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Original Article

EFFECT OF DIETARY INCLUSION OF NETTLE *(URTICA DIOICA L.)* ON HISTOLOGICAL PARAMETERS OF CAECAL TONSILS IN BROILERS

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ABSTRACT

Regarding the remarkable role of caecal tonsils as a basic lymphoid organ in poultry, this study aimed to evaluate the effect of long term administration of nettle *(Urtica Dioica)* 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 nettle (experimental groups) or basal diet (Control Group) for 45 days. On day 46 birds were slaughtered and caecal tonsils were dissected immediately. Six µm-thick transverse sections were made and stained with H&E for measuring villus height and villus basal width at the crypt-villus junction, as well as nodular unit width and height, follicular width and muscular layer width by using a linear graticule. Number of follicles per nodular unit was also determined under light microscope. Villi were more flattened in 1% nettle-treated group as compared to control. Although villi were shorter in other nettle-treated groups, their width was not changed appreciably ($p>0.05$). All groups showed decreased nodular unit width accompanied by increased height which was most prominent in the group fed with 1% nettle. Follicles were wider in all treated groups in comparison with control. Number of follicles per nodular unit showed a remarkable increase in all treated groups especially in 1% nettle-treated group. Muscular layer width decreased in treated groups as compared with control and the 1% nettle treated group had the lowest muscular layer width. In conclusion, dietary inclusion of *Urtica Dioica* during the rearing period of broilers especially at the dose of 1% can enhance histological features of immune structures in caecal tonsils.

Key words: caecal tonsil; histology; *Urtica Dioica*; broilers

INTRODUCTION

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A variety of antibiotics and growth promoters are now banned for use in food animals including poultry due to concerns about development of resistant strains of bacteria which may invade human or animal populations. On the other hand, intensive poultry farming as used today has increased the chance of development and spreading of microbial diseases which necessitates costly measures for prevention and/or treatment of infections. There is increasing global demand for effective and economical natural alternatives for antibiotics. Manipulation of immune responses by various agents in order to improve immunity has been considered as a recent approach. Agents with herbal origin have been examined extensively by different investigators for their immunomodulatory properties and many of them have demonstrated promising effects; as an example Shomali et al., demonstrated that dietary inclusion of *Zataria multiflora* improves histological features of immune structures of bursa of Fabricius in broiler chickens (1). Other examples are *Berberis lyceum* (2), bark of *Pinus radiata* (3), purple sweet potato (4), garlic and onion (5) and plum (6).

Nettle *(Utrica dioica L.)* belongs to the family Urticaceae and is a perennial plant which grows in temperate and tropical wasteland areas around the world. This plant has a long history of use both as a medicine and as a food source. It has been demonstrated that nettle leaf extract has immunomodulatory properties by reducing TNF-α and other inflammatory cytokines (7). Moreover, Gülçin et al., 2004 described a powerful antioxidant activity of water extract of nettle (8). As far as we know, possible immunomodulatory effects of nettle have not been reported in chickens. This motivated us to investigate the effect of inclusion of different doses of aerial parts of nettle in the diet of broilers histological parameters of caecal tonsils which are the largest lymphoid organs of avian gut-associated lymphoid tissue.

MATERIALS AND METHODS

Plant, birds and experimental design

U. Dioica was gathered from north of Tehran province during spring and summer and authenticated by the Institute of Medical 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 *U. dioica* (experimental groups) or basal diet with the composition mentioned in Table 1 as Control Group for 45 days. 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

At the end of the treatment period, all birds were slaughtered by decapitation and proximal part of caeca or caecal tonsils were removed immediately. 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 caecal tonsil of each bird and stained with H&E for measuring villus height and villus basal width at the crypt-villus junction, as well as nodular unit width and height, follicular width and muscular layer width by using a linear graticule. Number of follicles per nodular unit was also determined under light microscope. Arithmetic mean of 15 measurements of each parameter per section was calculated.

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

Clinical observations

No adverse clinical manifestations or mortalities were observed in chickens of different groups.

Histomorphometric parameters of cecal tonsils

As shown in table 2, inclusion of 1% nettle in the diet resulted in a significant decrease in both villus height and lengths as compared to control, in another word, villi were more flattened in this group. Although villi were shorter in other nettle-treated groups, their width was not changed appreciably ($p>0.05$). All groups showed decreased nodular unit width accompanied by increased height which was most prominent in the group fed with 1% nettle. Follicles were wider in all treated groups in comparison with control. Number of follicles per nodular unit showed a remarkable increase in all treated groups especially in 1% nettle-treated group. Muscular layer width decreased in treated groups as compared with control and the 1% nettle treated group had the lowest muscular layer width.

DISCUSSION

The caecum is exposed to continuous and constant invasion of bacterial or non bacterial antigens of extra caecal origin, since it receives the back flowing urine from the urodeum of the cloaca through the colon. Therefore, immunological surveillance against foreign microorganisms is necessary (9). The gut-associated lymphoid tissue in chickens includes organized lymphoid structures such as the bursa of Fabricius, caecal tonsils, Peyer's patch, Meckel's diverticulum, and lymphocyte aggregates scattered along the intra-epithelium and lamina propria of the gastrointestinal tract. In the caecal tonsils, the largest lymphoid organ of the avian gut-associated lymphoid tissue, both T and B cells are present in germinal centers (10). Caecal tonsils, on which nearly half of the lymph nodules are accumulated, are major lymphoid tissue in the avian cecum. Kitagawa et al. (1998) found that 45.7% of lymph nodules are accumulated in caecal tonsils of 6-months old white leghorn chickens (9). These characteristics describe the reason for involvement of caecal tonsils as an immune structure in some important infectious diseases of poultry including New castle disease, influenza *etc.* Therefore, enhancement of immune potential of this lymphoid organ may be beneficial in prevention of related diseases and/or reduction of mortality rates which clarifies the rationality for this study.

It is believed that the interdigitating meshwork of villi at the caecal entrance acts as a filter, excluding large particles and allowing only fluid and fine particles to be separated and pushed from the colonic contents into the caeca by colonic anti peristalsis (11). In the present study, the villi became shorter in all nettle-treated groups which were accompanied by increased villus width in 1% nettle-treated group. This possibly can set the scene for higher exposure of immune structures of caecal tonsils to potential antigens in the gut. On the other hand, thinning of muscular layer in nettle-treated groups which probably results in lower strength for peristaltic movements; may help in longer presence of microorganisms and other antigens in close proximity of immune structures in caecal tonsils. The resultant higher stimulation of immune structures may describe the prominent increase in follicular width as well as number of follicles per nodular unit especially in 1% nettle-treated group. Although this needs to be confirmed by more sophisticated methods including evaluation of cytokine profile and immunoglobulin content as well as possible changes in B and/or T cells population.

As stated previously, the most active dose of nettle in our study was 1%. Higher doses had statistically similar or even lower effects on above mentioned parameters. Compensatory mechanisms may be involved in this situation.

In conclusion, dietary inclusion of *Urtica Dioica* during the rearing period of broilers especially at the dose of 1% can enhance histological features of immune structures in caecal tonsils.

Table 2: Histomorphometric parameters (mean±SD) of cecal tonsils of birds in different groups at the end of the experiment

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

Parameters / Groups	Villus height (mm)	Villus width (mm)	Nodular unit width (mm)	Nodular unit height (mm)	Follicular width (mm)	Follicle number per nodular unit	Muscular layer width (mm)
control	0.12±0.01[a]	0.02±0.0[a]	0.17±0.01[a]	0.25±0.03[a]	0.02±0.03[a]	4.50±1.52[a]	0.08±0.0[a]
U.dioica 0.5%	0.07±0.01[b]	0.02±0.0[a]	0.13±0.03[b]	0.43±0.02[b,c]	0.03±0.0[b]	25.67±2.07[b]	0.06±0.0[b]
U.dioica 1%	0.05±0.0[c]	0.05±0.01[b]	0.13±0.03[b]	0.46±0.02[c]	0.03±0.0[b]	42.17±2.48[c]	0.04±0.0[c]
U.dioica 1.5%	0.07±0.01[b]	0.03±0.0[a]	0.12±0.0[b]	0.38±0.04[b,d]	0.03±0.0[b]	25.00±2.90[b]	0.07±0.00[b]
U.dioica 2%	0.08±0.02[b]	0.02±0.0[a]	0.12±0.01[b]	0.38±0.03[b,d]	0.03±0.0[b]	24.83±3.06[b]	0.06±0.01[b]

Table 1: Composition of basal diet

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

[a]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.

[b]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.

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