PALM VITAMIN E FOR AQUACULTURE FEEDS

WING-KEONG NG*; YAN WANG* and KAH-HAY YUEN**

ABSTRACT

In this overview, our current research on the use of palm oil-based vitamin E in aquaculture feeds will be highlighted. While most vegetable oils contain almost exclusively tocopherols, palm oil is notable because tocotrienols represent about 80% of the vitamin E content. Almost all vitamin E research in fish nutrition has focused on α-tocopherol, usually supplied as the synthetic all-rac-α-tocopherol acetate, as it is deemed the most potent of all the isoforms. Several feeding trials were carried out to investigate the deposition of vitamin E and their antioxidant activity in various tissues of tilapia and catfish fed various palm oil products and vitamin E sources. We were the first group of researchers to show that (1) the tocotrienol-rich fraction (TRF) extracted from palm oil is more potent than all-rac-α-tocopherol acetate as an antioxidant when used in tilapia diets; (2) fish tissues varied in their ability to accumulate tocotrienols with the highest concentrations being found in perivisceral adipose tissues, followed by liver, skin and muscle; (3) tissue concentrations of α-tocopherol, α-tocotrienol and γ-tocotrienol increased linearly in response to increasing dietary concentrations originating from added TRF. As a potent in vivo *antioxidant in fish tissues, palm vitamin E will have positive impacts on seafood quality such as prolonging shelf-life, maintaining colouration of pigmented seafood and enhancing the nutritional value of seafood.*

Keywords: palm oil, vitamin E, tocotrienols, aquafeeds, seafood quality.

Date received: 14 March 2008; **Sent for revision:** 21 March 2008; **Received in final form:** 29 May 2008; **Accepted:** 2 July 2008.

INTRODUCTION

The aquaculture industry is currently the fastest growing food production sector in the world. World aquaculture produces about 60 million tonnes of seafood worth more than USD 70 billion annually (FAO, 2006). Farmed fish accounts for about 50% of all consumed fish in the world, and this percentage is expected to continue to increase due to dwindling catches from capture fisheries. In recent years, technological advances in the aquafeed manufacturing industry have made possible the incorporation of high levels of dietary oils in fish feeds to produce energy-dense diets. Improvements in growth and feed utilization efficiency have been reported in fish due to the protein-sparing effect of dietary lipid (De Silva *et al*., 1991). However, feeding high levels of dietary fish oils, which contain a high proportion of polyunsaturated fatty acids (PUFA) which are highly susceptible to oxidation, can lead to increased oxidative stress for the fish that can result in pathological conditions (Sakai *et al*., 1998) and deterioration of fillet quality (Scaife *et al*., 2000). Farmed fish quality deteriorates rapidly after slaughter and affects the shelf-life, storage properties and quality of fish and surimi-based products. Increases in the lipid content of commercial fish feeds are usually not followed by appropriate antioxidant supplementation in order to maintain normal antioxidant status, and this further exacerbates the deleterious effects of lipid peroxidation, especially in cellular biomembranes which contain high amounts of PUFA.

Vitamin E is a potent antioxidant that inhibits lipid peroxidation in cell membranes. Vitamin E is the generic name given to a group of lipid-soluble compounds, which include four tocopherols, α-, β-, γ- and δ-T, and four tocotrienols, α-, β-, γ- and δ-T3,

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isoforms. Among them, α-T has the highest vitamin E activity (NRC, 1993). For dietary purposes, vitamin E activity is expressed as the α-tocopherol equivalent (α-TE) which is the activity of 1 mg RRR-α-tocopherol (Papas, 1999). On this basis, each of the natural vitamin E isoforms is assigned a biopotency factor (α-T, 1.0; β-T, 0.5; γ-T, 0.1; δ-T, 0.03; α-T3, 0.3; β-T3, 0.05; γ-T3, 0.01) according to the amount of vitamin E necessary to prevent fetal resorption in pregnant and vitamin E-deficient rats (Sheppard and Pennington, 1993; Drotleff and Ternes, 1999). The biopotency factor for δ-T3 is presently unknown. It is therefore not surprising that almost all vitamin E research in fish nutrition has focused on α-T, commonly supplied as the synthetic *all-rac*-α-tocopheryl acetate, as it is believed to be the most potent of all the isoforms (Frigg *et al*., 1990; Gatta *et al*., 2000; Huang *et al*., 2003). The synthetic *all-rac*-α-tocopheryl acetate is used worldwide in commercial fish feeds, and is a multi-million dollar industry located mainly in Europe. About 70% of all synthetic vitamin E produced globally ends up in vitamin premixes of animal feeds, including aquafeeds.

Recent *in vitro* research with isolated rat cells seems to indicate that the antioxidant activities among the various vitamin E isoforms are not necessarily correlated with their assigned biological activities. Serbinova *et al.* (1991) reported that *in vitro* α-T3 possesses 40-60 times higher antioxidant activity against lipid peroxidation and provides 6.5 times better protection of cytochrome P450 against oxidative damage than α-T in rat liver microsomal membranes. Ikeda *et al.* (2003) reported that in some tissues in rats fed equivalent dietary levels of α-T or α-T3, both isoforms provided equal protection against lipid peroxidation. The protective ability of tocotrienols from the tocotrienols-rich fraction (TRF) extracted from palm oil was reported to be significantly higher compared to α-T as effective inhibitors of protein oxidation and lipid peroxidation in rat liver microsomes (Kamat *et al*., 1997), with γ-T3 being the most effective.

The Fish Nutrition Laboratory at Universiti Sains Malaysia has successfully introduced palm oil as an alternative source of lipid and energy in aquaculture feeds for both cold-water and tropical fish species (Ng, 2006; Bahurmiz and Ng, 2007; Ng *et al*., 2007). Crude palm oil (CPO) is also one of the richest sources of natural vitamin E (600-1000 mg kg^{-1}), namely a unique mixture of tocopherols (18%-22%) and tocotrienols (78%-82%). Therefore, we conducted a series of feeding trials to investigate the use of palm vitamin E as a novel source of antioxidants for farmed fish.

PALM VITAMIN E FOR AQUACULTURE FEED

Tocotrienol Deposition in Fish Tissues

We first reported a linear increase in total vitamin E concentrations in the muscle of African catfish fed practical diets with increasing levels of palm fatty acid distillate (PFAD) at the expense of fish oil (Ng *et al*., 2004a). As far as we know, this represents the first reported data on the deposition of dietary palm tocotrienols in fish tissue. Muscle tocotrienol concentrations of African catfish were observed to increase significantly concomitant with increasing dietary PFAD. However, when tocotrienol concentrations were expressed as a percentage of total vitamin E, it was interesting to note that despite an increasing percentage of tocotrienols in the diet (1.8 % to 58.2%), tocotrienols constituted only 13.4% to 26.7% of the total vitamin E deposited in catfish muscle (*Figure 1*). In catfish fed PFAD-supplemented diets, an equilibrium in T:T3 ratio of about 7.5:2.5 in the muscle was reached in eight weeks irrespective of dietary vitamin E composition. In the catfish muscle, 68.5% to 80.2% of the total vitamin E deposited was present as α-T. Similar high ratios of α-T to the sum of other vitamin E isoforms in tissues have been reported also for laboratory mammals (Ikeda *et al*., 2003). Depending on the level of PFAD inclusion (0% to 100% added oil) in the catfish diet, total tocopherols and tocotrienols deposited in muscle ranged from 6.48 to 14.26 μg g^{-1} and 1.0 to 5.0 μg g^{-1}, respectively (*Figure 1*).

When we fed red hybrid tilapia with diets supplemented with a TRF extracted from CPO, α-T, together with α- and γ-T3 were found to be deposited into tilapia tissues, and their concentrations were observed to increase linearly in association with increasing levels of dietary TRF (Wang *et al.*, 2006). *Figure 2* shows this linear response in the adipose tissues and the actual amounts of the various vitamin E isoforms deposited. The α-T was the predominant isoform which accumulated in all tissues and plasma. Results from this study indicate that palm tocotrienols supplementation in tilapia diets could markedly enhance the tocotrienol concentration in various tissues, but the deposition is very tissue-specific. Tocotrienols constituted equilibriums of 46.7%-48.9%, 24.7%-33.1%, 21.6%-26.0%, 19.2%-22.2% and 8.0%-9.7% of the total vitamin E in adipose, liver, skin, muscle and plasma, respectively, of tilapia fed TRF-supplemented diets (E30 to E240), in spite of their high dietary compositions of about 80%. Thus, the adipose tissue of tilapia had the largest capacity to take up palm tocotrienols, followed by liver, skin, muscle and plasma.

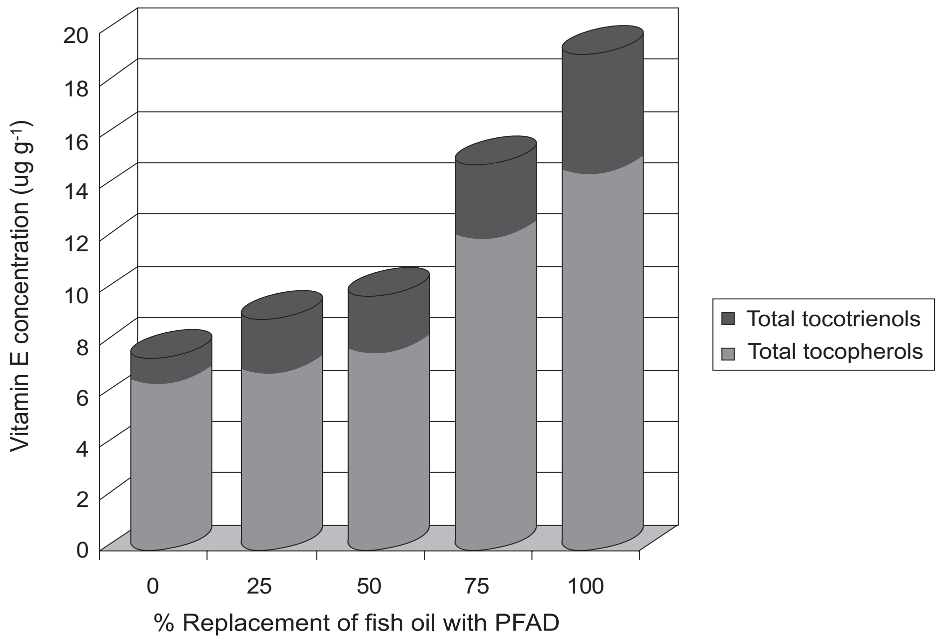

Figure 1. Deposition of palm tocopherols and tocotrienols in the muscle tissue (ug g^{-1}) of African catfish fed palm fatty acid distillate (PFAD)-based diets (modified from Ng et al.*, 2004a).*

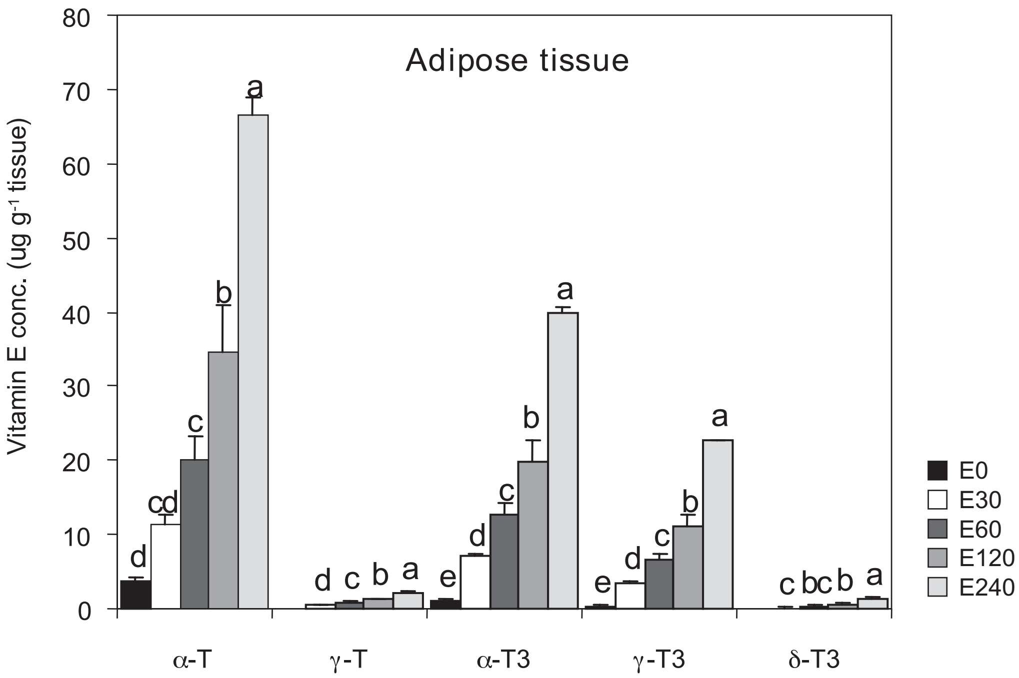

Figure 2. Vitamin E concentrations in the adipose tissue of red hybrid tilapia fed a diet without added vitamin E (E0) or with diets supplemented with 30 to 240 (E30, E60, E120 or E240) mg kg^{-1} total vitamin E derived from a tocotrienol-rich fraction (TRF) extracted from crude palm oil (CPO) (adapted from Wang et al.*, 2006). Bars having different alphabets within the same vitamin E isoform are significantly different (P<0.05).*

Oxidative Stability of Fish Fillets

The role of elevated levels of dietary α-T in improving fish flesh quality by maintaining oxidative stability has been well recognized in tilapia (Huang *et al*., 2003), rainbow trout (Frigg *et al*., 1990), Atlantic salmon (Scaife *et al*., 2000), sea bass (Gatta *et al*., 2000) and African catfish (Baker and Davies, 1996). All of these studies on the role of vitamin E in protecting membrane lipids from free radical attacks in fish tissues had relied on the application of synthetic *all-rac*-α-tocopheryl acetate as the sole dietary source of vitamin E. We were able to show that vitamin E concentrations in fish fillets increased in response to increasing dietary vitamin E originating from CPO (Lim *et al*., 2001), PFAD (Ng *et al*., 2004a) or palm TRF (Wang *et al*., 2006), and there was evidence to support the role of the accumulated palm vitamin E in enhancing oxidative stability of fish fillets. Lipid peroxidation (measured as TBARS) in muscle and liver of red hybrid tilapia fed low dietary TRF diets (E0 and E30) was significantly higher than those of fish fed high dietary TRF diets (E60 to E240) (*Figure 3*).

A Potent Antioxidant

A comparative study on the antioxidant potency of synthetic tocopheryl acetate compared to TRF was conducted in tilapia. Our research showed that there was no significant decrease in lipid peroxidation products beyond 50 mg *all-rac*-α-tocopheryl acetate/ kg diet, but the addition of dietary TRF at about 100 mg kg^{-1} diet caused a further decrease in lipid peroxidation as indicated by the concentrations of MDA in *Figure 4* (Ng *et al*., 2004b). This shows that TRF extracted from CPO is a more potent antioxidant compared to the conventional synthetic vitamin E when used in red hybrid tilapia feeds. For synthetic vitamin E esters such as acetates, they are chemically not an antioxidant until the animal hydrolyses it to α-T during the digestion process. Dietary vitamin E

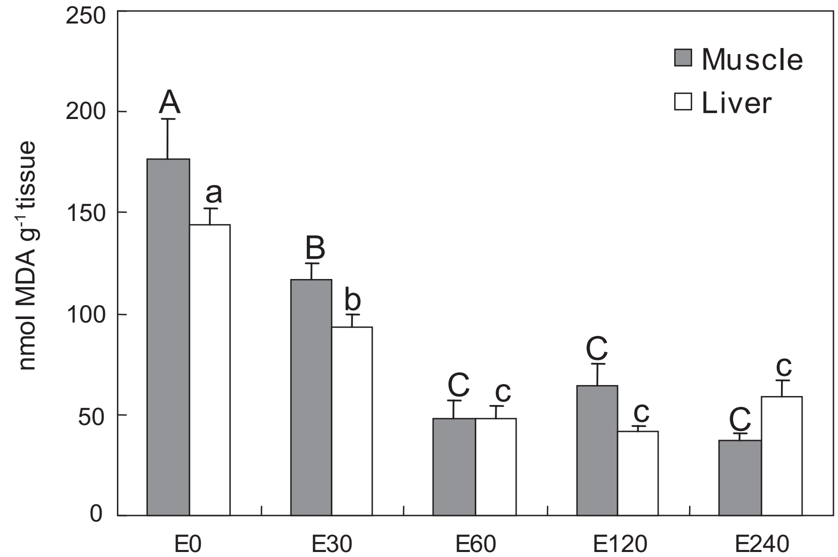

Figure 3. Effects of graded levels of total vitamin E derived from palm tocotrienol-rich fraction (TRF) on lipid peroxidation in red hybrid tilapia fillets. Bars not sharing a common letter are significantly different, P<0.05. MDA: malondialdehyde (adapted from Wang et al., *2006).*

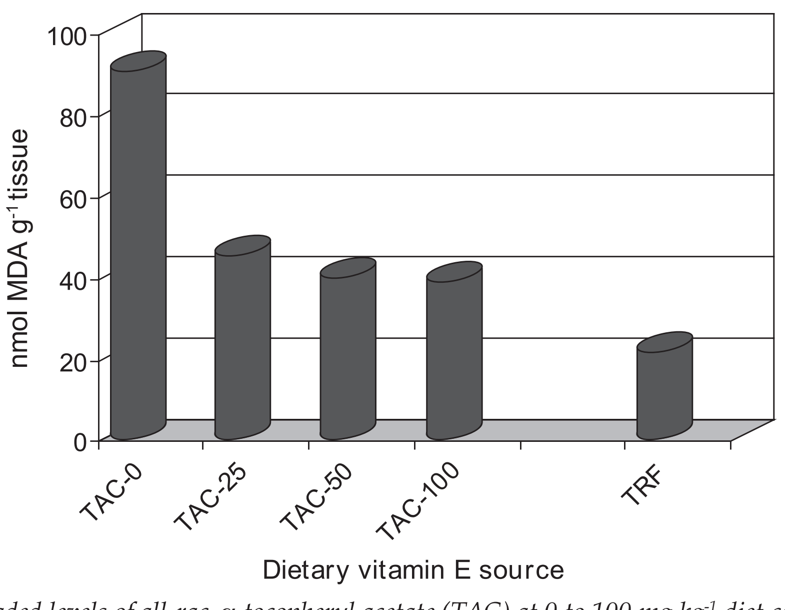

Figure 4. Effects of graded levels of all-rac-α-tocopheryl acetate (TAC) at 0 to 100 mg kg^{-1} diet compared to palm TRF on thiobarbituric acid-reactive substances from iron-vitamin C induced lipid peroxidation in muscle of red hybrid tilapia (modified from Ng et al. *2004b).*

isoforms are then taken up from the small intestine and re-assembled into chylomicrons by the Golgi body of the mucosa cells. Once the chylomicrons are carried to the liver, α- T will be selectively recognized by the α-tocopherol transfer protein (α-TTP), which has been identified in humans and rats. The high affinity of α-TTP for α-T basically ensures that this vitamin E isoform is the preferred form being transported to various tissues. Our results appear to imply that a similar α-T binding protein may exist in tilapia liver, although the isolation of such a protein has not been reported in fish. Based on the results obtained from our previous work (Ng *et al.*, 2004; Wang *et al.*, 2006), we assumed the existence of an α-TTP which expresses different relative affinities for the three tocotrienols with the relative affinity of α-T3 for α-TTP is the highest, followed by γ-T3 and then δ-T3. Despite the lower deposition of T3 in fish tissues, the lower levels of lipid peroxidation products measured compared to tissues from fish fed equivalent dietary concentrations of synthetic vitamin E esters, led us to conclude that tocotrienols has greater antioxidant potential when used in tilapia feeds.

POTENTIAL APPLICATION OF RESEARCH RESULTS

Longer Shelf-life and Quality of Fish Products

Elevated dietary levels of TRF resulted in marked increases in the deposition of vitamin E in fish tissues, improving oxidative stability which in turn can effectively prolong the storage duration or shelf-life of fresh and frozen fish fillets and surimi-based products. This will lead to increased profits for fish processors. As a potent natural antioxidant, palm vitamin E may enhance the deposition of carotenoids in pigmented seafood such as salmon and shrimp; thus, enhancing flesh quality, consumer acceptance and marketability. The accumulated palm vitamin E in these seafood products would slow down the oxidation of the natural pigments thereby maintaining a desirable colour for longer periods. The reddish colour in seafood products is often used as a quality parameter, and may increase the market value.

Human Health Benefits

The deposition of tocotrienols (and other non-α-T isoforms) in fish fillets also adds value to the product as the potential health benefits of tocotrienols in the human diet may include such beneficial effects as the prevention of cardiovascular diseases, cancer and stroke, among other degenerative diseases (Watkins *et al.*, 1999). Concentrations of tocotrienols in the final seafood product can be pre-determined by dietary manipulations of the diet fed to farmed fish. Japanese restaurants are mushrooming in most large cities of the world, and many urbanites are drawn to nicely-packaged ready-to-eat foods such as sushi and sashimi sold in major supermarkets. The full health benefits of tocotrienols to the human consumer will be obtained when seafood products are consumed raw as sashimi. Seafood products are already known for their health benefits, and reputable organizations such as the American Heart Association strongly endorse the use of omega-3 fatty acids (found in fatty fish) for cardiovascular disease prevention. Combined with the health benefits of tocotrienols found in farmed fish which are fed diets supplemented with palm TRF, the image of fish and seafood as healthy meat products will be further enhanced in the public's perception.

Fish Offal Oil

The perivisceral adipose tissue of tilapia was the major depot for vitamin E among the various tissues examined (Wang *et al*., 2006). At all dietary inclusion levels of TRF, total vitamin E concentrations found in the adipose tissue were the highest. Unlike other tissues, the adipose tissue of tilapia fed with palm TRF was rich in tocotrienols which made up almost 50% of the total vitamin E deposited. This makes fish offal oil a very useful by-product from the processing factories of farmed fish, and can be marketed as a tocotrienol-enriched fish oil targeting the health food sector. The concentrations of tocotrienols deposited in the perivisceral adipose tissue is dependent on dietary concentrations and have been shown to respond linearly (Wang *et al.*, 2006).

Commercial Potential

Plans are currently undertaken to conduct further pre-commercialization research on the use of a feed-grade TRF extracted and concentrated from CPO for use as a natural additive in aquaculture finishing feeds, especially for farmed fish with a high fat content. The product being all natural, the TRF can also be used as an antioxidant in organic aquaculture production. This is a novel concept of delivering tocotrienols to a wider variety of consumer products. It is anticipated that such tocotrienol-enriched fish and seafood products can be sold to niche markets, especially in developed countries and in large cities where health-conscious consumers are willing to pay a premium price for such products. A feed-grade TRF is currently not commercially available for the animal feed industry. Commercially available pharmaceutical grade TRF in the market is not price competitive compared to synthetic vitamin E currently used in the livestock and aquaculture feed industry.

CONCLUSION

The ever expanding oil palm cultivation in Malaysia and other tropical countries offers the possibility of a growing, cost-effective and sustainable alternative to synthetic vitamin E in fish feeds. Results from our research show that supplementation of TRF from CPO can result in significant deposition of palm tocopherols and tocotrienols into various fish tissues, which in turn can inhibit or slow down lipid peroxidation within these tissues. This is the first reported study on the deposition of tocotrienols in tilapia and catfish tissues. Naturally-occurring vitamin E found in vegetable oils such as palm oil can be an excellent alternative for synthetic *all-rac*-α-tocopheryl acetate as a dietary vitamin E source for fish. Further laboratory and commercial-scale studies are planned to fully exploit palm tocotrienols as a dietary vitamin E in aquafeeds.

ACKNOWLEDGEMENT

The first author would like to thank the Malaysian Palm Oil Board for the invitation to present the paper at the International Palm Oil Congress (PIPOC 2007).

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