



Beneficial Effects of Maternal Vitamin E Supplementation on the Antioxidant System of the Neonate Chick Brain

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ABSTRACT : Oxidative stress plays a crucial role in the laying stage which is a critical period for chick survival. We investigated the relationship of neonatal chick performance, brain antioxidant status and vitamin E supplementation level in hens. Starting at 17 weeks, hens were randomly divided into five groups. The control group received a basal diet without supplemental vitamin E (VE, dl- α -tocopherol acetate). Other groups received the same basal diet supplemented with vitamin E (40, 80, 120 and 160 mg/kg) through growth to egg production. Hens were artificially inseminated at 28 weeks of age and egg yolks were collected at day two. All remaining eggs were hatched. Yolk vitamin E content, hatchability and fertility of eggs were evaluated. Brains of the newly hatched chicks were further evaluated for their oxidative stress status, antioxidative status and vitamin E levels. Increased reproductive performance was observed in fertility and hatchability in the group supplemented at 40 mg/kg. Egg yolk and neonatal brain α -tocopherol was highest in eggs from hens fed 120 mg/kg and 80 mg/kg supplemental vitamin E, respectively. Brain MDA, ROS and iron levels were significantly higher in unsupplemented hens ($p < 0.01$). SOD activity was significantly higher in the group supplemented at 160 mg/kg than in all other groups. We concluded that maternal supplementation of vitamin E had beneficial effects on fertility, hatchability of eggs, neonatal brain oxidative status and SOD activity. (**Key Words :** Vitamin E, Antioxidation, Oxidative Stress, Brain, Hatching, Chick)

INTRODUCTION

Vitamin E is essential for preventing encephalomalacia and maintaining normal reproductive function of roosters and hens (Yoshida and Hoshii, 1976). Vitamin E is also recognized as the important cellular lipid-soluble chain-breaking antioxidant (Tappel, 1962) against oxidative stress.

Oxidative stress, the disturbance in the balance between oxidants and antioxidants, affects the reproductive function, fertilization and early embryonic development in animals and poultry (Tamate et al., 1995; Sikka, 1996). High levels of ROS causing protein, lipid oxidation and DNA damage has been reported (Freeman and Crago, 1982; Li et al., 2007). In the chick embryo developmental stage, it was characterized by a progressive increase in levels of polyunsaturated fatty acid (PUFA) for the optimal growth

and development of embryos (Speake and Thompson, 2000). During hatching and early postnatal periods the accumulating PUFA-producing tissues were vulnerable to peroxidation (Surai et al., 1996). Oxidative stress at that time might lead to essential nutrient deprivation or tissue damage. Therefore, the presence of an effective antioxidant (vitamin E) during embryonic and neonatal development is likely to be beneficial in protecting tissues against oxidative damage.

Several studies have reported that maternal diet affects the offspring status. Yessoufou et al. (2006) indicated that the n-3 PUFA diet improved hyperlipidemia and restored antioxidant status in streptozotocin (STZ)-induced diabetic dams and their offspring. Capper et al. (2005) found that neonatal and suckling lamb vitamin E status might be manipulated by vitamin E supplementation of the ewe during pregnancy and lactation. Martinez-Cruz et al. (2002) showed that administration of melatonin to hyperphenylalaninemia female rats prevented the oxidative damage in the pup rat brain and cerebellum.

Data concerning effects of maternal vitamin E supplementation on the antioxidant status of hatching chicks are rare. Previous studies have reported the beneficial effects of maternal vitamin E supplementation on

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Table 1. Composition of basal diets and weeks fed

Ingredients	Growing period			Laying period
	0 to 6 wk	6 to 12 wk	12 to 17 wk	17 to 28 wk
	----- % -----			
Ground yellow corn	64.6	65.1	70.9	67.0
Soybean meal (43.5%)	25.0	25.0	16.0	12.9
Fish meal (64%)	3.0	0.0	0.0	4.0
Wheat bran	4.5	6.8	10.0	6.4
Dicalcium phosphate	1.2	1.3	1.3	1.1
Calcium carbonate	1.2	1.3	1.3	8.1
Salt	0.3	0.3	0.3	0.3
Vitamin premix ¹	0.1	0.1	0.1	0.1
Mineral premix ²	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0
Calculated analysis				
Protein (%)	18.6	17.1	14.1	14.5
Metabolizable energy (kcal/kg)	2,858	2,827	2,862	2,719
Calcium (%)	0.92	0.88	0.85	3.53
Available phosphorus (%)	0.42	0.37	0.35	0.40
Total sulfur amino acids (%)	0.66	0.59	0.52	0.55
Linoleic acid (%)	1.60	1.65	1.79	1.64
Selenium (mg/kg)	0.12	0.10	0.12	0.14
Vitamin E (mg/kg)	15.50	15.80	17.30	16.10

¹ Provided per kilogram of diet: vitamin A (retinyl acetate), 6,300 IU (1.9 mg); cholecalciferol, 1,100 IU (27.5 µg); thiamine, 1.1 mg; riboflavin, 4.4 mg; pyridoxine 2.2 mg; vitamin B₁₂ 6.6 µg; menadione, 1.1 mg; biotin 0.11 mg; folic acid, 0.55 mg; niacin 44 mg; pantothenic acid, 12 mg.

² Provided per kilogram of diet: iron, 64 mg; copper, 12 mg; manganese, 64 mg; cobalt, 0.2 mg; zinc, 40 mg; iodine, 0.68 mg.

immune diseases of chicks and calves (Fuhrmann and Sallmann, 2000; Shinde et al., 2007). It has also been reported to benefit the regulation of antioxidant capacity in developing embryos and hatching (Bartholomew et al., 1998). Since brain is particularly susceptible to oxidative damage (Oi et al., 2002) and has the lowest antioxidant enzyme activities among all tissues (Mezes et al., 1997), the objective of this study was to evaluate the effect of maternal diets added with different levels of the antioxidant vitamin E on the embryonic stage, oxidative status and antioxidant abilities in the neonatal brains in relation to its early brain development during postnatal period.

MATERIALS AND METHODS

Animal and treatments

Taiwan native breeder chicks (day-old, n = 180) fed with corn-soybean diets without supplemental vitamin E until 17 weeks of age. After that, chicks were fed corn soybean diets and randomly assigned to five dietary treatments containing 0, 40, 80, 120, and 160 mg/kg of supplemental vitamin E (VE, dl- α -tocopherol acetate) (Roche, F. Hoffmann-La Roche Ltd, 4002 Basel, Switzerland) throughout the experimental period (Table 1). Hens were artificially inseminated with pooled semen (0.02 ml/hen) at 28 weeks of age. Six of the collected eggs in each treatment group were randomly sampled. Yolks were separated using an egg separator and stored at -20°C to determine vitamin E content later. The rest of the collected

eggs were placed in an incubator under standard commercial conditions for 21 d. Eggs were candled on d 5 and infertile eggs were removed. After 21 day incubation, the numbers of newly hatched chicks were recorded to determine hatchability. The unhatched eggs were cracked open to see if there were dead-in-shell chicks. Ten neonatal chicks (day-old, n = 10) from each treatment group, including the controls (0 mg/kg), were put to death with CO₂ gas. Brain tissues were quickly removed, weighed and homogenized in chilled 50 mM potassium phosphate buffer (pH 7.0).

Antioxidant vitamin E status

Vitamin E measurement (α -tocopherol in the brain and yolk) was determined by HPLC (Turner and Mathiasson, 2000). In brief, 1 ml of ethanol was added to 1 g of yolk or brain tissue and the mixtures were saponified with potassium hydroxide and tocopherols were extracted with hexane. Extract was dried under nitrogen, re-dissolved in methanol and injected into an HPLC system (HITACHI L-7480, Japan) fitted with a reverse phase HPLC column (Hypersil[®] HS C18 250×4.6 mm 5 µm, Thermo Quest, England). Chromatography was performed using methanol/water (98:2 v/v) as mobile phase at a flow rate of 1.5 ml/min followed by fluorescence detection of tocopherol at emission wavelength of 292 nm.

Reactive oxygen species measurements

ROS was measured using a modification of method by

Table 2. Reproductive performance of Taiwan Native Breeder hens supplemented with vitamin¹

	Added vitamin E ² (mg/kg)				
	0	40	80	120	160
Fertility (%) ³	78.4±5.1 ^b	93.8±2.2 ^a	91.9±2.0 ^a	91.8±2.3 ^a	86.8±3.2 ^{ab}
Hatch of all eggs set (%)	67.2±5.0 ^b	86.2±2.7 ^a	87.0±3.6 ^a	82.6±3.4 ^a	76.0±4.6 ^{ab}
Hatch of fertile eggs (%) ³	85.7±3.6	91.9±1.9	94.4±3.0	90.0±3.0	87.4±4.0
Dead in shell (%)	6.3±2.6	7.1±1.8	4.9±2.5	8.5±2.4	8.6±2.7

^{a, b} All values are Means±SE. Values in a row without the same superscript are significantly different (p<0.05).

¹ Starting at 17 wk, birds were fed corn soybean diets supplemented with five different levels of VE (dl- α -tocopherol acetate). All the birds during growing period (0 wk through 17 wk) were fed with same commercial diets without supplemental VE.

² Basal diet had 16.1 IU/kg VE.

³ Percent fertility is the percentage of fertile eggs of all eggs produced. Hatch of fertile eggs is the percentage of eggs hatched out of the fertile eggs.

LeBel et al. (1990). In brief, 1 ml brain homogenate was incubated with a final concentration of 5 μ M 2',7'-dichlorofluorescein diacetate in methanol for 15 min at 37°C. Fluorescence measurements were performed with a Hitachi 850 spectrofluorometer at 488 nm for excitation and at 525 nm for emission wavelengths.

Malondialdehyde (MDA) measurements

Lipid peroxidation of brain homogenates was measured as malondialdehyde (MDA) equivalents (Ohkawa et al., 1979). In brief, samples (100 μ l) were treated with sodium dodecyl sulfate (SDS) (0.2 ml, 8.1%), acetic acid solution (1.5 ml, 20%), and aqueous solution of thiobarbituric acid (TBA) (1.5 ml, 0.8%). The mixture was heated (95°C, 60 min) and cooled to room temperature. An equal volume of 1-butanol was added and shaken vigorously. The fluorescence intensity of the organic layer (upper layer) was measured at excitation and emission wavelengths of 515 and 553 nm, respectively. A standard curve was prepared using tetraethoxypropane, which would yield an equimolar amount of MDA.

Iron level measurements

Individual brain samples were dried for 24 h using a freeze-dryer. Dried brains were weighed. Each brain sample was wet-acid digested with concentrated nitric acid. Samples were analyzed by using the Varian Spectra AA220 iron flame atomic absorption spectrophotometer (Varian Pty Ltd, Victoria, Australia) (Morita et al., 1994). Values were expressed on the basis of dry weight.

Enzymatic antioxidant status

The activity of catalase (CAT) in the brain was measured using the method of Aebi (1984). In brief, H₂O₂ (100 μ l, 10 mM) and brain supernatant (100 μ l) were mixed well, incubated (20°C) and assayed immediately for the change of absorbance within 1 min at 240 nm by spectrophotometry (DU530, Beckmen, Fullerton, CA). One unit of CAT was defined as the amount required to decompose 1 μ mol of H₂O₂ within 1 min.

The activity of glutathione peroxidase (GPx) was determined using a modified Paglia and Valentine method (1967). In brief, 100 μ l of the brain supernatant was added to 200 μ l of GSSGR (5 units/ml), 50 μ l of glutathione (40 mM), and 620 μ l of K-P buffer (0.25 M). To this mixture were added 10 μ l of 20 mM NADPH (in 1% Na₂CO₃) and 20 μ l of 15 mM cumene hydroperoxide. Changes in absorbance were recorded at 340 nm for 3 min with a spectrophotometer and one unit of GPx was defined as μ mol NADPH oxidized/min.

The superoxide dismutase (SOD) activity was determined by colorimetry (Marklund and Marklund, 1974). One unit of SOD activity was defined as the amount of the enzyme inhibiting the autoxidation by 50%. The final assay mixture contained 10 μ l of the brain supernatant, 3 ml Tris-HCl buffer, and 6.1 μ l the pyrogallol (50 mM in 10 mM HCl). The solution was assayed immediately to monitor the change of absorbance measured within 1 min at 325 nm using a spectrophotometer.

Protein assay

Protein was measured using the BCA protein assay kit (Pierce, Rockford, IL). All enzyme activities were expressed as units per mg of protein.

Statistical analysis

Data were expressed as mean±SD. Treatment effects (diet) were statistically analyzed by ANOVA using the general linear model (SAS software, ver. 6.11; SAS Institute Cary, NC). Means were compared using Duncan's multiple-range test, and significance was determined at p<0.05.

RESULTS

The fertility and hatchability of eggs

The effects of dietary vitamin E supplementation on reproductive efficiency (including fertility and hatchability) were shown in Table 2. Significant improvements in fertility and hatch rate were found in 40 mg/kg of vitamin E fed as compared with those in control group (p<0.05). Our result

Table 3. Effects of supplemental vitamin E on α -tocopherol concentration of egg yolk and neonatal chick brain from Taiwan native breeder pullets¹

Items	Added vitamin E ² (mg/kg)				
	0	40	80	120	160
Egg yolk	----- $\mu\text{g/g}$ egg yolk -----				
Vitamin E	5.80 \pm 0.88 ^d	18.22 \pm 1.36 ^c	28.28 \pm 0.72 ^b	44.21 \pm 4.98 ^a	42.17 \pm 1.22 ^a
Brain	----- $\mu\text{g/g}$ brain -----				
Vitamin E	3.6 \pm 0.2 ^b	4.0 \pm 0.2 ^b	20.4 \pm 0.2 ^a	25.6 \pm 0.7 ^a	27.9 \pm 0.6 ^a

^{a, b, c, d} All values are Means \pm SE. Values in a row without the same superscript are significantly different ($p < 0.05$).

¹ Starting at 17 weeks of age, birds were fed corn soybean diets supplemented with five different levels of vitamin E (dl- α -tocopherol acetate). All birds were fed same practical diets without supplemental vitamin E during growing period (0 week through 17 weeks).

² Basal diet had 16.1 IU/kg vitamin E (calculated value).

Table 4. Effects of supplemental vitamin E on oxidative status of the brain of newly hatched chicks from Taiwan native breeder pullets¹

Oxidative indicators	Added vitamin E ² (mg/kg)				
	0	40	80	120	160
MDA (nM/mg)	7.27 \pm 0.55 ^a	4.95 \pm 0.17 ^b	4.21 \pm 0.19 ^b	1.98 \pm 0.12 ^c	1.78 \pm 0.05 ^c
ROS MFI	3.45 \pm 0.12 ^a	1.92 \pm 0.01 ^b	1.81 \pm 0.12 ^b	1.78 \pm 0.06 ^b	1.88 \pm 0.06 ^b
Iron ($\mu\text{g/g}$)	143.1 \pm 1.2 ^a	135.1 \pm 1.5 ^a	135.1 \pm 1.1 ^a	125.4 \pm 0.8 ^b	120.6 \pm 1.5 ^b

^{a, b, c} All values are Means \pm SE. Values within a row with different superscript letters are significantly different ($p < 0.01$).

¹ Starting at 17 weeks of age, birds were fed corn soybean diets supplemented with five different levels of vitamin E (dl- α -tocopherol acetate). All birds were fed same practical diets without supplemental vitamin E during growing period (0 week through 17 weeks).

² Basal diet had 16.1 IU/kg vitamin E (calculated value).

Table 5. Effects of supplemental vitamin E on antioxidant enzymes of the brain of newly hatched chicks from Taiwan native breeder pullets¹

Antioxidant enzyme	Added vitamin E ² (mg/kg)				
	0	40	80	120	160
SOD (U/mg protein)	1.06 \pm 0.12 ^b	0.98 \pm 0.21 ^b	1.18 \pm 0.10 ^{ab}	1.11 \pm 0.15 ^{ab}	1.69 \pm 0.08 ^a
CAT (U/mg protein)	0.22 \pm 0.05	0.15 \pm 0.01	0.19 \pm 0.02	0.24 \pm 0.05	0.28 \pm 0.04
GPx (mU/mg protein)	15.2 \pm 1.4	17.5 \pm 1.2	15.8 \pm 2.1	16.2 \pm 1.5	17.1 \pm 1.6

SOD = Superoxide dismutase; CAT = Catalase; GSH-Px = Glutathione peroxidase.

^{a, b} All values are Means \pm SE. Values within a row with different superscript letters are significantly different ($p < 0.01$).

¹ Starting at 17 weeks of age, birds were fed corn soybean diets supplemented with five different levels of vitamin E (dl- α -tocopherol acetate). All birds were fed same practical diets without supplemental vitamin E during growing period (0 week through 17 weeks).

² Basal diet had 16.1 IU/kg vitamin E (calculated value).

also indicated that fertility and hatch rates of all eggs set were more sensitive to supplemental vitamin E than the hatch rates of fertile eggs. Adding 80 mg/kg of vitamin E produced the trend of lowest dead-in-shell percentage and when supplemental vitamin E exceeded 80 mg/kg, dead-in-shell rates tended to increase again but with no statistical significance.

α -Tocopherol in egg yolk and neonatal brain

Maternal supplementations of vitamin E affected the vitamin E concentration in egg yolk and neonatal chick brain as shown in Table 3. Alpha-tocopherol concentrations in yolk significantly went up with increased vitamin E, up to 120 mg/kg ($p < 0.05$). Beyond that level (160 mg/kg) there was no corresponding increase in yolk content. The concentration of α -tocopherol was not found in brains of neonate chicks from hens supplemented at 0 and 40 mg/kg. However, higher levels (i.e., 80-160 mg/kg) vitamin E supplementation exhibited significant increasing the levels of α -tocopherol in the neonatal chick brains ($p < 0.05$).

Oxidative status in neonatal brain

Treatment with maternal vitamin E actually prevented the elevation of chick brain MDA levels (Table 4). We found significantly lower brain MDA levels in chicks from hens fed at 120, 160 mg/kg levels, compared with those fed at 40 and 80 mg/kg ($p < 0.01$). The effects of maternal vitamin E on brain ROS was similar to those in brain MDA. The ROS levels of neonatal chicks with maternal vitamin E supplementation were significantly lower than those in control group ($p < 0.01$). Brain iron levels were shown in Table 4. Iron was significantly lower in chicks from hens supplemented at 120 and 160 mg/kg, than at 0, 40 or 80 mg/kg ($p < 0.01$).

Antioxidant enzymes in neonatal brain

The activity of SOD in chick brain was increased from hens fed at the highest levels vitamin E (160 mg/kg) compared to those fed at 0 and 40 mg/kg. However, there were no significant differences in the brain GPx and CAT activities between each level of vitamin E supplementation

groups and the control group (Table 5).

DISCUSSION

Our results confirmed the existence of oxidative stress in chick brain without vitamin E supplementation by generating ROS and MDA which was disadvantageous for chick growth and survival. This result was in total agreement with previous reports in vitamin E deficiency (Fuhrmann and Sallmann, 1995). Two similar studies showed that overproduction of free radicals in newly-hatched chicks brain as hens were fed highly unsaturated and contained very low levels of vitamin E (Surai et al., 1999) and generated a high levels of free radicals than any other organ (Reiter, 1995). Our study showed that maternal vitamin E supplementation with low doses (40 mg/kg) was able to override the effect of oxidative stress (MDA and ROS) in the neonatal chick brains. This was in agreement with previous studies indicating a decreasing MDA level in the tissues as a result of vitamin E supplementation. Grune et al. (2001), who found that the hens fed feed levels with 80 mg/kg vitamin E prevented cytotoxic aldehydic lipid peroxidation (MDA) in PUFA-enriched eggs. Surai et al. (1999a) indicated that a high vitamin E supplementation (365 µg/g) in the maternal diet significantly decreased their susceptibility to MDA in liver, lung and brain. Sahin et al. (2002) also found that dietary vitamin E (250 mg) and vitamin C (250 mg) decreased serum MDA concentration ($p < 0.05$) in laying hens. It is well known that vitamin E is a very potent cellular scavenger of reactive oxygen species (ROS). In order to understand the protective role of vitamin E against brain ROS the levels of production, we determined the levels of ROS by using dichlorofluorescein fluorescence. Our results showed that maternal supplementation of vitamin E significantly decreased ROS levels in brain. Although no similar studies of ROS level and maternal vitamin supplementation have attested this, it was reported that the potential side effect was associated with antioxidant vitamin supplementation. For example, vitamin C might have behaved as a prooxidant and aggravated ROS-induced mutagenesis (Allen, 1989) while vitamin E supplements did not reduce ROS activity in the short term (Everett et al., 2002).

In our study, the level of iron in chick brain could be significantly counteracted by supplementation with 120 and 160 mg/kg vitamin E in hen diets. These results have reflected on brain lipid peroxidation indicating that MDA level in 120 and 160 mg/kg vitamin E were significantly lower than those in 40 and 80 mg/kg vitamin E groups. Brain was more susceptible to Fe^{2+} -induced lipid peroxidation than the liver in chicks (Surai et al., 1996). It was not clear how vitamin E lowered the levels of iron in brain. A possible explanation is that vitamin E recycle alpha

lipoic acid (Packer et al., 2001) which is metal chelator (Biewenga et al., 1997) leading to the decrease in level of brain iron by chelating Fe^{2+} .

The efficiency of maternal vitamin E supplementation in reducing the sensitivity of the developing brain to oxidative stress was found to be associated with an elevation of vitamin E levels in yolk and brain. Egg yolk α -tocopherol levels tended to increase and reached a plateau as vitamin E supplementation of 120 mg/kg was given. These results were not in agreement with those of Jiang et al. (1994), who observed that egg yolk vitamin E content went up linearly as dietary vitamin E was increased from 50 to 400 mg/kg. Maternal vitamin E supplementation brought about a significantly elevated brain α -tocopherol levels (20.4 µg/g) at a dose of 80 mg/kg. Surai (2000) observed that adding 40, 100, 200 mg/kg vitamin E of the maternal diet could increase 5.19, 8.33 and 15.22 µg/g α -tocopherol concentration, respectively, in the developing brain of the chicks. Alpha-tocopherol is a main form of tocopherol in the brain which can be transferred from feed to eggs. In this respect, the uptake of tocopherol into yolk and brain tissue in relation to their presence in the maternal diet indicated that tocopherol was efficiently absorbed and retained in neonatal brain.

The effects of vitamin E supplemented on enzymatic activities in newly hatched chicks, our results showed an insignificantly increasing the activity of SOD, but not GPx and CAT of the chick brain with increasing maternal supplementation (160 mg/kg). The results regarding the activities of antioxidant enzymes of chicks in response to vitamin E supplementation were not similar. The existence of tissue-specific antioxidant profiles has been reported in that an elevation of liver antioxidant system of the newly-hatched chick from maternal diet antioxidant vitamin E supplementation together with selenium (Surai et al., 2000), had increased SOD and CAT activities during embryonic development (Allen, 1989). In our results, the activities of GPx and CAT in the brain were not significantly different in supplemented groups. It was reported that the activities of GPx and CAT were very low in the brain compared with other tissues (Mezes et al., 1997). We speculated that the activities of the GPx and CAT in the brains of chicks might have been less responsive to vitamin E supplementation of the mother hen's diet. However, vitamin A supplementation in other study showed the activities of GPx and SOD were decreased in the chicken liver and brain when vitamin A was increased. The authors of that report speculated that an excess of vitamin A might have compromised the antioxidant defense system (Surai et al., 2000). Our study did not find high levels of vitamin E fed to the mother hens to compromise the antioxidant enzymes in the brain of their offsprings.

There have been very few studies reported on the

relationship of vitamin E with breeder hens' fertility or hatchability. The Fertility and hatchability of eggs were significantly improved with providing maternal 40mg/kg vitamin E in our study. Our result is consistent with other study which also suggests that 40 mg/kg vitamin E supplementation markedly enhanced in fertility and hatchability of eggs (Muduuli et al., 1982). However, this level of vitamin E supplementation was higher than previously study which recommended 20 mg/kg of supplemental vitamin E in the feed for breeding hens (Hennig et al., 1986), the efficiency of supplementation with different amounts of vitamin E may be associated with physiological stress variation of eggs. It should be noted that maternal vitamin E supplementation has beneficial effects on the fertility and hatchability of eggs.

CONCLUSION

We concluded that maternal supplementation of vitamin E at 40-120 mg/kg had beneficial effects on fertility, hatchability of eggs and neonatal brain oxidative status, and at 160 mg/kg had beneficial effects on brain SOD activity.

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