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Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick

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Abstract 1. The effects of selenium and vitamin E supplementation of the maternal diet on their transfer to the egg yolk and tissues of the newly hatched chick and on the development of the antioxidant system in the chick liver in early postnatal life were investigated.

2. One hundred Cobb broiler breeder hens were divided into 10 equal groups and housed in pens at 25 weeks of age. Each hen received 1 of the treatment diets which included 0·2 or 0·4 mg/kg selenium, 40, 100, 200 mg/kg vitamin E or their combination. After 6 weeks, the hens were artificially inseminated once per week. From week 8, eggs were collected and placed in an incubator. After hatching, chicks from each group were reared (under standard commercial conditions) to 10 d of age. The chicks were fed on a standard starter commercial broiler diet. At the time of hatching, and at 5 and 10 days old, 4 chicks from each group were sacrificed and blood, liver and brain were collected for the subsequent biochemical analyses.

3. The inclusion of organic selenium or vitamin E in the commercial diet significantly increased their concentration in the egg and in the liver of 1-d-old chicks obtained from the eggs enriched with these substances. A positive effect of such dietary supplementation was seen at d 5 and d 10 of postnatal development.

4. There was a positive effect of selenium supplementation of the maternal diet on glutathione concentration in the liver of 1-d-old and 5-d-old chicks. A combination of a dietary selenium supplementation with high vitamin E doses further increased glutathione concentration in the liver. Dietary selenium supplementation significantly increased selenium-dependent glutathione peroxidase (Se-GSH-Px) activity in the liver of the 1-d-old and 5-d-old chicks and decreased liver susceptibility to peroxidation.

6. It is concluded that the nutritional status of the laying hen determines the efficiency of the antioxidant system throughout embryonic and early postnatal development of the offspring.

INTRODUCTION

A substantial amount of the tissue lipids in the developing chick embryo is polyunsaturated (Speake *et al.*, 1998) requiring an effective system of antioxidant defence (Surai, 1999). Tissues of newly hatched chicks express a range of antioxidant defences including natural antioxidants (vitamin E, carotenoids, glutathione, ascorbic acid) and antioxidant enzymes including superoxide dismutase, glutathione peroxidase and catalase (Surai *et al.*, 1999*a*). While it is known that vitamin E and carotenoids are delivered from the maternal diet *via* the yolk and the others are synthesised in the tissues, little is known about the mechanism that regulates the antioxidant system during chick embryo and postnatal development.

A low oxygen pressure in the environment surrounding embryos during development seems to have been retained in the course of evolution to protect the vulnerable developing tissues from the damage caused by the action of reactive oxygen species (Ar and Mover, 1994) since the rate of free radical generation in cells depends on the ambient oxygen concentration (Turrens *et al.*, 1982). However, the increased oxygen tension in the embryonic tissues at hatching requires the protective effect of the natural antioxidants (Surai *et al.*, 1996).

Maternal diet composition is a major determinant of antioxidant system development in the chick during embryogenesis and in early postnatal development (Surai, 1999). Vitamin E is effectively transferred from food into egg yolk (Surai *et al.*, 1998). Our previous observations indicate that an increased vitamin E supplementation of the maternal diet can substantially increase vitamin E concentration in the developing tissues of the chick and significantly decrease their susceptibility to lipid peroxidation (Surai *et al.*, 1999). During the first 2 weeks posthatch, the concentration of vitamin E in the liver decreased by 10 times in chickens (Surai and Ionov, 1994), goslings and ducklings (Surai *et al.*, 1993) and more than 50 times in turkeys (Soto-Salanova *et al.*, 1993). The mechanisms of such changes are not clear. Nevertheless, the accumulation of natural antioxidants in the liver during embryogenesis is considered to be an adaptive mechanism to protect against lipid peroxidation

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during hatching time and early postnatal development (Surai, 1999).

Selenium is another important determinant of antioxidant system efficiency. Glutathione peroxidase plays a central role in antioxidant defence in the cell by removing hydrogen peroxide and lipid hydroperoxides formed during metabolism and superoxide radical dismutation (Jaeschke, 1995) and needs selenium as a cofactor (Combs and Combs, 1984). The selenium content of the egg depends on its concentration in the hen's diet, and also on the form of dietary selenium used, since organic selenium is more efficiently deposited in the egg yolk (Cantor, 1997). There is also an indication that selenium can be transferred to chick embryo tissues from the egg content (Hassan, 1986).

All the elements of the antioxidant system interact with each other, forming an efficient antioxidant defence. This interaction probably starts at the level of nutrient absorption and continues during their metabolism. For example, dietary selenium had a sparing effect on vitamin E, with the result that rats (Scott *et al.*, 1977), chickens (Thompson and Scott, 1970) and ducklings (Dean and Combs, 1981) given selenium supplements had significantly increased plasma vitamin E concentrations.

Data concerning effects of selenium and vitamin E supplementation of the maternal diet on the development of antioxidant system during chick embryo and early postnatal development are not available. Thus, the aim of the present study was to determine the effect of selenium and vitamin E supplementation of the maternal diet on their transfer to the egg yolk and their concentration in the tissues of newly hatched chick and to investigate the effect of increased selenium and vitamin E supply on the activity of Se-GSH-Px in the chick liver in early postnatal development.

MATERIALS AND METHODS

Birds

One hundred Cobb broiler breeder hens were divided into 10 equal groups and housed in pens at 25 weeks of age. Each hen received 1 of the treatment diets (Table 1). Selenium was supplemented in the form of Sel-Plex (Alltech Inc.), containing selenium mainly as selenomethionine. After 6 weeks, the hens were artificially inseminated once *per* week. From week 8, eggs were collected and placed in an incubator. After hatching, chicks from each group were reared separately (under standard commercial conditions) to 10 d of age. The chicks were fed on a standard commercial broiler starter diet (containing 20 mg/kg vitamin E and 0·1 mg/kg selenium). At the time of hatching, and at 5 and 10 days of age, 4 chicks from each group were sacrificed and blood, liver and brain were collected for subsequent analyses.

Analytical procedures

Vitamins A and E were determined by the method of McMurray *et al.* (1980) as described by Surai *et al.*, 1996. In brief, the samples were saponified with ethanolic potassium hydroxide in the presence of pyrogallol and the retinol and tocopherols were extracted from the mixture with hexane. The extract was dried under nitrogen, re-dissolved in methanol and injected into an HPLC system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co with JASCO Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb, type, S30DS2, 3 μ C_{18} reverse phase HPLC column, 15 cm×4·6 mm (Phase Separations, UK). Chromatography was performed using a mobile phase of methanol/water (97:3 v/v) at a flow rate of 1·1 ml/min. Fluorescence detection of retinol involved excitation and emission wavelengths of 330 and 480 nm respectively. The relevant wavelengths for tocopherol detection were 295 and 330 nm. Standard solutions of all-trans retinol and α-tocopherol in methanol were used for instrument calibration and tocol was used as an internal standard.

Carotenoids were determined spectrophotometrically (Surai and Speake, 1998). Two ml of tissue or yolk homogenate (20% in 0·01 M phosphate buffer, pH 7·4) were mixed with 2 ml of ethanol. Hexane (5 ml) was then added and the mixture was shaken vigorously for 5 min. The

Table 1. *Selenium and vitamin E supplementation of the basal diets*[a]

Dietary Group	Diet	Vitamin E (mg/kg)	Selenium (mg/kg)
1	Semi-synthetic	No	No
2	Commercial (CD)	No	No
3	CD	No	0·2
4	CD	No	0·4
5	CD	40	No
6	CD	100	No
7	CD	200	No
8	CD	40	0·2
9	CD	100	0·4

[a] The level of selenium in the semi-synthetic diet was 44 μg/kg and in commercial diet 171 μg/kg. Selenium was supplemented in the form of Sel-Plex. Semi-synthetic and commercial diets contained 4·86 and 10·05 mg/kg α-tocopherol. Both commercial and semi-synthetic diets were balanced in other nutrients.

hexane phase containing the carotenoids was separated by centrifugation and collected. The extraction was repeated twice more with 5 ml hexane. Hexane extracts were combined and carotenoids were determined from absorption at 446 nm using lutein as a calibration standard.

Reduced glutathione was determined using the method of Griffith (1980) by means of the determination of total glutathione following enzymatic recycling with glutathione reductase; oxidised glutathione was determined in the presence of 2-vinylpyridine and reduced glutathione was calculated as the difference between total and oxidised glutathione.

Selenium concentrations were determined using hydride generation atomic absorption spectrometry (Surai *et al.*, 1999*a*). The organic matter in the sample is destroyed by the action of the nitric/perchloric/sulphuric acids. The selenium in the residue is dissolved in 3 M hydrochloric acid and any selenate converted to selenite by gentle heating. The method used a hydride generator, fluorescence detector (Model 10·033), autosampler all from (Model 20·099) P.S. Analytical Ltd and Avalon software.

For GSH-Px determination, tissue samples were washed in potassium phosphate buffer (10 mM, pH7·4) at 4°C, homogenised in 9 volumes of the same buffer, supplemented with 30 mM potassium chloride as described by Wilson *et al.* (1992). The homogenate was centrifuged (3500 *g* for 30 min at 4°C) and the enzyme activities of the supernatant were determined.

Selenium-dependent GSH-Px activity was measured by a coupled reaction with excess glutathione reductase, monitoring the NADPH oxidation at 340 nm using hydrogen peroxide as a substrate (St Clair and Chow, 1996). Units of glutathione peroxidase activity were expressed as μmol NADPH oxidised/min/g fresh tissue.

Tissue susceptibility to lipid peroxidation was determined as previously described (Surai *et al.*, 1996). Tissue homogenates (10% w/v), in sodium phosphate buffer, 10 mM, pH 7·4, containing 1·15% (w/v) potassium chloride, were prepared and incubated at 37°C for 60 min in the presence of 0·1 mM ferrous sulphate under air with gentle shaking. At the end of the incubation, butylated hydroxytoluene was added to a concentration of 0·01% (v/v). The accumulation of malondialdehyde was determined by HPLC, (Halliwell and Chirico, 1993, with some modification). In brief, after incubation of tissue homogenate (1 ml) at 37°C, 0·2 ml of sodium dodecyl sulphate (8%) was added and samples were vortexed. Then, 1·5 ml of 20% acetic acid at pH 3·5 (adjusted by potassium hydroxide) and 1·5 ml of thiobarbituric acid (TBA, 0·8% in water) were added, samples were vortexed and incubated at 95°C for 60 min. After cooling, an aliquot of sample was mixed with methanol and centrifuged at 5000 rpm for 10 min. The clear supernatant was transferred to the vial and 5 μl of the solution was injected onto the 3 μ C_{18} reverse phase HPLC column (Phenomenex, Sphereclone ODS2, 100×3·2 mm, Cheshire). Separation of thiobarbituric acid-malondialdehyde adduct from other chromogens was achieved using a mobile phase of 50 mM phosphate buffer (pH 7·0) mixed with methanol in proportion 65:35 by volume and fluorescence detection with excitation at 515 and emission at 553 nm respectively. Results were expressed as μg malondialdehyde/g fresh tissue. 1,1,3,3-tetramethoxypropane was used as a standard for malondialdehyde determination.

Statistical analyses

Results are presented as mean (± se) of measurements on tissue from 4 replicate egg yolks or chicks. Statistical analysis was performed by 1-way ANOVA and *t* test.

RESULTS

As can be seen from Table 2 the inclusion of organic selenium in the commercial diet significantly ($P<0·01$) increased the selenium concentration in both the yolk and the albumen of the eggs. While there was a dose dependent response, the amount of selenium accumulated in individual eggs was variable and presumably reflects some features of selenium metabolism in laying hens. Selenium accumulated in the egg can be transferred to the developing embryo. As can be seen from Table 2, selenium concentration in the liver of 1-d-old chicks obtained from the eggs enriched with selenium was

Table 2. *Selenium concentration in eggs and in tissues of 1-d-old chicks, ng/g fresh tissue*

Group	Egg yolk	Egg albumen	Liver	Brain
1	190·7±25·6	39·7±7·2	323·0±65·8	190·7±20·4
2	298·3±35·4	50·7±8·1	382·3±71·6	196·7±31·9
3	605·3±23·95**	193·7±18·8**	733·3±43·6*	298·7±56·2
4	854·0±117·1**	403·7±22·2***	1399·7±249·8*	342·3±59·7
5	292·7±52·2	53·7±11·3	506·7±77·2	187·7±10·2
6	216·3±21·2	60·7±8·8	452·0±50·3	185·7±18·3
7	237·0±41·79	57·3±7·5	487·0±33·3	181·0±6·0
8	535·3±70·6*	213·0±42·0*	660·0±61·9*	270·7±18·7
9	656·0±78·5*	274·7±57·7*	1055·3±69·3**	337·3±5·2*

Values are mean±SEM (n=4). Significance compared to group 2: ***$P<0·001$; **$P<0·01$; *$P<0·05$.

Table 3. *Vitamin concentrations in the egg yolk and yolk sac membrane of old chicks, μg/g fresh tissue*

Group	Vitamin E		Vitamin A		Carotenoids	
	Egg yolk	Yolk sac membrane	Egg Yolk	Yolk sac membrane	Egg yolk	Yolk sac membrane
1	13·26±1·04**	73·1±8·2*	6·16±0·54	22·1±2·8	32·18±1·57*	44·2±3·6**
2	19·57±0·97	105·1±9·8	6·33±0·53	19·3±3·5	24·13±1·41	23·2±1·3
3	32·2±2·09**	122·2±4·7	5·96±0·46	18·8±2·0	22·18±1·34	25·2±1·7
4	45·51±4·0***	161·2±9·7**	6·11±0·34	20·3±2·1	26·17±2·07	28·4±2·8
5	153·32±15·15***	296·7±20·2***	5·65±0·26	20·0±2·2	22·11±3·44	20·2±1·2
6	298·97±13·01***	1022·1±133·7***	5·88±0·65	22·1±3·1	20·88±1·58	21·1±2·1
7	538·5±28·82***	1924·3±236·6***	6·57±0·37	23·7±2·7	19·66±2·77	22·2±1·6
8	188·22±17·40***	388·1±29·1***	6·16±0·45	21·2±1·8	24·11±2·44	23·2±1·8
9	308·93±18·44***	1123·2±138·3***	5·81±0·57	22·02±2·5	23·66±5·18	22·7±2·7

See Table 2.

significantly (P<0·05) higher than in those from the control eggs. The combination of selenium with high vitamin E doses did not enhance selenium accumulation. There was a trend in selenium accumulation in the chick brain depending on the selenium level in the diet which did not reach significance and only a combination of 0·4 mg/kg selenium with 100 mg/kg vitamin E significantly (P<0·05) increased selenium concentration in the brain.

Vitamin E accumulation in the egg yolk reflected its concentration in the breeder diet (Table 3). Inclusion of organic selenium in the maternal diet significantly increased the vitamin E level in the yolk. A combination of selenium and increased vitamin E supplementation did not further increase vitamin E accumulation in the egg yolk. Vitamin E and selenium supplementation did not have any effect on vitamin A and carotenoid concentration in the egg yolk (Table 3). The exception was group 1 (P<0·05), the semi-synthetic diet, which gave significantly higher yolk carotenoid concentration than in group 2.

The vitamin E concentration in the yolk sac membrane of 1-d-old chicks reflected its level in the egg yolk and there was a significant increase in vitamin E concentrations in this tissue in groups 5 to 9 compared to group 2 (Table 3). There was also a positive effect (P<0·05) of the highest dose of selenium supplementation (0·4 mg/kg) on the level of vitamin E in the yolk sac membrane. There were no significant differences in vitamin A and carotenoid concentrations between the groups except for group 1.

Vitamin E in the liver, plasma (Table 4) and brain (Table 5) of 1-d-old chicks also reflected its concentration in egg yolk. Again there was a positive effect (P<0.05) of selenium supplementation of the maternal diet on concentrations of vitamin E in the liver, brain and blood plasma of d-old chicks. There were no significant differences in vitamin A and carotenoid contents in the liver and plasma of 1-d-old chicks (data not shown). The exception was in group 1, where carotenoid concentration in the liver was significantly (P<0·01) higher than in other chick groups.

There was a substantial decrease in vitamin E concentration between d1 and 10 of posthatch development. A positive effect of sclenium and vitamin E supplementation of the maternal diet was seen at d 5 and d 10 of the postnatal development when vitamin E concentrations in the liver and plasma were significantly higher than those in the control group. Moreover, increased vitamin E concentrations in the chicken liver and plasma at d 1 and d5 of postnatal development were associated with a significant increase in vitamin E concentration in the brain (Table 5).

There was a positive effect of selenium supplementation of the maternal diet on the reduced glutathione concentration in the liver of 1-d-old and 5-d-old chicks (P<0·01) (Table 6). A combination of

Table 4. *Vitamin E concentration in chick liver and plasma*

Group	1-d-old		5-d-old		10-d-old	
	Liver, μg/g	Plasma, μg/ml	Liver, μg/g	Plasma, μg/ml	Liver, μg/g	Plasma, μg/ml
1	88·1±3·8*	6·19±0·45	29·3±5·7	5·02±0·45*	10·5±0·60*	7·21±0·63
2	119·9±9·1	8·17±0·46	38·9±3·7	6·54±0·28	12·3±0·36	7·59±0·44
3	144·2±3·4*	9·86±0·59*	56·2±2·7**	8·21±0·34**	15·1±0·65**	9·66±0·29**
4	166·1±9·9*	10·2±0·49*	67·5±5·1**	8·41±0·44*	16·8±1·2*	10·5±0·43**
5	398·4±27·0***	32·9±3·02***	69·6±3·0	10·6±0·38***	19·2±2·5*	11·5±0·86**
6	947·3±25·4***	44·7±1·98***	99·0±5·8***	13·6±0·34***	23·6±1·7***	12·1±0·78**
7	1636·4±75·0***	78·7±6·47***	125·2±1·7***	18·5±1·0***	30·3±2·5***	15·3±1·5**
8	522·17±35·4***	42·2±2·42***	88·3±5·2***	14·5±0·79***	22·4±1·8***	12·6±1·3**
9	950·4±35·4***	82·1±8·77***	113·4±6·6***	25·5±1·84***	25·2±1·7***	14·2±0·73**

See Table 2.

Table 5. *Vitamin E in the brain of chicks, μg/g fresh tissue*

Group	1-d-old	5-d-old
1	1·06±0·1*	0·83±0·10
2	1·47±0·1	1·06±0·10
3	1·91±0·13*	1·22±0·10
4	1·89±0·06**	1·44±0·17
5	5·19±0·48***	4·06±0·14***
6	8·33±0·74***	6·33±0·36***
7	15·22±0·77***	13·7±0·76***
8	6·11±0·44***	4·34±0·39***
9	9·26±0·41***	6·41±0·27***

See Table 2.

Table 6. *Glutathione in the liver of the chicks, μg/g fresh tissue*

Group	1-d-old	5-d-old
1	424·3±14·9	488·3±14·9
2	482·9±25·8	563·1±25·8
3	667·6±30·8**	691·1±30·8*
4	696·5±24·4**	744·2±24·5**
5	542·0±46·4	661·4±46·4
6	592·1±30·6	693·1±30·6*
7	631·2±40·8*	721·1±40·8*
8	648·0±54·1*	741·1±54·1*
9	846·6±41·9***	922·1±41·9***

See Table 2.

a dietary selenium supplementation with high vitamin E doses further increased glutathione concentration in the liver. The highest vitamin E dose (200 mg/kg) itself had a positive effect on glutathione concentration in the liver ($P<0·05$).

GSH-Px activity in the liver of newly hatched chicks depended on selenium supplementation and selenium concentration in the tissue (Table 7). Dietary selenium supplementation significantly ($P<0·01$) increased Se-GSH-Px activity in the liver of the d-old chicks. On the other hand, a low-selenium semi-synthetic diet caused a significant ($P<0·05$) decrease in the activity of Se-GSH-Px in the liver. There was no significant difference in Se-GSH-Px activity between 2 degrees of selenium supplementation (0·2 or 0·4 mg/kg). GSH-Px activity in the liver of 5-d-old chicks in groups 3, 4, 8 and 9 was significantly higher than in the control group. At the same time, in group 1, GSH-Px activity in the liver was significantly lower ($P<0·05$) than in the control group. There was no effect of selenium in maternal diet on GSH-Px activity in the chick liver at day 10.

Table 7. *Selenium-dependent glutathione peroxidose (Se-GSH-Px) activity in the chick liver, U/g fresh tissue*

Group	1-d-old	5-d-old	10-d-old
1	10·3±1·1*	16·7±1·1*	29·6±2·4
2	15·8±1·2	22·2±1·8	31·1±1·7
3	24·5±1·6**	29·0±1·3*	37·2±1·5
4	27·1±2·0**	33·2±1·3**	35·2±2·7
5	13·7±1·2	21·4±1·2	32·1±2·0
6	14·7±1·5	22·2±1·2	31·2±2·2
7	15·0±1·4	20·8±1·2	32·1±3·2
8	26·3±4·0*	30·2±2·0*	36·1±2·1
9	29·1±1·8***	30·8±2·0*	37·2±2·3

See Table 2.

Malondialdehyde accumulation in the liver as a result of Fe-stimulated lipid peroxidation is presented in Table 8. Tissue susceptibility to peroxidation significantly decreased between d 1 and 5 of development and then remained almost constant. Malondialdehyde accumulation in the liver of 1-d-old and 5-d-old chicks from groups 3 to 9 was significantly lower than in the control group. In the liver of chickens from groups 7 and 8 this significant difference was maintained up to d 10 of development.

DISCUSSION

The importance of selenium in poultry nutrition is associated with its participation in maintaining the antioxidant system of the cells. In the chicken, selenium deficiency causes exudative diathesis (Noguchi *et al.*, 1973) and pancreatic fibrosis (Cantor *et al.*, 1975) and in both cases lipid peroxidation is probably a major factor (Fraga *et al.*, 1987). For example, nutritional pancreatic atrophy in chicks may be overcome by feeding vitamin E at 15 to 20-fold excess over the levels normally regarded as nutritionally required (Whitacre *et al.*, 1987). It is also interesting to note that exudative diathesis was observed at hatching, indicating that the deficiency lesions had developed during the embryonic period (Hassan *et al.*, 1990).

Selenium concentration in the egg yolk and in the liver and brain of the newly hatched chicks depends on selenium dietary provision to the hen. However, there was a high individual selenium variation in the yolk and albumen. This may account in part for the lack of significant difference in selenium level in yolk and albumen between the groups fed on the semi-synthetic and commercial diets (Groups 1 and 2) even though the level of selenium in the diet differed substantially. It is also possible that the 8 week period on the semi-synthetic diet was not sufficient to deplete completely the selenium reserves in the hen's body. Selenium availability in foodstuffs depends on many factors and varies considerably (Combs and Combs, 1986). These factors include the amount and chemical form of the element ingested with food, solubilisation within the intestine, the physiological state of the organism, interactions

Table 8. *Malondialdehyde accumulation in the liver of chicks, μg/g fresh tissue*

Group	1-d-old	5-d-old	10-d-old
1	24·9±2·2	12·4±1·8	9·33±0·81
2	22·7±1·5	10·1±0·80	9·10±1·15
3	16·4±1·4*	6·49±0·95*	7·22±0·91
4	14·2±1·5**	6·15±0·93*	7·14±0·84
5	15·5±2·4*	6·22±1·1*	6·35±0·94
6	13·1±1·5**	5·14±0·70**	6·05±0·76
7	10·1±1·2***	5·22±0·70**	5·44±0·72*
8	12·1±2·3***	5·31±0·96**	5·66±0·52*
9	9·8±1·0***	5·12±0·43**	5·88±0·85

See Table 2.

with other elements, diseases, drug administration and age (Wolfram, 1999). In this respect the supplementation form of selenium used in this study (Sel-Plex) is characterised by high bio-availability in different animal species (Mahan, 1999, Yoshida *et al.*, 1999).

There is a tissue specificity in selenium transfer from egg to the embryo. For example, in contrast to the liver, there was only a trend (which did not reach significance) for selenium accumulation in the brain of chickens hatched from eggs enriched in selenium. Compared to other tissues, the brain is considered to be more resistant to compositional manipulation (Meydani *et al.*, 1988; Vatassery *et al.*, 1984). In general, there is a lack of information available on mechanisms of selenium delivery from the liver to peripheral tissues. Recently a selenium-binding protein from hepatic tissues of chick embryo has been isolated with an approximate Mr of 56 kD and rich in sulphydryl groups (Padmaja *et al.*, 1996).

This study has shown that the effect of selenium is still apparent at 5 and 10 d of postnatal development. This finding suggests that selenium accumulated in the liver of newly hatched chicks is actively used during the 1st days of postnatal development. It may well be that absorption is not sufficiently effective just after hatching and the chick relies on the reserves of the element accumulated during embryogenesis. Selenium is vital for chickens, as an essential component of Se-dependent GSH-Px, an enzyme that reduces peroxides and protects cells against the damaging effects of oxidation.

The most striking finding of this work was the 'sparing' effect of selenium on vitamin E metabolism and transfer to the egg yolk and the developing tissues. For example, an inclusion of organic selenium in the breeder diet significantly ($P<0{\cdot}05$) increased vitamin E concentration in egg yolk (Table 3). This is in agreement with previous work indicating an increased vitamin E level in the plasma of rats, chicken and ducklings as a result of selenium supplementation (Scott *et al.*, 1977; Thompson and Scott, 1970; Dean and Combs, 1981). The mechanisms for this sparing are not clear. For example the effect could be related to selenium antioxidant properties. It suggests that selenium as a component of glutathione peroxidase actively participates in lipid peroxide removal from the cell and therefore less vitamin E is used for a similar purpose. We can also speculate that selenium can have an effect on other aspects of vitamin E metabolism and transport to target tissues. For example, vitamin E is metabolised more rapidly in selenium deficient rats than in supplemented ones (Fisher & Whanger, 1977).

An increased vitamin E concentration in the yolk was associated with its accumulation in the liver of the newly hatched chick, in agreement with our observations (Surai, 1999; Surai *et al.*, 1997, 1999). The efficiency of vitamin E transfer from egg yolk to the liver of the developing embryo depends on the initial vitamin E concentration in the egg. For example, in groups 1 and 2, which were fed on a low vitamin E diet, about 30% of total egg yolk vitamin E was found in the liver of newly hatched chicks. In contrast, dietary vitamin E supplementation (40 to 200 mg/kg) decreased this efficiency by half (13·0% to 15·8%). These results indicate that in the developing embryo presumably there are metabolic mechanisms responsible for an increased vitamin E mobilisation from the egg yolk in the case of low vitamin E provision.

Considering vitamin E in the liver of chicks during postnatal development it is necessary to underline that its concentration substantially decreases during the first 10d of posthatch life. This is in agreement with our previous observations with chickens, turkey poults, goslings and ducklings (Surai *et al.*, 1998) and with other data obtained from turkey poults (Soto-Salanova, 1998; Soto-Salanova and Sell, 1995, 1996; Soto-Salanova *et al.*, 1993). Selenium supplementation of the maternal diet increased the vitamin E level in the liver and plasma of 1-d-old chicks and this difference was maintained during 10 d of postnatal development. An increased vitamin E supplementation of the maternal diet was even more effective, delaying vitamin E depletion not only in the liver but in the brain as well. These data explain why it is difficult to produce symptoms of vitamin E and selenium deficiency in chicken during postnatal development if the maternal diet contains sufficient vitamin E (Hassan *et al.*, 1990); maternal diets low in vitamin E and selenium are used to study exudative diathesis in postnatal chicks (Bartholomew *et al.*, 1998).

Another important finding in this study was the beneficial effect of selenium supplementation on the level of reduced glutathione in the liver of newly hatched chicks ($P<0{\cdot}01$). The highest vitamin E dose in the maternal diet increased the concentration of glutathione in the liver of newly hatched chicks as well. Similar results were obtained with rats fed on a high level of vitamin E (Scott *et al.*, 1977; Lii *et al.*, 1998). Glutathione is considered to be one of the most important water soluble antioxidants in the cell (Sastre *et al.*, 1996; Bains and Shaw, 1997) and its elevated concentration can be considered as an indication of an increased antioxidative protection of the tissues.

In chicken tissues, a balance between pro-oxidative and antioxidative activities plays a crucial role in development and chick viability. Natural antioxidants interact with each other to build the antioxidant system of the cell which protects the cell from the detrimental effect of free radicals and the toxic effect of products of lipid peroxidation (Surai, 1999). Because our data showed the protective effect of vitamin E against lipid peroxidation during embryo development and in postnatal life, and the possibility of enrichment of egg yolk and embryonic tissues by selenium and vitamin E,

an optimal supplementation of the maternal breeder diet by these antioxidants should be considered and investigated.

An understanding of the important role of vitamin E in the maternal diet and its possible beneficial effect on newly hatched chicks was probably a driving force for increasing the recommended level of vitamin E supplementation for broiler breeders from 28 mg/kg in 1988 up to 100 mg/kg in 1995 (Surai, 1999). Similarly, evidence is accumulating to support the hypothesis that the vitamin E status of turkey poults may be inadequate during the first 3 weeks after hatching (Sell, 1996) and there should be a reconsideration of vitamin E supplementation of turkey breeder diets as well.

GSH-Px activity in the liver of d-old chicks depends on the selenium of the maternal diet. A low-selenium diet was associated with decreased concentrations in the egg yolk and as a result Se-GSH-Px activity in the liver of newly hatched chicks significantly decreased. Similarly, chicks produced from hens fed a low selenium and low vitamin E diet had low activities of GSH-Px in plasma and pancreas at hatching (Bunk and Combs, 1981). On the other hand, selenium dietary supplementation increased Se-GSH-Px activity in the liver (Table 7) and pancreas (Bunk and Combs, 1981). An efficient carry-over of selenium and vitamin E from hens to their progeny was accompanied by a significant increase in muscle selenium, liver GSH-Px activity and vitamin E content at hatching (Hassan *et al.*, 1990). There was no difference in Se-GSH-Px activity in the liver in a response to further increase in selenium supplementation (from 0·2 to 0·4 mg/kg), which probably means that inclusion of 0·2 mg/kg selenium in the maternal diet provides sufficient to the egg and embryonic tissues to meet the requirement for maximum Se-GSH-Px activity. Inclusion of 0·3 mg/kg selenium from baker's yeast in diets for chickens from hatching to d 35 significantly increased GSH-Px activity in erythrocytes, plasma and liver (Arai *et al.*, 1994). GSH-Px activity in the liver increased throughout embryonic development, reaching its maximum at time of hatching (Surai, 1999*a*). In the liver of the newly hatched chick, Se-dependent GSH-Px is the major form of the enzyme, comprising about 61% of total activity (Surai *et al.*, 1999*a*). In the majority of the tissues of the newly hatched chick there was a highly significant correlation between selenium concentration and Se-GSH-Px activity (Surai *et al.*, 1999*a*). It is interesting that in chicken liver about 28% of GSH-Px activity is represented by the monomeric form of the enzyme (Miyazaki and Motoi, 1992). It has been suggested that the effect of selenium on the activity of GSH-Px is achieved through pretranslational mechanisms, including Se-GSH-Px gene expression and cytosolic mRNA stabilisation (Christensen and Burgener, 1992) and dietary selenium can also regulate the level of GSH-Px mRNA at the post-transcriptional stage (Toyoda *et al.*, 1990); therefore, GSH-Px mRNA is a primary target of the selenium regulatory mechanism (Weiss *et al.*, 1997).

There was no significant difference in GSH-Px activity in the liver of d-old chicks between 0·2 and 0·4 mg/kg selenium supplementation of the maternal diet. On the other hand, 0·4 mg/kg gave more protection against peroxidation due to higher levels of vitamin E and glutathione in the liver of 1-d-old and 5-d-old chicks compared to 0·2 mg/kg selenium supplementation. Since the process of selenium transfer from food to egg yolk, and subsequently to embryonic tissues, has received limited attention (Cantor, 1997), there is no clear answer as to which level of selenium supplementation is optimal for broiler breeders and more research is needed. Recently, it has been shown that supplementation at 0·4 mg/kg for White Leghorn type chickens reduced death or lesions from *E. coli* or sheep erythrocyte antigen challenge from 86% to 21% and dietary additions of selenium between 0·1 and 0·8 mg/kg resulted in a substantial (77%) antibody titre increase in chickens (Larsen *et al.*, 1997).

As can be seen from the data shown in Table 8, liver susceptibility to lipid peroxidation substantially decreased in postnatal development despite decreasing vitamin E and carotenoid concentration. This can be explained as a result of increased concentration of glutathione (Table 6) and GSH-Px activity (Table 7) as well as of lipid composition changes (Noble and Cocchi, 1990).

The major finding of this work is a beneficial effect of supplementation of the maternal diet with selenium and vitamin E on the antioxidant protection of the neonatal chick which is maintained well into postnatal development. The data clearly indicate that nutritional status of the laying hen determines the efficiency of the antioxidant system throughout early postnatal development of the offspring. An optimal antioxidant status of the newly hatched chick is an effective means for their protection against damaging effects of free radicals and products of their metabolism. Since natural antioxidants determine the redox potential of the cell, which is responsible for gene regulation (Primiano *et al.*, 1997), further research is needed to elucidate mechanisms for relationships between antioxidant compounds in the diet and their accumulation in the tissues and interactions with antioxidant enzymes.

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