Research Article

Effect of Inorganic Selenium on Blood Biochemistry under Dexamethasone Induced Stress in Broiler Chicken

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Abstract | This study was conducted to detect the effect of inorganic selenium on the liver enzymes, kidney function and changes in biochemical parameters of broiler chicken under dexamethasone (DEX) induced stress. The day old, two hundred (n=200) chicks were bought from commercial hatchery and randomly divided into five groups with eight replicas(n=5) in each group. Broiler chickens were fed with starter and finishing commercial corn based basal diet (BD) with different doses of inorganic Selenium (Se) powder. Group-A was kept negative control provided with BD twice a day. Group-B was positive control group fed with 15mg DEX/kg twice/day. The Group-C was fed with BD+0.2mgSe+15mg DEX/kg. Similarly, Group-D was fed with BD+0.3mgSe+15mgDEX. Group-E was fed with BD+0.4mgSe+15mg DEX/kg. Two chicks were randomly slaughtered from each replicate and blood glucose level, kidney function and liver enzyme were examined. After slaughtering, the collected blood was centrifuged for 15 minutes at 1000 rpm for serum analysis and anticoagulant added tube for whole blood. The serum was stored at -20˚C until further analysis. Biochemical parameters were measured through commercial kits. The result of the current study showed that the values of Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were higher significantly (P≤0.05) in negative control group (B) as compared to the group C, D and E. The level of uric acid, creatinine and blood urea were significantly higher (P≤0.05) in group A (negative control) (P≤0.05) as compared to the group B (positive control) C, D, and E. The blood cholesterol level and low-density lipoprotein (LDL) in group B (positive control) was significantly higher (P≤0.00) than the group A (negative control) and Se supplemented group C, D and E.

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Introduction

The poultry industry has many challenges to suppressed bird's immunity, growth and production performance. The emergence and re-emergence of diseases user confidence type of product safety are the major challenges to the current situation for the future of national and international industry (Hafez and Attia, 2020). There is huge pressure on poultry, market to establish equilibrium between demand and supply. Several types of stresses like transport, fasting, temperature, overcrowding and antibiotics etc., are the main causes behind this gap. These stresses affect the growth as well health of the broilers and sometime cause mortality (Hussain *et al*., 2015). In every stress condition the level of glucocorticoid suddenly arise. Both natural and synthetic derivatives steroid hormones are present in Glucocorticoids (GCs) which can cause several damages to birds like effecting kidney function, liver enzymatic production increase in blood creatinine, urea and uric acid and suppress the immune system (Duff *et al*., 2019). DEX is a synthetic form of the glucocorticoids. Therefore, the antibiotics growth promoters are banned internationally to protect the human health and environment. The use of alternative of antibiotics growth promoter is needed to enhance the health status of the birds. It has been reported that the commercially available alternative phytobiotic, probiotic and trace minerals have impact on the health, production and performance of poultry (Ren *et al*., 2019). Trace minerals (TMs) play important role in production of broiler chicks, it not only play role in health maintains but also in quality of meat production control of disease and reduce stress. TMs are also function on various biosynthetic, digestive and physiological process as a co-factors of several metallo-enzymes, including the MN-SOD, Zn/Cu-SOD and selenium containing enzymes (Wang *et al*., 2019). Currently inorganic selenium (Se) trace element attract the more attention of poultry farmer because of strong adsorbing ability, high bioavailability, low toxicity and high catalytic efficiency as compare with organic selenium on broiler poultry (Wang et al., 2019). Selenium is the important trace mineral element that needed for the body normal function and therefore on the broiler chickens and have important role in their health maintenance. The sign and symptom of Se deficiency on poultry has been a vital role in protection of antioxidant through the enzyme glutathione peroxidase (GPx). It has been recommended that the selenium supplemented feed of roosters can help as a nutritional food have low cholesterol and high selenium substances for both cardiovascular disorder patient and healthy people. Selenium is an essential trace mineral that plays vital role in productivity, growth, immune function and anti-stress. The different forms of selenium supplementation such as organic and inorganic selenium the concentration of selenium on broiler diet is different. In nature selenium is present in two chemical form inorganic and organic. Inorganic selenium can be found in several minerals in the form of, selenite, selenide and also in the metallic form. In other side oilseed, grain and forages meals the selenium is bond to the different amino acid such as cysteine and methionine. Therefore, the animal in nature receives the selenium primarily as seleno-methionine The majority of the selenium absorbed by the plant is in the form of selenite or selenate, or it is produced by the plant using seleno amino acids, which account for at least 50% of the selenium in cereal grains. Plants are Se-methyl-Selenocysteine, Selenocysteine, and Se-methyl-Seleno-methionine (Surai, 2002). However, the use of selenium on broiler industries is comparatively less therefore the current study was designed to find the impact of inorganic se supplementation, on the serum biochemistry in broiler chickens under dexamethasone induced stress. To examine the effect of inorganic Se supplementation on liver enzyme (ALT, AST, ALP), renal function (blood urea, creatinine and uric acid), lipid profile i.e; Total Cholesterol, High density lipoprotein (HDL) and Low density lipoprotein (LDL) and blood glucose level on broiler chickens under dexamethasone induced stress.

Materials and Methods

Experimental design, birds and rearing conditions

The trial was conduct in experimental poultry shed at College of Veterinary Sciences and Animal Husbandry (CVS and AH), Abdul Wali khan University, Mardan. The collected samples were processed in laboratory of physiology, CVS and AH. Two-hundred-day-old birds were allocated to five groups and having eight replicates in each group and five (5) birds per replica. The total duration of the experimental trial was five weeks (35 days), and the birds were kept in an environmentally controlled experimental shed. Temperature of 35°C was maintained on the day 1st and was decreased gradually (3°C/week) to 26°C by

the end of the 3rd week (21 day). From day 22nd to the end of the trial, the temperature was kept at 26°C. The relative humidity (RH) was kept at 65% during the whole experiment. Group-A was considered as a negative control group provided with basal diet (BD) only, Group-B was fed with BD and dexamethasone (DEX-15mg/kg), Group-C was supplemented with 0.2mg Se+DEX-15mg/kg in BD. Similarly, Group-D was supplemented with 0.3mg Se+DEX-15mg/kg) in BD and Group-E was fed with BD+0.4mgSe+15mg DEX/kg (Table 1). The freshwater was offered ad libitum during the whole research trial.

Sampling

Two chicks from each replicate were randomly selected and slaughtered. The blood was collected from jugular veins in vacutainer and centrifuged for 15 minutes at 1000 rpm for serum analysis. The parameters under observation were liver function test (ALT, AST, and ALP) and kidney function (blood urea, uric acid, creatinine, and blood glucose and lipid profile.

Urea, creatinine, uric acid, and glucose were determined from blood serum through available commercial kit. A total of 20µ liter of serum was taken in a calcium glass tube with the help of micropipette for determination of Urea. Then 1000µ liter of reagent 01 and 250µ liter of reagent 02 was mixed with it using DiaSys–Germany REF 12767726876. After mixing, the mixture was incubated for 50 seconds and the results were displayed on the screen and the values were noted. Creatinine assay kit was used for determination of creatinine and 50µ liter of the serum was obtained in calcium glass tube. Then, 1000µ liter of reagent 01 and 250µ liter of reagent 02 was mixed with it using DiaSys–Germany REF 12767726876. The tube containing mixture was incubated for 60 seconds and the obtained results were noted. Glucose was determined by taking 10µ liter serum and 1000µ liter of reagent 01 with 250µ liter of reagent 02 in calcium glass tube and was mixed using DiaSys–Germany REF 12767726876. The tube containing mixture was incubated for 5 minutes and the obtained results were noted

Statistical analysis

Statistical analysis was done through SPSS software (IBM SPSS Statistics 22), respectively and subjected to one-way ANOVA and displayed as mean standard error mean (SEM) (Version 20.0). The one-way analysis of variance (ANOVA) was employed to examine the means of different groups in the study. The Duncan post hoc test was employed to evaluate the disparities between groups, with the critical level set at a significance level of ($P \leq 0.05$).

Results and Discussion

According to the result of the current study, the values of ALT were significant (P≤0.05) in negative control group (A) as compare to the group C, D and E. While the value of AST is also higher (P≤0.05) in group A (negative control) as compare to the value of C, D and E. The values of ALP were competitively high (P≤0.05) in group A (negative control) (P≤0.05) as compared to group C, D and E (Table 2).

Table 1: *Feed composition of different groups during research trial.*

Groups	A	B	C	D	E
Feed composition	BD	BD+DEX-15mg/kg	0.2mg Se+DEX-15mg/kg	0.3mg Se+DEX-15mg/kg	0.4mg Se+DEX-15mg/kg

BD: Basal diet; Se: Selenium; DEX: Dexamethasone.

Table 2: *Effect of selenium (Se) on liver enzymatic function (ALT, AST, ALP) of broiler chickens under dexamethasone induced stress.*

Groups	A	B	C	D	E	SEM	P-Value
ALT(µ/l)	7.92^{bc}	13.90^{a}	10.62^{b}	5.75^{d}	6.62^{cd}	0.418	0.02
AST (µ/l)	152.80^{b}	300.62^{a}	151.30^{b}	150.60^{b}	153.10^{b}	5.59	0.03
ALP (µ/l)	1440.50^{a}	1802.2^{b}	1510.25^{a}	1480.70^{a}	1501.0^{a}	32.15	0.02
	* – data statistically significant at P<0.05						

ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

Table 3: *Effect of selenium (Se) on renal function (urea, uric acid, creatinine, glucose level) of broiler chicken under dexamethasone induced stress.*

Groups	A	B	C	D	E	SEM	p-value
UC(mg/dl)	4.33^{b}	5.562^{a}	4.82^{b}	4.65^{b}	4.31^{b}	0.0332	0.02
CrT (mg/dl)	0.20^{b}	0.287^{a}	0.175^{b}	0.20^{b}	0.162^{b}	0.0154	0.01
U(mg/dl)	5.25^{b}	7.50^{a}	5.25^{b}	4.62^{b}	4.37^{b}	0.4304	0.04
G (mg/dl)	177.46^{bc}	190.20^{a}	178.0^{b}	172.4^{bc}	152.36^{c}	4.614	0.03

UC: Uric Acid; CRT: Creatinine; U: Urea; G: Glucose.

Table 4: *Effect of selenium (Se) on lipid profile of broiler chicken under dexamethasone induced stress.*

Parameter	A	B	C	D	E	SEM	P-Value
Cholesterol (Mg/dl	127.00^{ab}	138.20^{a}	128.70^{ab}	118.30^{b}	113.30^{b}	2.44	0.026
Triglycerides (mg/dl)	59.30	62.80	60.25	72.66	70.42	2.84	0.344
HDL (mg/dl)	68.80	66.50	70.26	74.40	74.30	3.284	0.200
LDL (mg/dl)	86.38^{b}	99.58^{a}	89.75^{b}	83.36^{b}	88.42^{b}	2.478	0.008

HDL: high density lipoprotein; LDL: low density lipoprotein.

The result of current study concludes that the level of uric acid in blood was significantly higher (P≤0.05) in group A (negative control) (P≤0.05) as compared to the group B (positive control), C, D, and E group. While the blood creatinine level was also higher (P≤0.05) in group A (negative control group) than group B (positive control) and selenium supplementation group C, D and E. The blood urea was also comparatively high in group A (negative control) (P≤0.05) than positive control group B and selenium supplemented group C, D and E. The glucose concentration in blood was also higher (P≤0.05) in group A (negative control) than the group B (positive control) and group C, D and E (Table 3).

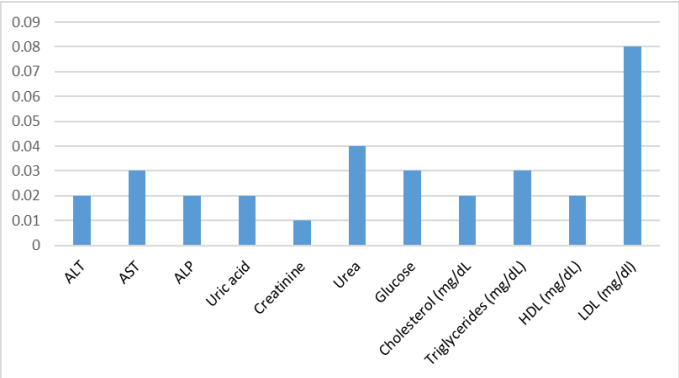

Figure 1: *Comparative analysis of blood biochemistry of broiler chicken.*

The lipid profile result showed that the blood cholesterol level in group A (negative control) significantly (P≤0.00) higher than the group B (positive control) and SE supplemented group C, D and E. The values of triglycerides were remained insignificant. The level of HDL was also recorded insignificant. The level of LDL in blood of broiler is higher (P≤0.05) in DEX treated group than the negative control group A and Se supplemented groups C, D and E (Table 4).

This study was designed to analyze the effect of selenium supplementation source of trace mineral and their relation on the antioxidant enzymatic activity, growth performance and serum concentration in the broiler chicken. Selenium is considered as a vital trace mineral play important role in growth performance antioxidant status and liver enzymatic activity. The current study showed that the ALT level is increase in the group B (positive control or DEX treated group) while the ALT level in group C, D and E is decrease due to selenium supplementation. Similarly, the level of AST and ALP also increase in positive control group B (DEX treated group) while their level decrease in Se supplemented group C, D and E. The previous study found that the Se supplementation improve the antioxidant activity, growth performance, liver and kidney function of broiler chickens. Another study of Boostani *et al*. (2014) supported our current study, to show the effect of selenium on serum biochemistry. They concluded that the Se supplements increase the liver enzymatic function, renal function and growth performance of broiler chickens.

In present study we investigate that the addition of Se on feed of broiler chicken can positively effect on the kidney function, blood urea level, level of uric acid, creatinine and glucose in blood. Selenium is a trace mineral plays important role in growth

performance liver and kidney function and blood glucose concentration. The current study showed that the uric acid level was increased in positive control group B (DEX treated group) while the level uric acid is decreased in Se supplemented group C, D and E. The same results were also reported by Zhang *et al.* (2015) and Liang *et al.* (2015) who suggested that the level of uric acid was reduced with the selenium supplementation in broilers. According to current study, the level of blood urea and creatinine level in blood were increased in DEX treated group while level of the same was remained normal in negative control group A. However, the level of urea and creatinine was become down in Se supplemented group C, D and E. The same study was conduct by Sun *et al.* (2015) who reported that the deficiency of selenium can increase the level creatinine and urea in blood while decrease the renal antioxidant capacity to control the level of blood urea and creatinine and sometime the selenium deficiency and use of high level of glucocorticoid increase the risk of damages in renal tissues due to which the level of urea and creatinine increase in blood.

In the current research findings, the blood cholesterol level in group B (positive control) was significantly higher in comparison to group A (negative control). The level of LDL in blood of broiler was higher ($P \leq 0.05$) in DEX treated group (positive control) than the negative control (group A) and Se supplemented groups. Dexamethasone induced oxidative stress and increased the glucocorticoid, cortisol and cholesterol level in blood circulation. Selenium supplements in the diet of broilers enhance the antioxidant activity and reduce the level of cholesterol when compared with dexamethasone treated group (Nwakpu *et al.*, 2016). Selenium are important trace mineral and improved the activity of antioxidant enzyme such as catalase, Plasma glutathione peroxidase (GHS-PX), Superoxide dismutase (SOD), HDL-Cholesterol and decrease serum cholesterol, Low density lipoprotein (LDL)-Cholesterol and Malondialdehyde (MDA) level in the broiler (Cai *et al.*, 2012).

Conclusions and Recommendations

In the current research investigation, DEX increased the serum creatinine level in chicks. The same has been reported in case of urea and uric acid and could be attributed to an initial state of stress induced by DEX affecting the kidneys. Similarly, the level of cholesterol was high in DEX treated chicks and lowered in negative control and chicks treated with Se supplementation. Similar to our findings that reduced serum creatinine levels was noted in birds when supplemented with Selenium is also reported previously. Selenium improves the level blood urea and uric acid. Similarly, Se due to its hypolipidemic properties increased the level of triglyceride while DEX induced birds showed decreased level of HDL. The serum cholesterol levels were also high in DEX treated birds compared to negative control group and selenium treated birds. Use of DEX also increased the level of glucose in blood of broiler and Se reduced the level of glucose in birds. Similarly, the level of ALT, AST and ALP is also increased by the use of DEX in broiler. While the supplementation of selenium decreased the level of ALT, AST and reduced the ALP significantly. It is recommended that further research work needs to be carried out on the effect of selenium (Se) on the broiler growth and health. Further, different awareness seminars and workshops are necessary for the poultry farmer about the addition of selenium in broiler feed. The researcher must use selenium on deferent animals and birds with different concentrations and show their effect and outcomes to the farmers, academia and industry.

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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

Irfan Ullah: Investigation, writing-original draft preparation.
Asad Ullah: Conceptualization, supervision.
Tahira Tayyeb: Methodology.
Rafiq Ullah: Project administration.
Muhammad Hanif and Faiza Khan: Validation.
Imad Khan: Resources.

Raheela Taj: Data curation.
Fatima Syed: Visualization.
Shumaila Gul: Formal analysis.
Muhammad Sadeeq: Software.
Muneeb Islam and Arsalan Khan: Writing-review and editing.
Khudija Ghani: Helped in collection of relevant literature.

Ethical approval
The study was approved by the ethical review committee, Department of Zoology, Abdul Wali Khan University Mardan.

Conflict of interest
The authors have declared no conflict of interest.

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