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# EFFECT OF ANTIOXIDANT FORMULATION SUPPLEMENTATION THROUGH WATER ON ANTIOXIDANT STATUS OF BROILER CHICKEN

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**ABSTRACT**
Oxidative stress in broiler chicken can result in damage to biomolecules, cells and tissues which thereby can decrease immunity, antioxidant system and result in poor growth rate and production of the bird's .Vitamin E & selenium play an important role in protecting cells from oxidative damage and hence improve the growth and performance of live stock. The effects of selenium, vitamin E and *Ocimum sanctum* extract (E-Care Se Herbal was the source of vitamin E, selenium and *Ocimum sanctum* extract) supplementation through water to broiler chickens on the antioxidant status in the liver was investigated. The trial was conducted with straight run broilers, the birds were randomly divided into 2 experimental treatments containing 6 replicates for each treatment with 20 birds per replicate. The treatments consisted of treatment - T1 (Control, without any supplementation in the water) and treatment-T2 (E-Care Se Herbal @ 0.5ml/20 birds, given continuously from day 1 till end of trial period). Birds were sacrificed after 42 days of age and subsequently liver and whole blood samples were collected (6 birds per treatment) for antioxidant enzymes, non enzymatic antioxidant and lipid peroxidation level analysis. All animal housing, care and experimental procedures were approved by and conformed to the requirements of the Institutional Animal Ethics Committee. The inclusion of selenium, vitamin E and *Ocimum sanctum* extract (T2) supplemented through water significantly increased catalase activity and decreased the lipid peroxidation levels by decreasing the formation of malondialdehyde in the liver tissues in comparison to the control (T1). There was no significant difference in the glutathione levels and glutathione peroxidase (GSH-Px) among the treatments. The present study indicates the synergistic effect of vitamin E, selenium and *Ocimum sanctum* extract preparation *in vivo* by protecting against oxidative damage and lipid peroxidation in the birds.

**KEY WORDS:** Oxidative stress, Vitamin E, Selenium, *Ocimum sanctum* extract, Antioxidant enzymes, lipid peroxidation..

## INTRODUCTION

Reactive oxygen species (ROS) are free radicals containing oxygen with one or more unpaired electron, which make them a very reactive chemical species and it is continuously generated in the body because of endogenous metabolic process or due to environmental stress condition[1, 2]. Oxidative stress is due to disturbance in the balance between the production of ROS and antioxidant defense mechanism in the biological system. To counter act this oxidative stress, cells have an antioxidant defense system against these reactive oxygen species [3,4].

Cells have several antioxidant molecules produced endogenously in the body such as ascorbate, α-tocopherol, carotenoids,glutathione *etc*. Apart from these antioxidant molecules there are numerous antioxidant enzymes involved in the quenching or removal of free radicals such as superoxide dismutase (SOD), catalase,glutathione peroxidase (GSH-Px) *etc*. The antioxidant enzymes are synthesized within the endogenous system, when the required cofactor is supplied through the diet otherwise there will be deficiency in synthesizing and maintaining the antioxidant enzyme level resulting in the oxidative stress [5,6].

Oxidative stress in poultry especially broiler chicken can results in damage to biomolecules, cells and tissues which decreases immunity, antioxidant status and can results in poor growth rate and production of birds[5,7].Vitamin E and selenium plays an important role in protecting cells from oxidative damage and hence improve the growth and performance of live stock. Vitamin E and selenium forms the integral part of antioxidant system present in cell membranes. Adequate concentration of vitamin E and selenium in cell membranes will improve integrity of cell membranes and protect them from free radical damage. Oxidative stress can be minimized by the use of vitamin E, selenium, *Ocimum sanctum* extract, which improves the antioxidant defense system in the birds and stress condition[8,9,10].

The present study was conducted to evaluate the effect of preparation containing vitamin E ,selenium, and *Ocimum sanctum* extract on antioxidant enzymes like catalase, glutathione peroxidase (GSH-Px) and non-enzymatic antioxidant molecule-reduced glutathione and lipid peroxidation levels in broilers.

## MATERIALS AND METHODS

Two hundred forty, day old straight run broiler chicks (Cobb 400, procured from a reputed breeder) were divided into 2 treatment groups with similar mean weight, comprising 20 birds each with six replicates for each group. Treatments were allocated as a completely randomized block design. Standard starter/grower diet (from hatching to 21 days, 22.5% CP and 3025 kcal/kg

ME), finisher diet (from 22 to 42 days, 19.57% CP and 3200 Kcal/kg ME) were used as the basal diet (Table 1).

The experimental treatments were imposed from Day 0 to 42 days of age. The first treatment group (T1) was taken as control was given basal diet and water without any supplements. The preparation E-Care Se Herbal (Each ml contains vitamin E: 100 mg, selenium: 0.5 mg, extract of *Ocimum sanctum*: Q.S.) was supplemented with the water in the treatment (T2) at-0.5ml/20 birds, given continuously from 0-42 days of age. The experimental unit in this trial was the pen.

The experiment was conducted following the guidelines of the Institutional Animal Ethics Committee. During the experiment, the chicks were housed individually in pens; each pen is considered as experimental unit and reared at a conventional ambient temperature with a relative humidity of 60-70%. Feed and water were made available. Light was provided 24 hours each day. The experiment lasted for 6 weeks, broiler growth performance was assessed by measuring feed intake every day, body weight gain and feed conversion rate every week. At the end of 42 days of age, 6 birds for each treatment were sacrificed and whole blood was taken, along with liver tissues samples. Liver samples collected were further rinsed in a buffer solution and then immediately stored in plastic containers with phosphate buffer saline (1x , pH 7.0) at -20 ºC for later analysis for estimating reduced glutathione (GSH) content, lipid peroxidation (TBARS) and catalase activity. The collected whole blood samples were analysed for Glutathione peroxidase activity.

**Analysis of antioxidant enzymes: Catalase and glutathione peroxidase activity**

**Catalase activity**

The activity of catalase was determined by the method of Ayo *et al.*, 1974[11]. The time taken to decrease the absorbance (ΔA) by 0.05 units at 240nm was recorded and Catalase activity was expressed as units per gram of liver.

**Glutathione peroxidase activity**

Activity of whole blood GSH-Px were determined by spectrometry using the Randox ransel kit (Randox Laboratories Ltd., UK) as per the method described by Paglia & Valentine (1967) using heparinized whole blood for the analysis[12]. The decrease in absorbance at 340nm is measured and GSH-Px activity was expressed as units per litre of blood.

**Analysis of non enzymatic antioxidant: Reduced glutathione**

GSH levels in the liver were estimated according to the modified method of Moron, 1979[13]. The amount of reduced glutathione (GSH) was expressed as nanomoles of reduced glutathione per gram of liver.

**Lipid peroxidation levels**

MDA levels in the liver was estimated according to the method of Wright *et al.*, 1981[14]. Determination of malondialdehyde (MDA) by thiobarbituric acid (TBA) is used as an index for the extent of lipid peroxidation. The absorbance was read at 535 nm and the concentration of MDA was calculated based on the formation of pink pigment due to reaction between the malondialdehyde with thiobarbituric acid (TBA).A series of standard solutions in the concentration range from 0.3045 to 3.046 nano moles were prepared by using 2,2,3,3-tetra methoxy propane. The results were expressed as nanomoles of malondialdehyde per gram of liver.

**Statistical analysis:** The data were analyzed statistically using the SAS software (JMP Software version 8) and means were separated using Tukey's Multiple Range Test [15].

**TABLE 1:** Ingredient and nutritional composition of the starter diet (0-21 days) and grower/finisher diets (22-42 days).

| Ingredients (in Kg) | Starter Diet | Finisher Diet |
|---|---|---|
| Maize | 56.906 | 61.139 |
| Soyabean meal 45% CP | 36.921 | 31.016 |
| Sunflower Oil | 2.58 | 4.643 |
| DL-Methionine | 0.253 | 0.172 |
| L-Threonine 98% | 0.043 | 0.036 |
| L-Lysine HCl | 0.086 | 0.005 |
| Choline Chloride 60% Veg | 0.1 | 0.1 |
| Lime stone | 1.388 | 1.132 |
| Sodium bicarbonate | 0.251 | 0.172 |
| Salt | 0.197 | 0.256 |
| Dicalcium Phosphate.$2H_2O$ | 1.065 | 1.119 |
| Phytase 5000 IU | 0.01 | 0.01 |
| Broiler Vitamin Premix | 0.1 | 0.1 |
| Broiler Mineral Premix | 0.1 | 0.1 |
| Total (kg) | 100 | 100 |
| Nutrients(Calculated Value) | | |
| Crude Protein % | 22 | 19.57 |
| Ether extract | 5.41 | 8.04 |
| Crude Fibre % | 3.48 | 3.22 |
| Lysine % | 1.25 | 1.03 |
| Methionine % | 0.58 | 0.58 |
| Calcium % | 1 | 0.9 |
| Available Phosphorus % | 0.45 | 0.45 |
| Metabolizable energy, Kcal/Kg | 3025 | 3200 |
| M+C % | 1.83 | 1.5 |

**RESULTS**

The response to various treatments on feed intake, weight gain, and feed conversion ratio (FCR) is summarized in There were no differences in feed intake, weight gain, and FCR among the treatments during 0-42 days trial on the growth performance of broiler chicken.

The response to treatments on antioxidant enzyme (Catalase, GSH-Px), Non enzymatic antioxidant (Reduced glutathione) and lipid peroxidation (TBARS) levels is summarized.

Lipid peroxidation levels which is measured in terms of malondialdehyde formation showed a statistical difference ($P<0.05$) among the treatments in the liver tissues of broiler chicken at 42 days of age. The supplemented treatment (T2) showed a reduction in Malondialdehyde (MDA) formation among the treatments.

There were differences in enzyme catalase levels at 42 days of age, in the water supplemented treatments against the control. The increase in reduced glutathione levels in liver and enzyme glutathione peroxidase in the whole blood was not statistical significant difference among the treatments in at 42 days of age.

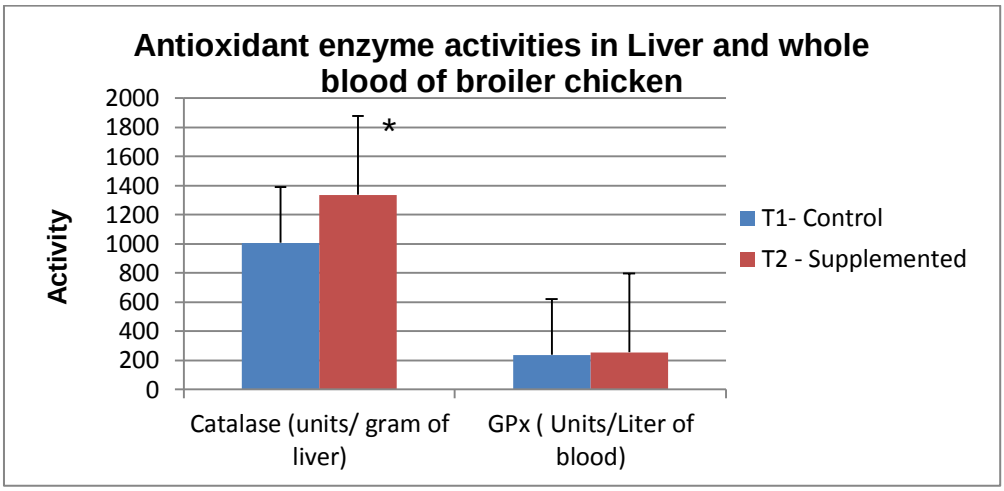


**FIGURE 1:** Antioxidant enzyme activities in the liver and the whole blood of Broiler chickens at 42 days of age. (*Indicates significant difference($P<0.05$)

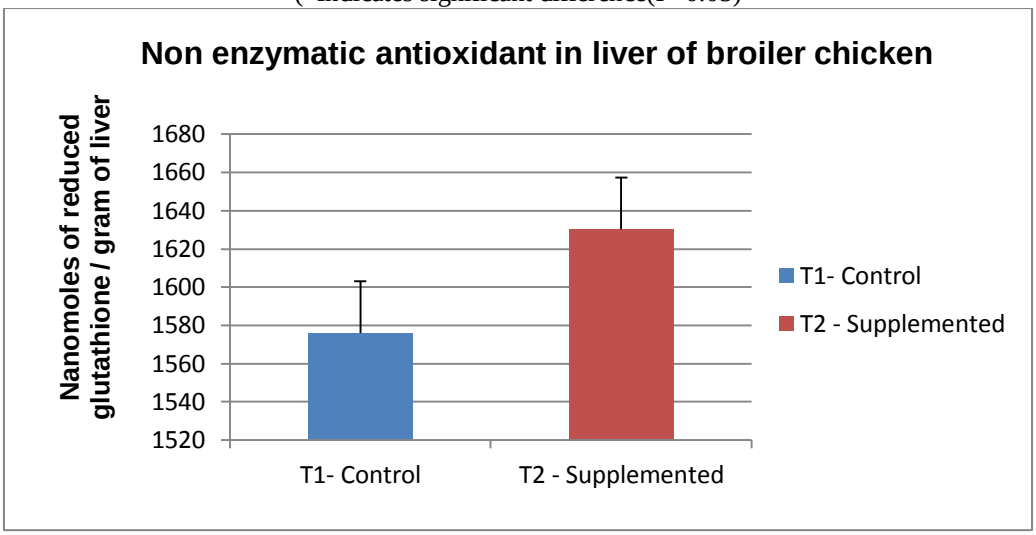


**FIGURE 2:** Non enzymatic Antioxidant enzyme activities in the liver of Broiler chickens at 42 days of age

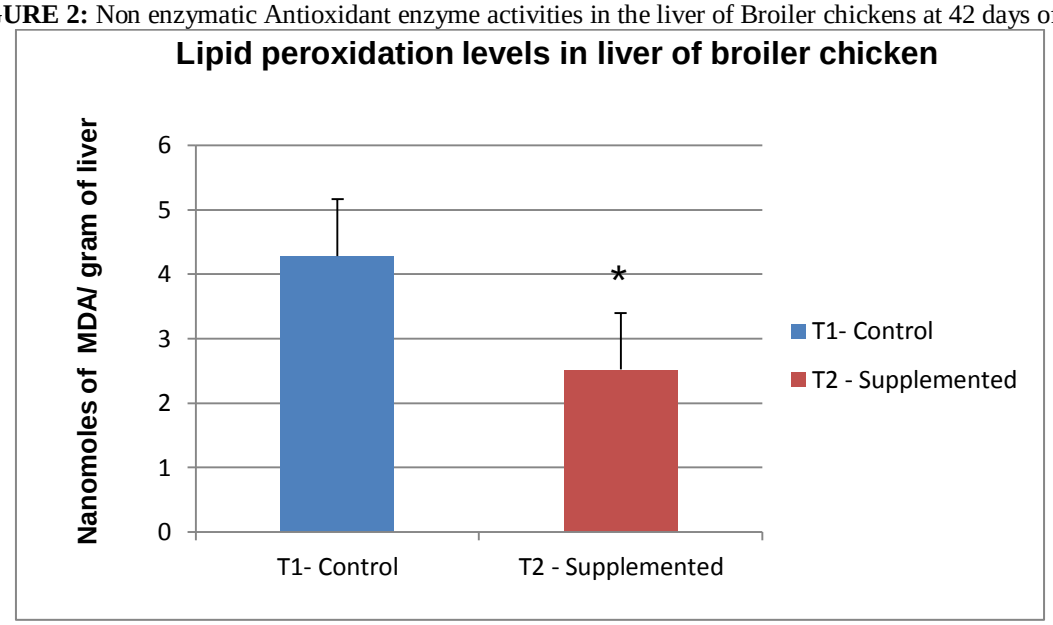


**FIGURE 3**: Lipid peroxidation levels in the liver of Broiler chickens at 42 days of age.(* Indicates significant difference)

**DISCUSSION**

A trial was conducted to evaluate the vitamin E,selenium preparation and *Ocimum sanctum* (E-Care Se herbal) when supplemented through water to broiler chicken on antioxidant enzyme levels and lipid peroxidation under non-challenged conditions.

Catalase is an ubiquitous antioxidant enzymes present in the animal cells which catalyse the decomposition of hydrogen peroxide ($H_2O_2$) in to water and oxygen.

Removal of the $H_2O_2$ from the cell by catalase provides protection against oxidative damage to living cells by hydrogen peroxide toxicity.

In the present study there were significant difference between the treatments in enzyme catalase activity at 42 days of age, the water supplemented treatment (T2) showed a increase in catalase activity against the control treatment (T1) (Figure 1). The synergistic effect of *Ocimum sanctum* extract and selenium shown to increase the levels of catalase activity in the liver compared to control(T1) was also reported by Vara Prasad Reddy *et al*., 2009 [9].

Glutathione peroxidase GSH-Px is a major selenium containing enzymes found in mammalian cells which catalyses the degradation of various peroxides by oxidizing glutathione and protect the cells from oxidative damage. Supplementation of selenium have a profound influence on GSH-Px activities, indicating the importance of mineral selenium as it enhances the antioxidant status of the bird by increase in the activity of GSH-Px in the serum and liver tissues of broilers. The synergistic effect of vitamin E and selenium has an *in vivo* antioxidant effect, which can protect against oxidative damage and lipid peroxidation of PUFA(Polyunsaturated Fatty Acids)[16-19].

In the present study there was no significant difference ($P>0.05$) in whole blood glutathione peroxidase(GSH-Px) activity levels in broiler chicken between the treatments (Table 3 and Figure 1).

Glutathione is the major free thiol in most living cells and is involved in many biological processes such as removal of toxic compounds and maintenance of the oxidation state of protein sulfhydryls .Maintaining the levels of glutathione concentration in liver tissue is important in balancing the antioxidant status in the body [19].Glutathione reductase activity helps in maintaining the reduced glutathione (GSH) levels in the liver based on the ability to reduce the oxidized glutathione. These glutathione reductase activities are enhanced by the inclusion of selenium and vitamin E, when these antioxidant compounds are supplemented there is an increase in the GSH levels in the liver tissues[20,21,22].

In the present study there was no significant difference between control and treatment groups with respect to Glutathione levels in the liver tissues of broiler chicken ( Figure 2).

Lipid peroxidation of poly unsaturated fatty acids (PUFA) results in the formation of byproducts like malondialdehyde which is measured in terms of TBARS (Thiobarbituric acid reactive substance). Lipid peroxidation is an index for the oxidative stress which results in higher malondialdehyde (MDA) formation in the birds when oxidative stress is high. The mean MDA values in the liver tissues of broilers at 42 days of age as influenced by supplementing vitamin E, selenium and *Ocimum sanctum* extract is represented in table 3 and figure 3.

In the present study, there was significant ($P<0.01$) decrease in the malondialdehyde levels in the liver tissues with water supplemented treatment (T2), while there were minimal reduction in the control group. This results indicated a positive effects of antioxidant compounds (vitamin E, selenium, *Ocimum sanctum*) to combat the effects of oxidative stress, which was evident in an decrease in the lipid peroxides formation.

Similar findings were also reported by K Sahin *et al*., 2009 showing that supplementing vitamin E can reduce the formation of lipid peroxides under heat stress condition in broilers. Vitamin E and selenium plays an important role in reducing the lipid peroxidation by protecting the liver from any free radical damage, as it prevents peroxidation of poly unsaturated lipids in cell membrane by free radicals scavenging effects and their supplementation has proven to decrease the formation of malondialdehyde (MDA) levels in the liver [23-26]

## CONCLUSION

Antioxidant preparation containing vitamin E, selenium and *Ocimum sanctum* extract supplemented through water significantly increased antioxidant parameters like Catalase and lipid peroxidation but did not affect performance and other parameters like glutathione peroxidase and reduced glutathione in broiler chicken. The positive effect of the preparation on antioxidant status of the bird may be attributed to its antioxidant properties.

In conclusion, oxidative stress which is the major cause of concern as result of rapid growth rate in broiler chicken can be minimized by the supplementation of antioxidant preparation through water thereby ameliorating the stress and thus be safely used as seen in the present study.

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- **Metric System**
  ml-milliliter
  kg-kilogram
  mg-milligram
  nm- nanometer

- **List of abbreviations used**
  1. GSH -Reduced glutathione
  2. GSH-Px-Glutathione peroxidase
  3. ROS- Reactive oxygen species
  4. CP – Crude protein
  5. CF – Crude fiber
  6. ME- Metabolizable Energy
  7. TBARS- Thio barbituric acid reactive substances
  8. MDA - Malondialdehyde
  9. TBA - thiobarbituric acid
  10. PUFA-polyunsaturated fatty acids.
  11. FCR-Feed conversion ratio.
  12. TCA –Tri chloro acetic acid