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MUCIN2 GENE EXPRESSION IN THE CHICKEN INTESTINAL GOBLET CELLS ARE AFFECTED BY DIETARY ESSENTIAL OILS

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Abstract

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The mucous layer that covers the intestinal absorptive surface acts as a diffusive barrier between the intestinal lumen and absorptive cells. In this report, we studied the effect of changing the body weight, intestinal mucin2 gene expression and number of goblet cells by feeding 1-d-old chicks a control diet or a diet containing either an antibiotic growth promoter or dietary essential oils (clove, coriondrum, artemisia sieberi and myrtle) at three levels. On day 42 of age two birds from each replicate were randomly selected, weight and then slaughtered. Goblet cell number was analyzed from each preparation. Mucin2 mRNA expression in intestinal jejunum tissue was quantitated using real-time quantitative PCR. We found that body weights were increased in chicks fed antibiotic and essential oil treatments compared with those fed control diet at 42 days of age ($P<0.01$). Dietary essential oils increased the mucin2 gene expression in the jejunum compared with the control treatment ($P<0.05$). In dietary essential oils fed chicks, the number of goblet cells was decreased in the small intestine compared with control treatment ($P<0.05$). These results indicate that essential oils altered processes of mucin biosynthesis via changes in the intestinal mucin2 gene expression and number of goblet cells. These modifications in mucin dynamics influence gut function and health and may change nutrient uptake.

Key words: essential oil; gene expression; mhucin2
Abbreviations: mRNA – Messenger RNA; PCR – polymerase chain reaction; cDNA – complementary DNA; RT – Reverse transcription

Introduction

The entire surface of the chicken gastrointestinal tract is covered by a layer of mucus that functions as a diffusive barrier between the intestinal lumen and absorptive cells. The mucins are the main component of the mucus layer, which are produced and secreted by goblet cells. The mucus layer is part of the innate host response, protecting against luminal microflora, preventing gastrointestinal pathologies and participating in the processes of nutrient digestion and absorption by Forstner et al. (1995). In humans, mucins are now categorized into three distinct families according to the structure of the protein product which are gel-forming (Mucin2, Mucin5AC, Mucin5B and Mucin6), soluble (Mucin7) and membrane-bound (Mucin1, Mucin3, Mucin4 and Mucin12) (Moniaux et al., 2001). Mucin2 is the major intestinal mucin gene that was initially isolated from a human jejunum cDNA library (Sadasivan et al., 2011).

The addition of antibiotics to broiler diets has long been used to promote growth-however the emergence of antibiotic-resistant bacteria has led to the banning of the use of antibiotics in animal diets in the European Union (Marshall and Levy, 2011). In accordance with these restrictions, the use of phytogenic feed additives, which comprise a wide variety

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of herbs, has recently gained increasing interest. Numerous studies have demonstrated anti-oxidative and anti-microbial efficacy of phytogenic compounds (Denli et al., 2004). In addition, it was hypothesized that phytogenic compounds may specifically enhance the activities of digestive enzymes and promote intestinal mucus production (Moghadam et al., 2011).

Dietary factors (fiber, phytate, protein and anti-nutritional) might affect both the synthesis and secretion of mucin from the goblet cells and the recovery of mucin in digesta (Montagne et al., 2003). Feed deprivation for the first 48h after hatch resulted in delayed development of the mucosal layer and perturbed processes of mucin synthesis and secretion in the small intestine of young chicks (Uni et al., 2003). Changes in mucin secretion and synthesis following interaction with dietary components might be accompanied by changes in the composition of mucin and in mucus properties, including mucus thickness (Montagne et al., 2004).

Thus, four experiments were conducted to determine whether dietary essential oils (*clove, Coriondrumsativum L, Artemisia sieberi and MyrtusCommunis*) alter body weight, the number of small intestine goblet cells and mucin2 mRNA concentration in broiler chicks.

Materials and Methods

Birds and Housing

Experiments took place at the poultry research station at the University of Tehran, Aburaihan Campus, Pakdasht, Tehran, Iran, in 2013. Four separate experiments on 4 essential oils (*clove, Coriondrumsativum L, Artemisia sieberi and MyrtusCommunis*) were conducted. In each experiment, two hundred one day-old Ross 308 male broiler chicks were weighed and randomly assigned to 5 treatments, 4 replicates with 10 birds per cage by a completely randomized design. The birds were kept in 20 cages (1*1.1m) and a photoperiod of 24 h light /d was maintained days 1-3 and 23 h light and 1h of darkness during the trial (42 days of age). The ambient temperature was gradually decreased from 33 to 20°C on day 42. The experimental diets were based on corn-soybean meal with vegetable oil. In each experiment, treatments included a basal diet (Ross 308 recommendation) considered as control, an antibiotic treatment receiving 600 mg/kg recommended level for growth promotion) of flavophospholipol and diets supplemented with essential oil at three levels. Essential oils consisted of clove, coriondrum, *Artemisia sieberi* and myrtle. The ingredients and the composition of control diet are presented in Table1.

Table 1
Composition of basal diet and analyzed contents of main nutrients (%)

Feed ingredients (g/kg)	Starter (1–10 days)	Grower (11–24 days)	Finisher (25–42 days)
Corn, Grain	58.8	59.9	65.3
Soybean Meal-44	35.6	33.6	28.3
Dical. Phos.	1.74	1.50	1.56
Soybean Oil	1.44	2.96	2.81
Calcium CO_3	1.34	1.11	1.15
Mineral Premix	0.25	0.25	0.25
Vitamin Premix	0.25	0.25	0.25
DL-Methionine	0.24	0.16	0.14
Common Salt	0.20	0.20	0.20
L-Lysine HCl	0.15	–	–
Total	100	100	100
Energy (MJ/kg)	12.14	12.64	12.85
Protein	21.12	20.16	18.28
Calcium	1.00	0.86	0.87
Phosphorus	0.48	0.43	0.43
Lysine	1.22	1.06	0.93
Methionine	0.56	0.47	0.42
Met + Cys	0.90	0.80	0.73
Threonine	0.78	0.75	0.67

Dicalcium phosphate contained: 16% phosphorous and 23% calcium. [a]Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; Antioxidant 100 mg. [b] Mineral premix per kg of diet: Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10 mg; I (KI, 58% I), 1 mg; Se ($NaSeO_3$, 45.56% Se), 0.2 mg

Myrtle and coriondrum essential oils were obtained from zardband pharmaceutical company (Tehran, Iran) and *Artemisia sieberi* and clove essential oils were obtained from Barije kashan pharmaceutical company (Kashan, Iran). Chemical composition of these essential oils had been reported by Mohammadi et al. (2014) and Mahmoodi Bardzardi et al. (2014b). The use of essential oils (artemisia sieberi

and coriondrum) chemical composition information was accepted by the authors. The essential oils were mixed with a carrier (soybean oil), which was then added to the basal diet. All diets were prepared freshly every week and diets were in mash form. All chicks were fed starter diets from 0 to10 days of age, grower diets from 11 to 24 days of age and finisher diets from 25 to 42 days of age. At 42 days of age, 10 birds in each cage were weighted and body weight mean values were calculated.

Goblet cell number measurement

On day 42 of age, two birds from each replicate (n= 8 birds/treatment) were randomly selected and then killed by cervical dislocation. Digestive tract was carefully excised. After removing the intestinal contents, approximately 3 cm lengths of duodenum (midpoint of the pancreatic loop), jejunum (midpoint of jejunum) and ileum (5 cm after Meckel's diverticulum) were removed for gut goblet cell number measurement. The Intestinal samples were flushed with physiological saline and fixed in 10% buffered neutral formalin solution (8.25 g/mol). Examination was carried out according to the method of (Iji et al., 2001). Paraffin sections at 6 μm thickness were made from each sample, stained with hematoxylin and eosin, and examined by light microscopy. Goblet cell number was analyzed from each preparation. The number of goblet cells (per 100 μm villus height) was measured using a 25 squared graticule.

RNA Extraction and Reverse Transcription PCR Assay for mucin2 Gene Expression

Total RNA was isolated from the chicken gut jejunum tissue using the RNXTM (Plus) (RN7713C, Cinnagen Inc., Tehran, Iran) according to the manufacturer's instructions. The quantity and integrity of isolated RNA were determined for each sample by using both UV absorbance (260/280) as well as by 1% agarose gel electrophoresis. Then, RNA samples were stored at −80°C until use. Ribonucleic acid was treated with DNase using Ambion's DNA-free kit (Fermentas/Life Science/Isogene Co., Taiwan) to remove any possible DNA contamination. Samples were stored at −80°C until use. Reverse transcription (RT) PCR was performed using a RevertAid first-strand cDNA synthesis kit (Fermentas/Life Science/Isogene Co.) containing RNA (5 μg), 20 pmol of gene specific primer, and diethylpyrocarbonate-treated water. The mixture was incubated at 65°C for 5 min. Then, 5× reaction buffer, 20 U/μL of RiboLockRNase Inhibitor (Fermentas/Life Science/Isogene Co.), 10 m*M*deoxynucleoside triphosphate mix, and 200 U/μL of RevertAid M-MuLV Reverse Transcriptase (Fermentas/Life Science/Isogene Co.) were added to the above mixture. After incubation (42°C, 60 min), the mixture was heated (70°C, 5 min) and then stored at −20°C. Specific sets of primer pairs used are shown in Table 2. Chicken mucin2 primer sequences and 18S primers as an internal control were reported by (Moghadam et al., 2011). All of the PCR products were sequenced.

Table 2
Oligonucleotide primer sequences for reverse transcription PCR amplification in experiments

Primer	(5́-3́) Sequence	Product size (bp)
Mucin2		
Forward	TCA CCC TGC ATG GAT ACT TGC TCA	200
Reverse	TGT CCA TCT GCC TGA ATC ACA GGT	
18S		
Forward	CGA TGC TCT TAA CTG AGT GT	148
Reverse	CAG CTT TGC AAC CAT ACT C	

Mucin2 mRNA Quantitation in gut jejunum Tissue by Real-Time RT-PCR

A master mix containing SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), 10 pmol of forward primer, 10 pmol of reverse primer, cDNA, and water was prepared to perform real-time PCR. The following PCR protocol was used on the LightCycler® 96 Instrument: initial steps were 10 min at 95°C, followed by a 3-step amplification program (15 s at 95°C, 15 s at 60°C followed by 30 s at 72°C) repeated 46 times. The 18S ribosomal RNA was chosen as a reference gene. Each PCR run included a no-template control and replicates of control and unknown samples. Runs were performed in duplicate.

Statistical Analysis

All analyses were conducted using the GLM procedures of SAS. Significant differences among individual group means were determined with Duncan's multiple range test SAS (2002). Relative expression of mucin2 mRNA was determined using the ($2^{-\Delta\Delta Ct}$) method and then analyses were conducted using GLM of SAS.

Results

This is the first report to demonstrate mucin2 gene expression influence by different essential oil levels in broiler chickens. The purpose of these experiments was to extend our studies on the relationship of essential oils with intestinal tissue goblet cell number and mucin2 gene expression in broiler chickens.

Fig. 1. Expression of mucin2 and 18s ribosomal RNA in jejunum in broiler chicken. RT-PCR amplified fragments of mucin2 and 18s ribosomal RNA were separated by gel electrophoresis (1%) and produced a 200 and 148 bp fragment of the mucin2 and 18s RNA, respectively

List a sample name of each lane from left to right: Negative control-antibiotic-control-control- clove-coriondrum- artemisia sieberi- myrtle

Mucin2 mRNA expression in the broiler chicken jejunum was detected (Figure 1). The specificity of the amplified cDNA fragment of chicken mucin2 was verified by agarose gel 1% and sequencing PCR product mucin2 fragment (200 bp).

Broilers of all treatments had a normal intestinal structure. Photomicrograph of a cross section of jejunum full thickness from a control animal is shown in Figure 2.

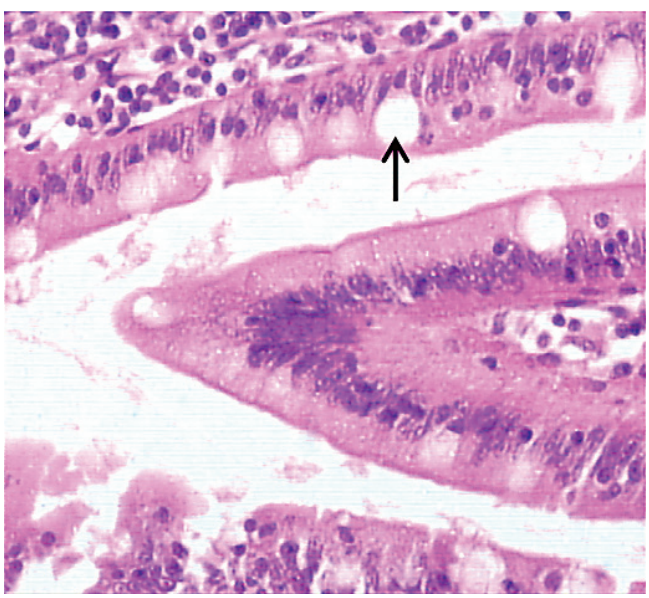

Fig. 2. Photomicrograph of a cross section of jejunum full thickness from a control animal (arrow: indicating Goblet cell as measured) H & E, X 400 (bar= 500 µm)

The major components of clove essential oil are β–caryophyllene (12.2%), eugenol (73.4%) and acethyleugenol (8.99%) Mohammadi et al.(2014) and the major components of myrtle essential oil are α-pinene (30.1%), limonene (20.4%) and 1, 8-cineole (18.1%) Mahmoodi Bardzardi et al. (2014a). The major components of artemisia sieberi essential oil are α-thujone (28.06%), β-thujone (10.56%), camphor (8.37%), borneol (6.92%) and carvacrol (4.38%). The major components of coriondrum essential oil are linalool (67.6%), α-pinene (7.1%), camphor(4.4%) and terpinene gamma (7.2%).

In experiment 1, the results indicated that antibiotic and clove essential oil in the diet at a level of 300 mg/kg increased body weight compared with other treatments ($P<0.01$) (Table 3). Also, dietary clove essential oil at levels of 100, 300 and 500 mg/kg affected the number of intestinal goblet cells and mucin2 gene expression in broiler chickens (Table 3). Dietary clove essential oil at levels of 100 and 500 mg/kg significantly increased mucin2 gene expression compared with control and antibiotic treatments ($P<0.001$).The number of goblet cells of the duodenum, jejunum and ileum significantly decreased for broiler chickens fed the clove essential oil at levels of 300 and 500 mg/kg supplementation compared with control treatment ($P<0.01$).

In experiment 2, dietary coriondrum essential oil at level of 300 mg/kg and antibiotic increased body weight compared with control and coriondrum essential oil at level of 100 mg/kg treatments ($P<0.01$) (Table 4). Also, intestinal mucin-2gene expression was increased in the coriondrum essential oil treatment compared with control and antibiotic treatments ($P<0.0001$). Coriondrum essential oil at levels of 200 and 300 mg/kg decreased the number of goblet cells of the duodenum, jejunum and ileum compared with the control treatment ($P<0.01$) (Table 4).

In experiment 3, the results indicated that antibiotic and artemisia sieberi essential oil in the diet at a level of 100 mg/kg increased body weight compared with the control treatment ($P<0.01$) (Table 5). Also, artemisia sieberi essential oil had the highest mucin2 gene expression at the level of100 mg/kg compared with other treatments ($P<0.0001$). Artemisia sieberi essential oil at levels of 200 and 300 mg/kg decreased the number of goblet cells of the duodenum, jejunum and ileum compared with control and antibiotic treatments ($P<0.001$) (Table 5).

In experiment 4, dietary myrtle essential oil and antibiotic increased body weight compared with the control treatment ($P<0.01$) (Table 6). Also, at the levels of 200 and 300 mg/kg myrtle essential oil mucin2 gene expression was increased compared with other treatments ($P<0.001$) (Table 6). Also, myrtle essential oil at levels of 300 mg/kg in duodenum, 200 mg/kg in jejunum and all levels in ileum decreased the number of goblet cells compared with control treatment ($P<0.01$) (Table 6).

Table 3
Effect of clove essential oil on mucin2 gene expression and the number of goblet cells in intestinal tissue of broiler chicken

Treatments	Mucin2 gene expression	Number of goblet cells Duodenum	Number of goblet cells Jejunum	Number of goblet cells Ileum	Body weight
antibiotic	0.53 ^{c}	8.62^{bc}	8.87^{ab}	7.62^{cb}	2356^{a}
control	1.27 ^{c}	10.62^{a}	10.37^{a}	10.75^{a}	2175^{b}
100mg/kg	15.25^{ab}	8.87^{b}	9.12^{a}	8.62^{b}	2248^{b}
300mg/k	10.34 ^{b}	7.37 ^{cd}	7.62^{bc}	7.62^{bc}	2349^{a}
500mg/kg	32.92 ^{a}	7.12 ^{d}	7.12 ^{c}	6.12 ^{c}	2216^{b}
SEM	0.12	0.43	0.49	0.5	32.4
P value	<0.0001	0.0003	0.0028	0.0002	0.003

^{a,b,c}Means within a column with different superscripts are significantly different (P<0.05)
SEM: standard error of means

Table 4
Effect of coriondrum essential oil on mucin2 gene expression and the number of goblet cells in intestinal tissue of broiler chicken

Treatments	Mwucin2 gene expression	Number of goblet cells Duodenum	Number of goblet cells Jejunum	Number of goblet cells Ileum	Body weight
antibiotic	0.53 ^{c}	8.62^{b}	8.87^{b}	7.62^{cb}	2356^{a}
control	1.27 ^{b}	10.62^{a}	10.37^{a}	10.75^{a}	2175^{b}
100mg/kg	4.43 ^{a}	9.12^{ab}	8.12^{cb}	8.87^{b}	2215^{b}
200mg/kg	3.86 ^{a}	7.62^{bc}	7.37 ^{c}	7.12 ^{c}	2264^{ab}
300mg/kg	3.73^{a}	6.37 ^{c}	7.12 ^{c}	6.37 ^{c}	2354^{a}
SEM	0.11	0.51	0.49	0.46	31
P value	<0.0001	0.0005	0.0012	0.0001	0.002

^{a,b,c}Means within a column with different superscripts are significantly different (P<0.05)
SEM: standard error of means

Table 5
Effect of *Artemisia sieberi* essential oil on mucin2 gene expression and the number of goblet cells in intestinal tissue of broiler chicken

Treatments	Mucin2 gene expression	Number of goblet cells Duodenum	Number of goblet cells Jejunum	Number of goblet cells Ileum	Body weight
antibiotic	0.53^{bc}	8.62^{b}	8.87^{b}	7.62^{bc}	2313^{a}
control	1.27 ^{b}	10.62^{a}	10.37^{a}	10.75^{a}	1999^{c}
100mg/kg	23.20 ^{a}	7.00^{bc}	7.75^{bc}	7.75^{b}	2192^{ab}
200mg/kg	1.33 ^{b}	6.25 ^{c}	6.50 ^{c}	7.00 ^{c}	2037^{bc}
300mg/kg	0.51 ^{c}	6.00 ^{c}	6.50 ^{c}	6.00 ^{c}	2085^{bc}
SEM	0.14	0.56	0.47	0.51	50
P value	<0.0001	0.0002	0.0001	0.0002	0.003

^{a,b,c}Means within a column with different superscripts are significantly different (P<0.05)
SEM: standard error of means

Discussion

The prohibition of antibiotic use in poultry feed has forced investigators to research growth promoting alternatives (Marcinčák et al., 2011). These alternatives are greatly favored in the poultry industry. Essential oils (clove, *Coriondrum sativum L, Artemisia sieberi* and *Myrtus Communis*) are alternatives to growth promoting antibiotics. It was expected that supplementation of diets with herbs would stimulate the growth performance of broilers (Mahmoodi Bardzardi et al., 2014b; Toghyani et al., 2011). The bioactive substances of these herbs may improve feed digestibility, the gut microbial balance and excitation of digestive enzymes and thus improve growth performance in broilers (Ghaza-

Table 6
Effect of myrtle essential oil on mucin2 gene expression and the number of goblet cells in intestinal tissue of broiler chicken

Treatments	Mucin2 gene expression	Number of goblet cells Duodenum	Number of goblet cells Jejunum	Number of goblet cells Ileum	Body weight
antibiotic	0.53 [c]	8.62[b]	8.87[ab]	7.62[b]	2163[a]
control	1.27 [b]	10.62[a]	10.37[a]	10.75[a]	1999[b]
100mg/kg	2.18 [b]	9.00[ab]	8.75[ab]	8.50[b]	2174[a]
200mg/kg	15.38 [a]	9.00[ab]	8.00 [b]	7.50 [b]	2192[a]
300mg/kg	26.67 [a]	8.25 [b]	8.25[ab]	7.00 [b]	2263[a]
SEM	0.09	0.51	0.46	0.45	33.4
P value	0.0002	0.0005	0.0012	0.0001	0.009

[a,b,c]Means within a column with different superscripts are significantly different (P<0.05)
SEM: standard error of means

nfari et al., 2014). These herbs also may improve immunity in host animals and increase availability of nutrients in the intestine for absorption; thereby resulting in animals that grow better and decrease incidence of disease and mortality (Sadeghi et al., 2012).

Our experiments indicate significantly improved body weights of birds fed the diets containing essential oils and antibiotic which agree with results reported by Bassett (2000). He observed that the supplementation of oregano essential oil increased the body weight of birds. Similarly, an improved performance in broilers was reported by Ather and Mujeeb (2000) when using a poly herbal premix and liquid which contained five herbs. Alçiçek et al. (2004) showed that birds fed a diet containing 48 mg essential oil/kg had the best performance, followed by those receiving the diets containing 72 mg essential oil/kg, the antibiotic, the negative control and the 24 mg essential oil/kg at day 42, respectively.

In experiment 1, body weight improvement was not linear. Body weight decreased in broiler chickens fed 500 mg/kg clove oil than using 300 mg/kg clove oil. Emami et al. (2012) reported that 200 mg/kg peppermint oil in broiler's diet improved the performance. But, the treatment with 400 mg/kg did not affect performance. Jouany and Morgavi (2007) reported that essential oils are part of the defense mechanism used by plants against pathogens. An excessive dosage can be toxic to animals or at least induce a negative response. Also, they found a significantly lowered absorption of alanine from the rat intestine when eugenol and cinnamaldehyde of clove with concentrations 850–1000 mg/kg were added to the diet.

In this study, the use of essential oils (*MyrtusCommunis, Artemisia sieberi,* clove and *Coriondrum sativum L*) in poultry feed resulted in an increase in mucin2gene expression in intestinal jejunum tissue in broiler chickens (P<0.05). We indicated mucin2 gene expression only in jejunum. Because of most absorption occurs in the intestinal jejunum. Mucin is the major constituent of the mucus layer and serves a crucial role in protecting the gut from acidic chyme, digestive enzymes and pathogens. In addition to its protective functions, mucin is involved in filtering nutrients in the gastrointestinal tract and can influence nutrient digestion and absorption (Montagne et al., 2004). Carbohydrates, proteins and specific amino acids such as threonine have been demonstrated to alter mucin secretion and may interact directly with goblet cells or with the enteric nervous system to elicit changes in mucin secretion (Horn et al., 2009; Moghadam et al., 2011; Smirnov et al., 2006 and Smirnov et al., 2005). Mucin 2 gene expression enhanced after starvation in chickens (Smirnov et al., 2004). There was no effect of threonine on intestinal goblet cell density or mucin 2 mRNA abundance for broilers (Horn et al., 2009; Moghadam et al., 2011). The expression 5 pattern of the mucin 2 gene in chickens fed antibiotic growth promoter or a probiotic product were greater than the observation in controls (Smirnov et al., 2005). Supplementation of turmeric, thyme and cinnamon to both basal diets increased the expression of mucin 2 mRNA in jejunum of chickens (Kamali Sangani and Masoudi, 2014). Any component, dietary or environmental, that induces changes in mucin gene expression has the potential to affect the integrity of the mucus layer and nutrient absorption. Cytokines, growth factors and bacterial products or any conditions that affect differentiation of goblet cells can also affect mucin2 gene expression. It is possible that bioactive substances of these

herbs may influence the HapA concentration that is an extracellular proteinase and increases secretion and accumulation of mucin 2 gene in the gastrointestinal tract. The bioactive substances also may alter the activity of transcription factors such as GATA4 and Fox1 that regulates mucin 2 gene expression in broiler chickens (van der Sluis et al., 2008; van der Sluis et al., 2004). Carvacrol (thyme extract), cinnamaldehyde (cinnamon extract), and capsicum oleoresin (chamomile) increased mucin seretion in the intestinal villis in broiler chicken (Jamroz et al., 2006).

Our results showed that the number of goblet cells in small intestinal tissue was decreased in the essential oils treatments compared with the control treatment ($P<0.05$). The epithelium of the intestinal tract is covered mainly by a layer of mucus composed of mucin glycoproteins that are synthesized and secreted by goblet cells. Medical plants, through their effects on intestinal morphology such as: bowel wall thickness, surface villous, high and crypt depth, the density and size of goblet cells, affects the synthesis and secretion of mucin. In fact, eugenol in clove extracts and other active ingredients can affect goblet cells, and the growth rate and synthesis and secretion of mucin. In turn, goblet cells compensate for mucin losses by increasing mucin synthesis. Our experiments showed that the number of goblet cells in the small intestine of broilers fed essential oils declined. Also, intestinal mucin2 gene expression increased in broilers fed essential oils. Our results indicate that dietary essential oils help to compensate for decreased mucin secretion. These experiments confirm the hypothesis that by reducing the number of goblet cells, mucin gene expression is increased. Smirnov et al. (2005) showed that feeding dietary probiotics and antibiotic growth promoter supplementation increased the expression of mucin mRNA in the jejunum and ileum compared with controls. The dietary probiotic increased the goblet cell "cup" area throughout the small intestine compared with the other groups. Uni et al. (2003) found that a 48h starvation period increased goblet cell density when compared with fed chicks. Changes in goblet cell density may be indicative of changes in mucin degradation and synthesis. Also, Uni et al. (2003) reported that feed intake significantly increased the percentage of mucin-containing goblet cells, suggesting changes in mucin dynamics. Such changes in the mucus layer have implications for gut protective functions and may affect intestinal nutrient absorption. Smirnov et al. (2006) showed that providing carbohydrates as an energy source to late-term embryos had a trophic effect on the small intestine and enhanced goblet cell development. The mechanisms by which dietary components influence mucin dynamics in developing chicken intestines are poorly understood. Changes in the mucin type and mucin gene expression in goblet cells of the chicken small intestine may be due to the direct and indirect effects of dietary supplementation.

Conclusions

Our results showed that supplementation with essential oils (*MyrtusCommunis, Artemisia sieberi,* clove and *Coriondrumsativum L*) enhanced the mucin2 gene expression in jejunum of broiler chickens and decreased intestinal of goblet cell numbers, and thus it may influence its protective properties and nutrient absorption. Also, we found that body weight was increased in chicks fed antibiotics or essential oils treatments compared with those fed control diets at 42 days of age. More work is needed to demonstrate whether manipulating mucin, through feed or feeding strategies, prevents or enhances gastrointestinal function. This may represent a new interesting possibility for enhancing gastrointestinal function. The challenge is to find nutritional strategies that maximize the protective effect of mucus and minimize the metabolic costs associated with mucin production.

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