IJBPAS, May, 2015, 4(5): 2458-2467

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF DIFFERENT LEVELS OF ZATARIA MULTIFLORA IN DIET ON MUCOSA MEMBRANOUSHISTOLOGY OF SMALL INTESTINE OF BROILER CHICKS

ALI SHADMANESH[*1] AND ALI AKBARAZADI[2]

1, 2: Department of veterinary, Eghlid branch, Islamic Azad University, Eghlid, Iran

***Corresponding Author: E Mail: shad_39606@yahoo.com; Tel.: 987144552600; Fax: 987144552212**

ABSTRACT

An experiment with fifty day-old broiler chicks in farm of Teheran Azad university research science was conducted to evaluate the effect of different levels of Zataria multiflora on mucous membranous histology of intestine small of broiler chicks. The experimental design was a complete randomized with five treatments. Chicks divided to 5 groups with 10 replications in each pen. Then, a series of iso-caloric and iso-nitrogenous broiler starter and grower diets were formulated in which five percentages (0,1%,1.5%,2% & 5%) of Zataria multiflora. Diets were assigned randomly to every group of chickens and they were under 40 days of experimental diet. The end of trial, chickens weighted and slaughtered. After slaughtering, samples were taken of duodenum, jejunum& ileum sections. Then the samples placed in 10%formalin solution and transmitted to the histology lab of the faculty. Then, in the histology section, the normal stages of fixation, preparation, molting, cutting, coloring (using of E&H method) and paste the coverslip on the slide was performed, and the slides were studied using a light microscope. The height of villi, width of villi, depth of crypt, thickness of sub-mucosa and muscular layer were measured by a linear graticule.

Data obtained in a completely randomized design were analyzed using the SAS 2009.The means were compared by method multivariate of Duncan at the 5% level.In the present study, the result showed that consumption of Zataria Multiflora in doses had not effect significant on the height

of villi and depth of crypt and the ratio of the height of villi to the depth of crypt in the mucous of small intestine chickens (p>0,05).The results showed that body weight gain was not significantly affected by increase in the level of Zataria Multiflora (p>0.05). Somewhat, this indicatesthe lack of any change in intestinal absorption, and can justify the lack of a significant difference in the weight of broilers fed with this plant compared to the control group. In general, it seems that the values used ofZataria Multiflorain this study, at least through effect on intestinal histology does not affect the intestinal absorption.

Keyword: Zataria Multiflora, Intestinal Histology, Broiler

INTRODUCTION

Prohibiting the use of antibiotics, especially in European countries, efforts to appropriate alternatives has been started and also has continuous **(Najafi *et al*., 2010)**. Results shows use of medical vegetables and derivatives of them, by improved feed intake, feed efficiency, poultry health and digestive system, were authored in improved efficiency of poultry production, which is almost comparable with antibiotics **(Hashemi & Davoodi, 2005; Grashron, 2010)**. On the other hand, the phenolic extracts of Zataria multiflora has anti-fungus and anti-bacterial property and also it improved carcass quality in poultry.

Unlike antibiotics stimulus growth, natural feed additives don't reduce microbial load. Against colonization of nocuous bacteria is restrict and change intentional natural flora load and increase active of favorite bacteria in gut. While, antibiotics stimulus growth with reduce of bacteria load can increase growth of broiler by reduce energy and protein requirements for maintenance and growth of intestinal tissue. Maintain intestinal health by natural food additives will be carried out by different mechanisms: changes in intestinal PH, maintaining the protective intestinal mucosa, selection of intestinal useful organisms against pathogenic, increase acidity fermentation, increase receive nutrient and increase security response. Level interior of intestine is circular crimps that covered of villus and the mouth of glands are open in it **(Simon *et al*., 2004)**.

Zataria multiflora is a suffruticose, perennial shrub with 40–80 cm height which belongs to Labiateae (Lamiaceae) family and is known by the common Persian name "Avishane Shirazi". This plant grows wild, on rocky and gravelly slopes, from southern to central parts of Iran and also in Pakistan and Afghanistan. *Z. multiflora* is extensively used in the traditional medicine as antiseptic, analgesic

and carminative **(Zarei Mahmoudabadi *et al.*, 2007)**. It is also a condiment. Recent *in vivo* studies have clearly demonstrated the immunomodulatory effects of essential oil of this plant **(Khosravi *et al.*, 2007; Soltani *et al.*, 2007; Shokri *et al.*, 2006)**. Aerial branches of thyme (Zataria multiflora) is contain at least 6.0 percent essence, fatty acids, β-sytositrol and Betolin. Essence of plant is contain 69%phanol such as Carvacrol, and containing non-phenol composition is such as para-symene. Main constituents inIranian essence are Carvacrol and Thymol, and has some Linalool and para-symene. Plant is contain 29, 25.18, 1.96 and 1.90% respectively **(Rezayian, 2005)**. Carvacrol is main constituent of thyme and Marjoram that its antibacterial and antifungal effects has been proved on various microorganisms **(Mousavi *et al.*, 2010; Kim *et al.*, 1995)**.

Analgesic effects of this herb surveyed on mice and found that it is effective on chronic pain **(Rezayian, 2005)**. Thyme has anti-spasmodic effect on smooth muscle that of the property essence is used for treatment of IBS (irritable bowel syndrome). Also it has an antitussive effect **(Grashorn, 2010;Joven *et al.*, 1994; Katayama *et al.*, 1960)**. According to **Luna *et al.*, (2010)**, Thymol supplementation improved meat quality. Mode of action of thyme can be explained by antimicrobial activity **(Lee *et al.*, 2003)** or an improvement of intestinal nutrient digestibility **(Amad *et al.*, 2011), but Peric *et al.*, (2010)** indicated that stimulatory effects of phytogenic additives on growth performance in broiler chickens are not connected with the gut morphology and cecum microbial concentrations.

Understanding the digestive system of poultry, histological changes associated with the consumption of different foods, its effects on digestibility and intestinal absorption can be modified as a result of diet and help raise poultry production potential. This led us to study the histological changes of the mucosa of the small intestine of broiler chickens by the inclusion of this medicinal herb in diet. Although studies on the use of Thymus vulgaris and its effects on chemical parameters and immune blood has been done, but heretofore, no research has been done for histological. The aim of this study was to evaluate the effects of adding different amounts of Thymus vulgaris in broiler diets on broiler small intestine histological characteristics.

MATERIALS AND METHODS

Birds, Management and Experiment Design

An experiment with fifty day-old Ross broiler chicks in farm of Teheran Azad university

research science was conducted to evaluate the effect of different levels of Zataria multiflora on mucous membranous histology of intestine small of broiler chicks. The experimental design was a complete randomized with five treatment. After ensuring apparently healthy chickens, Chicks divided to 5 groups with 10 replications in each pen. Then, a series of iso-caloric and iso-nitrogenous broiler starter and grower diets were formulated in which five percentages (0, 1%, 1.5%, 2% & 5%) of Zataria multiflora. All of corn-soybean based dietary treatments were formulated to meet the NRC (1994) for starter (1 to 21 days) and grower (22 to 40 days) periods. Diets were assigned randomly to every group of chickens and the duration of the experiment was 40 days.

Sampling Analysis

The end of trial, chickens weighted and slaughtered. After slaughtering, with a distance of 2 cm, samples were taken of duodenum, jejunum & ileum sections. Then, the sample was placed in a jar containing 10% buffered formalin, and the related information of each sample were written on each jar, and samples transmitted to the histology lab of the faculty. Then, in the histology section, the normal stages of fixation, preparation, molting, cutting, coloring(using of E&H method) and paste the coverslip on the slide was performed, and the slides were studied using a light microscope. The height of villi, width of villi, depth of crypt, thickness of sub-mucosa and muscular layer were measured by a linear graticule.

Statistical Analysis

A completely randomized design with 5 treatments was used. Data were analyzed by using the GLM procedures of SAS 9.1 (SAS Institute Inc. 2009). Differences between means were tested using Duncan's multiple range test at the5% level

RESULTS AND DISCUSSION

The results of the duodenum of broilers in **Table 1 and Figures 1 to 5** are shown. The results showed that the length of villi in levels 0.5 and 1% of thyme were increased but this increase was not statistically significant (P>0.05). The highest width of villi was in level 1% of thyme that this increase was statistically significant (P>0.05).

There was no significant difference in depth of crypt between experimental groups and the control (P>0.05). Proportion length of villus to depth of crypt was increased in the experimental group than control but this increase was not statistically significant (P>0.05). There was no significant difference in the submucosa and layer in between the experimental groups (P>0.05).

The results of the jejunum of broilers in **Table 2 and Figures 1 to 5** are shown. The results showed that there were no significant difference in length of villus, the thickness of submucosa and the thickness of muscle between the experimental groups (P>0.05).There was significant difference in width of villi between the experimental group and the control group (P>0.05). Depth of crypt in levels 0.5% and 1% of thyme increased and was statistically significant different with others groups (P>0.05). Proportion length of villus to depth of crypt was increased in the experimental group than control but this increase wasnot statistically significant (P>0.05).

Results from the ileum of broiler chickens in **Table 3 and Figures 1-5** are shown. The results were showed that there were no significant difference in length and width of villi and proportion length of villus to depth of crypt between the control group and others groups(P>0.05). There was no significant difference in depth of crypt, the thickness of submucosa and muscle layer between the experimental groups (P>0.05).

Results of body weight gain of broilers are shown in **Table 4**. The results show that body weight gain was not significantly affected by increase in the level of thyme (P>0.05).

Table 1: Histological parameters (mean ±SD) of duodenum of birds in different groups at the end of the experiment

Parameters groups	Villus length (mm)	Villus width (mm)	Villus length (mm)	Ratio of Villus length To crypt length	Submucosa (mm)	thickness of muscle(mm)
Control	0.17±0.01^{a}	0.02±0.00^{a}	0.02±0.00^{a}	7.11±0.89^{a}	0.01±0.00^{a}	0.02±0.00^{a}
Z.multifora(0.5%)	0.18±0.02^{a}	0.02±0.00^{a}	0.02±0.01^{a}	8.34±2.11^{a}	0.01±0.00^{a}	0.03±0.01^{a}
Z.multifora(1%)	0.18±0.01^{a}	0.03±0.01^{b}	0.02±0.00^{a}	8.76±1.11^{a}	0.01±0.00^{a}	0.03±0.01^{a}
Z.multifora(1.5%)	0.17±0.02^{a}	0.02±0.00^{a}	0.02±0.00^{a}	8.45±1.30^{a}	0.01±0.00^{a}	0.02±0.00^{a}
Z.multifora(2%)	0.17±0.02^{a}	0.02±0.00^{a}	0.02±0.00^{a}	8.27±1.58^{a}	0.01±0.00^{a}	0.02±0.00^{a}

Different superscript letters demonstrate significant difference in a column (p<0.05)

Table 2: Histological parameters (mean ±SD) of jejunum of birds in different groups at the end of the experiment

Parameters groups	Villus length (mm)	Villus width (mm)	Villus length (mm)	Ratio of Villus length To crypt length	Submucosa (mm)	thickness of muscle(mm)
Control	0.16±0.01^{a}	0.02±0.00^{a}	0.02±0.00^{a}	7.30±1.46^{a}	0.01±0.00^{a}	0.02±0.00^{a}
Z.multifora(0.5%)	0.18±0.01^{a}	0.03±0.01^{b}	0.03±0.01^{b}	7.92±2.17^{a}	0.01±0.00^{a}	0.03±0.01^{a}
Z.multifora(1%)	0.18±0.01^{a}	0.03±0.00^{b}	0.03±0.00^{b}	7.83±2.61^{a}	0.01±0.00^{a}	0.03±0.01^{a}
Z.multifora(1.5%)	0.17±0.02^{a}	0.03±0.00^{b}	0.02±0.00^{a}	7.73±1.76^{a}	0.01±0.00^{a}	0.02±0.00^{a}
Z.multifora(2%)	0.16±0.02^{a}	0.03±0.00^{b}	0.02±0.00^{a}	7.54±1.91^{a}	0.01±0.00^{a}	0.02±0.00^{a}

Different superscript letters demonstrate significant difference in a column (p<0.05)

Table 3: Histological parameters (mean ±SD) of ileum of birds in different groups at the end of the experiment

Parameters groups	Villus length (mm)	Villus width (mm)	Villus length (mm)	Ratio of Villus length To crypt length	Submucosa (mm)	thickness of muscle(mm)
Control	0.15±0.01^{a}	0.02±0.00^{a}	0.02±0.00^{a}	7.01±1.34^{a}	0.01±0.00^{a}	0.02±0.00^{a}
Z.multifora(0.5%)	0.16±0.02^{a}	0.02±0.00^{a}	0.02±0.01^{a}	7.44±2.36^{a}	0.01±0.00^{a}	0.03±0.01^{a}
Z.multifora(1%)	0.16±0.01^{a}	0.03±0.01^{a}	0.02±0.00^{a}	7.56±1.47^{a}	0.01±0.00^{a}	0.03±0.01^{a}
Z.multifora(1.5%)	0.16±0.02^{a}	0.03±0.00^{a}	0.02±0.00^{a}	7.37±1.38^{a}	0.01±0.00^{a}	0.02±0.01^{a}
Z.multifora(2%)	0.16±0.03^{a}	0.03±0.00^{a}	0.02±0.00^{a}	7.54±1.91^{a}	0.01±0.00^{a}	0.02±0.00^{a}

Different superscript letters demonstrate significant difference in a column (p<0.05)

Table 4: Comparison average body weight between different groups at the end the experiment

treatments	control	z.multifora(0.5%)	z.multifora(1%)	z.multifora(1.5%)	z.multifora(2%)
Weight(g)	1866.7±57.7[a]	1865.7±67.7[a]	1800±100[a]	1866.3±57.7[a]	1866.2±57.7[a]

Different superscript letters demonstrate significant difference in a column (p<0.05)

Figure 1: Duodenum, jejunum and ileum of broilers fed the control diet (left to right)

Figure 2: Duodenum, jejunum and ileum of broiler chickens fed with thyme 0.5% (left to right)

Figure 3: Duodenum, jejunum and ileum of broiler chickens fed with thyme 1% (left to right)

Figure 4: Duodenum, jejunum and ileum of broiler chickens fed with thyme1.5% (left to right)

Figure 5: Duodenum, jejunum and ileum of broiler chickens fed with thyme 2% (left to right)

DISCUSSION

With study the results of the above table, we know that with increasing levels of Zataria multiflora diet, histological characteristics of cells and intestinal villus height and width change is not significant. In application is not chiefly meaningful, because changes in the duodenal villi and absorption of nutrients is not improved in the intestine, and there was no significant difference in body weight between the control and other treatments. The results of this was adverse with the results **Neyestanak *et al.*, 2011 and Safa & Al-Bietawi (2009)**, and but was almost consistent with results of **Toghyani (2010) and Haftman & wu (2010)**. Inadequate material

medicine in thyme, use method incorrect, density inadequate of thyme in diet and different responses chicks in experiment can be reason Lack of improvement in characteristics upon and efficiency of broiler in this experiment. So far, most research has been done on the effects of herbal medicines on broiler performance, their stimulatory effects on the processes of digestion and absorption, stimulate the secretion of digestive enzymes **(Nasiroleslmi & Torky, 2010)**. many studies have been done on the effects of thyme products on the performance, the histologic features of immune system and antimicrobial effects, that we can mention the following: **Khosravi (2007)** reported that the use of essence thyme enhances the cellular immune responses and humoral in the rabbit. **Shokri (2006)** showed that extracts of thyme can improve the innate immune responses in mice. **Soltani (2010)** showed that extracts of thyme can improve the immune responses in fish.

Shomaly (2013) showed that consumption of Thyme in different dose improves histological parameters of the bursa of Fabricius in chickens. **Mousavi (2010)**, effects of essence thyme and naysin on the growth of *S. typhimurium* were studied in barley soup,and stated that the phenolic substances in essence thyme in affect reaction with the bacterial outer membrane , cause change in the cell membrane, cause disorder in permeability the membrane,and reduces the number of bacteria.

Van Leeuwen (2004), stated that the morphology of the small intestine mucosal layers broilers depends to age, diet formulated and intestinal bacterial flora. They were investigated mucosal layer morphology at different ages with different diets (soybean and wheat and wheat with enzyme) and infected with *Salmonella typhimurium*. And found that the shape of intestinal villi changes by factors upon. One of these changes is an increase villi in the jejunum of poultry fed diets soy bean with enzyme. And size of villi is reduce by broilers infected with *Salmonella typhimurium*. Villi of small intestine increased surface of intestine. Finally, by increasing the villi is increased the intestinal absorption. Also, length villus to crypt depth ratio is another important index to show the level of intestinal absorption. Increasing in length villus to crypt depth ratio is caused increasing absorption rate.

REFERENCES

[1] Amad AA, Männer K, Wendler KR, Neumann K and Zentek J (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler

chickens. *Poultry Science* 90 2811–2816.

[2] Effatpanah, H., Sabokbar, A., Kordbacheh, P., bahonar,A., Bayat, M., &Saeednejad, L.(2010). Antifungal effect of Zataria multiflora: An invitroevaluation.Globalveterinaria, 4(2), 140-143.

[3] Eisemann, (2004). A close-up looks at the small intestines. In: Amino Acid Metabolism Course - NTR 701-001. North Carolina State University, Raleigh, North Carolina.

[4] Grashorn,M.A. (2010). Use of phytobiotics in broiler nutrition – an alternative to infeed antibiotics, Journal of Animal and Feed Science, Vol 19, 338-347.

[5] Hashemi,S.R., and Davoodi. H. (2010). Phytogenic as new class of feed additive in poultry industry, Journal of Animal and veterinary Advances, Vol 9, 17: 2295-2304.

[6] Jafari, S., Amanlou,M., Borhan-Mojabi,K., &Farsam,H.(2003). Comparative study of zataria multiflora extracts with *Myrthus communis* preparation in the treatment of recurrent aphthous stomatitis, Daru.11, 1.

[7] Joven, B.J. (1994). Factors that intract with the antibacterial action of thyme essential oil and its active constituents, J. Appl. Bacteriol, 76, 626-631.

[8] Katayama. T., & Nagai. I. (1960). Chemical significance of volatile components of specie from the food preservative viewpoint IV: Structure and antibacterial activity of some terpens: Nippon: Suisan caktalli., 26, 29-32.

[9] Khosravi, A., Franco, M., Shokri, H. &Yahyarayeat, R. (2007). Evaluation of the effects of Zataria multiflora, Geranium pelargonium, Myrth and Lemon essences on immune system function in experimental animals, Journal of Veterinary Research, 62, 119-123.

[10] Kim. J. M. et al. (1995). Antibacterial activity of carvacrol, citral, geraniol, against salmonella typhimorium in culture medium and fish cubes, J. food. Sci. 60, 1346-1374.

[11] Hoffman. P.D., and Wu. C. (2010). Effect of thymol and thyme oil feed supplementation on growth performance, serum antioxidant levels and cecal Salmonella population in broilers. The Journal of

Applied Poultry Research, 19: 432-443.

[12] Luna A, Lábaque MC, Zygadlo JA and Marin RH (2010). Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. *Poultry Science* 89 366–370.

[13] Mousavi, M., Akhoundzadehbasti, A., Misaghi, A., Jabbarikhameneh, H.,Karim, G., &Zahraeisalehi, M.T. (2010). Survey of effect of A ZATARIA MULTIFLORA BOISS. The Journal of Applied Poultry Research, 20 314-320.

[14] Nasiroleslami, M., and Torki. M. (2010). Including essential oils of fennel (Foeniculumvulgare) and ginger (Zingeberofficinale) to diet and eva;uating performance of laying hens, white blood cell count and egg quality characteristics. Journal of Advances in Environmental Biology, Vol 4, 3: 341-345.

[15] Neyestanak et al., (2011), "The effect of drinking thyme essential oils on performance, energy efficiency and protein in broiler chickens", Journal of Animal Science (research building), No. 52,56-62

[16] Peric L, Milosevic N, Žikic D, Bjedov S, Cvetkovic D, Markov S, Mohnl M and Steiner T (2010). Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. *ArchivTierzucht*53(3) 350-359.

[17] Rezayian, M. (2005), "Histology poultry", Tehran University Press

[18] Safa et al., (2009). The effect of feeding of crushed thyme (Thymus valgaris) on growth, blood constituents, gastrointestinal tract and carcass characteristics of broiler chickens, The Journal of Poultry Science, Vol 4, 2: 100-108.

[19] Shokri, H., Asadi, F., Bahonar, A. R., &Khosravi, A. R. (2006). The Role of Zataria Multiflora Essence (Iranian herb) on Innate Immunity of Animal Model, Iran journal of Immunology, 3 (4):164-168.

[20] Shomali T, Hamedi S, Paryani MR, Mohseni SM, &Farzaneh M. (2013). Effect of Dietary Inclusion of Zataria multiflora on Histological Parameters of Bursa of Fabricius in Broilers, J.World.poult. Res, 3(1):35-37.

[21] Shomali T, Mosleh N, &Hamedi S. (2011). The effects of green tea

powder on performance parameters, D-xylose absorption and jejunum (histology) in broilers, Online J Vet Res., 15 (2): 147-154.

[22] Soltani, M., Sheikhzade, N., Ebrahimzademousavi, H. A. &Zargar A. (2010). Effects of Zataria multiflora essential oil on innate immune responses of common carp (Cyprinuscarpio), Journal of Fisheries and Aquatic Science, 5: 191-19.

[23] Toghyani, M., Tohidi, M., Gheisari, A.B., &Tabeidian .S.A. (2010). Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme asalternative for an antibiotic growth promoter, African journal ofbiotechnology. Vol 9, 40: 6819-6825.

[24] Ultee. A., Kets. E. P. W. & Smid. E. J. (1999). Mechanisms of action of carvacrol on food borne pathogen Bacillus cereus, Applied and environmental microbiology, 65 (10): 4606-10.

[25] VanLeeuwen, P., Mouven, J.M.V.M., Vander Klis, J.D., & Verstegen, M.W.A. (2004). Morphology of the small intestinal mucosal surface of broiler in relation to age, diet formulation, small intestinal microflora and performance, British Poultry Science, 45, 1: 41-48.