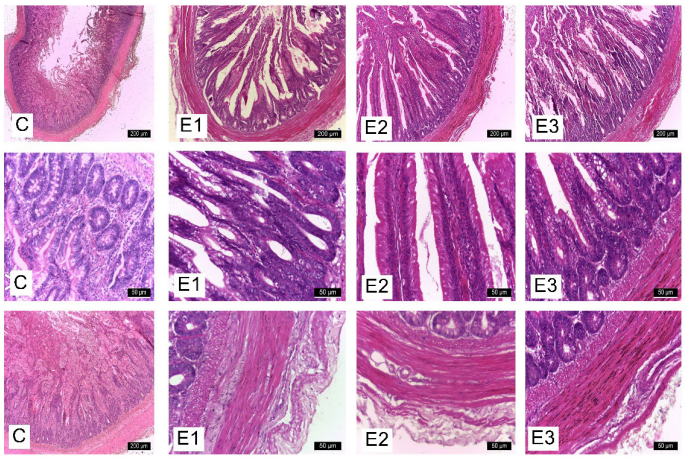

Figure 1 – Histological particularities of the duodenum in chickens from the control and experimental groups (HE staining).

The summarized results of the duodenal morphometric analysis are shown in Table 1. Morphometric parameters that characterize mucosal architecture underwent variations between the control group and the other groups. The morphometric parameters of the duodenal mucosa showed differences between the control group and the experimental groups.

In group E1, by adding 0.5% turmeric powder to the feed, the height of the villi increased by 23.24 % compared to the control group. In the case of group E2 (1% turmeric powder), the mean height of the villi increased by 35.17 % compared to the control group and 9.67 % compared to group E1. Group E3 is the only group in which a deepening of the intestinal crypts is observed, unlike group E1 or E2, where a decrease in the mean crypt depth is observed (Figure 1). Turmeric powder supplementation tended to reduce crypt depth.

The best villi height: crypt depth ratio was identified in group E2. In case of the control group, fed only with

the basic diet, a much lower ratio of villi height to crypt depth was observed.

The villi height to crypt depth ratio is an indicator of the digestive capacity of the small intestine. An increase in this ratio corresponds to an increase in digestion and absorption. An increase in the height of the intestinal villi and the ratio of their height to crypt depth is an indicator of intense nutrient absorption area and better absorption function.

The largest diameters of the duodenal villi were identified in group E3, by adding 2% turmeric powder in the diet. Differences in the diameter of the duodenal villi between groups E1, E2 and E3 can only be observed at the base of the villi. The values increased with the addition of turmeric powder up to 2% in the diet.

In another study, the mean values of villus length, villus width and ileal crypt depth in birds ranged from 943.81 to 1148.20 micrometers (villi length), 192.42 to 213.29 micrometers (villi width) and 184.65 to 213.79 micrometers (crypt depth), respectively. There was a significant increase (p<0.05) in the mean values of villi length, from 947.94 micrometers in the control group to 1145.77 micrometers in the treatment group fed with the highest level of turmeric powder (1.0%) (Kosti *et al.*, 2018).

Some studies have already shown that the ileum was the most responsive site, where villi changed significantly with nutritional changes or an increase in nutrient content in the intestinal lumen was observed (Yamauchi *et al.*, 1992).

The results of the experiment conducted by Kosti *et al.* (2018) showed that crypt depths also increased with villi height. There was a significant (p<0.05) increase in crypt depth from 189.32 micrometers in the control group to 213.79 micrometers in the highest dose in the experimental group (1.0% turmeric powder). Thus, it can be observed that the inclusion of turmeric powder in the diet resulted in an improvement in the villi length, villi width and crypt depth in the gastrointestinal tract of birds.

As shown in Table 2, the relationship between the turmeric dose added to feed and the villi height, as well as crypt depth, is best described by a quadratic regression model, based on the value of R^2. The relationship between the turmeric dose added to feed and the villi height to crypt depth ratio, as well as the diameter of the intestinal villi is best described by a cubic regression model, based on the value of R^2. In all cases, the coefficients of determination (R^2s) are relatively low, so the proposed regression models partially predict the outcome.

Supplementation of the diet with turmeric powder also positively influenced weight gain (Table 3). The highest increase in body weight was observed for

Table 1 – Effect of adding different levels of turmeric (*Curcuma longa*) in broilers diet on duodenal histology (mean ± standard error).

Group	Diameter of duodenal villi (at base) (µm)	Villous height to crypt depth ratio	Villi height (µm)	Crypts depth (µm)
C	139.65 ± 40.14^{a}	6.52 ± 0.32	880.33 ± 73.14^{ab}	135,33 ± 13.57
0.5 % turmeric (E1)	131.47 ± 38.98^{b}	8.71 ± 2.00	1085.00 ± 79.56^{a}	128.33 ± 24.58
1 % turmeric (E2)	155.25 ± 26.13	10.09 ± 1.50^{a}	1190.00 ± 8.54^{bc}	119.66 ± 17.03
2 % turmeric (E3)	199.51 ± 60.99^{ab}	5.62 ± 2.06^{a}	980.66 ± 130.90^{c}	187.66 ± 56.63

$^{a, b, c, d}$ – within a column, means with the same letter differ significantly at 0.05.

Table 2 – Regression analysis between levels of added turmeric (*Curcuma longa*) in broilers diet and parameters concerning duodenal histology.

Independent variable	Dependent variable	Regression model	Regression equation	R^2	Standard error of the estimate
Turmeric level in diet	Villi height	Linear	$Y = 34.21X + 1004.06$	0.043	130.15
Turmeric level in diet	Villi height	Quadratic	$Y = -254.03X^2 + 560.41X + 877.05$	0.902	43.83
Turmeric level in diet	Villi height	Cubic	$Y = -40.11X^3 – 139.16X^2 + 488.94X + 880.33$	0.869	45.96
Turmeric level in diet	Crypts depth	Linear	$Y = 27.21X + 118.93$	0.215	34.82
Turmeric level in diet	Crypts depth	Quadratic	$Y = 37.72X^2 – 50.93X + 137.79$	0.369	31.22
Turmeric level in diet	Crypts depth	Cubic	$Y = 30.11X^3 – 48.50X^2 + 2.72X + 135.33$	0.308	32.69
Turmeric level in diet	Villous height to crypt depth ratio	Linear	$Y = -0.62X + 8.28$	0.044	2.36
Turmeric level in diet	Villous height to crypt depth ratio	Quadratic	$Y = -3.79X^2 + 7.23X + 6.38$	0.626	1.55
Turmeric level in diet	Villous height to crypt depth ratio	Cubic	$Y = -1.59X^3 + 0.77X^2 + 4.39X + 6.51$	0.637	1.62
Turmeric level in diet	Diameter of duodenal villi	Linear	$Y = 33.07X + 127.52$	0.251	43.37
Turmeric level in diet	Diameter of duodenal villi	Quadratic	$Y = 18.83X^2 – 5.94X + 136.94$	0.280	43.09
Turmeric level in diet	Diameter of duodenal villi	Cubic	$Y = -33.06X^3 + 113.51X^2 – 64.85X + 139.65$	0.289	43.40

the 1-14 days interval in broilers that received 0.5% turmeric powder (group E1). At the end of the experiment, it was observed that the addition of 1% turmeric powder in the diet had the best effects, which is in agreement with literature data (Mondal *et al.*, 2015).

The significantly increased body weight in the group fed 0.5% turmeric powder may be due to the optimal antioxidant activity of turmeric. This level could stimulate protein synthesis due to the bird's enzyme system. One study showed that the inclusion of 0.5 or 1% turmeric powder resulted in significant improvement in body weight gain and feed conversion ratio compared to the control group (p<0.05) (Al-Sultan & Gameel, 2004).

The significant effect of turmeric powder on body weight by day 28 was consistent with the findings of a previous report (Namagirilakshmi *et al.*, 2010). They had found that inclusion of turmeric at the rate of 5g/kg significantly increase body weight of broiler.

Another study also showed that chickens fed with turmeric did not significantly increase in weight compared to the control group (Emadi *et al.*, 2006). Negative effects on feed intake, live body weight and feed conversion ratio were observed by Abbas (2010) when turmeric was fed to birds at levels of 2.0 g/kg, 5.0 g/kg (Yamauchi *et al.*, 1992), 10.0 g/kg and 30.0 g/kg.

At the end of the 42-day experiment, 1% curcumin in the diet resulted in the highest body weight.

Table 3 – Effect of adding different levels of turmeric (*Curcuma longa*) in broilers diet on weight gain (mean± standard error).

	Time (days interval)				
	1-14	15-28	1-28	28-42	1-42
Control	302.50±7.04[a]	776.20±35.51	1079.00±41.70	1590.00±43.20[a]	2668.70±44.10[a]
0.5 % turmeric (E1)	358.10±10.64[a]	920.70±14.34	1279.00±20.73	1720.00±32.00[a]	2998.80±32.30[a]
1 % turmeric (E3)	325.40±11.96	811.50±15.35	1137.00±27.19	1800.00±35.20[a]	2936.90±38.40[a]
2 % turmeric (E3)	336.50±8.32	814.80±5.57	1151.00±14.18	1680.00±20.04	2831.30±22.90

[a, b, c, d] – within a column, means with the same letter differ significantly at 0.05.

Inclusion of more than 50 g/kg feed of turmeric powder is not recommended, in order to avoid induction of hyperemia and infiltration of the parenchyma and portal space with mononuclear cells (Al-Sultan & Gameel, 2004).

In the current experiment, the presence of IL-6 is positive in the control and E3 groups.

Presence of TNFα is more intense in the control, E1 and E3 groups and moderate in the E2 group. Pax-7 staining is positive in all groups (Figure 2).

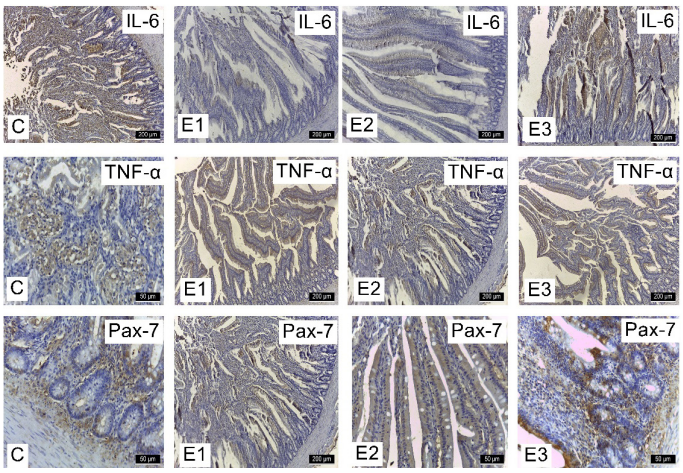

Figure 2 – Presence of IL-6, TNFα and Pax-7 in the duodenum of chickens from the control and experimental groups.

Broilers are exposed to more than one stressor during animal farming. Inflammation could directly lead to compromised intestinal integrity (Olkowski *et al.*, 2006). This contributes to impaired digestion and nutrient absorption. In addition, inflammation and gut damage will lead to an increase in nutrient consumption, in order to alleviate the inflammatory status and repair the gut. This cascade of events is responsible for reduced animal performance and severe economic losses due to disease (Lu *et al.*, 2014).

Cytokines are proteins secreted by cells that play an important role in activating and regulating other cells and tissues during inflammation and immune response. In recent years, advances in avian immunology and genetics have led to the discovery of a number of cytokines, mainly in chickens but also in turkey and other avian species.

IL-6 is a multifunctional cytokine that has proinflammatory activity through induction of acute phase protein synthesis and is important in the development of adaptive immune responses leading to differentiation of B lymphocytes, cytotoxic T cells and T cell growth (Kaiser *et al.*, 2000).

In this experiment, positive staining for IL-6 was observed in the control and E3 groups, suggesting the existence of an inflammatory process. Turmeric powder reduced inflammation, thus in the E1 and E2 groups immunostaining was negative for IL-6. In

another experiment, Alphamune® (an innovative dietary supplement rich in beta glucans) significantly reduced IL-6 expression in the ileum on both day 21 and day 42, compared to the negative control group. Alphamune® contains β-glucans which have been shown to reduce the expression of pro-inflammatory cytokines such as IL-4, IL-6, IL-8 and IL-18 (Yoon *et al.*, 2009).

Yoon *et al.* (2009) showed that essential oils significantly reduce the production of IL-6 and TNFα. In the current experiment it was observed that TNFα presence is reduced in the E2 group and higher in all other groups, which can imply that turmeric powder reduces its expression. In agreement with the results of another study (Lu *et al.*, 2014), in which various preparations with antioxidant effects were used, in the current experiment, by adding turmeric powder in the diet, a reduction in the expression of IL-6 in the intestine was observed; the best results were observed for a level of 1 % turmeric powder.

A previous study (Al-Sadi *et al.*, 2016) has shown that proinflammatory cytokines, including tumor necrosis factor TNF-α, IL-1β, IL-6 and interferon-γ, cause an increase in intestinal junctional permeability and contribute to the inflammatory process, by allowing luminal antigenic penetration.

Tumor necrosis factor TNF-α, a key mediator of intestinal inflammation, causes an increase in intestinal epithelial tight junction permeability, through activation of the myosin light chain kinase gene (MLCK; official name MYLK3). It is also a pleiotropic cytokine that plays critical roles in host defense and in acute and chronic inflammation. Tumor necrosis factor TNF-alpha-induced protein 8 (TNFAIP8/TIPE) family of proteins are known to be involved in the maintenance of immune homeostasis. Deficiency in these proteins increased splenic CD4+ cell levels, serum cytokine levels (IL-17A, TNF and IL-6) and expression of active caspase-3 in the small intestine, while expression of the cell survival factor Ki-67 was significantly decreased in small intestinal epithelial cells, suggesting that TNFAIP8 (TNF) might be involved in gastrointestinal tract pathology. However, the exact function of TNFAIP8 in regulating the immune response in chronic inflammatory diseases is still unknown. The biological role of modulating inflammation, altering the immune response and regulating cell survival or death appears to be dependent on the cellular context and disease (Yoon *et al.*, 2009).

The mediated signaling also plays an important role in maintaining the colonic epithelial barrier and wound healing. The signaling promotes cell proliferation and wound repair, by activating epithelial Wnt/β-Catenin signaling, which is an important pathway for maintaining intestinal villi/ crypt architecture (Flanagan *et al.*, 2018).

In general, elevated levels of TNFα are induced during inflammation or infection, possibly perpetuating intestinal inflammation. In the present study, by adding turmeric powder to the diet, a reduction in the presence of TNFα was observed, which again indicates the positive effects of turmeric powder on the intestinal structure and ultimately on the whole body (Visek, 1978).

Paired box (Pax) proteins 3 and 7 are members of the PAX gene family and are important for the commitment of cells to a myogenic lineage during muscle development or regeneration (Charytonowicz *et al.*, 2014). It has been shown that the Pax genes also play key roles in the formation of tissues and organs during embryogenesis in mammals (Buckingham & Relaix, 2007). In chicken, Pax3 and Pax7 negatively regulate the expression of each gene in the dermomyotome and have essential roles in the development of chick somites and limbs (Galli *et al.*, 2008).

The expression of Pax3/7 and MyoD genes occurs in different bird tissues. Besides skeletal muscle tissues, these three genes are also expressed in the heart, spleen, fat, brain etc. They have coordinative roles in heart, spleen and brain, which is similar with the situation found in skeletal muscle. Moreover, the tissue expression patterns of Pax3 and Pax7 also have differences in other tissues such as gut tissues (Relaix *et al.*, 2005).

In duck, both Pax3 and Pax7 are expressed in breast muscle, heart, spleen, kidney, small intestine, abdomen fat, brain and pancreas, and their expression levels are significantly correlated, indicating the overlapping roles of Pax3 and Pax7 (Vorobyov & Horst, 2006).

Thinner intestinal epithelium improves nutrient absorption and reduces the metabolic needs of the gastrointestinal system. The thinning of the gastrointestinal wall might be due to an inhibition of microbial production of polyamines and volatile fatty acids, known to increase the rate of cellular turnover and enterocyte activity (Ferket *et al.*, 2002). It was found that the diameter of the duodenal villi was significantly larger in birds fed turmeric at 200 mg/kg at 21 days, while at 42 days, the control group and the group to which additionally turmeric was fed at 100 mg/kg showed greater width (Durrani *et al.*, 2006).

CONCLUSIONS

Our experiment demonstrated that the addition of *Curcuma longa* powder at 0.5-1 % in feed of broiler chickens exerted intestinal anti-inflammatory effects, revealed by decreased expression of some interleukins (IL6 and TNFα) and positively influenced weight gain, by increasing the absorption surface of the duodenal mucosa.

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