JWPR
Journal of World's Poultry Research

J. World's Poult. Res. 3(1): 35-37, 2013

© 2011, Scienceline Publication

Original Article

Effect of Dietary Inclusion of *Zataria multiflora* on Histological Parameters of Bursa of Fabricius in Broilers

Tahoora Shomali[1, *], Somayeh Hamedi[2], Mohammad Reza Paryani[2], Seyed Mehdi Mohseni[3], Mostafa Farzaneh[3]

[1]*Division of Pharmacology and Toxicology, Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*
[2]*Department of Basic Sciences, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran*
[3]*Department of Basic Sciences, Faculty of Specialized Veterinary Sciences, Science & Research Branch, Islamic Azad University, Tehran, Iran*

*Corresponding author's email: Tahoora.shomali@yahoo.com

ABSTRACT

Regarding the remarkable role of bursa of Fabricius as a primary lymphoid organ in poultry, this study aimed to evaluate the effect of long term administration of *Zataria multiflora* as an herbal immunomodulatory agent on histological features of this organ in broiler chickens. To this end, fifty, one-day old chickens were randomly divided into five equal groups and fed with diets contained 0.5, 1, 1.5, and 2% of *Z. multiflora* (experimental groups) or basal diet (control group) for 45 days. On day 46, birds were slaughtered and bursa of Fabricius was dissected immediately. 6µm-thick transverse sections were made and stained with H&E for measuring height of pelicae, follicular width as well as thickness of follicular cortex and medulla using a linear graticule. Number of follicles in plicae was also counted under light microscope. The results showed a dose dependent increase in all histomorphometric parameters due to *Z. multiflora* administration and the highest increase was in the thickness of follicular cortex of birds treated with 2% *Z. multiflora*. In conclusion, dietary inclusion of *Z. multiflora* during the rearing period of broilers, dose dependently affects histological structures of bursa of Fabricius in a way that may enhance its role as a lymphoid organ.

Key words: Bursa of Fabricius; Histology; *Zataria multiflora*; Broilers.

INTRODUCTION

The health risks for birds including the chance of development and spreading of microbial diseases have increased in intensive poultry farming due to large flock sizes in high stocking densities. Different approaches including vaccination and antibiotics are used to reduce this chance. However even these solutions have their own pitfalls, for example vaccination stress or more importantly incomplete protection after vaccination may be problematic. Although it is well documented that antibiotics have beneficial effects on animal growth, performance and health; concerns arise when regarding issues like development of antimicrobial resistant strains and the presence of drug residues in edible tissues. Therefore, extensive investigations are in progress to find alternatives especially for antibiotic use. Manipulation of immune responses by various agents in order to improve immunity has been considered, recently.

Among immunomodulatory agents, those with plant origin have been examined extensively by different investigators and some of them have shown promising results in chickens including *Berberis lyceum* (Chand et al., 2011), bark of *Pinus radiata* (Park et al., 2011), purple sweet potato (Hanieh et al., 2010), garlic and onion (Hanieh et al., 2010) and plum (Lee et al., 2008).

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).

Regarding the immunomodulatory effects of *Z. multiflora*, the aim of the present study was to evaluate the effect of dietary inclusion of different doses of this

To cite this paper: 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's Poult. Res.* 3(1): 35-37.
Journal homepage: http://jwpr.science-line.com/

plant on histological parameters of bursa of Fabricius as a major primary lymphoid organ in broiler chickens.

MATERIALS AND METHODS

Plant, birds and experimental design

Z. multiflora was collected from Shiraz Province, Iran and was identified by the Institute of Medicinal Plants, Tehran, Iran. Aerial parts of the plant were air-dried and then roughly ground. Fifty, one-day old chickens (Arbor Acres) were housed individually in cages in a temperature-controlled room (24±2°C) with 16:8 h light/dark cycle. Each cage was fitted with an individual feeder and a nipple drinker. Birds had free access to food and tap water during the experimental period. Birds were randomly divided into five equal groups and fed with diets contained 0.5, 1, 1.5, and 2% of *Z. multiflora* (experimental groups) or basal diet with the composition mentioned in Table 1 as Control Group for 45 days. On day 46, birds were slaughtered by cervical dislocation and bursa of Fabricius was dissected immediately. All methods used in the study were in compliance with the institutional ethical guidelines for use of animals in research.

Sample preparation and histological evaluation

Samples were fixed in 10% buffered formalin. Routine histological laboratory methods were used and 6µm-thick transverse sections were made by a rotary microtome. A total number of 10 sections were made from each bursal segment of each bird and stained with H&E and studied under light microscope for measurement of height of pelicae, follicular width as well as thickness of follicular cortex and medulla by using a linear graticule. The number of follicles in each plica was also determined. Arithmetic mean of 15 measurements of each parameter per section was calculated.

Table 1. Composition of the basal diet

Composition	Percentage
Corn	50.00
Wheat	13.00
Soybeen	20.00
Barley	7.40
Vitamin[1]	0.25
Minerals[2]	0.25
Dicalcium Phosphate	1.70
Salt	0.30
Shell	7.00
DL-Methionin	0.10

[1]The vitamin supplied per 2.5 kg premix: Vitamin A: 9500000 IU; Vitamin D3: 2000000 IU; Vitamin E: 18000 IU; Vitamin K3: 2000 mg; Vitamin B6: 3000 mg; Vitamin B9 1000 mg; Vitamin B12: 15 mg; Vitamin B1: 1800 mg; Biotin: 100 mg; Vitamin B2: 6600 mg; Vitamin B3: 10000 mg; Vitamin B5: 30000 mg; Cholin Chloride: 250000 mg. [2]The mineral supplied per 2.5 kg premix : Mn: 100000 mg; I: 1000 mg; Fe: 50000 mg; Se: 200 mg; Zn: 100000 mg; Cholin Chloride: 250000 mg; Cu: 10000 mg

Statistical analysis

Data expressed as mean±SD. Data comparisons performed by one-way ANOVA method followed by Tukey's multiple comparison test and differences considered statistically significant at $p<0.05$.

RESULTS

As shown in table 2 a dose dependent increase was observed in all histomorphometric parameters due to *Z. multiflora* administration. The highest increase was in the thickness of follicular cortex of birds treated with 2% *Z. multiflora* which was more than two times the follicular cortex thickness of control group, followed by follicular width, follicular medulla thickness, follicular number per plica and height of plicae, respectively.

Table 2. Histomorphometric parameters (mean±SD) of bursa of Fabricius of birds in different groups at the end of the experiment

Parameter / groups	Pelica height (µm)	Follicular width (µm)	Follicular cortex thickness (µm)	Follicular medulla thickness (µm)	Number of follicles per pelica (µm)
Control	0.62 ± 0.03^{a}	0.16 ± 0.02^{a}	0.04 ± 0.01^{a}	0.12 ± 0.01^{a}	23.33 ± 2.42^{a}
Z.multiflora (0.5%)	0.63 ± 0.04^{a}	0.20 ± 0.03^{a}	0.04 ± 0.01^{a}	0.16 ± 0.03^{a}	23.17 ± 2.40^{b}
Z.multiflora (1%)	$0.67\pm0.03^{a,b}$	0.25 ± 0.02^{b}	$0.05\pm0.01^{a,b}$	0.20 ± 0.03^{b}	33.67 ± 2.31^{b}
Z.multiflora (1.5%)	$0.71\pm0.13^{a,b}$	$0.27\pm0.03^{b,c}$	0.06 ± 0.01^{b}	0.21 ± 0.03^{b}	33.4 ± 4.22^{b}
Z.multiflora (2%)	0.74 ± 0.03^{b}	0.31 ± 0.03^{c}	0.09 ± 0.02^{c}	0.22 ± 0.02^{b}	40.75 ± 3.77^{c}

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

DISCUSSION

The bursa of Fabricius plays a central role in the development of the antibody-producing B-lymphocyte lineage in birds. It consists of more than 10,000 follicles surrounded by connective tissue. Follicular B cells undergo very rapid cell division and the follicles provide an appropriate microenvironment for generating a primary repertoire of antibodies (Mustonen, 2012). Regarding the remarkable role of bursa of Fabricius as a primary lymphoid organ in poultry, this study aimed to evaluate the effect of long term administration (from day one post hatch to slaughter at day 46) of *Z. multiflora* as an immunomodulatory agent on histological features of this organ in broiler chickens. Our data clearly demonstrate that *Z. multiflora* is able to induce dose dependent changes in histomorphometric parameters of bursa of Fabricius; where bursal follicles of birds treated with *Z. multiflora* especially at the highest dose

To cite this paper: 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's Poult. Res.* 3(1): 35-37.
Journal homepage: http://jwpr.science-line.com/

were wider and had thicker cortex and medullary structures. More over; these birds had more follicles in each plica.

At around the time of hatch, the structure of bursal follicles changes and the mature follicle develops. Contact with gut derived contents may be required for normal bursal development after hatch. Cells of the follicle associated epithelium actively transport the contents of the bursal lumen into the lymphoid compartment of the bursa. Since the bursal lumen is connected to the gut lumen by the bursal duct, this provides a mechanism by which the development of bursal B cells after hatch occurs in the presence of materials derived from the gut (Ratcliffe, 2006). So administration of *Z.multiflora* by oral route in our study may have provided a good situation for making the active chemical constituents available to bursal B cells.

It is in the follicular cortex that most cell division occurs after hatch (Ratcliffe, 2006); moreover most peripheral B cells are derived from the follicular cortex (Paramithiotis and Ratcliffe, 1994). Therefore, the obvious increase that was observed in follicular cortex width in our study may be due to higher cell proliferation and/or lower cell migration from cortex to periphery. Regarding this fact that only about 5% of the bursal cells generated each day immigrate to the periphery (Lassila, 1989) and there is clearly no major migration of cells from the cortex to the medulla (Paramithiotis and Ratcliffe, 1994); it is more probable that higher cell proliferation is the cause behind the increased follicular cortex width which was observed in our study, especially when considering higher follicular width and thicker follicular medulla. On the other hand, there are significant levels of B cell death in the bursa, especially after hatch (Motyka and Reynolds, 1991; Paramithiotis, 1995) and we cannot exclude that the increase in above parameters may be due to inhibition of apoptosis of B-lymphocytes by *Z.multiflora* in treated birds.

In conclusion, dietary inclusion of *Z .multiflora* during the rearing period of broilers, dose dependently increases the number and width of follicles and the thickness of follicular cortex and medullary structures of bursa of Fabricius in broilers which may positively affect the ability of bursa of Fabricius for encountering the antigens.

REFERENCES

Chand, N, Durrani FR, Ahmad S and Khan A, 2011. Immunomodulatory and hepatoprotective role of feed-added Berberis lycium in broiler chicks. *J. Sci. Food Agric.*, 91(10):1737-1745.

Hanieh, H, Gerile C, Narabara K, Gu Z, Abe A and Kondo Y, 2010. In vivo immunomodulatory effects of dietary purple sweet potato after immunization in chicken. *Anim. Sci. J.*, 81(1):116-121.

Hanieh, H, Naraba K, Piao M, Gerile C, Abe A and Kondo Y, 2011. Modulatory effects of two levels of dietary Alliums on immune response and certain immunological variables, following immunization, in White Leghorn chickens. *Animal. Sci. J.*, 81(6): 673–680.

Khosravi, A, Franco M, Shokri H and Yahyaraeyat 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(4): 119-123.

Lassila, O, 1989. Emigration of B cells from chicken bursa of Fabricius. *Eur. J. Immunol.*, 19(5):955–958.

Lee, SH, Lillehoj HS, Lillehoj EP, Cho SM, Park DW, Hong YH, Chun HK and Park HJ, 2008. Immunomodulatory properties of dietary plum on coccidiosis. *Comp. Immunol. Microbiol. Infect. Dis.*, 31(5):389-402

Motyka, B and Reynolds JD, 1991. Apoptosis is associated with the extensive B cell death in the sheep ileal Peyer's patch and the chicken bursa of Fabricius: a possible role in B cell selection. *Eur. J. Immunol.*, 21(8):1951–1958.

Mustonen, L, Alinikula J, Lassila, O and Nera, KP, 2010. Bursa of Fabricius. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net

Paramithiotis, E, Jacobsen KA and Ratcliffe MJH, 1995. Loss of surface immunoglobulin expression precedes B cell death by apoptosis in the bursa of Fabricius. *J. Exp. Med.*, 181(1):105–113.

Paramithiotis, E and Ratcliffe MJH, 1994. B cell emigration directly from the cortex of lymphoid follicles in the bursa of Fabricius. *Eur. J. Immunol.*, 24(2):458–463.

Park, IJ, Cha SY, Kang M, So YS, Go HG, Mun SP, Ryu KS and Jang HK, 2011. Effect of proanthocyanidin-rich extract from Pinus radiata bark on immune response of specific-pathogen-free White Leghorn chickens. *Poult. Sci.*, 90(5):977-982.

Ratcliffe, MJ, 2006. Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development. *Dev. Comp. Immunol.*, 30(1-2):101-118.

Shokri, H, Asadi F, Bahonar AR and Khosravi AR, 2006. The Role of *Zataria multiflora* essence (Iranian herb) on innate immunity of animal model. *Iran. J. Immunol.*, 3(4): 164-168.

Soltani, M, Sheikhzade N and Ebrahimzadeh Mousavi HA and Zargar A, 2010. Effects of *Zataria multiflora* essential oil on innate immune responses of common carp *(Cyprinus carpio)*. *J. Fisheries Aquatic. Sci.*, 5: 191-199.

Zarei Mahmoudabadi, A, Dabbagh MA and Fouladi Z, 2007. In Vitro Anti-Candida Activity of *Zataria multiflora* Boiss. *Evid. Based Complement. Alternat. Med*., 4(3): 351–353.