High dietary concentrations of methionine reduce the selenium content, glutathione peroxidase activity and oxidative stability of chicken meat

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ABSTRACT: Three experiments (EXP) were conducted using two hundred seventy male chicks Ross 308 in each (90 cockerels per treatment; 3 replications, 30 chickens per pen) for 42, 38 and 35 days. The basal diets (treatment 1) for three EXPs contained the identical ingredients, and the concentration of selenium (Se), methionine (Met) and total sulphur amino acids (TSAA) in the control diet was 0.11 mg/kg, 5.65 g per kg and 9.25 g/kg, respectively. Chicks in dietary treatment 2 were fed a basal diet supplemented with 0.3 mg/kg of Se (EXP 1, 2 and 3) and in dietary treatment 3 they were fed a basal diet with the addition of 0.3 mg/kg of Se and 1 g/kg (EXP 1 and 2) or 2.5 g/kg of DL-methionine (EXP 3). In EXP 1, sodium selenite and in EXP 2 and 3 Se-enriched yeast were used as sources of supplemental selenium, respectively. The results indicated that the addition of Se or Se and Met into the diet of broilers did not significantly affect the final live weight of chickens and the feed consumption. Moreover, the concentration of Met ($P = 0.004$), Cys ($P = 0.01$) and tyrosine ($P < 0.001$) in breast muscle increased with an increase in dietary Met content, and the isoleucine concentration decreased ($P < 0.001$). Moreover, the addition of inorganic and organic sources of Se increased the Se content of breast meat ($P < 0.001$). On the other hand, the addition of Met decreased the concentration of Se ($P < 0.001$) in breast meat and reduced glutathione peroxidase activity and oxidative stability of raw breast muscle ($P = 0.019$, $P < 0.001$) and breast meat stored for 3 days ($P = 0.016$, $P = 0.006$) in EXP 2 and 3.

Keywords: surplus of methionine; selenium; chicken diet; meat quality; performance; male broiler

Selenium (Se) is a well-known antioxidant, and selenoproteins can catalyse the oxidation of sulphhydryl groups and/or reduction of disulphides. Se is isomorphous with sulphur (Hawkes and Alkan, 2010); thus, many sulphur-containing molecules possess a selenium homologue. Glutathione peroxidase (EC 1.11.1.9; GSH-Px), which plays a crucial role in the cellular antioxidant system (Zhao et al., 2009), is the best-known selenium-based enzyme. Sulphur-containing amino acids are antioxidants (Mosharov et al., 2000) that can control the oxidative status of the cell and are involved in the synthesis of intracellular antioxidants (Wu et al., 2004). Moreover, sulphur-containing amino acids are the precursors of cellular components that alter the oxidative status of the cell and participate in various signalling pathways. For instance, sulphur-based amino acids are the precursors to glutathione, taurine, hydrogen sulphide and sulphates and can be metabolised into hydrogen sulphide (Tang et al., 2010), which strongly increases the activity of enzymes. Methionine has a strong effect on the activity of GSH-Px, glutathione reductase and glutathione transferase (Blaszcyk et al., 2010). Due to the mech-

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anism of sulphur-based amino acid metabolism, changes in the dietary levels of Met and Cys may have a beneficial or deleterious effect on mammals; thus, recommendations for Met and cysteine (Cys) intake must be re-evaluated (Tesseraud et al., 2009). Bunchasak (2009) reviewed the role of dietary Met in poultry nutrition, and Atencio et al. (2004) suggested that the total sulphur amino acid (TSAA) content in the diet of 1 to 20 day-old and 24 to 38 day-old chicks would be 9.07 g/kg and 8.44 g/kg, respectively. However, the aforementioned TSAA requirements of chickens for commercial production are higher than the NRC (1994) recommendations. Han and Baker (1993) demonstrated that the dietary concentration of Met 7 g/kg was not harmful to young broiler chicks fed diets based on maize and soybean meal. In contrast, an increase in the dietary Met content from 5.9 g/kg to 18.6 g/kg reduced the final weight of 4 to 11 day-old chicks by 20% (Acar et al., 2001). The knowledge of interaction effects between Met and Se is limited. In poultry diets based on maize and soybean meal, methionine is the limiting amino acid, and Se is a trace element. Selenomethionine, the Se analogue of Met, is the main form of Se found in plants. Selenium yeast is the common supplement of organic Se in the diet of animals. Selenite, molybdate and sulphate are absorbed by passive diffusion and absorbed selenite can induce antagonistic responses (Underwood and Suttle, 1999). Selenomethionine is absorbed by a sodium transport system that is specific for neutral amino acids and is inhibited by Met. Moreover, inorganic selenium is gradually reduced, resulting in the formation of hydrogen selenide. Thus, prior to incorporation, inorganic Se is transformed into selenosulphate, and selenocysteinyl-tRNAs (from UGA codon) are integrated to form selenocysteines (Se-Cys) (Lobinski et al., 2000). Alternatively, selenomethionine is demethylated and converted into Se-Cys. Selenomethionine, which is used to create selenoproteins, contributes to forming tissues capable of rapid protein synthesis (Schrauzer, 2000). Experiments conducted on rats revealed that methionine supplementation may increase the bioavailability of Se from foods with a low Se content; however, Se-Met bioavailability may not be affected by the higher Se concentrations (Waschulewski and Sunde, 1988). The antioxidant activity of dietary Met and Se is optimal at a specific Met to Se ratio and concentration range. Deviations from the optimal ratio of dietary Met to Se alter GSH-Px activity and deposition of Se in tissues. The results of previous studies revealed that the addition of 0.6 mg of Se/kg to the diet of breeding hens led to egg yolk with lower GSH-Px activity than that of hens fed a diet supplemented with 0 and 0.3 mg of Se/kg (Wang et al., 2010). Moreover, excess dietary Met may lead to the preferential incorporation of

Table 1. Composition of the basal diet[a]

Ingredients	(g/kg)
Wheat	290
Maize	302.2
Soybean meal	320
Fish meal	20
Rapeseed oil	40
Limestone	10
Dicalcium phosphate	9
Sodium chloride	2
Vitamin-mineral premix[b]	5
DL-Methionine	1.8
Analysed chemical composition (EXP 1)[c]	
Dry matter	892
Crude protein	217
Crude fat	63
Crude fibre	34
Calcium	8.4
Phosphorus	5.6
Selenium	11×10^{-5}
AME_N by calculation (MJ/kg)	12.35

[a]experimental diets were supplemented with 0.3 mg/kg of Se and 1 g/kg (EXP 1 and 2) or 2.5 g/kg (EXP 3) of DL-methionine

[b]premix provided 3.8 mg/kg of retinyl acetate, 0.13 mg/kg of cholecalciferol, 50 mg/kg of α-tocopheryl acetate, 3 mg/kg of menadione, 3 mg/kg of thiamine, 6 mg/kg of riboflavin, 4 mg/kg of pyridoxine, 15 μg/kg of cyanocobalamin, 50 mg per kg of niacin, 20 mg/kg of calcium pantothenate, 0.2 mg per kg of biotin, 1.8 mg/kg of folic acid, 250 mg/kg of choline chloride, 17 mg/kg of copper, 50 mg/kg of iron, 1 mg/kg of iodine, 100 mg/kg of manganese, 80 mg/kg of zinc and 0.8 mg/kg of cobalt

[c]dietary concentration of amino acids: Met = 5.65; Cys = 3.60; Thr = 8.07; Val = 9.19; Ile = 8.56; Leu = 15.27; Phe = 10.14; His = 5.13; Lys = 11.50; Arg = 13.63; Gly = 8.62; Tyr = 6.23

Se-Met into the body in a form other than GSH-Px (Zhao et al., 2009). Data concerning this issue in broilers are still missing.

The objective of the present study was to determine the effect of a high dietary concentration of Met (6.7 or 8.2 g/kg) in combination with a limited content of Se (0.4 mg/kg) on the performance of broiler chickens, GSH-Px activity in breast muscle, Se and TBARS content in meat.

MATERIAL AND METHODS

All experimental procedures were approved by the Institute of Animal Science in Prague and the Animal Care and Use Committee. In total, three experiments were performed. Two hundred seventy Ross 308 male chicks were randomly assigned to 3 dietary treatments with 3 replicate pens (30 chickens per pen) in each experiment. The chicks were housed in nine environmentally controlled pens with continuous lighting, and the effects of three different dietary treatments were determined. Water and experimental diets were available *ad libitum*, and the body weight of individual chicks and the feed intake were measured at 0 and 21 days, and on the final day of the experiment. The mortality and health of chickens were examined daily. The basal diet, which contained 0.11 mg/kg of Se, 5.65 g/kg of Met and 9.25 g/kg of TSAA (Table 1), was used as a control (treatment 1) for all three EXPs. Chicks in treatment 2 were fed a basal diet with the addition of 0.3 mg/kg of Se from sodium selenite (EXP 1) or 0.3 mg/kg of Se from Se-enriched yeast (EXP 2 and 3). Broilers in dietary treatments 3 were fed a basal diet supplemented with 0.3 mg/kg of Se and 1 g/kg of DL-methionine (EXP 1 and 2) or 2.5 g of DL-Met (EXP 3). The experiments were terminated at 42 (EXP 1), 38 (EXP 2) and 35 days (EXP 3) of broiler chickens age. The birds were weighed and nine broilers of the mean body weight were selected from each dietary treatment and subjected to a 12 h fasting. Consequently, they were electrically stunned, manually slaughtered, eviscerated, cold-water washed and chilled at 4°C. The carcass, carcass parts and organs were weighed. Breast meat was weighed without bone and leg meat (thigh and drumstick) with bone. Carcass characteristics were expressed as a percentage share of carcass weight.

Analyses

In EXP 1, the concentration of amino acids and Se in breast meat (n = 9 per treatment) was determined. Alternatively, in EXP 2 and 3 (n = 9 per treatment), the dry matter, fat, crude protein, Se and MDA content and GSH-Px activity in breast meat stored in plastic bags at –20°C were evaluated. Dietary selenium concentration and Se content of breast muscle were determined by a fluorometric method (Surai, 1999) with a Millenium Excalibur apparatus (PSAnalytical, England). The activity of GSH-Px in the meat was measured immediately after mincing. A coupled assay based on tert-butyl hydroperoxide was conducted, and the amount of oxidised NADPH was determined by monitoring the absorbance at 340 nm. The activity was expressed in μmol of oxidised NADHP min/g of tissue (DeVore and Greene, 1982). To determine the amino acid content, the samples were homog-

Table 2. Growth traits and concentration of selenium in breast meat (EXP 1)

Characteristics	Basal	SS	SS + Met	SEM	Significance
BW (0 day; g)	43.8	43.5	41.4		
BW (21st day; g)	628.1	638.5	625.2	6.25	NS
BW (42nd day; g)	2669.2	2760.6	2684.0	17.50	NS
F:G (42nd day; g/g)	1.65	1.58	1.59	0.026	NS
Mortality (%)	0	4	4		
Selenium content (μg/kg DM)	391.5^{c}	560.7^{a}	500.5^{b}	18.59	< 0.001

SS = the basal diet was supplemented with sodium selenite (0.3 mg Se/kg)

Met = the basal diet was supplemented with 1 g of DL-methionine/kg

BW = body weight; F:G = feed:gain; DM = dry matter

^{a,b,c} values with different superscripts differ significantly; NS = not significant

Table 3. Concentration of amino acids in breast meat (EXP 1)

Amino acid (AA)		Basal	SS	SS + Met	SEM	Significance
Essential (g/kg DM)	Thr	39.8	38.7	40.2	0.48	NS
	Val	43.6	40.9	39.4	0.87	NS
	Ile	39.1[a]	38.8[a]	35.5[b]	0.43	< 0.001
	Leu	70.5	69.7	68.5	0.51	NS
	Phe	35.5	34.9	34.5	0.24	NS
	His	31.2	30.9	29.5	0.44	NS
	Lys	77.3	75.9	75.3	0.53	NS
	Arg	57.0	53.1	52.4	0.87	NS
	Met	22.8[b]	23.6[b]	26.8[a]	0.55	0.004
Nonessential (g/kg DM)	Cys	14.9[b]	16.0[ab]	17.3[a]	0.33	0.010
	Asp	82.8	81.0	82.0	0.71	NS
	Ser	35.0	34.2	35.3	0.38	NS
	Glu	124.0	123.8	121.8	0.72	NS
	Pro	35.5	37.7	34.5	0.75	NS
	Gly	37.4	36.7	36.9	0.32	NS
	Ala	51.0	49.7	50.9	0.48	NS
	Tyr	29.9[b]	30.5[b]	42.3[a]	1.31	< 0.001
Total		827.4	815.9	823.1	5.09	NS
Σ Essential AA		416.9	406.4	402.1	2.67	NS
Σ Nonessential AA		410.5	409.5	421.0	2.99	NS

SS = the basal diet was supplemented with sodium selenite (0.3 mg Se/kg)

Met = the basal diet was supplemented with 1 g of DL-methionine/kg

DM = dry matter

[a,b] values with different superscripts were significantly different; NS = not significant

enised, and 0.3 g of material was hydrolysed in 6M hydrochloric acid for 23 h at 110°C. The resulting hydrolysate was cooled, filtered and evaporated in a vacuum evaporator at 53°C. The dry residue was dissolved in Na-citric buffer at a pH of 2.2. To determine the concentration of sulphur-containing amino acids, the samples were treated with performic acid prior to protein hydrolysis, and the concentration of amino acids was determined by an automatic Amino Acid Analyser AAA-400 (INGOS Ltd., Prague, Czech Republic) equipped with an Ostion LG ANB ion-exchange column. The fat content in the basal diet and breast meat was determined by extraction with petroleum ether using a Tecator Extraction System 1045 Soxtec (Foss Tecator AB, Höganäs, Sweden), and the crude protein content was evaluated by a Kjeltec Auto 1030 Analyser (Foss Tecator AB, Höganäs, Sweden). Lipid oxidation in breast meat was measured according to the method of Piette and Raymond (1999), and the results were expressed as thiobarbituric acid-reactive substances – TBARS in mg of malondialdehyde – MDA/kg muscle. Prior to analysis, breast meat was thawed in thermostat for 1 h at 18°C (MDA 0) or stored in a refrigerator at 2.5 to 4°C for 3 days (MDA 3).

The results were analysed by an analysis of variance (ANOVA) using the GLM procedure of SAS (SAS Institute Inc., 2003).

RESULTS

The final live weight of the chickens and the relative feed consumption were not significantly affected by the supplementation of Se and Met (Tables 2, 4

Table 4. Growth traits (EXP 2)

Characteristics	Basal	SY	SY + Met	SEM	Significance
BW (0 day; g)	45.9	45.5	45.4		
BW (21st day; g)	692.2[a]	659.7[b]	592.3[c]	7.06	< 0.001
BW (38th day; g)	2381.6	2332.2	2302.7	16.08	NS
F:G (38th day; g/g)	1.72	1.75	1.67	0.029	NS
Mortality (%)	3	4	0		

SY = the basal diet was supplemented with Sel-Plex (0.3 mg of Se/kg)
Met = the basal diet was supplemented with 1 g of DL-methionine/kg
BW = body weight; F:G = feed:gain
[a,b,c] values with different superscripts were significantly different; NS = not significant

Table 5. Carcass characteristics (percentage share in carcass weight) and chemical analysis of breast meat (EXP 2)

Characteristics	Basal	SY	SY + Met	SEM	Significance
Carcass weight (g)	1692.2[a]	1605.0[b]	1593.2[b]	11.19	< 0.001
Breast (%)	23.4	22.8	22.4	0.27	NS
Skin from breast (%)	2.2	2.1	2.1	0.05	NS
Leg (%)	27.2	27.2	27.2	0.19	NS
Skin from leg (%)	3.0	3.1	2.8	0.08	NS
Liver (%)	2.8	2.8	2.7	0.07	NS
Heart (%)	0.7	0.7	0.8	0.02	NS
Gizzard (%)	1.5	1.6	1.5	0.04	NS
Abdominal fat (%)	0.6[b]	0.7[a]	0.7[a]	0.01	< 0.001
Fat (g/kg DM)	24.7	26.6	28.7	1.10	NS
Crude protein (g/kg DM)	916.8	915.3	912.0	1.27	NS

SY = the basal diet was supplemented with Sel-Plex (0.3 mg of Se/kg)
Met = the basal diet was supplemented with 1 g of DL-methionine/kg
DM = dry matter
[a,b] values with different superscripts were significantly different; NS = not significant

Table 6. Selenium content, glutathione peroxidase (GSH-Px) activity and malondialdehyde content in raw (MDA 0) breast meat and breast meat stored for 3 days (MDA 3), (EXP 2)

Characteristics	Basal	SY	SY + Met	SEM	Significance
Selenium content (μg/kg DM)	317.8[c]	982.5[a]	790.9[b]	53.00	< 0.001
GSH-Px (U/g)	0.337[a]	0.334[a]	0.271[b]	0.0107	0.011
MDA 0 (mg/kg)	0.240[b]	0.227[b]	0.294[a]	0.0106	0.019
MDA 3 (mg/kg)	0.329[b]	0.325[b]	0.421[a]	0.0161	0.016

SY = the basal diet was supplemented with Sel-Plex (0.3 mg of Se/kg)
Met = the basal diet was supplemented with 1 g of DL-methionine/kg
DM = dry matter; U = 1 μmol of NADPH oxidised per min/g meat tissue
[a,b,c] values with different superscripts were significantly different

Table 7. Growth traits (EXP 3)

Characteristics	Basal	SY	SY + Met	SEM	Significance
BW (0 day; g)	41.5	41.6	41.1		
BW (21st day; g)	970,2[b]	997,9[ab]	1010,2[a]	6.67	0.043
BW (35th day; g)	2528.4	2517.7	2613.6	18.66	NS
F:G (35th day; g/g)	1.64	1.57	1.52	0.029	NS
Mortality (%)	4[b]	3[c]	8[a]	0.8	< 0.001

SY = the basal diet was supplemented with Sel-Plex (0.3 mg of Se/kg)
Met = the basal diet was supplemented with 2.5 g of DL-methionine/kg
BW = body weight; F:G = feed:gain
[a,b,c] values with different superscripts were significantly different; NS = not significant

and 7). Significantly higher mortality in the second experimental group of EXP 3 was associated with a high growth rate. In particular, rapid growth was observed when the chicks were 3 and 5 weeks of age, and an average weight of 1010 g and 2614 g was reached, respectively. Carcass characteristics (Table 5 and 8) were not significantly affected by the supplementation of Se and Met; however, in experiment 3, 2.5 g/kg of Met and 0.3 mg/kg of Se were supplied, and a reduction in abdominal fat was observed ($P < 0.015$). Moreover, high dietary concentrations of Met increased the content of Met ($P = 0.004$), Cys ($P = 0.01$) and tyrosine ($P < 0.001$) in breast meat and decreased the isoleucine content ($P < 0.001$), Table 3. Moreover, the addition of inorganic and organic sources of Se increased the Se concentration in breast muscle ($P < 0.001$). But Met supplementation significantly ($P < 0.001$) decreased the Se content of muscle (Table 2, 6 and 9), and the oxidative stability and glutathione peroxidase activity of breast meat were also reduced (Table 6 and 9).

DISCUSSION

The Met and TSAA requirements of commercial broiler chickens proposed by the NRC (1994) are

Table 8. Carcass characteristics (percentage share in carcass weight) and chemical analysis of breast meat (EXP 3)

Characteristics	Basal	SY	SY + Met	SEM	Significance
Carcass weight (g)	1780.0[b]	1776.0[b]	1856.0[a]	14.33	0.031
Breast (%)	28.2	27.6	28.7	0.27	NS
Skin from breast (%)	2.1	2.4	2.2	0.06	NS
Leg (%)	26.6	26.2	28.5	0.50	NS
Skin from leg (%)	2.6	2.7	2.5	0.09	NS
Liver (%)	3.2	3.5	3.2	0.11	NS
Heart (%)	0.7	0.6	0.7	0.01	NS
Gizzard (%)	1.5[a]	1.1[b]	1.2[b]	0.05	0.013
Abdominal fat (%)	1.3[a]	1.4[a]	0.8[b]	0.08	0.015
Fat (g/kg DM)	40.1	44.4	47.0	2.59	NS
Crude protein (g/kg DM)	883.5	890.4	890.1	2.48	NS

SY = the basal diet was supplemented with Sel-Plex (0.3 mg of Se/kg)
Met = the basal diet was supplemented with 2.5 g of DL-methionine/kg
DM = dry matter
[a,b] values with different superscripts were significantly different; NS = not significant

Table 9. Selenium content, glutathione peroxidase (GSH-Px) activity and malondialdehyde content of raw breast meat (MDA 0) and breast meat stored for 3 days (MDA 3) (EXP 3)

Characteristics	Basal	SY	SY + Met	SEM	Significance
Selenium content (μg/kg DM)	513.5[c]	882.3[a]	750.7[b]	28.72	< 0.001
GSH-Px (U/g)	0.279[b]	0.333[a]	0.285[b]	0.0062	< 0.001
MDA 0 (mg/kg)	0.219[a]	0.088[b]	0.123[b]	0.0133	< 0.001
MDA 3 (mg/kg)	0.279[b]	0.246[b]	0.331[a]	0.0116	0.006

SY = the basal diet was supplemented with Sel-Plex (0.3 mg of Se/kg)
Met = the basal diet was supplemented with 2.5 g of DL-methionine/kg
DM = dry matter; U = 1 μmol of NADPH oxidised per min/g meat tissue
[a,b,c] values with different superscripts were significantly different

low, and the recommendations are often exceeded. In the present study, the Met and TSAA concentrations of the control treatment were similar to those recommended by Zelenka et al. (2007). The TSAA content of the basal diet was higher approximately by 0.18 g/kg than the recommendations of Atencio et al. (2004) for broiler chickens up to 20 days of age, by 0.25 g/kg higher than the NRC recommendations for chicks up to 21 days of age (1994), 1.05 g/kg lower than that proposed by Zelenka et al. (2007) for chicks up to 10 days of age and by 0.15 g/kg higher than that proposed by Zelenka et al. (2007) for 11 to 27 day-old chicks. The dietary Met concentration used in the control was chosen to reach a small surplus of the limiting amino acid. When 1 g/kg or 2.5 g/kg of Met and inorganic or organic sources of Se were supplied, symptoms of Met and Se imbalance were observed. In particular, the Se content of the muscle and the activity of GSH-Px decreased in all three experiments. Moreover, MDA production increased, and the storage stability of the meat was reduced. However, significant differences in the growth traits of chickens were not observed among treatments, and Met supplementation increased the concentration of Met and Cys in breast muscle.

In contrast, the high dietary concentration of Met (7 g/kg) without Se supplementation was not harmful to broiler chickens in an experiment of Han and Baker (1993). Wang et al. (2009, 2010), Zhao et al. (2009) found out that Met and Se in the maternal diet of hens had a positive effect on the antioxidant capacity of progeny. The synergistic effect of Met and Se on TBARS and on the concentration of Se in muscle was significant only when high concentrations (0.6 mg Se/kg and 5.4 g Met/kg) or low concentrations of Met and Se were added to the maternal diet (0 mg Se/kg and 3.2 g Met/kg). Moreover, synergistic effects of high Met concentrations on GSH-Px activity were observed only in the absence of supplemental Se (Sel-Plex was as a source of Se). This phenomenon was attributed to the incorporation of Se-Met into tissues (or eggs) in a form other than GSH-Px (Zhao et al., 2009).

After demethylation, Se-Met (EXP 2 and 3) from Se yeast is converted into Se-Cys and is incorporated into tissues capable of rapid protein synthesis, as demonstrated by Schrauzer (2000). Similarly, the results of the present study indicated that Se-Met from Se yeast was incorporated into breast meat. However, in both experiments, the addition of Met significantly decreased the Se content of muscle. Moreover, GSH-Px activity was not enhanced; thus, high concentrations of Met may have inhibited GSH-Px. In EXP 3, the dietary concentration of Met was high, and the Se content was moderate. However, a high dietary intake of Met can lead to higher Met catabolism and can disrupt the optimal Met to Se ratio. Moreover, the sulphur present in amino acids or endogenous sulphide ions may affect the production of GSH-Px. Se is isomorphic with sulphur (Hawkes and Alkan, 2010), and both sulphate and selenite are absorbed by passive diffusion and may induce antagonistic reactions (Underwood and Suttle, 1999). Thus, the observed decrease in the amount of Se incorporated into muscle in EXP 1 was likely due to interaction effects between sulphur and Se.

The results of the present study indicate that high dietary concentrations of Met (6.7 g/kg or 8.2 g/kg) and Se concentration of 0.4 mg/kg (from selenite or Se-enriched yeast) reduce Se deposition in muscle, GSH-Px activity and oxidative stability of broiler chicken meat.

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