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Original Article

Comparative analysis of various sources of selenium on the growth performance and antioxidant status in broilers under heat stress

Análise comparativa de várias fontes de selênio no desempenho de crescimento e status antioxidante em frangos de corte sob estresse térmico

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Abstract

The supplementation of Selenium-enriched probiotics is effective in reducing oxidative stress and maintaining meat quality stability in broiler chicken especially under heat stress. An experimental study was conducted to perform Comparative analysis of Selenium yeast with inorganic Se in broilers under heat stress. A total of 120 broilers chicks of one day were assigned to 4 groups each consisting 30 chicks fed on same basal diet but different selenium sources. The basal diet of group D1 was not supplemented with Se source (Negative control), group D2 basal diet was supplemented with inorganic selenium (Sodium selenite 0.22mg/Kg starter phase and 0.15mg/Kg finisher phase), group D3 basal diet was supplemented with commercially available organic selenium (Seleno-methionine 0.22mg/Kg starter phase and 0.15mg/Kg finisher phase) and group D4 basal diet was supplemented with self-developed organic selenium (Se-enriched yeast 0.22mg/Kg starter phase and 0.15mg/Kg finisher phase). The performance parameters i.e. feed intake (FI), live body weight (BW) and FCR were not significantly ($p>0.05$) effected by selenium supplementation in the starter phase but were significantly ($p<0.05$) effected in the finisher phase. Selenium supplementation significantly ($p<0.05$) effected serum Se level in different supplemented groups. Higher serum Se value (58.20±0.06) was recorded in D4 group. Similarly significantly lower selenium value was recorded for D4 and higher was recorded for D1 (11.36±0.08). However lower serum Paraoxonase (PON) value was recorded for D4 (13.24±0.01) and higher for D1 (13.33±0.03). Comparatively self-developed Se enriched yeast increased the Se accumulation and improved antioxidant system. Glutathione peroxidase (GPx) was found higher in D4 (12.333±0.03) followed by D3, D2 and D1 respectively. Whereas superoxide dismutase (SOD) was significantly lower ($p<0.05$) in D4 (0.1437±0.003) followed by D3 (0.1457±0.002). Selenium supplementation increased the bird's survival rate. Birds fed on Se enriched yeast showed higher Se deposition and better antioxidant capacity as compared to other sources of selenium. Se-enriched yeast displayed an improved result on Se deposition in tissues, and oxidative capacity, meat tenderness and immune response level as compared to other sources of selenium.

Keywords: broilers, Se-enriched yeast, sodium selenite, oxidative stress, seleno-methionine.

Resumo

A suplementação de probióticos enriquecidos com selênio é eficaz na redução do estresse oxidativo e na manutenção da estabilidade da qualidade da carne em frangos de corte, especialmente sob estresse por calor. Um estudo experimental foi conduzido para realizar uma análise comparativa da levedura selênio com o Se inorgânico em frangos de corte sob estresse térmico. Um total de 120 pintos de um dia foi dividido em 4 grupos, cada um consistindo de 30 pintos alimentados com a mesma dieta basal, mas com diferentes fontes de selênio. A dieta basal do grupo D1 não foi suplementada com fonte de Se (controle negativo), a dieta basal do grupo D2 foi suplementada com selênio inorgânico (selenito de sódio 0,22 mg / kg fase inicial e 0,15 mg / kg fase finalizadora), a dieta basal do grupo D3 foi suplementada com selênio orgânico disponível comercialmente (fase inicial de seleno-metionina 0,22 mg / kg e fase finalizadora de 0,15 mg / kg) e a dieta basal do grupo D4 foi suplementada

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com selênio orgânico autodesenvolvido (fermento enriquecido com Se 0,22 mg / kg fase inicial e 0,15 mg / kg fase finalizadora). Os parâmetros de desempenho, ou seja, consumo de ração (FI), peso corporal vivo (PC) e FCR não foram significativamente ($p > 0,05$) afetados pela suplementação de selênio na fase inicial, mas foram significativamente ($p < 0,05$) afetados na fase final. A suplementação de selênio afetou significativamente ($p < 0,05$) o nível de Se sérico em diferentes grupos suplementados. O maior valor de Se sérico (58,20 ± 0,06) foi registrado no grupo D4. Da mesma forma, valor de selênio significativamente menor foi registrado para D4 e maior foi registrado para D1 (11,36 ± 0,08). No entanto, um valor mais baixo de Paraoxonase (PON) sérica foi registrado para D4 (13,24 ± 0,01) e mais alto para D1 (13,33 ± 0,03). A levedura enriquecida com Se comparativamente autodesenvolvida aumentou o acúmulo de Se e melhorou o sistema antioxidante. A glutationa peroxidase (GPx) foi encontrada maior em D4 (12,333 ± 0,03) seguido por D3, D2 e D1 respectivamente. Enquanto a superóxido dismutase (SOD) foi significativamente menor ($p < 0,05$) em D4 (0,1437 ± 0,003) seguido por D3 (0,1457 ± 0,002). A suplementação de selênio aumentou a taxa de sobrevivência da ave. Aves alimentadas com levedura enriquecida com Se apresentaram maior deposição de Se e melhor capacidade antioxidante em comparação com outras fontes de selênio. A levedura enriquecida com Se apresentou um resultado melhorado na deposição de Se nos tecidos, capacidade oxidativa, maciez da carne e nível de resposta imune em comparação com outras fontes de selênio.

Palavras-chave: frangos, levedura enriquecida com Se, selenito de sódio, estresse oxidativo, seleno-metionina.

1. Introduction

Selenium is one of the utmost significant trace elements. Irrespective of selenium high quantities toxicity, its deficiency is a global issue lead to adversely affect animals performance and efficiency, with more vulnerability to diseases (Malavolta and Mocchegiani, 2018). Selenium is identified as one of the essential nutrients and vital trace mineral for several biological functions in poultry production, including antioxidant defense, growth performance, egg and meat quality, enzyme function, stimulation of immune system and reproduction (Saad et al., 2009). Selenium was considered primarily to be lethal for animals, but later it was reported to be essential for proper functioning of "glutathione peroxidase", an antioxidant enzyme which eliminates free radicals from the body during normal metabolism (Heindl et al., 2010; Huang et al., 2019). It is vital in poultry diet in order to protect them from antioxidant stress as it is an important part of antioxidant enzymes. The deficiency of Se can cause stress by causing certain biochemical changes in the body which ultimately leads to various diseases (Richards et al. 2010). The heavy metals which are conventionally defined as the elements which have metallic properties (ductility, conductivity, ligand specificity, and stability with cationic forms, etc.) along with an atomic number >20 are usually characterized as heavy metals (Ali et al., 2020; Asif et al., 2020). The metal ions as most common soil, water, air and food contaminants are usually Ar, Fr, Cu, Pb, Mn, Cd, Hg, Cr, Ni, Au and Zn (Balqees et al., 2020; Haseeb et al., 2020). A number of the heavy metals are usually required by plants in the form of micronutrients as the metal ions are usually found as the natural soil component (Nadeem et al., 2020; Nazir et al., 2020; Nazir *et al*., 2020). While the human activities and industrialization has caused increase of these heavy metals in the soil, food chain and water to drink, which is causing a number of diseases in animals, plants and human, the presence of selenium in food helped to detoxified the effects of heavy metal toxicity besides the other abiotic stress on living organisms (Zubair et al., 2016; Ishaque et al., 2020; Yousef et al., 2020).

Selenium exists in inorganic (selenite and selenate) and organic (seleno-methionine and seleno-cysteine) forms. Organic selenium is the most bioavailable, safer and less toxic as compared to inorganic selenium. Selenium yeast is the best source of organic selenium (Fan and Vinceti, 2015). By growing *Saccharomyces cerevisiae* (Baker's yeast) in Se-enriched medium, can accumulate greater amount of Se and incorporate them mainly to produce Selenomethionine (Se-Met) . Sodium selenite (Na_2SeO_3) is bio-transformed to organic form absorbing by the yeast. Through this practice it can be converted to highly bioactive and safer organic source with better nutritive properties (Esmaeili and Khosravi-Darani, 2014). Yeasts have a high amount of protein as compared to other plant sources. It can integrate Se by replacing sulphur atom in protein. Yeast can easily be cultured in the laboratory to get biomass with high protein content as it utilizes soluble sugars and organic acids. The ultimate product is used to fortify food and feed and to make supplements (Esmaeili et al., 2012).

To avoid excessive losses due to environmental extremes, standardizing feed and supplements formulation in poultry production is unavoidable. Heat stress makes poultry production uneconomical by reducing body weight, growth rate and feed efficiency. Poor quality feed can lead to slow growth, lower egg and meat production, poor immune response and even results in huge losses in the form of mortality (Lara and Rostagno, 2013). Preparation of proper poultry feeds is one of the best techniques to sustain quality and to lowers production cost. Nutritional antioxidant supplementation in general and Selenium yeast supplementation in particular are considered major defensive measures for upholding high productive and reproductive performance in poultry. Broilers need different rations in different phases of their growth, where their energy, minerals and vitamins requirement varies accordingly. Selenium as a part of seleno protein is required by poultry for maintaining optimum health, egg and meat quality (Wang and Xu, 2008).

The aim of the study was comparative analysis of selenium yeast and inorganic Se sources on the growth performance and antioxidant status of broilers under heat stress.

2. Material and Methods

This experimental work was a part of NRPU Project No. 20-3386 approved by Higher Education Commission of Pakistan. Research work was carried out in Department of Poultry science, The University of Agriculture Peshawar and Centralized Resource Laboratory (CRL), University of Peshawar.

2.1. Four dietary treatments

The study was conducted to equate the significance of selenium yeast with an inorganic Se on the growth performance and immune response in broilers under heat stress. A total of 120 broilers chicks were obtained on the day of hatching at poultry science department, university of Agriculture, Peshawar. They were distributed into 4 groups of 30 birds each, with three replicate each of 10 chicks kept in large cages with four compartments. From day first till week sixth the chicks were fed with basal diet differing in source of selenium supplementation.

- Group 1 was given the basal diet with no selenium supplementation; it was the negative control group (D1);
- Group 2 was given the same basal diet with sodium selenite as a source of selenium (D2);
- Group 3 received same basal diet supplemented with commercially available seleno-methionine (D3);
- The diet for Group 4 chicks comprised of same basal diet supplemented with self-developed selenium enriched yeast (D4);

Vitamin and Mineral premix for mixing into basal diet was purchased from Kepro poultry Pakistan (Faisalabad). The composition of basal diet for starter and finisher is presented in Table 1 and 2 respectively (Dalia et al., 2017).

- Mineral premix provided the following per kg diet: iron 120 mg, manganese 150 mg, copper 15 mg, zinc 120 mg, iodine 1.5 mg, and cobalt 0.4 mg;
- Vitamin premix provided the following per kg diet: Vitamin A (retinyl acetate) 10.32 mg, cholecalciferol 0.250 mg, vitamin E (DL-tocopheryl acetate) 90 mg, vitamin K 6 mg, cobalamin 0.07 mg, thiamine 7 mg, riboflavin 22 mg, folic acid 3 mg, biotin 0.04 mg, pantothenic acid 35 mg, niacin 120 mg and pyridoxine 12 mg (Abdulla et al., 2017).

Table 1. Basal feed formulation (g/kg) broiler starter rations with different sources of Selenium.

S.No	Ingredients (%)	D1	D2	D3	D4
1	Whole maize	52.5	52.5	52.5	52.5
2	Soya bean meal	32.5	32.5	32.5	32.5
3	Fishmeal	5.1	5.1	5.1	5.1
4	Vitamin Premix	0.10	0.10	0.10	0.10
5	Mineral Premix	0.15	0.15	0.15	0.15
6	Amino acids (lysine and threonine)	0.25	0.25	0.25	0.25
7	Lime	0.60	0.60	0.60	0.60
8	Salt	0.30	0.30	0.30	0.30
9	Selenium source	No selenium	Sodium Selenite 0.22mg/Kg	Seleno-methionine 0.22mg/Kg	Selenium enriched Yeast 0.22mg/Kg

Table 2. Basal feed formulation (g/kg) broiler finisher rations with different sources of Selenium.

S.No	Ingredients gm/ Kg	D1	D2	D3	D4
1	Whole maize	56.2	56.2	56.2	56.2
2	Soya bean meal	30.0	30.0	30.0	30.0
3	Fishmeal	3.2	3.2	3.2	3.2
4	Vitamin Premix	0.10	0.10	0.10	0.10
5	Mineral Premix	0.15	0.15	0.15	0.15
6	Amino acids (lysine and threonine)	0.25	0.25	0.25	0.25
7	Lime	0.35	0.35	0.35	0.35
8	Salt	0.30	0.30	0.30	0.30
9	Selenium source	No selenium	Sodium Selenite 0.15mg/Kg	Seleno-methionine 0.15mg/Kg	Selenium enriched Yeast 0.15mg/Kg

2.2. Parameters analyzed

Blood and tissue samples were taken from each group in the starter and finisher phases to determine different parameters. The parameters were analyzed using the already developed methods with some modifications according to our needs and available facilities.

2.3. Growth parameters

For growth performance feed intake and body weight gain were recorded to calculate feed efficiency of the experimental birds.

2.4. Selenium concentration (mg/kg)

Selenium concentration in blood and muscle was determined by the method as described by Habibian et al. (2014) with some modifications. Samples were taken during starter and finisher phases from each dietary group. Muscles were freeze dried and then crushed into small fractions using sterilized blender. The samples were digested in 50% nitric acid using microwave. These samples were filtered by using syringe filters of 0.45µm pore size. The filtrate was further digested with sub-distilled 20% nitric acid. Total Se concentration was measured using atomic absorption spectrometry (Habibian et al., 2014).

2.5. Determination of Serum Malondialdehyde (MDA)

Malondialdehyde was determined in serum by the technique described by Ohkawa *et al*. (1979) with few modifications. Serum samples of 200µl was added into a reaction mixture containing 1500µl of citric acid (20%) having a pH 3.5, 200µl of (8.1%) sodium dodecyl sulfate (SDS), and 1500µl (0.8%) thiobarbituric acid (TBA). The mixture was mildly heated at 95°C for one hr in water bath and was allowed to cool at room temperature. After this, a mixture containing 500µl of pyridine and n-butanol (1:15, v/v) was added along with one ml distilled water and shaken vigorously. The mixture was centrifuged at 400 rpm for 10 min. The organic layer was separated and absorbance was measured at 532nm using UV-visible spectrophotometer (Valenzuela, 1991).

2.6. Determination of Paraoxonase (PON1)

Paraoxonase 1 plays a defensive role in diseases linked with oxidative stress. Serum PON1, predominantly produced by the liver, is chiefly linked with serum High-Density Lipoproteins (HDL). It has been affirmed that it play a vital role in the antioxidant activity of HDL by defending Low-Density Lipoproteins (LDL) against the lipid peroxidation therefore, reduces the chances of atherosclerosis. In point of fact, PON1 deficiency can cause increased oxidative stress (Litvinov et al., 2012). The PNO1 was determined with commercially available "Chicken Paraoxonase (PON1) ELISA Kit". This commercially available kit practices the Sandwich-ELISA principle. ELISA plate micro wells present in this set have been pre-coated with Chicken PON1 specific antibody. Samples were added to the ELISA plate wells and joint by the fixed antibody. At that same time a biotinylated sensing antibody, specific for ChickenPON1 and Avidin-Horseradish Peroxidase (HRP) conjugate were added sequentially to each micro ELISA plate well and incubated. Plates were washed to remove any free components. The substrate solution was added separately to each well. Wells having Chicken PON1, Avidin-HRP and biotinylated detection antibody fusion appeared blue in color. The reaction of enzyme-substrate ended by adding stop solution and ultimately the color turned yellow.

2.7. Total oxidant Status (TOS)

The TOS was determined by using the method described by Erel (2005), which is an automated colorimetric measurement process with fairly modifications (Erel, 2005). In the afore-mentioned method, oxidants present in the blood serum sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. This reaction takes place in acidic environment where the ferric ion produces a colored complex with xylenol orange. The results were measured spectrophotometrically where the intensity showed the total sum of oxidant molecules existing in the sample.

2.8. Superoxide dismutase (SOD)

Superoxide dismutase in muscles and blood samples were determined with the help of commercially available kits, (Superoxide dismutase (SOD) typed assay kit (Hydroxylamine method), A001-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The procedure given in the leaflet was followed for determining the SOD. Superoxide dismutase is a class of enzymes having a catalytic role in the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide is the major responsive oxygen species in the body. Superoxide dismutase has a significant role in antioxidant defense mechanism. The decline of 440nm absorption is relational to SOD activity. The commercially available kit is performed in a convenient 96-well micro titer-plate format.

2.9. Glutathione peroxidase (GPx)

Glutathione peroxidase is an enzymes family which plays an important role to protect organisms from oxidative stress. Glutathione peroxidase transforms reduced glutathione (GSH) to oxidized glutathione (GSSG) whereas reducing lipid hydro-peroxides to their resultant alcohols and/or free hydrogen peroxide to water (Birben et al., 2012). Glutathione peroxidase in muscle and blood was determined with the help of commercially available kits. The procedure given in the leaflet was followed.

Glutathione peroxidase ELISA kit relates the competitive enzyme immunoassay practice using a monoclonal anti-GSH-Px antibody and a GSH-Px- Avidin-Horseradish Peroxidase (HRP) conjugate. The result of the enzyme-substrate reaction gave blue colored compound. Addition of stop solution turned the solution yellow. Spectrophotometer at 340nm was used to measure the intensity of color in the micro ELISA plate. The intensity of the color and GSH-Px concentration has an inverse relationship.

3. Results

The present study evaluate the supplementation potential of selenium enriched yeast on growth performance, serum biochemistry and antioxidant biomarker in muscles of broiler both in starter and finisher phases under summer stress. The results are presented as under.

3.1. Effect of various sources of selenium supplementation on the growth performance of broilers in starter phase under summer stress

Overall mean feed intake, body weight, feed conversion ratio and mortality are presented in Figure 1. In starter phase all the performance parameters feed intake, body weight and feed conversion ratio was not significantly ($p>0.05$) affected with the supplementation of selenium enriched yeast. However, numerical difference in feed intake, body weight and feed conversion ratio was found among the supplemented groups. No significant differences ($p>0.05$) was recorded in percent mortality in all supplemented groups.

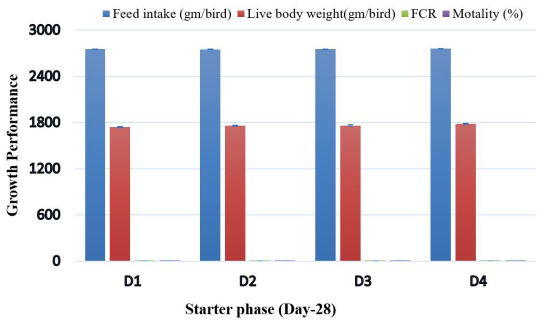

Figure 1. Effect of various sources of selenium supplementation on the growth performance of broilers in starter phase under summer stress. where D1 (control) basal diet without selenium supplementation, D2, basal diet supplemented with sodium selenite (0.22 mg/kg), D3, basal diet supplemented with commercially available organic selenium (0.22 mg/kg) and D4; basal diet supplemented with selenium enriched yeast (0.22 mg/kg).

3.2. Effect of various sources of selenium supplementation on the serum profile of broilers during summer stress in starter under summer stress

Selenium supplementation significantly ($p<0.05$) affected serum selenium level of broiler in different supplemental groups in starter phase presented in Table 3. Significantly higher ($p<0.05$) serum selenium values (58.20 ±0.06) was recorded in D4 group followed by D3 (50.71^{b}± 0.27) and least value (36.28±0.12) was recorded in D1. Significantly lower ($p<0.05$) serum MDA was recorded for D4 (6.63 ±0.11) followed by D3 (7.46 ±0.13), D2 (10.26±0.03) and higher serum values was recorded for D1 (11.36±0.08). However, significantly lower ($p<0.05$) serum Paraoxonase (PON) was recorded for D4 (13.24 ±0.01) followed by D2 (13.29±0.04) and higher serum PON values was recorded for D1 (13.33±0.03).

Again, similar trends was found for total anti-oxidant status (TOS), Superoxide dismutase (SOD), significantly lower ($p<0.05$) for TOS (0.230±0.02) and SOD (0.149±0.003) was recorded for D4 and higher TOS (0.340± 0.01) and SOD (0.153±0.002) values was recorded for D1 while significantly, higher ($p>0.05$) serum glutathione peroxidase (GPx) was recorded for D4 (15.07±0.03) and least was recorded for D1 (12.10±0.01) in broiler starter phase under summer stress.

3.3. Effect of various sources of selenium supplementation on selenium concentration and antioxidant biomarker in muscles of broiler

Selenium concentration and antioxidant biomarker in broilers muscles was presented in the Table 4. The p value was significantly reported in the in D4 (70.23±0.04), followed by D3 (69.12±0.35), D2 (45.91±0.26) and lower values was recorded for D1 (42.20±0.02), while similar trend was noticed for glutathione peroxidase (GPx) in broiler muscles, significantly, higher ($p>0.05$) glutathione peroxidase (GPx) in muscles was found in D4 (12.33 ±0.03), followed by D3 (10.33±0.06), D2 (8.413±0.09) and lower values was recorded for D1 (5.170±0.04). However, superoxide dismutase (SOD) in muscles a significantly lower ($p<0.05$) SOD (0.1437±0.003) was recorded for D4, followed by D3 (0.1457±0.002), D2 (0.1480^{b} ±0.003) and higher SOD (0.1517± 0.004) values was recorded for D1 in broiler meat at starter phase under summer stress.

Table 3. Effect of selenium supplementation on the serum profile of broilers during summer stress in starter under summer stress.

Diets	Serum profile of broiler in starter phase (Day- 28)					
	Se (Conc.) (µg/L)	MDA nmol/ml	PON (U/L)	TOS (µmol (H_2O_2) Equi/L)	SOD (U)	GPx (U/L)
	Means± SE	Means± SE	Means± SE	Means± SE	Means± SE	Means± SE
D1	36.28^{d} ±0.12	11.36^{a}±0.08	13.33^{a}±0.03	0.340^{a} ± 0.01	0.153^{a}±0.002	12.10^{d}±0.01
D2	42.28^{c} ±0.01	10.26^{b}±0.03	13.29^{ab}±0.04	0.300^{b}±0.03	0.151^{b}±0.001	13.78^{c}±0.01
D3	50.71^{b}± 0.27	7.46^{c} ±0.13	13.28^{ab}± 0.01	0.270^{c} ±0.01	0.150^{b}±0.002	14.28^{b}±0.02
D4	58.20^{a} ±0.06	6.63^{d} ±0.11	13.24^{b} ±0.01	0.230^{d} ±0.02	0.149^{c}±0.003	15.07^{a}±0.03
p. value	0.000	0.000	0.0318	0.000	0.0003	0.0000

SE = Standard error, p. value = Probability level, D1 (Control), D2 (Sodium selenite=0.22 mg/kg), D3 (Seleno-methionine =0.22 mg/kg), D4 (Selenium enriched yeast=0.22 mg/kg), a,b,c,d Mean with different superscript in the same column are significantly different at $\alpha = 0.05$.

3.4. Effect of various sources of selenium supplementation on the growth performance of broilers in finisher phase

Overall mean feed intake, body weight, feed conversion ratio and mortality of finisher phase are presented in Figure 2. The performance parameters feed intake, body weight and feed conversion ratio was significantly ($p<0.05$) affected with various sources of selenium supplementation in diets. Significantly ($p< 0.05$) higher feed intake was recorded in D1 (4390.6±2.20) followed by D3 (4369.7 ±0.26) and lowest was recorded in D2 (4334.9±1.63) while significantly ($p<0.05$) higher body weight was recorded in D4 (2066.1 ±2.95), followed by D1 (2034.3±1.18) and least was recorded for D2 (2029.2±1.74). Similarly, feed conversion ratio and percent mortality was also found significant. Mean comparison of FCR demonstrated that significantly ($p<0.05$) higher (poor) FCR was recorded in D1 (2.160±0.05), followed by D3 (2.146±0.03) and least FCR value (better) was recorded in D4 (2.113±0.03). Mean percent mortality was significantly ($p<0.05$) lower in D4 (2.84±0.04) while higher percent mortality was recorded in D1 (3.71± 0.07) in finisher phase of broiler under summer stress.

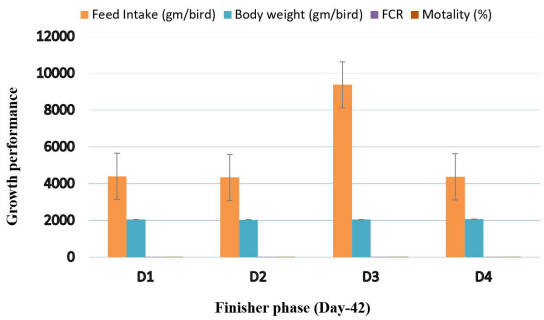

Figure 2 . Effect of various sources of selenium supplementation on the growth performance of broilers in finisher phase. Where D1 (Control), D2 (Sodium selenite=0.22 mg/kg), D3 (Seleno-methionine =0.22 mg/kg), D4 (Selenium enriched yeast=0.22 mg/kg).

3.5. Effect of various sources of selenium supplementation on the serum profile of broiler during summer stress in finisher phase

Selenium supplementation significantly ($p<0.05$) affected serum selenium level of broiler in different supplemental groups of finisher phase are presented in Table 5. Significantly higher ($p<0.05$) serum selenium values (61.33±0.16) was recorded in D4 group followed by D3 (53.34±0.14) and least value (37.36±0.15) was recorded in D1. Significantly lower ($p<0.05$) serum MDA was recorded for D4 (8.26 ±0.03) followed by D3 (9.50 ±0.05), D2 (13.20±0.05) and higher serum MDA values was recorded for D1 (14.26±0.16). However, significantly lower ($p<0.05$)

Table 4. Effect of various sources of selenium supplementation on selenium concentration and antioxidant biomarker in muscles of broiler.

Diets	Selenium concentration and antioxidant biomarker in broilers muscles		
	Se (Conc.) (mg/kg)	GPx (U/L)	SOD (U)
	Means± SE	Means± SE	Means± SE
D1	42.20^{d} ±0.02	5.170^{d} ±0.04	0.1517^{a} ± 0.004
D2	45.91^{c} ±0.26	8.413^{c} ±0.09	0.1480^{b} ±0.003
D3	69.12^{b} ±0.35	10.33^{b} ±0.06	0.1457^{c} ±0.002
D4	70.23^{a} ±0.04	12.33^{a} ±0.03	0.1437^{d} ±0.003
p. value	0.0000	0.0000	0.0001

SE = Standard error, p. value = Probability level, D1 (Control), D2 (Sodium selenite=0.22 mg/kg), D3 (Seleno-methionine =0.22 mg/kg), D4 (Selenium enriched yeast=0.22 mg/kg), ^{a,b,c,d} Mean with different superscript in the same column are significantly different at $\alpha = 0.05$

Table 5. Effect of various sources of selenium supplementation on the serum profile of broiler during summer stress in finisher phase.

Diets	Serum profile of broiler in finisher phase (Day- 42)					
	Se (Conc.) (µg/L)	MDA nmol/ml	PON (U/L)	TOS (µmol(H_2O_2) Equi/L)	SOD (U)	GPx (U/L)
	Means± SE	Means± SE	Means± SE	Means± SE	Means± SE	Means± SE
D1	37.36^{d}±0.15	14.26^{a}±0.16	11.50^{a}±0.03	0.453^{a}±0.002	0.168^{a}±0.03	12.85^{d}±0.02
D2	48.12^{c}±0.03	13.20^{b}±0.05	11.44^{b}±0.02	0.420^{b}±0.004	0.166^{b}±0.01	15.65^{c}±0.04
D3	53.34^{b}±0.14	9.50^{c} ±0.05	11.39^{b}±0.03	0.390^{c}±0.002	0.165^{c}±0.04	16.72^{b}±0.05
D4	61.33^{a}±0.16	8.26^{d} ±0.03	11.31^{c}±0.01	0.373^{c}±0.001	0.165^{c}±0.02	17.41^{a}±0.02
p. value	0.0000	0.0000	0.0003	0.0001	0.0000	0.0000

SE = Standard error, p. value = Probability level, D1 (Control), D2 (Sodium selenite=0.22 mg/kg), D3 (Seleno-methionine =0.22 mg/kg), D4 (Selenium enriched yeast=0.22 mg/kg), ^{a,b,c,d} Mean with different superscript in the same column are significantly different at $\alpha = 0.05$.

serum Paraoxonase (PON) was recorded for D4 (11.31±0.01) followed by D2 (11.44±0.02) and higher serum PON values was recorded for D1 (11.50±0.03).

The total anti-oxidant status (TOS), Superoxide dismutase (SOD), significantly lower ($p<0.05$) for TOS (0.373±0.001) and SOD (0.165±0.02) was recorded for D4 and higher TOS (0.453±0.002) and SOD (0.168±0.03) values was recorded for D1 while significantly, higher ($p>0.05$) serum glutathione peroxidase (GPx) was recorded for D4 (17.41±0.02) and least was recorded for D1 (12.85±0.02) in the finisher phase of broiler under summer stress.

3.6. Effect of various sources of selenium supplementation on selenium concentration and antioxidant biomarker in muscles of broiler at finisher phase

Selenium concentration and antioxidant biomarker in broilers muscles in finisher phase are presented in Table 6. Significantly, higher ($p>0.05$) muscle selenium concentration was found in D4 (82.26 ±0.05), followed by D3 (74.39±0.08), D2 (50.13±0.03) and lower values was recorded for D1 (45.17±0.07), while comparable inclination was noted for glutathione peroxidase (GPx) in broiler muscles, significantly, higher ($p>0.05$) glutathione peroxidase (GPx) in muscles was found in D4 (16.13±0.02), followed by D3 (14.31± 0.03) and least values was recorded for D1 (06.81± 0.02).

Although, superoxide dismutase (SOD) in muscles with a significantly lower ($p<0.05$) SOD (0.1623± 0.001) was recorded for D4, followed by D3 (0.1640± 0.004), D2 (0.1667±0.003) and higher SOD (0.1670±0.004) values was recorded for D1 in broiler meat at finisher phase of broiler under summer stress.

4. Discussion

A study of similar nature conducted in 2008 showed that there was a significant difference between the two experimental groups and control birds while exhibited no significant difference between the two different groups of experimental birds. According to the study no effect of dietary selenium was observed on the final weight, survival rate and Daily Gain of the birds (Wang and Xu, 2008). A study based on selenium supplementation effect on broilers growth performance showed that organic selenium supplementation is being superior to that of inorganic selenium. Further increased dietary selenium supplementation markedly reduces FCR as a result of lower feed intake while maintained the same weight gains (Choct et al., 2004). In the current study somewhat similar results have been observed for feed intake and body weight gain, organic selenium supplementation has an improved impact on the body weight gain.

Findings of the current study are in accordance to a study conducted in 2011 to determine selenium supplementation effect on broiler chicks' performance, which reported that no mortality was observed during the experimental phases. This study also determined that selenium and vegetable oil supplementation in broiler diets considerably enhanced final body weight and meat quality of birds (Ibrahim et al., 2011). On the other hand Ševčíková et al. (2006) recorded different results when supplemented chicken with different sources of selenium i.e. selenium enriched yeast and selenium enriched alga, the results showed that there were no substantial differences between the groups in FCR and mortality rate. Selenium enriched alga had the finest feed conversion as compared to selenium enriched yeast, and the selenium supplementation somewhat increased mortality rate in both experimental groups (Ševčíková et al., 2006). In the current study it was observed that heat stress increased mortality rate and a decreased feed conversion was recorded during heat stress. A study of somehow similar nature was conducted in 2009, the results of which indicated that heat stress negatively affect the growth performance and immune response of broilers, however these can be improved by dietary supplementation of Selenium under heat stress (Niu et al., 2009).

Study conducted in 2003 by Schrauzer concluded that Selenium enriched yeast suuplementation showed a higher concentration of selenium in muscles and serum so, these finding are in agreements to the current results (Schrauzer, 2003). Similarly Payne & Southern, 2005, also revealed that absorption and rentention of selenium in the body depends on the quantity and chemical form of selenium (Payne and Southern, 2005b). Selenium enriched

Table 6. Effect of various sources of selenium supplementation on selenium concentration and antioxidant biomarker in muscles of broiler at finisher phase.

Diets	Selenium concentration and antioxidant biomarker in broiler muscles (Day- 42)		
	Se (Conc.) (mg/kg)	GPx (U/L)	SOD (U)
	Means± SE	Means± SE	Means± SE
D1	45.17^{d} ±0.07	06.81^{d} ± 0.02	0.1670^{a} ±0.004
D2	50.13^{c} ±0.03	12.29^{c} ± 0.04	0.1667^{a} ±0.003
D3	74.39^{b} ±0.08	14.31^{b} ± 0.03	0.1640^{b}± 0.004
D4	82.26^{a} ±0.05	16.13^{a} ±0.02	0.1623^{b} ± 0.001
p. value	0.0000	0.0000	0.0020

SE = Standard error, p. value = Probability level, D1 (Control), D2 (Sodium selenite=0.22 mg/kg), D3 (Seleno-methionine =0.22 mg/kg), D4 (Selenium enriched yeast=0.22 mg/kg), ^{a,b,c,d} Mean with different superscript in the same column are significantly different at $\alpha = 0.05$.

(organic form) dietatry supplements are readily available as comapared to inorganic form (Kubachka et al., 2017). Recent study findings are also in accordance to the one conducted in 2008 which discovered that organic form of selenium (Se-enriched yeast) has far more better results than inorganic form (Wang and Xu, 2008). Likewise a study conducted in the same area concluded that Se- enriched yeast supplementation lowered MDA concentration as compared to the controlled group (Boostani et al., 2015). Supplementing poultry diet with organic selenium reduced MDA concentration (Skrivan et al., 2008). Chicken fed with selenium supplementation had the lowest MDA level (Ghazi Harsini et al., 2012). So, all these findings support the finding of the current study.

Consulting to a study which showed that antioxidant activity of PON 1 in the serum improved when selenium supplementation was given in diet (Safiullah et al., 2019), these findings are in accordance to our results. In contrast study conducted on the effect of selenium supplementation on rats revealed that selenium treatment reduced Paraoxonase activity in them. Paraoxonase activity when tested under heat stress along with dietary supplementations showed an improved PON 1 activity, these supplementations offer a prospective defensive administration practice in averting heat stress in birds effecting their growth performance (Harisa, 2013).

Previously an experimental study was conducted to check the effects of dietary sodium selenite and selenium yeast on oxidative stability of chicken meat concluded that Selenium enriched yeast increased the oxidative stability of chickens meat (Ahmad et al., 2012). Similarly a number of studies have shown positive response to dietary supplementation of selenium in poultry (Paton et al., 2002). Furthermore, other studies specified that the organic source of selenium have far more better results in oxidative stability of chicken as compare to inorganic sources (Kuricova et al., 2003). Research has shown that organic form of selenium supplementation to poultry diet not only increases FCR and total yield but also quality of meat. This enhanced performance is proved to be related to better antioxidant status of chickens (Upton et al., 2008). Similar results have been shown by different experimental studies concluding organic form of selenium as best source for enhancing SOD activities in chicken's tissues (Hu et al., 2012; Suchý et al., 2014). A study of relevant nature conducted to find the combined effect of vitamin E and selenium on superoxide dismutase level confirmed an increase SOD level in broilers (Traş et al., 2000). The findings that SOD activity was considerably improved in all three experimental groups of chickens fed on Se-supplemented diets for 4 weeks supports results of the current study (Holovská Junior et al., 2003).

Previously effect of different sources of selenium was tested in broilers and results concluded that glutathione peroxidase activities in broilers plasma and tissues of the selenium yeast treatment groups was considerably different ($p<0.05$) from control group, it was even higher that of inorganic selenium treatment group (Wang and Xu, 2008). Other similar nature study results showed that selenium yeast supplementation in broiler feed resulted in better muscles Se concentrations and glutathione activity than those supplemented with inorganic form of selenium (Payne and Southern, 2005a). Likewise to this current experimental study, many researchers described the effect of selenium supplementation on chickens. Heindl et al. (2010) found that the selenium source and level, including inorganic sources significantly ($P \leq 0.001$) affect the glutathione peroxidase activity in broilers muscles (Heindl *et al*., 2010), further in 2008 it was described the organic selenium have a positive influence on GPx activities (Skrivan et al., 2008). But other authors observed no significant difference in GPx activities in birds fed on different sources of selenium supplementations (Woods et al., 2020).

5. Conclusion

Dietary selenium supplementation in the form of Se-enriched yeast is capable of enhancing the growth performance of broilers, selenium accumulation in the muscles beneficial for secondary consumers. Further the valuable impacts of Se-enriched yeast on growth performance and enhanced immune responses in broiler chickens support the conclusion that Se-enriched yeast is one the essential source of Se supplementation in poultry diet now a days. Better performance as specified by enhanced body weight gain, feed conversion ratio, TOS, GPx, SOD activities in broilers proposes that Se-enriched yeast supplementation is better to other sources of selenium. Consequence of the Se-enriched yeast supplementation, the antioxidant status was improved that positively affect the overall growth performance of broilers.

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