

A Study of the Effect of Vitamin C and *Ocimum Sanctum* Supplementation on Antioxidant Enzyme Levels in Broilers Under Heat-Stress

Research Article

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Abstract

This experiment was conducted in heat-stressed broiler chicken to evaluate the effect of dietary supplementation of Vitamin C and *Ocimum sanctum* on antioxidative enzyme levels. A total of forty broiler chickens of day old age were divided into 4 groups of 10 each, were used for this study. Vitamin C (300 mg/kg), *Ocimum sanctum* leaf powder (0.5%) and their combination were added to the basal diet. Superoxide dismutase (SOD), glutathione peroxidase (GSH-px), catalase, reduced glutathione (GSH) and lipid peroxidation levels in plasma were measured at the end of 6 weeks of age. Dietary supplementation of Vitamin C itself increased SOD, GSH-px, catalase enzyme levels significantly ($p < 0.05$). However, supplementation of both Vitamin C and *Ocimum sanctum* effectively enhanced the levels of SOD, GSH-px, catalase, and GSH with concomitant decrease in lipid peroxidation levels in plasma. It is concluded that dietary supplementation of Vitamin C and its combination with *Ocimum sanctum* at 0.5% level can combat oxidative stress caused by high environmental temperature in broilers, by enhancing antioxidative enzyme levels.

Key Words: Antioxidants; Vitamin C; *Ocimum sanctum*; SOD; GSH-Px; Catalase; GSH; Lipid Peroxidation; Broiler Chickens.

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Introduction

Poultry production suffers significant losses because of heat stress. Heat stress adversely affects broiler performance and livability. Body temperature and metabolism rate of birds are relatively high compared to mammals, which make birds vulnerable to oxidative stress under high environmental temperature, which is the major cause of sudden death syndrome [1]. Heat stress not only adversely affects production performance but also increases the incidence of infectious and metabolic diseases in poultry. This can be minimized by the use of anti-stress compounds. Antioxidants are substances, present biologically lower concentra-

tions and significantly delay or prevent oxidation of substances like protein, lipids, DNA and carbohydrates [2]. External sources of antioxidant are essential for the control of oxidation process, which include vitamin C & E, carotenoids, phytoflavonoids etc.

Vitamin C also commonly known as ascorbic acid is an important antioxidant. Although birds synthesize vitamin C in their body, however, its supplementation in the poultry diet has been recommended during stressful conditions [3]. Several herbal plants are found to possess anti oxidant properties and the ubiquitous herb i.e., *Ocimum sanctum* which belongs to Lamiaceae family is a fairly economic therapeutic agent for several pathological conditions as well as anti-stress [4] and antioxidant agent [5].

The present study was conducted to evaluate the effect of Vitamin C and *Ocimum sanctum* on antioxidant enzyme levels in broiler chicken under heat stress.

Materials and Methods

A forty broiler chicks of day old age were randomly divided into 4 groups comprising of 10 birds in each group with following dietary regimes.

Group I: Standard diet (Control)

Group II: Standard diet + Vitamin C (300mg/kg)

Group III: Standard diet + *Ocimum sanctum* (0.5%)

Group IV: Standard diet + Vitamin C (300mg/kg) + *Ocimum sanctum* (0.5%)

The birds were reared from day old to 6th week of age in cages under standard managerial practices during months of April & May. The temperature inside the house is ranged from 35 – 38°C. Freshly collected *O. sanctum* leaves were shade dried and powdered. Vitamin C and *Ocimum sanctum* leaf powder were sup-

plemented as above to the standard broiler diet. The broiler starter and finisher diets were fed *ad libitum* to the birds. The *Ocimum sanctum* leaf powders were used at 0.5% level of inclusion based on the reports of [6], along with Vitamin C (300mg/kg).

Blood samples were collected at the end of 6th week from the wing vein using heparinised vacutainer for separating plasma by standard procedures of centrifugation. The levels of superoxide dismutase (SOD) activity in plasma was measured by the method of [7], glutathione peroxidase (GSH-Px) activity as per the method described by [8] and catalase activity as per the method of [9]. Reduced glutathione levels in plasma were estimated by the method of [10]. Lipid peroxidation assay was carried out according to [11]. Statistical analysis of the data was analyzed by randomized block design as per [12].

Results & Discussion

Superoxide dismutase is considered as critical antioxidative enzyme that acts as a scavenger of oxygen anion to form hydrogen peroxide and hence diminishes the toxic effects due to the free radical. This primary defense widely distributed in oxygen metabolizing cells to protect aerobic cells from deleterious actions of free radicals [13]. The mean plasma SOD values of broilers at 6 weeks of age as influenced by dietary supplementation of Vitamin C and *O. sanctum*, is presented in Table 1. In the present study, dietary supplementation of Vitamin C (300mg/kg), *O. sanctum* at 0.5% level and their combination significantly ($p < 0.05$) enhanced plasma SOD activity when compared control group. The results of our study are in accordance with the earlier reports of [14], who observed that SOD activity significantly ($P < 0.05$) increased with dietary supplementation of Vitamin C in summer stressed broilers. Similarly, [6] reported significant ($P < 0.05$) increase in SOD activity with dietary supplementation of *O. sanctum* in broilers.

The Glutathione peroxidase, present in the cytosol and mitochondria matrix, catalyses the degradation of various peroxides by sacrificial oxidation of glutathione. Glutathione peroxidase enzyme reduces peroxides and protects cells against the damaging effects of oxidation. The mean plasma GSH-Px values of broiler chickens at 6 week of age as influenced by dietary supplementation of Vitamin C and *O. sanctum* is presented in Table 1.

In the present study, there is a significant increase in plasma GSH-Px activity with Vitamin C supplementation and its combination with of *O. sanctum* at 0.5% level. Similar findings were observed by [6], that oxidative stress was effectively inhibited by the dietary supplementation of *O. sanctum* and selenium by increasing plasma GSH-Px activity in broiler chicken.

The catalase causes the reduction reaction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals.

During stress the accumulated free radicals causes the inactivation of this enzyme by simple glycation which reduces structural abnormality and thus limits the activity of catalase [11]. The mean plasma catalase values of broiler chickens of 6 weeks of age, influenced by dietary supplementation of Vitamin C and *O. sanctum* are presented in Table 1.

In the present study, catalase activity was highest with Vitamin C supplemented with *O. sanctum* followed by Vitamin C supplemented alone and *O. sanctum* alone supplemented group when compared to control group. The enhanced catalase activity in the group with combination of Vitamin C and *Ocimum sanctum* indicates synergistic effect of Vitamin C and *O. sanctum* on scavenging free radicals and hydrogen peroxides.

The findings of increased plasma catalase levels in the present study were in agreement with the reports of [6], they observed increases in plasma catalase enzyme levels by supplementation of *O. sanctum* in broilers. Similarly, [14] reported that catalase activity significantly ($P < 0.05$) increased with dietary supplementation of Vitamin C in summer stressed broilers.

GSH (reduced glutathione) offers protection against oxygen derived free radicals and cellular lethality following exposure to oxidative stress. Its high electron donating capacity combined with higher intracellular concentrations generate great reducing power [15]. Dietary supplementation of Vitamin C and *O. sanctum* and their combinations on plasma GSH levels is presented in Table 2.

The findings of increased plasma GSH (reduced glutathione) levels in the present study were in agreement with the reports of [16], they observed increases in plasma GSH enzyme levels by supplementation of *O. sanctum* in broilers.

Lipid peroxidation occurs as a consequence of increased oxidative stress primarily due to disruption of pro-oxidant/antioxidant balance. Increased oxidative stress has been associated with increased lipid peroxidation in many diseases [17]. In the present study, plasma malondialdehyde (MDA) levels were decreased with dietary supplementation of Vitamin C and *Ocimum sanctum*. This may be due to conversion of lipid peroxides to alcohol derivatives rather than MDA in the presence of higher GSH levels with Vitamin C and *Ocimum sanctum*.

The findings of decreased plasma malondialdehyde (MDA) levels in the present study were in agreement with the reports of (14), they observed decreased plasma malondialdehyde (MDA) levels by supplementation of Vitamin C in broilers. Similarly, [18] observed that there was significant decrease in plasma malondialdehyde (MDA) levels by supplementation of Vitamin C in broilers. [16] observed that there was significant decrease in plasma malondialdehyde (MDA) levels by supplementation of *Ocimum sanctum*

Table 1. Effect of dietary supplementation of Vitamin C, *Ocimum sanctum* and their combinations on SOD, GSH-Px and Catalase enzyme levels in broilers.

Groups	SOD (50% pyrogylol auto-oxidation/ min/mg)	GSH-Px (μM of GSH utilized/ min/mg)	Catalase (μM of H_2O_2 decomposed /min/mg)
I	2.23 ^a \pm 0.03	2.46 ^a \pm 0.01	41.79 ^a \pm 0.11
II	2.85 ^c \pm 0.02	2.88 ^b \pm 0.02	44.54 ^c \pm 0.18
III	2.64 ^b \pm 0.03	2.85 ^b \pm 0.02	43.87 ^b \pm 0.07
IV	3.15 ^d \pm 0.02	3.28 ^c \pm 0.01	45.15 ^d \pm 0.09

Means bearing common superscripts in column do not differ significantly ($p < 0.05$)

Table 2. Effect of dietary supplementation of Vitamin C, *Ocimum sanctum* and their combinations on Reduced glutathione and Lipid peroxidation levels in broilers.

Groups	Reduced glutathione (mg GSH / ml)	Lipid peroxidation (nM of MDA / ml)
I	1.05 ^a ±0.03	6.20 ^c ±0.03
II	1.15 ^a ±0.02	5.23 ^{ab} ±0.03
III	1.13 ^a ±0.03	5.39 ^b ±0.03
IV	1.23 ^b ±0.02	5.08 ^a ±0.04

Means bearing common superscripts in column do not differ significantly (p<0.05)

in broilers.

Hence it is concluded that the combination of both Vitamin C and *O. sanctum* can combat oxidative stress caused by rapid growth rate in heat stressed broilers there by effectively enhancing the SOD, GSH-Px, catalase activities and GSH levels with concomitant decrease in lipid peroxidation levels in plasma of heat stressed broilers.

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