

# Effect of dietary supplementation of selected trace elements and ascorbic acid on serum protein patterns of pre-immunized broiler chickens

## Abstract

**Introduction:** Improvement of the immune response of broiler chickens is an important topic in broilers industry. The use of trace elements and vitamins for such purpose is still interesting issue in the biochemical research arena.

**Aim:** The current study aimed to compare between the effect of selected trace elements and that of ascorbic acid on serum protein patterns of broiler chickens.

**Materials and Methods:** Seventy-five broiler chickens were divided into 5 equal groups. Birds in the group 1 kept on basal diet only and served as a control group. Birds in the groups 2-4 were kept on basal diet mixed with same dose (100mg/kg diet) of ascorbic acid, copper sulphate or zinc sulphate, respectively, whereas birds in the group 5 kept on basal diet mixed with 10mg/kg diet of potassium iodide. Birds were immunized through wing vein injection with 1ml of 5% sheep red blood cells suspension after two weeks from the start of the experiment. The other wing vein was used for blood samples collection at 2 and 4 weeks post immunization. The harvested sera were used for colorimetric determination of serum total protein, albumin and total globulin. In addition, serum globulin fractions ( $\alpha$ ,  $\beta$  and  $\gamma$ ) were determined by salt fractionation.

**Results:** The current findings indicated that, zinc sulphate or ascorbic acid were potent immunostimulents as evidenced by increased concentrations of serum total proteins, total globulins and  $\gamma$ -globulin fraction of preimmunized chickens fed diet mixed with either zinc sulphate or ascorbic acid compare to control. However, the immunostimulant effect of zinc sulphate was more pronounced. Copper sulphate was immunosuppressive as reflected on decreased total globulins and  $\gamma$ -globulin fraction in the serum of preimmunized chickens compare to control. Potassium iodide did not affect the serum protein patterns of the examined birds.

**Conclusion:** The current study recommended the addition of the examined dose of zinc sulphate or ascorbic acid to the diet of broiler chickens. However, addition of the examined dose of copper sulphate to the diet of broiler chickens is not recommended.

**Keywords:** ascorbic acid, zinc sulphate, potassium iodide, copper sulphate, broiler chickens

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**Abbreviations:** AA, ascorbic acid; LSD, least significant difference; PHA, phyto-hemagglutinin-p

## Introduction

Enhancing the overall performance of broiler chickens is the main objective of economic field production units. Diets for birds are supplemented with trace elements and vitamins to avoid deficiencies that can lead to a wide variety of clinical and pathological disorders. Trace minerals fulfill a central role in many metabolic processes throughout the body and are essential for correct growth and development of all animals. Deficiency symptoms are typically manifested as disturbances in multiple metabolic processes, resulting in lower production performance, loss of appetite, reproductive disorders, and impaired immune response.<sup>1</sup> Zinc is an essential mineral needed for physiological processes in many enzymes.<sup>2</sup> Through these enzymes, zinc is required for bone formation, cell mediated immunity, sex maturity and protein metabolism. Therefore, zinc deficiency is accompanied by changes in the biochemical profile of serum constituents.<sup>3</sup> The antioxidant effect of zinc compound

has been documented in broiler chickens.<sup>4</sup> Iodine affects the metabolism of other nutrients through its synergistic and antagonistic relationships with other elements. The occurrence of this element depends on geographical distribution. A dose of 5mg/kg feed was safe for a given group of animals. However, the maximum authorized limit of iodine in the feed of broiler chickens 10mg/kg feed does not represent a health risk.<sup>5</sup> The importance of iodine in broilers industry is attributed to its role in human health. Iodine deficiency attenuates the thyroid hormone biosynthesis, causes hypothyroidism. However, exceeding the human requirement is mainly associated with health risk. Therefore, avoidance of excessive or insufficient consumption of iodine is necessary for human health.<sup>5</sup> Interestingly, birds as well as mammals respond to iodine deficiencies by enlargement of the thyroid that means Goiter in poultry is very common.<sup>6,7</sup> Copper (Cu) is a vital trace mineral for broiler chicks. It acts as a co-enzyme for cuproenzymes (cytochrome c oxidase and lysyl oxidase). Although, NRC recommends 8mg of Cu/kg of diet as the minimum requirement for broiler chicks,<sup>8</sup> higher levels of Cu (125 to 250ppm) from cupric sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) are added to poultry diets.<sup>9</sup>

Ascorbic acid (AA) is important for many biochemical processes.<sup>10</sup> Dietary supplementation of AA has been selected as a suitable and successful method to ameliorate the detrimental effects of heat stress in broiler chicken.<sup>4</sup> It has been reported that, ascorbic acid is also included in diets to improve feed intake, egg production and egg quality in poultry.<sup>11</sup> Ascorbic acid is not typically added to poultry diets<sup>12</sup> because they can synthesize it to meet their physiological needs. However, under a heat stress the metabolic need for ascorbic acid exceeds the synthesizing capacity of the birds.<sup>13</sup> Therefore, ascorbic acid supplementation plays a major role in the improvement of poultry production performance. Because of, biologically active substances in poultry meat can be increased by supplementing broilers feed with vitamins and minerals,<sup>14</sup> the current study aimed to compare the effect of selected trace elements with ascorbic acid on protein patterns of broiler chickens.

## Materials and methods

### Experimental design

This study was carried out on seventy-five Hubbard broiler chickens at 12weeks of age. The birds were obtained from El-Nobarya Co. They were fed a basal diet for 2weeks before starting the experiment. The system of light, temperature and humidity were adjusted as recommended for broiler production.<sup>15</sup> The birds were classified into 5 groups (15 birds for each). Birds in the group 1 kept on basal diet only and served as a control group. Birds in the groups 2-4 were kept on basal diet mixed with same dose (100mg/kg diet) of either ascorbic acid,<sup>16</sup> copper sulphate<sup>9</sup> or zinc sulphate,<sup>17</sup> respectively whereas birds in the group 5 kept on basal diet mixed with 10mg/kg diet of potassium iodide.<sup>5</sup> Birds were immunized through wing vein injection with 1ml of 5% sheep red blood cells suspension after two weeks from the start of the experiment.

### Blood sampling and the analytical methods

Blood samples were collected from wing vein two and four weeks post immunization. The obtained sera were kept frozen at -20°C until used for quantitative determination of serum total serum protein and albumin.<sup>18</sup> Serum globulins were fractionated by salt fraction method<sup>19</sup> by using sodium sulphate to precipitate the  $\beta$  and  $\alpha$ -globulins and an ammonium sulphate/sodium chloride solution to precipitate  $\gamma$ -globulins.

### Statistical analysis

The data analysis of biochemical constituents in serum was carried out using a General Linear Model (GLM) procedure and means were compared by Least Significant Difference (LSD) using SPSS 16.0 statistical software.<sup>20</sup> The statistical indices has been based on triplicate analysis.

## Results

Albumin concentration in the serum of all preimmunized chickens (group 2-5) was not affected by dietary supplementation of examined trace elements and ascorbic acid 2weeks (Table 1) or 4weeks (Table

2) post immunization compare to control (group 1). The situation was different for serum total protein concentration which increased significantly after dietary supplementation of either zinc sulphate (group 5) 2weeks (Table 1) and 4weeks (Table 2) post immunization. Moreover, total proteins has been increased significantly in the serum of birds fed basal diet mixed with ascorbic acid (group 2) but only 4weeks post immunization compare to control (Table 1) (Table 2). However, potassium iodide had no effect on the concentration of serum total protein concentration of broiler chickens (group 3) neither 2weeks nor 4weeks post immunization compare to control (group 1) (Table 1) (Table 2). In the contrary, total protein concentration has been reduced significantly in birds fed basal diet mixed with copper sulphate (group 4) 2weeks (Table 1) and 4weeks (Table 2) post immunization compare to control (group 1). The concentration of total globulin increased significantly in the serum of birds fed basal diet mixed with zinc sulphate (group 5), 2weeks (Table 1) or 4weeks (Table 2) post immunization compare to control (group 1). Furtherly, total globulins has been increased significantly in the serum of birds fed basal diet mixed with ascorbic acid (group 2) but only 4weeks post immunization compare to control (Table 1) (Table 2). However, potassium iodide had no effect on the concentration of serum total globulins concentration of broiler chickens (group 3) neither 2weeks nor 4weeks post immunization compare to control (group 1) (Table 1) (Table 2). In the contrary, total globulins concentration has been reduced significantly in birds fed basal diet mixed with copper sulphate (group 4) 2weeks (Table 1) and 4weeks (Table 2) post immunization compare to control (group 1). The concentration of  $\alpha$  and  $\beta$ -globulin fraction in the serum of all primmunized chickens (group 2-5) was not affected by dietary supplementation of examined trace elements and ascorbic acid 2weeks (Table 1) post immunization compare to control (group 1). However, after 4weeks post immunization, the concentration of  $\alpha$  and  $\beta$ -globulin increased significantly in the serum of birds fed basal diet mixed with either zinc sulphate (group 5) or ascorbic acid (group 2) compare to control. Potassium iodide had no effect on the concentration of serum  $\alpha$  and  $\beta$ -globulin concentration of broiler chickens (group 3) neither 2weeks nor 4weeks post immunization compare to control (group 1) (Table 1) (Table 2). In the contrary,  $\alpha$  and  $\beta$ -globulin concentration has been reduced significantly in birds fed basal diet mixed with copper sulphate (group 4) 2weeks (Table 1) and 4weeks (Table 2) post immunization compare to control (group 1). The concentration of  $\gamma$ -globulin fraction in the serum of all primmunized chickens (group 2-5) was not affected by dietary supplementation of examined trace elements and ascorbic acid 2weeks (Table 1) post immunization compare to control (group 1). However, after 4weeks post immunization, the concentration of  $\gamma$ -globulin increased significantly in the serum of birds fed basal diet mixed with either zinc sulphate (group 5) or ascorbic acid (group 2) compare to control. Potassium iodide had no effect on the concentration of serum  $\gamma$ -globulin concentration of broiler chickens (group 3) neither 2weeks nor 4weeks post immunization compare to control (group 1) (Table 1) (Table 2). In the contrary,  $\gamma$ -globulin concentration has been reduced significantly in birds fed basal diet mixed with copper sulphate (group 4) 2weeks (Table 1) and 4weeks (Table 2) post immunization compare to control (group 1).

**Table 1** Serum total protein, albumin, total globulin,  $\alpha$  and  $\beta$ -globulin and  $\gamma$ -globulin concentrations (g/dl) of broiler chickens following 2weeks of oral administration of same dose (100mg/kg diet) either of ascorbic acid, potassium iodide, copper sulphate or zinc sulphate

Parameters	Total protein	Albumin	Total globulin	$\alpha$ and $\beta$ -globulin	$\gamma$ -globulin
Control	3.55±0.10b	2.08±0.08a	1.47±0.15b	0.84±0.13a	0.63±0.01b
Ascorbic acid	3.58±0.03b	2.02±0.14a	1.56±0.16b	0.80±0.14a	0.76±0.04a

Table Continued..

Parameters	Total protein	Albumin	Total globulin	α and β-globulin	γ-globulin
Potassium iodide	3.50±0.05b	2.01±0.14a	1.57±0.10b	0.87±0.08a	0.68±0.02b
Copper sulphate	3.17±0.20c	2.00±0.13a	1.20±0.15c	0.66±0.12a	0.54±0.03c
Zinc sulphate	4.00±0.03a	2.20±0.05a	1.80±0.43a	1.00±0.35a	0.80±0.02a

Means in the same column with no common letter differ significantly (P<0.05)

**Table 2** Serum total protein, albumin, total globulin, α and β-globulin and γ-globulin concentrations (g/dl) of broiler chickens following 4weeks of oral administration of same dose (100mg/kg diet) either of ascorbic acid, potassium iodide, copper sulphate or zinc sulphate

Parameters	Total protein	Albumin	Total globulin	α and β-globulin	γ-globulin
Control	3.84±0.13b	2.30±0.07a	1.54±0.11b	0.84±0.16b	0.70±0.02b
Ascorbic acid	4.09±0.16a	2.00±0.13a	2.09±0.08a	0.99±0.09a	1.10±0.13a
Potassium iodide	3.80±0.09b	2.22±0.11a	1.58±0.07b	0.75±0.04b	0.83±0.06b
Copper sulphate	3.06±0.12c	2.16±0.05a	0.90±0.04c	0.39±0.05c	0.51±0.03c
Zinc sulphate	4.42±0.05a	2.46±0.19a	1.96±0.09a	0.90±0.07a	1.06±0.10a

Means in the same column with no common letter differ significantly (P<0.05)

## Discussion and conclusion

Human nutrition science proposed the concept of “functional food”. The functional foods have nutritional value beside its supplementary effects to the human physiology and immunology.<sup>21</sup> Broilers meat can contribute to the increasing range of functional food.<sup>14</sup> Biologically active substances, normally contained in poultry meat in variable quantities, can be increased by supplementing broilers feed with vitamins and minerals.<sup>14</sup> The non-significant changes in serum albumin and significant increase in serum total proteins, total globulins and γ-globulin in ascorbic acid supplemented birds, 4weeks post immunization disagree with previous study in quails.<sup>10</sup> The authors demonstrated that, quails who fed on ration containing ascorbic acid in different doses (150, 250 and 500mg/kg diet) caused significant increase of albumin concentration while serum globulin was not affected. This confiction perhaps attributed to the species and dose difference. The significant increase of serum γ-globulin of birds supplemented with ascorbic acid 2 and 4weeks post immunization indicated the immunostimulant effect of ascorbic acid at the examined dose. The observed immunostimulant effect of ascorbic acid in the current study come in accordance with previous work in broilers.<sup>16</sup> The estimated non-significant effect of potassium iodide (10mg/kg diet) on protein patterns of the broilers chickens agree with previous report in broilers.<sup>22</sup> The authors reported that, protein content was not affected by inclusion of different forms of iodine in the broiler ration. The negative effect of copper sulphate on serum protein patterns of examined broilers as evidenced by significant reduction of total proteins and all globulin fractions may attribute to administration of high dose of this element. Parallel to the current assumption, other work,<sup>9</sup> stated that, caution is advised when considering higher levels of Cu (250ppm) as a feed supplement in broiler diets. In addition, tribasic copper chloride was preferable over copper sulphate in broilers chicken at safety and performance point of view.<sup>23</sup> However, other works reported the beneficial effects of copper sulphate on reduction of lipids and cholesterol<sup>24</sup> and production performance and plasma biochemical characteristics of broiler chickens.<sup>25</sup> The non-significant changes in serum albumin and significant increase in serum total proteins, total globulins and γ-globulin of zinc sulphate supplemented birds, 2 and 4weeks post immunization agree with previous study<sup>26</sup> reporting the elevation of serum total protein in ewes suffering from zinc deficiency and supplemented with zinc sulphate.

The present data come in accordance with previous work in broilers<sup>27</sup> which reported that, serum total protein was significantly increased in broilers fed the same dose of zinc sulphate (100ppm) for the same period (2 and 4weeks). The current findings agree with other work<sup>28</sup> reported the increase in serum total protein level as a result of zinc sulphate supplementation in broiler chickens affected by heat stress. The increment of serum total protein in zinc supplemented broilers 2 and 4weeks post immunization reflects the role of zinc in protein biosynthesis.<sup>3</sup> The increment of γ-globulin induced by zinc administration indicated the immunostimulant effect of zinc which achieved at the dose of 100mg/kg diet and for short (2weeks) or long (4weeks) period post immunization. These findings come in agreement with previous reports that reported the immunostimulant effect of zinc in rats<sup>26</sup> and broilers.<sup>27</sup> The present results come in accordance with earlier study,<sup>29</sup> which reported that the immunresponse of broilers was significantly increased by high dietary zinc (181mg/kg ration). In the contrary, the present findings disagree with other study<sup>30</sup> which reported that, broiler chicks fed on various levels (8-88ug/gm diet) of zinc caused no influence on the primary or secondary immune response to sheep red blood cells or delayed hypersensitivity to phyto-hemagglutinine-p (PHA) or human gamma globulin.<sup>31,32</sup> The current study recommended the addition of zinc sulphate or ascorbic acid to the diet of broiler chickens at the examined dose due to their immunostimulant effect. Further, the addition of copper sulphate to the diet of broiler chickens at the examined dose is not recommended due to its immunosuppressive effect. The examined dose of potassium iodide did not affect the serum protein patterns of broiler chickens. Further studies are needed to evaluate the effect of combined administration of examined elements and ascorbic acid on serum protein patterns of broiler chickens and other chicken lines.

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## Conflict of interest

Author declares that there is no conflict of interest.

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