

# Asian Journal of **Poultry Science**

ISSN 1819-3609



www.academicjournals.com

#### **Asian Journal of Poultry Science**

ISSN 1819-3609 DOI: 10.3923/ajpsaj.2016.134.140



## Research Article Effect of Certain Phytobiotics on the Immune Response of Newcastle Disease Vaccinated Broiler Chickens

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### Abstract

**Objective:** In this study, the immunopotency of dried coriander seed (Cr), turmeric (Tu) and thyme (Th) powders feed additives in broilers was investigated. **Methodology:** Three hundred, day old broiler chicks were divided into 5 groups, each of 3 replicate and kept for a period of 35 days. A basal diet was used to which Cr, Tu and Th were added either solely at level of 0.75% or in mixture (Mx), while control fed basal diet (C). Haemagglutinating Inhibiting (HI) antibody titre against Newcastle Disease (ND) was measured in sera of all birds. By the end of the experimental period, three birds from each replicate were weighed and killed then bursa (Br), thymus (Thy) and spleen (Sp) were extracted to calculate weight indices and fixed in formol buffer for morphometric measurements. **Results:** The HI titres were higher (p<0.05) in Cr and Tu compared with others. The Br, Thy and Sp weight indices were higher (p<0.05) in all groups than C, while those of Tu, Th and Mx groups were higher (p<0.05) than Cr group. The morphometric measurements were higher (p<0.05) in all treated groups compared to C, while values of Tu were higher (p<0.05) than Th and Cr groups. **Conclusion:** Dried Cr and Tu feed additives had an immunomodulatory effect in broilers.

Key words: Herbal feed additives, broilers, immunity, Newcastle disease vaccine, HI

Received: April 02, 2016

Accepted: May 18, 2016

Published: June 15, 2016

Citation: M.M. Zaki, Wafaa A. Abd El-Ghany, Maha M. Hady and Reda M.S. Korany, 2016. Effect of certain phytobiotics on the immune response of Newcastle disease vaccinated broiler chickens. Asian J. Poult. Sci., 10: 134-140.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

Nowadays, using of alternative phytobiotics feed additives in the form of plant extracts or essential oils for broilers is encouraged due to European banning of using antibiotics as growth promoters<sup>1</sup>. Diseases especially immune-suppressive ones, vaccination failure and misuse of antibiotics cause great financial losses for poultry production all over the world. It was found that natural herbs contain variety of biologically active phytochemicals as flavonoids, terpenoids, lignans, linalool and other essential oils that proved antimicrobial, antioxidant, digestive and immune-stimulatory effects<sup>2-4</sup>. However, lately, the immuno-modulation mechanisms of herbs and their derivatives in poultry have been studied previously with successful results<sup>5-11</sup>.

Coriander (*Coriandrum sativum* L.) is a medicinal plant from the Umbeliferae family and the seeds contain mainly essential oil linalool. Coriander proved potential antibacterial<sup>12</sup>, antioxidant<sup>13</sup> and stimulatory effect of digestive process<sup>14</sup>.

The *Curcuma longa* L. of the family Zingiberaceae named turmeric is an herb. Curcumin a major component in turmeric has a potent antioxidant and anti-carcinogenic activities as well as immune potency effect<sup>15,16</sup>.

*Thymus vulgaris* is a herb in the Lamiaceae family and the major components of thyme essential oil are thymol and carvacrol that possess antioxidant properties<sup>17</sup>, while phenolic constituent shows great antimicrobial activities<sup>18</sup> and improve performance parameters<sup>19,20</sup>.

The effect of herbs either singly or in combination on the bird's immune response was not previously fully studied. Accordingly, the aim of the present study trial was to investigate the immunopotency of dried coriander seed, turmeric and thyme powders feed additives in broilers.

#### **MATERIALS AND METHODS**

**Experimental design:** A total of 300 days old Cobb 500 broiler chicks were divided into 5 groups, each of 3 replicate and reared on floor pens for a period of 35 days. Cobb broiler's specified basal diet were used to which coriander (Cr), turmeric (Tu) and thyme (Th) were added either solely at level of 0.75% or in mixture (Mx) at level of 0.25%, while control birds were fed untreated basal diet (C). Haemagglutinating Inhibiting (HI) antibody titers against Newcastle Disease (ND)

vaccination were measured in sera of all birds to evaluate the humoral immune response. By the end of the experimental period, three birds from each replicate were selected randomly then weighed and marked to determine live body weight. Each bird was slaughtered then bursa (Br), thymus (Thy) and spleen (Sp) were extracted and weighed individually. Tissue samples from extracted organ were fixed in 10% formol buffer to be used in morphometric measurements. The weight indices of Br, Thy and Sp were calculated and subjected to statistical analysis. All the birds were maintained as per the guidelines and approval was taken from the ethical committee of the faculty.

**Lymphoid organs weight index:** The organ weight indexes were calculated according to Zhang *et al.*<sup>21</sup>.

**Postmortem and histopathological examination:** Birds and their organs were subjected to careful postmortem examination and gross abnormalities were recorded. The fixed tissue specimens were processed and embedded in Paraffin wax, sectioned at 4  $\mu$ m and then stained with Hematoxylin and Eosin (H and E)<sup>22</sup>.

**Morphometric measurements:** Histoquantitative studies were performed by counting the number of bursal (Br), thymic (Thy) and splenic (Sp) lymphocytes according to morphometric method of Biljana *et al.*<sup>23</sup>, the test areas of Br, Thy and Sp were three random fields under light microscope, lymphocytes in these fields were counted and then the mean values were calculated for each sample.

**Statistical analysis:** The SPSS 18 software was used to analyze data output from the study, while significant differences among means were detected using LSD described by Snedecor and Cochran<sup>24</sup>.

#### **RESULTS AND DISCUSSION**

Immuno-modulation can be defined as a change (stimulating or suppressing) in the indicators of cellular, humoral and non-specific defense mechanisms<sup>25</sup>. Herbal plants exert immunological effects through their secondary metabolites<sup>26</sup>.

Phytogenic plants proved their immune-stimulatory activity through cytokine production, macrophage activation and lymphocyte activity<sup>27</sup> or anti-inflammatory activity<sup>28</sup>.



Fig. 1: HI antibody titers against ND vaccination

The mechanism of action of phytogenic feed additives as immuno-stimulating agents in poultry needs more focusing and explanations.

Herein, the results of HI antibody titers against ND vaccination (Fig. 1) indicated significant high levels in Cr, Tu and Mx groups compared with control and Th groups, while the titers of Cr and Tu groups were significantly higher than Mx group. No significant difference were detected in HI titers of both Th and control groups. The (Br), (Thy) and (Sp) weight indices were significantly (p<0.05) higher in all groups than control, while those indices were significantly (p<0.05) higher in Tu, Th and Mx groups as compared to Cr group (Table 1). The morphometric measurements of Br, Thy and Sp obtained from the histoquantitative studies (Fig. 2) revealed that all treated groups were significantly higher than control, while values of Tu and Mx groups were significantly (p<0.05) higher than Th and Cr groups (Table 1).

The effect of mixture of different medical plants added to the broilers ration to improve the humoral immune status of the birds was evaluated with successful results<sup>29,30</sup>, whoever, other study of Daneshmand *et al.*<sup>31</sup> reported no significant effect of herbal plants with or without probiotic on immune response to vaccination.

Scarcely or limit reports are available regarding the effect of coriander seed on poultry immunity. Recently, the positive effect of coriander on immune response has been demonstrated already<sup>32</sup>, although Jang<sup>33</sup> could not detect any significant effects on immune parameter. It was observed that supplementation of coriander seed 0.3% to poultry diet significantly improve the mean values of the total serum protein<sup>34,14</sup> and this may accordingly increase the level of circulating antibodies by indirect way.

Curcumin shows several pharmacological effects including anti-inflammatory, antimicrobial, antiviral, antifungal and antioxidant activities<sup>35</sup>. Considering the immuno-stimulatory effect of turmeric, the finding of this study is on accordance with earlier finding of Antony *et al.*<sup>36</sup>

who have observed that curcumin increased the circulating antibody titers against sheep red blood cells in bulb c mice. As well, Kurkure et al.37 detected that turmeric has restored the reduced humoral response of aflatoxicosis induced immune-suppression, thus carry the humoral immune-stimulating potential. More recently, many investigations<sup>38-41</sup> provided that turmeric feed additive had a significant improvement effect on immune organs weight index as well as antibody response against Newcastle disease vaccination in broiler chickens due to its antioxidant activity. Emadi and Kermanshahi42 found that serum immunologlobulins of chickens were affected by addition of different turmeric turmeric powder levels into the feed, correspondingly, IgA and IgM at 21 days of age and IgG at 21 and 42 days of age significantly increased in birds. The potent immuno-modulatory mechanism of turmeric powder on immune response was explained by Jagetia and Aggarwal<sup>43</sup> who demonstrated that turmeric powder can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells and dendritic cells, therefore, elevated antibody titer production and consequently better immune responses were expected.

On the contrary, Mehala and Moorthy<sup>44</sup> recorded that combination of Curcuma longa with Aloe vera plant feed additive reduced the haemagglutination inhibition antibodies titers values against Newcastle disease compared to control. Nouzarian et al.45 found that inclusion of turmeric powder had no significant impact on antibody titer production against ND and influenza viruses. The lack of satisfactory effects of turmeric on immune responses might be related to the inclusion levels of the additive in the bird's ration. Where in rats curcumin at a dose of 10 and 20 mg kg<sup>-1</sup> b.wt. could not enhance the IgG levels in the NK cells, whereas a higher dose (40 mg kg<sup>-1</sup>) did elevate lgG levels significantly, as well none of the three doses of curcumin significantly enhanced either delayed-type hypersensitivity or NK cell activity<sup>46</sup>. The health status of birds, hygienic status of experimental site, external challenges, basal diets composition and digestibility may to a great extent account for the contradictory reports in the literature regarding the immuno-modulatory effect of turmeric powder<sup>45</sup>.

Regarding thyme, it has been demonstrated to have bactericidal and fungicidal effect<sup>47,48</sup> as essential oil as thymol and carvacrol have been reported to possess antioxidant activity and accordingly, elevated immune responses of birds were predictable. In accordance with the results of this study Hosseini *et al.*<sup>49</sup> found that inclusion of ground thyme

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Fig. 2: Morphometric measurement of bursa, thymus and spleen in different groups (H and E 10X), C: Control, Cr: Coriander, Tu: Turmeric, Th: Thyme, Mx: Mixture, Br: Bursa, Sp: Spleen and Ty: Thymus

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	Treatments*			
Aspect Control	Coriander 1	Furmeric	Thyme Mixtur	e organ
Organ weight index				
Bursa 2.80±0.1° 4	3.64±0.26 <sup>bc</sup> 4.	.47±0.20ª	5.05±0.54ª 4.01	$\pm 0.34$ ab
Thymus 4.02±0.25 <sup>c</sup>	5.43±0.46 <sup>bc</sup> 7.	23±0.54ª	7.38±0.50ª 6.58	$\pm 0.47$ ab
Spleen 3.20±0.32 <sup>c</sup>	4.20±0.26 <sup>b</sup> 6.	.04±0.38ª	5.57±0.49 <sup>a</sup> 5.02	±0.29 <sup>ab</sup>
Morphometric measurement				
Bursa 153.00±3.54 <sup>c</sup> 86	1.00±26.87 <sup>b</sup> 366.	.50±3.54 <sup>bc</sup> 1743	3.00±31.11 <sup>ª</sup> 596.75	±23.69 <sup>b</sup>
Thymus 570.00±14.14 <sup>c</sup> 73	3.75±8.84° 597.	.50±10.61 <sup>b</sup> 672	2.50±14.14 <sup>b</sup> 650.75	±8.13 <sup>b</sup>
Spleen 1179.25±5.3 <sup>c</sup> 270	5.50±56.6 <sup>bc</sup> 3339	25±75.3 <sup>b</sup> 6413	3.00±5.66ª 4230.00	±9.90 <sup>b</sup>

Table 1: Organ weight index and morphometric measurement of bursa, thymus and spleen

\*Means in the same row with different superscripts are significantly different (p<0.05)

in diets of broilers immunological response to sheep red blood cells. It was observed that thyme not only act as immune-stimulator but also thyme essence alleviate the post vaccinal reactions to Newcastle disease<sup>50</sup>. Contrary results were obtained by Abdulkarimi<sup>4</sup>, Mansoub and Myandoab<sup>51</sup> and Toghyani *et al.*<sup>52,53</sup> who detected that application of thyme in broiler diets failed to have favorable statistically significant impact on humoral immune responses of the broilers to vaccinations. These inconsistent results considering the effect of thyme on the immune response might be related to the dose, type and preparation method of the additive and also vaccination program used.

#### CONCLUSION

Dried coriander and turmeric powders feed additives at level of 0.75% or a mixture of both of them had an immuno-modulatory effect in broilers and could be used in areas with high risk of viral infection as it will help in maximizing the benefits of vaccines. Moreover, further investigations on the interaction between the tested herpes should be carried out with special attention to recommended dose of each.

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