Asian-Aust. J. Anim. Sci.
Vol. 23, No. 4 : 501 - 507
April 2010

www.ajas.info

Evaluation of a Dietary Organic Selenium Supplement at Different Dietary Protein Concentrations on Growth Performance, Body Composition and Antioxidative Status of Broilers Reared under Heat Stress

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ABSTRACT : Three hundred chicks were randomly assigned among four treatments to evaluate the effect of an organic selenium supplement at various levels of dietary protein. Two levels of supplemental selenium (0 and 0.3 mg/kg) from zinc-L-selenomethionine were tested at two levels of dietary protein (normal and reduced) in a completely randomized design with 2×2 factorial layout. The experiment lasted up to 49 d of age during which all birds were exposed to 31±1°C. The effects of selenium or its interaction with CP on growth performance and carcass characteristics were not significant. However, feeding the reduced-CP diet decreased weight gain in the starting period and increased liver and abdominal fat weights relative to body weight. Ferric reducing ability of plasma (FRAP) was not significantly affected by dietary CP and Se or their interaction though FRAP values were numerically higher in the Se-supplemented group. Dietary CP content did not affect the activity of plasma glutathione peroxidase (GSHPx), though Se significantly elevated plasma GSHPx activity. The interaction of CP and Se was not significant for FRAP and plasma GSHPx activity. (**Key Words** : Broiler, Heat, Protein, Selenium)

INTRODUCTION

High environmental temperatures have deleterious effects, reducing the feed intake, live weight gain and feed efficiency of poultry. Feed intake and growth of broilers have been estimated to decrease about 3.6 and 1.5%, respectively, per degree increase between 22 and 32°C (Baziz et al., 1996). Heat stress causes a negative impact on growth and immunity due to induced alterations in physiological, hormonal, and molecular status as well as lipid peroxidation (Donker et al., 1990). Research has shown that supplementing broiler diets with antioxidants alleviated metabolic changes and lipid peroxidation caused by heat stress (Sahin et al., 2002).

Poultry nutrition research on the use of organic and inorganic Se sources has yielded significant improvements in growth performance (Surai and Dvorska, 2001) and antioxidant capacity (Surai, 2002) of broiler chickens. Improvement in antioxidant capacity is attributed to inducible Se dependent antioxidant enzymes (Surai, 2002). Selenium is an integral component of glutathione peroxidase (EC 1.11.1.9; GSHPx). GSHPx, in alliance with vitamin E, forms part of the cell's defence against reactive metabolites of oxygen which are produced excessively under stress conditions (Surai, 2002).

Many works have examined the effect of dietary CP on broiler performance under heat stress (Gonzalez-Esquerra and Leeson, 2005). However, combined effects of Se supplement and dietary CP level have not been studied. The interaction of selenium supplement with dietary fat has been recently studied and reported by Upton et al. (2009). They reported that blood GSHPx was significantly influenced by both fat and selenium, but the fat×selenium interaction was not significant. The objective of the present experiment was to evaluate the effect of dietary Se supplement at two levels of dietary CP in broiler chickens under continuous thermal stress.

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Received August 26, 2009; Accepted November 10, 2009

MATERIALS AND METHODS

Treatments and bird husbandry

Three hundred and fifty day-old male broiler chicks (Ross 308) were obtained from a local hatchery. Obvious runts and chicks in extreme weighs were eliminated within first week post-hatch. On day 7, following 6 h fasting, 300 chicks were randomly assigned to 20 groups of 15 birds kept on floor pens. Five such groups (replicates) were randomly assigned to one of four treatments. Treatments were two levels of dietary protein (normal and reduced) and two levels of supplemental selenium (0 and 0.3 mg/kg) from zinc-L-selenomethionine in a 2×2 factorial layout.

Zinc-L-selenomethionine was provided by ZINPRO Animal Nutrition (Avila-Se 1000), MN, USA. The reduced-CP diet was supplemented with DL-Met and L-Lys in a way that the ratio of TSAA:Lys was similar to that in the control diet in each feeding stage (Khajali et al., 2008). The experiment was divided in three stages of starter (7 to 21 d), grower (22 to 42 d), and finisher (42-49 d). Within a time period, all diets were isoenergetic. Prior to addition of Zn-L-selenomethionine, Se level in the experimental diets met or marginally exceeded minimum requirements of broilers as advocated by NRC (1994). The reduced-CP diet was prepared in a manner such that none of the essential amino acids was limiting and met the NRC (1994) recommendation. Table 1 depicts the composition of the experimental diets in different stages.

All chicks were maintained on a 23 L:1 D of light program with intensity of 15 lux and exposed to a high constant temperature (31±1°C) throughout the experiment. The experimental animals were kept, maintained and treated in accepted standards for the human treatment of animals.

Measurements

Prior to starting the experiment, protein content of corn, soybean meal, and fish meal as well as mixed diets were determined to assure of accurate level of dietary CP. Dietary protein and selenium contents of the experimental diets were also determined (AOAC, 1995). Body weight and feed consumption were recorded periodically and feed conversion ratio was calculated taking into account the weights of mortality within each period. Two birds from each replicate pen (10 per treatment), were wing banded for blood collection in heparinized tubes, which performed on Day 20 and Day 40. Plasma samples were obtained by centrifuging the blood at 3,000 g for 10 minutes and freezed at -80°C until the time they were analyzed for antioxidant power of the plasma using FRAP method (Benzie and Strain, 1996) as well as for assay of glutathione peroxidase activity (Mates et al., 2000). FRAP assay monitored the subsequent changes in absorbance as a result of the

Table 1. Composition of the experimental diets

Feed ingredient	Starter diet (%)		Grower diet (%)		Finisher diet (%)	
	Normal-CP	Reduced-CP	Normal-CP	Reduced-CP	Normal-CP	Reduced-CP
Corn	58.5	62.5	67.5	70.7	74.0	76.8
Soybean meal (42% CP)	37.0	31.5	29.0	24.2	16.3	12.7
Fish meal	-	-	-	-	5.0	4.0
Soya refined oil	1.2	1.1	-	-	-	-
Wheat bran	-	-	-	-	1.8	3.0
Dicalcium phosphate	1.4	1.6	1.2	1.2	0.8	0.8
Oyster shell	1.1	1.3	1.3	1.3	1.0	1.0
Salt	0.3	0.3	0.3	0.3	0.16	0.17
Trace mineral premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.07	0.15	-	0.06	0.01	0.09
L-HCl-lysine	-	0.14	-	1.0	-	0.13
Sand	-	0.91	0.2	1.67	0.43	0.81
Total	100	100	100	100	100	100
ME (Kcal/kg)	2,900	2,900	2,900	2,900	3,000	3,000
CP (%)	20.7	18.7	18.1	16.6	16.8	15.1
Met+cys (%)	0.82	0.82	0.69	0.69	0.70	0.64
Lys (%)	1.15	1.15	0.96	0.96	0.98	0.98
Se (mg kg^{-1})	0.2	0.21	0.20	0.20	0.20	0.2

[1] Supplied per 2.5 kg mineral premix: Fe, 50,000 mg; Mn, 100,000 mg; Zn, 100,000 mg; Cu, 10,000 mg; I, 1,600 mg; Se, 200 mg.

[2] Supplied per 2.5 kg vitamin premix: A, 9,000,000 IU; B_1, 1,800 mg; B_2, 8,250 mg; B_3, 1,000 mg; B_6, 3000 mg; B_{12}, 1,500 mg; D_3, 2,000,000 IU; E, 18,000 IU; K_3, 4,000 mg; B_9, 1,250 mg; B_5, 30,000 mg; H_2, 5,000 mg; Choline, 500,000 mg.

Zinc-L-selenomethionine was added at 100 mg/kg in the Se-supplemented treatments to provide 0.3 ppm Se.

reduction of ferric ions to ferrous form by electron donating of antioxidants. Two hundred and fifty microliters of plasma sample was incubated with 750 µl of FRAP reagent for 5 min at 37°C and the absorbance was read at 593 nm. FRAP reagent was 10 mM 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) in HCl, 2.5 mM chloroferric and 0.3 M potassium acetate, pH 3.6.

Activity of GSH-Px was assessed by measuring the oxidation of NADPH to $NADP^+$ by a decrease in absorbance at 340 nm. Two hundred microliters of plasma sample was incubated with 0.2 M phosphate buffer, pH 7.2, 15 mM GSH, and 50 U of Glutathione reductase for 15 min at 37°C. Following addition of 1.5 mM NADPH and 2 mM H_2O_2, the reduction in the absorbance was measured spectrophotometrically at 340 nm (Jenway spectrophotometer, Model 6505, UK).

At the end of experiment (day 49), after an overnight starvation, 10 chicks from each group were wing banded, weighed, killed by decapitation and their organs weighted. Before the time of slaughter, the live body weight of each bird was measured. When the head, shanks, and feathers were removed, the carcass was eviscerated by cutting around the vent to remove all of the viscera. Once eviscerated, the carcass without giblets was weighed and expressed as a percentage of its initial live weight and considered as the carcass yield (CY). Breasts were weighted and expressed as percentages of the live weight. The weights of the liver and abdominal fat were measured to the nearest 0.01 g and expressed as percentage of the live body weight.

Statistical analysis

Data were subjected to ANOVA in a completely randomized design with 2×2 factorial arrangements of treatments using SAS software (SAS Institute, 1996). In case where there was sampling within pens, data were subjected to a nested design. Tukey's test was used to separate the means at 0.95 probability.

RESULTS

Body weight gain of treatments is shown in Table 2. During the starting stage (7-21 d), weight gain was significantly decreased as dietary CP content reduced. The reduction in weight gain continued up to the end of experiment so that birds on reduced-CP diet gained less weight than those on the normal-CP diet during the growing and finishing periods. Nevertheless, birds fed reduced-CP diet had lower weight gain than the control throughout the experiment (1,658.8 vs. 1,822.2 g). Supplementing diet with Se had no significant improvement in weight gain in different stages of the experiment. The interaction of dietary CP and Se was not significant.

Table 2 depicts the effects of dietary treatments and their interaction on feed consumption during different periods of the experiment. Except for the growing period (21-42 d), birds fed significantly less feed on reduced-CP diet than the normal diet. There was no significant effect of supplemental Se with respect to feed intake. Analysis of variance showed no significant interaction between dietary CP and Se. The main effect of dietary CP content and Se

Table 2. Effect of protein level and organic selenium supplement on broiler performance

Variable	CP	Se	7-21 d	21-42 d	42-49 d	7-49 d
Weight gain (g/b)						
	Normal	w/o Se	318.2	864.2	618.5	1,800.9
	Normal	w Se	334.9	945.2	563.3	1,843.5
	Reduced	w/o Se	308.2	857.6	521.4	1,687.2
	Reduced	w Se	290.5	864.4	472.4	1,630.4
SEM (n = 10)			6.58	23.53	34.5	27.71
Feed intake (g/b)						
	Normal	w/o Se	525.1	1,677.0	1,367.9	3,569.9
	Normal	w Se	532.9	1,760.2	1,234.5	3,527.7
	Reduced	w/o Se	507.6	1,664.1	1,112.4	3,284.1
	Reduced	w Se	500.2	1,677.2	1,047.0	3,224.3
SEM (n = 10)			7.20	22.03	54.35	61.00
Feed:gain (g:g)						
	Normal	w/o Se	1.65	1.96	2.27	1.98
	Normal	w Se	1.59	1.86	2.19	1.91
	Reduced	w/o Se	1.65	1.94	2.14	1.95
	Reduced	w Se	1.72	1.94	2.23	1.98
SEM (n = 10)			0.03	0.04	0.06	0.02

w/o Se: Without organic selenium supplement.

Table 3. Effect of protein level and organic selenium supplement on broiler carcass characteristics (49 days of age)

	CP	Se	CY (%)	BY (%)	Liver (%)	AFP (%)
CP main effect						
Normal-CP			71.8	21.9[a]	2.09[b]	2.99[b]
Reduced-CP			71.7	21.1[b]	2.37[a]	3.32[a]
SEM (n = 10)			1.04	1.66	0.16	0.15
Se main effect						
w/o Se			71.4	21.7	2.17	3.34[b]
with Se			72.0	21.9	2.29	2.97[a]
SEM (n = 10)			1.04	1.66	0.16	0.15
Interaction						
	Normal	w/o Se	72.3	21.9	2.11	3.11
	Normal	w Se	71.3	21.9	2.07	2.86
	Reduced	w/o Se	71.9	21.7	2.40	3.50
	Reduced	w Se	71.6	21.8	2.28	3.10
SEM (n = 20)			0.74	1.17	0.12	0.11
Probability						

[a,b] Values within an age group comparison are different ($p<0.05$).
w/o Se: Without organic selenium supplement. CY = Carcass yield; BY = Breast yield; AFD = Abdominal fat percentage.

supplement as well as their interaction was not significant in terms of feed conversion ratio (Table 2).

Effects of dietary protein content, supplementary Se and their interaction on broiler carcass characteristics are presented in Table 3. Carcass yield was not significantly affected by dietary CP and Se levels or their interaction. Breast yield was significantly reduced in birds fed reduced-CP diet. No obvious impact of supplemental Se or its interaction with dietary CP was found in terms of breast yield. However, percentages of liver and abdominal fat pad relative to live body weight were significantly increased by reducing dietary CP content. Interestingly, addition of Se from Zn-selenomethionine caused significant reduction in abdominal fat deposition.

Ferric reducing ability of plasma (FRAP) was not significantly affected by dietary CP and Se or their interaction measured on 20 and 4o days of age (Table 4). Supplementation of organic Se tended to enhance FRAP. However, the main effect of Se was not significant. No consistent result of FRAP was observed attributable to dietary CP content. The interaction of dietary CP and Se was not significant. The activity of plasma GSHPx was not

Table 4. Effect of protein level and organic selenium supplement on plasma antioxidant status of broiler chickens

	CP	Se	GSH-Px activity		FRAP (total antioxidant)	
			20 d	40 d	20 d	40 d
CP main effect						
Normal-CP			2.40	2.41	536.8	586.5
Reduced-CP			2.40	2.39	581.5	531.0
SEM (n = 10)			0.10	0.12	46.9	49.0
Se main effect						
w/o Se			2.38	2.17[b]	556.8	543.1
with Se			2.43	2.62[a]	561.5	606.5
SEM (n = 10)			0.1	0.12	46.9	49.0
Interaction						
	Normal	w/o Se	2.40	2.40	460.2	566.3
	Normal	w Se	2.41	2.64	633.4	607.8
	Reduced	w/o Se	2.37	2.16	489.6	515.9
	Reduced	w Se	2.44	2.61	653.2	542.2
SEM (n = 20)			0.07	0.08	33.2	34.7

[a,b] Values within an age group comparison are different ($p<0.05$).
w/o Se: Without organic selenium supplement. GSH-Px: Plasma glutathione peroxidase activity (moles of NADPH oxidized per min per gram of protein). FRAP = Ferric reducing ability of plasma (μMol L^{-1}).

affected by CP content at 20 or 40 d of age. However, inclusion of the organic Se source significantly ($p<0.05$) elevated plasma GSHPx activity when measured at 40 days of age (Table 4). At 20 days of age, the activity of the enzyme was higher in Se-supplemented group compared to the control but the difference was not significant.

DISCUSSION

Experimental diets without addition of organic selenium supplement provided almost 0.2 mg/kg Se, which satisfied the minimal requirements of poultry (0.15 mg/kg) according to National Research Council (1994). Nutrient requirements advocated by NRC (1994) have been established under thermoneutral conditions and they are minimal requirements. There are several reports indicating nutrient requirements of broilers are influenced by ambient temperature (Brake et al., 1998; Chamruspollert et al., 2004). Jianhua et al. (2000) have recommended dietary Se level of 0.3 mg/kg diet for broiler chickens for maximum productivity. Se has been used up to level of 1.5 mg/kg in broiler diets (Placha et al., 2008). The levels beyond 1.5 may cause toxicity or clinical signs of selenosis.

Decreased weight gain especially during the starting period as a result of feeding the reduced-CP diet is because broiler chicks have high demand for protein to attain the fast growth rate at this critical stage. Amino acids are known as anabolic factors, which induce protein gain by stimulating protein synthesis while inhibiting proteolysis. These effects on protein turnover have been clearly demonstrated *in vitro* and *in vivo* (Grizard et al., 1995; Muramatsu, 1990). The other possibility is that birds on the reduced-CP diet consumed less feed and as a result gained less compared to the control. The reduction in weight gain as a result of feeding reduced-CP diet did not compensate up to the end of experiment and this can be attributed to lower feed intake in all feeding periods. The results reported herein are consistent with previous reports (Malheiros et al., 2003; Gonzales-Esquerra and Leeson, 2005). Supplementing diet with Se had no significant improvement in weight gain in different periods of the experiment. This might have occurred because dietary Se level has met the needs of birds. Similar results reported by Upton et al. (2009), who indicated that addition of 0.2 ppm Se either as selenium selenite or selenium yeast did not significantly improve body weight gain. Se has been reported to improve the growth performance of broilers when supplemented to Se-deficit diets (Jianhua et al., 2000). Jianhua et al. (2000) indicated that Se deficiency depresses growth of broilers by inhibiting hepatic 5-deiodinase activity which caused lower plasma T_3 concentration. Choct et al. (2004) demonstrated that improved weight gain due to Se nutrition was in part related to higher feathering as selenocystein is necessary for feather formation. In the study conducted by Yoon et al. (2007) under thermoneutral condition, effects of different doses of dietary Se from different sources ranged from 0 to 0.3ppm was evaluated. They reported that Se supplementation did not significantly improve broiler weight gain. The interaction of dietary CP and Se was not significant for growth rate. The effects of interaction of Se with CP on performance, as well as physiological parameters measured in the present study in broilers reared under heat stress, are not available in the literature.

Birds consumed less feed from the reduced-CP diet than the normal diet. The reduced-CP diet had proportionally higher crystalline forms of essential amino acids (Met and Lys) which absorbed faster into the blood and might have created an aminostatic feedback signal for reducing feed intake in birds (Jensen, 1989). There was no significant effect of supplemental Se with respect to feed intake. Analysis of variance showed no significant interaction between dietary CP and Se with respect to feed intake.

The main effect of dietary CP content and Se supplement as well as their interaction was not significant in terms of feed conversion ratio. Feed conversion ratio composed of two components: weight gain and feed intake. Since the similar trend occurred in these components, feed conversion ratio has not showed any significant changes. Similar results were reported by Ozkan et al. (2007), who found no significant difference with respect to feed conversion ratio between the birds received Se at 0.15 and 0.35 mg/kg under normal or cold conditions. In addition, Upton et al. (2009) indicated that addition of 0.2 ppm Se either as selenium selenite or selenium yeast had no significant effect on FCR. Conversely, Choct et al. (2004) in a normal temperature indicated that dietary Se supplementation up to 0.25 mg/kg markedly reduced feed conversion ratio as a result of significantly lower feed intakes of birds while maintaining the same weight gains.

Carcass yield was not significantly affected by dietary CP and Se levels or their interaction. This implies that CY is a trait that hardly influenced by nutritional modulations. Breast yield was significantly reduced in birds fed reduced-CP diet. This is consisted with previous report which showed that increase in dietary CP would result in higher breast yield (Bartov and Plavnik, 1998). Indeed, breast yield is more influenced by dietary manipulations compared to CY. No obvious impact of supplemental Se or its interaction with dietary CP was found in terms of breast yield.

Percentages of liver and abdominal fat pad relative to live body weight were significantly ($p<0.05$) increased by reducing dietary CP content. Feeding the reduced-CP diet or decreased protein intake increased hepatic lipid accretion because of decreased energy supply to muscle protein synthesis. Research has proven that an increase in dietary protein content has been associated with reduced activity of

key hepatic lipogenic enzyme fatty acid synthase (Bannister et al., 1983). In chickens, the liver is the main site of lipogenesis (Rosebrough et al., 1999). This can also explained the reason why feeding the reduced-CP diet resulted in increased abdominal fat deposition. Accordingly, elevation of liver weight in relation to whole body weight can speculate the intensive lipogenesis occurring in this organ. The other explanation for increased abdominal fat in the reduced-CP group is attributed to the lower production of uric acid. Chickens are uricotelic and excrete uric acid as the end product of dietary protein metabolism. The lower the dietary CP content, the lower uric acid excretion (Swennen et al., 2005). Each molecule of uric acid excretes 8.22 kcal energy per gram of N (Hill and Anderson, 1958). Hence, the birds on the reduced-CP diet are characterized by lower uric acid levels (indicative for reduced protein breakdown and lower protein ingestion) and this speculated as increased abdominal fat deposition (Malheiros et al., 2003). Interestingly, addition of Se from Zn-selenomethionine declined abdominal fat deposition ($p<0.05$). This can be explained by the results reported by Vadhanavikit and Ganther (1994), who indicated that Se supplementation declined the activity of cytosolic malic enzyme.

Ferric reducing ability of plasma (FRAP) employs reduction of ferric ions to ferrous form by electron donating of antioxidants. The higher the value of FRAP, the more powerful the antioxidant capacity. The inclusion of organic Se tended to enhance FRAP though insignificantly. The experimental diets used in this trial had little or no supplemental unsaturated oil. If unsaturated oil could be used in the diets, the difference between the dietary treatments could be more distinguishable. This speculates that organic Se tends to enhance the antioxidant power. FRAP was not significantly affected by dietary CP, the interaction of CP and Se though no consistent result was observed attributable to dietary CP content. The activity of plasma GSHPx was not affected by CP content measured on 20 or 40 d of age. However, inclusion of the organic Se significantly elevated plasma GSHPx activity, which can be regarded as an improvement of antioxidant status. Improvement in the antioxidant status as a result of selenomethionine supplementation is because Se plays a critical role as Se is an essential part of the selenoenzyme GSHPx. In this regard, Choct et al. (2004) showed that Se from sodium selenite but not from selenized yeast (an organic source) at 0.1 and 0.25 mg/kg increased the activity of GSHPx in the erythrocytes. In addition, Payne and Southern (2005) showed that Se from Se-enriched yeast enhanced the activity of plasma GSHPx in broiler chickens. Se supplementation has also reported to significantly elevate GSHPx activities in blood, liver and kidney of broiler chickens (Placha et al., 2008). At normal and low temperatures, supplementation with organic Se alone increased plasma GSHPx activity (Ozkan et al., 2007; Yoon et al., 2007). Likewise, methionine moiety can be converted to cysteine, which, in turn, converts to GSH. Both cysteine and GSH can function as direct scavengers of reactive oxygen species (ROS). GSH and cysteine can also protect proteins from irreversible oxidative damage through interactions between these thiols and proteins and the formation of mixed disulfides, such as glutathiolated proteins (Mallis et al., 2002).

In conclusion, the results reported herein found no significant interaction between dietary CP and Se with regard to growth, carcass characteristics and antioxidant status. Reducing dietary CP resulted in impaired broiler performance under heat stress conditions of the experiment. Supplementation of organic Se caused no significant improvement in growth performance. However, use of the organic Se may have advantage through increased antioxidant power as assessed through numerically higher FRAP values and higher activity of plasma GSHPx. In addition, supplementing the organic Se supplement reduced abdominal fat deposition.

ACKNOWLEDGMENTS

This research was funded by Shahrekord University, Shahrekord, Iran.

REFERENCES

AOAC. 1995. Official methods of analysis. 16th ed. Association of Official Analytical Chemists, Arlington, Virginia.

Bannister, D. W., I. E. Onell and C. C. Whitehead. 1983. The effect of biotin deficiency and dietary protein content on lipogenesis, gluconeogenesis and related enzyme activities in chick liver. Br. J. Nutr. 50:291-302.

Bartov, I. and I. Plavnik. 1998. Moderate excess of dietary protein increases breast meat yield of broiler chicks. Poult. Sci. 77: 680-688.

Baziz, H. A., P. A. Geraert, J. C. F. Padilha and S. Guillaumin. 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. Poult. Sci. 75:505-513.

Benzie, I. F. F. and J. J. Strain. 1996. The reducing ability of plasma as a measure of antioxidant power- the FRAP assay. Anal. Biochem. 239:70-76.

Brake, J., D. Balnave and J. J. Dibner. 1998. Optimum dietary arginine: lysine ratio for broiler chickens is altered during heat stress in association with changes in intestinal uptake and sodium chloride. Br. Poult. Sci. 39:639-647.

Chamruspollert, G., M. Pesti and R. I. Bakalli. 2004. Influence of temperature on the arginine and methionine requirements of young broiler chicks. J. Appl. Poult. Res. 13:628-638.

Choct, M., A. J. Naylor and N. Reinke. 2004. Selenium supplementation affects broiler growth performance, meat yield and feather coverage. Br. Poult. Sci. 45:677-683.

Donker, R. A., M. G. B. Nieuwland and A. J. Van der Zijpp. 1990. Heat-stress influences on antibody production in chicken lines selected for high and low immune responsiveness. Poult. Sci. 69:599-607.

Gonzalez-Esquerra, R. and S. Leeson. 2005. Effects of acute versus chronic heat stress on broiler response to dietary protein. Poult. Sci. 84:1562-1569.

Grizard, J., D. Dardevet, I. Papet, L. Mosoni, P. Patureau-Mirand and D. Attaix. 1995. Nutrient regulation of skeletal muscle protein metabolism in animals. The involvement of hormones and substrates. Nutr. Res. Rev. 8:67-91.

Hill, F. W. and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587-603.

Jensen, L. S. 1989. Relationship between protein and amino acid requirements of poultry. Proceedings of Georgia Nutrition Conference for Feed Industry. Nov. 8-10. Atlanta, GA.

Jianhua, H., A. Ohtsuka and K. Hayashi. 2000. Selenium influences growth via thyroid hormone status in broiler chickens. Br. J. Nutr. 84:727-732.

Khajali, F., E. Asadi Khashouie, S. K. Dehkordi and M. Hematian. 2008. Production performance and egg quality of Hy-line W36 laying hens fed reduced-protein diets at constant total sulfur amino acids to lysine ratio. J. Appl. Poult. Res. 17:390-397.

Malheiros, R. D., V. M. B. Moraes, A. Collin, G. P. J. Janssens, E. Decuypere and J. Buyse. 2003. Dietary macronutrients, endocrine functioning and intermediary metabolism in broiler chickens: Pair wise substitutions between protein, fat and carbohydrate. Nutr. Res. 23:567-578.

Mallis, R. J., M. J. Hamann, W. Zhao, T. Zhang, S. Hendrich and J. A. Thomas. 2002. Irreversible thiol oxidation in carbonic anhydrase III: protection by S-glutathiolation and detection in aging rats. Biol. Chem. 383:649-662.

Mates, J. M., J. C. Aledo, A. Perez-Gomez, E. Del Valle and M. Segura. 2000. Intrrelationship between oxidative damage and antioxidant enzyme activities: an easy and rapid experimental approach. Biochem. Educ. 28:93-95.

Muramatsu, T. 1990. Nutrition and whole-body protein turnover in the chicken in relation to mammalian species. Nutr. Res. Rev. 3:211-228.

National Research Council. 1994. Nutrient requirements for poultry. 9th Rev. Edi, National Academy Press, Washington, DC.

Ozkan, S., H. Basmacioglu, S. Malayoglu, S. Yalcin, F. Kardas, S. Kosturk, M. Cabuk, G. Oktay, S. Ozdemir, E. Ozdemir and M. Ergul. 2007. Dietary vitamin E (α-toopherol acetate) and selenium supplementation from different sources: performance, ascites-related variables and antioxidant status in broilers reared at low and optimum temperatures. Br. Poult. Sci. 48:580-593.

Payne, R. L. and L. L. Southern. 2005. Changes in glutathione peroxidase and tissue selenium concentrations of broilers after consuming a diet adequate in selenium. Poult. Sci. 84:1268-1276.

Placha, I., R. Borutova, L. Gresakova, V. Petrovic, S. Faix and L. Leng. 2008. Effects of excessive selenium supplementation to diet contaminated with deoxynivalenol on blood phagocytic activity and antioxidative status of broilers. J. Anim. Physiol. Anim. Nutr. 92:1-8.

Rosebrough, R. W., J. P. McMurtry and R. Vasilatos-Younken. 1999. Dietary fat and protein interactions in the broiler. Poult. Sci. 78:992-998.

Sahin, K., N. Sahin, M. Sar and M. F. Gursu. 2002. Effects of vitamins E and A supplementation on lipid peroxidation and concentration of some mineral in broilers reared under heat stress (32°C). Nutr. Res. 22:723-731.

Surai, P. F. 2002. Selenium in poultry nutrition 1. Antioxidant properties deficiency and toxicity. World's Poult. Sci. J. 58: 333-347.

Surai, P. F. and J. E. Dvorska. 2001. Is organic selenium better than inorganic sources? Feed Mix 9:8-10.

Swennen, Q., G. P. Janssens, S. Millet, G. Vansant, E. Decuypere and J. Buyse. 2005. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: endocrine functioning and intermediary metabolism. Poult. Sci. 84:1051-1057.

Upton, J. R., F. W. Edens and P. P. Ferket. 2009. The effects of dietary oxidized fat and selenium source on performance, glutathione peroxidase, and glutathione reductase activity in broiler chickens. J. Appl. Poult. Res. 18:193-202.

Vadhanavikit, S., and H. E. Ganther. 1994. Increased malic enzyme activity in selenium-deficient rat liver. J. Nutr. Biochem. 5:314-316.

Yoon, I., T. M. Werner and J. M. Butler. 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poult. Sci. 86:727-730.