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Effect on Feeding Thymolina Powder in The Carcass Characteristics and Morphology of Small Intestine of Ross 308 Broiler Chickens

(PENGIMBUHAN BUBUK THYMOLINA DALAM RANSUM MEMPERBAIKI PERFORMANS DAN MORFOLOGI USUS HALUS AYAM PEDAGING)

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ABSTRACT

This study was performed to elucidate the association effect on feeding thymolina powder in the carcass characteristics and morphology of small intestine of Ross 308 broiler chickens. In the present experiment, 320 one day old Ross 308 broiler chickens were used by using completely randomized design with 4 treatments, 4 replicates, in which each replicate contained 20 broiler chickens. Experimental treatments groups were: control, 0.5, 1, and 2% of Thymolina powder in chickens' diet. Thymolina® is an anti-bacterial powder drug which is made by composing 4 medicinal plants. A significant difference observed among treatments in each parameter of the intestinal morphology (villus height, villus width, crypt depth and epithelial thickness. The number of goblet cells were not affected. Results of this findings indicated that using Thymolina® in the diet of broiler chickens causes improvements in carcass characteristics. Therefore, it may improve the performance and creation of the favorable broiler chickens.

Keywords: broiler; morphology; Ross 308; small intestine; thymolina®

ABSTRAK

Penelitian ini dilakukan dengan tujuan untuk mencari hubungan antara pengimbuhan tepung thymollina dalam ransum terhadap karakteristik karkas dan morfologi usus halus ayam pedaging Ross 308. Pada penelitian ini sebanyak 320 ekor anak ayam umur satu hari digunakan, dan dalam penelitian ini digunakan rancangan acak lengkap, dengan empat perlakuan, empat ulangan, dan setiap ulangan terdiri dari 20 ekor anak ayam. Perlakuan terdiri dari pemberian ransum yang mengandung thymolina 0,5%; 1,0%; 2,0%; dan kontrol. Thymolina adalah bubuk antibakteri yang dibuat dari campuran empat tanaman obat. Perbedaan yang nyata diamati antar perlakuan pada morfologi usus (tinggi villus, lebar villus, kedalaman kripta epitel usus halus ayam perlakuan). Namun. jumlah sel Goblet pada usus tidak terpengaruh. Hasil dari temuan ini menunjukan bahwa pengimbuhan thymolina dalam ransum ayam pedaging menyebabkan perbaikan karakteristik karkas. Simpulan yang dapat ditarik bahwa thymolina dapat memperbaiki performans dan membuat ayam pedaging menjadi lebih nyaman.

Kata-kata Kunci: ayam pedaging, morfologi, Ross 308, usus halus, thymolina®

INTRODUCTION

Medicinal plants, due to having suitable effects on intestinal palatability and performances (Jugl-Chizzola *et al.,* 2006), antibacterial effects (Ozer *et al.,* 2007), wide antioxidant activities (Wei and Shibamoto, 2007), stimulation of the immune system (Roth-Maier *et al.,* 2009) and improvement in nutrient absorption (Windisch *et al.,* 2008) are added to

diets of broiler chickens.

Several studies indicate that medicinal plants result in improving growth and weight gain (Jamroz *et al.*, 2003; Cross *et al.*, 2007 and Hernández, 2004), improving feed conversion ratio (Ciftci *et al.*, 2009), reducing *Escherichia Coli* bacteria and population growth of *Lactobacillus* (Jamroz *et al.*, 2005), preventing fat oxidative corruption (Stoni *et al.*, 2006), intestinal morphological characteristics (Garcia *et al.*, 2007), and antimicrobial activity (Farag *et al.*, 1989). Therefore, in the present study, an antibacterial medicinal plant feed additive called Thymolina® in powder form was used to find the effects on carcass characteristics and small intestin morphology. In making this drug, 4 medicinal plants *Salvia officinalis, Matricaria chamomilla, Teucrium polium* and *Origanum majorana* were composed. Therefore, this study was performed to elucidate the association effect of feeding thymolina powder on the carcass characteristics and morphology of small intestine in Ross 308 broiler chickens.

among IGF-I genotypes, endocrine factors, and egg productivity and to estimate the possibility of selection for improvement of egg productivity by IGF-I genotype in KNOC.

RESEARCH METHODS

Animals, Experimental Design and Procedure

In the present experiment, 320 one day old Ross 308 broiler chickens were used based on a completely randomized design with 4 treatments, 4 replicates, each replicate was a floor pen contained 20 broiler chickens. Experimental treatments include basal diet containing no supplemented Thymolina® (control), basal diet supplemented with 0.5% Thymolina®, basal diet supplemented with 1% Thymolina® and basal diet supplemented with 2% Thymolina® in chickens' diet. Thymolina® (Sinafaravar Spadana Co., Iran) is an anti-bacterial powder drug which is made by composing 4 medicinal plants (*Salvia officinalis, Matricaria chamomilla, Teucrium polium* and *Origanum majorana*). This composition contains 1%active ingredient Thymol (Table 1).

In the present experimental study, the corn-wheat-soybean meal based diet was used to supply chickens' nutritional needs in different breeding periods (Starter (0-15 d), Grower (16-28 d), Finisher (29-42 d)), experimental diets were prepared and regulated based by the UFFDA software, and diets were formulized based on Ross 308 broiler chickens nutritional requirements (Table 2).

Carcasses traits, measured as the amount of abdominal fat and pectoral muscle, at the age 42 days old, from each replicate, 2 chickens with the lowest mean difference in weight were selected, and then they were slaughtered from the cervical vertebrae and dry feather picking methods (Valizadeh *et al*., 2014).

Assessment of Intestinal Morphology

At age 42 days old, from each replicate, 1 chicken having the nearest weight to the average weight of the herd was selected and after 12 hours of food deprivation, they were slaughtered, and as 2 cm samples from the three parts of the intestine, i.e. the duodenum (the middle part of the duodenum), jejunum (the middle part of the jejunum) and ileum (5 cm after Meckel's diverticulum) was taken.

After being washed by soluble phosphate buffered saline (PBS), samples were transmitted into plastic containers containing 6-7 ml 10% formalin. For the preparation of thin tissue slides, the paraffin wax treatment was used.

Table 1. The chemical composition of Thymolina®

Ingredient	*Important constituents*%
Salvia officinalis	camphor (37.17), 1,8 cineole (31.1), á -Thujone (20.34), â-thujene (3.37), borneol (2.02)
Matricaria chamomilla	(*E*)- â -farnesene (24.19), guaiazulene (10.57), á -bisabolol oxide A (10.21), á -farnesene (8.7) and á -bisabolol (7.27)
Teucrium polium	â –caryophyllene (29.5), farnesene-cis-b (11.2), â -pinene (5.2), carvacrol (8.3), bicyclogermacrene (6.4), â-pinene (5.2)
Origanum majorana	Trans-Caryophillene (19.08), Gamma-Cadinene (10.91), Trans-Beta-Farnesene (8.65), Gamma-Terpinene (6.29), Apiol (5.62)

Table 2. Ingredient composition of basal diet

Ingredient (g/Kg unless noted)	Starter (0-15 d)	Grower (16-28 d)	Finisher (29-42 d)
Yellow Corn	441.55	441.52	393.53
Wheat	100.00	200.00	300.00
Soybean Meal	380.98	283.92	232.77
Tallow/animal fat	22.96	23.86	25.23
L-Lysine Hcl	3.21	2.47	2.05
DL-Methionine	2.90	1.95	2.09
DCP	20.19	17.54	15.83
CaCo3	11.28	11.95	11.81
NaCl	2.73	2.59	2.49
Minerals premix*	2.50	2.50	2.50
Vitamin premix**	2.50	2.50	2.50
L-Threonine	2.80	2.80	2.80
Zeolite	2.00	2.00	2.00
Sodium bicarbonate	4.40	4.40	4.40
Total	1000	1000	1000
Analyzed composition			
ME, kcal/kg	*3025*	3150	3200
Crude protein,%	22	21	19
Calcium,%	1.05	0.9	0.85
P available,%	0.5	0.45	0.42
Methionine,%	0.51	0.45	0.41
Lysine,%	1.43	1.24	1.09
Methionine + Cystine,%	1.07	0.95	0.86
Threonine	0.94	0.83	0.74

* Mineral premix provided per kilogram of diet, manganese, 55 mg;zinc, 50 mg; iron, 80 mg; copper, 5 mg; selenium, 0.1 mg; iodine, 0.36mg; sodium, 1.6 g.

** Vitamin premix provided per kilogram of diet, retinylacetate, 8,250 IU; cholecalcipherol 1,000 IU; dl-á-tocopherol, 11 IU; cyanocobalamin,0.012 mg; phylloquinone, 1.1 mg; niacin, 53 mg; choline,1,020 mg; folacin, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg.

Morphological experiments were conducted with the Iji *et al.*, (2001) method. Cutting form the paraffin mold was conducted using device microtome (Leitz 1512, Germany). The conducted cuttings had thickness of 6 ìm *and after staining, optical microscope equipped with CCD Camera (Olympus, CX31-P, USA)* was used for measuring parameters.

Statistical Analysis

The experimental data were analyzed using MSTATC software in a completely randomized design and means were compared using Duncan's multiple range test at the significance level 5%.

RESULTS AN DISCUSSION

Carcass Characteristics

The results related to adding different levels of Thymolina® on the characteristics of carcasses of broiler are indicated in table 3.

Intestinal Morphology

The results obtained from measuring intestinal morphological characteristics are represented in table 4. Significant difference was observed among treatments in different parts of the intestine including villi length, villi width, crypt depth, and thickness of the epithelium ($P<0.05$). The maximum height of villi was

Table 3. Effects of Thymolina® on carcass characteristics

parameter	treatment				SEM	P. Value
	T1 (0)	T2 (0.5%)	T3 (1%)	T4 (2%)		
Carcass yield (%)	69.75[ab]	66.07[b]	64.62[b]	70.57[a]	1. 64	0.0287
Abdominal fat (%)	3.687	3.227	3.323	2.987	0.648	0.0913
Breast (%)	37.30[a]	31.72[b]	33.12[b]	26.53[c]	2.49	0.0005

[a,b,c]Means values within a row with different superscripts different significantly ($P < 0.05$).

Table 4. Effects of Thymolina® on intestinal morphology

parameter	treatment				SEM	P. Value
	T1 (0)	T2 (0.5%)	T3 (1%)	T4 (2%)		
Villus Height (VH) (µm)	1845[a]	1667[b]	1983[a]	1658[b]	102.32	0.0019
Villus Width (VW) (µm)	130[a]	94.33[c]	111.7[b]	118.3[b]	6.23	<0.0001
Crypt Depth (CD) (µm)	331.7[b]	501.7[a]	258.3[c]	546.7[a]	30.97	<0.0001
Goblet Cells Number	4.667	5.00	4.333	4.00	0.9718	0.5213
Epithelial Thickness (µm)	45.00[b]	26.33[c]	51.00[a]	26.67[c]	2.81	<0.0001
Ratios of VH:CD	5.577[a]	3.325[c]	7.75[b]	3.050[b]	0.542	<0.0001
Ratios of VH:VW	14.22[b]	17.65[a]	17.795[c]	14.03[a]	0.923	<0.0001

[a,b,c]Means values within a row with different superscripts different significantly ($P < 0.05$).

related to the 0.5% Thymolina® treatment and the minimum one was related to the 2% Thymolina® treatment. The maximum width of villi was related to the control treatment and the least was related to the 0.5% Thymolina® treatment. The maximum depth of crypt was related to the 2% Thymolina® treatment and the minimum one was related to the 1% Thymolina® treatment. The number of goblet cells was not affected and no significant difference was observed among treatments (P>0.05). The most number of these cells was related to the 0.5% Thymolina® treatment and the lowest number was related to the 2% Thymolina® treatment. In addition, there was no significant difference among treatments (P<0.05). The highest villi's length to width ratio was related to the 1% Thymolina® treatment and the lowest one was related to the 21% Thymolina® treatment. In addition, there was significant difference among treatments in this regard (P<0.05).

Prepared sections of the small intestine tissue were stained by Hematoxylin-Eosin are illustrated as follows (figure 1):

Carcass Characteristics

The percentage of carcass yield and pectoral muscle were significantly affected (P<0.05). Experiment groups receivers of medicinal plant of Thymolina® enjoy numerically higher percentage of carcass than the control group. The relative increase in the percentage of carcass can be related to the antibacterial effects of the medicinal plant because according to t Lee *et al.*, (2003), among the deficits of the existence of harmful bacteria in digestive systems, the increase in the breakdown of protein and amino acids by the deamination activity of digestive microbes on consumed proteins and amino acids as well as the increase in their breakdown due to the secretion of substances such as urease enzymes by microbes can be referred to. Therefore, regarding the fact that the use of medicinal plants reduce the intestinal microbial population; therefore, the speed of the breakdown of protein and amino acids in digestive juices reduces and more amount of them are absorbed and stored in the body; as a result, by improving the percentage of organs of carcasses, it causes

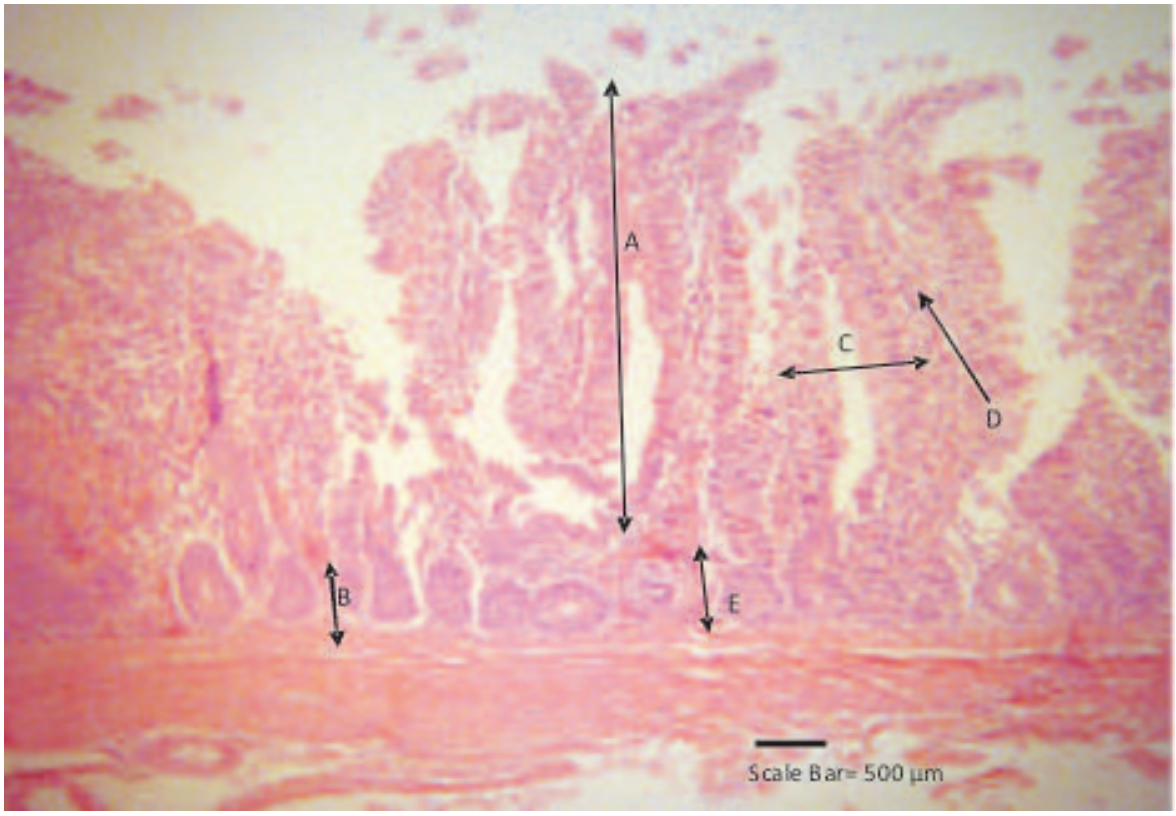

Figure 1. Histological figure of intestinal morphology, (HE, X100,) (Bar=500 ìm)
A: Villus height, B: Crypt depth, C:Villus width, D:Goblet cells number, E:Epithelial thickness

the reduction in the conversion of protein to fat and lower amounts of fat can be accumulated in the body.

According to different researchers, the efficiency of the weight of pectoral muscle and carcass in treatments which used Thymolina® reduced. The reason can be due to the composition of the basic diet (diets with low digestibility), the feed consumption ratio as well as hygiene and environmental standards.

In addition, the use of medicinal plants mainly due to antibacterial effects and effective materials available in them cause the improvement in digestion and absorption efficiency of different nutritional materials such as amino acids and consequently, they causes the improvement in carcass characteristics of broiler chickens.

The results obtained from this experiment is consistent with the results of Alcicek *et al.*, 2003; Bassett, 2000; Jayaprakasha, 2000; but inconsistent with those of Sarica *et al.*, 2005; *Mandal et al.*, 2000. The difference available between the results can be due to the difference in the ratio of using medicinal plants, the type of medicinal plants, the studied chickens, and management conditions.

Intestine Morphology

A significant difference can be observed among groups in villus height, villus width, crypt depth and epithelial thickness of the intestine ($P<0.05$). The highest villi height was related to the 0.5% Thymolina® treatment and the lowest one is related to the 2% Thymolina® treatment. The highest degree of villi width was related to the control treatment and the lowest one was related to the 0.5% Thymolina® treatment. The highest crypt depth was related to the 2% Thymolina® treatment and the least one was related to the 1% Thymolina® treatment. The results obtained from this experiment are consistent with the results of Loddi, 2004; Garcia *et al.*, 2007. The number of goblet cells was not affected and no significant difference was observed among treatments ($P>0.05$). The most number of these cells was related to the 0.5% Thymolina® treatment and the lowest number

was related to the 2% Thymolina® treatment. The results obtained from this experiment are consistent with the results of Haghighi Khoshkhoo (2010).

It seems that Thymolina®, with the reduction of harmful bacteria, the decrease in the production of toxic compounds and harmful intestinal bowel wall, and creating a favorable environment of intestinal villi growth, causes the growth of intestinal villi.

Longer villi cause the prevention of more rapid passage, the lower feces moisture content and lower feed conversion ratio. Therefore, the longer the villi are, the more absorption capacity the small intestine has. Villi height in chickens fed with medicinal plants causes that these compounds reduce the accumulation of pathogenic bacteria in the walls of the small intestine and causes the reduction in the production of toxic compounds by bacteria and causes the conversion in the morphology of the wall of intestines of broiler chickens. As a result, they prevent the destruction and damages to epithelial cells of the intestinal wall. Crypt depth contains specialized cells including absorption and goblet cells. Goblet cells secrete glycoprotein compounds that are known as mucous membranes which protect the intestine from the damages of bacteria and environmental toxins (Forstner, 1978). Therefore, mucous membranes have important roles in protecting the intestine against pathogenic bacteria. The essence of medicinal plants stimulates the activity of intestinal mucosa and pancreatic digestive enzymes (Platel and Srinivasan, 2000). It is likely that the reduction in the depth of crypt in the experiment group was related to the reduction of volatile fatty acids in the intestinal lumen and antibacterial effects. Medicinal plants, by reducing the activity of harmful microorganisms and the reduction in intestine inflammation and causes the reduction in the degree of reconstruction (Walton, 1988). In case of the reduction of goblet cells, it seems that the reduction of the number of these cells probably causes the reduction in the production of mucin, and at last it causes the reduction of wasting indigenous protein.

In general, it seems that effects of medicinal plants on the intestine morphology depend on the equivalence between stimulating tissues and the useful effects on the intestine health. However, some part of the conversion in the activity of digestive enzymes created at the time of using medicinal plants may be due to the increase in the amount of enzyme secretion which is affected by some of the parts of these feed additives during intestinal stress (Steiner, 2009).

CONCLUSION

Results indicated that using Thymolina® in the diet of broiler chickens causes improvements in carcass characteristics and Morphology of Small Intestine. Therefore, it can be effective on the carcass characteristics and Morphology of Small Intestine of broiler chickens and so may cause the improvement in performance. In general, the results of experiments of the present study indicated the use of Thymolina® as herbal additives which May replace the growth promoting antibiotics without side effects.

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