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**Effect of different dietary vitamin E levels on growth, fish  
composition, fillet quality and liver histology of meagre  
(*Argyrosomus regius*)**

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**Abstract (150-200 Words)**

Seven experimental isonitrogenous (50%) and isolipidic (16%) diets with different  
levels of  $\alpha$ -tocopherol acetate (16, 100, 190, 285, 430, 880 and 1300 mg kg<sup>-1</sup>) were  
tested during 72 days to evaluate growth performance, tissue composition, fillet  
oxidation and liver histology in meagre juveniles, *Argyrosomus regius*. Growth  
performance, feed conversion ratio (FCR) and tissue composition were similar among  
treatments ( $P > 0.05$ ). In the liver, no major differences were recorded in lipid and fatty  
acid composition but higher lipid vacuolization were observed in diets E100, E190 and  
E880. Muscle fatty acid profiles showed an increment of the highly unsaturated fatty  
acid (HUFA) and a decrease of the saturated fatty acid with the increase of dietary  
vitamin E, which was accompanied with a reduction of the muscle TBARS responses.

Therefore, is suggested that diets for this species should be supplemented with  
451 mg kg<sup>-1</sup> of DL- $\alpha$ -tocopherol acetate (496 UI of vitamin E), as determine by broken-

31 line regression analysis of muscle TBARS, to provide good overall growth performance  
32 and improved fish quality and storage stability. Moreover, results suggest that vitamin E  
33 deficiency or excess may deteriorate fish health.

34 **Keywords:** Growth performance, vitamin E and muscle TBARS.

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## 1.Introduction

Meagre *Argyrosomus regius* is a teleost fish of the family Sciaenidae, that can be found from 15 to about 200 m depth, in subtropical waters of the Mediterranean and Black Sea, along the Atlantic coasts of Europe and the East coast of Africa (Whitehead *et al.*, 1986). Meagre has been accredited as a potential candidate for the diversification of European aquaculture, mainly due to its fast growth (around 1kg in 18 months), flesh lipid quality (Poli *et al.*, 2003, Piccolo *et al.*, 2008, Hernández *et al.*, 2009, Grigorakis *et al.*, 2011) and high economic value, with retailers prices ranging between 7 and 12€/ kg depending on the areas (Montfort, 2010). Moreover, this species is easily adapted to captivity, displaying high capacity to tolerate wide ranges of temperature and salinity (Quémener *et al.*, 2002, Suquet *et al.*, 2009). Commercial ongrowing of meagre in the Mediterranean is presently carried out on both land-based tanks and offshore floating cages, with stocking densities between 10 and 15 kg m<sup>-3</sup> and total production reaching around 5000 tonnes (FEAP, 2105).

From the nutritional point of view, meagre is a carnivorous species with a natural diet based on Mysidacea, Decapoda, Echinoderms, Polychaetous, molluscs and teleostei (Cabral and Ohmert, 2001). Until now, no specific diet has been used to produce this species, being currently feed with commercially available seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) diets. However these diets may be inadequate due to the higher growth potential of meagre. To achieve competitive meagre culture, knowledge on specific dietary requirements are required. However, only a few works have been reported in the last years, mainly regarding dietary protein and lipid requirements. The protein and lipid requirement to achieve optimum growth in this species has been estimated to be around 50 % and 17%, respectively (Chatzifotis *et al.*, 2012, Chatzifotis *et al.*, 2010). Additionally, it was observed that it was possible to



include up to 75% of total dietary protein content as a mix of plant ingredients (Estévez *et al.*, 2011) or replace up to 20% of fishmeal by carob seed germ meal (Couto *et al.*, 2016) with no major impacts on growth performance of meagre juveniles. In terms of body contents, meagre is a lean fish, showing less than half of the whole body lipid content of European seabream and seabass.

Until now, there are no studies focusing on the vitamin requirement of *A. regius*. The vitamin E ( $\alpha$ -tocopherol) is one of the most studied vitamins due to its important physiological implications in all species. Vitamin E exists in groups of eight lipid soluble compounds, four tocopherols and four tocotrienols (NRC, 2011), and represents one of the most important natural antioxidants to prevent the deleterious effect caused by reactive oxygen (ROS) and free radicals (Hamre, 2011, Mourente *et al.*, 2007, Di Mascio *et al.*, 1991). Vitamin E is usually supplied in fish diets as  $\alpha$ -tocopherol acetate, due to its higher stability and oxidation resistance during feed processing and storage (Hamre and Lie, 1995, Peng *et al.*, 2008). It's the ability to donate their phenolic hydrogen atoms to lipid-free radicals (Burton, 1989, Mourente *et al.*, 2007), acting as quenchers of singlet oxygen free radicals, that renders these substances the capacity to protect tissues from damage, especially the unsaturated fatty acids (PUFA) of the cellular membrane that are more susceptible to oxidation. Due to this fact, several authors have demonstrated that by supplementing diets with vitamin levels well above the requirement, approximately 50 mg kg<sup>-1</sup> for most fish species (NRC, 2011), fillet quality was improved by increasing oxidative stability and its shelf-life (Bell *et al.*, 2000, Ruff *et al.*, 2003, Ruff *et al.*, 2002, Gatta *et al.*, 2000, Hamre *et al.*, 2004, Jittinandana *et al.*, 2006, Peng and Gatlin III, 2009).

Apart providing protection from oxidation, some studies demonstrated that vitamin E deficiency impair growth performance in Atlantic salmon (Hamre and Lie,

1995), channel catfish fingerlings (Wilson *et al.*, 1984), rainbow trout (Cowey *et al.*, 1984), black sea bream (Peng *et al.*, 2009), sea bream (Tocher *et al.*, 2002) and spotted murrel juveniles (Abdel-Hameid *et al.*, 2012). However, in some studies the influence of vitamin E on growth was not observed (Cowey *et al.*, 1983, Cowey *et al.*, 1981, Wilson *et al.*, 1984, Baker and Davies, 1996a, Bell *et al.*, 2000, Gaylord *et al.*, 1998, Gatlin *et al.*, 1992). The effect of vitamin E on growth performance is still unclear and its importance needs to be assessed, particularly in a fast growing fish species such as meagre.

The aims of present work was to study the impact of different vitamin E dietary levels during the ongrowing phase of *A. regius* on growth performance and feed utilization parameters, as well as on the biochemical composition of fish fillet and liver histology, muscle and liver fatty acid profile and muscle TBARS.

## **2. Materials and Methods**

### **2.1. Diets**

Seven isonitrogenous (50% protein) and isolipidic (16% lipid) fish meal and oil experimental diets (Table 1) were prepared by adding different levels of vitamin E (0, 100, 200, 300, 500, 1000 and 1500 mg kg<sup>-1</sup>) at expense of the  $\alpha$ -cellulose. The vitamin E was provided as DL- $\alpha$ -tocopherol acetate (Sigma-Aldrich, Madrid, Spain), due to its higher stability and oxidation resistance during feed processing and storage (Peng et al., 2008). Diets were named from E16 (no vitamin E addition) to E1300 (1500 mg of vitamin E) according to its vitamin content. All diets were prepared by mixing the ingredients carefully in a horizontal mixer (DANAMIX BM 330, Azpeitia, Gipuzcua, Spain) and then cold pelletized (California Pellet mill, CPM de 2HP mod 8.3, USA), throughout a 3mm matrix diameter, and dried in an air-oven at 35°C during the night. Diets were bulk stored in a dark and refrigerated chamber at 10 °C. Daily, the amount required was removed in order to preserve the diet quality throughout the trial. A sample of each diet was taken and stored at -80°C for subsequent biochemical analysis. The formulation and proximate composition of diets are shown in Table 1.

### **2.2. Fish and Culture Conditions**

Meagre juveniles were obtained from broodstock-induced spawning at the Fundación Parque Científico y Tecnológico facilities (Telde, Canary Island, Spain). After anesthetized with clove oil (4 ml/100 L), fish initial weight (62.90±12.95 g) and length were recorded and randomly distributed in circular fiberglass tanks of 500 L in triplicate groups of 50 fish. All tanks were net covered to prevent escapes of fish, and supplied with natural seawater and air injection, being the experiment carried out under natural photoperiod of about 11h light/13h dark according to season (October to

December). The temperature and dissolved oxygen concentration were measured twice a week with an oxymeter (Oxy Guard, Handy Polaris V 1.26), with values from 21.1 to 23.6°C and 6.1 to 6.9 mg L<sup>-1</sup> for temperature and dissolved oxygen, respectively. Fish were carefully hand-fed three times per day (08:00, 11:00 and 14:00 h), six days per week for 72 days. After feed distribution, remaining feed in the bottom of the tanks was collected and dry to correct feed intake.

### 2.3. Theory/calculation

At days 23, 48 and 72 from the start of the trial, fish were anesthetized and individual whole body weight and standard length recorded. Fish were unfed for 24 hours before all samplings. Obtained data were then analysed according to the subsequent equations to study fish response for survival, growth and feed utilization parameters. Means and standard deviations of each triplicate were calculated for each treatment.

Survival (%) = 100 x (final number fish – initial number fish)/ initial number fish

Growth (%) = 100 x ((final mean weight – initial mean weight)/initial mean weight)

Weight gain (g) = final mean weight- initial mean weight.

SGR: Specific growth rate (%) = 100 x (ln final mean weight – ln initial mean weight)/ number of days.

FI: Feed intake (g) = feed intake for 72 days experiment (g)/number of fish

FCR: Feed conversion ratio= feed intake (g)/weight gain (g)

PER: Protein efficiency ratio = weight gain (g)/protein intake (g) (dry matter)

K: Condition factor (%)= 100 x (final weight /(final length)<sup>3</sup>)

HSI: Hepatosomatic index (%)= 100 x (liver weight/final weight)

VSI: Visceral index (%)= 100 x ((final weight-final eviscerated fish weight)/final weight)

The index of atherogenicity and thrombogenicity, related to effects of different fatty acids on human's health, were calculated according to Ulbricht and Southgate (1991):

Index of atherogenicity (AI)

$$AI = [(12:0) + (4 \times 14:0) + (16:0)] \times [(PUFA \text{ } n-6 \text{ and } n-3) + MUFA]^{-1}$$

Index of thrombogenicity (TI)

$$TI = [(14:0) + (16:0) + (18:0)] \times [(0.5 \times MUFA) + (0.5 \times n-6) + (3 \times n-3) + (n-3/n-6)]^{-1}$$

#### 2.4. Sample Collection and Biochemical Analysis

At the end of the trial fish were anesthetized and weight and length were recorded. Nine fish per treatment were used for whole body composition. Liver and muscle from 4 fish per tank were removed for biochemical analyses and the opposite muscle side recovered for TBARS analysis. All samples were weighted and stored at -80°C until analysed. For histological evaluation, livers from 5 fish per tank were collected and fixed in 10% buffer formaldehyde. Fish were fasted for one day before all samplings and sacrificed by immersion in iced seawater.

Feed samples and whole fish and muscle pools from all tanks were analysed in triplicate. In case of liver pools samples, only total lipid and moisture analysis were carried out. All samples were homogenized for crude protein, moisture and ash content analyses according to AOAC (2006). Totals lipids analysis were performed by the method described by Folch *et al.* (1957) and fatty acids methyl esters were determined by trans-esterification of the total lipids with 1% sulphuric acid in methanol according to Christie (1982). Fatty acid methyl ester (FAMES) were diluted in hexane and

separated, identified and quantified by gas chromatography under the conditions described by Izquierdo *et al.* (1990). Individual methyl esters were identified by comparison with external standard (EPA 28, Nippai, Ltd. Tokyo, Japan).

## **2.5. Measurement of thiobarbituric acid-reactive substances (TBARS)**

The 2-thiobarbituric acid-reactive substances (TBARS) analysis were performed in the muscle according to Shahidi and Zhong (2005).

Muscle samples (1g) were first homogenized with 2 mL of 10% (w/v) trichloroacetic acid (TCA). Then, samples and two blanks homogenates were centrifuged at 4000g for 30 min at 4°C. Once centrifuged, the supernatant was filtered and mixed with the same volume of thiobarbituric acid (TBA, 0.02M). Then, the samples and blanks were stirred and heated at 90°C for 20 minutes. Finally, the absorbance of the supernatant was measured at 532 nm by UV/Vis spectrophotometer (Thermo Scientific, Evolution 300 model, Chicago, USA) compared with two blanks. A standard solution with malonaldehyde (T-1642, Sigma-Aldrich, Munich, Germany) was used to obtain a calibration curve and absorbance values were correlated with this curve in order to calculate the amount of malonaldehyde (MDA) in fillets. TBARS values were expressed as mg of malonaldehyde per kg of fillet.

## **3.6. Histological analysis**

For histological analysis, five liver samples per tank (n=15 per treatment) were fixed in 10% buffered formaldehyde dehydrated through graded alcohol, then xylene, and finally embedded in paraffin. The paraffin blocks were serially cut at 5µm and stained with haematoxylin and eosin (H&E) (Martoja and Martoja-Pierson, 1970) before examination under a light microscope. Stained sections of liver were assessed for

cytoplasmic lipid vacuolization using a four graded score: 0, not observed; 1, few; 2, medium; 3, and severe.

### **3.7. Statistical analysis**

All data was tested for normality of distribution and homogeneity of variance (Zar, 1999). Data were analysed with one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons when significant differences were observed among groups ( $P < 0.05$ ). When data did not display normal distribution and homogeneity of variance a non-parametric analysis and multiple-range test (Kruskal-Wallis) was applied followed by a multiple comparisons of mean ranks. Analyses were performed using the SPSS 15.0 (IBM Corp., New York, USA) statistical package. Significant differences were considered when  $p \leq 0.05$ . The optimum dietary vitamin E level was estimated by broken-line regression analysis (Figure 1) of muscle TBARS (Robbins *et al.*, 1979).

### **3.Results**

#### **3.1. Biochemical composition of the experimental diets**

Biochemical and fatty acid composition of the experimental diets are shown in Table 1. The concentration of the vitamin E of all experimental diets differed slightly from the added content mainly due to losses during feed processing.

#### **3.2. Growth performance and feed utilisation**

The inclusion of dietary vitamin E did not affect growth performance, feed utilisation and biometric parameters of meagre juveniles fed the different levels of vitamin E for 72 days (Table 2). Thus, no significant differences ( $P > 0.05$ ) were found for final weight and length, weight gain and SGR, fish survival, feed intake, FCR and PER. Moreover, no differences were recorded on condition factor (K) and hepatosomatic and visceral indexes for different levels of dietary vitamin E.

#### **3.3. Whole body, muscle and liver proximate composition**

Whole body proximate composition was significantly ( $P < 0.05$ ) affected by vitamin E addition (Table 3). Whole body protein of fish fed on E16 and E285 diets were higher than E100 and E430 treatments. Moisture of fish fed on the E16 diet was the lowest (73.35 % wet weight). Whole body lipid and ash showed no significant differences ( $P > 0.05$ ) among treatments.

Regarding liver composition (Table 3), no significant differences were found in moisture and lipid among the different groups. Nonetheless, the lowest values were recorded in fish fed E190 and 285 diets.



Muscle composition did not show significant differences either in protein, moisture or ash, while the lipid content was affected by different treatments (Table 3). Muscle lipid content was significantly higher in meagre fed with no addition of vitamin E (E16) compared with the E190 group.

### **3.4. Liver and muscle fatty acid composition**

Liver fatty acids profile (Table 4) was not affected by dietary vitamin E. The saturated fatty acids levels ranged from 28.52 to 30.58 % (g/100g of fatty acids) and no significant differences were observed. Monounsaturated fatty acids levels varied between 39.92 and 41.12 % (g/100g of fatty acids) and were the most predominant fatty acid class, with oleic acid corresponding to more of 50% of the total unsaturated fatty acids. Long-chain polyunsaturated were not affected by vitamin E levels. DHA and EPA were similar between diets.

Muscle fatty acids were affected by the increase of dietary vitamin E (Table 5), showing a decrease of the saturated and monounsaturated fatty acids as the dietary vitamin E increased, although without significant differences. Muscle LC-PUFA content was higher in fish fed on E880 and E1300 diets, but without significant differences. Nonetheless, LC-PUFA levels were increased more than two-fold from the low vitamin diet (11.64 g/100g of fatty acids) to the high vitamin diets (E880 and E1300, 25.64 and 23.87 g/100g of fatty acids, respectively), especially due to the increase of DHA and EPA levels. EPA was significantly higher in the muscle of the meagre fed with the E 880 diets, compared with other treatments.

### **3.5. Muscle thiobarbituric acid reactive substances (TBARS)**

Vitamin E had a significant ( $P < 0.005$ ) effect on lipid muscle oxidation (Table 6). Muscle TBARS levels were significantly higher in fish fed the E16 and E100 diets compared to fish fed the highest levels.

The recommended level of vitamin E to avoid lipid peroxidation is around 451 mg kg<sup>-1</sup> in the form of DL- $\alpha$ -tocopherol acetate, as determined by broken-line regression analysis (Figure 1).

### 3.6. Liver histology

A higher degree of cytoplasmic vacuolization was observed in the hepatocytes of fish fed E16, E100 and E880 diets (Figure 2), showing large hepatocytes with a displacement of cell nuclei. However, fish fed E285 and E430 diets showed regular hepatocyte morphology with cytoplasmic lipid vacuoles that did not alter hepatocyte size (Figure 2). Scoring of liver lipid vacuolization was slightly higher in fish fed the lowest and highest dietary vitamin E ( $1.98 \pm 0.74$  for diet E16;  $2.01 \pm 0.14$  for diet E100;  $2.00 \pm 0.12$  for diet E190;  $1.96 \pm 0.18$  for diet E880;  $1.87 \pm 0.38$  for diet E1300) although not significantly different with those found in fish fed E285 and E430 diets ( $1.86 \pm 0.38$  and  $1.81 \pm 0.40$ , respectively).

#### 4. Discussion

Meagre is seen as a new candidate for the Mediterranean aquaculture, but to our best knowledge, there is little data on nutrient requirements for this species. In the present work, all diets formulated on basal fishmeal and fish oil with different vitamin E levels were well accepted. This was reflected in feed intake (1.9 to 2.1 g fish<sup>-1</sup> day<sup>-1</sup>), specific growth rate (1.4 to 1.5 %) and feed conversion ratios (0.73 to 0.95), which were identical among groups irrespective of dietary vitamin E level. Similar responses have been reported in other species such as catfish (Gaylord et al., 1998, Wilson et al., 1984), red drum (Peng and Gatlin III, 2009), African catfish (Baker and Davies, 1996a), turbot (Stéphan *et al.*, 1995a, Tocher et al., 2002) or rainbow trout (Cowey et al., 1981). Nonetheless, this response seems to be species dependent as it was observed that growth can be affected by low vitamin E levels in Atlantic salmon (Hamre and Lie, 1995), common carp, (Watanabe *et al.*, 1970), spotted murrel (Abdel-Hameid et al., 2012), grouper (Lin and Shiau, 2005), hybrid striped bass juveniles (Kocabas and Gatlin, 1999) or Korean rockfish (Bai and Lee, 1998). The lack of differences in growth performance can be related to the trial time (72 days) and the high amount of vitamin C supplemented in the diets (5000 mg kg<sup>-1</sup>). Tappel (1972) was the first to hypothesize that vitamin C could promote a sparing effect on vitamin E, where oxidized vitamin E could be regenerated to its reduced form by ascorbate. In some fish species this sparing action has been suggested (Yildirim-Aksoy *et al.*, 2008, Betancor *et al.*, 2012, Hamre, 2011, Mourente et al., 2007, Ortuño *et al.*, 2001) having an influence on growth, tissue composition or immune responses. The duration of the trial is also an important factor affecting vitamin E deposition or depletion. Fish were fed with a commercial diet before the trial, which supplemented with vitamin E, thus the 72 days were probably not enough to induce growth impairment. Nonetheless, in some studies where trial duration

was superior to 72 days (300 days (Gaylord et al., 1998) and 140 days (Wilson et al., 1984)), no effects on growth were also observed. It is also worth notice that studies where growth differences were recorded used either purified or semi-purified diets, suggesting that practical diets contain other substances that can counteract the effects of vitamin E deficiency. The combined effect of the trial duration and the presence of high amounts of vitamin C in the diets probably resulted in lack of differences in growth performance, even in the fish fed the lowest vitamin E level (E16). Is quite difficult to assess all the factors involved in vitamin E requirement and further studies should be performed to understand vitamin E needs in this species. Nonetheless, this was one of the best growth performance recorded so far for this species, in line with the results attained by Couto et al. (2016). Indeed, most of the growth trials undertaken with this species (Chatzifotis *et al.*, 2006, Chatzifotis et al., 2012, Chatzifotis et al., 2010, Estévez et al., 2011, Martínez-Llorens *et al.*, 2011) obtained poorer growth performances and lower feed intake even when compared with the lowest vitamin E level (E16) used in the present trial. The differences in growth performance were possible attributed to the lower dietary protein (47% protein %DM) (Estévez et al., 2011, Martínez-Llorens et al., 2011) and lower rearing temperature (Chatzifotis et al., 2006, Chatzifotis et al., 2012, Chatzifotis et al., 2010, Estévez et al., 2011, Martínez-Llorens et al., 2011), which was less 2-4 degrees compared to the present trial. The optimum dietary protein and lipid for the species has been estimated to be around 50% and 17% (Chatzifotis et al., 2012), respectively, which are in line with the levels used in this trial. Moreover, temperature is one major abiotic factor that affects fish growth and survival (Ibarz *et al.*, 2010, Imsland *et al.*, 2007, Brett, 1979). In the future, optimum temperatures should be tested to improve rearing conditions for this species.

Diet composition affected whole body protein and moisture contents, but it does not seem related to dietary vitamin E level since higher protein levels were found in the vitamin E-deficient group. In previous studies (Chatzifotis et al., 2012, Martínez-Llorens et al., 2011), meagre fed with similar diets or with different protein/lipid ratios displayed similar whole body moisture and lipid content. Muscle lipid content was significantly higher in fish fed the low vitamin E diet, but only when comparing with diet E190, with similar values found in fish fed the other diets. In turbot (Ruff et al., 2003) and European seabass (Gatta et al., 2000) no significant differences were recorded in muscle composition, but an increasing trend in lipid content was observed in groups fed with high vitamin E diets (approximately 1000 mg kg<sup>-1</sup>). Similar results were recorded in trout (Jittinandana et al., 2006).

In the present trial, different inclusion levels of vitamin E did not lead to significant differences in the liver or viscera to body weight ratios among treatments. Liver fatty acid composition was constituted mainly by the monounsaturated (40.1 to 41.1%), followed by the saturated (28.5 to 30.6%), the n-3 (15.9 to 18.7%) and the n-6 (5.9 to 7.5%) classes, but in the overall no major effects have been observed. Despite the lack of significant differences, fish fed either with low or high vitamin E diets presented higher liver lipid vacuolization than the fish fed with diets E285 and E430. In Atlantic salmon, Bell et al. (2000) observed that a deficiency of vitamin E and astaxanthin in the diet caused a stimulation of hepatic fatty acid desaturation and elongation activities promoting hepatic tissue damage such as vacuolated hepatocytes and ceroidosis. Bai and Lee (1998) showed that a total lack of vitamin E (0 mg kg<sup>-1</sup>) exerts a negative effect on liver lipid peroxidation in Korean rockfish (*Sebastes schlegeli*). In the present trial, only at intermediate levels (285 and 430 mg kg<sup>-1</sup>) of dietary vitamin E liver presented normal morphology, with fish fed with either low or

high vitamin E levels developing high degree of liver lipid vacuolization. Likewise, in sea bream (Tocher et al., 2002) reported a significant decrease on liver long-chain polyunsaturated acids at vitamin E intermediate levels (100 mg kg<sup>-1</sup> of  $\alpha$ -tocopherol) compared with the other treatments.

The slight increase in liver lipid vacuolization observed in fish fed the high vitamin E diets can be related to its pro-oxidant effect. It has been shown that an excess of dietary vitamin E and low amount of vitamin C can lead to an increase of tissues tocopheroxyl radicals that can abstract hydrogen atoms from PUFA, starting lipid peroxidation (Ingold *et al.*, 1993, Bowry *et al.*, 1992) and leading to tissues damage in fish (Gatta et al., 2000; Tocher et al., 2002; Hamre 2011). However, as already pointed out, in the present trial vitamin C was in excess, suggesting that even in this condition the pro-oxidant effect could prevail.

Slight differences were recorded in muscle EPA proportion when dietary vitamin E increased. Despite the lack of significant differences, DHA and long chain PUFA also exhibited an increasing trend, with muscle levels increasing two-fold from the vitamin E deficient group (6.1 and 11.6 %) to the E1300 group (22.7 and 23.9 %). The atherogenic (0.53 to 0.79) and thrombogenic (0.33 to 0.84) indexes exposed this difference, with fish fed high vitamin diets presenting lower AI and TI (Table 5). In carp, Watanabe and Takashima (1977) observed the same effect on muscle fatty acids while in Atlantic salmon the opposite effect was recorded, but probably due to the low feed intake in this trial (Hamre and Lie, 1995). Watanabe and Takashima (1977) suggested that dietary deficiency of  $\alpha$ -tocopherol could exert some effects on fatty acid composition, but when exceeding the requirement level had little effect on fatty acid composition of fish. Likewise, dietary  $\alpha$ -tocopheryl acetate levels and length of feeding did not alter liver and muscle fatty acids composition in Atlantic salmon fed with levels above the

requirement (Scaife *et al.*, 2000). In a study performed in gilthead seabream, turbot and halibut (Tocher *et al.*, 2002), it was observed that not all species responded similarly with the increase in dietary vitamin E, probably owing it to the different capacity to store vitamin E. Coherently, there were significantly lower percentages of EPA, DHA and PUFA only in the liver of halibut, which presented the lowest levels of liver vitamin E and the highest PUFA to vitamin E ratio compared to the other species. In turbot (Stéphan *et al.*, 1995b) it was demonstrated that the appearance of TBARS was correlated with the disappearance of *n*-3 and *n*-6 fatty acids with 4, 5, or 6 double bonds. Furthermore, dietary fish oil increased the susceptibility to *in vivo* and *in vitro* fatty acid peroxidation but vitamin E supplementation was able to mitigate this phenomenon. However, in rats fed vitamin E deficient diets (Buttriss and Diplock, 1988) there is an increase of 22:6n-3 and 20:4n-6 in mitochondrial and microsomal membranes, possibly as the result of an increased activity of fatty acid desaturation and elongation mechanisms. A similar effect has also been found in African catfish fed oxidized oil (Baker and Davies, 1996b). The effect of vitamin E on fatty acid composition is unclear but the intrinsic capacity to store lipids from each species and the tissue where is stored seems to be a key factor.

The most common criteria used to determine the vitamin E requirement used have been based on weight gain or liver microsomal peroxidation. The dietary requirement obtained in different species range between 27 to 153 mg kg<sup>-1</sup> as DL-tocopherol acetate: in red drum (Peng and Gatlin III, 2009) was 27 mg kg<sup>-1</sup>, 54 to 100 mg kg<sup>-1</sup> for grouper (Lin and Shiau, 2005), depending on the dietary lipid level, 20-30 mg kg<sup>-1</sup> for Atlantic salmon (Hamre and Lie, 1995), 50 mg kg<sup>-1</sup> for rainbow trout (Cowey *et al.*, 1983), 15 to 45 mg kg<sup>-1</sup> for channel catfish (Gaylord *et al.*, 1998, Wilson *et al.*, 1984), depending of fish size, 100 mg kg<sup>-1</sup> for common carp (Watanabe *et al.*,

1970), 40 mg kg<sup>-1</sup> for Korean rockfish (Bai and Lee, 1998) and 127 to 154 mg kg<sup>-1</sup> for *Channa punctatus* (Abdel-Hameid et al., 2012). The different responses to dietary vitamin E could be relate to fish species, size and development stage, culture condition, different levels and organ storage of vitamin E, as well as the added effect of different antioxidant nutrient presented in diets. Dietary vitamin E requirement can be increased by dietary factors such as polyunsaturated fatty acids, oxidized lipid (Cowey et al., 1984, Zhong *et al.*, 2008), as well as presence and abundance of other antioxidant nutrients such as selenium and vitamin C (Betancor et al., 2012, Bell *et al.*, 1985), or astaxanthin (Hamre, 2011). The optimum dietary vitamin E level in meagre was 451 mg kg<sup>-1</sup> diet, as determine by the broken-line regression method, based on muscle TBARS levels. Muscle TBARS level is not commonly used as a criteria to establish the optimum dietary vitamin E level, although it is a good indicator of the overall quality of the fish, especially of the muscle that is the edible portion. Currently, commercial diets are already being supplemented with 150-300 mg of tocopherols in order to take advantage of the antioxidant effect of vitamin E and its health effects. Thus, our intention was not only assess the level needed to avoid any sign of vitamin E deficiency but also improve fillet quality by increasing its storage stability. Due to the poikilothermic nature of fish, their cell membranes are rich in polyunsaturated fatty acids and hence more prone to peroxidation (Tocher, 2003). An increase of the fillet quality and protection from lipid oxidation by an increment of the vitamin E in the diet has been reported in different studies. Jittinandana and co-workers (2006) observed that trout fed with 5000 mg kg<sup>-1</sup> of vitamin E (DL- $\alpha$ -tocopheryl acetate) displayed a significantly lower muscle TBARS compared to fish fed diets with 200 mg kg<sup>-1</sup> of vitamin E. In turbot (Ruff *et al.*, 2003; Stéphan et al., 1995), muscle TBARS level was significantly ( $P < 0.01$ ) lower in fish fed high  $\alpha$ -tocopheryl acetate level diets, being this



phenomenon accentuated after six months of freeze storage or in fish oil based diets (Stéphan et al., 1995). In sea bream, (Gatta et al., 2000) similar results were obtained. Nonetheless, it is worth noticing that in the present trial, even at the lowest dietary level of vitamin E, muscle TBARS were below the maximum values ( $1.51 \text{ mg kg}^{-1}$  of MDA) to deem fish as rancid and unacceptable for consumption (Ke *et al.*, 1984).

In conclusion, dietary vitamin E levels did not influence growth performance and whole body composition of meagre. Nonetheless, fish fed the intermediate levels (285 and  $430 \text{ mg kg}^{-1}$ ) presented normal liver histology when compared to fish fed the lower ( $16$  and  $190 \text{ mg kg}^{-1}$ ) and higher ( $880$  and  $1300 \text{ mg kg}^{-1}$ ) levels, where a higher degree of lipid vacuolization was observed. Accordingly, it is suggested the inclusion of  $451 \text{ mg kg}^{-1}$  of DL- $\alpha$ -tocopherol acetate (496 UI of vitamin E) in meagre diets, as determined by broken-line regression analysis of muscle TBARS, to provide good overall growth performance and improved flesh quality and storage stability.

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#### Reference

- Abdel-Hameid, N.-a. H., Abidi, S. F. & Khan, M. A. 2012. Dietary vitamin E requirement for maximizing the growth, conversion efficiency, biochemical composition and haematological status of fingerling *Channa punctatus*. *Aquac. Res.*, 43, 226-238.
- Aoac 2006. *Official methods of analysis of AOAC International*, Maryland, USA, AOAC International.

454 Bai, S. C. & Lee, K.-J. 1998. Different levels of dietary dl- $\alpha$ -tocopheryl acetate affect the  
455 vitamin E status of juvenile Korean rockfish, *Sebastes schlegeli*. *Aquaculture*, 161, 405-  
456 414.

457 Baker, R. T. M. & Davies, S. J. 1996a. Changes in tissue  $\alpha$ -tocopherol status and degree of lipid  
458 peroxidation with varying  $\alpha$ -tocopheryl acetate inclusion in diets for the African catfish.  
459 *Aquac. Nutr.*, 2, 71-79.

460 Baker, R. T. M. & Davies, S. J. 1996b. Increased production of docosahexaenoic acid (22:6 n-3,  
461 DHA) in catfish nutritionally stressed by the feeding of oxidized oils and the  
462 modulatory effect of dietary  $\alpha$ -tocopheryl acetate. *J. Fish Biol.*, 49, 748-752.

463 Bell, J. G., Cowey, C. B., Adron, J. W. & Shanks, A. M. 1985. Some effects of vitamin E and  
464 selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in  
465 rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.*, 53, 149-157.

466 Bell, J. G., Mcevoy, J., Tocher, D. R. & Sargent, J. R. 2000. Depletion of  $\alpha$ -Tocopherol and  
467 Astaxanthin in Atlantic Salmon (*Salmo salar*) Affects Autoxidative Defense and Fatty  
468 Acid Metabolism. *The Journal of Nutrition*, 130, 1800-1808.

469 Betancor, M., Caballero, M. J., Terova, G., Corà, S., Saleh, R., Benítez-Santana, T., Bell, J. G.,  
470 Hernández-Cruz, C. & Izquierdo, M. 2012. Vitamin C Enhances Vitamin E Status and  
471 Reduces Oxidative Stress Indicators in Sea Bass Larvae Fed High DHA Microdiets.  
472 *Lipids*, 47, 1193-1207.

473 Bowry, V. W., Ingold, K. U. & Stocker, R. 1992. Vitamin E in human low-density lipoprotein.  
474 When and how this antioxidant becomes a pro-oxidant. *Biochem. J.*, 288, 341-344.

475 Brett, J. R. 1979. Environmental factors and growth. In: HOAR, W. S., RANDALL, D. J. &  
476 BRETT, J. R. (eds.) *Fish Physiol.* New York: Academic Press.

477 Burton, G. W. 1989. Antioxidant Action of Carotenoids. *The Journal of Nutrition*, 119, 109-  
478 111.

479 Buttriss, J. L. & Diplock, A. T. 1988. The relationship between  $\alpha$ -tocopherol and phospholipid  
480 fatty acids in rat liver subcellular membrane fractions. *BBA- Lipid Lipid Met*, 962, 81-  
481 90.

482 Cabral, H. & Ohmert, B. 2001. Diet of juvenile meagre, *Argyrosomus regius*, within the Tagus  
483 estuary. *Cah. Biol. Mar.*, 42, 289-293.

484 Chatzifotis, S., Martin-Prat, A., Limberis, N., Papandroulakis, N. & Divanach, P. 2006. First  
485 data on growth of cultured brown meagre *Sciaena umbra* using diets with different  
486 protein and fat contents. *Fisheries Sci*, 72, 83-88.

487 Chatzifotis, S., Panagiotidou, M. & Divanach, P. 2012. Effect of protein and lipid dietary levels  
488 on the growth of juvenile meagre (*Argyrosomus regius*). *Aquac. Int.*, 20, 91-98.

489 Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I. & Mylonas, C. C.  
490 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and  
491 serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture*, 307, 65-70.

492 Christie, W. W. 1982. *Lipid Analysis*, Oxford, UK, Pergamon Press.

493 Couto, A., Barroso, C., Guerreiro, I., Pousão-Ferreira, P., Matos, E., Peres, H., Oliva-Teles, A.  
494 & Enes, P. 2016. Carob seed germ meal in diets for meagre (*Argyrosomus regius*)  
495 juveniles: Growth, digestive enzymes, intermediary metabolism, liver and gut histology.  
496 *Aquaculture*, 451, 396-404.

497 Cowey, C. B., Adron, J. W., Walton, M. J., Murray, J., Youngson, A. & Knox, D. 1981. Tissue  
498 Distribution, Uptake, and Requirement for  $\alpha$ -Tocopherol of Rainbow Trout (*Salmo*  
499 *gairdneri*) Fed Diets with a Minimal Content of Unsaturated Fatty Acids. *The Journal*  
500 *of Nutrition*, 111, 1556-1567.

501 Cowey, C. B., Adron, J. W. & Youngson, A. 1983. The vitamin E requirement of rainbow trout  
502 (*Salmo gairdneri*) given diets containing polyunsaturated fatty acids derived from fish  
503 oil. *Aquaculture*, 30, 85-93.

504 Cowey, C. B., Degener, E., Tacon, A. G. J., Youngson, A. & Bell, J. G. 1984. The effect of  
505 vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*)  
506 grown at natural, varying water temperatures. *Br. J. Nutr.*, 51, 443-451.

507 Di Mascio, P., Murphy, M. E. & Sies, H. 1991. Antioxidant defense systems: the role of  
508 carotenoids, tocopherols, and thiols. *The American Journal of Clinical Nutrition*, 53,  
509 194S-200S.

510 Estévez, A., Treviño, L., Kotzamanis, Y., Karacostas, I., Tort, L. & Gisbert, E. 2011. Effects of  
511 different levels of plant proteins on the ongrowing of meagre (*Argyrosomus regius*)  
512 juveniles at low temperatures. *Aquac. Nutr.*, 17, e572-e582.

513 Feap 2105. European Aquaculture Production Report 2005-2014. Liege, Belgium.

514 Folch, J., Lees, M. & Sloane Stanley, G. H. 1957. A simple method for the isolation and  
515 purification of total lipids from animal tissues. *J. Biol. Chem.*, 226, 497-509.

516 Gatlin, D. M., Bai, S. C. & Erickson, M. C. 1992. Effects of dietary vitamin E and synthetic  
517 antioxidants on composition and storage quality of channel catfish, *Ictalurus punctatus*.  
518 *Aquaculture*, 106, 323-332.

519 Gatta, Pirini, Testi, Vignola & Monetti 2000. The influence of different levels of dietary vitamin  
520 E on sea bass *Dicentrarchus labrax* flesh quality. *Aquac. Nutr.*, 6, 47-52.

521 Gaylord, T. G., Rawles, S. D. & Gatlin Iii, D. M. 1998. Re-evaluation of vitamin E  
522 supplementation of practical diets for channel catfish, *Ictalurus punctatus*, production.  
523 *Aquac. Nutr.*, 4, 109-114.

- Grigorakis, K., Fountoulaki, E., Vasilaki, A., Mittakos, I. & Nathanailides, C. 2011. Lipid quality and filleting yield of reared meagre (*Argyrosomus regius*). *Int. J. Food Sci. Technol.*, 46, 711-716.
- Hamre, K. 2011. Metabolism, interactions, requirements and functions of vitamin E in fish. *Aquac. Nutr.*, 17, 98-115.
- Hamre, K., Christiansen, R., Waagbø, R., Maage, A., Torstensen, B. E., Lygren, B., Lie, Ø., Wathne, E. & Albrektsen, S. 2004. Antioxidant vitamins, minerals and lipid levels in diets for Atlantic salmon (*Salmo salar*, L.): effects on growth performance and fillet quality. *Aquac. Nutr.*, 10, 113-123.
- Hamre, K. & Lie, Ø. 1995. Minimum requirement of vitamin E for Atlantic salmon, *Salmo salar* L., at first feeding. *Aquac. Res.*, 26, 175-184.
- Hernández, M. D., López, M. B., Álvarez, A., Ferrandini, E., García García, B. & Garrido, M. D. 2009. Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. *Food Chem.*, 114, 237-245.
- Ibarz, A., Padrós, F., Gallardo, M., Fernández-Borràs, J., Blasco, J. & Tort, L. 2010. Low-temperature challenges to gilthead sea bream culture: review of cold-induced alterations and 'Winter Syndrome'. *Rev. Fish Biol. Fish.*, 20, 539-556.
- Imsland, A. K., Bjørnsson, B. T., Gunnarsson, S., Foss, A. & Stefansson, S. O. 2007. Temperature and salinity effects on plasma insulin-like growth factor-I concentrations and growth in juvenile turbot (*Scophthalmus maximus*). *Aquaculture*, 271, 546-552.
- Ingold, K. U., Bowry, V. W., Stocker, R. & Walling, C. 1993. Autoxidation of lipids and antioxidation by alpha-tocopherol and ubiquinol in homogeneous solution and in aqueous dispersions of lipids: unrecognized consequences of lipid particle size as exemplified by oxidation of human low density lipoprotein. *Proc. Natl. Acad. Sci. U. S. A.*, 90, 45-49.
- Izquierdo, M. S., Watanabe, T., Takeuchi, T., Arakawa, T. & Kitajima, C. 1990. Optimum EFA levels in *Artemia* to meet the EFA requirements of red sea bream (*Pagrus major*). In: TAKEDA, M. & WATANABE, T. (eds.) *the Current Status of Fish Nutrition in Aquaculture*. Tokyo, Japan.
- Jittinandana, S., Kenney, P. B., Slider, S. D., Kamireddy, N. & Hankins, J. S. 2006. High Dietary Vitamin E Affects Storage Stability of Frozen-refrigerated Trout Fillets. *J. Food Sci.*, 71, C91-C96.
- Ke, P. J., Cervantes, E. & Robles-Martinez, C. 1984. Determination of thiobarbituric acid reactive substances (TBARS) in fish tissue by an improved distillation-spectrophotometric method. *J. Sci. Food Agric.*, 35, 1248-1254.
- Kocabas & Gatlin, Iii 1999. Dietary vitamin E requirement of hybrid striped bass (*Morone chrysops* female × *M. saxatilis* male). *Aquac. Nutr.*, 5, 3-7.

- Lin, Y.-H. & Shiau, S.-Y. 2005. Dietary vitamin E requirement of grouper, *Epinephelus malabaricus*, at two lipid levels, and their effects on immune responses. *Aquaculture*, 248, 235-244.
- Martínez-Llorens, S., Espert, J., Moya, J., Cerdá, M. J. & Tomás-Vidal, A. 2011. Growth and nutrient efficiency of meagre (*Argyrosomus regius*, Asso1801) fed extruded diets with different protein and lipid levels. *Int. J. Fish. Aquac.*, 3, 195-203.
- Martoja, R. & Martoja-Pierson, M. 1970. *Técnicas de Histología Animal*, Barcelona, Spain, Toray-Masson S.A.
- Montfort, M. C. (ed.) 2010. *Present Market Situation and Prospects of Meagre (Argyrosomus regius), as an Emerging Species in Mediterranean Aquaculture*, Rome: FAO.
- Mourente, G., Bell, J. G. & Tocher, D. R. 2007. Does dietary tocopherol level affect fatty acid metabolism in fish? *Fish Physiol Biochem*, 33, 269-280.
- Nrc 2011. *Nutrient Requirements of Fish*, Washington, D.C., National Academy Press.
- Ortuño, J., Cuesta, A., Angeles Esteban, M. & Meseguer, J. 2001. Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (*Sparus aurata* L.) innate immune system. *Vet. Immunol. Immunopathol.*, 79, 167-180.
- Peng, L., Wang, X. & Gatlin Iii, D. M. 2008. RRR- $\alpha$ -Tocopheryl succinate is a less bioavailable source of vitamin E than all-rac- $\alpha$ -tocopheryl acetate for red drum, *Sciaenops ocellatus*. *Aquaculture*, 280, 165-169.
- Peng, L. I. & Gatlin Iii, D. M. 2009. Dietary vitamin E requirement of the red drum *Sciaenops ocellatus*. *Aquac. Nutr.*, 15, 313-319.
- Peng, S., Chen, L., Qin, J. G., Hou, J., Yu, N., Long, Z., Li, E. & Ye, J. 2009. Effects of dietary vitamin E supplementation on growth performance, lipid peroxidation and tissue fatty acid composition of black sea bream (*Acanthopagrus schlegeli*) fed oxidized fish oil. *Aquac. Nutr.*, 15, 329-337.
- Piccolo, G., Bovera, F., De Riu, N., Marono, S., Salati, F., Cappuccinelli, R. & Moniello, G. 2008. Effect of two different protein/fat ratios of the diet on meagre (*Argyrosomus regius*) traits. *Ital. J. Anim. Sci.*, 7, 363-371.
- Poli, B. M., Parisi, G., Zampacavallo, G., Iurzan, F., Mecatti, M., Lupi, P. & Bonelli, A. 2003. Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits and freshness changes in refrigerated commercial-size fish. *Aquac. Int.*, 11, 301-311.
- Quéméner, L., Suquet, M., Mero, D. & Gaignon, J.-L. 2002. Selection method of new candidates for finfish aquaculture: the case of the French Atlantic, the Channel and the North Sea coasts. *Aquat. Living Resour.*, 15, 293-302.
- Robbins, K. R., Norton, H. W. & Baker, D. H. 1979. Estimation of Nutrient Requirements from Growth Data. *The Journal of Nutrition*, 109, 1710-1714.

- Ruff, N., Fitzgerald, R. D., Cross, T. F., Hamre, K. & Kerry, J. P. 2003. The effect of dietary vitamin E and C level on market-size turbot (*Scophthalmus maximus*) fillet quality. *Aquac. Nutr.*, 9, 91-103.
- Ruff, N., Fitzgerald, R. d., Cross, T f., Teurtrie, G. & Kerry, J p. 2002. Slaughtering method and dietary  $\alpha$ -tocopheryl acetate supplementation affect rigor mortis and fillet shelf-life of turbot *Scophthalmus maximus* L. *Aquac. Res.*, 33, 703-714.
- Scaife, Onibi, Murray, Fletcher & Houlihan 2000. Influence of  $\alpha$ -tocopherol acetate on the short- and long-term storage properties of fillets from Atlantic salmon *Salmo salar* fed a high lipid diet. *Aquac. Nutr.*, 6, 65-71.
- Shahidi, F. & Zhong, Y. 2005. Lipid oxidation: measurement methods. *In: SHAHIDI, F. (ed.) Bailey's Industrial Oil and Fat Products*. John Wiley & Sons.
- Stéphan, G., Guillaume, J. & Lamour, F. 1995a. Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary n - 6 or n - 3 polyunsaturated fatty acids. *Aquaculture*, 130, 251-268.
- Stéphan, G., Guillaume, J. & Lamour, F. 1995b. Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary n – 6 or n – 3 polyunsaturated fatty acids. *Aquaculture*, 130, 251-268.
- Suquet, M., Divanach, P., Hussenot, J., Coves, D. & Fauvel, C. 2009. Marine fish culture of "new species" farmed in Europe. *Cah. Agric.*, 18, 148-156.
- Tappel, A. L. 1972. Vitamin E and free radical peroxidation of lipids. *Ann. N. Y. Acad. Sci.*, 203, 12-28.
- Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.*, 11, 107-184.
- Tocher, D. R., Mourente, G., Van Der Eecken, A., Evjemo, J. O., Diaz, E., Bell, J. G., Geurden, I., Lavens, P. & Olsen, Y. 2002. Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). *Aquac. Nutr.*, 8, 195-207.
- Ulbricht, T. L. V. & Southgate, D. a. T. 1991. Originally published as Volume 2, Issue 8773 Coronary heart disease: seven dietary factors. *The Lancet*, 338, 985-992.
- Watanabe, T. & Takashima, F. 1977. Effect of alpha-Tocopherol Deficiency on Carp VI: Deficiency Symptoms and Changes of Fatty Acid and Triglyceride Distributions in Adult Carp. *Nippon Suisan Gakkaishi*, 43, 819-830.
- Watanabe, T., Takashima, F., Ogino, C. & Hibiya, T. 1970. Requirement of Young Carp for alpha-Tocopherol. *Nippon Suisan Gakkaishi*, 36, 972-976.
- Whitehead, P. J. P., Bauchot, M. L., Hureau, J. C., Nielsen, J. & Tortonese, E. 1986. *Fishes of the North-eastern Atlantic and the Mediterranean*, Paris, France, UNESCO.

634 Wilson, R. P., Bowser, P. R. & Poe, W. E. 1984. Dietary Vitamin E Requirement of Fingerling  
635 Channel Catfish. *The Journal of Nutrition*, 114, 2053-2058.

636 Yildirim-Aksoy, M., Lim, C., Li, M. H. & Klesius, P. H. 2008. Interaction between dietary  
637 levels of vitamins C and E on growth and immune responses in channel catfish,  
638 *Ictalurus punctatus* (Rafinesque). *Aquac. Res.*, 39, 1198-1209.

639 Zar, J. H. 1999. *Biostatistical Analysis*, London, Prentice Hall.

640 Zhong, Y., Lall, S. P. & Shahidi, F. 2008. Effects of dietary oxidized oil and vitamin E on the  
641 growth, blood parameters and body composition of juvenile Atlantic cod *Gadus morhua*  
642 (Linnaeus 1758). *Aquac. Res.*, 39, 1647-1657.

643

**Table 1.** Ingredients (g/kg of diet) and proximate (% dry weight) composition of the experimental diets

	Diets						
	E16	E100	E190	E285	E430	E880	E1300
<b>Ingredients (g kg<sup>-1</sup>)</b>							
FM <sup>1</sup>	680	680	680	680	680	680	680
Fish Oil <sup>1</sup>	85	85	85	85	85	85	85
Gelatinized corn starch <sup>2</sup>	187.3	187.2	187.1	187	186.8	186.3	185.8
Vitamin Premix <sup>3</sup>	20	20	20	20	20	20	20
Mineral Premix <sup>4</sup>	20	20	20	20	20	20	20
CMC <sup>5</sup>	5	5	5	5	5	5	5
Choline	2.7	2.7	2.7	2.7	2.7	2.7	2.7
DL-alpha-tocopherol <sup>6</sup>	0	0.1	0.2	0.3	0.5	1	1.5
<b>Proximate composition (% dry weight)</b>							
Crude protein	49.97	48.75	49.17	48.79	49.25	48.86	49.29
Crude lipid	14.4	15.19	15.6	15.17	14.5	14.12	14.94
Ash	16.07	15.69	15.76	15.7	15.76	15.96	15.19
Moisture	8.39	6.89	7.45	6.9	6.73	7.1	7.55
Vitamin E (mg/kg)	16.45	98.15	193.94	284.2	431.57	880	1306.3
<b>Fatty acid composition (g/100 g fatty acids)</b>							
14:0	4.37	4.26	4.31	4.18	4.37	4.33	4.46
16:0	18.17	17.86	17.9	18.58	18.66	18.44	19.03
18:0	4.46	4.37	4.38	4.64	4.58	4.50	4.65
18:1n-9	17.95	17.93	17.88	17.33	17.78	17.48	17.03
18:2n-6	3.72	3.85	3.7	3.75	3.8	3.76	3.57
18:3n-3	1.18	1.2	1.2	1.14	1.18	1.18	1.1
20:4n-6	1.22	1.24	1.23	1.34	1.29	1.29	1.28
20:5n-3	6.37	6.53	6.57	6.66	6.51	6.63	7.14
22:6n-3	13.39	13.62	13.72	14.27	13.12	13.82	13.92
Saturated	28.42	27.87	28.01	28.77	29.03	28.58	29.41
Monounsaturated	38.26	38.2	37.99	36.49	37.53	36.91	35.99
Polyunsaturated	33.32	33.93	34	34.74	33.44	34.51	37.60

<sup>1</sup> Fish meal and oil, South American origin, BioMar Iberia S.A., Spain.

<sup>2</sup> Merigel 100 Amylum Group, Barcelona, Spain.

<sup>3</sup> Vitamin premix contains (mg/kg or IU/kg of dry diet): thiamine 40 mg, riboflavin 50 mg, pyridoxine 40 mg, calcium pantothenate 117 mg, nicotinic acid 200 mg, biotin 1 mg, folic acid 10 mg, cyanocobalamin, 0.5 mg, choline chloride 2700 mg, Myo-inositol 2000 mg, ascorbic acid 5000 mg, menadione 20 mg, cholecalciferol 2000 IU, ethoxyquin 100 mg, retinol acetate 5000 IU. Vitamin E (DL-alpha-tocopherol acetate) was added at 0, 100, 200, 300, 500, 1000 or 1500 mg/kg for each diet.

<sup>4</sup> Mineral premix contains (g/kg of dry diet): calcium orthophosphate 1.60 g, calcium carbonate 4 g, ferrous sulphate 1.5 g, magnesium sulphate 1.6 g, potassium phosphate 2.8 g, sodium phosphate 1 g, aluminium sulphate 0.02 g, zinc sulphate 0.24 g, copper sulphate 0.20 g, manganese sulphate 0.08 g, potassium iodate 0.02 g.

<sup>5</sup> Carboxymethyl cellulose (sodium salt, Sigma-Aldrich, Munich, Germany).

<sup>6</sup> Vitamin E = The dietary vitamin E content was analysed by high-performance liquid chromatography (HPLC).



**Table 2.** Growth performance and feed utilization of meagre fed different vitamin E levels for 72 days  
(mean  $\pm$  SD, n=3).

	Diets						
	E16	E100	E190	E285	E430	E880	E1300
Initial weight (g)	63.00 $\pm$ 1.08	62.94 $\pm$ 1.55	62.10 $\pm$ 0.98	63.10 $\pm$ 2.33	63.16 $\pm$ 0.93	62.61 $\pm$ 0.85	62.60 $\pm$ 1.40
Initial lenght (cm)	13.70 $\pm$ 0.19	13.71 $\pm$ 0.16	13.75 $\pm$ 0.09	13.84 $\pm$ 0.17	13.73 $\pm$ 0.02	13.76 $\pm$ 0.17	13.68 $\pm$ 0.09
Final weight (g)	174.88 $\pm$ 4.59	183.48 $\pm$ 7.66	174.58 $\pm$ 1.48	179.26 $\pm$ 7.85	175.72 $\pm$ 4.15	180.50 $\pm$ 7.02	182.62 $\pm$ 9.50
Final lenght (cm)	19.84 $\pm$ 0.28	20.27 $\pm$ 0.27	19.94 $\pm$ 0.31	20.30 $\pm$ 0.33	20.11 $\pm$ 0.47	20.17 $\pm$ 0.10	20.30 $\pm$ 0.20
FI <sup>1</sup>	2.07 $\pm$ 0.05	1.99 $\pm$ 0.12	1.87 $\pm$ 0.12	1.99 $\pm$ 0.05	1.94 $\pm$ 0.37	1.93 $\pm$ 0.03	2.01 $\pm$ 0.07
SGR <sup>2</sup>	1.45 $\pm$ 0.06	1.49 $\pm$ 0.04	1.44 $\pm$ 0.03	1.45 $\pm$ 0.01	1.41 $\pm$ 0.06	1.48 $\pm$ 0.02	1.44 $\pm$ 0.00
FCR <sup>3</sup>	0.86 $\pm$ 0.09	0.76 $\pm$ 0.04	0.74 $\pm$ 0.03	0.79 $\pm$ 0.03	0.95 $\pm$ 0.22	0.73 $\pm$ 0.01	0.80 $\pm$ 0.08
PER <sup>4</sup>	1.24 $\pm$ 0.04	1.37 $\pm$ 0.04	1.35 $\pm$ 0.05	1.31 $\pm$ 0.05	1.21 $\pm$ 0.11	1.37 $\pm$ 0.04	1.33 $\pm$ 0.01
K <sup>5</sup>	2.22 $\pm$ 0.03	2.19 $\pm$ 0.02	2.19 $\pm$ 0.08	2.15 $\pm$ 0.06	2.15 $\pm$ 0.09	2.19 $\pm$ 2.19	2.16 $\pm$ 2.16

Significant differences between treatments are indicated with different letter ( $P < 0.05$ ).

<sup>1</sup> **Feed intake (g)** = feed intake for days experiment (g)/ number of fish

<sup>2</sup> **Specific growth rate**= 100x(ln final weight-ln initial weight)/n° days

<sup>3</sup> **Feed conversion ratio** = feed intake (g)/weight gain (g)

<sup>4</sup> **Protein efficiency ratio** = weight gain (g)/protein intake (g) (dry matter)

<sup>5</sup> **Condition factor (%)** = 100 x (final weight /(final length)<sup>3</sup>)

**Table 3.** Biochemical composition (% wet weight) of whole body, muscle, and liver in meagre fed different vitamin E levels for 72 days (means  $\pm$  SD, n=3).

	Diets						
	E16	E100	E190	E285	E430	E880	E1300
HSI <sup>1</sup>	1.57 $\pm$ 0.13	1.57 $\pm$ 0.12	1.56 $\pm$ 0.04	1.51 $\pm$ 0.07	1.56 $\pm$ 0.07	1.58 $\pm$ 0.03	1.70 $\pm$ 0.17
VSI <sup>2</sup>	10.18 $\pm$ 0.53	10.21 $\pm$ 1.06	10.31 $\pm$ 1.28	10.34 $\pm$ 0.97	10.42 $\pm$ 1.51	10.14 $\pm$ 0.37	10.22 $\pm$ 0.89
<b>Whole body composition (WW%)</b>							
Protein	17.95 $\pm$ 0.74 a	16.24 $\pm$ 0.12 b	17.68 $\pm$ 1.00 ab	18.40 $\pm$ 0.99 a	16.26 $\pm$ 0.05b	17.60 $\pm$ 0.90 ab	17.12 $\pm$ 0.33 ab
Lipid	4.87 $\pm$ 0.48	4.99 $\pm$ 0.53	4.26 $\pm$ 0.83	5.49 $\pm$ 0.30	4.02 $\pm$ 0.91	5.42 $\pm$ 0.48	5.06 $\pm$ 0.25
Moisture	73.35 $\pm$ 2.05 b	76.44 $\pm$ 0.53 a	76.01 $\pm$ 0.10 ab	74.37 $\pm$ 0.26 ab	74.46 $\pm$ 0.37 ab	74.55 $\pm$ 0.42 ab	75.18 $\pm$ 0.02 ab
Ash	3.22 $\pm$ 0.02	2.01 $\pm$ 0.65	2.71 $\pm$ 0.35	2.74 $\pm$ 0.05	2.95 $\pm$ 0.07	2.39 $\pm$ 0.73	2.54 $\pm$ 0.95
<b>Muscle (WW%)</b>							
Protein	21.36 $\pm$ 1.35	20.74 $\pm$ 0.78	20.64 $\pm$ 0.31	20.30 $\pm$ 0.13	20.57 $\pm$ 0.60	20.41 $\pm$ 0.08	20.17 $\pm$ 0.36
Lipid	1.89 $\pm$ 0.18 a	1.72 $\pm$ 0.21 ab	1.03 $\pm$ 0.14 b	1.12 $\pm$ 0.25 ab	1.27 $\pm$ 0.36 ab	1.29 $\pm$ 0.04 ab	1.48 $\pm$ 0.10 ab
Moisture	78.74 $\pm$ 0.63	79.23 $\pm$ 0.50	78.59 $\pm$ 0.32	78.13 $\pm$ 0.52	78.36 $\pm$ 2.37	78.73 $\pm$ 0.45	78.64 $\pm$ 0.18
<b>Liver (WW%)</b>							
Lipid	20.06 $\pm$ 3.84	19.08 $\pm$ 5.07	17.83 $\pm$ 2.46	17.47 $\pm$ 4.09	23.32 $\pm$ 4.25	22.11 $\pm$ 0.09	26.79 $\pm$ 2.39
Moisture	58.06 $\pm$ 1.44	55.09 $\pm$ 8.21	58.59 $\pm$ 3.65	58.79 $\pm$ 2.02	57.44 $\pm$ 1.42	61.46 $\pm$ 0.56	59.12 $\pm$ 1.89

Significant differences between treatments are indicated with different letter ( $P < 0.05$ ).

<sup>1</sup> **Hepatosomatic index (%)** = 100 x (liver weight/final weight)

<sup>2</sup> **Visceral index (%)** = 100 x ((final weight-final eviscerated fish weight)/final weight)

**Table 4.** Liver fatty acid composition (% total identified fatty acids) of meagre fed different vitamin E levels for 72 days (means  $\pm$  SD, n=3).

	Diets						
	E16	E100	E190	E285	E430	E880	E1300
<b>Fatty acids</b>							
14:0	2.56 $\pm$ 0.06	2.27 $\pm$ 0.18	2.48 $\pm$ 0.11	2.49 $\pm$ 0.11	2.44 $\pm$ 0.16	2.34 $\pm$ 0.03	2.41 $\pm$ 0.40
16:0	19.31 $\pm$ 0.79	19.99 $\pm$ 0.24	19.09 $\pm$ 0.18	20.43 $\pm$ 0.90	20.23 $\pm$ 0.55	20.37 $\pm$ 0.63	19.38 $\pm$ 0.92
18:0	5.33 $\pm$ 0.28	5.24 $\pm$ 0.00	5.54 $\pm$ 0.51	5.35 $\pm$ 0.09	5.80 $\pm$ 0.16	5.73 $\pm$ 0.27	6.03 $\pm$ 0.07
18:1n-9	23.19 $\pm$ 0.77	22.65 $\pm$ 0.50	23.00 $\pm$ 0.26	23.48 $\pm$ 0.45	23.50 $\pm$ 0.22	23.52 $\pm$ 0.03	22.74 $\pm$ 0.84
18:2n-6	3.88 $\pm$ 0.11	3.61 $\pm$ 0.05	4.08 $\pm$ 0.00	3.98 $\pm$ 0.19	3.97 $\pm$ 0.18	3.67 $\pm$ 0.09	3.48 $\pm$ 0.53
18:3n-3	0.90 $\pm$ 0.01	0.86 $\pm$ 0.00	0.96 $\pm$ 0.01	0.91 $\pm$ 0.04	0.91 $\pm$ 0.01	0.85 $\pm$ 0.03	0.81 $\pm$ 0.10
18:4n-3	0.74 $\pm$ 0.02	0.68 $\pm$ 0.01	0.77 $\pm$ 0.02	0.71 $\pm$ 0.05	0.69 $\pm$ 0.02	0.68 $\pm$ 0.00	0.69 $\pm$ 0.13
20:00	0.24 $\pm$ 0.01	0.24 $\pm$ 0.00	0.24 $\pm$ 0.01	0.25 $\pm$ 0.01	0.25 $\pm$ 0.00	0.25 $\pm$ 0.00	0.24 $\pm$ 0.01
20:1n-9	0.95 $\pm$ 0.01	0.86 $\pm$ 0.00	0.90 $\pm$ 0.05	0.97 $\pm$ 0.02	0.94 $\pm$ 0.03	0.88 $\pm$ 0.02	0.82 $\pm$ 0.12
20:4n-6	0.81 $\pm$ 0.01	0.83 $\pm$ 0.05	0.89 $\pm$ 0.03	0.86 $\pm$ 0.04	0.84 $\pm$ 0.04	0.82 $\pm$ 0.06	0.81 $\pm$ 0.08
20:5n-3	3.46 $\pm$ 0.05	3.42 $\pm$ 0.22	3.75 $\pm$ 0.12	3.34 $\pm$ 0.10	3.35 $\pm$ 0.09	3.54 $\pm$ 0.14	3.77 $\pm$ 0.38
22:5n-3	2.42 $\pm$ 0.72	3.66 $\pm$ 2.22	2.06 $\pm$ 0.03	1.88 $\pm$ 0.09	1.84 $\pm$ 0.12	1.90 $\pm$ 0.00	3.15 $\pm$ 1.51
22:6n-3	9.22 $\pm$ 0.14	8.93 $\pm$ 0.87	9.37 $\pm$ 0.36	8.52 $\pm$ 0.35	8.60 $\pm$ 0.35	8.53 $\pm$ 0.26	9.34 $\pm$ 1.82
Saturate	28.63 $\pm$ 1.02	28.84 $\pm$ 0.02	28.52 $\pm$ 0.81	30.45 $\pm$ 0.93	30.58 $\pm$ 0.71	30.45 $\pm$ 0.37	29.19 $\pm$ 1.00
Monounsaturates	41.12 $\pm$ 0.78	39.92 $\pm$ 0.81	40.72 $\pm$ 0.55	40.98 $\pm$ 0.57	40.85 $\pm$ 0.30	40.98 $\pm$ 0.02	40.12 $\pm$ 0.21
Polyunsaturated	30.24 $\pm$ 1.79	31.24 $\pm$ 0.84	30.76 $\pm$ 0.26	28.57 $\pm$ 1.04	28.57 $\pm$ 0.49	28.57 $\pm$ 0.35	30.69 $\pm$ 1.21
LC-PUFA	18.65 $\pm$ 1.69	20.48 $\pm$ 0.91	19.00 $\pm$ 0.46	16.68 $\pm$ 0.59	16.70 $\pm$ 0.56	17.51 $\pm$ 0.51	19.86 $\pm$ 0.26
AI	0.49 $\pm$ 0.02	0.48 $\pm$ 0.01	0.49 $\pm$ 0.02	0.53 $\pm$ 0.02	0.52 $\pm$ 0.02	0.51 $\pm$ 0.01	0.48 $\pm$ 0.02
TI	0.37 $\pm$ 0.02	0.37 $\pm$ 0.02	0.37 $\pm$ 0.00	0.42 $\pm$ 0.02	0.42 $\pm$ 0.02	0.41 $\pm$ 0.00	0.37 $\pm$ 0.02

$\Sigma$  SFA (saturated fatty acids) include 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0

$\Sigma$  MUFA (monounsaturated fatty acids) include 14:1, 15:1, 16:1, 17:1, 18:1, 20:1.

$\Sigma$  PUFA (polyunsaturated fatty acids) include 16:2, 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:6

$\Sigma$  LC-PUFA (polyunsaturated fatty acids) include 20:3, 20:4, 20:5, 22:6

**Table 5.** Muscle fatty acid composition (% total identified fatty acids) of meagre fed different vitamin E levels for 72 days (means  $\pm$  SD, n=3).

	Diets						
	E16	E100	E190	E285	E430	E880	E1300
<b>Fatty acids</b>							
14:0	2.90 $\pm$ 0.43	2.86 $\pm$ 0.18	2.88 $\pm$ 0.19	2.58 $\pm$ 0.12	3.02 $\pm$ 0.41	2.86 $\pm$ 0.00	2.64 $\pm$ 0.33
16:0	27.28 $\pm$ 1.90	23.61 $\pm$ 2.33	23.78 $\pm$ 1.37	24.33 $\pm$ 3.20	24.43 $\pm$ 2.17	20.53 $\pm$ 0.18	22.19 $\pm$ 3.23
18:0	10.23 $\pm$ 1.28	7.86 $\pm$ 0.69	8.17 $\pm$ 0.75	8.70 $\pm$ 1.32	8.47 $\pm$ 2.13	6.28 $\pm$ 0.12	7.38 $\pm$ 1.14
18:1n-9	20.11 $\pm$ 0.67	18.97 $\pm$ 1.08	18.98 $\pm$ 0.42	18.52 $\pm$ 1.50	19.41 $\pm$ 0.74	17.78 $\pm$ 0.10	17.77 $\pm$ 1.79
18:2n-6	4.35 $\pm$ 0.15	4.14 $\pm$ 0.06	4.36 $\pm$ 0.15	4.34 $\pm$ 0.18	4.60 $\pm$ 0.27	4.35 $\pm$ 0.10	4.27 $\pm$ 0.27
18:3n-3	0.64 $\pm$ 0.10	0.76 $\pm$ 0.09	0.77 $\pm$ 0.06	0.69 $\pm$ 0.08	0.78 $\pm$ 0.15	0.88 $\pm$ 0.02	0.78 $\pm$ 0.06
18:4n-3	0.31 $\pm$ 0.09	0.51 $\pm$ 0.12	0.48 $\pm$ 0.07	0.43 $\pm$ 0.14	0.47 $\pm$ 0.16	0.70 $\pm$ 0.00	0.57 $\pm$ 0.13
20:00	0.40 $\pm$ 0.03	0.34 $\pm$ 0.03	0.34 $\pm$ 0.03	0.35 $\pm$ 0.05	0.36 $\pm$ 0.05	0.28 $\pm$ 0.00	0.30 $\pm$ 0.05
20:1n-9	0.73 $\pm$ 0.01	0.70 $\pm$ 0.05	0.70 $\pm$ 0.04	0.67 $\pm$ 0.08	0.71 $\pm$ 0.01	0.64 $\pm$ 0.00	0.63 $\pm$ 0.01
20:4n-6	1.21 $\pm$ 0.08	1.39 $\pm$ 0.14	1.42 $\pm$ 0.14	1.46 $\pm$ 0.21	1.27 $\pm$ 0.15	1.48 $\pm$ 0.00	1.54 $\pm$ 0.26
20:5n-3	2.09 $\pm$ 0.64 b	3.57 $\pm$ 0.79 ab	3.30 $\pm$ 0.55 ab	3.42 $\pm$ 1.30 ab	3.02 $\pm$ 0.70 ab	4.89 $\pm$ 0.01 a	4.30 $\pm$ 0.90 ab
22:5n-3	0.66 $\pm$ 0.20	1.16 $\pm$ 0.30	1.06 $\pm$ 0.18	1.09 $\pm$ 0.41	0.96 $\pm$ 0.21	1.54 $\pm$ 0.02	1.36 $\pm$ 0.36
22:6n-3	6.13 $\pm$ 2.48	11.62 $\pm$ 3.33	10.91 $\pm$ 2.33	14.27 $\pm$ 3.68	10.24 $\pm$ 1.58	15.68 $\pm$ 0.30	14.72 $\pm$ 6.17
Saturate	41.58 $\pm$ 2.94	35.41 $\pm$ 3.21	35.91 $\pm$ 2.37	36.67 $\pm$ 4.48	37.04 $\pm$ 4.08	30.66 $\pm$ 0.08	33.19 $\pm$ 4.82
Monounsaturates	37.98 $\pm$ 1.77	36.54 $\pm$ 2.00	36.71 $\pm$ 1.02	34.97 $\pm$ 2.51	37.77 $\pm$ 2.09	34.83 $\pm$ 0.04	34.29 $\pm$ 3.21
Polyunsaturated	20.44 $\pm$ 3.47	28.05 $\pm$ 4.90	27.38 $\pm$ 3.36	28.36 $\pm$ 6.82	34.51 $\pm$ 3.47	34.51 $\pm$ 0.12	32.51 $\pm$ 8.04
LC-PUFA	11.64 $\pm$ 3.52	19.59 $\pm$ 4.74	18.56 $\pm$ 3.36	17.31 $\pm$ 10.80	13.80 $\pm$ 7.21	25.64 $\pm$ 0.31	23.87 $\pm$ 7.92
AI	0.79 $\pm$ 0.07	0.63 $\pm$ 0.09	0.64 $\pm$ 0.07	0.68 $\pm$ 0.18	0.72 $\pm$ 0.15	0.53 $\pm$ 0.01	0.57 $\pm$ 0.12
TI	0.84 $\pm$ 0.22	0.49 $\pm$ 0.13	0.51 $\pm$ 0.10	0.72 $\pm$ 0.54	0.77 $\pm$ 0.48	0.33 $\pm$ 0.00	0.40 $\pm$ 0.16

$\Sigma$  SFA (saturated fatty acids) include 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0

$\Sigma$  MUFA (monounsaturated fatty acids) include 14:1, 15:1, 16:1, 17:1, 18:1, 20:1.

$\Sigma$  PUFA (polyunsaturated fatty acids) include 16:2, 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:6

$\Sigma$  LC-PUFA (polyunsaturated fatty acids) include 20:3, 20:4, 20:5, 22:6

**Table 6.** Fillet lipid oxidation (MDA: Malonaldehyde, mg kg<sup>-1</sup>) of meagre fed different vitamin E levels for 72 days (means  $\pm$  SD, n=9)

	TBARS (mg MDA kg <sup>-1</sup> )
<b>Diets</b>	
E16	1.09 $\pm$ 0.18 a
E100	0.98 $\pm$ 0.06 ab
E190	0.87 $\pm$ 0.26 abc
E285	0.73 $\pm$ 0.23 bcd
E430	0.53 $\pm$ 0.15cd
E880	0.48 $\pm$ 0.04cd
E1300	0.46 $\pm$ 0.00 d

**Figure 1.** TBARS concentration in muscle of juvenile meagre fed diets containing graded levels of vitamin E for 72 days. Minimum dietary requirement was established by broken-line regression analysis.

**Figure 2.** a) liver from fish fed E16 diet; b) liver from fish fed E430 diet; c) liver from fish fed E880 diet. H&E 40X.

Figure 1.

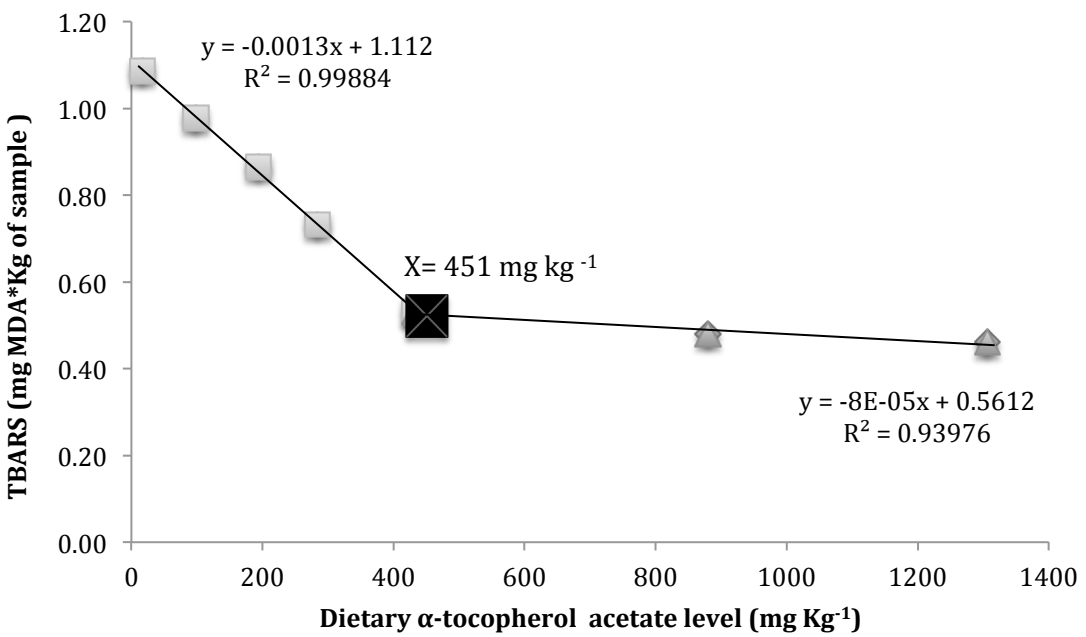


Figure 2.

