

ANTIOXIDANT ENZYME STATUS IN BROILERS: ROLE OF DIETARY SUPPLEMENTATION OF TULASI (*Ocimum sanctum*) AND SELENIUM

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ABSTRACT

An experiment was conducted in broiler chickens to evaluate the effect of dietary supplementation of Tulasi (*Ocimum sanctum*) and selenium on antioxidative enzyme levels. Total forty-two broiler chicks of day-old divided into six groups of seven each were used for this study. *Ocimum sanctum* leaf powder (0.25% and 0.5%), organic selenium (0.3 ppm) and their combinations were added to the basal diet. Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) and Catalase levels in plasma were measured at the end of 3rd and 6th week of age. Dietary selenium (0.3 ppm) supplementation in itself significantly ($P < 0.01$) increased GSH-Px activity and supplementation of only *Ocimum sanctum* leaf powder (0.5%) significantly ($P < 0.01$) increased SOD and Catalase levels. However, *Ocimum sanctum* leaf powder (0.5%) and its combination with selenium (0.3 ppm) more effectively enhanced the levels of SOD, GSH-Px and Catalase. It is concluded that dietary supplementation of *Ocimum sanctum* at 0.5% level and its combination with selenium (0.3 ppm) can combat oxidative stress in broilers there by increasing the antioxidative enzyme levels.

Key words: Antioxidants, *Ocimum sanctum*, selenium, SOD, GSH-Px, Catalase, broiler chicken.

INTRODUCTION

Oxidative stress is the major cause of reduction in growth rate in broilers and increase incidence of infectious and metabolic diseases in poultry, which can be minimized by the use of anti-stress compounds. The choice of anti-stress compounds should aim not only to ameliorate the stress and also to be safe and economical.

Antioxidants are substances present in lower concentrations and significantly delay or prevent oxidation of substrates such as protein, lipids, carbohydrates and DNA (Sen, 1995). Of the several plants which are found to possess antioxidant properties, the ubiquitous herb, Tulasi (*Ocimum sanctum*) is a fairly economic therapeutic agent for several pathological conditions as well as anti-stress (Bhargava and Singh, 1981) and antioxidant agent (Gupta *et al.*, 2006). External sources of

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antioxidant nutrients essential for antioxidant protection include antioxidant vitamins E and C and the mineral selenium. Organic selenium is a natural seleno-aminoacid (selenomethionine) which possesses antioxidant properties and improves resistance against oxidative stress (Mahmoud and Edens, 2003).

The present study was conducted to assess the effect of various levels of *Ocimum sanctum* leaf powder, organic selenium and their different combinations on Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) and Catalase levels in broilers.

MATERIALS AND METHODS

A total of 42 Cobb broiler chicks of day-old were randomly divided into six groups comprising of seven birds in each group with the following dietary regimens.

- Group I (control) -Standard diet
- Group II -Standard diet + *Ocimum sanctum* 0.25%
- Group III -Standard diet + *Ocimum sanctum* 0.5%
- Group IV -Standard diet + Organic Selenium 0.3 ppm
- Group V -Standard diet + *Ocimum sanctum* 0.25% + Organic Selenium 0.3 ppm
- Group VI -Standard diet + *Ocimum sanctum* 0.5% + Organic Selenium 0.3 ppm

The birds were reared in cages under standard managerial practices from day-old to six weeks of age. Freshly collected, shade dried *Ocimum sanctum* leaf powder and organic selenium were supplemented as above to the standard broiler

diet. The broiler starter and finisher diets were fed *ad libitum* to the birds. Institutional animal ethical committee approved this experiment.

Blood samples were collected at the end of 3rd and 6th week from the wing vein in sterile heparinized tubes. Plasma was separated by centrifugation. The levels of superoxide dismutase in plasma was measured by the method of Marklund and Marklund (1974), glutathione peroxidase activity was assessed as per the method of Rotruck *et al.* (1973) and catalase activity was determined by the method of Caliborne (1985). Statistical analysis of the data was analyzed by completely randomized block design as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Dismutation of superoxide ion to hydrogen peroxide by SOD is often called as the primary defense. SOD, is widely distributed in oxygen metabolizing cells and protect aerobic cells against deleterious actions of superoxide radicals and other reactive oxygen species (ROS) (Yamaguchy, 1991). The mean plasma SOD values of broilers at 3rd and 6th week of age as influenced by dietary supplementation of *Ocimum sanctum* and selenium is presented in Table. 1. In the present study, dietary supplementation of *Ocimum sanctum* at 0.5% level (Group III) and its combination with selenium at 0.3ppm (Group VI) significantly ($P < 0.01$) enhanced plasma SOD activity. *Ocimum sanctum* alone (Group II & III) found to be more effective in scavenging superoxide ion by increasing SOD activity when compared to selenium alone (Group IV). However, *Ocimum sanctum* at 0.5% level and selenium at 0.3ppm combination (Group VI) found to possess a superior effect on SOD activity.

The results of our study are in accordance with the earlier reports of Holovska *et al.* (2003), who observed that SOD activity significantly

Table 1
Effect of dietary supplementation of *Ocimum sanctum*, organic selenium and their combinations on plasma SOD levels in broilers

Groups	SOD (50% Pyrogylol auto-oxidation / min / mg)	
	3 rd week	6 th week
I	1.26 ^a ± 0.08	2.43 ^a ± 0.06
II	1.62 ^c ± 0.03	2.71 ^c ± 0.02
III	1.93 ^d ± 0.06	3.19 ^e ± 0.03
IV	1.48 ^b ± 0.04	2.61 ^b ± 0.03
V	1.85 ^d ± 0.03	2.97 ^d ± 0.03
VI	2.08 ^e ± 0.04	3.24 ^e ± 0.02

Means bearing same superscripts in a column do not differ significantly (P<0.05)

Table- 2
Effect of dietary supplementation of *Ocimum sanctum*, organic selenium and their combinations on plasma GSH-Px levels in broilers

Groups	GSH-Px (nM GSH utilized / min / mg)	
	3 rd week	6 th week
I	2.01 ^a ± 0.03	2.73 ^a ± 0.05
II	2.10 ^a ± 0.01	2.84 ^b ± 0.03
III	2.25 ^b ± 0.04	2.98 ^c ± 0.02
IV	2.55 ^c ± 0.03	3.24 ^d ± 0.05
V	2.59 ^c ± 0.02	3.25 ^d ± 0.03
VI	2.72 ^d ± 0.03	3.35 ^e ± 0.04

Means bearing same superscripts in a column do not differ significantly (P<0.05)

Table 3
Effect of dietary supplementation of *Ocimum sanctum*, organic selenium and their combinations on plasma Catalase levels in broilers

Groups	Catalase (nM of H ₂ O ₂ decomposed / min / mg)	
	3 rd week	6 th week
I	30.25 ^a ± 0.64	42.69 ^a ± 0.51
II	31.34 ^{ab} ± 0.39	43.38 ^{ab} ± 0.30
III	32.07 ^b ± 0.36	44.80 ^c ± 0.44
IV	31.35 ^{ab} ± 0.28	43.74 ^{abc} ± 0.25
V	31.78 ^b ± 0.15	43.93 ^{bc} ± 0.24
VI	32.49 ^b ± 0.18	44.75 ^c ± 0.32

Means bearing same superscripts in a column do not differ significantly (P<0.05)

(P<0.05) increased with dietary supplementation of organic selenium in chicken. Similar findings were also recorded with dietary supplementation of selenium which significantly increased activity of SOD in the erythrocytes and tissues to minimize oxidative stress in chicken by inhibiting the oxygen free radical production and scavenging the superoxide ions (Ozturk-urek *et al.*, 2001).

The glutathione peroxidase, present in the cytosol and mitochondrial matrix, catalyses the degradation of various peroxides by oxidizing glutathione. Selenium is an essential component of selenium-dependent glutathione peroxidase enzyme, which reduces peroxides and protects cells against the damaging effects of oxidation. The mean plasma GSH-Px values of broilers at 3rd and 6th week of age as influenced by dietary supplementation of *Ocimum sanctum* and selenium is presented in Table.2.

In the present study, there is a significant (P<0.01) increase in the plasma GSH-Px levels with selenium supplementation (Group IV) and its combination with *Ocimum sanctum* at 0.25% and 0.5% level (Group V & VI). Fidler *et al.* (1980) reported that hydrogen peroxide induced oxidative stress was effectively inhibited with dietary selenium supplementation by increasing plasma GSH-Px activity in Leghorn chicken. Payne and Southern (2005) and He-Jianhua *et al.* (2000) also observed that dietary selenium supplementation increased the plasma GSH-Px activity in the broiler chicken which played a vital role in the detoxification of hydrogen peroxide and protect the cell from injury caused by peroxides.

In the cell catalase reacts with generated hydrogen peroxide to form water and molecular oxygen thereby protecting the cells against hydrogen peroxide toxicity and lipid peroxidation (Yamaguchi, 1991). The mean plasma catalase values of broilers at 3rd and 6th week of age as influenced by dietary supplementation of *Ocimum sanctum* and selenium is presented in Table.3. In the present study, catalase activity was significantly ($P < 0.01$) increased in 0.5% *Ocimum sanctum* supplemented group (Group III). The present study revealed that the plasma catalase levels in broilers were not influenced by the dietary supplementation of selenium at 0.3ppm level as compared to *Ocimum sanctum* at 0.5% level. Similar finding was reported by Aydemir *et al.* (2000) in erythrocytic catalase activity in chicken supplemented with selenium. However, combination of selenium with *Ocimum sanctum* (Group VI) significantly ($P < 0.05$) increased the catalase activity. This may be due to synergistic effect of *Ocimum sanctum* and selenium on scavenging free radicals and hydrogen peroxides.

Wang *et al.* (1998) and Holovska *et al.* (2003) observed age-related increase in plasma antioxidant enzyme levels by antioxidants supplementation in broilers. Even in our study, increased plasma SOD, GSH-Px and Catalase levels at 6th week as compared to 3rd week in all groups could be due to *Ocimum sanctum* and organic selenium supplementation; this supplementation might have enhanced enzyme levels to scavenge ROS and free radicals which are produced more during rapid growth period (from 3rd to 6th week).

Hence, it is concluded that the combination of *Ocimum sanctum* (0.5% level) with organic selenium (0.3ppm level) can combat oxidative stress caused by rapid growth rate in broilers, there by effectively enhancing the SOD, GSH-Px and catalase activities in the body.

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