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

Tahoora Shomali¹*, Somayeh Hamedi², Mohammad Reza Paryani³, Seyed Mehdi Mohseni², Mostafa Farzaneh³

¹Division of Pharmacology and Toxicology, Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
²Department of Basic Sciences, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran
³Department 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 lycium (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 Labiatae (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
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.

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.

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

Table 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>50.00</td>
</tr>
<tr>
<td>Wheat</td>
<td>13.00</td>
</tr>
<tr>
<td>Soybeens</td>
<td>20.00</td>
</tr>
<tr>
<td>Barley</td>
<td>7.40</td>
</tr>
<tr>
<td>Vitamin 1</td>
<td>0.25</td>
</tr>
<tr>
<td>Minerals 2</td>
<td>0.25</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>1.70</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
</tr>
<tr>
<td>Shell</td>
<td>7.00</td>
</tr>
<tr>
<td>DL-Methionin</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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: 50000 mg; Cholin Chloride: 250000 mg. The mineral supplied per 2.5 kg premix : Mn: 100000 mg; Fe: 50000 mg; Se: 200 mg; Zn: 100000 mg; Cholin Chloride: 250000 mg; Cu: 10000 mg

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Z.multiflora (0.5%)</th>
<th>Z.multiflora (1%)</th>
<th>Z.multiflora (1.5%)</th>
<th>Z.multiflora (2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelica height (µm)</td>
<td>0.62±0.03a</td>
<td>0.63±0.04a</td>
<td>0.67±0.03ab</td>
<td>0.71±0.13bc</td>
<td>0.74±0.03b</td>
</tr>
<tr>
<td>Follicular width (µm)</td>
<td>0.16±0.02a</td>
<td>0.20±0.03a</td>
<td>0.25±0.02b</td>
<td>0.27±0.03bc</td>
<td>0.31±0.03c</td>
</tr>
<tr>
<td>Follicular cortex thickness (µm)</td>
<td>0.04±0.01a</td>
<td>0.04±0.01a</td>
<td>0.05±0.01ab</td>
<td>0.06±0.01b</td>
<td>0.09±0.02c</td>
</tr>
<tr>
<td>Follicular medulla thickness (µm)</td>
<td>0.12±0.01a</td>
<td>0.16±0.03a</td>
<td>0.20±0.03b</td>
<td>0.21±0.03b</td>
<td>0.22±0.02b</td>
</tr>
<tr>
<td>Number of follicles per plica (µm)</td>
<td>23.33±2.42a</td>
<td>23.17±2.40b</td>
<td>33.67±2.31b</td>
<td>33.4±4.22b</td>
<td>40.75±3.77c</td>
</tr>
</tbody>
</table>

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


