Research Article

Effect of Inorganic Selenium Supplementation on Thyroid Hormone and Redox Status in Broiler under Dexamethasone Induced Stress

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Abstract | Poultry industries are facing to many challenges which have made it a big problem for humans and birds. One of the major problems is oxidative stress which causes free radical damage and imbalance of thyroid functions. The research experiment was performed to check the effect of orally inorganic selenium on the redox status and thyroid hormones of broilers under dexamethasone induce stress. Day old 100 chicks were purchased from trading hatchery and divided randomly into five different groups having four replicates (n=5) in each group. Broiler chickens feed with starter and finishing commercial corn based basal diet with different concentration of selenium powder. Basal diet (BD) was fed to Group A, which kept as negative control group and no dexamethasone (DE) was offered. Group B was considered as (+ve control group) fed with (BD+15mg DE/kg). The group C, was fed with (BD+0.2mg Se + 15mg DE/kg). Similarly, group D was fed with (BD+0.3mg Se+15mg DE). Group E, fed with (BD+0.4 Se+15mg/kg feed). At the end of the trial, two birds were randomly selected for slaughtering and samples were collected from liver, muscles, kidney and whole blood. The whole blood was centrifuged for further analysis. The redox status and thyroid hormones were measured through commercially available kits. The current study resulted that selenium supplementation improved($P \leq 0.05$) the catalase level in the liver and muscles which fight against the free radical and reduce oxidative stress. In the blood serum the catalase and Malondialdehyde (MDA) both shows significant ($P \leq 0.05$). In conclusion I assume that 0.3mg/kg of selenium supplementation in redox status and thyroid hormone show better results in comparison to the control group.

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Introduction

In poultry farming, broiler chickens' entails encountering various stressors, such as immunological stress, oxidative stress, and transportation-related stress. These stressors have been observed to have detrimental effects on broiler. For instance, they can impact body weight, increase vulnerability to diseases, and impair immune functions (Lin *et al*., 2006). During regular metabolism of cells typically produce peroxides and reactive oxygen species (ROS) as free radicals. ROS can have some beneficial effects, as in phagocytes they protect the broilers from bacterial and parasitic attack. Particularly pro-oxidants and antioxidants regulate the balance, with chronic oxidative stress arising after a prolonged imbalance in pro-oxidants and antioxidants (Finkel and Holbrook, 2000). If natural antioxidant mechanism is not sufficient to extinguish high reactive oxygen species, and pro-oxidants are increased in body, they are able to cause oxidative damage and react with all the components of the cell organelles and attack proteins, lipids, carbohydrates and nucleic acid, and causes the lipid peroxidation a condition called oxidative stress (Surai *et al*., 2019). When an animal experiences stress, it triggers the activation of the hypothalamus-pituitary-adrenal (HPA) axis. As a result, the adrenal gland secretes glucocorticoids. The excess amount of glucocorticoid is the hallmark of stress (Ohu and Adeola, 2019). Stimulation of glucocorticoid, which is also analogues of dexamethasone has been used (Caldefie-Chezet *et al*., 2005). Dexamethasone, a synthetic glucocorticoid, suppresses the immune system and causes oxidative stress in some species of chicken (Osho and Adeola, 2020).

Antibiotic supplementation in the diet of chicken has a negative impact on public health, and the European Union (EU) has banned it, because it poses a risk to human health due to the causing of antibiotic resistant (Castanon, 2007). Therefore, researchers try to introduce some alternatives which are cheap, easily available and healthier for both birds and humans. Probiotics and the prebiotics in the feed of broilers chicks and trace minerals give alternative sources for the feed instead of using antibiotics (Griggs and Jacob, 2005). In order to maintain the homeostatic state that all living things have, minerals are essential nutrients.

Selenium is considered as a very potent element with a very precise extent between the lower limit effective doses and lower limit toxic dose so great care must be kept during its use. A low enough dosage of selenium is useless, but a high enough quantity may cause heart disease, neurodegenerative illness, infertility, prostate cancer, weakened immune systems, and infectious disorders in humans (Kursvietiene *et al*., 2020).

The supplements of selenium in chicken fed are a safe way to use for reducing the detrimental effect of the oxidative stress. Research studies shows that selenium is very important element to increases animal health and animal production. Antioxidants scavenging the free radical which was formed in body of broilers due to lipid peroxidation, hence the selenium supplements in the diet increase the activity of antioxidant defense system and help the biological system to boost immunity (Abbas *et al*., 2013). Selenium improved the growth traits parameters of broiler chicks (Kinal *et al*., 2012). On the other hand, micro-flora is beneficial micro-organisms found in the intestines of broiler chicken hence healthy chicken needs a healthy micro-flora. Selenium supplementation also enhances growth of micro-flora. Selenium with combination of some vitamins play antioxidant role in protecting cell membrane from per-oxidative damage.

This research was carried out to find out how broiler redox status was affected by selenium supplementation during dexamethasone-induced stress, as well as how broiler thyroid hormone was affected by selenium supplementation.

Materials and Methods

Experimental design, birds and feeding

The trial was conducted in an experimental poultry shed at the College of Veterinary Sciences and Animal Husbandry (CVS and AH), Abdul Wali Khan University, Mardan. The collected samples were processed in the Physiology laboratory. Two-hundred-day-old chick were distributed into five groups having eight replicates in each group and five (5) chick per replicate. The total duration of the experimental trial was five weeks (35 days), Moreover, the chicks were reared in standard managemental conditions. On the first day of the trial, the temperature was kept 35°C and was reduced progressively (3°C/week) at the end of the third week (21 days) and then kept constant till the end of the trial. The temperature remained at 26°C from day 22 until the trial's conclusion.

Table 1: *Grouping of chicks.*

Groups	A	B	C	D	E
Feed composition	BD	BD+DE 15mg/kg	Se-0.2+DE-15mg/kg	Se0.3+D-15mg/kg	Se0.4+D-15mg/kg

BD = Basal Diet, Se = Selenium, DE = Dexamethasone.

For the duration of the experiment, the relative humidity (RH) was maintained at 65%. Group A was considered as a (negative control group) provided with basal diet (BD) only. Group B was fed with BD and dexamethasone (DE-15mg/kg). The group C was supplements with Se-0.2+DE-15mg /kg in basal diet. Similarly, Group D was fed with (Se-0.3mg + De-15mg/kg) in BD (Table 1). The freshwater and feed (ration) were offered ad libitum every group.

At the 35 day of the trial two birds were randomly selected from each replicate and were slaughtered to determine the effect on redox status and thyroid hormone. Muscle, liver, kidney and blood samples were collected. The whole blood was centrifuged and serum was stored at -37°C for further analysis. The oxidant and antioxidant enzymes i.e., catalase and Malondialdehyde (MDA) were measured through commercially available kits. Thyroid hormones (T3, T4) were measured with the help of commercially available kit.

Statistical analysis

With SPSS (Version 20.0), the data were statistically analyzed and presented as mean standard error mean (SEM). The group means were examined using the one-way ANOVA. Group differences were evaluated using Tukey's test, with a critical threshold of ($P < 0.05$) shown.

Results and Discussion

Effect of dietary selenium on MDA and Catalase level in the blood serum

In serum the supplemented groups showed great variance Catalase (CAT) level in the blood serum was recorded highest in group E ($P \leq 0.05$) in comparison to the positive control (Group-B). However, the catalase level was also higher ($P \leq 0.05$) in groups A, C and D in comparison to positive control. The MDA level in blood serum was higher ($P \leq 0.05$) in positive control (Group-B) in comparison to all other supplemented groups. However, the MDA level was also higher ($P \leq 0.05$) in group A (BD), and E (BD+0.4) in comparison to group C, D (Table 2).

Table 2: *Result of selenium supplementation on antioxidant and oxidant levels on the serum of broilers under dexamethasone induce stress.*

Parameters	A	B	C	D	E	SEM	P value
CAT (KU/l)	9.64^{b}	6.80^{c}	8.98^{b}	10.47^{b}	12.32^{a}	4.15	0.026
MDA(nmol/ml)	3.2^{ab}	3.64^{a}	3.00^{b}	2.79^{b}	3.11^{ab}	0.26	0.007

CAT= Catalase, MDA= Malondialdehyde.

Effect of dietary selenium on MDA and Catalase level in the liver

In the current study the catalase value in liver showed a higher ($P \leq 0.05$) value in group D (Se0.3+D-15mg/kg) and E (Se0.4+D-15mg/kg,) as compared to positive control (Group-B). Group A (BD) and C (Se-0.2+DE-15mg/kg) also showed higher ($P \leq 0.05$) value as compared to positive control (Group-B). The MDA value did not vary among the supplemented, positive and negative control groups (Table 3).

Table 3: *Result of selenium supplementation on antioxidant and oxidant levels on the liver of broilers under Dexamethasone induce stress.*

Parameters	A	B	C	D	E	SEM	P value
CAT (KU/l)	63.35^{b}	55.30^{c}	62.08^{b}	70.36^{a}	68.75^{a}	15.52	0.004
MDA (nmol/ml)	4.48	4.72	4.50	4.12	3.90	0.19	0.170

Effect of dietary selenium on MDA and Catalase level in the muscle

In the current study the catalase value in the muscle of broiler showed higher ($P \leq 0.05$) value in group D (Se0.3+D-15mg/kg) as compared to positive control (Group-B). However, in group A, C, and E showed higher level as compared to positive control (Group-B). The MDA concentration did not vary among the supplemented and positive and negative control groups (Table 4).

Effect of dietary selenium on MDA and Catalase level in the kidney

In the current study the catalase value in the kidney of the broiler showed did not vary among the groups. The MDA value shows significance ($P \leq 0.05$) value.

The value of MDA in the kidney of the broiler is highest in group B. However, group A and C also had higher value as compared to group D and E (Table 5).

Table 4: *Influence of selenium supplementation on antioxidant and oxidant levels on the muscle of broilers under Dexamethasone induce stress.*

Parameters	A	B	C	D	E	SEM	P value
CAT (KU/l)	14.80^{ab}	13.22^{b}	14.70^{ab}	16.10^{a}	15.87^{ab}	5.452	0.004
MDA (nmol/ml)	1.90	2.40	2.30	2.22	1.90	0.05	0.120

CAT= Catalase; MDA= Malondialdehyde.

Table 5: *Effects of selenium supplementation on antioxidant and oxidant levels on the kidneys of broilers under Dexamethasone induce stress.*

Parameters	A	B	C	D	E	SEM	P value
CAT (KU/l)	19.71	18.10	20.69	23.49	23.71	9.804	0.064
MDA (nmol/ml)	7.10^{b}	9.40^{a}	7.20^{b}	5.72^{c}	5.30^{c}	6.952	0.020

CAT= Catalase, MDA= Malondialdehyde.

Effect of dietary selenium on the level of thyroid hormone in the blood

Effect of selenium on thyroid hormones shows that the T4 value did not vary among the treated and the positive and negative control groups. In group C and D value is also higher (P≤0.05) as compared to group C and D (Table 6).

Table 6: *Effect of selenium supplementation on T3, T4 and TSH hormone.*

Parameters	A	B	C	D	E	SEM	P value
T4	22.5^{a}	13^{c}	16^{b}	24.38^{a}	21.6^{a}	3.67	0.046
T3	4.19^{ab}	3.34^{b}	4.5^{b}	5.3^{a}	5.9^{ab}	0.88	0.030
TSH	0.0085	0.0055	0.005	0.0055	0.011	000	0.100

T4= Thyroxin, T3= Triiodothyronine, TSH= Thyroid stimulating hormone. The superscript a-c within the same row, means with different superscripts are significantly different (P<0.05) and represent the Means of five replicates.

The present study was designated to determine the effect of selenium on redox status (level of MDA and catalase) and thyroid hormone (T3, T4) of broiler under dexamethasone stress. Selenium is a tracing mineral that is important for well-functioning of many organs. The selenium easily absorbed through gut mucosa by the process active transport (Kuršvietienė *et al.*, 2020). Therefore, Se was used in the diet of broilers to avoid the detrimental effects the oxidative stress when the birds were freely exposed. In cell-mediated immunity and antibody-mediated immunity, Se has beneficial role at several points.

Effect of selenium on redox status in the blood serum

In the current study the effect of selenium in blood serum the catalase level show higher (P≤0.05) in group E in comparison to positive control. A similar experiment was conducted by Muhammad *et al.* (2022) who reported that the Se is an integral part of different antioxidants enzymes. The antioxidants capability of Se, which reduce the proceed of lipid peroxidation and scavenging the free radicles. The Se reduces the level of MDA and increasing the level of catalase in serum (Chung *et al.*, 2017).

Effect of selenium on redox status in the liver

In the current study we found that the effect of selenium supplementation on the liver catalase level is significant (P≤0.05) higher shown in groups D and E in comparison to positive control. Selenium supplements, respectively. A similar result was conducted that supplementation of selenium (selenite) improved the antioxidant status of broilers under DE stress (Li *et al.*, 2018). The MDA level in the liver resulted in improvement in them. The Se act as antioxidant, these antioxidants play an important role to protect the cell from reactive oxygen species (ROS) by inhibiting the generation of free radicles and thus preventing the process of lipid peroxidation.

Effect of selenium on redox status in the muscles

In the current study we found that the effect of selenium supplementation on the muscle catalase level is significant (P≤0.05). Highest significance shown in group D which are fed by BD+0.3mg Se+15mg DE. A similar result was documented by Muhammad *et al.* (2022) wherein the scientist used Se in the diet of the broilers. The supplements showed highest Se concentration in muscles tissue. The current study showed the insignificance in the selenium supplemented group hence resulting in simply improvement in them. The MDA level in the muscle of the broiler shows a significant (P≤0.05) level however, they used Se in fed. Dietary selenium increases the level Se in muscles and serum which enhance the oxidative capability of these tissues by

improving the GSH-PX catalase, and SOD activities and reduce the concentration of MDA in birds (Ghazi Harsini *et al*., 2012).

Effect of selenium on redox status in the kidney

In the current study we found that the effect of selenium supplementation in the kidney catalase level is significant (P≤0.05). Highest significance shown in group E which are followed by group A, C and D. The MDA level in the kidney in views significant (P≤0.05) in group B. It is concluded that selenium is effective for the inhibition of lipid peroxidation which occurs in birds during stress. Similar findings showed that the most effective lipid oxidation inhibitor was a combination of 0.5 mg/kg selenium and 125 mg/kg vitamin E. It was also shown by the findings that broilers under heat stress had much more skeletal muscle when they were fed vitamin E via feed (Habibian et al., 2012).

Effect of dietary selenium on the thyroid hormone of the broiler

The current study shows the effect of selenium on the thyroid hormone of a broiler under oxidative stress. In T4 group E showed more significance (P≤0.05) as compared to control group. However, C and D also showed significance (P≤0.05) as compared to the control group. Another study conducted that in fact, Se-deficient chickens showed low levels of T3 in blood serum and increased blood T4 concentrations (Jianhua *et al*., 2000). They also concluded that there was a decrease in plasma T3 after the injection of corticosterone which was assigned to increased circulating corticosterone concentration. It means that the T3 level is remained low after the injection of corticosterone.

Conclusions and Recommendations

From this study it was concluded that the dietary inorganic selenium supplements significantly influenced the growth hormones and redox status of the broilers under oxidative stress. In summary, the study finding indicates that the 0.3mg/kg of selenium supplementation in the basal diet of the broilers enhanced the catalase (CAT) level in serum, muscle, kidney, and liver. The 0.3 and 0.3mg/kg of selenium supplementation also enhanced the growth hormones levels (T3, T4) in birds under dexamethasone-induce stress.

The study further encourages the investigation of the suppression of growth trace elements, or their nano size will be to alleviate the harmful effects of the oxidative. Different trace elements or their nano size will be to alleviate the harmful effects of oxidative stress.

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Novelty Statement

The research and experimental work on the subject title is original and new in the field of poultry feed supplementation in Khyber Pakhtunkhwa, Pakistan.

Author's Contribution

Afshan: Investigation, writing-original draft preparation; writing-review and editing.
Asad Ullah: Conceptualization, Supervision.
Imad Khan: Methodology.
Rafiq Ullah and Tahira Tayyab: Project administration.
Fatima Syed and Raheela Taj: Visualization.
Shumaila Gul: Data curation.
Faiza Khan: Software.
Ibad Ullah Jan: Validation.
Syed Weqas Ali and Assad Ullah: Formal analysis.

Conflict of interest

The authors declared no potential conflicts of interest with respect to research, authorship, and/or publication with the work submitted.

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