The role of selenium in ameliorating the impact of heat stress on growth, hormones, minerals and tibia measurements of broiler chickens

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Abstract

The study was conducted to investigate the potent capacity of selenium to curtail the adverse effects of heat stress in broiler chickens. A total of 256, day old Cobb 500 broiler chicks were randomly allotted to four experimental treatments (0, 0.1, 0.2 and 0.3 mgSe/kg), with four replicates in a completely randomized design. Initial weight, daily feed intake, daily weight gain, final weight, feed conversion ratio, daily water intake, feed cost/kg gain, mortality, serum cortisol and thyroxine levels, calcium, phosphorus and ash levels in tibia bone and faeces and weight, length and robusticity index of tibia bone were determined. Results indicated that Initial weight, daily feed intake, daily weight gain, final weight, feed conversion ratio, daily water intake and mortality were similar ($p > 0.05$) across the treatment groups and feed cost/kg gain was lower in broilers birds fed 0.3 mgSe/kg. Selenium supplementation at 0.1 and 0.2 mgSe/kg lowered ($p < 0.05$) cortisol and increased ($p > 0.05$) thyroxine level, respectively. Mineral deposition in tibia bone was similar ($p > 0.05$), calcium excretion was higher ($p < 0.05$) in the control group and higher phosphorus was observed in the faeces of broilers fed the control diet, 0.1 and 0.3 mgSe/kg. Tibia measurements were similar ($p > 0.05$) in all treatments.

Keywords: Calcium, Phosphorus, hormone, Bone, Robusticity, broiler bird

Le rôle du sélénium dans l'amélioration de l'impact du stress thermique sur la croissance, les hormones, les minéraux et les mesures du tibia des poulets de chair

Résumé

L'étude a été menée pour étudier la puissante capacité du sélénium à réduire les effets néfastes du stress thermique chez les poulets de chair. Un total de 256 poussins de poulets Cobb 500 âgés d'un jour ont été attribués au hasard à quatre traitements expérimentaux (0, 0,1, 0,2 et 0,3 mgSe / kg), avec quatre réplicats dans un plan complètement randomisé. Poids initial, apport alimentaire quotidien, gain de poids quotidien, poids final, taux de conversion alimentaire, apport quotidien en eau, coût de l'aliment / gain de kg, mortalité, taux de cortisol et de thyroxine sériques, taux de calcium, de phosphore et de cendres dans les os et les fèces du tibia et poids, la longueur et l'indice de robustesse de l'os du tibia ont été déterminés. Les résultats ont indiqué que le poids initial, l'apport alimentaire quotidien, le gain de poids quotidien, le poids final, le ratio de conversion alimentaire, l'apport quotidien en eau et la mortalité étaient similaires ($p > 0,05$) dans les groupes de traitement et le coût de l'alimentation / gain en kg était plus faible chez les poulets de chair nourris 0,3 mgSe / kg. Une supplémentation en sélénium à 0,1 et 0,2 mgSe / kg a abaissé ($p < 0,05$) le cortisol et augmenté ($p > 0,05$) le taux de thyroxine, respectivement. Les dépôts de minéraux dans l'os du tibia étaient similaires ($p > 0,05$), l'excrétion de calcium était plus élevée ($p < 0,05$) dans le groupe témoin et une plus grande quantité de phosphore a été observée dans les fèces des poulets de

chair nourris avec le régime témoin, 0,1 et 0,3 mgSe / kg. Les mesures du tibia étaient similaires ($p > 0,05$) dans tous les traitements.

Mots clés: calcium, phosphore, hormone, os, robustesse, poulets de chair

Introduction

As global warming is consistently increasing, the environmental temperature will continue to rise (Richter *et al.*, 2010). Seasonal variations in ambient temperature is an important stressor that affects broiler production in many regions of the world, and huge losses on capital of investment may accrue due to reduced production performance, depressed immunity and mortality (Melesse *et al.*, 2011). The hot-dry season, characterized by high atmospheric temperature (AT) and high relative humidity (RH), in the Northern Guinea Savannah zone has been shown to be thermally stressful to poultry (Sinkalu *et al.*, 2015). Heat stress causes an increase in free radicals and reactive oxygen species (ROS) which can lead to oxidative damage (Zhang *et al.*, 2014). Heat stress can negatively affect bone development and drastically reduce available calcium and phosphorus in broiler chickens (Sgavioli *et al.*, 2016; Persia *et al.*, 2003). Heat stress disrupts the productive performance, physiological and behavioural attributes of chickens, hence antioxidants are required in micro quantities to counter the deleterious effect of reactive oxygen species (ROS) during heat stress (Abdulrashid *et al.*, 2010). Selenium (Se) is an antioxidant that protects the body cells against damage caused by free radicals and oxidative stress (Abdel-Azeem, 2010). The enzyme glutathione peroxidase protects cells and cell membranes from oxidation, and Se is an important part of its structure (Oliveira *et al.*, 2014). Selenium plays vital roles in animal health and productivity (Zhou and Wang, 2011). The use of antioxidant minerals such as selenium in broiler diets can minimize the effects of stress caused by

high temperatures due to the impact of free-radical and consequently improve the growth performance and physiological characteristics of birds (Choct *et al.*, 2010; Habibian *et al.*, 2014). The objective of this study was to determine the effect of Selenium during heat stress on growth, hormones, bone and faecal minerals and tibia measurements of broiler chickens.

Materials and methods

The experiment was conducted at Ahmadu Bello University, Samaru, Zaria. Two hundred and fifty six, one day old *Cobb 500* broiler chicks were randomly allotted to four experimental treatments (0, 0.1, 0.2 and 0.3 mgSe/kg), with four replicates each in a Completely Randomized Design. A maize/soybean meal based broiler starter and finisher diets was formulated according to NRC (1994) nutrient requirement. Feed and water was provided *ad libitum*. All standard routine management practices were strictly adhered to.

Initial weight was taken at the beginning of the experiment, while feed intake and weight gain were taken weekly using a digital precision weighing balance (Satorius ENTRIS). Water was measured and offered daily to the birds, left over was also recorded at the end of each day, specified amount of water was poured into an open container and placed at intervals along the passage within the building and evaporation losses was accounted for daily by measuring the quantity of water left in the container and subtracted from the initial quantity measured using a calibrated measuring cylinder. The ort and evaporation loss was then subtracted from the quantity of water offered to the birds to determine the amount of water consumed daily. Mortality records were taken as they

occurred.

Hormonal assay

Thyroxine was determined according to the procedure by Chopra *et al.* (1971) while **cortisol** this was determined according to the procedure by (Foster and Dunn 1974).

Determination of tibia geometric properties

During carcass analysis at day 50, the left tibia of two birds per replicate was removed. Tibia was labeled and immersed in boiling water (100°C) for 15 minutes according to the procedure described by Applegate and Lilburn, (2002), in order to completely remove the tissue. Length of each bone was measured using a meter rule, and the distance from proximal to distal extremities of each tibia was taken as the tibia length. The bone weight was obtained using a digital precision weighing balance (Satorius ENTRIS). The bone weight/length index was computed by dividing the tibia weight by its length (Seedor *et al.*, 1991), while the Robusticity index was determined using the formula described by Reisenfeld (1972).

Robusticity Index = $\frac{\text{Bone length}}{\text{Cube root of boneweight}}$

Cube root of boneweight

To determine bone ash, the bones were oven-dried at 100°C for 24 hours and then burnt to ash in a muffle furnace at 600°C for 6 hours according to the procedure described by A.O.A.C. (1990). The percentage ash was then determined relative to dry weight of the tibia. Calcium and Phosphorus in bone and faeces was also analyzed after digestion of the bone and faeces and then read in a spectrophotometer (A.O.A.C. 1990).

Data analysis

Data obtained from this experiment were statistically analyzed using General Linear

Model Procedure of Statistical Analysis System software package while significant difference among means were compared using the Tukey Procedure (SAS, 2002).

Result and discussion

The THI in the morning averaged 26.85% and 35.65% at afternoon. The results obtained indicated that THI in the afternoons was higher than THI in the mornings which revealed the absence of heat stress in the morning and the presence of severe heat stress in the afternoon (Ndubuisi *et al.*, 2020). The growth performance of broiler chickens fed diets supplemented with different levels of selenium from day 1-49 of the study (Table 1) showed that daily feed intake, daily weight gain, final weight, feed conversion ratio, daily water intake and mortality were similar ($p>0.05$) across all the treatment groups. Broilers fed 0.3 mg selenium supplemented diet had the least feed cost per kg gain. The similarity observed in growth performance of broiler chickens throughout the experimental period could be due to the ability of broiler chickens to adapt and maintain regular homeostasis during prolonged stress. The result obtained in this study is in agreement with the report of Guoshun *et al.* (2013) and Bakhshalinejad *et al.* (2018). Stress weakens the immune system of animals, making them porous to diseases. Chronic respiratory disease, Newcastle disease and severe heat stress was reported during post mortem examination and may be responsible for the high mortality rate especially in the 0.3 selenium supplemented group which may be due to the increased level of selenium, although diets are shown to be safe for poultry when they contain 0.15 - 4.0 mg/kg selenium (NRC, 1994).

The role of selenium in ameliorating the impact of heat stress

Table 1: Effect of selenium supplementation on performance of broiler chickens (day 1-49)

Parameters	Dietary levels of Selenium (mg/kg diet)				SEM	P value
	0	0.1	0.2	0.3		
Initial weight (g/bird)	41.41	39.69	43.44	39.54	1.13	0.0982
Daily feed intake (g/b/d)	82.13	80.08	86.90	80.08	2.26	0.1646
Daily weight gain (g/b/d)	44.600	44.123	45.990	44.428	0.960	0.5434
Final weight (g/bird)	2226.80	2201.75	2296.89	2216.46	46.81	0.5108
Feed conversion ratio (g)	1.84	1.82	1.89	1.80	0.03	0.2437
Daily water intake (ml/b/d)	257.93	261.92	267.64	269.33	6.90	0.6401
Feed cost/kg gain (₹/kg)	219.91	217.43	226.63	216.68	-	-
Mortality (%)	14.06	12.50	10.94	20.31	4.96	0.5777

Figure 1 and 2 shows the cortisol and thyroxine levels of broiler chicks fed varying levels of selenium supplemented diets during 28 days of the study. Cortisol was higher ($p < 0.05$) in broilers fed the control diet (10.40) and 0.3 mgSe/kg (12.20) but broilers fed 0.1 mgSe/kg (5.00) had the least ($p < 0.05$) cortisol level. Broilers fed 0.2 and 0.3 mgSe/kg supplemented diet had higher ($p > 0.05$) thyroxine level (41.68) while the least (35.05) was recorded in broilers fed the control diet. Glucocorticoids (cortisol, corticosterone, dexamethasone, prednisolone) are released from the adrenal cortex in response to stress, which affects metabolic pathways, immune function, and endocrine systems (Fan *et al.*, 2009; Shokrane *et al.*, 2019). The decreased level of cortisol in broilers fed 0.1 and 0.2 mg supplemented diet is an indication that these levels were adequate for the antioxidant role of selenium against ROS and pro-oxidants during heat stress. Fan *et al.* (2009) reported a decrease in corticosterone level in broilers fed diet supplemented with 0.1 and 0.4 mg of sodium selenite compared to the control. The high level of cortisol in broilers fed 0.3 mgSe/kg diet might be due to a pro-oxidant

effect of selenium caused by chemical interaction. Antioxidants or polyphenols can act as pro-oxidants under certain conditions, such as the presence of metal ions, the concentration of antioxidant in matrix environment and its redox potential (Sotler *et al.*, 2019).

Thyroid hormone plays a significant role on metabolism and growth (Fan *et al.*, 2009; Shokrane *et al.*, 2019). Preter (2000) revealed that selenium deficiency can lead to a decrease in triiodothyronine (T3) synthesis and this may be due to the fact that selenium is an important auxiliary factor for the key enzyme of 5- deiodinase. The iodothyroxine deiodinase enzymes convert the pro-hormone thyroxine (T4) to the active form triiodothyronine (T3) (Selim *et al.*, 2015). T3 is the main hormone that regulates growth by controlling the body's energy and protein anabolism (Preter, 2000). The higher thyroxine level observed in selenium supplemented groups is an indication that selenium protected the thyroid gland from ROS (Ujang, 2008). The result of thyroid hormone observed in this study is consistent with El-Samra *et al.* (2014) and Fan *et al.* (2009) but in contrast to Shokrane *et al.* (2019).

Fig. 1: Effect of varying levels of selenium on cortisol level of starter broiler chickens (day 28)

Fig. 2: Effect of varying levels of selenium on thyroxine level of starter broiler chicks (day 28)

Table 2 shows the calcium, phosphorus and ash contents of bone and faeces of broiler chickens fed varying levels of selenium supplemented diet during the study. Calcium, Phosphorus and ash content of the tibia bones of the various groups ranged from 30.83 – 33.75, 10.04 – 10.42 and 0.37 – 0.46 respectively and were similar ($p>0.05$). Calcium and Phosphorus

excreted in the faeces ranged from 16.67 – 22.50 and 0.89 – 1.12 respectively. Broilers fed the control diet had higher ($p<0.05$) faecal calcium deposition, while higher ($p<0.05$) faecal Phosphorus was observed in chickens fed the control diet, 0.1 and 0.3 mgSe/kg diet respectively. Phosphorus is the second most abundant mineral in the body with immense importance in poultry

diets for efficient growth and development (Shahid *et al.*, 2018). Although, Calcium, Phosphorus and Ash content of tibia bones of broiler chickens in the different groups were similar, the higher excretion of calcium in the faeces of broilers fed the control diet and Phosphorus in the faeces of broilers fed the control diet, 0.1 and 0.3 mgSe/kg feed respectively could be due to the impact of heat stress which led to decreased binding of minerals to protein for proper intestinal absorption and utilization (Shahid *et al.*, 2018).

Sahin *et al.* (2009) further stated that heat stress also increases mineral excretion and decreases serum and liver concentration of minerals. This agrees with the report of Persia *et al.* (2003) who indicated that there is a reduction in the available calcium and phosphorus utilization during chronic heat stress due to reduced growth. Persia *et al.* (2003) also stated that reduction in feed

intake during heat stress in poultry compromises the nutrient intake hence decreases the mineral uptake as well as its subsequent utilisation.

The tibia measurements of broiler chickens fed varying levels of selenium supplemented diets are presented in Table 3. Tibia weight, length, weight/length and robusticity index among chickens in all treatment groups were similar ($p>0.05$). In general, the result increased ($p>0.05$) with increase in selenium supplementation up to 0.2 mg with a decline at 0.3 mg selenium supplementation. Zeng *et al.* (2013) reported that insufficient Se intake can lead to retarded growth and modification of bone metabolism; the involvement of Se in bone health has mainly been its preventive function against oxidative stress and inflammation, boost of immune system and its involvement in cell division, proliferation and apoptosis.

Table 2: Effect of selenium supplementation on calcium, phosphorus and ash content in bone and faeces of broiler chickens

Parameters (%)	Dietary levels of Selenium (mg/kg diet)				SEM	P value
	0	0.1	0.2	0.3		
Bone						
Calcium	33.67	33.75	30.83	33.75	2.84	0.8443
Phosphorus	10.42	10.04	10.32	10.27	0.18	0.4995
Ash	0.46	0.42	0.37	0.39	0.03	0.1327
Faeces						
Calcium	22.50 ^b	18.75 ^a	17.92 ^a	16.67 ^a	1.34	0.0483
Phosphorus	1.06 ^b	1.12 ^b	0.89 ^a	1.07 ^b	0.05	0.0421

^{ab} Means with different superscript on the same row differ significantly ($p<0.05$)

Table 3: Effect of selenium supplementation on tibia measurements of broiler chickens

Tibia Measurements	Dietary levels of Selenium (mg/kg diet)				SEM	P value
	0	0.1	0.2	0.3		
Tibia Weight (g)	4.63	4.76	5.14	4.84	0.22	0.4396
Tibia Length (cm)	7.13	7.08	7.38	7.20	0.12	0.3354
Tibia Weight/Length Index (g/cm)	0.65	0.67	0.70	0.67	0.02	0.6402
Robusticity Index (cm/g ^{1/3})	4.64	4.47	4.35	4.47	0.16	0.6641

The increase in bone weight and length observed in the selenium treated groups is an indication of selenium's involvement in growth. This agrees with Moreno-Reyes *et*

al. (2009) who reported that selenium deficiency impaired bone metabolism and osteopenia in rats. The decrease observed in tibia development of broilers fed diet

supplemented with 0.3 mg of selenium could be due to the increased cortisol level recorded in this group, and may have led to increased ROS activity which may have affected bone metabolism.

The decrease in robusticity index reveals that selenium is involved in bone turgidity. Robusticity index is an indication of bone strength, and a low robusticity index obtained in birds fed the Selenium diets indicates a strong bone structure. The similar results obtained in this study agree with the work of Adem (2018) who observed non-significant values for robusticity index in broilers fed betaine supplemented diet. Lameness was also observed in broilers fed the control diet which reveals that heat stress could have impeded bone metabolism.

Conclusion

The supplementation of selenium 0.1 and 0.2 mg/kg in the diets of broiler chickens during heat stress alleviated the deleterious effect of heat stress through improved thyroxine level and reduced cortisol level. It also enhanced mineral retention by reducing its losses through faeces and improved bone strength.

References

A.O.A.C. 1990. Association of Official Analytical Chemists, Official Methods of Analysis. 15th Ed Washington DC.

Abdel-Azeem, S. A. 2010. Improving Reproductive Efficiency of Rabbit Does using Hormonal, Nutritional and Acetic Intra-Vaginal Washing Treatments, *A PhD thesis*.

Abdulrashid, M., Agwunobi, L. N. and Hassan, M. R. 2010. Ascorbic Acid and Heat Stress in Domestic Chicken Nutrition: A Review. *Journal of Agriculture, Forestry and the Social Sciences*, 8(2): 249-

257.

Adem, L. A. 2018. Responses of broiler chickens fed betaine hydrochloride supplementation under dexamethasone induced stress condition. *A PhD thesis*, Ahmadu Bello University, Zaria.

Applegate, T. J. and Lilburn, M. S. 2002. Growth of the femur and tibia of a commercial broiler line. *Poultry Science*, 81: 1289-1294.

Bakhshalinejad, R., Akbari, M. K. R. and Zoidis E. 2018. Effects of different dietary sources and levels of selenium supplements on growth performance, antioxidant status and immune parameters in Ross 308 broiler chickens. *British Poultry Science*, 59: 81-91.

Choct, M., Naylor, A. J. and Reinke, N. 2010. Selenium supplementation affects broiler growth performance. *British Journal of Poultry Science*, 45: 677-683.

Chopra, I. J., Solomon, D. H. and Ho, R. S. 1971. A radioimmunoassay of thyroxine. *Journal of Clinical Endocrinology*, 33: 865.

El-Samra, H. A. A., Kalaba, Z. M., Tolba, A. A. H. and El-Deeb, M. A. I. 2014. Alleviating adverse effects of heat stress by using organic selenium and chromium for local laying hens. *Journal Animal and Poultry Production, Mansoura University*, 5(7): 397-411.

Fan, C., Yu, B. and Chen, D. 2009. Effects of different sources and levels of selenium on performance, thyroid function and antioxidant status in stressed broiler chickens. *International Journal of Poultry Science*, 8(6): 583-587.

Foster, L. and Dunn, R. 1974. Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma.

- Clinical Chemistry*, 20: 365.
- Guoshun, C., Jinfeng, W. and Chong, L. 2013.** The effect of Different Selenium Levels on Production Performance and Biochemical Parameters of Broilers. *Italian Journal of Animal Science*, 12 (4) : 79. DOI: 10.4081/ijas.2013.e79.
- Habibian, M., Ghazi, S., Moeini, M. M, and Abdol-Mohammadi, A. 2014.** Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. *International Journal of Biometeorology*, 58: 741-752.
- Melesse, A., Maak, S., Schmidt, R. and Von-Lengerken, G. 2011.** Effect of long-term heat stress on key enzyme activities and T3 levels in commercial layer hens. *International Journal of Livestock Production*, 2: 107-116.
- Moreno-Reyes, R., Egrise, D., Neve, J. and Pasteels, J. L. 2009.** Selenium deficiency induced growth retardation is associated with an impaired bone metabolism and osteopenia. *Journal of Bone and Mineral Research*, 18(8): 1-7.
- National Research Council. 1994.** Nutrient requirement of poultry, National Academy Press, Washington, D.C. Ninth Edition.
- Ndubuisi, D. I., Abdurashid, M. and Daudu, O. M. 2020.** The role of selenium in ameliorating heat stress on thermoregulatory, growth and haematological indices of broiler chickens. *Nigerian Journal of Animal Science and Technology*, 3(3):
- E. and Bertechini, A. G. 2014.** Effect of different sources and levels of selenium on performance, meat quality, and tissue characteristics of broilers. *Journal of Applied Poultry Research*, 23(1): 15-22.
- Peric, L., Milosevic, N., Zikic, D., Kanacki, Z., Dzinic, N., Nollet, L. and Springs, P. 2009.** Effect of selenium sources on performance and meat characteristics of broiler chickens. *Journal of Applied Poultry Research*, 18: 403-409.
- Persia, M. E., Utterback, P. L., Biggs, P. E., Koelkebeck, K. W. and Parsons, C. M. 2003.** Interrelationship between environmental temperature and dietary non-phytate phosphorus in laying hens. *Poultry Science*, 82: 1763-1768.
- Preter, F. S. 2000.** Organic selenium benefits to animal and humans. A biochemist view. In: Proceedings of Alltech's 16th symposium. Nottingham University Press, Nottingham, Pp. 205-213.
- Reisenfeld, A. 1972.** Metatarsal robusticity in bi-pedal rats. *American Journal of Physiology and Anthropology*, 40: 229-234.
- Richter, K., Haslbeck, M. and Buchner, J. 2010.** The heat shock response: life on the verge of death. *Molecular Cell*, 40(2): 253-266.
- S.A.S., 2002.** Statistical Analysis System Institute, User's Guide. Version 9 for Windows. North Carolina, U.S.A.
- Sahin, K., Sahin, N., Kucuk, O., Hayirli, A. and Prasad, A. S. 2009.** Role of dietary zinc in heat-stressed poultry: A review. *Poultry Science*, 88: 2176-2183. Doi: 10.3382/ps.2008-00560.
- Seedor, J. G., Quarruccio, H. A. and**

- Thompson, D. D. 1991. The bio-phosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. *Journal of Bone Mineral Research*, 6: 339-346.
- Selim, N. A., Radwan, N. L., Youssef, S. F., Salah-Eldin, T. A. and Abo-Elwafa, S. 2015. Effect of inclusion inorganic, organic or nano selenium forms in broiler diets on: 2-physiological, immunological and toxicity statuses of broiler chicks. *International Journal of Poultry Science*, 14(3): 144-155.
- Sgavioli, S., Domingues, C. H. F., Santos, E. T., Quadros, T. C. O., deBorges, L. L., Garcia, R. G., Louzada, M. J. Q. L. and Boleli, I. C. 2016. Effect of In-Ovo Ascorbic Acid Injection on the Bone Development of Broiler Chickens Submitted to Heat Stress During Incubation and Rearing. *Revista Brasileira de Ciência Avícola*, 18(1): 153-162.
- Shahid, H. M., Ravi, P. P., Veena, M., Tariq, A. M., Lamella, O. and Shimla, Y. 2018. Role of dietary minerals in heat-stressed poultry: A review. *Journal of Entomology and Zoology Studies*, 820-826.
- Shokraneh, M., Sadeghi, A. A., Mousavi, S. N., Esmaeilkhani, S. and Chamani, M. 2019. Effects of *in ovo* injection of nano-selenium and nano-zinc oxide and high eggshell temperature during late incubation on antioxidant activity, thyroid and glucocorticoid hormones and some blood metabolites in broiler hatchlings. *Acta Scientiarum. Animal Sciences*, 42: 1-9.
- Sinkalu, V. O., Ayo, J. O., Abimbola, A. A and Ibrahim, J. E. 2015. Effect of melatonin on cloacal temperature and erythrocyte osmotic fragility in layer hens during the hot-dry season. *Journal of Applied Animal Research*, 43(1): 52-60.
- Sotler, R., Poljšak, B., Dahmane, R., Jukić, T., Jukić, D. P., Rotim, C., Trebše, P and Starc, A. 2019. Pro-oxidant activities of antioxidants and their impact on health. *Acta Clin Croat*, 58(4): 726-736.
- Ujang, T. (2008). Selenium: its role as antioxidant in human health. *Environmental Health and Preventive Medicine*, 13(2): 102-108.
- Zeng, H., Cao, J. J. and Combs, G. F. 2013. Selenium in bone health: Role in antioxidant protection and cell proliferation. *Nutrients*, 5(1): 97-110.
- Zhang, B., Guo, X., Chen, J., Zhao, Y., Cong, X., Jiang, Z., Cao, R., Cui, K., Gao, S. and Tian, W. 2014. Saikosaponin-D attenuates heat stress-induced oxidative damage in 11c-pk1 cells by increasing the expression of anti-oxidant enzymes and hsp72. *American Journal of Chin. Medicine*, 42(5): 1261-1277.
- Zhou, X. and Wang, Y. 2011. Influence of dietary nano elemental selenium on growth performance, tissue selenium distribution, meat quality, and glutathione peroxidase activity in Guangxi Yellow chicken. *Journal of Poultry Science*, 90: 680-686.

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