Effects of Selenium-Enriched Probiotics on Lameness and Growth Improvement in Broiler Chickens Under Heat Stress Condition

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Abstract

This study describes the anti-heat stress benefits of Selenium-enriched probiotics (SP). A total of 200 1-d-old male broiler-chickens were subjected to heat stress due to natural high ambient temperature of summer. The broilers were randomly categorized into 4 treatments with 5 replicates per treatment and 10-broiler-chickens per replicate. They were fed a basal control diet (Con) and basal diet with probiotics (Pr), sodium-selenite (SS), and SP for 42 days. Se concentration/kg of diet for Con, Pr, SS, and SP groups was 0.10, 0.11, 0.41, and 0.44 mg/kg, respectively. The birds were graded for % age-lameness and body weight gain (BWG). The blood samples and bone marrows were collected after 42 days and analyzed through radioimmunoassay, rt-PCR, and atomic fluorescent spectrophotometer. It was found that the BWG was improved with no lameness in SP group. SP decreased serum oxidative markers, malondialdehyde, and T4 and increased Ca and P levels, T3, glutathione peroxidase, superoxide dismutase, and catalase (p<0.05). Furthermore, the SP treatment upregulated the mRNA expression of DIO2 and MCT-8, whereas downregulated DIO3. It concludes that SP as a feed additive with Se concentration of approximately 0.4 mg/kg improves growth performance and lameness by improving skeletal system through the upregulation of DIO2 and T3 in heat-stressed broilers.

Keywords: Bone health, broiler chickens, Se-probiotics

Introduction

Heat stress is a major challenging environmental problem which often causes severe economic loss by decreasing egg production, growth rate, metabolic activity, and feed consumption (Attila et al., 2010). Among other challenges, a metabolic disorder tibial dyschondroplasia (TD) affects the bone growth in young poultry and has become a leading cause of increased carcass condemnation in commercial poultry (Świątkiewicz and Arczewskaja-Wlosek, 2012). The responsible factor for TD is decreased production of thyroid hormones by reducing plasma T3 and increasing T4 concentration in broiler chickens. T3 regulates bone resorption and mineral deposition to maintain optimal bone strength (Williams, 2009).

Thyroid hormones play essential role in the development and function of bones (Williams et al., 2008; Williams, 2009). In target cells, DIO2 converts thyroxin (T4) to tri-iodothyroxin (T3), whereas iodothyronine deiodinase type-3 (DIO3) inactivates T3 and also inhibits the expression of T4. Therefore, DIO2 and DIO3 levels determine local T3 availability and its impact on bone tissue metabolism (Cheng et al., 2010; Galton et al., 2009). According to previous studies, high temperature conditions (Lara and Rostagno, 2013) and oxidative stress (Boostani et al., 2015) can significantly affect the production of thyroid hormones by reducing plasma T3 and increasing T4 concentration in broiler chickens. T3 regulates bone resorption and mineral deposition to maintain optimal bone strength (Williams, 2009). Supplementation of dietary Se promoted conversion of T4 to T3, with an elevated antioxidiant activity and decreased lipid peroxidation in stressed broilers (Fan et al., 2009). Selenium potency was...
further increased by converting inorganic Se into organic Se using various probiotic strains (Özsoy and Yalcìn, 2011; Gan et al., 2014; Liu et al., 2015). Lactic acid bacteria convert inorganic Se (sodium selenite) to organic selenium i.e., selenomethionine and selenocysteine (Lee et al., 2019). SP produce organic selenium which is equipped with a stronger antioxidant capability. As a feed additive, SP ameliorated the deleterious effects of heat stress in pigs by reducing the free radical load (Gan et al., 2013). It also improved liver function by reducing oxidative stress in rats (Liu et al., 2015; Nido et al., 2016). SP is used in heat-stressed poultry birds with favorable outcomes. SP has been found to improve liver function and protection (Khan et al., 2019), meat quality (Khan et al., 2018), egg production (Pan et al., 2011) and heart health (Khan et al., 2016) in poultry birds reared under high ambient temperature. However, to date, its effect on lameness, body growth, and underlying mechanisms related to bone health i.e., thyroid hormones regulation in broiler chickens raised under high ambient temperature of summer.

Materials and Methods

**SP, sodium selenite, and probiotics**

The fermented form of SP and probiotics (Pr) were obtained from Institute of Nutritional and Metabolic Disorders of Domestic Animals and Fowls, Nanjing Agricultural University, Jiangsu, China. They were comprised of 2 types of probiotic strains *L. acidophilus* and *S. cerevisiae* having $0.25 \times 10^{11}$/mL and $0.25 \times 10^{10}$/mL of colony-forming units, respectively. Approximately 5% of bacteria were put in to a 50 L fermentation tank with sodium selenite for fermentation at 30°C for 36 h to obtain SP product. The Se concentration was measured by AF-610A atomic fluorescence spectrometer to be approximately 10 mg/L (Lv et al., 2015).

**Experimental design**

A day-old male broiler chicks (n=200, Ross 308) with an average body weight of 45.5 g were randomly distributed in 4 treatment groups. The groups were named according to their diet as control group (Con): birds were given a basal diet without Se supplementation, SS group: basal diet with selenium supplementation using inorganic sodium selenite, Pr group: basal diet mixed with probiotics, Sp group: SP were added to basal diet. The birds were kept for an experimental period of 42 days. National Research Council (1994) guidelines were used to formulate the basal diet as shown in Table 1. Se concentration in the diet was measured by hydride generation atomic fluorescence spectrometry (HG-AFS) (Beijing Titan Instrument Co., Ltd, Beijing, China) according to the methods described by Gamiz-Gracia and De Castro (1999) (Table 2). The Se concentration measured in the basal diet was 0.11 mg/kg feed. Approximately 0.30 mg of Se/kg feed was added to SS and Sp groups where their final Se concentration increased up to 0.41 mg/kg feed (Table 1).

**Management practices**

Experimentation involving animals was permitted by Animal Care and Use Committee of Nanjing Agricultural University (ACUC: SYXX (Su) 2011-0036). The experiments were carried out for 42 days. During the first 14 days, the broiler chickens were kept in a temperature-controlled shed where the temperature was maintained between 34 and 36°C. Afterwards, for the rest of the trial, all the broilers were exposed to natural cyclic high ambient temperature of summer i.e., 31 to 38°C and 26 to 30°C during day and night, respectively. The interior humid-

### Table 1. Composition of basal diet for heat-stressed broiler chickens.

| Ingredients                        | Starter (0 to 21 d) | Grower (22 to 42 d) |
|------------------------------------|---------------------|---------------------|
| Corn (g/kg)                        | 58.12               | 65.31               |
| Soybean meal (g/kg)                | 31.61               | 23.31               |
| Corn gluten meal (g/kg)            | 3.90                | 4.50                |
| Vegetable oil (g/kg)               | 1.61                | 2.51                |
| Limestone (g/kg)                   | 1.32                | 1.22                |
| Dicalcium phosphate (g/kg)         | 1.76                | 1.59                |
| Sodium chloride (g/kg)             | 0.42                | 0.32                |
| L-Lys (g/kg)                       | 0.15                | 0.16                |
| DL-methionine (g/kg)               | 0.15                | 0.10                |
| Premix (g/kg)                      | 1.00                | 1.00                |

**Calculation of nutrients**

| Metabolizable energy (MJ/kg)       | 11.27               | 11.77               |
| Crude protein (g/kg)               | 220                 | 193                 |
| Calcium (g/kg)                     | 10                  | 9.1                 |
| Available phosphorus (g/kg)        | 4.39                | 3.69                |
| Lys (g/kg)                         | 10.7                | 9.6                 |
| Met (g/kg)                         | 5.1                 | 4.39                |
| Met + Cys (g/kg)                   | 8.1                 | 7.2                 |

*Provided per kilogram of diet: Fe, 60 mg; Cu, 7.5 mg; Zn, 65 mg; Mn, 110 mg; I, 1.1 mg; bacitracin Zn, 30 mg; vitamin A, 4500 IU; vitamin D3, 1000 IU; vitamin E, 30 IU; vitamin K1, 1.3 mg; vitamin B12, 2.2 mg; vitamin B2, 10 mg; vitamin B3, 10 mg; choline, 400 mg; vitamin B5, 50 mg; vitamin B6, 4 mg; biotin, 0.04 mg; vitamin B11,1 mg; vitamin B12, 1,013 mg.

### Table 2. Calculated and analyzed selenium concentrations in diets for heat-stressed broiler chickens (mg/kg)

| Treatments                      | Supplemental | Calculated | Analyzed |
|---------------------------------|--------------|------------|----------|
| Basal diet                      | 0.00         | 0.10       |          |
| Basal diet + probiotics         | 0.00         | 0.11       | 0.11     |
| Basal diet + sodium selenide    | 0.30         | 0.41       | 0.41     |
| Basal diet + SP                 | 0.30         | 0.41       | 0.42     |

*selenium concentration for the basal diet and supplement samples were analyzed by hydride generation atomic fluorescence spectrometry (HG-AFS) method to confirm the calculated concentration.*
ity of shed ranged from 60 to 80%. All broilers were vaccinated against viral diseases (Newcastle disease & infectious bursal disease). During the entire course of experimental period, feed and water were provided ad libitum. Mash feed was provided for first 20 days and pellet feed for the rest of the experimental period. All the birds were provided with light for 23 h during the 0-7 days age range, 20 h during the 8-30 days age range, and 23 h during the 31-45 days age range, with decreasing intensity of light from 30 lx to 5 lx (Olanrewaju et al., 2006).

**Growth performance and mortality**

The daily food consumption was recorded every day over the course of the experiment. After starting the experiment, the broiler chicks were weighed individually every day during the experimental period. From these data, final body weight, daily weight gain (DWG), total feed intake, and feed conversion ratio (FCR) were determined at the end of experiment. The abnormal behavioral responses during the hot hours of the day were recorded on daily basis. The percent mortality was recorded by formula; % Mortality = (the number of dead birds in a group/initial birds in a group × 100).

**Gross examination and percentage of lameness**

The gross observations were performed on the basis of leg abnormalities and thickness of tibia ring in broiler chickens. The birds were sacrificed and their leg, previously identified with a ring, was removed. Legs were dissected with a scalpel; the tibiae were cut axially 5 cm from the proximal epiphysis using a steel saw, and placed in a flask containing 10% buffered formalin. 24 h later, the specimens were submitted to gross examination and scored as 0-3; 0: no change in the thickness of growth plates, 1: a mild growth plate thickening (1-3 mm approximately), 2: growth-plate thickening of 3-6 mm, 3: growth plates thicker than 6 mm (Pelicia et al., 2012).

The percentage of lameness was determined by the following formula:

% Lameness = (the number of effected birds in a group/initial number of birds in a group × 100).

**Sample collection and processing**

At the 42nd day, blood samples (2 mL) were collected through left wing venipuncture from randomly selected ten broiler birds (2 per replicate) of each treatment. The blood samples were stored in heparinized and non-heparinized tubes, centrifuged at 1008 × g for 10 min, 4°C to obtain serum. The serum was stored at −20 °C for further analysis. The remaining RBC sediment was washed three times, lysed with ten volumes of ice-cold distilled water to prepare erythrocyte lysates and stored at −70°C for further analysis (Gan et al., 2014). The birds were sacrificed at the end of experimental period. Femur bones were collected, and broken and bone marrows were aspirated. Bone marrows collected were perfused with isotonic saline (ice cold), snap-frozen in liquid nitrogen before storing at −70°C.

**Biochemical analysis of serum enzymes, minerals, and T3 and T4**

Serum alkaline phosphatase (ALP), Ca, P, Glucose, total cholesterol (TC), Triglyceride, and uric acid (UA) were measured as previously described (Liu et al., 2015) using Hitachi BS-300 Automated Chemistry Analyzer (High-tech Industrial Park, Shenzhen, China). Serums T3 and T4 concentration were determined by the Radio Immuno Assay (RIA) kits (North Institute of Biological Technology, Beijing, China) using the manufacturer guidelines. The intra- and inter-assay coefficients of variation were less than 10%.

**Selenium concentration in serum**

An equipment atomic fluorescence spectrometer (AFS-930A) was used to determine Se concentration in serum (Jitian Analysis Instrument Co., Beijing, China) (Gan et al., 2013).

**Analysis of GSH-Px, SOD and CAT activities, and MDA levels**

Erythrocyte lysate was used for the analysis of glutathione per-oxidase (GSH-Px), serum oxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels using spectrophotometer as described elsewhere (Ahmad et al., 2012) by following the manufacturer’s guidelines (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

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**Table 3. Primers used in real-time quantitative PCR**

| Target gene | Gene bank accession no. | Primer sequence (5'−3') |
|-------------|-------------------------|--------------------------|
| GAPDH       | K01458                  | Forward: TGAAGTGAGGCTGCAACGATT<br>Reverse: ACGCTGATGGAAGATAGTAGTGA |
| DIO2        | NM_2041143              | Forward: TGGTTTCGTGCGCCCTCAA<br>Reverse: ACACTGGAAGTTCCGTTTCTC |
| DIO3        | NM_001122648.1          | Forward: CAGGGAGGAAAGGTGATGTACC<br>Reverse: TCCGGAGCGGGGTTTTGTACT |
| MCT8        | XM_426274.4             | Forward: CAACTCCCCGCGATCATCTACA<br>Reverse: AGCCAACCCCATGCTTTTAAA |

GAPDH, Glyceraldehyde phosphate Dehydrogenase; ID2, iodothyronine deiodinase type 2; ID3, iodothyronine deiodinase type 3; MCT8, Mono-carboxylate Transporter-8
Real-Time Quantitative PCR (rtPCR)

The mRNA expression of DIO2, DIO3, and mono carboxylate transporter-8 (MCT8) were quantitatively determined by rtPCR. GAPDH gene was kept as the reference gene. Gene sequence of the desired targets was determined by Primer 5.0 online software (Table 3).

mRNA was isolated from frozen cartilage of tibial growth plate using RNAiso Plus reagent (Takara Biotechnology, Dalian, China). The resulting pellets of isolated mRNA were resuspended in 30 μL diethyl- pyrocarbonate-treated water. mRNA concentration was measured by measuring its absorbance at 260/280 nm. 1 μg of mRNA was used to prepare cDNA using Oligo-dT primers and RNase M-MLV. cDNA was mixed with SYBR Green I PCR Master Mix (Takara Bio Inc, Kusatsu, Japan), desired primer (forward and Reverse) (Table 3), and PCR grade water. The mixture was amplified through ABI Prism 7300 Detection System (Applied Bio-systems, MA, USA). Relative mRNA expression of DIO2, DIO3, and MCT8 in the bone marrow was detected by 2-ΔΔCT method relative to GAPDH expression. All reactions were performed in triplicate.

Statistical analysis

The results were expressed as mean and standard error. Statistical Package for Social Sciences version 19 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Significant differences were analyzed by one-way analysis of variance followed by the Tukey's post hoc test. A p-value <0.05 was considered as significant.

Results

Growth improvement

Final body weight, daily weight gain (DWG), total feed intake, and feed conversion ratio (FCR) were more significantly improved (Figure 1, Table 4, p=0.025) in SP group as compared to Con, Pr, and SS groups. In addition, SS group had no significant difference in final body weight, DWG, and FCR with Con group. Furthermore, Pr group had a better effect as compared to Con and SS groups (Table 4, p=0.021). Both Pr and SS groups were statistically same regarding all values however they were better than the Con group (Table 4).

The percentage of mortality was lower (p=0.025) in Pr and SP groups as compared to Con and SS groups. When comparing SP and Pr group, the percentage of mortality was lower in the SP group (p=0.03). There was no statistical difference in percentage of mortality between Con and SS groups (Figure 1, Table 4).

Table 4. Effects of SP on growth performance (final Body weight and daily weight gain), feed utilization, and mortality rate in Ross male broiler chickens under heat stress condition

| Parameters                | Con        | Pr         | SS         | SP         |
|---------------------------|------------|------------|------------|------------|
| Final body weight (g)     | 1480±48.13 | 1787.5±14.93 | 1430±25.50 | 2190±57.15 |
| Total feed intake (g/bird)| 5622±127   | 4765±33    | 4898±157   | 3955±181   |
| Daily weight gain (g/d)   | 34.5±0.7   | 42.2±0.4   | 33.2±0.4   | 51.1±1.1   |
| FCR                       | 3.2±0.2    | 2.3±0.1    | 3.0±0.1    | 1.5±0.0    |
| Mortality rate (%)        | 16         | 08         | 10         | 02         |

**Mean values bearing the same superscripts in a row do not differ significantly (p<0.05).**
Effect of SP on gross lesions of tibia growth plate and percentage of lameness

Table 5 indicates gross lesion scores and lameness percentage. Pr and SS groups showed (p<0.05) moderate (small) lesions as compared to the Con group. However, no gross lesions were observed in SP group. Pr and SS supplementation reduced the percentage of lameness more significantly as compared to the Con group. In addition, no lameness was observed in SP treatment group (Table 5, p<0.05).

Effect of SP on serum biochemical parameters

The serum ALP, glucose, TC, Triglyceride, and UA decreased, whereas Ca and P increased more significantly with SP treatment as compared to other treatments (Table 6; p<0.02). No significance was noted between Pr and SS treatments; however, it was significantly different with Con and SP treatments (Table 6; p<0.01).

Effect of SP on plasma T3, T3/T4, and T4

The Plasma T3 concentration and T3/T4 ratio was increased, whereas T4 was significantly decreased in all treatment groups, except the Con group. In addition, the SP treatment showed a better effect in increasing T3 and T3 to T4 ratio and decreasing T4 than Pr and SS treatments (Figure 2a-c; p<0.003).

Table 5. Effects of SP on the gross and radiographic lesions of tibia growth plate and percentage of lameness in male broiler chickens at 42 days of age under heat stress condition

| Treatment | Gross lesion scores | Lesion severity | Percentage of lameness (%) |
|-----------|---------------------|-----------------|----------------------------|
| Con       | 3                   | Extensive lesion| 18                         |
| Pr        | 1                   | Moderate (small) lesion | 12                         |
| SS        | 1                   | Moderate (small) lesion | 10                         |
| SP        | 0                   | No lesion        | 00                         |

Effects of Pr, SS, and SP on tibia growth plate lesion. The severity of lesion depends on the lesion score as indicated. Gross lesion scores: 0 (zero) absence of lesion; 1 indicates small lesion; 2 indicates intermediate lesion; and 3 indicates extensive lesion.

Table 6. Effects of SP on serum biochemical parameters of broiler chickens under heat stress condition

| Blood biochemical parameters | Con     | Pr      | SS      | SP      |
|------------------------------|---------|---------|---------|---------|
| Alkaline phosphatase (U/L)   | 542±25.7 | 441±14.5 | 471±14.1 | 218±17.9 |
| Ca (mg/dL)                   | 5.07±0.1  | 7.13±0.1  | 7.16±0.2  | 9.01±0.2  |
| P (mg/dL)                    | 2.72±0.2  | 5.80±0.2  | 6.00±0.1  | 6.59±0.1  |
| Glucose (mg/dL)              | 243±7.1  | 154±6.1  | 156±6.4  | 97±6.5   |
| Total cholesterol (mg/dL)    | 161±8.3  | 110±3.4  | 108±4.0  | 77±5.9   |
| Triglyceride (mg/dL)         | 160±5.4  | 120±4.2  | 124±3.1  | 94±5.3   |
| Uric Acid (mg/dL)            | 9.23±0.3  | 4.89±0.1  | 5.00±0.1  | 2.33±0.3  |

**Means and standard errors. Mean values bearing the same superscripts in a row do not differ significantly (p<0.05).**
Effect of SP on serum Se concentration
Among all the treatments, the highest serum Se concentration was observed for SP treatment (Table 5; p<0.001). In addition, the Se concentration of SS treatment was higher than those of Con and Pr treatments; although it was lower than that of the SP treatment. Se concentration was not statistically different between Pr and Con treatments (Table 5; p<0.008).

Effect of SP on blood antioxidant enzyme activities and lipid peroxidation
The Pr, SS, or SP supplementation increased the level of antioxidant enzymes (GSH-Px, SOD, CAT) whereas decreased lipid peroxidation (MDA level) compared to Con (Table 7; p<0.001). In addition, SP treatment showed better performance as compared to P and SS treatments. Furthermore, the SOD activity and GSH levels were higher in SS and P treatments compared to the Con treatment. The level of GSH-Px was higher with SS treatment compared to Con and Pr treatments; however, it was not statistically different between Con and P treatments. Regarding the expression of SOD and CAT, no difference was observed between Pr and SS treatments. The serum MDA level was more significantly reduced with SP treatment as compared to other treatments (Table 7; p<0.01). Its level was same with Pr and SS treatments but lower than that of the Con treatment (Table 7; p<0.01).

mRNA expression of selenoproteins and MCT8 in bone marrow
Among all the treatments, the mRNA expression of selenoproteins DIO2 and DIO3 was highest in case of SP treatment. Their expression was higher both in SS and SS treatments compared to Con and P treatments (Figure 3; p<0.01). However, no statistical difference was observed between Con and P treatments (Figure 2a, b). When compared with other treatments, the mRNA expression of MCT8 was highest (Figure 3c; p<0.01) with SP treatment. It was followed by the higher expression with SS treatment as compared to Con and Pr treatments. However, no significant difference was observed between Con and Pr treatments (Figure 3c).

Discussion
High ambient temperatures could reduce growth performance by reducing feed intake and nutrient utilization in broilers (Harsini et al., 2012). Various Se supplemented diets have improved FCR and antioxidant capacity in heat-stressed birds (Woods et al., 2020); however, organic selenium is found to be better than inorganic Se in terms of feed utilization and growth performance (Yang et al., 2014). Similar results were obtained previously, where SP as a feed additive significantly improved

Table 7. Effect of SP on serum selenium (Se), glutathione peroxidase (GSH-Px), serum oxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) concentration in broiler chickens under heat stress

| Antioxidants | Con       | Pr        | SS        | SP        |
|--------------|-----------|-----------|-----------|-----------|
| Selenium concentration (ng/g) | 96.53±6.0<sup>c</sup> | 99.50±4.9<sup>c</sup> | 155.92±5.5<sup>b</sup> | 186.37±3.9<sup>a</sup> |
| GSH-Px (u/mg) | 337.80±8.2<sup>c</sup> | 370.30±10.6<sup>c</sup> | 501.62±14.6<sup>a</sup> | 609.45±16.4<sup>a</sup> |
| SOD (u/mg) | 91.81±6.0<sup>c</sup> | 122.51±2.3<sup>b</sup> | 118.25±5.1<sup>b</sup> | 143.32±2.4<sup>a</sup> |
| CAT (mg/g) | 2.38±0.1<sup>c</sup> | 3.75±0.1<sup>c</sup> | 3.74±0.1<sup>b</sup> | 4.29±0.1<sup>a</sup> |
| MDA (nmol/mg) | 3.14±0.1<sup>c</sup> | 2.84±0.1<sup>b</sup> | 2.89±0.1<sup>b</sup> | 1.28±0.1<sup>c</sup> |

<sup>a-c</sup>Mean and standard errors. Mean values bearing the same superscripts in a row do not differ statistically (p<0.05).
the body weight and FCR by consuming less feed as compared to SS, P, and Con groups in rats (Liu et al., 2015; Nido et al., 2016) and in pigs under high heat stress conditions (Gan et al., 2014). Our results are in accordance with the previous findings. We observed a similar increase in final body weight and daily weight gain (DG) by utilizing less feed as compared to Con, SS, P groups.

Ca and P are mainly involved in the mineralization and development of bone (Proszkowiec-Weglarz and Angel, 2013). Heat stress negatively alter the Ca and P level in bones which predisposes hens to tibial bone dyschondroplasia (TD) (Hoseini-Vashan et al., 2016). The incidence can be reduced with dietary supplementation of Ca and P (Attia and Hassan, 2017). Earlier studies showed that different sources of Se improve calcium deposition in the tibia of hens (Attia et al., 2010). Se improved the cartilage integrity and reversed the mycotoxin-induced cartilage necrosis (Medeiros, 2016). Probiotics, as a feed supplement, has been shown to improve the bone metabolism and bone mineral density in laying hens (Yan et al., 2019). We found similar increase in Ca and P levels with Pr and SS supplementation. However, the increase was more profound with the combination of Se and Pr as compared to SP, which indicates that it more favorably promoted the bone health by increasing the availability of Ca and P.

Heat stress negatively regulates Se uptake and metabolism, which may lead to skeletal muscle disorders and cartilage chondrocyte differentiation (Sun et al., 2011). Only 50% of the broilers showed normal gait score i.e, 0. A higher gait score (abnormal) in the rest of birds was due to the stress of higher ambient temperature (Cordeiro et al., 2009). We found that SP increased the Se-associated Ca deposition and improved the bone growth and quality. We found no lame bird in the SP group, which indicates that SP as a feed supplement eliminates those pathological process due to heat stress which affect normal conformation of bones and bone growth.

The serum ALP enzyme is elevated in various bone pathologic conditions such as osteitis deformans (Balani and Marda, 2016) osteomalacia, Fanconi’s syndrome, and hyperparathyroidism. In certain bone conditions such as TD in animals, the ALP level was not altered; it was comparable to the levels in healthy animals (Rath et al., 2005). In calves, Se had no effect on the expression of ALP except at higher level of T3 (Mudgal et al., 2012). Similarly reduced expression of ALP was observed in layers fed with SS (Mohapatra et al., 2014). The finding of this study regarding the expression of serum ALP were in agreement with the findings of Rath et al. (2005).

The environmental stress negatively affects the performance, relative asymmetry, and tonic immobility. Various blood constituents (glucose, cholesterol, and triglycerides) are altered in response to environmental stress (Attia et al., 2018; Attia and Hassan, 2017). High environmental temperature elevates the plasma level of cholesterol, glucose, and MDA and decreases glutathione peroxidase (Tawfeek et al., 2014). Se supplementation has favorable effects upon blood biochemical markers. It reduces the glucose and uric acid concentration in blood serum of broilers reared in high environmental temperature (Harsini et al., 2012). Dietary supplementation with vitamin E and Se reduced glucose, triglycerides, cholesterol, and urea contents in heat-stressed Japanese quails. Se and vitamin E supplementation showed inverse relation with the expression of serum biochemical markers (Ferit Gursu et al., 2003). Similar trend was observed in heat-stressed broilers fed with Se supplemented diet (Harsini et al., 2012) and probiotic supplemented diet (Sohail et al., 2010). We found comparable results with Se supplementation i.e., a reduced expression of triglycerides and glucose. However, the effect was more profound with SP supplementation than SS and Pr supplementation solely.

The production of reactive oxygen species (ROS) is increased during heat stress; this negatively influences the metabolism of bone cells (osteoclasts, osteoblasts, and osteocytes) (Demience et al., 2015). Antioxidant enzymes are produced in response to ROS, which require Se as a co-factor for their synthesis (Jang et al., 2014; Tawfeek et al., 2014). In animals, Se deficiency caused skeletal abnormalities such as osteopenia, osteoporosis, chondronecrosis, and endemic osteoarthropathy due to defective bone and cartilage metabolism (Tawfeek et al., 2014). Higher MDA level in serum indicates oxidative stress in broiler chickens affected by heat stress. It is produced due to ROS-induced cell membrane lipid peroxidation (Abou-Zeid et al., 2015). Lipid peroxidation decreased, whereas antioxidant activity in terms of glutathione peroxidase and glutathione reductase increased in broiler’s plasma with Se supplementation (Dalia et al., 2017). Similar results were seen in broilers fed with probiotic Bacillus subtilis. There was an increased level of serum immunoglobulins and antioxidant enzymes, and a decreased level of serum MDA and ROS (Bai et al., 2017). SP feed supplementation reversed the deleterious effects of high-fat diet on the liver of mice. It reduced the expression of liver enzymes, total cholesterol, GSH-Px, SOD, CAT, and MDA (Nido et al., 2016). In this study, our results were in agreement with SS and Pr supplementation found in other animals. However, we found most profound antioxidant activity and lowest serum MDA level with the meldon use of Se and P in heat stress broilers.

Various clinical observations suggest that thyroid hormones are actively involved in bone metabolism. T3 causes bone remodeling through osteoblastic proliferation, differentiation, and apoptosis and probably through the activation of osteoclasts formation and activation (Gan et al., 2013). Therefore, hypothyroidism in humans causes retarded bone growth and remodeling due to impaired functions of chondrocytes, osteoblasts, and osteoclasts (Bassett et al., 2010). Previous studies reported that T3 plasma concentration is consistently decreased during high-temperature conditions (Lara and Rostagno, 2013). Supplementation of dietary Se promotes the conversion of T4
to T3; this elevates antioxidant activity and decreases lipid peroxidation in broilers during heat stress (Fan et al., 2009). The high level of Se in serum increases T3 and T3:T4 ratios, whereas T4 level in broilers under oxidative stress due to corticosterone administration (Lin et al., 2004). The antioxidant functions of Se are mediated by the induction of GSH-Px, SOD, and CAT (Nido et al., 2016). The antioxidant activity is increased with increasing Se supplementation in broilers’ diet, as indicated by decreased expression of serum MDA (Hala and Fathy, 2014). In this study, we found that combined Se and probiotics strengthened the antioxidant system (GSH-Px, SOD, CAT) of SP group more profoundly than Pr and SS group. We found an increased plasma T3 concentration and T3:T4 ratio and a decreased T4 level with SP supplementation.

The selenoenzyme type DIO2 is required for normal bone development by regulating the intracellular T3 transportation through MCT8 transportation system (Williams et al., 2008). Inside the target cells, T4 is converted to T3 by DIO2. In contrast, T3 is inactivated by the activity of DIO3. Thus, the local T3 availability depends on the balanced activity of DIO2 and DIO3 (Waung et al., 2015). In this study, SP supplementation more significantly up-regulated the DIO2 and down-regulated DIO3 in bone marrow of heat-stressed broiler chicken as compared with Pr and SS treatments. These data provide essential insight into thyroid hormone action in bone, revealing a crucial role of DIO2 in optimizing mineralization and toughness of the skeleton. We anticipated that high level of DIO2 is essential for normal cartilage and bone formation. We observed low T4 and high T3 level in blood with SP supplementation in diet, which indicated that SP is actively involved in the conversion of T4 to T3. Our results are in agreement with the weanling piglets indicated that SP is actively involved in the conversion of T4 to T3 by DIO2 and DIO3 (Lv et al., 2015).

Nowadays, a close housing system i.e., control shed, which is equipped with a temperature and humidity regulatory system is utilized for commercial farming. However, its high cost of installation limits its access; it cannot be accessed by small-scale traditional farmers. Therefore, the broiler birds are reared in a semi-close housing system, where the likelihood of summer/heat stress affection is higher in summer. From the findings of this study, we inferred that Se and P combination as SP has mutual synergistic effects and their use as a dietary supplementation could be a primary approach to ameliorate summer stress in broilers. The combination of SP produces abundant organic Se, which is more potent than inorganic Se and has least toxic effects.

**Conclusion**

The present data suggests that SP as a feed additive with Se concentration of 0.4 mg/kg improves growth performance and skeletal development by increasing T3 transportation and enhancing Ca and P agglomeration. However, further study is required to assess the effects of SP on bone health and development.

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