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Effect of Selenium on Morphology, Mineral Retention of Tibia Bone and Muscle Health of Broiler under Dexamethasone-Induced Stress

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ABSTRACT

In current research study, the antioxidant activity of inorganic selenium (Se) for broilers under induced stress was determined to improve the muscle and tibia bone health as well as tissue mineral retention. The poultry industry is playing a vital role in fulfilling the protein requirement globally. This was obtained by rearing broilers with standard farming which produce good quality meat sometimes the meat quality is affected when the broilers are coming produce good quality meat. Sometimes the meat quality is affected when the broilers are coming into stressed during farming and transportation. To minimize the effect of stress over the broilers growth the free radicles in the body are neutralized with the help of antioxidants. This research study was designed to introduce the broilers by induced stress by inclusion of dexamethasone in their diet. To cope with their detrimental effect, inorganic selenium was provided in different concentrations to birds. A day-old 200 were distributed in five groups having eight replicas with 05 chicks per replicate. The dietary plan for the chicks was as follows. Group A (Negative control) was supplemented with only a basal diet. Group B (Positive control) was supplemented with dexamethasone 15mg /kg diet. Group C, D and E were supplemented with 0.2mg, 0.3mg, and 0.4mg Se /kg of the diet, respectively. This research study concluded that adding 0.3mg of inorganic selenium/kg of diet improved the calcium and phosphorous deposition in tibia bone, tibia bone morphology, as ash content, and muscle histomorphometry.

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Key words
Broilers, Bone, Dexamethasone, Muscle, Stress, Trace mineral

INTRODUCTION

Protein is the major component of meat. This is obtained mostly from the meat-producing industry. The poultry industry is among those that produce broilers production (Abbas *et al*., 2016). Globally the poultry industry is facing several challenges in fulfilling the protein requirement for maintenance and combating various diseases and harsh conditions. Pakistan obtains most of the protein requirement from the poultry industry. Farming in Pakistan

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also has several challenges in which the stress in poultry sector or during transportation affects the growth traits of broilers. The huge demand due to population growth and chicken meat consumption, palatability and taste puts pressure on production. This also reduces the quality of meat as well as the chicken's health. Despite the huge demand for rearing chickens on farms also have several health problems like diseases, infections, starvation, heat, cold, crowding, and suffocation, these stresses bring the broilers or other chickens in oxidative stress as a result of their growth and health effects adversely (Aggrey *et al*., 2010).

When the broilers face environmental stresses, they respond to them and initiate a chemical response by secreting the glucocorticoids. The abrupt change in the level of glucocorticoid has a certain negative effect on the muscle as well as on bone health (Godfray *et al*., 2010). The growth and health of broilers affected by stress indirectly reduce the quality of meat as well as its nutritional value. For this purpose, the remedy and neglecting of stresses during farming or transportation is necessary (Xueting *et al*., 2018).

Induced stress with inclusion of dexamethasone raises the abrupt changes in the body of the broilers which results in the production of free radicals. These reactive radicle leads to the degeneration of the muscle cell membrane which causes their losses of water content. By the water or incapability to hold water the meat quality of broilers is decreased. This physiological process can be paused by neutralizing the free radicles with antioxidants. Previously this was achieved by growth promoters and drugs. But later due to its negative effect on the broiler and consumers health various growth promoters like antibiotics were banded globally (Cannizzo *et al*., 2010).

Recent broiler farming is looking for the usage of photobiotics as well as selected feed designs that have sufficient concentrations of antioxidants. Being a trace mineral and component of the antioxidant enzyme se has a vital role in normalizing the free radicle produced as result of stress. It initiates a chemical reaction to normalize the reactive species which causes damage to cells (Kieliszek and Błażejak, 2016). Se catalyze to enhance the performance of antioxidant enzyme as a result the cells are protected from harmful substance and improve the health as well as tissues (Skřivan *et al*., 2011). Se can be supplements in the diet in three ways. It is available in natural products or organic form, synthesized inorganic, and is designed specially in nano size (Briens *et al*., 2013). The inorganic form of Se has wide availability as well it is favorable economically for the industry. The Nano size particle can also be easily designed from the inorganic form of Se (Wang *et al*., 2011).

Protecting the muscle cells from free radicles antioxidant enzymes serves in the presence of Se. The Se is integral part of antioxidant enzymes. It initiates the synthesis as well as production of seleno-protein enzymes (Surai *et al*., 2016). Some studies suggest that Se is boosts the immune system and it accumulates in muscles, blood plasma, and well liver mostly (Habibian *et al*., 2016). Glutathione peroxidase enzymes are the main component of Se. This enzyme protects the body cells from oxidative stress as well it boosts immunity against certain infections. This also leads to the accumulation of Se in various tissues of the broilers. It causes to increase in the nutrient value of broilers (Yang *et al*., 2012).

Several studies have been conducted on the investigation of Se on broilers' growth performance, but no such study identified the effect of inorganic Se and their liable dose for supplementation to broilers is not identified. This research work will find out the performance of different doses of inorganic Se on pectoral muscle histo-morphometry. This research will also find the performance of different concentrations of inorganic Se on tibia bone morphology and mineral retention.

MATERIALS AND METHODS

Birds grouping and feeding

This study was performed in the managemental control poultry shed, Collage of Veterinary Sciences and Animal Husbandary, Abdul Wali Khan University, Mardan.

A day-old 200 broiler chicks were randomly allotted in five groups each having eight replicas per group and five birds were present in per replica. Group A was negative control having only basic diet supplementation, group B was positive control by inducing stress with supplementing 15mg of dexamethasone per kg diet. Groups C, D and E were supplemented with 0.2mg Se, 0.3mg Se, and 0.4mg Se/kg of the diet, however, 15mg dexamethasone per kg of diet were also supplemented in the Se supplemented groups.

On day 35, two chicks from each replicate were slaughtered and obtained their tibia bone. The bone was boiled for 10 min to remove the meat. A muscle sample was taken from the breast to determine the histo-morphometry.

Tissue processing for muscle morphometric parameters

The paraffin embedding method was used for tissue processing and hematoxylin and eosin (H and E) were used for staining the histological sections of muscle (Canene-Adams, 2013).

Muscle fascicle and fiber diameter were measured by using the method of Luqman *et al*. (2020). Using a 4X

objective lens, images were taken at random locations on the slide to measure the facile diameter. The diameter of five muscle fibers in three fascicles were measured in millimeters. The diameter of the muscle fibers was used to calculate the cross-sectional area using the histomorphometry program Progress Capture Pro 2.7.7. Labomed USA. Muscle cells and muscle fibers were counted in a 0.5mm radius circle and then converted into 1mm as explained by Khan *et al*. (2022).

Tibia bone morphometric characteristics

Tibial bones were taken from two birds from each replicate and detached as drumsticks with their flesh intact. The drum sticks were submerged in boiling water for 10 min. After that drumsticks were allowed to cool for 42 h. Using a weighing scale and digital Vernier Calipers, the tibial bone's breadth, length, and weight were assessed. Each bone's diameter was measured externally from medial to lateral and at the midpoint. The diameter of the medullary canal (MC) was measured using a vernier caliper after the bone was broken in the middle point in the shaft. The bone weight by its length, the bone weight/ length index was calculated according to Mabelebele *et al*. (2017) as weight/length index= weight (mg)/ length (mm).

The robusticity index was measured bone length (mm)/ cube root of bone weight (mg).

The index of tibiotarsal bone was determined accourding to Yusof *et al*. (2023) by the following formula.

(Diaphysis diameter-medullary canal diameter/ diaphysis diameter) × 100

A sample of dry tibia bone fragments was collected in a China crucible and burnt for 24 h in a muffle furnace at 560ºC to measure the content of bone ash. The bone ash percentage was calculated relative to dry tibia weight.

Tissue mineral retention

For determining tissue mineral retention tibia bone, was ground to form powder which was subjected to flame photometry (Biotech Engineering Management Co. Ltd. UK). By using UV-spectrophotometer (V-1100, Thermo Fischer Scientific, USA) to determine the Phosphorus and ash content (Sunder *et al*., 2013).

Statistical analysis

The data regarding the samples were analyzed via SPSS software 20.1 version. The group difference was determined by one-way ANOVA post hock Tukey's test by considering the significant value $P \leq 0.05$.

RESULTS AND DISCUSSION

The impact of Se on histomorphometry of muscle is significant as shown in Table I. Se supplementation has improved muscle histomorphometry in various ways. The muscle fiber diameter was improved in Se-supplemented groups Se0.2 mg, and Se0.4mg as compared to the non-supplemented groups. The muscle fiber cross-section area was also found significant in Se-supplemented groups concerning non-supplemented groups. The muscle fiber density has improved by Se supplementation concerning non-Se-supplemented groups. Muscle growth, bone mineralization, and overall health all reflect broiler growth. Rapid growth in 35 days with a growth promotercauses broiler oxidative stress. In this study, broilers were given inorganic Se to reduce stress and improve growth. It improved muscle histomorphometry noticeably. The cross-sectional area and density of muscle fibers increased significantly in the Se-supplemented group. Se is required for the synthesis of glutathione peroxidase, and other antioxidant enzymes, and this enzyme works efficiently in the presence of Se in the diet. Glutathione peroxidases are an antioxidant enzyme family. Their primary function in the body is to neutralize hydrogen peroxide and organic hydroperoxides in the intracellular and extracellular environments. As a result, it protects the cell and cell membrane from induced oxidative stress. It scavenging the free radicals and also inhibit the production of free radicals (Li *et al*., 2018).

Table I. Performance of inorganic Se of muscle histomorphometry.

Parameters	A (NC)	B (PC)	C (0.2 mg Se)	D (0.3 mg Se)	E (0.4 mg Se)	SEM	P value
Muscle fascicles diameter (mm)	0.39^{ab}	0.18^{c}	0.38^{c}	0.42^{ab}	0.52^{a}	0.12	0.001
MFSCA (mm^2)	0.12^{b}	0.27^{c}	0.11^{b}	0.14^{ab}	0.21^{a}	0.56	0.003
MFSD (mm)	6.33	5.10	6.17	4.27	6.06	0.74	0.516
MFSCSA (mm^2)	22.97	20.4	29.96	30.15	29.87	6.66	0.564
Muscle fiber density	121.5	94.0^{b}	104.0^{ab}	103.0^{ab}	117.0^{ab}	4.1	0.034

MFSCA, muscle fiber diameter cross-sectional area; MFSD, muscle fascicles diameter; MFSCSAm muscle fascicles cross-sectional surface area. ^{a,b,c} Means with different superscript letters in the same row differed significantly ($P < 0.05$).

Table II. Performance of inorganic Se of tibia bone morphometric parameters.

Parameter	A (NC)	B (PC)	C (0.2mg Se)	D (0.3mg Se)	E (0.4mg Se)	SEM	P value
Bone length (mm)	85.62	81.37	85.12	87.12	88.87	3.08	0.333
Bone weight (mg)	7000.0^{b}	4125.0^{d}	5500.0^{cd}	7250.0^{b}	9750.0^{a}	348.0	0.000
DD (mm)	1.058	0.90	0.940	0.950	1.091	0.26	0.16
MCD (mm)	0.4250	0.3750	0.40	0.38	0.42	0.22	0.530
MWD	0.2^{ab}	0.21^{ab}	0.18^{b}	0.2^{ab}	0.26^{a}	0.17	0.026
LWD	0.1	0.1	0.1	0.1	0.11	00	0.421
RI	44.49^{bc}	51.39^{a}	48.27^{ab}	45.07^{bc}	41.83^{c}	1.43	0.001
TI	59.7	57.44	57.39	59.54	60.51	2.46	0.874
W/LI	82.43^{b}	50.54^{c}	64.57^{c}	84.78^{b}	109.88^{a}	4.86	0.000

NC, negative control; PC, positive control; DD, diaphysis diameter; MCD, medullary can diameter; MWD, medial wall diameter; LWD, lateral wall diameter; RI, rubustitcity index; TI, tibiotarsal index; W/LI, weight/length index. ^{a,b,c} Means with different superscript letters in the same row differed significantly ($P < 0.05$).

Table III. Performance of inorganic Se on calcium, phosphorus, and ash content of tibia bone.

Parameter	A(NC)	B(PC)	C(0.2mg Se)	D(0.3mg Se)	E(0.4mg Se)	SEM	P value
Ash %	47.25^{bc}	41.75^{c}	46.5^{bc}	56^{ab}	61.2^{a}	3.04	0.004
Calcium %	24.25^{ab}	13.0^{b}	21.5^{ab}	21.5^{ab}	26.7^{a}	2.44	0.031
Phosphorus %	12.7^{a}	6.25^{b}	9.0^{ab}	11^{ab}	10.7^{ab}	1.11	0.038

^{a,b} Means with different superscript letters in the same row differed significantly ($P < 0.05$).

The same study found that Se supplementation improves muscle fiber density and cross-sectional area (Hu *et al*., 2012). Comparison of organic and inorganic Se and concluded that inorganic Se was the most potent antioxidant (Wang *et al*., 2011). Inorganic Se has improved the growth traits, and Se supplementation also has a significant effect on bird feathering, weight gain, drip loss, and breast muscle yield. The optimal supplementation for chicks was 0.3 mg/kg Se (Cai *et al*., 2012). This revealed that the muscles and liver of chickens contained significant amounts of Se.

The performance of Se on morphometric parameters of the tibia bone is also significant (Table II). Inorganic Se supplementation significantly improved the bone weight, bone length as well weight length index as compared to the non-supplemented groups. The performance of Se was found significant on the medial wall diameter, and lateral wall diameter in the 0.3 mg Se-supplemented group as compared to the non-supplement groups. This represents that inorganic Se have a role in improving the bone morphometric parameter. Dexamethasone causes stress, which reduces feed efficiency and increases mineral excretion. Se is a trace mineral that has been positive effect on oxidative stability and antioxidant ability. The availability of minerals such as ash, phosphorus, and calcium is used to calculate bone-breaking strength. When these minerals are present in sufficient quantities, they can improve bone strength as well as their nutritional value. This showed that Se causes the retention of vital minerals in different tissue such as bone and muscle (Shaw *et al*., 2010). Dexamethasone is a synthetic form of glucocorticoid that causes tibial bone necrosis and inhibits growth hormones. Trace element supplementation increases tibia bone length, width, and strength (Bao *et al*., 2010). Heat stress reduces tibia bone length, weight, tibiotarsal index, and mineral retention in birds (Hafeez *et al*., 2014). Similarly, feeding birds trace minerals increased tibia bone length, diaphysis diameter, and weight/length index. The trace elements supplementation in bird diet has a significant effect on wall thickness, Tibiotarsal index, and ash content due to high bioavailability, mineral retention, and improved integrity and bone density (Sahraei *et al*., 2012).

The performance of inorganic Se on the calcium, phosphorus, and ash content in the tibia bone of broilers is represented in Table III. It is found that the 0.4mg of inorganic Se has been the most significant dose for calcium and phosphorus for deposition in the tibia bone as it also increased the ash content in the tibia bone compared to other doses.

Bone mineralization is critical for reducing bone fractures as well as abnormalities. Bone strength is obtained when several minerals like calcium, phosphorus, and ash are deposited into the bone (Briens *et al*., 2013). In this current research study, it was found that the

mineral content of bones has significantly improved in the Se-supplemented groups, according to this research study. These findings are in line with Khan *et al*. (2022), who found that supplementing with Se improves tissue mineral retention. The selenocysteine glutathione peroxidase (GSH-Px) enzyme is found in the phospholipid peroxidation of muscle cell membranes. As a result, muscle cells retain more water, muscle fiber diameter, and fascicle cross-sectional area increase and meat quality improves.

CONCLUSION

The 0.3mg inorganic Se per kg of the diet has good performance to improve the tibia bone morphology as well as calcium, phosphorous, and ash content. Moreover, the broiler muscle histomorphometry was also improved with 0.3mg inorganic Se supplementation. In the future, the effect of stress on growth gene performance needs to be identified, their regulation as well with the help of a formulated diet with antioxidants should be focused on for better meat production.

DECLARATIONS

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Approval of ethical committee

This research project was duly approved by the Ethical Review Committee of the College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University, Mardan Khyber Pakhtunkhwa, Pakistan.

Statement of conflict of interest

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

REFERENCES

Abbas, Y., Sahota, A., Akram, M., Javed, K., Younus, M., Mehmood, S., Ahmad, S. and Jatoi, A., 2016. Effect of different dietary lysine regimens on the growth performance and economic efficiency of Japanese quails. *J. Anim. Pl. Sci.*, **26**: 315-319.

Aggrey, S.E., Karnuah, A.B., Sebastian, B. and Anthony, N.B., 2010. Genetic properties of feed efficiency parameters in meat-type chickens. *Genet. Sel. Evol.*, **42**: 1–5. https://doi.org/10.1186/1297-9686-42-25

Bao, Y.M., Choct, M., Iji, P. and Bruerton, K., 2010. Trace mineral interactions in broiler chicken diets. *Br. Poult. Sci*., **51**: 109–117. https://doi.org/10.1080/00071660903571904

Briens, M., Mercier, Y., Rouffineau, F., Vacchina, V. and Geraert, P.A., 2013. Comparative study of a new organic selenium source v. seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. *Br. J. Nutr.,* **110**: 617–624. https://doi.org/10.1017/S0007114512005545

Cai, S., Wu, C., Gong, L., Song, T., Wu, H. and Zhang, L., 2012. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. *Poult. Sci*., **91**: 2532–2539. https://doi.org/10.3382/ps.2012-02160

Canene-Adams, K., 2013. Preparation of formalin fixed paraffin-embedded tissue for immunohistochemistry. *Meth. Enzymol.,* **533**: 225–233. https://doi.org/10.1016/B978-0-12-420067-8.00015-5

Cannizzo, F.T., Spada, F., Benevelli, R., Nebbia, C., Giorgi, P., Brina, N., Bollo, E., Biolatti, B., 2010. Thymus atrophy and regeneration following dexamethasone administration to beef cattle. *Vet. Rec.,* **167**: 338–343. https://doi.org/10.1136/vr.c3303

Godfray, H.C.J., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Nisbett, N., Pretty, J., Robinson, S., Toulmin, C. and Whiteley, R., 2010. The future of the global food system. *Philos. Trans. R. Soc. B Biol. Sci*., **365**: 2769–2777. https://doi.org/10.1098/rstb.2010.0180

Habibian, M., Ghazi, S. and Moeini, M.M., 2016. Effects of dietary selenium and vitamin E on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. *Biol. Trace Elem. Res.* **169**: 142–152. https://doi.org/10.1007/s12011-015-0404-6

Hafeez, A., Mader, A., Boroojeni, F.G., Ruhnke, I., Röhe, I., Männer, K. and Zentek, J., 2014. Impact of thermal and organic acid treatment of feed on apparent ileal mineral absorption, tibial and liver mineral concentration, and tibia quality in broilers. *Poult. Sci*., **93**: 1754–1763. https://doi.org/10.3382/ps.2013-03750

Hu, C., Li, Y., Xiong, L., Zhang, H., Song, J. and Xia, M., 2012. Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. *Anim. Feed Sci.*

Technol., **177**: 204–210. https://doi.org/10.1016/j.anifeedsci.2012.08.010

Khan, I., Zaneb, H., Masood, S., Ashraf, S., Rehman, H.F., Tahir, S.K., Rehman, H.U., Khan, A., Taj, R. and Rahman, S.U., 2022. Supplementation of selenium nanoparticles-loaded chitosan improves production performance, intestinal morphology, and gut microflora in broiler chickens. *J. Poult. Sci*., **59**: 272–281. https://doi.org/10.2141/jpsa.0210026

Kieliszek, M. and Błażejak, S., 2016. Current knowledge on the importance of selenium in food for living organisms: A review. *Molecules,* **21**: 609. https://doi.org/10.3390/molecules21050609

Li, J., Zhang, L., Yang, Z., Zhang, Z., Jiang, Y., Gao, F. and Zhou, G., 2018. Effects of different selenium sources on growth performance, antioxidant capacity and meat quality of local Chinese Subei chickens. *Biol. Trace Elem. Res.,* **181**: 340–346. https://doi.org/10.1007/s12011-017-1049-4

Luqman, Z., Ali, H.M. and Raza, Q., 2020. In-ovo effects of lysine amino acid on histomorphometry of thigh muscles, cecal tonsils and pH in Japanese Quail. *Pak-Eur. J. Med. Life Sci*., **3**: 1–5.

Mabelebele, M., Norris, D., Siwendu, N., Ng'ambi, J., Alabi, O.J. and Mbajiorgu, C., 2017. Bone morphometric parameters of the tibia and femur of indigenous and broiler chickens reared intensively. *Appl. Ecol. environ. Res.*, **3**: 1-5.

Sahraei, M., Janmmohamdi, H., Taghizadeh, A. and Cheraghi, S., 2012. Effect of different zinc sources on tibia bone morphology and ash content of broiler chickens. *Adv. biol. Res.,* **6**: 128–132.

Shaw, A., Blake, J. and Moran, E., 2010. Effects of flesh attachment on bone breaking and of phosphorus concentration on performance of broilers hatched from young and old flocks. *Poult. Sci*., **89**: 295–302. https://doi.org/10.3382/ps.2009-00402

Skřivan, M., Englmaierová, M., Dlouhá, G., Bubancová, I. and Skřivanová, V., 2011. High dietary concentrations of methionine reduce the selenium content, glutathione peroxidase activity and oxidative stability of chicken meat. *Czech J. Anim. Sci.,* **56**: 398–405. https://doi.org/10.17221/1289-CJAS

Sunder, G.S., Kumar, C.V., Panda, A., Raju, M. and Rao, S.R., 2013. Effect of supplemental organic Zn and Mn on broiler performance, bone measures, tissue mineral uptake and immune response at 35 days of age. *Curr. Res. Poult. Sci*., **3**: 1–11. https://doi.org/10.3923/crpsaj.2013.1.11

Surai, P.F., Fisinin, V.I. and Karadas, F., 2016. Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. *Anim. Nutr*., **2**: 1–11. https://doi.org/10.1016/j.aninu.2016.01.001

Wang, Y., Zhan, X., Yuan, D., Zhang, X. and Wu, R., 2011. Effects of selenomethionine and sodium selenite supplementation on meat quality, selenium distribution and antioxidant status in broilers. *Czech J. Anim. Sci*., **56**: 305–313. https://doi.org/10.17221/1296-CJAS

Xueting, L., Rehman, M.U., Mehmood, K., Huang, S., Tian, X., Wu, X. and Zhou, D., 2018. Ameliorative effects of nano-elemental selenium against hexavalent chromium-induced apoptosis in broiler liver. *Environ. Sci. Pollut. Res*., **25**: 15609–15615. https://doi.org/10.1007/s11356-018-1758-z

Yang, Y., Meng, F., Wang, P., Jiang, Y., Yin, Q., Chang, J., Zuo, R., Zheng, Q. and Liu, J., 2012. Effect of organic and inorganic selenium supplementation on growth performance, meat quality and antioxidant property of broilers. *Afr. J. Biotechnol*., **11**: 3031–3036. https://doi.org/10.5897/AJB11.3382

Yusof, H.M., Mohamad, R., Zaidan, U.H. and Samsudin, A.A., 2023. Influence of dietary biosynthesized zinc oxide nanoparticles on broiler zinc uptake, bone quality, and antioxidative status. *Animal,* **13**: 115. https://doi.org/10.3390/ani13010115