Effect of feeding Thymolina® powder on the gene expression IGF-1 in Ross 308 broiler chickens

S.M. Hosseini[1], M. Chamani[1]*, A. Seidavi[2], A.A. Sadeghi[1] and Z. Ansari-Pirsaraei[3]

[1] Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran, [2]Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran, [3]Department of Animal Science, Faculty of Animal and Fishery Sciences, Agricultural Science and Natural Resources University of Sari, Sari, Iran

*Corresponding Author: Email- m.chamani@srbiau.ac.ir

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Abstract

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, and each replicate contained 20 broiler chickens. Experimental treatments include 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. In addition, to the gene expression of IGFI, the same birds were selected and at the end of the period, the amount of 1-2 ml blood were taken under the wing of venous vessel, and then the investigation of the amount of the gene expression of IGF-1 was conducted using specific primers® GEN, and Real - Time PCR Reaction. The gene expression of IGF-1 increased and significant differences among different treatments were observed ($P<0.05$). In general, results indicated that using Thymolina® in the diet of broiler chickens causes improvements in the immune system by increasing the gene expression of IGF-1. Therefore, it can be effective on the immune responses of broiler chickens and cause the improvement creation of the favorable and effective immune system in broiler chickens.

Keywords: Broiler; Gene expression; IGF-1; Ross 308; Thymolina.

Introduction

There is currently considerable controversy regarding the use of antibiotics as growth promoters in poultry production, what has led to restriction or even a complete ban of these substances in some countries. Considering the simple removal of antibiotic growth promoters might have a negative economic impact, search for alternative additives has been incentivized. Nutritional products used as additives/supplements to bulk feedstuffs (e.g. grains, oilseeds, forage, etc.) are meant to improve performance, or in certain cases to cure nutritional deficiency and/or metabolic disorders. It has, however, been established that phytogenic feed additives can be used with similar efficacy, since they are capable of influencing important physiological processes in the animal organism **(**Günther, 1990): Intensification of the impulses sent by the taste and smelling-nerves in the nasal cavity area towards the central nervous system, Increasing the secretion of digestive juices, e.g. saliva, gastric juice, gall, pancrease and intestinal secretion, Intensification of the activity of digestive enzymes in the gastro-intestinal area, Increasing nutrient absorbtion by activating the transport mechanisms, Inhibition of oxidation-processes of intermediary metabolism, e.g. amino acids, Inhibiting the growth of bacteria and fungi within the alimentary-tract and stabilization of the microbial flora, Inhibition of mould growth on feedstuffs (fungicide effect).

The insulin-like growth factors (IGFs) are proteins with high sequence similarity to insulin. That has an important functional in growth and development different tissue and transcribe. IGFs are part of a complex system that cells use to communicate with their physiologic environment. IGF-1 consists of 70 amino acids in a single chain with three intermolecular disulfide bridges. Insulin-like growth factor 1 (IGF-1) is mainly secreted by the liver as a result of stimulation by growth hormone (GH) (Abdolmohammadi, 2008). Researchers in previous studies demonstrated the positive effects of IGF-1 on growth performance in broiler chickens (Lazar, 1993; McNabb and King, 1993; Huybrechts *et al.,* 1985). Today, some feed additives such as phytogenic extracts and probiotics are used as growth promoters in poultry nutrition (Abdulkarimi *et al.,* 2011; Al-Kassie, 2009; Lee *et al*., 2004; Peric *et al*., 2010).

Generally, the studies reviewed herein support the notion that phytogenic compounds could serve as natural non-antibiotic growth promoters in broiler nutrition. The efficacy of phytogenic applications in broiler nutrition depends on many factors such as composition and feed inclusion level of phytogenic preparations, bird genetics, overall diet composition and overall farm management. To improve the efficiency and profitability of animal production by means of animal feeding (nutrition) remains to be an economic priority. To achieve these goals, by virtue of their biologically active principles, several essential oil plants can offer a natural and healthy alternative. Therefore, in the present study, an antibacterial medicinal plant called Thymolina® in powder was used. In making this drug, 4 medicinal plants *Salvia Officinalis, Matricaria Chamomilla, Teucrium Polium* and *Origanum Majorana* were composed. The aim of this study was to evaluate the potential of Effect of Feeding Thymolina® Powder on the Gene Expression IGF-1 in Ross 308 Broiler Chickens.

Materials and 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).

Table 1. The chemical composition of Thymolina®

Botanical name	*Important constituents*%
Salvia officinalis	camphor (37.17), 1,8 cineole (31.1), α -Thujone (20.34), β-thujene (3.37), borneol (2.02)
Matricaria chamomilla	(*E*)- β -farnesene (24.19), guaiazulene (10.57), α -bisabolol oxide A (10.21), α -farnesene (8.7) and α -bisabolol (7.27)
Teucrium polium	β –caryophyllene (29.5), farnesene-cis-b (11.2), β -pinene (5.2), carvacrol (8.3), bicyclogermacrene (6.4), β-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)

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).

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	2.296	2.386	2.523
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-α-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.

Quantitative Real-Time PCR Analysis

To investigate the gene expression of IGF-1, a bird was selected from each replicate and at the end of the period, as 1-2 ml of blood samples were taken from the venous vessel under the wing. Samples were transferred to the laboratory immediately after being coded and were kept there in the temperature -20 C° until DNA extraction. DNA extraction was conducted using the modified salting-out method developed by Miller *et al.*, (1998). To extract RNA, Accuzol TM (Cat. No. K-2102, Korea BIONEER) kit was used. To normalize data and also delete the effect of possible errors, the housekeeping gene (Beta-actin) was used. To design IGF-1 gene-specific primers, firstly the full sequence and length of the cDNA fragment was extracted from the NCBI website. Then, using the Online Primer 3 software program, for amplification of fragments with a length of 127 bp, required premiers were designed (Table 3).

Table 3. Primer sequences (5→3) used in real-time PCR

Primer		Sequence	Products (bps)
IGF-1	Forward	GACTATACAGAAAGAACCCAC	127
	Reverse	TATCACTCAAGTGGCTCAAGT	
ß-Actin	Forward	CCGCTCTATGAAGGCTACGC	128
	Reverse	CTCTCGGCTGTGGTGGTGAA	

For making cDNA, Quanti Nova™ SYBR® Green PCR, QIAGEN kit was used. To draw the standard curve, six different dilutions of cDNA were prepared. For each target sample gene, a sample of Beta-actin was considered. Then, the target gene, beta-actin and standards were put in the device Corbett Rotor-Gene® 3000.

Thermal cycling has stages of primary activation (95 °C for five min.), string isolation (95 °C for 15 sec.) and amplification (60 °C for 40 sec.). To ensure the existence of the desired amplified fragments made in PCR, at the end, amplified products were loaded on agarose gel 2% (figure 1).

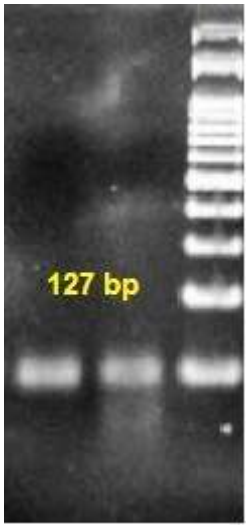

Figure 1. The expression qRT- PCR product on agarose gel 2% and the 135 bp fragment of the IGF-1

To determine the Real-Time quantity, colors such as SYBR®Green was used. Levels of the IGF-1 gene is calculated with Livak's method (Livak and Schmittgen, 2001). In this method, firstly, the mean CT of target genes and beta-actin genes was calculated. Then, the value of ΔC_T ($\Delta C_{T\ (test)}$) was obtained from the difference of CT of target genes from the CT of the beta-actin genes. The first treatment (control 1, without injection) was considered as calibrator and the value of the ΔC_T related to this treatment was subtracted from the value of the ΔCT of other treatments. The obtained value is the $\Delta\Delta C_T$. then, the relative gene expression of IGF-1 with the formula $2^{-\Delta\Delta CT}$ was calculated in the Excel software program. The software was used in the device Real–Time PCR (Rotor – Gene 6000 Series Software 1.7) for primary analysis of the data of the gene expression.

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 and discussion

The results obtained from the gene expression of IGF-1 are illustrated in table 4. The highest degree of the gene expression of IGF-1 was related to the 2% Thymolina® treatment (2.2020) and the lowest was related to the control treatment (0.0478). The results indicate that experiment treatments are influenced and significant difference was observed among treatments (P<0.05).

Table 4. Effects of Thymolina® on Gene expression IGF-1

parameter	treatment				SEM	P. Value
	T1 (0)	T2 (0.5%)	T3 (1%)	T4 (2%)		
IGF-1	0.0478 [b]	0.4927 [b]	0.6933 [b]	2.2020 [a]	0.142585	<0.0001

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

The gene expression of IGF-1 was significantly affected and there was significant differences among treatments (P<0.05). The most degree of gene expression of IGF-1 was related to the 2% Thymolina® treatment and the lowest one was related to the control treatment.

During recent years, phytogenic feed additives have attracted increasing interest as an alternative growth promoter to replace the use of antibiotic feed additives. In principal, the primary mode of action of growth promoting feed additives arises from beneficially affecting the ecosystem of gastrointestinal microbiota through controlling

potential pathogens. This applies especially to critical phases of the animals' production cycle or hygienic disorders of the environment. Sensitive phases for digestive disorders are characterized at the weaning phase of piglets or the early life span of poultry. Due to a more stabilized intestinal health, animals are less exposed to microbial toxins and other undesired microbial metabolites, such as ammonia and biogenic amines. Consequently, growth promoting feed additives relieve the host animal from immune defense stress during critical situations, raise the intestinal availability of essential nutrients for absorption, and thus, assist the animal to grow better within the framework of its genetic potential (Windisch *et al.,* 2008).

IGF-1 is a primary mediator of the effects of growth hormone (GH). Growth hormone is made in the anterior pituitary gland, is released into the blood stream, and then stimulates the liver to produce IGF-1. IGF-1 then stimulates systemic body growth, and has growth-promoting effects on almost every cell in the body, especially skeletal muscle, cartilage, bone, liver, kidney, nerves, skin, hematopoietic cell, and lungs. In addition to the insulin - like effects, IGF-1 can also regulate cell growth and development, especially in nerve cells, as well as cellular DNA synthesis (Yakar *et al*., 2002).

The physiological mechanisms of action of the avian IGFs are slightly different compared to mammals; high amounts of IGFs are present in free form in chickens. Several reports indicate that administration of exogenous IGF exerts negative effects on growth rate and body fat decline (Goodridge *et al.,* 1989; McMurtry, 1998). Florini *et al.,* (1991) reported that muscle determination genes may play a pivotal role in controlling myogenesis as IGF-1 stimulates terminal myogenic differentiation in L6A1 cells by inducing a large increase in expression of the myogenin gene (myogenin mRNA is elevated by IGF-1). IGF leads to a rise in ornithine decarboxylase activity, DNA, RNA and protein synthesis and finally to cell replication. IGF has distinct effects on the differentiation of cells of mesodermal origin; thus, erythroid cells in the mouse, precursors of muscle cells in the chick and precursors of osteoblasts in the rat undergo differentiation in the presence of IGF (Froesch *et al.,* 1986). According to some in vivo studies, IGF-1 infusion is considered more effective in malnourished animals compared to healthy ones, and IGF-1 administration is not thought to stimulate muscle protein synthesis in well-fed mammals and birds (Kita *et al.,* 2002). In the growing chicken, IGF-I mRNA was detected not only in the liver but also in the spleen, lung, brain, kidney, heart, intestine, thymus and muscle.

Conclusion

Results indicated that using Thymolina® in the diet of broiler chickens causes improvements in the immune system by increasing the gene expression of IGF-1. Therefore, it can be effective on the immune responses of broiler chickens and cause the improvement and effective immune system in broiler chickens. In general, the results of experiments of the present study indicated the use of Thymolina® as herbal additives which can be replaced by the growth promoting antibiotics without side effects.

References

1) Abdolmohammadi, A., Moradishahrebabak, M., Mehrabani Yeganeh, H. 2008. Study of Genetic Variation for Four Candidate Genes Using PCR-RFLP and HRM and Their Association with Reproduction and Production Traits in Holstein Cows of Iran. Thesis of Ph.D. University of Tehran.
2) Abdulkarimi, R., Aghazadeh, A., Daneshyar, M. 2011. Growth performance and some carcass charactristics in broiler chickens supplemented with thymus extract (*Thymus vulgaris*) in drinking water. Journal of animal science, 7, 400-405.
3) Al-Kassie, G.A.M. 2009. Influence of two plant extracts derived from thyme and cinnamon on broiler performance. Pakistan Veterinary Journal, 29, 169-173.
4) Florini, J.R., Ewton, D.Z., Roof, S.L. 1991. Insulin-like growth factor-I stimulates terminal myogenic differentiation by induction of myogenin gene expression. Molecular Endocrinology, 5, 718–724.
5) Froesch, E.R., Schmid, C., Zangger, I., Schoenle, E., Eigenmann, E., Zapf, J. 1986. Effects of IGF/somatomedins on growth and differentiation of muscle and bone. Journal of Animal Science, 63, 57-75.
6) Goodridge, A.G., Crish, J.F., Hillgartner, F.B., Wilson, S.B. 1989. Nutritional and hormonal regulation of the gene for avian malic enzyme. Journal of Nutrition, 119, 299–308.
7) Günther, K.D. 1990. Gewürzstoffe können die Leistung erhöhen. Kraftfutter, 73,469–474.
8) Huybrechts, L.M., King, D.B., Lauterio, T.J., Marsh, J., Scanes, C.S. 1985. Plasma concentrations of somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as Determined by heterologous radioimmunoassay. Journal of Endocrinology, 104, 233-239.

9) Kita, K., Shibata, T., Yaman, M.A., Nago, K., Okumura, J. 2002. Response of muscle protein synthesis to the infusion of insulin-like growth factor-1 and fasting in young chickens. Asian Australian Journal of Animal Science, 15, 1760–1764.
10) Lazar, M.A. 1993. Thyroid hormone receptors: Multiple forms, multiple possibilities. Endocrine Reviews, 14, 184-192.
11) Lee, K.W., Everts, H., Beynen, A.C. 2004. Essential Oils in Broiler Nutrition. International Journal of Poultry Science, 3(12), 738-752.
12) Livak, K.J., Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods, 25, 402–408.
13) McMurtry, J.P. 1998. Nutritional and developmental roles of insulin-like growth factors in poultry. American Society for Nutritional Sciences, 128, 302S–305S.
14) McNabb, F.M., King, D.B. 1993. Thyroid hormones effects on growth, development and metabolism. In: The Endocrinology of Growth, Development, and Metabolism of Vertebrates, edited by Schreibman MP, Scacanes CG, Pang PKT (Academic Press) New York, pp: 393-417.
15) Miller, S.A., Dykes, D.D., Paletsky, H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Research, 16(3), 1215.
16) Peric, L., Milosevic, N., Žikic, D., Bjedov, S., Cvetkovic, D., Markov, S., Mohnl, M., Steiner, T. 2010. Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. Archives Animal Breeding, 53(3), 350-359.
17) Steiner, t. 2009. Phytogenics in Animal Nutrition Natural Concepts to Optimize Gut Health and Performance, Erber AG, Austria.
18) Windisch, W., Schedle, K., Plitzner, C., Kroismayer, A. 2008. Use of phytogenetic products as feed additives for swine & poultry. Journal of Animal Science, 86, 140-148.
19) Yakar, S., Rosen, C.J., Beamer, W.G., Ackert-Bicknell, C.L., Wu, Y., Liu, J.L., Ooi, G.T., Setser, J., Frystyk, J., Boisclair, Y.R., LeRoith, D. 2002. Circulating levels of IGF-1 directly regulate bone growth and density. Journal of Clinical Investigation, 110(6), 771–781.