British Poultry Science (1999) **40:** 406–410

Relationship between vitamin E content and susceptibility to lipid peroxidation in tissues of the newly hatched chick

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Abstract 1. The effect of supplementing the diet of the parent hen with vitamin E on the vitamin E content of the yolk and of embryonic and neonatal tissues was evaluated and the effects of elevated tissue concentrations of vitamin E on peroxidation susceptibility was examined.

2. Laying hens (Ross 1 broiler-breeder strain) were maintained on diets containing either 147 (control diet) or 365 (high vitamin E diet) μg vitamin E/g feed.

3. In the day-16 embryo, the concentrations of of vitamin E in the yolk sac membrane, liver, brain and lung were respectively 5·0, 4·3, 1·7 and 5·6 times greater for those derived from the hens on the high vitamin E diet compared with those from the control group.

4. In the day-old chick, the concentrations of vitamin E in the yolk sac membrane, liver, brain and lung were respectively 14·8, 2·8, 3·0 and 5·1 times greater for those derived from hens on the high vitamin E diet compared with those from the control group.

5. Homogenates of tissues from the day-old chick were incubated in the absence and presence of Fe^{2+} in order to determine the extent of spontaneous and iron-stimulated peroxidation as measured by the generation of thiobarbituric acid reacting substances. For the chicks derived from hens on the control diet, the brain was markedly more susceptible to both spontaneous and iron-stimulated peroxidation than were the other tissues. Tissues from the chicks derived from the hens on the high vitamin E diet exhibited significantly reduced susceptibilities to peroxidation. In particular, the susceptibility of the brain was reduced to the same level as that of the other tissues.

6. It is concluded that the high peroxidative susceptibility of the chick's brain can be normalised by supplementation of the parent hen with vitamin E.

INTRODUCTION

During the development of the chick embryo, the differentiating tissues acquire characteristic lipid profiles which, in many cases, are distinguished by very high proportions of polyunsaturated fatty acids (Noble and Cocchi, 1990; Speake *et al.*, 1993; Maldjian *et al.*, 1996). Although such fatty acids are believed to play crucial roles in the functional development of certain embryonic tissues (Neuringer *et al.*, 1988), their highly polyunsaturated structures are extremely susceptible to free-radical induced peroxidative damage (De Man, 1992). Moreover, the risk of lipid peroxidation is likely to be enhanced by the high rates of oxidative metabolism displayed by various embryonic tissues (Noble and Cocchi, 1990) and also by the increasing rate of oxygen uptake across the shell as development proceeds (Freeman and Vince, 1974). The hatching process may be particularly hazardous because the emergent chick not only becomes exposed to atmospheric oxygen but also undergoes a further dramatic increase in metabolic rate (Freeman and Vince, 1974). Thus it would appear that in the newly hatched chick metabolic conditions which favour the generation of reactive oxygen species coincide with the attainment of a highly polyunsaturated lipid profile in sensitive tissues such as the brain and retina.

Defence against peroxidation in the avian embryo is provided by the concerted action of a range of antioxidant components (Wilson, 1990; Surai *et al.*, 1996). Primary amongst these is vitamin E, a lipid-soluble antioxidant which converts fatty acid peroxyl radicals to their less reactive hydroperoxides and, thereby, curtails the chain reaction of peroxidation (MacPherson, 1994). One consequence of inadequate provision of vitamin E to the chick embryo is the development of encephalomalacia from peroxidative damage to the cerebellum area of the brain (Mezes *et al.*, 1997). The aim of the present work was to increase the vitamin E concentration in sensitive tissues (brain and lung) of the embryo by supplementation of the diet of the parent hen and to assess any resultant changes in the susceptibility of these tissues to peroxidation. Previous studies have reported the effects of dietary vitamin E supplementation on the concentration of this vitamin in chicken tissues (Sheehy *et al.*, 1991; Cherian *et al.*, 1996), egg yolk (Cherian *et al.*, 1996; Qi and Sim, 1998; Jiang *et al.*, 1994) and the newly hatched chick (Cherian and Sim, 1997).

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Accepted for publication 19th January 1999.

ISSN 0007-1668 (print)/ISSN 1466-1799 (online)/99/030406–05

MATERIALS AND METHODS

Birds

Laying hens of the Ross 1 broiler-breeder strain were provided *ad libitum* with a proprietary breeder's wheat barley-based diet supplemented with maize oil (50 g/kg). This basal diet contained 115 μg vitamin E/g of which about 80% was derived from the maize oil; this vitamin E was mainly present as α-tocopherol and α-tocotrienol. The diets were further supplemented with α-tocopherol acetate to provide a concentration of either 30 μg/g (control diet) or 250 μg/g (high vitamin E diet). The quantities of α-tocopherol (the sum of that present naturally in the dietary constituents plus the α-tocopherol acetate supplement) were 65 μg/g in the control diet and 285 μg/g in the supplemented diet. The hens (10 per group) which were provided with these diets from 30 weeks of age were fertilised by artificial insemination (Kelso *et al.*, 1997). The fertile eggs laid by the hens over 7 d from 35 weeks of age were collected. Five eggs from each dietary group were used to determine the vitamin E content of the yolks. The remaining eggs were incubated at 37·8°C and 60% relative humidity in a forced draught incubator with automatic egg turning. Tissues from day 16 embryos and from 1-d-old chicks were obtained and washed rapidly in an aqueous solution (9 g/kg) of sodium chloride at 4°C, blotted with filter paper and weighed. The yolk sac membrane samples were washed particularly thoroughly to ensure that all adherent yolk was removed.

Analytical procedures

The vitamin E content of the diets, yolks and tissues were determined using the method of Gaal *et al.* (1995). In brief, the samples were saponified with ethanolic potassium hydroxide in the presence of pyrogallol and the vitamin E was extracted from the mixture with petroleum spirit. The extract was dried under nitrogen, re-dissolved in methanol and injected on to a Spherisorb S30DS2, 3 μ C_{18} reverse phase HPLC column, 150 mm×4.6 mm (Phase Separations, Clwyd, 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 utilised excitation and emission wavelengths of 295 and 330 nm, respectively. The different isoforms of vitamin E (α-tocopherol, β+γ tocopherol, α-tocotrienol, β+γ tocotrienol) were identified by comparison with the retention times of standard tocopherols (Sigma, Poole, UK) and tocotrienols (Merck, Darmstadt, Germany). Between 80% and 90% of the total vitamin E of yolks and tissues consisted of α-tocopherol. Values for total vitamin E (the sum of the different isoforms) are presented.

In order to measure susceptibility to lipid peroxidation, yolk and tissue samples were homogenised in 9 volumes of sodium phosphate buffer, 10 mM, pH 7·4, containing 11·5 g/l potassium chloride. Homogenates were incubated for 1 h at 37°C with gentle shaking in the presence or absence of 0·1 mM ferrous sulphate. At the end of the incubation, butylated hydroxytoluene was added to a concentration of 0·1 ml/l. Thiobarbituric acid reacting substances (TBARS) were determined spectrophotometrically according to the method of Ohkawa *et al.* (1979).

Statistical analysis

Results are presented as the means (± SE) of measurements on 5 replicate samples. Statistical analyses were performed by Student's *t* test.

RESULTS

The hens on the vitamin E-supplemented diet produced eggs which were substantially enriched with the vitamin (Table 1). Thus, the concentration of vitamin E in the yolk achieved by the dietary supplementation was 3·8 times greater than that observed in eggs from control birds. This enhancement was reflected to various extents in the amounts of vitamin E accumulated by the developing tissues of the chick (Table 1). Thus the concentration of vitamin E in the liver of the 16-d-old embryo was 4·3 times greater in the supplemented group than in the controls. Similarly, the amount of the vitamin in the lung of the day-16-old embryo was increased 5·6 times by dietary supplementation. The brain, at this stage, appeared less responsive, with only a 1·6 times increase over the control values. However, in the day-old chick, the concentration of vitamin E in the brain was 3 times higher in those derived from the supplemented group compared with the appropriate controls. The yolk sac membrane of the day-old chicks from the supplemented group contained exceptionally high concentrations of vitamin E.

Lipid peroxidation in tissue homogenates from the day-old chick was assessed by the generation of TBARS during incubation in the absence and presence of ferrous sulphate (Table 2). The rate of spontaneous peroxidation in the absence of exogenous iron was relatively low in homogenates of initial yolk, yolk sac membrane, liver and lung from the control group. A dramatic exception to this pattern was provided by the brain samples of the day-old control birds which exhibited much higher rates of spontaneous peroxidation. Tissue homogenates from chicks derived from vitamin E-supplemented hens displayed significantly reduced susceptibilities to spontaneous peroxidation compared with the controls. The most marked reduction in this susceptibility was observed for the brain. Thus, in total contrast to the control samples, the brains from the vitamin E-supplemented group were similar to the other tissues in their rates of spontaneous peroxidation.

Table 1. *Effect of dietary supplementation of the hen with vitamin E on the concentration of vitamin E in the initial yolk and of tissues of the developing chick*

	Vitamin E (μg/g fresh wt)		
	Control	Supplemented	*P*<
Parent's feed	147·3 ± 1·0	365·5 ± 1·8	0·001
Initial yolk	183·9 ± 12·9	705·4 ± 61·4	0·001
Day-16 embryo			
YSM	148·8 ± 12·5	743·4 ± 53·3	0·001
Liver	122·4 ± 10·5	528·4 ± 18·5	0·001
Brain	3·5 ± 0·2	5·8 ± 0·9	0·05
Lung	5·2 ± 0·6	29·1 ± 0·6	0·001
Day-old chick			
YSM	268·2 ± 21·6	3972·9 ± 247·6	0·001
Liver	751·8 ± 50·4	2110·5 ± 195·8	0·001
Brain	7·7 ± 0·5	23·1 ± 2·3	0·001
Lung	25·5 ± 1·7	129·8 ± 10·2	0·001

Results are means ± SE of measurements on 5 yolks, on tissues from 5 embryos/chicks, and on 5 replicate portions of food. Abbreviation: YSM, yolk sac membrane.

Table 2. *Effect of dietary supplementation of the parent hen with vitamin E on the susceptibility of aqueous homogenates of tissues of the day-old chick to lipid peroxidation*

	μg TBARS/g tissue/h		
	Control diet	Supplemented diet	*P*<
Fe^{2+} absent			
Initial yolk	4·4 ± 0·4	2·7 ± 0·3	0·01
YSM	6·1 ± 0·6	4·2 ± 0·3	0·05
Liver	7·3 ± 0·7	5·1 ± 0·3	0·01
Brain	43·1 ± 5·1	7·1 ± 0·9	0·05
Lung	8·2 ± 0·7	5·0 ± 0·5	0·001
			0·01
Fe^{2+} present			
Initial yolk	46·6 ± 5·9	20·2 ± 2·9	0·01
YSM	30·1 ± 3·8	18·3 ± 2·1	0·05
Liver	21·1 ± 1·7	16·6 ± 1·2	0·05
Brain	110·2 ± 10·2	19·7 ± 2·1	0·001
Lung	24·6 ± 2·1	18·4 ± 1·1	0·05

Results are means ± SE of 5 yolks or of tissue samples obtained from 5 chicks from each dietary group. Abbreviation: YSM, yolk sac membrane.

The addition of iron greatly stimulated the rates of peroxidation in tissue homogenates from the control group. Again, the extent of TBARS generation was far greater for the brain compared with the other tissues. Tissue homogenates from the vitamin E-supplemented group were significantly less prone to iron-stimulated peroxidation compared with the control group. Once more, the most dramatic effect was observed in the case of the brain with the result that, for the supplemented group, the brain was similar to the other tissues with regard to susceptibility to iron-induced peroxidation.

DISCUSSION

The brain of the newly-hatched chick appears to be particularly at risk from peroxidative damage. For example, the clinical condition termed encephalomalacia results from peroxidative events in the cerebellum and is associated with inadequate concentrations of vitamin E (Mezes *et al.*, 1997). This particular sensitivity of the brain derives from the conjunction of 3 factors. Firstly, the phospholipids of the neuronal membranes contain especially large amounts of highly polyunsaturated fatty acids, such as docosahexaenoic and arachidonic acids (Maldjian *et al.*, 1996). Secondly, the brain generates free radicals at a greater rate than other tissues, partly because of its very high rate of energy metabolism and oxygen consumption and partly because the synthesis, action and degradation of certain neurotransmitters promotes free radical formation (Reiter, 1995). Thirdly, and somewhat surprisingly, the amounts of many of the major antioxidant components are very low in the brain of the chick. For example, the concentration of vitamin E in the brain of the day-old chick is only about 1% of that in the liver and is considerably less than those in the heart, lung, adipose tissue, kidney and skeletal muscle (Surai *et al.*, 1996). Other

lipid-soluble antioxidants such as vitamin A and carotenoids are almost undetectable in the brain (Gaal *et al.*, 1995; Surai *et al.*, 1996). The activities of glutathione peroxidase and catalase as well as the concentration of selenium are also very low in the brain compared with other tissues (Gaal *et al.*, 1995). A key exception to this pattern is provided by ascorbic acid which is maintained at very high concentrations in the brain throughout the 2nd half of the embryonic period (Wilson, 1990; Surai *et al.*, 1996). It is possible that ascorbic acid in the brain may function in the regeneration of vitamin E by promoting the re-cycling of the tocopheroxyl radical back to the active antioxidant from (Surai *et al.*, 1996). However, previous work has shown that the brain is far more susceptible than the other tissues of the chick to *in vitro* lipid peroxidation (Surai *et al.*, 1996).

In an effort to reduce the sensitivity of the developing brain to peroxidative damage, the diet of the parent hen was supplemented with vitamin E. This readily resulted in increased amounts of vitamin E in the yolk and, subsequently, in the tissues of the embryo and chick, consistent with previous work (Surai *et al.*, 1997, 1998*a*, 1998*b*). However, in the embryo, the brain displayed a degree of resistance to such enhancement. Thus, the concentration of vitamin E in the brain of the day-16 embryo was only increased 1·6 times as a result of dietary supplementation of the hen whereas the concentrations in the liver and lung were increased by 4·3 times and 5·6 times, respectively. More encouragingly, the vitamin E content of the brain of the day-old chick exhibited an impressive 3·0-fold enhancement as a result of dietary supplementation of the parent compared with 2·8- and 5·1-fold increases in the liver and lung, respectively. The most striking increase (14·8-fold) in tissue vitamin E content, as a result of the supplementation, was observed in the yolk sac membrane of the day-old chick. This exceptional responsiveness of the yolk sac membrane may be related to its role in the transfer of vitamin E from the yolk to the embryo although a definitive explanation is not possible at this stage. In the case of both dietary groups, the concentrations of vitamin E in the various tissues were markedly greater in the day-old chick than in the day-16 embryo, reflecting the continuous transfer of the vitamin from the yolk to the embryo during the final period of development (Noble *et al.*, 1993; Surai *et al.*, 1996).

The supplemented diet, which contained 2·4 times more vitamin E than the control diet, produced a 3·8-fold increase in the concentration of vitamin E in the egg yolk. This apparently exaggerated response may be explained as follows. The main forms of vitamin E in the control diet were γ-tocopherol and γ-tocotrienol because these forms predominate in the maize oil and grain components (Hess, 1993; White and Xing, 1997; Yoshida *et al.*, 1998) whereas the supplemented diet was relatively enriched in α-tocopherol from the addition of α-tocopherol acetate. The preferential incorporation of α-tocopherol into the developing yolk, as reported by several previous studies (Cherian *et al.*, 1996; Cherian and Sim, 1997; Qi and Sim, 1998; Surai and Speake, 1998), would explain the enhanced effectiveness of the supplemented diet in increasing the total vitamin E content of the yolk.

The elevated concentration of vitamin E in the brain of the chick, brought about by supplementation of the diet of the parent hen, was highly effective in reducing the susceptibility of brain homogenates to peroxidation. A prominent observation for samples from the control group was the exceptional sensitivity to peroxidation, as measured by TBARS formation, displayed by the brain in comparison with the other tissues. Thus the rates of both spontaneous and iron-stimulated peroxidation were far greater for the brain than for the liver, lung, yolk and yolk sac membrane. Vitamin E supplementation of the parent hen produced significant reductions in peroxidation susceptibility for all the tissues of the chick in both the absence and presence of iron in the incubations. The key finding was that supplementation of the maternal diet with vitamin E brought the peroxidative susceptibility of the chick brain into line with that of the other tissues.

Thus, in conclusion, the anomalous status of the brain in enduring an especially high peroxidative susceptibility compared with the other chick tissues, is completely rectified as a result of supplementation of the maternal diet with vitamin E. The potential implications of this result for the prevention of neural and other impairments during avian development await investigation.

ACKNOWLEDGEMENTS

The authors are grateful to the Scottish Office Agriculture and Fisheries Department for financial support and to the Royal Society for a research fellowship to PFS.

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