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

Rodríguez Lozano, A.[a], Borges, P. [a*], Robaina, L. [a], Betancor, M.[b], Hernández-Cruz, C.M. [a], Romero García, J. [a], Caballero, M.J. [a], Vergara, J.M. [a], Izquierdo, M. [a]

[a] Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain
[b] Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

Corresponding author: Pedro Alexandre Coelho Borges. (*Grupo de Investigación en Acuicultura), University of Las Palmas de Gran Canaria, carretera de Taliarte s/n, P.O. Box 56, E-35200 Telde, Las Palmas, Spain.*
Tel.: +34 928 13 29 00; E-mail: pedroacborges@live.com

Abstract (150-200 Words)

Seven experimental isonitrogenous (50%) and isolipidic (16%) diets with different levels of α-tocopherol acetate (16, 100, 190, 285, 430, 880 and 1300 mg kg^{-1}) 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^{-1} of DL-α-tocopherol acetate (496 UI of vitamin E), as determine by broken-

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

Keywords: Growth performance, vitamin E and muscle TBARS.

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 (Quéméner *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^{-3} 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 (α-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 α- 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^{-1} 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^{-1}) at expense of the α-cellulose. The vitamin E was provided as DL-α-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 -80ºC 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 Fundácion Parque Cientifico y Tecnologico 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 23.6ºC and 6.1 to 6.9 mg L^{-1} 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)^3)

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

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

151 The index of atherogenicity and thrombogenecity, related to effects of different
152 fatty acids on human's health, were calculated according to Ulbricht and Southgate
153 (1991):

154 Index of atherogenicity (AI)

155 $AI= [(12:0) + (4 \times 14:0) + (16:0)] \times [(PUFA\ n\text{-}6\ and\ n\text{-}3) + MUFA]^{-1}$

156 Index of thrombogenicity (TI)

157 $TI= [(14:0) + (16:0) + (18:0)] \times [(0.5 \times MUFA) + (0.5 \times n\text{-}6) + (3 \times n\text{-}3) + (n\text{-}3/n\text{-}6)]^{-1}$

158

159 **2.4. Sample Collection and Biochemical Analysis**

160 At the end of the trial fish were anesthetized and weight and length were
161 recorded. Nine fish per treatment were used for whole body composition. Liver and
162 muscle from 4 fish per tank were removed for biochemical analyses and the opposite
163 muscle side recovered for TBARS analysis. All samples were weighted and stored at -
164 80ºC until analysed. For histological evaluation, livers from 5 fish per tank were
165 collected and fixed in 10% buffer formaldehyde. Fish were fasted for one day before all
166 samplings and sacrificed by immersion in iced seawater.

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

214 ## 3.Results

215

216 ### 3.1. Biochemical composition of the experimental diets

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

220

221 ### 3.2. Growth performance and feed utilisation

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

228

229 ### 3.3. Whole body, muscle and liver proximate composition

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

235 Regarding liver composition (Table 3), no significant differences were found in
236 moisture and lipid among the different groups. Nonetheless, the lowest values were
237 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)

262 Vitamin E had a significant (P <0.005) effect on lipid muscle oxidation (Table
263 6). Muscle TBARS levels were significantly higher in fish fed the E16 and E100 diets
264 compared to fish fed the highest levels.
265 The recommended level of vitamin E to avoid lipid peroxidation is around 451
266 mg kg^{-1} in the form of DL-α-tocopherol acetate, as determine by broken-line regression
267 analysis (Figure 1).
268
269 **3.6. Liver histology**
270 A higher degree of cytoplasmic vacuolization was observed in the hepatocytes of
271 fish fed E16, E100 and E880 diets (Figure 2), showing large hepatocytes with a
272 displacement of cell nuclei. However, fish fed E285 and E430 diets showed regular
273 hepatocyte morphology with cytoplasmic lipid vacuoles that did not alter hepatocyte
274 size (Figure 2). Scoring of liver lipid vacuolization was slightly higher in fish fed the
275 lowest and highest dietary vitamin E (1.98±0.74 for diet E16; 2.01±0.14 for diet E100;
276 2.00±0.12 for diet E190; 1.96±0.18 for diet E880; 1.87±0.38 for diet E1300) although
277 not significantly different with those found in fish fed E285 and E430 diets (1.86±0.38
278 and 1.81±0.40, respectively).

279 **4. Discussion**

280 Meagre is seen as a new candidate for the Mediterranean aquaculture, but to our
281 best knowledge, there is little data on nutrient requirements for this species. In the
282 present work, all diets formulated on basal fishmeal and fish oil with different vitamin E
283 levels were well accepted. This was reflected in feed intake (1.9 to 2.1 g $fish^{-1}$ day^{-1}),
284 specific growth rate (1.4 to 1.5 %) and feed conversion ratios (0.73 to 0.95), which were
285 identical among groups irrespective of dietary vitamin E level. Similar responses have
286 been reported in other species such as catfish (Gaylord et al., 1998, Wilson et al., 1984),
287 red drum (Peng and Gatlin III, 2009), African catfish (Baker and Davies, 1996a), turbot
288 (Stéphan *et al.*, 1995a, Tocher et al., 2002) or rainbow trout (Cowey et al., 1981).
289 Nonetheless, this response seems to be species dependent as it was observed that growth
290 can be affected by low vitamin E levels in Atlantic salmon (Hamre and Lie, 1995),
291 common carp, (Watanabe *et al.*, 1970), spoted murrel (Abdel-Hameid et al., 2012),
292 grouper (Lin and Shiau, 2005), hybrid striped bass juveniles (Kocabas and Gatlin, 1999)
293 or Korean rockfish (Bai and Lee, 1998). The lack of differences in growth performance
294 can be related to the trial time (72 days) and the high amount of vitamin C
295 supplemented in the diets (5000 mg kg^{-1}). Tappel (1972) was the first to hypothesize
296 that vitamin C could promote a sparing effect on vitamin E, where oxidized vitamin E
297 could be regenerated to its reduced form by ascorbate. In some fish species this sparing
298 action has been suggested (Yildirim-Aksoy *et al.*, 2008, Betancor *et al.*, 2012, Hamre,
299 2011, Mourente et al., 2007, Ortuño *et al.*, 2001) having an influence on growth, tissue
300 composition or immune responses. The duration of the trial is also an important factor
301 affecting vitamin E deposition or depletion. Fish were fed with a commercial diet before
302 the trial, which supplemented with vitamin E, thus the 72 days were probably not
303 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 where 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.

328 Diet composition affected whole body protein and moisture contents, but it does
329 not seem related to dietary vitamin E level since higher protein levels were found in the
330 vitamin E-deficient group. In previous studies (Chatzifotis et al., 2012, Martínez-
331 Llorens et al., 2011), meagre fed with similar diets or with different protein/lipid ratios
332 displayed similar whole body moisture and lipid content. Muscle lipid content was
333 significantly higher in fish fed the low vitamin E diet, but only when comparing with
334 diet E190, with similar values found in fish fed the other diets. In turbot (Ruff et al.,
335 2003) and European seabass (Gatta et al., 2000) no significant differences were
336 recorded in muscle composition, but an increasing trend in lipid content was observed in
337 groups fed with high vitamin E diets (approximately 1000 mg kg^{-1}). Similar results were
338 recorded in trout (Jittinandana et al., 2006).

339 In the present trial, different inclusion levels of vitamin E did not lead to
340 significant differences in the liver or viscera to body weight ratios among treatments.
341 Liver fatty acid composition was constituted mainly by the monounsaturated (40.1 to
342 41.1%), followed by the saturated (28.5 to 30.6%), the n-3 (15.9 to 18.7%) and the n-6
343 (5.9 to 7.5%) classes, but in the overall no major effects have been observed. Despite
344 the lack of significant differences, fish fed either with low or high vitamin E diets
345 presented higher liver lipid vacuolization than the fish fed with diets E285 and E430. In
346 Atlantic salmon, Bell et al. (2000) observed that a deficiency of vitamin E and
347 astaxanthin in the diet caused a stimulation of hepatic fatty acid desaturation and
348 elongation activities promoting hepatic tissue damage such as vacuolated hepatocytes
349 and ceroidosis. Bai and Lee (1998) showed that a total lack of vitamin E (0 mg kg^{-1})
350 exerts a negative effect on liver lipid peroxidation in Korean rockfish (*Sebastes*
351 *schlegeli*). In the present trial, only at intermediate levels (285 and 430 mg kg^{-1}) of
352 dietary vitamin E liver presented normal morphology, with fish fed with either low or

353 high vitamin E levels developing high degree of liver lipid vacuolization. Likewise, in
354 sea bream (Tocher et al., 2002) reported a significant decrease on liver long-chain
355 polyunsaturated acids at vitamin E intermediate levels (100 mg kg^{-1} of α-tocopherol)
356 compared with the other treatments.

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

365 Slight differences were recorded in muscle EPA proportion when dietary vitamin
366 E increased. Despite the lack of significant differences, DHA and long chain PUFA also
367 exhibited an increasing trend, with muscle levels increasing two-fold from the vitamin E
368 deficient group (6.1 and 11.6 %) to the E1300 group (22.7 and 23.9 %). The atherogenic
369 (0.53 to 0.79) and thrombogenic (0.33 to 0.84) indexes exposed this difference, with
370 fish fed high vitamin diets presenting lower AI and TI (Table 5). In carp, Watanabe and
371 Takashima (1977) observed the same effect on muscle fatty acids while in Atlantic
372 salmon the opposite effect was recorded, but probably due to the low feed intake in this
373 trial (Hamre and Lie, 1995). Watanabe and Takashima (1977) suggested that dietary
374 deficiency of ∞-tocopherol could exert some effects on fatty acid composition, but
375 when exceeding the requirement level had little effect on fatty acid composition of fish.
376 Likewise, dietary ∞-tocopheryl acetate levels and length of feeding did not alter liver
377 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^{-1} as DL-tocopherol acetate: in red drum (Peng and Gatlin III, 2009) was 27 mg kg^{-1}, 54 to 100 mg kg^{-1} for grouper (Lin and Shiau, 2005), depending on the dietary lipid level, 20-30 mg kg^{-1} for Atlantic salmon (Hamre and Lie, 1995), 50 mg kg^{-1} for rainbow trout (Cowey et al., 1983), 15 to 45 mg kg^{-1} for channel catfish (Gaylord et al., 1998, Wilson et al., 1984), depending of fish size, 100 mg kg^{-1} for common carp (Watanabe et al.,

403 1970), 40 mg kg^{-1} for Korean rockfish (Bai and Lee, 1998) and 127 to 154 mg kg^{-1} for
404 *Channa punctatus* (Abdel-Hameid et al., 2012). The different responses to dietary
405 vitamin E could be relate to fish species, size and development stage, culture condition,
406 different levels and organ storage of vitamin E, as well as the added effect of different
407 antioxidant nutrient presented in diets. Dietary vitamin E requirement can be increased
408 by dietary factors such as polyunsaturated fatty acids, oxidized lipid (Cowey et al.,
409 1984, Zhong *et al.*, 2008), as well as presence and abundance of other antioxidant
410 nutrients such as selenium and vitamin C (Betancor et al., 2012, Bell *et al.*, 1985), or
411 astaxanthin (Hamre, 2011). The optimum dietary vitamin E level in meagre was 451 mg
412 kg^{-1} diet, as determine by the broken-line regression method, based on muscle TBARS
413 levels. Muscle TBARS level is not commonly used as a criteria to establish the
414 optimum dietary vitamin E level, although it is a good indicator of the overall quality of
415 the fish, especially of the muscle that is the edible portion. Currently, commercial diets
416 are already being supplemented with 150-300 mg of tocopherols in order to take
417 advantage of the antioxidant effect of vitamin E and its health effects. Thus, our
418 intention was not only assess the level needed to avoid any sign of vitamin E deficiency
419 but also improve fillet quality by increasing its storage stability. Due to the
420 poikilothermic nature of fish, their cell membranes are rich in polyunsaturated fatty
421 acids and hence more prone to peroxidation (Tocher, 2003). An increase of the fillet
422 quality and protection from lipid oxidation by an increment of the vitamin E in the diet
423 has been reported in different studies. Jittinandana and co-workers (2006) observed that
424 trout fed with 5000 mg kg^{-1} of vitamin E (DL-α-tocopheryl acetate) displayed a
425 significantly lower muscle TBARS compared to fish fed diets with 200 mg kg^{-1} of
426 vitamin E. In turbot (Ruff *et al*., 2003; Stéphan et al., 1995), muscle TBARS level was
427 significantly ($P < 0.01$) lower in fish fed high α-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 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 mg kg $^{-1}$) presented normal liver histology when compared to fish fed the lower (16 and 190 mg kg $^{-1}$) and higher (880 and 1300 mg kg $^{-1}$) levels, where a higher degree of lipid vacuolization was observed. Accordingly, it is suggested the inclusion of 451 mg kg^{-1} of DL-α-tocopherol acetate (496 UI of vitamin E) in meagre diets, as determine 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

561 Lin, Y.-H. & Shiau, S.-Y. 2005. Dietary vitamin E requirement of grouper, *Epinephelus*
562 *malabaricus*, at two lipid levels, and their effects on immune responses. *Aquaculture,*
563 248**,** 235-244.
564 Martínez-Llorens, S., Espert, J., Moya, J., Cerdá, M. J. & Tomás-Vidal, A. 2011. Growth and
565 nutrient efficiency of meagre (*Argyrosomus regius*, Asso1801) fed extruded diets with
566 different protein and lipid levels. *Int. J. Fish. Aquac.,* 3**,** 195-203.
567 Martoja, R. & Martoja-Pierson, M. 1970. *Técnicas de Histología Animal,* Barcelona, Spain,
568 Toray-Masson S.A.
569 Montfort, M. C. (ed.) 2010. *Present Market Situation and Prospects of Meagre (Argyrosomus*
570 *regius), as an Emerging Species in Mediterranean Aquaculture,* Rome: FAO.
571 Mourente, G., Bell, J. G. & Tocher, D. R. 2007. Does dietary tocopherol level affect fatty acid
572 metabolism in fish? *Fish Physiol Biochem,* 33**,** 269-280.
573 Nrc 2011. *Nutrient Requirements of Fish,* Washington, D.C., National Academy Press.
574 Ortuño, J., Cuesta, A., Angeles Esteban, M. & Meseguer, J. 2001. Effect of oral administration
575 of high vitamin C and E dosages on the gilthead seabream (Sparus aurata L.) innate
576 immune system. *Vet. Immunol. Immunopathol.,* 79**,** 167-180.
577 Peng, L., Wang, X. & Gatlin Iii, D. M. 2008. RRR-α-Tocopheryl succinate is a less bioavailable
578 source of vitamin E than all-rac-α-tocopheryl acetate for red drum, *Sciaenops ocellatus*.
579 *Aquaculture,* 280**,** 165-169.
580 Peng, L. I. & Gatlin Iii, D. M. 2009. Dietary vitamin E requirement of the red drum *Sciaenops*
581 *ocellatus*. *Aquac. Nutr.,* 15**,** 313-319.
582 Peng, S., Chen, L., Qin, J. G., Hou, J., Yu, N., Long, Z., Li, E. & Ye, J. 2009. Effects of dietary
583 vitamin E supplementation on growth performance, lipid peroxidation and tissue fatty
584 acid composition of black sea bream (*Acanthopagrus schlegeli*) fed oxidized fish oil.
585 *Aquac. Nutr.,* 15**,** 329-337.
586 Piccolo, G., Bovera, F., De Riu, N., Marono, S., Salati, F., Cappuccinelli, R. & Moniello, G.
587 2008. Effect of two different protein/fat ratios of the diet on meagre (Argyrosomus
588 regius) traits. *Ital. J. Anim. Sci.,* 7**,** 363-371.
589 Poli, B. M., Parisi, G., Zampacavallo, G., Iurzan, F., Mecatti, M., Lupi, P. & Bonelli, A. 2003.
590 Preliminary results on quality and quality changes in reared meagre (Argyrosomus
591 regius): body and fillet traits and freshness changes in refrigerated commercial-size fish.
592 *Aquac. Int.,* 11**,** 301-311.
593 Quéméner, L., Suquet, M., Mero, D. & Gaignon, J.-L. 2002. Selection method of new
594 candidates for finfish aquaculture: the case of the French Atlantic, the Channel and the
595 North Sea coasts. *Aquat. Living Resour.,* 15**,** 293-302.
596 Robbins, K. R., Norton, H. W. & Baker, D. H. 1979. Estimation of Nutrient Requirements from
597 Growth Data. *The Journal of Nutrition,* 109**,** 1710-1714.

598 Ruff, N., Fitzgerald, R. D., Cross, T. F., Hamre, K. & Kerry, J. P. 2003. The effect of dietary
599 vitamin E and C level on market-size turbot (Scophthalmus maximus) fillet quality.
600 *Aquac. Nutr.,* 9**,** 91-103.
601 Ruff, N., Fitzgerald, R d., Cross, T f., Teurtrie, G. & Kerry, J p. 2002. Slaughtering method and
602 dietary α-tocopheryl acetate supplementation affect rigor mortis and fillet shelf-life of
603 turbot *Scophthalmus maximus* L. *Aquac. Res.,* 33**,** 703-714.
604 Scaife, Onibi, Murray, Fletcher & Houlihan 2000. Influence of α-tocopherol acetate on the
605 short- and long-term storage properties of fillets from Atlantic salmon Salmo salar fed a
606 high lipid diet. *Aquac. Nutr.,* 6**,** 65-71.
607 Shahidi, F. & Zhong, Y. 2005. Lipid oxidation: measurement methods. *In:* SHAHIDI, F. (ed.)
608 *Bailey's Industrial Oil and Fat Products.* John Wiley & Sons.
609 Stéphan, G., Guillaume, J. & Lamour, F. 1995a. Lipid peroxidation in turbot (Scophthalmus
610 maximus) tissue: effect of dietary vitamin E and dietary n - 6 or n - 3 polyunsaturated
611 fatty acids. *Aquaculture,* 130**,** 251-268.
612 Stéphan, G., Guillaume, J. & Lamour, F. 1995b. Lipid peroxidation in turbot (*Scophthalmus*
613 *maximus*) tissue: effect of dietary vitamin E and dietary n − 6 or n − 3 polyunsaturated
614 fatty acids. *Aquaculture,* 130**,** 251-268.
615 Suquet, M., Divanach, P., Hussenot, J., Coves, D. & Fauvel, C. 2009. Marine fish culture of
616 "new species" farmed in Europe. *Cah. Agric.,* 18**,** 148-156.
617 Tappel, A. L. 1972. Vitamin E and free radical peroxidation of lipids. *Ann. N. Y. Acad. Sci.,*
618 203**,** 12-28.
619 Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish.*
620 *Sci.,* 11**,** 107-184.
621 Tocher, D. R., Mourente, G., Van Der Eecken, A., Evjemo, J. O., Diaz, E., Bell, J. G., Geurden,
622 I., Lavens, P. & Olsen, Y. 2002. Effects of dietary vitamin E on antioxidant defence
623 mechanisms of juvenile turbot (Scophthalmus maximus L.), halibut (*Hippoglossus*
624 *hippoglossus L.*) and sea bream (*Sparus aurata L.*). *Aquac. Nutr.,* 8**,** 195-207.
625 Ulbricht, T. L. V. & Southgate, D. a. T. 1991. Originally published as Volume 2, Issue
626 8773Coronary heart disease: seven dietary factors. *The Lancet,* 338**,** 985-992.
627 Watanabe, T. & Takashima, F. 1977. Effect of alpha-Tocopherol Deficiency on Carp VI:
628 Deficiency Symptoms and Changes of Fatty Acid and Triglyceride Distributions in
629 Adult Carp. *Nippon Suisan Gakkaishi,* 43**,** 819-830.
630 Watanabe, T., Takashima, F., Ogino, C. & Hibiya, T. 1970. Requirement of Young Carp for
631 alpha-Tocopherol. *Nippon Suisan Gakkaishi,* 36**,** 972-976.
632 Whitehead, P. J. P., Bauchot, M. L., Hureau, J. C., Nielsen, J. & Tortonese, E. 1986. *Fishes of*
633 *the North-eastern Atlantic and the Mediterranean,* Paris, France, UNESCO.

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

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

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

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

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

	Diets						
	E16	E100	E190	E285	E430	E880	E1300
Ingredients (g kg^{-1})							
FM[1]	680	680	680	680	680	680	680
Fish Oil[1]	85	85	85	85	85	85	85
Gelatinized corn starch[2]	187.3	187.2	187.1	187	186.8	186.3	185.8
Vitamin Premix[3]	20	20	20	20	20	20	20
Mineral Premix[4]	20	20	20	20	20	20	20
CMC[5]	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[6]	0	0.1	0.2	0.3	0.5	1	1.5
Proximate composition (% dry weight)							
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
Fatty acid composition (g/100 g fatty acids)							
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

[1] Fish meal and oil, South American origin, BioMar Iberia S.A., Spain.
[2] Merigel 100 Amylum Group, Barcelona, Spain.
[3] 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.
[4] 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.
[5] Carboxymethyl cellulose (sodium salt, Sigma-Aldrich, Munich, Germany).
[6] 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 ± SD, n=3).

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

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

[1] **Feed intake (g)** = feed intake for days experiment (g)/ number of fish

[2] **Specific growth rate=** 100x(ln final weight-ln initial weight)/nº days

[3] **Feed conversion ratio =** feed intake (g)/weight gain (g)

[4] **Protein efficiency ratio =** weight gain (g)/protein intake (g) (dry matter)

[5] **Condition factor (%) =** 100 x (final weight /(final length)^3)

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

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

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

[1] **Hepatosomatic index (%) =** 100 x (liver weight/final weight)

[2] **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 ± SD, n=3).

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

Σ SFA (saturated fatty acids) include 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0
Σ MUFA (monounsaturated fatty acids) include 14:1, 15:1, 16:1, 17:1, 18:1, 20:1.
Σ PUFA (polyunsaturated fatty acids) include 16:2, 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:6
Σ 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 ± SD, n=3).

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

Σ SFA (saturated fatty acids) include 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0
Σ MUFA (monounsaturated fatty acids) include 14:1, 15:1, 16:1, 17:1, 18:1, 20:1.
Σ PUFA (polyunsaturated fatty acids) include 16:2, 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:6
Σ LC-PUFA (polyunsaturated fatty acids) include 20:3, 20:4, 20:5, 22:6

Table 6. Fillet lipid oxidation (MDA: Malonaldehyde, mg kg^{-1}) of meagre fed different vitamin E levels for 72 days (means ± SD, n=9)

	TBARS (mg MDA kg^{-1})
Diets	
E16	1.09 ± 0.18 a
E100	0.98 ± 0.06 ab
E190	0.87 ± 0.26 abc
E285	0.73 ± 0.23 bcd
E430	0.53 ± 0.15cd
E880	0.48 ± 0.04cd
E1300	0.46 ± 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.

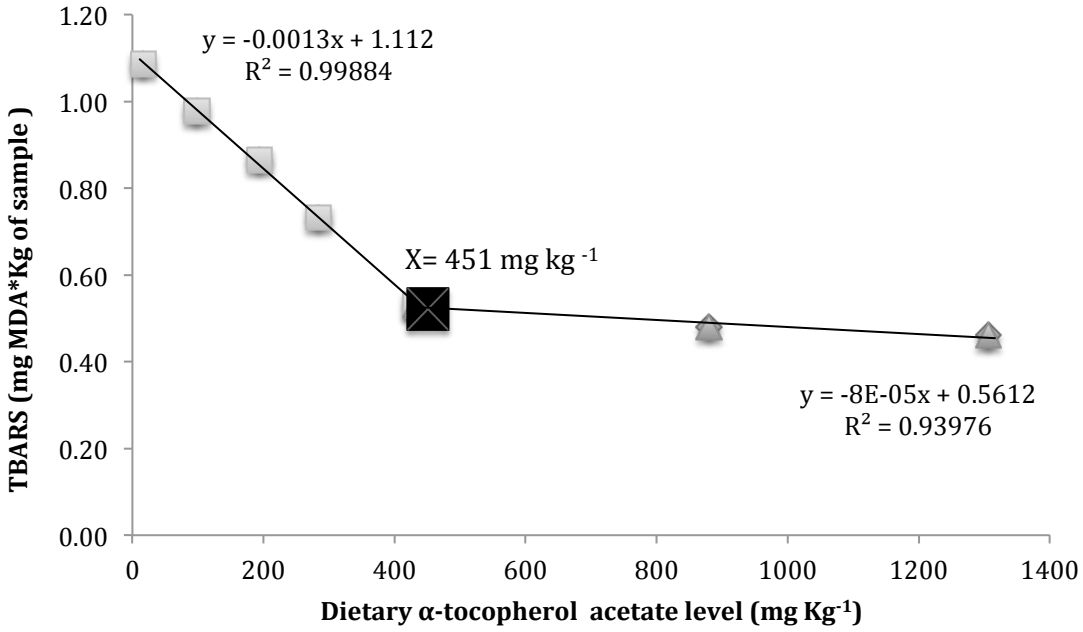
y = -0.0013x + 1.112
R² = 0.99884
X= 451 mg kg -1
y = -8E-05x + 0.5612
R² = 0.93976
TBARS (mg MDA*Kg of sample)
Dietary α-tocopherol acetate level (mg Kg-1)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
200
400
600
800
1000
1200
1400

Figure 2.