Research Article

Effect of Inorganic Selenium Supplementation on Gut Morphology and Growth Performance in Broilers under Stress Conditions in Khyber Pakhtunkhwa, Pakistan

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Abstract | Poultry industry is confronted to different hurdles which are blamed for the decline in the nutrients supplements for humans. One of the hurdles is the oxidative stress, which is responsible for the reduction of gut morphology and growth performance in the poultry birds. The present study was performed to explore the impact of inorganic selenium (sodium selenite). Day old chicks were kept in a control environment. At day 7, five groups were made with four replicas in each group based on the diet being offered. Group A, the positive control group fed with normal basal diet group B was termed as negative control group offered basal diet + dexamethasone and group C, D and E was offered 0.2mg/kg, 0.3mg/kg, and 0.4mg/kg of basal diet, respectively. The trial lasted for 35-days and on the last day two chickens from each replicate were slaughtered (a cumulatively eight chickens from each group). Samples from different regions (duodenum, jejunum, and ileum) of the small intestine were collected and kept in the fixatives. Slices from each part were stained with H and E stain to make it fit for microscopy. Picture were taken with different magnifications, i.e., 4X, 10X and 40X and histo-morphometry was done through Pixel-Pro (Labomed America Inc). The statistical analysis was through IBM SPSS (Statistics.V22.X86). The present study concluded that selenium improves the intestinal morphology i.e. villus height, villus width, villus surface area showed better growth($P \leq 0.05$) in the selenium supplemented groups. The FCR initially did not differ ($P \geq 0.05$) among the groups but toward the end it was comparatively better ($P \leq 0.05$) in selenium supplemented groups. The relative body weight of different organs i-e liver, gizzard, heart, pancreas, and proventriculus etc. showed significant ($P \leq 0.05$) results in each of selenium-supplemented groups. Overall, 0.3 mg/kg (Basal diet) of selenium showed better results with respect to the positive control group.

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Introduction

Several biological, dietary, and environmental stresses are thought to be to blame for weakened reproductive and productive capacities as well as weakened overall health (Surai and Fisinin, 2014). When cells undergo normal metabolism, they naturally create reactive oxygen species (ROS), sometimes referred to as oxidative stress, free radicals, and peroxides. ROS cause lipids, nucleic acids, and proteins to oxidize (Erin *et al*., 2000). They are essential for a variety of metabolic functions, including the production of cytokines, immune regulation, and ion transport. The physiological detoxification processes carried out by the cells themselves completely eliminate the copious ROS generated within cells (Rehman *et al*., 2018). When the environment is thermo-neutral, the transcriptional factor Nrf2 is activated, which stimulates the increased manufacture of antioxidant molecules to combat excessive ROS generated within the cell (Surai *et al*., 2019). The polarity between the two system either by the increase production of the peroxides or decrease in the effectiveness of the antioxidant is called oxidative stress (Mishra and Jha, 2019).

Literature has indicated that Heat stress and cellular oxidative stress are connected (Estévez, 2015). All of the cells' constituents, including proteins, lipids, and DNA, are damaged by free radicals created during oxidative stress. According on the intensity of the oxidative stress, the results might range from minor, reversible changes to cell death in the event of extreme oxidative stress (Ivanova and Yaneva, 2020). In poultry, oxidative stress is linked to biological damage, serious health issues, slow development rates, and financial crises (Wasti *et al*., 2020) During all these stress conditions the glucocorticoid level elevates and causes different malfunctions in the body as discussed in the earlier sections. We can also use glucocorticoids to induce stress (physiological) in the body and one of them is dexamethasone (Palme, 2019).

In recent times, there has been a significant surge of interest and concern within the scientific community regarding the utilization of trace minerals nanoparticles. Based on previous research findings, it has been observed that mineral nanoparticles exhibit significant promise in enhancing poultry growth and maintaining the integrity of the intestinal barrier due to the uniform spherical shape with biological properties along with small size. Generally, the trace minerals manufactured through the biological ways are used because they are more safe, cheap and, easy to use (Ali *et al*., 2022).

Selenium is a key component of about 25 various selenoproteins and majority of them are enzymes for example glutathione peroxidase, thioredoxin reductases and others. Two forms i.e., organic and inorganic selenium are generally used as supplements for poultry. It also has importance in animals and human health due to the antioxidant activity, chemo preventive, anti-inflammatory, antiviral and enhancing immunity (Zoidis *et al*., 2014). It is also associated to the protection against cancer, thyroid physiology and preventing the muscular and cardiovascular disorders (Mao and Teng, 2013). Selenium is generally offered in inorganic salt forms such as sodium selenate or in organic form such as S-enriched yeast (SY) selenocysteine, selenomethionine. Insufficient intake of selenium results in various diseases such as Kashin-Beck disease and goiter in humans, week immunity, liver necrosis, reduced egg production, poor fertility, muscular dystrophy, white muscles and exudative diathesis in the livestock animals. On the other hand, excessive intake is toxic and the less availability of the inorganic discourages its toxicity (Muhammad *et al*., 2021).

This research study was designed to investigate the effect of inorganic selenium (Sodium selenate) which is the chief and easily available antioxidant on commercial level.

Materials and Methods

Experimental design, birds and feeding

The trail was conducted in experimental poultry shed at College of Veterinary Sciences and Animal Husbandry (CVS and AH), Abdul Wali Khan University, Mardan. The samples were processed in histology laboratory of the same college. Two hundred-day-old birds were divided into five groups, with five (5) birds per replicate and eight (8) copies in each group (Lyon and Lyon, 1997). The experimental trail lasted for 35 days, or five weeks overall, and the birds were housed in an experimental shed with regulated environmental conditions. On day 1, the temperature was kept at 35°C, and by the end of the third week (21 days), it had dropped progressively (3°C each week) to 26°C. The trail's reached to day 22 had a constant temperature of

26°C. For the duration of the experiment, the relative humidity (RH) was maintained at 65%. Group A received just the baseline diet (BD) and was regarded as a negative control group. Fed to Group B was BD with DE-15 mg/kg of dexamethasone. The baseline diet of Group C was supplemented with Se-0.2+DE-15 mg/kg. In a similar manner, Group D received (Se-0.3 mg + De-15 mg/kg) in BD (Table 1). The freshwater and feed (ration) were offered ad libitum every group (Khan *et al*., 2022).

Two chicks from each replica (randomly selected) were slaughtered (Shabani *et al*., 2015). After the slaughter 3cm fragments that were taken from each part of the Intestine i.e., duodenum, jejunum, and ileum were cleaned and kept in the preservatives (formalin). The tissues were processed through formalin-fixed paraffin-embedding technique (Al-Sabaawy *et al*., 2021).

Statistical analysis

Slides pictures and statistical analysis were done through Pixel-Pro and 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 Tukey's 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

In duodenum the selenium supplemented groups showed great variance in the respective parameters especially in the replica D and E as compared to the non-supplemented groups (negative and positive control group) as shown in Tables 2, 3 and 4. Almost all of the parameters showed significance but some the parameters i.e. villus surface area and crypt depth ratio did not show any variance. In jejunum the selenium supplemented groups showed great variance in the respective parameters especially in the replica D and E as compared to the non-supplemented groups (negative and positive control group) as shown in Table 1. Almost all of the parameters showed significance but some the parameters i.e., crypt depth ratio did not show significant value in Table 2 and Figure 1.

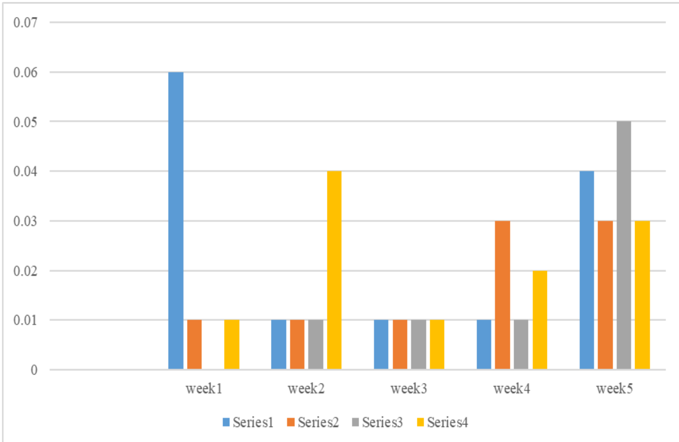

Figure 1: *Chart showing the weekly growth performance.*

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.

Table 2: *Effect of Se on the intestinal morphology (duodenum).*

Group	A	B	C	D	E	P- VALUE
VH	3131±14.34^{a}	2175.4±23.78^{a}	3525±99.21^{a}	3661±269.99^{a}	3669±160.69^{a}	.10
VW	291±0.7^{ab}	186±25.32^{b}	188±0.9^{b}	222±5.66^{ab}	322±37.48^{a}	.01
VSA	2864±20^{ab}	1823±261^{b}	2084±68^{b}	2563±234^{ab}	3697±380^{b}	.07
CD	382±34^{b}	158±56^{b}	445±0.39^{a}	504±0.28^{a}	606±64^{a}	.04
LPT	129.9±1.6^{ab}	99.8±1.2^{b}	143.4±19.1^{ab}	111.2±3.35^{b}	170.0±3.65^{a}	.01
CD RATIO	8.26±0.79^{a}	22.4±7.82^{a}	7.90±0.22^{a}	7.26±0.53^{a}	6.08±0.37^{a}	.09
MM	88.34±6.54^{ab}	67.91±6.07^{c}	87.91±3.58^{ab}	159±22.38^{ab}	212.1±16.6^{a}	.02
ME	306.9±4.5^{a}	248.9±20.2^{a}	592.2±9.52^{a}	592.3±80.9^{a}	363.84±8.91^{a}	.03

VH, Villus Height; VW, Villus Width; VSA, Villus Surface Area; CD, Crypt Depth; LPT, Lamina Propria Thickness; CD ratio, Crypt Depth Ratio; MM, Mucosa Muscularis; ME, Mucosa Externa.

Table 3: *Effect of Selenium supplementation on the intestinal morphology (Jejunum).*

Group	A	B	C	D	E	P-value
VH	1878±187.3^{bc}	1421.7±159.6^{c}	2451±78^{ab}	2609±72.5^{ab}	3166±260^{a}	0.05
VW	180±1.24	141±16.94	280±27.8^{ab}	232±14.2	352±9.2^{a}	0.02
VSA	1774±4.11	1386±176	3094±220^{ab}	2665±33.9	4067±285^{a}	0.01
CD	317±51	326±31.5	450±70.1^{ab}	356±49.4	634±51.42^{a}	0.03
LP	93.9±0.17	91.2±1.53	125±1.60	164±7.06	114±7.54^{a}	0.01
VH:CDR	8.26±0.79	22.4±7.8	7.90±0.22	7.26±0.53	6.08±0.37	0.09
MM	83.9±0.35	75.7±3.9	108±0.20^{b}	99.5±1.21	130±4.7^{a}	0.01
ME	306±4.5	248±20.2	592.2±9.5	592.3±80.9	363±8.91	0.03

VH= Villus Height, VW=Villus Width, VSA= Villus Surface Area, CD= Crypt Depth, LPT=Lamina Propria Thickness, CD ratio=Crypt Depth Ratio, MM= Mucosa Muscularis, ME= Mucosa Externa.

Table 4: *Effect of selenium supplementation on the intestinal morphology (Ilium).*

Group	A	B	C	D	E	P-value
VH	2189±31.3bc	1695±201c	2714±168^{ab}	2485±50ab	3112±34^{a}	.03
VW	217.1±6.14b	180±20.5b	398±112.1ab	270±5.9ab	612±101a	.03
VSA	1493±63.5b	972±223.2b	3456±1166ab	2114±89.3ab	5998±1055^{a}	.01
CD	367±42.9	285±21	487±10.17	441±30.09	785±97.02a	.06
LP	120±8.29bc	87±6.00c	179±12.02b	132±2.7ab	345±24.62a	.01
VH:CDR	4.8±0.26bc	3.1±0.43c	6.5±0.06ab	5.75±0.42ab	7.7±0.7a	.05
MM	88±2.5^{ab}	81±1.15^{b}	141±1.31ab	100±6.7	245±56.4a	.02
ME	485±92.6	283±142	741±107.9	364±69.1	653±67.3	.09

VH= Villus Height, VW=Villus Width, VSA= Villus Surface Area, CD= Crypt Depth, LPT=Lamina Propria Thickness, CD ratio=Crypt Depth Ratio, MM= Mucosa Muscularis, ME= Mucosa Externa.

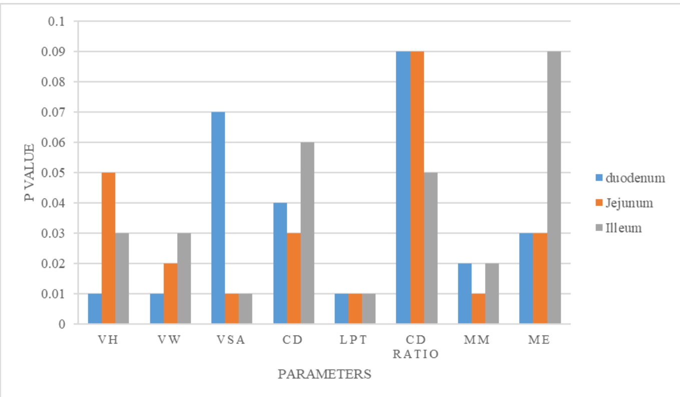

Figure 2: *Chart showing growth performance in S.I.*

In the Ileum selenium supplemented groups showed great variance in the respective parameters especially in the replica D and E as compared to the non-supplemented groups (negative and positive control group) as shown in Table 3. Almost all of the parameters showed significance but some the parameters i.e., Villus height to crypt depth, crypt depth and *Mucosa externa* did not show significant value as shown in Table 3. The overall chart of the P values is given. Based on the observed changes in body weight gain and feed conversion ratio, it is evident that the groups receiving selenium supplementation exhibited the most notable results during the third and fourth weeks of the experimental trial (Figure 2). In the aforesaid weeks the selenium supplemented groups showed greater variance in term of feed intake, bodyweight gain; feed conversion ratio and significance is shown in Tables 4 and 5.

The current study reflects that in the whole trial the selenium supplemented groups showed significant body weight indicating that selenium contributing to the final body weight and weight gain in the broilers. Similar study also documented that that selenium supplementation significantly improved the body weight gains. Selenium is a supporting factor and activator of the vital enzyme iodothyronine 5′-deiodinase. Finally converts thyroxine to its active metabolite triiodothyronine, which is necessary for the growth traits (Woods *et al*., 2020). The value of FCR varied among the supplemented and control group. According to previous study selenium supplementations has multiple favoring impact on the growth of the broiler in the unhealthy oxidative stress situations. The bioavailability of inorganic Se is more that the organic selenium due to the absorption

Table 5: *Overall results of body weight gain, feed intake and FCR amongst the groups during research trial.*

No. of weeks	Group	A	B	C	D	E	P value
Week1	Body weight	121±1.54	132±3.47	125±2.54	128±2.72	143±9.6	0.06
	Feed intake	395±6.82[a]	365±2.34	352±2.39	342±1.70	340±3.12	0.01
	Feed conversion ratio	2.34±0.1	2.65±0.01	2.77±0.02	2.87±0.17	3.25±.067[a]	0.01
Week2	Body weight	339±0.57[ab]	301±19.7[b]	375±1.58[a]	362±3.56[a]	346±1.93[a]	0.01
	Body weight gain	202±3.01[ab]	165±25.14[b]	253±1.31[a]	236±6.26[a]	215±2.05[ab]	0.01
	Feed intake	1063±14[c]	404±137[b]	980±7.34[ab]	1182±40[ab]	1335±26[a]	0.01
	Feed conversion ratio	5.38±0.52[a]	3.65±0.36[b]	5.97±0.29[a]	5.29±0.36[a]	4.39±0.27[ab]	0.04
Week3	Body weight	471±21[c]	558±32[bc]	582±13[b]	612±21[ab]	701±22[a]	0.01
	Body weight gain	140±16[c]	220±33[bc]	246±13[ab]	267±20[ab]	326±32[a]	0.01
	Feed intake	444±26[b]	472±30[b]	361±28[b]	846±118[a]	483±34[b]	0.01
	Feed conversion ratio	3.31±0.47[a]	2.24±0.22[ab]	1.4±0.22[b]	3.1±0.28[a]	1.51±0.9[b]	0.01
Week4	Body weight	957±46[b]	932±37.52[b]	1028±56.93[a]	1086±63[ab]	1207±45[ab]	0.01
	Body weight gain	375±52[ab]	356±52[c]	439±53[bc]	506±46[abc]	556±32[a]	0.03
	Feed intake	415±23[b]	452±73[b]	693±27[a]	783±49[a]	712±16[a]	0.01
	Feed conversion ratio	1.42±0.1[b]	2.0±0.38[a]	1.72±0.25[ab]	1.24±0.24[ab]	0.75±0.13[b]	0.02
Week5	Body weight	2045±93[a]	1482±87[b]	1505±51[b]	1782±102[ab]	1767±86[ab]	0.04
	Body weight gain	891±112[ab]	547±84[b]	1049±104[a]	696±137[ab]	560±126[ab]	0.03
	Feed intake	708±40[ab]	764±62[ab]	605±41[ab]	635±68[a]	541±32[ab]	0.05
	Feed conversion ratio	0.7±0.14[ab]	0.45±0.05[b]	1.38±0.19[a]	1.03±0.14[ab]	1.11±0.32[ab]	0.03

pathway (Ali *et al*., 2022). A researcher Deng (2022) documented supportive results and reported that selenium supplemented broilers showed weekly increasing FI and FCR ratio indicating a relatively better production performance in these supplemented group indicated by the high FCR ratio (Deng *et al*., 2022). Similar results were also reported indicated that the lowest feed conversion ratio in selenium supplemented group showing that the growth performance is maximum with respect to the feed intake. Selenium supplementation in different amount did not make any difference for the final body weight and weight gains, However the birds final body weight was significance increases. Dietary Se supplements in the feeds of birds has been documented to effectuate noteworthy enhancements in the traits of growth (Yoon *et al*., 2007).

The current study shows that villus height, and villus surface area showed significantly better marking lines for the duodenum, jejunum and ileum in the selenium supplemented groups as compared the control group. Another study conducted by (Khan *et al*., 2022) showed results in agreement to our ones that VH was higher in the Se groups than in the control group in the duodenum and ileum. The Se induces the enterocytes hyperplasia which lead to increase the villus surface area for absorption of nutrients. In previous study stated that Se yeast inclusion in the diets encouraged enterocytes hyperplasia of in the small intestine segments (duodenal and jejunal) mucosa which augmented absorption of nutrients and enhanced growth in broilers (Dalia *et al*., 2020).

In comparison to the control group, the Se group had higher VSA and VH/CD in all segments of the small intestine. Additionally, the VH/CD was higher in the Se groups' duodenum and ileum compared in the control group. The VW (all small intestinal segments), LP (duodenum and ileum), and CD (duodenum) did not differ between the experimental groups. A similar agreeing result was shown by He *et al*. (2019), who stated that the effect of Se deprivation on the small intestine's morphological makeup. The villi in the duodenum and ileum were noticeably higher in the Se group than in the control group. The intestinal villi were noticeably shorter in the jejunum but did not stand out as being different between the Se group and control group. The crypt depth and mucosal thickness in the duodenum, jejunum, and ileum were become shorter to different degrees in the Se-group as compared to in the control group. Antioxidant impact of Se enhance the VH, which might delay the process of apoptosis and increases viability of enterocyte. Dietary Se regulates antioxidant defense mechanisms by regulatory different antioxidant enzymes, protecting the integrity of the intestinal epithelial layer by scavenging the reactive oxygen species (ROS). This stability between apoptosis and enterocytes proliferation helps in homeostasis of intestine (Moghaddam *et al*., 2017).

The relative organ weight showed significance in the selenium supplemented groups, this indicates that the stress is countered in the supplemented groups however the stressed birds did not show any reasonable growth in the visceral organ i.e., liver, gizzard, heart and proventriculus. However, Lochi presented an agreeing result stating that weight of the bursa and spleen increased significantly, in terms of relative organ weight (Lochi *et al*., 2023). The Se increase the number of lymphocytes by its proliferation in immune organs. The lowest value, however, was found in stressed group. Additionally, the weights of the gizzard, proventriculus, and cecum, both full and empty, were raised. Similar studies were documented by Boostani *et al*. (2015) who observed the positive effect of Se supplementation of immune organs (bursa of Fabricius, thymus, and spleen). The developing status of the lymphoid organs is usually assessed from their RW measurements.

Other findings were reported by Alagbe (2019), observed that the non-significant variations in the relative weights of organs can be simply observed as a reflection that the Se material were nontoxic.

Conclusions and Recommendations

The addition of 0.3mg/kg of inorganic selenium (sodium selenite) in the supplementation has synergic effects on the intestinal histo-morphometry, FCR and relative body weight of the visceral organs with no extra expenses as in organic selenium is require in trace amount. Furthermore, the selenium supplementation has a better impact on the body of broiler to cope the oxidative stress cause by glucocorticoid i.e., dexamethasone. I would recommend that in future the poultry farmer and other personal concerned with poultry industry must add selenium in their supplements to save their time in terms of better growth performance and gut morphology improves resulting in high body weight gain thereby earn much better revenue.

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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

Shafqat Ullah: Investigation, writing-original draft preparation; writing-review and editing.
Asad Ullah: Conceptualization, supervision, methodology.
Imad Khan and Rafiq Ullah: Project administration, validation.
Raheela Taj: Resources.
Fatima Syed: Validation.
Shumaila Gul: Software.
Faiza Khan: Visualization.
Ibad Ullah Jan: Data curation.
Muneeb Islam and Sumaira: Formal analysis.

Ethical approval
The animal welfare committee of the Department of Zoology, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan approved the animal care ethical protocol used for this experiment.

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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