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**EFFECTOS DE LOS DIFERENTES NIVELES DE
VITAMINA E EN DIETAS DE ENGORDE PARA
CORVINA (*Argyrosomus regius*)**

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Trabajo realizado en el Instituto Canario de Ciencias Marina (ICCM) y en la Universidad de Las Palmas de Gran Canaria, España, bajo la dirección de la Dra. Lidia Esther Robaina Robaina y Dra. Carmen María Hernández.

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ÍNDICE

Agradecimientos -----	1
Índice tablas -----	2
Índice figuras -----	4
Introducción -----	6
Bibliografía-----	27
Objetivos -----	38
Resumen-----	39
Paper-----	41
Abstract-----	41
Introduction -----	43
Objectives-----	46
Materials and methods-----	47
Results-----	52
Discussion-----	64
Conclusions-----	77
Conclusiones-----	80
References-----	82

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Índice tablas.

Tabla I. Producción de la corvina (<i>Argyrosomus regius</i>) procedente de la acuicultura Mediterránea 1997-2010 (FAO, 2012d). Las cantidades son expresadas en toneladas.---	16
Tabla II. Niveles dietéticos de vitamina E y signos de deficiencia en los animales -----	23
Tabla III. Niveles dietéticos de vitamina E y signos de exceso en los animales.-----	24
Table IV. Formulation and proximate analysis of the experimental diets ($\text{g}\cdot\text{kg}^{-1}$) -----	48
Table V. Fatty acid profile (mean \pm SD) of the experimental diets (g/100g fatty acid) ----	52
Table VI. Main fatty fatty acid and relations (mean \pm SD) of the experimental diets (g/100g fatty acid) -----	53
Table VII. Fish growth performance, feed utilization and biometric parameters (mean \pm SD) of juveniles meagre fed different levels of vitamin E for 72 day of experimental -----	53
Table VIII. Biochemical composition (mean \pm SD) of whole body, muscle and liver in juvenile meagre (% wet weight) -----	54
Table IX. Whole body fatty acid composition (means \pm SD) of meagre at the end of the trial (g/100g fatty acid) -----	57
Table X. Main fatty acid and relations of the whole body (means \pm SD) of meagre at the end of the trial (g/100 g fatty acid) -----	58
Table XI. Muscle fatty acid composition (means \pm SD) of meagre (g/100g fatty acid) --	59
Table XII. Main fatty acid and relations of muscle (means \pm SD) of meagre at the end of the trial (g/100 g fatty acid) -----	60

Table XIII. Fatty acid composition (means±SD) of meagre liver at the end of the trial
(g/100g fatty acid) ----- 61

Table XIV. Main fatty acid and relations of liver (means±SD) at the end of the trial (g/100
g fatty acid) ----- 61

Table XV. Thiobarbituric acid reactive substance (TBARS) in fillet of meagre fed graded
levels of vitamin E ----- 62

Listas de figuras

Figura 1. La producción de la acuicultura y de capturas en los países Mediterráneos incluyendo las algas desde el año 1.950 al 2010 (FAO, 2012d) -----	8
Figura 2. La producción de la acuicultura en los países del Mediterráneo desde 1984 al 2010 (FAO, 2012d) -----	10
Figura 3. Producción de mercado de los alimentos acuícolas por países de pescado, moluscos y crustáceos (FAO, 2012d) -----	11
Figura 4. Exportación de pescado, crustáceos y marisco frescos, congelado y enlatado procedente de los países Mediterráneos (FAO, 2012d) -----	12
Figura 5. Importación de pescado, molusco y crustáceos frescos, congelado y enlatado procedente de los países Mediterráneos (FAO, 2012d) -----	13
Figura 6. Evolución de la producción acuícola de corvina en Europa y Mediterráneo entre 1990-2010, basados sobre datos de APROMAR, FAO y FEAP (APROMAR, 2011). ----	15
Figura 7. Evolución de la producción mundial procedente de las capturas y de la acuicultura de corvina (<i>Argyrosomus regius</i>) para el periodo 1997-2010 (FAO, 2012d) -	15
Figura 8. Producción de captura y de acuicultura en los países Mediterráneo (FAO, 2012d) -----	16
Figura 9. Imágenes de corvina (<i>Argyrosomus regius</i>) en su medio natural-----	17
Figura 10. Mapa de distribución de corvina (<i>Argyrosomus regius</i>) -----	17
Figura 11. Imágenes de corvina (<i>Argyrosomus regius</i>) cultivadas en tanques y en jaulas-----	19

Figure 12. TBARS concentrations in muscle of juvenile meagre fed diets containing graded levels of vitamin E ----- 62

Figure 13. Liver of meagre fed with different levels of vitamin E. (a) and (b) Livers of the diet 1500. Granulomes and migration of the nuclei in hepatocytes (H&E). (c) Diet E1000. Granulomes and migration of the nuclei in hepatocytes (H&E). (d) and (e) Diet E0. Lipid vacuoles accumulation (H&E) ----- 63

INTRODUCCIÓN

La acuicultura comprende una serie de actividades cuyo objetivo se centra en la reproducción y cría, crecimiento, protección contra los depredadores, control de enfermedades, genética, sistemas de cultivo, técnicas de producción y en el comercio de los organismos acuáticos, incluyendo peces, moluscos, crustáceos y algas, tanto de agua dulce, como salada y salobre. En los últimos años, esta actividad se ha basado en controlar la reproducción y mejorar el conocimiento biológico de las especies cultivadas e innovar en tecnología para producir especies de rápido crecimiento, así como en el desarrollo de productos alimenticios seguros y de alta calidad (OESA, 2010).

La acuicultura, más allá de representar al sector emergente de producción de alimento de mayor crecimiento anual, es considerada como una actividad del presente y del futuro, debido a su potencial de desarrollo capaz de contribuir a reducir la pobreza y la malnutrición, proporcionando alimentos ricos en proteínas, ácidos grasos insaturados, vitaminas y minerales que contribuyen a mejorar el bienestar y la salud de las personas. Esta actividad genera ingresos y nuevos puestos de trabajo cualificados, promoviendo así el desarrollo socioeconómico de las regiones en la que continua su expansión (FAO, 2012a). Así, el sector de producción de organismos acuáticos crece más rápido que el sector de la ganadería terrestre con una tasa media anual de un 8,8% desde 1970 en acuicultura, frente a un 2,8% para aves, cerdos y ganado de corral. En la actualidad la acuicultura representa más de un tercio de la producción global de pescado (47%), de la cual la producción Europea sólo representa un 4% de la producción de la acuicultura global (General Fisheries Commission for the Mediterranean, 2010).

Acuicultura Mediterránea: Producción de la Acuicultura Mediterránea

La producción total de peces, moluscos, crustáceos y algas de la acuicultura en los países Mediterráneos han alcanzado 1,815.338 toneladas en 2010, las cuales representan aproximadamente un 2,32% de la producción mundial en acuicultura (78,065.388 toneladas) en ese mismo año (**Figura 1**). La industria de la acuicultura de los países Mediterráneos ha crecido tremendamente en los últimos años (17,76% entre 1970 y 2010), debido principalmente a que las capturas se han estabilizado y las especies convencionales están totalmente explotadas o sobreexplotadas. Ya desde principios del 2000 algunos trabajos como el de Basurco, B. y Lovatelli, A. (2003), señalan que la mayor parte de la producción de algunas especies tales como el mejillón (*Mytillus galloprovincialis*), almeja (*Ruditapes philippinarum*), ostra (*Ostrea edulis*), corvina (*Argyrosomus regius*), dorada (*Sparus aurata*), lubina (*Dicentrarchus labrax*), trucha (*Oncorhynchus mikiss*), tilapia (*Orochromis niloticus*) y carpa (*Cyprinus carpio*) proviene casi totalmente de la acuicultura; lo que según estos autores se atribuye, principalmente a los siguientes factores: un incremento de la población mundial, unido al incremento de consumo per capita de pescado que se ha duplicado en los últimos 50 años (Ahmed, M. y Delgado, C., 2000; Muir, J., 2005), con valores de 14,4 kg en 1990 a 18,8 kg per cápita/año en el 2011; y a la acotación de las zonas pesqueras de cada país así como al incremento en los precios de fuel. Por todo lo indicado, la acuicultura es entendida como una estrategia de futuro importante en la Unión Europea y en los países Mediterráneos, ya que proporciona seguridad alimentaria, una fuente de nutrición de alta calidad a los consumidores y proteínas seguras y relativamente baratas.

La acuicultura en los países mediterráneos ha estado cuantitativamente más focalizada en la producción de moluscos, pero en la actualidad la producción de moluscos ha decaído de 649.706 Tm en 1999 a 499.886 Tm en el 2010. Por el contrario, la parte de

la producción acuícola relativa a las especies de peces marinos se ha incrementado constantemente de 1.280 Tm en 1970 hasta 412.600 Tm en el 2010. En el mismo periodo, la producción de peces de agua dulce presenta un aumento constante desde las 60.529 a las 901.709 Tm, mientras que la producción de crustáceos ha disminuido de 3.281 en 1987 a 1.022 Tm en el 2010. Finalmente, la producción de algas ha descendido de 5.062 Tm en 1997 a 121,25 Tm en 2010 (FAO, 2012d).

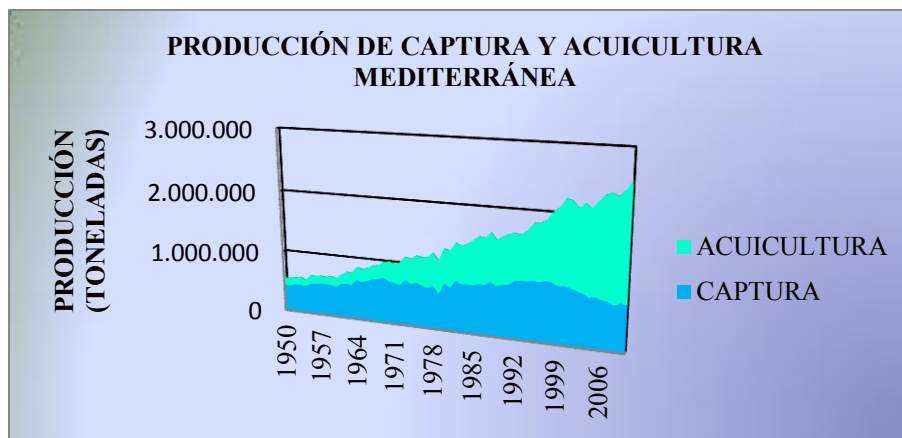


Figura 1. La producción de la acuicultura y de capturas en los países Mediterráneos incluyendo las algas desde el año 1950 al 2010 (FAO, 2012d)

El crecimiento de la industria acuícola en los países Mediterráneos es favorecido por su geografía (condiciones ideales físico químicas y temperatura) y por su proximidad a los mercados viables. La producción de la acuicultura en el Mediterráneo es dominada por seis países: Egipto, España, Francia, Italia, Grecia y Turquía. La producción total de la acuicultura en los países del Mediterráneo desde 1984 al 2010 se muestra en la **figura 2**.

La producción a escala comercial en Egipto según los datos expuesto por la FAO (2012c), esta basada principalmente en especies de agua dulce como la tilapia (*Oreochromis aureu*, *Oreochromis niloticus*), carpa (*Cyprinus carpio*, *Ctenopharygodon idellus*, *Hypophthalmichthy hobilis* y *Mylopharyngodon piceus*) y camarón gigante (*Macrobrachium rosenbergii*). También, produce a nivel comercial especies marinas tales como la lubina (*Dicentrarchus labrax*), dorada (*Sparus aurata*), corvina (*Argyrosomus*

regius), mugil (*Mugil cephalus*) y el crustáceo decápodo (*Litopenaeus vannamei*). Por el contrario, la mayor producción en Grecia, está basada en diferentes especies de peces marinos como dorada, lubina, trucha arcoiris (*Onchorynchus mykiss*), anguila europea (*Anguila anguila*), sargo picudo (*Diplodus puntazo*), breca (*Pagellus erythrinus*), sargo (*Diplodus sargus*), atún (*Thynnus thynnus*), lenguado (*Solea solea*), mugil y dentón (*Dentex dentex*). En el caso de Turquía, la producción está dividida entre especies marinas tales como mejillón (*Mytilus galloprovinciales*), camarón (*Penaeus japonicus*), dorada, lubina, la trucha arcoiris (*Oncorhynchus mykiss*), lenguado del Mar Negro (*Scophthalmus maeoticus*), salmón del Atlántico (*Salmo salar*), sargo picudo, pargo común (*Pagrus pagrus*), dentón, mero (*Epinephelus spp.*) y el engorde del atún rojo del Atlántico (*Thunnus thynnus thynnus*) y otras especies de agua dulce como la carpa (*Cyprinus carpio*). La producción a escala comercial en Francia está centrada principalmente en ostras japonesas o del pacífico (*Cassostrea gigas*), trucha arcoiris, lubina, dorada, rodaballo (*Psetta maxima*), y camarón (*Penaeus stylirostris*). En Italia la producción está basada fundamentalmente en mejillones y almejas (*Ruditapes philippinarum* y *Ruditapes decussatus*), aunque también, se producen especies eurihalinas tales como lubinas, doradas, anguila, diferentes especies de mugílidos (*Mugil cephalus*, *Chelon labrosus*, *Liza ramada*, *Liza saliens* y *Liza aurata*), breca, verrugato (*Umbrina cirrosa*), dentón y corvina, y especies de agua dulce como diferentes especies de carpa, trucha (*Oncorhynchus mykiss*, *Salmo trutta* y *Salmo trutta marmoratus*), lucio norteño (*Esox lucius*), bagre (*Coregonus lavarettus*) y esturión (*Acipenser naccarii*, *A. transmontanus* y *A. baerii*).

En España, las especies marinas criadas actualmente a escala comercial según la FAO (2012c) son: mejillón, dorada, lubina, rodaballo, atún rojo (*Thunnus thynnus*), besugo (*Pagellus bogaraveo*), lenguado (*Solea vulgaris*), sargo (*Diplodus sargus*), mugil (*Mugil spp.*), corvina (*Argyrosomus regius*), anguila europea, almeja japonesa (*Ruditapes*

philippinarum), salmón del Atlántico, seriola o pez de limón (*Seriola dumerilii*), dentón, abadejo (*Pollachius pollachius*) y baila (*Dicentrarchus punctatus*); y respecto a peces continentales y de aguas salobres, las especies más importantes producidas son: la trucha arcoiris, esturión (*Acipenser naccarii*) y la tenca (*Tinca tinca*). Atendiendo a los datos de la FAO (2012c) podríamos decir que la producción en España está especialmente basada en mejillón, trucha arco iris, lubina, rodaballo y corvina. Por otro lado, y según el informe de APROMAR 2011, las especies marinas de crianza a nivel comercial en España serían: dorada, lubina, rodaballo, anguila, besugo, corvina, lenguado y langostinos, siendo la dorada, la lubina, el rodaballo y la corvina las especies de mayor producción en el año 2010, con un notable incremento de esta dos últimas, que son dos de las especies de mayor rentabilidad por kilogramo producido en los últimos años.

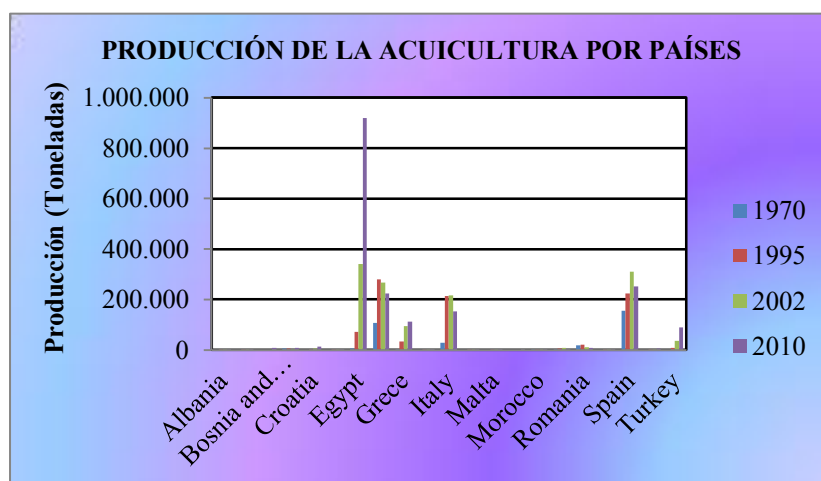


Figura 2. La producción de la acuicultura en los países del Mediterráneo desde 1984 al 2010 (FAO, 2012d).

El mercado de los productos de la acuicultura de los países Mediterráneos está adecuado, como el mercado de cualquier otro sector, a sus potenciales consumidores, considerando sus necesidades, hábitos alimenticios y economía para responder y satisfacer su demanda. Por otro lado, el mercado debe tener en cuenta los cambios estructurales de la familia actual y el desarrollo de los nuevos hábitos alimenticios, debido a que en los últimos años han incrementado el número de personas que viven solas y también el

consumo de comidas rápidas y pre-cocinadas. Estos cambios, llevan a las industrias a incrementar la producción de productos elaborados, bien mediante eviscerado y corte en pequeñas proporciones o bien directamente producir productos precocinados (General Fisheries Commission for the Mediterranean, 2010).

El abastecimiento del mercado de alimentos acuáticos procedentes de la acuicultura se ha incrementado significativamente desde una producción de 267.227 toneladas en 1976 a 899.725 toneladas en el 2009 (**Figura 3**). Este incremento en la producción de alimentos acuáticos, está ligado a la mejora de la eficiencia del pienso y a una disminución del factor de conversión (FCR) según lo informado por Fernández, A. *et al.* (2005). Los mayores países productores de alimentos procedentes de la acuicultura en dicho año eran: España, Francia, Marrueco, Italia y Portugal, de los cuales suponen un 97,05% de la producción global de los países Mediterráneos.

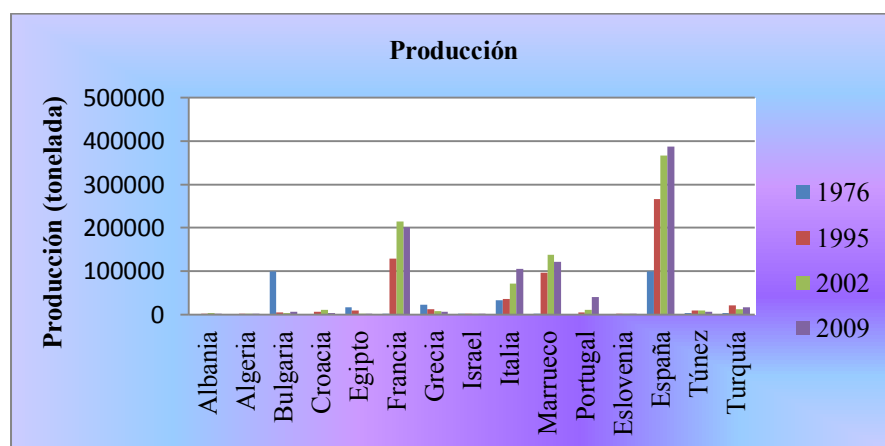


Figura 3. Producción de mercado de los alimentos acuícolas por países de pescado, moluscos y crustáceos (FAO, 2012d).

El consumo doméstico de los alimentos acuáticos ha sido extrapolados a producciones, importaciones y exportaciones de los datos proporcionados por la FAO (2012d) para la acuicultura de los países Mediterráneos. El consumo aparente (producción+importaciones–exportaciones) de pescado, crustáceos y moluscos frescos, congelados y enlatados en el área del Mediterráneo es aproximadamente de 1,516.757 Tm, de las cuales se produjeron unas 899.725 Tm en el año 2009 (**Figura 3**) y se exportaron

unas 737.517 Tm con un valor económico de 3,159.352 €, de los cuales España, Marruecos, Francia, Grecia, Portugal, Italia y Turquía son los mayores países exportadores con un 96,59% de la exportación global (**Figura 4**). Sin embargo, la cantidad importada es aproximadamente 1,354.549 Tm con un valor económico de 6,073.588 €, siendo España, Italia, Francia, Portugal, Grecia, Egipto, Marruecos y Bulgaria los mayores países importadores, representando un 92,35% de las importaciones globales del Mediterráneo (**Figura 5**).

En España el consumo aparente de pescado, crustáceos y moluscos frescos, congelados y enlatados ronda alrededor de 454.132 Tm, de las cuales se producen 387.942 Tm y se exportan 312.927 Tm con un valor económico de 905.113€. Sin embargo, la cantidad importada está alrededor de 379.117 Tm con un valor económico de 1,702.181€. Según los datos de la FAO (2012c), la tercera parte de la producción española de mejillones es destinada a consumo interno y el resto es exportada a Italia y Francia. La producción de peces marinos es destinada mayormente a los mercados nacionales, mientras que las importaciones de pescado proceden de los alevines criados en Grecia, Francia, Turquía, Chipre, Croacia y Malta, y que en su mayoría fueron exportados previamente desde criaderos españoles hasta esos países.

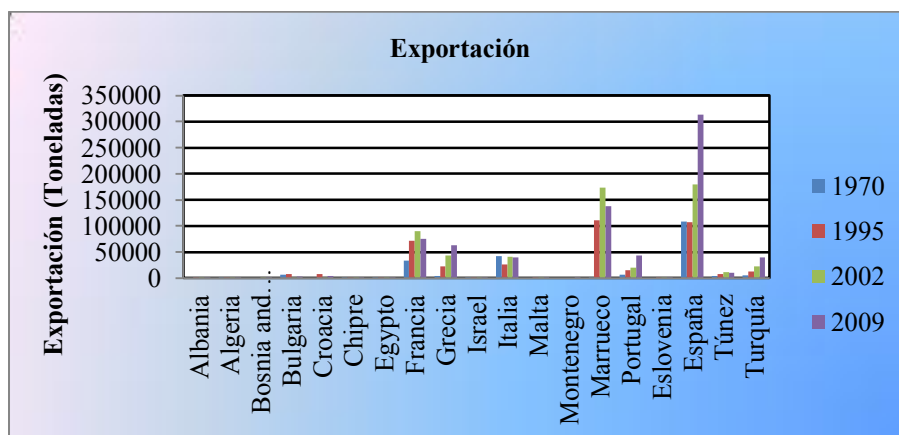


Figura 4. Exportación de pescado, crustáceos y marisco frescos, congelado y enlatado procedente de la acuicultura de los países Mediterráneos (FAO, 2012d).

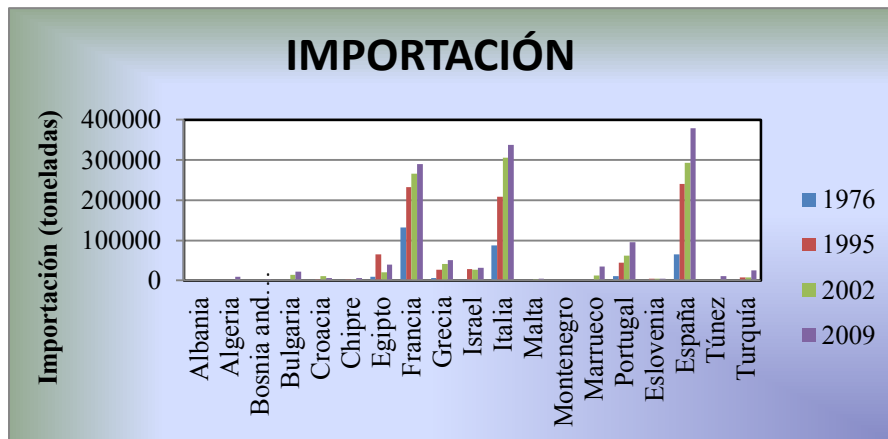


Figura 5. Importación de pescado, molusco y crustáceos frescos, congelado y enlatado procedente de los países Mediterráneos (FAO, 2012d).

Efectos de la crisis económica actual sobre la acuicultura en el Mediterráneo

El efecto de la crisis en el sector de la acuicultura según APROMAR (2011), ha sido avisado desde hace tiempo y en la actualidad está plasmada en las estadísticas con un estancamiento general de las producciones. Esta situación fue causada por una continuada pérdida de competitividad de las empresas de acuicultura radicadas en la Unión Europea por retos endógenos y una serie de graves causas exógenas.

Los retos endógenos en los que las empresas deben trabajar para paliar dicho problema son: reducir los costes, mejorar la eficiencia productiva, la apertura de nuevos mercados y en la búsqueda de fórmulas de concentración empresarial. Para ello, las empresas en los últimos años, se han centrado en el desarrollo de productos de valor añadido, en orientar al cliente, avanzar en innovación, en abrir nuevos mercados, utilización de certificados de calidad o medioambientales, personal altamente cualificado, mejorar la comunicación y promoción al exterior y en la producción de nuevas especies con potencial, debido a que en tiempos de crisis la diversificación de especies ayuda a amortiguar los efectos de estancamiento de la producción de dorada y lubina sin implicar el futuro de dichas producciones. Estas nuevas especies tales como la corvina y el lenguado

convierten a la acuicultura en un negocio empresarial abierto a otros campos económicos ya que serían las únicas que reportan beneficios reales a las empresas según lo informado por APROMAR (2011). Por otro lado, las pérdidas de competitividad debidas a causas exógenas, es originada por la desigualdad en las condiciones de participación en un mercado global y por la inexistencia de un marco normativo apropiado.

El avance en estrategias empresariales para paliar esa pérdida de competitividad de las empresas, se ha visto afectado en los últimos años por la crisis debido a que ha ocasionado una disminución en los préstamos a las empresas por las instituciones financieras nacionales, una disminución de la inversión extranjera directa y una reducción de la asignación presupuestaria para el sector (en términos de inversiones, investigación, servicios de extensión y capacitación). Todos estos factores, han llevado a mayores costes de producción a las empresas por una subida de los piensos, una disminución de las demandas y una bajada de los precios del pescado en el mercado, originando que muchas de las pequeñas empresas desaparezcan y sólo persistan las grandes empresas y las multinacionales según la FAO (2011).

Relevancia del cultivo de especies alternativas de interés: caso de la corvina (*Argyrosomus regius*)

La corvina (*Argyrosomus regius*) es una especie que ha comenzado a ser producida regularmente y de forma extendida en acuicultura a partir del año 2005. La producción a escala comercial de esta especie se lleva a cabo en Europa, en diversos países mediterráneos y en numerosas empresas, debido a que presenta un crecimiento mucho más rápido que la dorada y la lubina. Por este motivo, la corvina se coloca más rápido en el mercado, ya que pueden alcanzar 1 kg en 12 meses, contando además con que su rango de talla comercial es bastante amplio entre 1 y 4 kg aproximadamente.

La corvina es un pescado muy apreciado en aquellas regiones en las que se ha venido consumiendo tradicionalmente, no obstante dada su escasa pesca y su reciente producción, es poco conocida en la mayor parte de los mercados (APROMAR, 2011). La producción actual de corvina en Europa, según el informe de APROMAR (2011), es de 3.855 toneladas, un 77% más que en el 2009. Los principales países productores de corvina a nivel Europeo son: España con una producción de 3.250 toneladas (84,3% del total), Italia, Francia y Grecia (**Figura 6**).

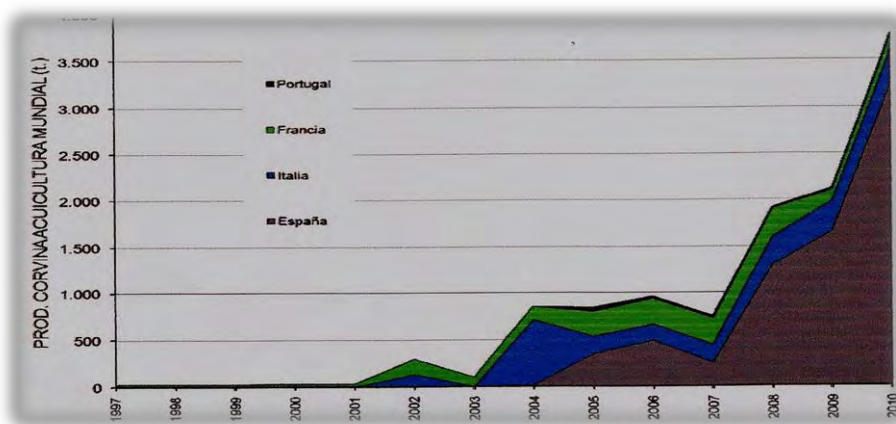


Figura 6. Evolución de la producción acuícola de corvina en Europa y Mediterráneo entre 1990-2010, basados sobre datos de APROMAR, FAO y FEAP (APROMAR, 2011).

Según los datos de la FAO (2012d), los países que capturan actualmente mayor cantidad de corvina son Ghana, Mauritania, Egipto y Francia con 5.134 Tm a nivel mundial en el 2010; mientras las capturas procedentes del Mediterráneo fueron de 2.077 Tm en ese mismo año (**Figura 8**). La producción total de las capturas de corvina a nivel mundial en el 2010 fue de 5.676 Tm (**Figura 7**).

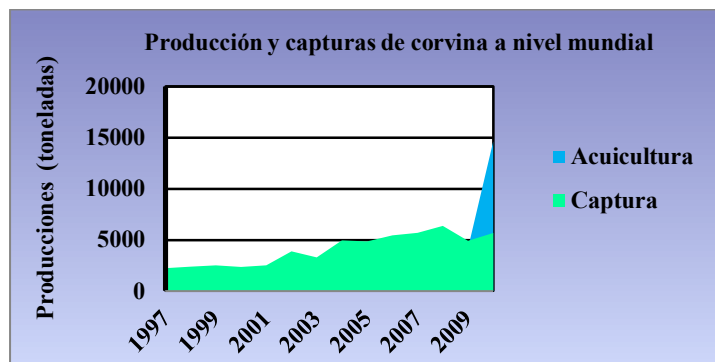


Figura 7. Evolución de la producción mundial procedente de las capturas y de la acuicultura de corvina (*Argyrosomus regius*) para el periodo 1997-2010 (FAO, 2012d).

Por otro lado, la producción mundial de corvina procedente de la acuicultura en el 2010 fue de 14.634 Tm (FAO, 2012d) (**Figura 8**). Egipto es, con diferencia, el mayor productor de corvina del Mediterráneo, con una cantidad de 12.246 Tm, seguido de España con una producción de 1.853 Tm (**Tabla I**).

Tabla I. Producción de la corvina (*Argyrosomus regius*) procedente de la acuicultura en los países Mediterráneos 1997-2010 (FAO, 2012d). Las cantidades son expresadas en toneladas.

País	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Croacia	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Chipre	0	0	0	0	0	0	0	0	0	0	0	0	0	12
Egipto	0	0	0	0	0	0	0	0	0	0	0	2.031	2.272	12.246
Francia	30	30	30	33	35	165	100	147	267	282	282	555	418	400
Italia	0	0	0	0	0	131	0	696	186	172	192	109	102	45
Portugal	0	0	0	0	0	0	0	0	47	23	27	15	44	76
España	0	0	0	0	0	0	3	16	347	489	251	1.123	1.348	1.853
Total	30	30	30	33	35	296	103	859	847	966	752	3.833	4.184	14.634

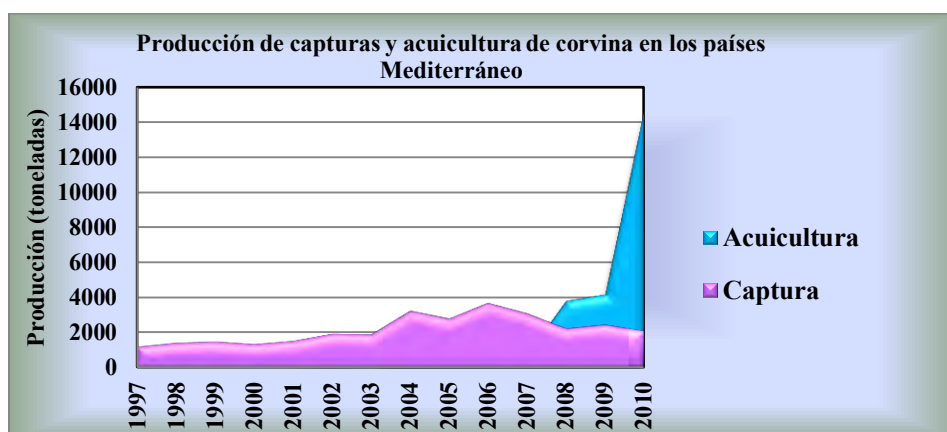


Figura 8. Producción de captura y de acuicultura en los países Mediterráneo (FAO, 2012d).

La corvina como especie de interés para la acuicultura. Biología y estado actual de cultivo

La corvina es un pez teleósteo de la familia Scianidae (Chao, L., *et al.*, 1986). Esta especie, se distribuye por el Atlántico, desde el Sur de Suecia y Noruega hasta las desembocaduras del río Congo, incorporando Canarias y Madeira y en todo el Mediterráneo pero de forma escasa (Chao, L., *et al.*, 1986; Cárdenas, S., 2010) (**Figura 9 y 10**).



Figura 9. Imágenes de corvina (*Argyrosomus regius*) en su medio natural.

Es una especie, eurihalina y euriterma que habita generalmente en fondos rocosos o campos de *Posidonia sp.* y suelen encontrarse en profundidades de entre 15 y 200 metros. Se desplaza normalmente en pequeños grupos hacia la desembocadura de los ríos y lagunas para reproducirse (Catalán, I., *et al.*, 2006; Cárdenas, S., 2010). Los juveniles exploran diferentes hábitats hasta que llegan a adultos y alcanzan la madurez sexual, que aparece entre los 4 y 5 años.



Figura 10. Mapa de distribución de corvina (*Argyrosomus regius*).

La corvina, tal y como ha sido anteriormente expuesto se ha convertido en una especie potencial para la diversificación de la acuicultura en el Mediterráneo y en el este de la costa Atlántica (Poli, B., *et al.*, 2003; Hernández, M., *et al.*, 2009; Chatzifotis, S., *et al.*, 2010), por su rápido crecimiento y buenas características nutritivas del filete (Poli, B., *et al.*, 2003; Piccolo, G., *et al.*, 2008; Hernández, M., *et al.*, 2009; Grigokaris, K., *et al.*,

2011). Esas características, han hecho que el cultivo de corvina se desarrolle rápidamente, siendo actualmente la cuarta especie más importante en la acuicultura marina española (Cárdenas, S., 2010). Sin embargo, a pesar de su buena calidad en la composición bioquímica del filete y del pez entero, y ser altamente apreciada en el mercado, es una especie no ampliamente conocida y, por tanto, su precio medio actual en el mercado Europeo es de 7 a 12 €/kg dependiendo de las áreas de consumo (FAO, 2012b). Por otro lado, los productores para dar a conocer la corvina y abrir nuevos mercados llevan desde el año 2002 tratando de diferenciar los productos de corvina en el mercado: en peces pequeños (600 g a 1 kg), los cuales son vendidos enteros o fileteados. Mientras que los peces grandes (1 kg a 3-5 kg) son troceados o fileteados y ahumados (FAO, 2012b).

El engorde de *Argyrosomus regius* en el Mediterráneo y en las Islas Canarias se realiza principalmente en tanques circulares o rectangulares y en jaulas flotantes (offshore) con densidades desde 10 a 15kg/m³, adaptándose fácilmente a esas condiciones de cautividad y tolerando amplios rangos de temperatura y salinidad (Quémèner, L., 2002; Suquet, M., *et al.*, 2009; Cárdenas, S., 2010; Chatzifotis, S., *et al.*, 2012) (**Figura 11**). Por otro lado, desde el punto de vista productivo, presenta elevadas tasas de crecimiento y bajas tasas de conversión (Jiménez, M., *et al.*, 2005; Cárdenas, S., 2010), y mortalidades muy bajas ya que la corvina no tiene problemas de enfermedades porque la transferencia de las mismas en pequeñas poblaciones, es limitada (Duncan, N., *et al.*, en prensa). Aunque, hay estudios patológicos que afirman que las corvinas presentan enfermedades patológicas tales como úlceras localizadas en la cabeza y el vientre causadas por el hongo *Penicillium digitatum* (Manuali, E., *et al.*, 2005), infecciones por especies de gusanos de la Clase Trematodo, como por ejemplo; *Benedenia sciaenae* (Toksen, E., *et al.*, 2007), *Calceostoma spp.* (Hayward, C.J., *et al.*, 2007; Duncan, N., *et al.*, 2008) y *Sciaenocotyle spp.* (Hayward *et al.*, 2007, Ternengo *et al.* 2010), y de la Clase Nematoda, como *Philometra sp.*

(Moravec *et al.*, 2007). Por otro lado, se ha descrito la presencia de granulomatosis en la fase de engorde de la corvina, que ha sido asociadas a las carencias nutricionales de vitamina C y del complejo vitamínico B (Ghittino, C., *et al.*, 2004).



Figura 11. Imágenes de corvina (*Argyrosomus regius*) en tanques y en jaulas.

Atendiendo a sus hábitos alimenticios, la corvina es una especie de hábitos nocturnos y carnívora, en su medio natural la alimentación está basada en misidáceos, poliquetos, crustáceos decápodos, equinodermos, moluscos y teleósteos (Cabral, H. y Ohmert, B., 2001; Jiménez, M., *et al.*, 2005; Cárdenas, S., 2010). Es por este motivo, de sus hábitos alimenticios carnívoros, en cierta forma similares a los de la dorada y la lubina, por lo que bajo condiciones de cultivo se las ha estado alimentando hasta hace poco tiempo con piensos comerciales usados para estas dos especies, con niveles dietéticos de entre un 45% a 48% de proteínas y de 20 a 24% de lípidos.

De los pocos estudios nutricionales realizados en corvina, actualmente se sabe que esta especie parece tener unos requerimientos más bajos de lípidos y más altos de proteínas que los de la dorada y lubina. Así, el requerimiento óptimo de lípidos para esta especie según Chatzifotis, S., *et al.* (2010), es alrededor de un 17%, ya que estos autores encontraron que tanto niveles de lípidos superiores (21%) como inferiores (13%) ocasionaron una reducción en la tasa de crecimiento de la corvina. En un estudio posterior de Estévez, A., *et al.* (2011), demostraron que la tasa de crecimiento de las corvinas no se vio afectada con una dieta comercial que incluía 315 g kg⁻¹ de proteínas vegetales (o 76,2% del contenido total de proteínas en dieta), utilizada en peces con un peso medio de 20,47g.

Por último, un estudio reciente realizado también por Chatzifotis, S., *et al.* (2012), muestra que los requerimientos óptimos encontrados para corvina son de un 50% de proteína y un 17% de lípidos, con los cuales se obtuvieron mejores resultados de conversión del alimento (FCR) y crecimiento (SGR) que en los estudios mencionados anteriormente.

Importancia del pienso en el incremento sostenible de la producción de peces. Caso de la corvina

La dieta seca es un componente ideal para el desarrollo de una acuicultura sostenible ya que bajo unos requerimientos nutritivos adecuados en la dieta para peces marinos y de agua dulce, se produce una bajada de los factores de conversión (FCR), mejora la palatabilidad, hay una mayor eficiencia proteica (PER), un mayor crecimiento, ya que incrementa la respuesta inmune y la resistencias a enfermedades, debido a la adición de nutrientes esenciales como aminoácidos, fosfolípidos y antioxidantes, ocasionando además un menor impacto medioambiental de los sistemas de cultivo.

La calidad del pienso repercute de forma positiva en la calidad composicional y nutricional del pescado, en el sabor y la apariencia de los peces (Hasan, M., 2001; Fernández, A., *et al.*, 2005; Muir, J., 2005; General Fisheries Commission for the Mediterranean, 2010). El aumento de la producción y esas mejoras de la calidad del producto, favorecen que el pescado de crianza sea más competitivo en el mercado, permitiendo a las empresas mejorar sus costes de producción y obtener mayores beneficios (Fernández, A., *et al.*, 2005; General Fisheries Commission for the Mediterranean, 2010).

En los últimos años, la tendencia de las compañías de pienso e instituciones investigadoras, se ha basado en trabajar sobre la reducción de harina y aceite de pescado en los piensos, usando sustituciones de harina y aceite vegetales tales como soja, girasol, cereales de gluten, colza, etc. (Fernández, A., *et al.*, 2005; FAO, 2012 a), a causa de la escasez de la harina y el aceite de pescado. Esos estudios han suministrado información

más detallada tanto sobre los procesos digestivos y las necesidades nutricionales de muchas especies cultivadas como el procesamiento de las materias primas de los piensos para lograr que sean más adecuadas a la hora de su inclusión en los mismos. Esta mejora en el conocimiento ha permitido disminuir los factores de conversión y a reducir la cantidad de residuos de la industria (Fernández, A., *et al.*, 2005; FAO, 2011; FAO, 2012a).

Existe, como ha sido comentado en el apartado anterior, muy poca información sobre las dietas óptimas para corvina, lo cual resulta cuanto menos extraño dado el incremento mostrado en la producción de esta especie en los últimos años. Así y todo, las estadísticas y varios trabajos de investigación muestran que la producción de corvina es rentable, debido a su rápido crecimiento y a la buena calidad del filete (Poli, B., *et al.*, 2003; Piccolo, G., *et al.*, 2008; Hernández, M., *et al.*, 2009; Grigokaris, K., *et al.*, 2011) proporcionando a los animales el doble de ración que a la dorada y pudiendo ser alimentados al 1 o 2% de su peso corporal/día según Duncan, N., *et al.* (en prensa) y Cárdenas, S. (2010) respectivamente. Todos estos factores, hacen que actualmente la investigación este orientada al desarrollo de dietas optimizadas para corvinas y en sus diferentes fases de desarrollo ya que resultan indispensables por las repercusiones que tendrían para mejorar las tasa de crecimiento y la eficiencia de los nutrientes según Martínez-Llorens, S. *et al.* (2011).

Vitamina E en las dietas para engorde de peces: Niveles y repercusiones

La vitamina E es un grupo de ocho componentes liposolubles formado por cuatro tocoferoles y cuatro tocotrienoles (NRC, 1993). Está vitamina es añadida en las dietas de engorde en forma de α -tocoferol acetato, debido a que es la forma más estable del α -tocoferol, ya que los peces son incapaces de sintetizarla, es importante incorporarla en la dieta para satisfacer los requerimientos nutricionales de los animales (Puangkaew, J., *et al.*, 2004; Hamre, K., 2011).

Diversos estudios nutricionales y fisiológicos han mostrado el importante papel de la vitamina E sobre las mejoras en el crecimiento en varias especies acuáticas (Cowey, C., *et al.*, 1984; Wilson, R., *et al.*, 1984; Hamre, K y Øyvind, L., 1995; Baker, R. y Davies, S., 1997; Bai, S.C y Lee, K., 1998; Kocabas, A. y Gatlin III, D.M., 1999; Mourente, G., *et al.*, 2000; Tocher, D.R., *et al.*, 2003; Lin, Y. y Shiau, S., 2005; Jittinandana, S., *et al.*, 2006; Peng, S., *et al.*, 2009; Abdel-Hameid, H., *et al.*, 2012), debido a que mejora el sistema inmunitario en los peces según estudios previos realizados (Murray, T. y Andrews, J., 1974; Sealey, W. y Gatlin III, M., 2002; Puangkaew, J., *et al.*, 2004; Lin, Y. y Shiau, S., 2005; Puangkaew, J., *et al.*, 2005; Yildirim-Aksoy, M., *et al.*, 2008; Zhong, Y., *et al.*, 2008; Kashani, Z., *et al.*, 2011; Abdel-Hameid, H., *et al.*, 2012). Sin embargo, hay por el contrario otros importantes números de trabajos que demuestran que la vitamina E no afecta al crecimiento, algunos de estos aspectos quedan bastante menos claros en el caso de especies de rápido crecimiento como la corvina (Stéphan, G., *et al.*, 1995; Gaylord, T., *et al.*, 1998; Bell, G., *et al.*, 2000; Gatta, P.P., *et al.*, 2000; Lygren, B., *et al.*, 2000; Tocher, R., *et al.*, 2002 ; Ruff, N., *et al.*, 2002 ; Ruff, N., *et al.*, 2003; Tocher, D.R., *et al.*, 2003; Puangkaew, J., *et al.*, 2004 ; Puangkaew, J., *et al.*, 2005; Xiao-dong, Z., *et al.*, 2007; Peng, L., *et al.*, 2008; Yildirim-Aksoy, M., *et al.*, 2008 ; Zhong, Y., *et al.*, 2008).

Entre las múltiples funciones de la vitamina E en los parámetros productivos de los animales son muchos los autores que relacionan los efectos antioxidantes del α -tocoferol sobre las mejoras en el crecimiento y la calidad del producto, sobre todo en la prevención que ejercería esta vitamina sobre el daño oxidativo de los tejidos y de los ácidos grasos. Este efecto protector ocurre mediante la donación que realiza el α - tocoferol de sus átomos de hidrógeno fenólicos a los radicales libres de los lípidos, resultando de esta manera en la terminación de la reacción en cadena de la peroxidación lipídica (Burton, G. y Ingold, K., 1989 ; Mourente, G., *et al.*, 2007).

Se presenta a modo de resumen (**tablas II y III**) los trabajos que han sido realizados con niveles de vitamina E en dietas para acuicultura y sus respuestas fisiológicas y productivas en los animales. Se muestran tanto los signos de deficiencia como el exceso de esta vitamina.

Tabla II. Niveles dietéticos de vitamina E y signos de deficiencia en los animales.

Especies	Niveles (mg/kg)	Vitamina E	Señales	Autores
<i>Cyprinus carpio</i>	0-50	DL- α -tocopherol acetate	Pobre crecimiento, distrofia muscular, atrofia, trastorno de las fibras musculares, ceroides en el bazo, incremento de proteínas en el suero	Watanabe, T., et al. (1970)
<i>Salmo gairdneri</i>	0-40	DL- α -tocopheryl acetate	Mayor fragilidad de los eritrocitos, peroxidación lipídica en el músculo, hígado y branquias.	Cowey, C., et al. (1983)
<i>Ictalurus punctatus</i>	0-40	DL- α -tocopheryl acetate	Alta peroxidación lipídica, alta fragilidad de los eritrocitos, hemosiderosis en el bazo y en el páncreas, cerodiosis hepática.	Wilson, R., et al. (1984)
<i>Oreochromis aureus</i>	0	DL- α -tocopheryl acetate	Descoloración del hígado, acumulación de ceroides acompañada de necrosis hepática.	Roem, A., et al. (1990)
<i>Seriola quinqueradiata</i>	0	All-rac- α -tocopheryl acetate	Tejido elipsoidal hipertrófico en bazo, macrófagos hiperplásicos de retículoendotelio, melano-macrófagos centrados, pigmento amarillos (hemosiderina y ceroides), núcleos picnóticos, núcleos cariolíticos, citoplasmas basofílicos, vacuolas y ceroides, aumento de la TBAR en hígado.	Shimeno, S., (1991)
<i>Salmo salar</i>	0	DL- α -tocopheryl acetate	Descoloración del hígado, acumulación de ceroides acompañada de necrosis hepática, distrofia muscular, vacuolas en el hígado, altos nivel de eritrocitos y oxidación lipídica.	Hamre, K. y Øyvind, L. (1995)
<i>Sebastes schlegeli</i>	0-20	α -tocopherol	Disminución del crecimiento, bajos hematocritos, baja hemoglobina y oxidación lipídica.	Bai, S. y Lee, K., (1998)
<i>Morene chrysops</i>	0-10	DL- α -tocopheryl acetate	Disminución del crecimiento, baja tasa de eficiencia proteica, bajos hematocritos (0mg/kg de DL- α -tocopheryl acetate)	Kocabas, A. y Gatlin III, D.M., (1999)
<i>Cirrhinus mrigala</i>	19-66	DL- α -tocopheryl acetate	Disminución del crecimiento, baja tasa de eficiencia proteica, bajo SGR y mayor fragilidad de los eritrocitos,	Paul, B., et al. (2004)
<i>Ephinehus malabaricus</i>	0-50	DL- α -tocopheryl acetate	Disminución del crecimiento, altos valores de los TBARS hepática, bajo nivel de los leucocitos blancos, baja tasa de producción O ₂ ⁻ , baja actividad de la lisozima y baja actividad del complemento alternativo.	Lin, Y. y Shiau, S., (2005)
<i>Scianop ocellatus</i>	0-20	All-rac- α -tocopheryl acetate	Disminución del crecimiento, baja eficiencia alimentaria, edema en el corazón, baja eficiencia alimentaria, incremento en la producción de anión superóxido	Peng, L. y Gatlin III, D.M., (2009)
<i>Channa punctatus</i>	0-100	DL- α -tocopheryl acetate	Mayor índice hepatosomático, retardo en el crecimiento y bajo factor de condición, bajos niveles de hemoglobina, bajos hematocritos, alta fragilidad de eritrocitos y altos valores hepáticos de los TBARS.	Abdel-Hameid, H., et al. (2012)

Tabla III. Niveles dietéticos de vitamina E y signos de exceso en los animales.

Especies	Niveles (mg/kg)	Vitamina E	Señales	Autores
<i>Salmo gairdneri</i>	100	DL- α -tocopheryl acetate	Ligero aumento de la fragilidad de los eritrocitos.	Cowey, C., et al. (1983)
<i>Ictalurus punctatus</i>	60-80	DL- α -tocopheryl acetate	Aumento de la peroxidación lipídica.	Wilson, R., et al. (1984)
<i>Oreochromis aureus</i>	80	DL- α -tocopheryl acetate	Incrementa la oxidación lipídica.	Roem, A., et al. (1990)
<i>Seriola quinqueradiata</i>	300-500	All-rac- α -tocopheryl acetate	Ligero aumento de las TBARS.	Shimeno, S., (1991)
<i>Salmo salar</i>	200-800	DL- α -tocopheryl acetate	Peroxidación lipídica	Hamre, K. y Øyvind, L., (1995)
<i>Sebastes schlegeli</i>	120-500	α -tocopherol	Bajos hematocritos, baja hemoglobina (500), baja tasa de eficiencia proteica (500), y oxidación lipídica.	Bai, S. y Lee, K., (1998)
<i>Morene chrysops</i>	40-80	DL- α -tocopheryl acetate	Bajos hematocritos a un nivel de 60mg/kg vitamina E y sube a niveles de 80mg/kg de vitamina E	Kocabas, A. y Gatlin III, D.M., (1999)
<i>Cirrhinus mrigala</i>	216	DL- α -tocopheryl acetate	Menor fragilidad de los eritrocitos,	Paul, B., et al. (2004)
<i>Ephineelus malabaricus</i>	400-800	DL- α -tocopheryl acetate	Altos valores de la TBARS hepática, disminución de leucocitos blancos, alta tasa de producción O ₂ ⁻ , alta actividad de la lisozima y alta actividad del complemento alternativo.	Lin, Y. y Shiau, S., (2005).
<i>Scianop ocellatus</i>	40-80 IU	all-rac- α -tocopheryl acetate	Baja concentración de ácido ascórbico y peroxidación lipídica.	Peng, L. y Gatlin III, D.M., (2009)
<i>Channa punctatus</i>	220-260	DL- α -tocopheryl acetate	Reducción del crecimiento, pobres factores de condición, altos índices hepatosomáticos, reducción hemoglobina y bajada hematocrito.	Abdel-Hameid, H., et al. (2012)

El estrés oxidativo es producido por un exceso de moléculas de oxígeno reactivas (ROS) y de radicales libres tales como superóxidos (O₂⁻), hidroxilos (OH[·]), peróxido de hidrógeno (H₂O₂), ambos radicales de oxígeno centrados, tioles (RS[·] un radical de sulfuro centrado), triclorometilo (CCl₃[·], un radical de carbono centrado formado por el metabolismo del tetraclorometano en el hígado) y óxido nítrico (NO[·] en el cual el electrón no apareado es deslocalizado entre ambos átomos), las cuales son continuamente producidas por reacciones metabólicas, por transporte de electrones y actividades enzimáticas

(oxigenasas y la citocromo P450) en la mitocondrias, microsoma, membranas nucleares y fagocitos de los organismos aeróbicos (Halliwell, B. y Gutterried, J., 1996; Tocher, D.R., *et al.*, 2002). Estas moléculas de oxígeno reactivas a altas concentraciones pueden reaccionar con una amplia variedad de biomoléculas y de una manera bastante inespecífica, produciendo oxidación en los lípidos de la membrana, proteínas, ácido nucleicos y alterar de esta manera los estados redox celulares, dando como resultado patologías de los tejidos, carcinogénesis química y envejecimiento (Ames, B., 1989; Mourente, G., *et al.*, 2007). Mientras que pequeñas cantidades de estas moléculas de oxígeno reactivas y radicales libres son beneficiosas e incluso indispensables para la regulación del crecimiento celular y el desarrollo (Nordberg, J. y Arner, E., 2001; Zingg, J., 2007; Hamre, K., 2011).

El estrés oxidativo en los peces causa un deterioro oxidativo en los ácidos grasos altamente insaturados, el cual es conocido como peroxidación lipídica. La peroxidación lipídica comienza por las moléculas de oxígeno reactivas tales como la abstracción de hidroxilos de un átomo de hidrógeno de un metileno procedente de un ácido graso altamente insaturado, cediendo el paso a un radical lipídico como producto primario. Ese radical lipídico puede estabilizarse por reorganización molecular a un radical dieno conjugado, el cual reacciona fácilmente con el O₂ para producir el radical peroxilo (Mourente, G., *et al.*, 2007; Hamre, K., 2011), dando lugar a productos secundarios tales como aldehídos, cetonas, alcoholes, hidrocarburos, ácidos volátiles orgánicos y compuestos epóxidos. Esos radicales peroxilos formados por una vuelta del ciclo reaccionan con un nuevo ácido graso altamente insaturado. La terminación de esta reacción en cadena, es realizada cuando dos radicales lipídicos se combinan para formar una especie no-radical (HØlmer, G., 1993; Frankel, E., 1998; Hamre, K., 2011).

En diferentes ensayos, se ha demostrado que una deficiencia y un exceso de la vitamina E en los peces provoca un incremento de la peroxidación lipídica, causando un

incremento del proceso de elongación y desaturación de los ácidos grasos (Cowey, C., *et al.*, 1983; Wilson, R., *et al.*, 1984; Shimeno, S., 1991; Hamre, K. y Øyving, L., 1995; Baker, R. y Davies, S., 1997; Bai, S. y Lee, K., 1998; Bell, G., *et al.*, 2000; Peng, L. y Gatlin III, D.M., 2009), quedando reflejado en una disminución de los ácidos grasos altamente insaturados tales como el ácido eicosapentanoico (EPA) y ácido docohexanoico (DHA) y en un aumento de los ácidos grasos saturados y monoinsaturados.

Por otro lado en innumerables trabajos, se ha demostrado, que un incremento de la vitamina E en la dieta conserva los tejidos frente a dicha peroxidación lipídica, debido a que origina una disminución de los niveles de las sustancias reactivas del ácido tiobarbitúrico (TBARS) del músculo e hígado de los peces; aumentando así la estabilidad oxidativa de los tejidos almacenados en el congelador (Cowey, C., *et al.*, 1984; Gattlin, P.P., *et al.*, 1992; Bai, S. y Gatlin, D.M., 1993; Stéphan, G., *et al.*, 1995; Olsen, R. y Henderson, J., 1997; Gaylord, T., *et al.*, 1998; Sakai, T., *et al.*, 1998 b; Bell, G., *et al.*, 2000; Gatta, P.P., *et al.*, 2000; Ruff, N., *et al.*, 2002; Tocher, D.R., *et al.*, 2002; Ruff, N., *et al.*, 2003; Tocher, DR., *et al.*, 2003; Hamre, K., *et al.*, 1994; Lin, Y. y Shiau, S., 2005; Jittinandana, S., *et al.*, 2006; Xiao-dong, Z., *et al.*, 2007; Peng, L., y Gatlin III, D.M., 2009; Abdel-Hameid, H., 2012).

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OBJETIVOS

A la vista de lo anteriormente descrito y considerando la necesidad de optimizar las dietas para el cultivo de corvina (*Argyrosomus regius*), se plantea el presente trabajo cuyo objetivo general consiste en determinar los niveles óptimos dietéticos de vitamina E durante la fase de engorde de esta especie.

Para la consecución de este objetivo general se plantea un experimento en el que se probarán niveles crecientes entre 0 y 1.500 mg/kg de vitamina E en dieta con el fin de abordar los siguientes objetivos concretos en esta especie:

- 1) Evaluar las repercusiones en la vitamina E sobre el crecimiento y los parámetros productivos .
- 2) Determinar el impacto de la vitamina E sobre la composición bioquímica proximal y de ácidos grasos en el animal entero, en hígado y filete.
- 3) Estudiar el efecto de los niveles de vitamina E en dietas sobre la estabilidad oxidativa de los filetes.

RESUMEN

La corvina (*Argyrosomus regius*), es una especie potencial para la diversificación de la acuicultura Mediterránea por su rápido crecimiento y buenas características nutritivas del filete, así como su alto valor en el mercado, lo que ha propiciado un notable incremento de su producción en los últimos años. Sin embargo, y a pesar de las muchas toneladas de esta especie que se producen actualmente, existen muy pocos trabajos que aborden aspectos relacionados con sus requerimientos nutritivos, sobre todo en la etapa de engorde de los animales. Para engorde de corvina se tiene constancia de que sus requerimientos óptimos son de un 50% de proteína dietaria y un 17% de lípidos, y que parece soportar además niveles elevados de harinas vegetales en dieta (76.2% del total de la proteína). No existe sin embargo referencia alguna sobre requerimientos en otro tipo de nutrientes para esta especie, como vitaminas y minerales. La corvina es una especie magra de rápido crecimiento, por lo que sus requerimientos nutritivos distan de aquellos para la dorada y la lubina sobre las que se basan sus dietas comerciales actualmente. La determinación de requerimientos nutricionales es a día de hoy completamente necesaria para el incremento adecuado y sostenible de la corvina.

En este contexto se plantea el presente trabajo, con el objetivo de evaluar el efecto de la inclusión en dieta de niveles crecientes de 0 a 1500 mg/kg de vitamina E, sobre el crecimiento de los animales y parámetros productivos de las dietas, la composición bioquímica de los tejidos del animal y la estabilidad oxidativa de los filetes. Para ello se utilizaron corvinas de un peso medio inicial de $62,90 \pm 12,95$ g que fueron alimentadas con siete dietas isoproteicas (50%) e isocalóricas (16%), conteniendo diferentes niveles de inclusión de vitamina E (0, 100, 200, 300, 500, 1000 y 1500 mg/kg) en la forma de α -tocoferol acetato, siendo cada una de ellas evaluadas por triplicado. Las corvinas fueron muestreadas individualmente de peso y talla al inicio, 23, 48 y 72 días de alimentación.

Los resultados obtenidos muestran que la inclusión dietética de vitamina E a los niveles ensayados no afectó a los índices de supervivencia, crecimiento, y parámetros biométricos de los animales, ni a la utilización del alimento, La inclusión creciente de esta vitamina en la dieta causó una alteración de los lípidos y de la humedad del pez entero y del músculo, mientras que no alteró el contenido del hígado. Los perfiles de los ácidos en el pez entero y en el músculo mostraron un incremento de los ácidos grasos altamente insaturados (HUFA), una disminución de los ácidos grasos saturados y monoinsaturados con un incremento de la vitamina E en la dieta, acompañados de una disminución de la TBARS del músculo, mientras que en el hígado se observó una disminución de los ácidos grasos altamente insaturados y un aumento de los ácidos grasos saturados y monoinsaturados cuando los niveles de vitamina E estaban entre 300-500 mg/kg. Por otro lado, y para estos dos mismos niveles, 300-500 mg/kg, no se observaron vacuolas lipídicas ni granulomas en los cortes histológicos de hígados, mientras que sí aparecieron vacuolas lipídicas para los niveles de 100, 200 y 1000 mg/kg, observándose además granulomas en las dietas más extremas 0, 1000 y 1500 mg/kg de vitamina E.

Atendiendo a los resultados obtenidos, niveles de entre 300 y 500 mg/kg de vitamina E resultan adecuados en dietas para engorde de corvinas, si bien el requerimiento óptimo fue de 509,32 mg/kg de vitamina E, estimado por el método de regresión de la línea quebrada aplicada a los valores de las TBARS del músculo. Finalmente, un defecto o un exceso de la vitamina E en la dieta origina un deterioro en la salud de *Argyrosomus regius*, evidente en los tejidos hepáticos de los animales tras 72 días de alimentación, debido a la peroxidación lipídica que origina daños en los tejidos del animal e incrementa la estabilidad oxidativa del filete durante el almacenamiento.

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

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Abstract

Seven experimental isoproteic (50%) and iscaloric (16%) diet with different inclusion levels of vitamin E (0, 100, 200, 300, 500, 1000 and 1500 mg/kg) as α -tocopherol acetate were tested in triplicate groups, to evaluate fish growth responses, whole body and fillet and liver composition, fillet oxidation and liver histology in meagre fish, *Argyrosomus regius*. Juveniles meagre with an initial average weight of 62.90 ± 12.95 g were hand fed until apparent satiation for 10 weeks, along which fish were individually sampled for weight and length at days 0, 23, 48 y 72. Results did not show significant differences ($P > 0.05$) in final weight, length, specific growth rate (SGR), fish survival, feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and moreover not in the condition factor (K) and hepatosomatic and visceral indexes between treatments along the trial. The different levels of vitamin E inclusion caused on the other hand an alteration of the lipid and moisture in whole body and in the total muscle lipids; no effect were observed in the liver proximate composition. The fatty acid profiles in the whole body and muscle showed an increment of the highly unsaturated fatty acid (HUFA) and a

decrease of the saturated and monounsaturated fatty acid, with an increment of dietary vitamin E which was accompanied with a reduction of the muscle TBARS responses. In the livers, a decrease of the unsaturated fatty acid and an increment of the saturated and monounsaturated fatty acids were observed for the 300 and 500 mg/kg of vitamin E dietary levels. In these same diets, 300 and 500 mg/kg, did not appear lipid vacuoles at high extent or granulomes; lipid vacuoles were observed, in livers from diets 100, 200 and 1000 mg/kg and moreover granulome at the extreme diets levels, 0, 1000 and 1500 mg/kg of vitamin E, which demonstrate the prooxidant effect of this vitamin in meagre tissues at levels over 1000 mg/kg of vitamin E while oxidant effects were observed for levels under 300 mg/kg act as oxidant.

According to the above results, an optimum dietary inclusion level between 300 and 500mg/kg of vitamin E (DL- α -tocopherol acetate) during the on-growing of meagre was established. Moreover, a requirement of 509.32 mg/kg vitamin was obtained by the broken-line regression analysis applied to muscle TBARS responses. Results also demonstrate how a defect and an excess of vitamin E in the diet of meagre fish, although without differences in growth and feed conversion, caused a deteriorated fish health after 72 days feeding.

KEY WORDS: growth, requirement, liver damage and fillete quality.

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Introduction

Meagre (*Argyrosomus regius*) is a teleost fish of the family Sciaenidae (Chao, L., *et al.*, 1986) founded in subtropical water of the Mediterranean and Black Sea, along the Atlantic coasts of Europe and the East coast of Africa. This specie lives inshore and shelf water close to bottom, as well as in surface and midwaters from 15 to about 200 m (Poli, B., *et al.*, 2003; Hernández, M., *et al.*, 2009; Chatzifotis, S., *et al.*, 2010). Meagre has been accredited as a great candidate for the diversification of the aquaculture in the Mediterranean and Eastern Atlantic coasts and also, for commercial production, mainly due to their fast growth and high lipid quality of the flesh (Poli, B., *et al.*, 2003; Piccollo, G., *et al.*, 2008; Hernández, M., *et al.*, 2009; Grigokaris, K., *et al.*, 2011).

Meagre specie is easily adapted to captivity, exhibiting the capacity of tolerate wide ranges of temperature and salinity (Quèmèner, L., 2002; Suquet, M., *et al.*, 2009;. Cárdenas, S., 2010; Chatzifotis, S., *et al.*, 2012); presenting high growth rates of around 1kg in 18 months (Cárdenas, S., 2010) and low feed conversion ratio. According to Chatzifotis, S., *et al.*, (2012) feed conversion values of 0.9 to 1.2 (fish mean weight 21.8 ± 3.7) were reported, being previously argued from Monfort, M. (2010) that feed conversion ratio of course depends on the used feed quality.

Ongrowing of meagre in the Mediterranean and Canary Island is carried out mainly in land-based circular or rectangular tank and in floating cages (offshore) with density from 10 to 15 kg m⁻³; thus meagre is a species that often has no disease problems because the transfer at low densities is limited (Duncan, N., *et al.*, in press). However, in pathological studies performed on meagre were showed that present pathological problems such as ulcer localized in the head and abdomen caused by the fungus *Penicillium digitatum* (Manueli, E., *et al.*, 2005), infection by species of worm of the Trematodes

Class, such as *Benedenia sciaenae* (Toksen, E., *et al.*, 2007), *Calceostoma* spp. (Hayward, C.J., *et al.*, 2007; Duncan, N., *et al.*, 2008) y *Sciaenocotyle* spp. (Hayward *et al.*, 2007, Ternengo *et al.*, 2010), and of the Clase Nematoda, as *Philometra* sp. (Moravec *et al.*, 2007). Also, were described the presence of granulomatosis in meagre in the on-growing phase, which have been associated with nutritional deficiencies of vitamin C and vitamin B complex (Ghittino, C., *et al.*, 2004). It specie has moreover many good market qualities being highly appreciated within the market, but with the exception that is a species not widely known thus having an average price in the European markets of around 7 to 12€/ kg depending on the consumer areas (FAO, 2012).

Because of the above facts, the meagre culture is being rapidly increasing up, being actually the fourth most important species in the Spanish marine aquaculture (APROMAR, 2011) and according to FAO (2012), current production data of meagre is of 1853 tonnes, being Spain the higher European producer.

Increasing interest around specific culture conditions and feeds for higher growth rate fish species like meagre, which support higher fish production and fish quality are focused nowadays. According to their feeding habits, meagre is a carnivorous species and their feeding in the wild is based on Mysidacea, Decapoda, Echinoderms, Polychaetous, mollusk and teleostei (Cabral, H. and Ohmert, B., 2001, Jiménez, M., *et al.*, 2005; Cárdenas, S., 2010). Thus, its species has been currently feed under culture conditions with feeds close to those for sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) since they are also carnivorous species. Specific dietary requirements are needed in order to optimize meagre culture and only a few works has been reported in the last years, mainly regarding dietary proteins and lipid. Thus, an optimum level of 50% protein (Chatzifotis, S., *et al.*, 2012) and 17% lipid (Panagiotidou *et al.*, 2007; Chatzifotis, S., *et al.*, 2010; Chatzifotis, S., *et al.*, 2012) have being stated for meagre. Furthermore, some

other studies showed no effect on fish specific growth rates by feeding commercial diets with 315 g* kg⁻¹ of vegetable proteins (or 76.2% of the total protein content) in meagre with an average weight of 20.47 g (Estévez, A., *et al.*, 2011). By other hand, meagre fish, contrary to sea bream and sea bass, is a lean fish showing less than half of the lipid content of those.

To our knowledge no experiences on dietary vitamin levels and their implications in *Argyrosomus regius* fish have been reported. Vitamin E (α -tocopherol) represents one of the vitamins widely studied under aquaculture conditions due to its important physiological implications in all species. The vitamin E exists in group of eight lipid soluble compounds, four tocopherols and four tocotrienols (NRC, 1993), and represent one of the most important natural antioxidant to prevent the effect caused by reactive oxygen (ROS) and free radicals (Mascio, P., *et al.*, 1991; Mourente, G., *et al.*, 2007; Hamre, K., 2011). Vitamin E is usually supplied in fish diets as α - tocopherol acetate, due to its higher stability and oxidation resistance respect to that of the α -tocopherol during feed processing and storage (Hamre, K. and Øyvind, L., 1995; Peng, L., *et al.*, 2008) and by other hand, because it has being demonstrated that fish stored vitamin E in the free form of α -tocopherol in the liver, due to that the acetate ester is hydrolyzed in the lumen of the gut by pancreatic carboxyl ester hydrolase (Muller, D., *et al.*, 1976; Peng, L., *et al.*, 2008) and by the concurrent presence of bile salts (Hung, S., *et al.*, 1982; Traber, M.G., *et al.*, 1993; Rigotti, A., 2007; Peng, L., *et al.*, 2008; Hamre, K., 2011).

Research in different aquatic species has reported a clear effect of the vitamin E supplement in the diets on the fish growth performance improvement (Cowey, C., *et al.*, 1984; Wilson, R., *et al.*, 1984; Hamre, K. and Øyvind, L., 1995; Baker, R. and Davies, S., 1997; Bai, S.C. and Lee, K., Lin, Y. and Shiau, S., 2005; Jittinandana, S., *et al.*, 2006; Peng, S., *et al.*, 2009; Abdel-Hameid, H., *et al.*, 2012). Although, according to some other

references the influence of vitamin E on growth was not shown (Stéphan, G., *et al.*, 1995; Gaylord, T., *et al.*, 1998; Bell, G., *et al.*, 2000; Lygren, B., *et al.*, 2000; Gatta, P.P., *et al.*, 2000; Tocher, D.R., *et al.*, 2002; Ruff, N., *et al.*, 2002; Ruff, N. *et al.*, 2003; Tocher, D.R., *et al.*, 2003; Puangkaew, J., *et al.*, 2004; Puangkaew, J., *et al.*, 2005; Xiao-dong, Z., *et al.*, 2007; Peng, L., *et al.*, 2008; Yildirim-Aksoy, M., *et al.*, 2008; Zhong, Y., *et al.*, 2008). Thus, nowadays is still unclear the effect of vitamin E on fish growth performance and also appears less unclear in fast growing fish species.

Nevertheless, an important effect of the vitamin E on other productive aspects as fish quality parameters and oxidation of the fillets, have been extensively demonstrated in different species. The reported effects are related to the antioxidants effects of the α -tocopherol which prevent the oxidative damage of the tissues and the oxidation of the unsaturated fatty acids, regarded the ability of the α -tocopherol to donate their phenolic hydrogen atoms to lipid-free radicals, affecting the peroxidation chain reaction (Burton, G., and Ingold, K., 1989; Mourente, G., *et al.*, 2007). Acting as quenchers of singlet oxygen free radicals prevent damage in the tissues, mainly in the unsaturated fatty acids of the cellular membrane (Mascio, P. *et al.*, 1991; Mourente, G. *et al.*, 2007; Hamre, K., 2011) and thus, increasing oxidative stability of the fish fillet to lengthen storage time (Cowey, C. *et al.*, 1984; Gattlin, D.M. *et al.*, 1992; Bai, C. and Gatlin, D.M., 1993; Stéphan, G., *et al.*, 1995; Olsen, R. and Henderson, R., 1997; Gaylord T., *et al.*, 1998; Bell, G., *et al.*, 2000; Gatta, P.P., *et al.*, 2000; Ruff, N., *et al.*, 2002; Ruff, N., *et al.*, 2003; Hamre, K., *et al.*, 2004; Jittinandana S., *et al.*, 2006; Xiao-dong, Z., *et al.*, 2007; Peng, L. and Gatlin III, D.M., 2009).

The aims of present work was to study the impact of the vitamin E dietary levels during of ongrowing state of *Argyrosomus regius* on fish growth and feed utilization

parameter: besides biochemical compositions in fish fillet and liver tissues, as well as the storage stability of the fillet were also evaluated.

Materials and methods

Experimental Diets

Based on an isocaloric (16% lipid) and isoproteic (50% protein) fish meal and fish oil based diet, seven experimental diets were prepared by adding different levels of vitamin E (0, 100, 200, 300, 500, 1000 and 1500 mg/kg). The vitamin E was provided as DL- α -tocopherol acetate (Sigma-Aldrich, Madrid, Spain), being premixed and diluted with α -cellulose and the rest of the vitamin premix for each of the diets to obtain the final desired concentration. Diets were coded from E0 (no vitamin E addition) to E 1500 (1500 mg vitamin E).

The diets were prepared using a mixer (DANAMIX BM 330, Azpeitia, Gipuzcua, Spain); once carefully homogenized all the ingredients and premixes, the resultant mixtures were pelletized (California Pellet mill, CPM de 2HP mod 8.3, USA), throughout a 3mm diameter to obtain same size of granules for all the diets. After dry down in the oven at 38°C during the night, diets were bulk stored in a dark and refrigerated chamber at 10 °C from where the needed amount to feed the fish was taking every day. A subsample of each diet was also taken and stored at -80°C for the subsequent biochemical analysis. Formulation of the basal diets and their correspondent biochemical analysis are show in **table IV**.

Table IV. Formulation and proximate analysis of the experimental diets (g*kg⁻¹)

INGREDIENTS	(g*kg ⁻¹)						
FM ¹	680						
Fish Oil ¹	85						
Gelatinized corn starch ²	190						
Vitamin Premix ³	20						
Mineral Premix ⁴	20						
CMC	5						
Choline	2.70						
Proximate composition (% dry weight)	E0	E100	E200	E300	E500	E1000	E1500
Crude protein	49.97	48.75	49.17	48.79	49.25	48.86	49.29
Crude lipid	14.40	15.19	15.60	15.17	14.50	14.12	14.94
Ash	16.07	15.69	15.76	15.70	15.76	15.96	15.19
Carbohydrate ⁵	19.56	20.37	19.47	20.34	20.48	21.05	20.58
Moisture	8.39	6.89	7.45	6.90	6.73	7.10	7.55
Gross energy (kJ kg ⁻¹) ⁶	20.29	20.25	20.52	20.25	20.11	19.90	20.19

¹ High quality fish meal and fish oil from South America and provided by Biomar Spain.

²Merigel 100 Amylum Group.

³Vitamin Premix (mg/kg⁻¹ diet): Vit B1 (tiamin): 40; Vit B2 (riboflavin): 50; Vit B6 (piridoxin): 40; Calcium Pantotenat: 116.9; Nicotinic acid: 200; Biotin (Vit H): 1; Folic acid: 10; Vit B12 (cyanocobalamin): 0.5; Myo-Inositol: 2000; Vit C: 5000; Vit K3 (menadione): 20; Vit D3 (cholecalciferol): 5; Vit A (retinol acetate): 25; Etoxiquin: 100. Vit E (DL- α -tocopherol acetate) was added at 0, 100, 200, 300, 500, 1000 or 1500 mg/kg. for each diet.

⁴Mineral Premix (g/kg⁻¹ diet): (H₂PO₄)₂Ca: 1.605; CaCO₃: 4; FeSO₄*7H₂O: 1.5; MgSO₄*7H₂O: 1.605; K₂HPO₄:2.8; Na₂PO₄*H₂O:1; Al(SO₄)₃*6 H₂O: 0.02; ZnSO₄*5H₂O: 0.24; CuSO₄*5H₂O: 0.12; MnSO₄*H₂O: 0.08; KI: 0.02; CoSO₄*7H₂O: 0.08

⁵Carbohydrate = 100 – (protein + lipid + ash)

⁶ Gross energy = (23.6 KJ*Kg⁻¹ x % protein + 39.8 KJ*Kg⁻¹ x % lipid +17.2KJ*Kg⁻¹ x % carbohydrate)/100.

Fish and feeding

For the feeding trial, meagre juveniles (*Argyrosomus regius*) obtained by broodstock induced spawnings at the Instituto Canario de Ciencias Marinas facilities (Telde, Canary Island, Spain), were used. Fish of initial mean weight of 62.90±12.95 g were anesthetized with clove oil (4 ml/100 L salt water), standard length measured and randomly distributed in circular fiberglass tanks of 500 L in groups of 50 fish each. All tanks were net covered to prevent leaks of fish, and were supplied with natural seawater and air injection, being the experiment lasted 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 (Monday and Thursday) with an oxy-meter (Oxy Guard, Handy Polaris V 1.26), with values from 21.1 to 23.6°C (considered as

an acceptable temperature range for the growth of meagre) and 6.1 to 6.9 mg L⁻¹ for temperature and dissolved oxygen, respectively.

Experimental diets were tested in triplicate groups of fish for 72 days along which fish were hand fed until apparent satiation, three times per days (08:00, 11:00 and 14:00 h), 6 days week. Corrected daily feed intake was calculated after recovering and dryer the uneaten feeds from the bottom of the tanks.

Growth performance and feed utilization

Every proximately 20 days, from the initial of the trial, fish were anesthetized for individual whole body weight and standard length measurement (days 23, 48 and 72 days of feeding); being total length also measured at 72 days. Fish were unfed for one day before all samplings.

Obtained data were then analyzed according to the subsequent equations to study fish response for survival, growth, biometric and feed utilization parameters, by performing means and standard deviations of each triplicate by treatment.

Survival (%) = $100 * (\text{final number fish} - \text{initial number fish}) / \text{initial number fish}$

Growth (%) = $((\text{final mean weight} - \text{initial mean weight}) / \text{initial mean weight}) * 100$.

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

SGR (specific growth rate) = $100 \times (\ln \text{ final mean weight} - \ln \text{ initial mean weight}) / \text{number of days}$.

FI = feed intake (g)/fish per day.

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

PER (protein efficiency ratio) = weight gain (g)/ protein intake (g)

K (condition factor (%)) = $100 * (\text{fish weight} / (\text{fish length})^3)$.

HSI (hepatosomatic index (%)) = $100 \times (\text{liver weight} / \text{fish weight})$.

VSI (viscerosomatic index (%)) = $100 \times (\text{fish weight} - \text{fish eviscerated fish weight}) / \text{fish weight}$.

Sample collection and analysis

At the end of the trial 9 fish per treatment were killed by immersion in ice seawater and used for whole fish analyses. Liver and muscle (a complete side) from other 4 fish per tank were obtained for biochemical analyses and the remained muscle side recovered for oxidation analysis (TBARS). All samples were weighted and stored at -80°C until analyzed. For histological evaluation, livers from 5 fish per tank sacrificed in similar conditions as above were fixed in 10% buffer formaldehyde.

Feed sample and whole fish and muscle pools from all tanks were analyzed in triplicate to exception of the liver pool, which were analyzed in duplicate. Furthermore, in the liver samples only the total lipid and moisture analysis were carried out due to the lower amount of sample. Obtained samples were homogenized for crude protein, moisture and ash content analyses according to AOAC (2000). Total lipids content were performed by the method described by Folch, J., *et al.* (1957) and fatty acids methyl esters were determined by trans-esterification of the total lipids with 1% sulfuric acid in methanol according to the methodology of Christie, W., *et al.* (1982). Fatty acid methyl ester (FAMES) were diluted in hexane and separated, identified and quantified by gas chromatography under the conditions described by Izquierdo, M.S., *et al.* (1990). Individual methyl esters were identified by comparison with external standard (EPA 28, Nippai, Ltd. Tokyo, Japan).

Measurements of thiobarbituric acid reactive substances (TBARS) were carried out by the methods described by Shaidi, F. and Hong, C., (1991), where muscle sample (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 is filtered and mixed with the same volume of thiobarbituric acid (TBA, 0.02M). Then, the samples and two blanks were stirred and heated at 90°C for 20 minutes. After that, the tubes were immediately on ice to stop the reaction. Once cooling the down, the tubes were outstand left to reach room temperature. Finally, the absorbance of the supernatant was measured at 532 nm compared with two blanks. The concentration obtained was expressed as µg of oxidated lipid per kilogram of tissue from a calibration curve.

For histological analysis of the livers, the five samples per tank already fixed in 10% buffered formaldehyde were dehydrated in an ethanol series and embedded in paraffin. Next, the samples were serially cut at 5µm and fixed to the microscope slide. Finally, the sample was stained with haematoxilyn and eosin (H&E) (Martoja, R. and Martoja-Pierson, M., 1970; Garcia Del Moral, R., 1993).

Statistical analysis

All data from the feeding trial were checked to see if they followed a for normal distribution and homogeneity of variance (Sokal, R.R. and Rohlf, E.J., 1995). For determinate significant differences between treatments, the parametric and homoscedastic data were analysed with one-way analysis of variance (ANOVA) using Tukey's test for multiple comparisons. A non-parametric analysis and multiple-range test (Kruskal-Wallis) for data which not displaying a normal distribution and homogeneity of variance were applied. Significant different performed was evaluated with a 0.05 significance levels the SPSS (15.0) statistical package.

RESULTS

Diets biochemical analysis

Diets biochemical analysis is showed in table IV and their correspondent fatty acid composition in **tables V and VI**. As it was expected according to the similar formula feeds, all experimental diets proximate compositions showed similar profiles.

Table V. Fatty acid profile (mean±SD) of the experimental diets (g/100g fatty acid).

Fatty acid	DIETS						
	E0	E100	E200	E300	E500	E1000	E1500
14:0	4.37±0.09	4.26±0.00	4.31±0.00	4.18±0.17	4.37±0.04	4.33±0.12	4.46±0.21
14:1n-7	0.06±0.01	0.06±0.00	0.06±0.00	0.07±0.02	0.06±0.00	0.06±0.00	0.06±0.01
14:1n-5	0.19±0.01	0.18±0.00	0.17±0.00	0.19±0.01	0.18±0.00	0.18±0.00	0.18±0.01
15:0	0.61±0.01	0.59±0.00	0.60±0.00	0.60±0.00	0.62±0.01	0.60±0.01	0.60±0.01
15:1n-5	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
16:OISO	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
16:0	18.17±0.41	17.86±0.00	17.90±0.04	18.58±1.06	18.66±1.09	18.44±0.70	19.03±1.87
16:1n-7	5.54±0.06	5.55±0.00	5.55±0.03	5.35±0.25	5.58±0.12	5.52±0.12	5.59±0.03
16:1n-5	0.26±0.01	0.27±0.00	0.26±0.01	0.27±0.01	0.27±0.01	0.27±0.01	0.28±0.03
16:2n-6	0.33±0.00	0.35±0.00	0.35±0.01	0.33±0.03	0.35±0.02	0.34±0.00	0.37±0.05
16:2n-4	0.65±0.00	0.67±0.00	0.65±0.02	0.72±0.09	0.72±0.07	0.70±0.08	0.39±0.45
17:0	0.43±0.01	0.42±0.00	0.43±0.00	0.42±0.00	0.43±0.00	0.33±0.14	0.31±0.12
16:3n-4	0.06±0.00	0.05±0.00	0.06±0.00	0.05±0.00	0.06±0.00	0.24±0.26	0.24±0.26
16:3n-3	0.21±0.01	0.21±0.00	0.20±0.00	0.21±0.00	0.21±0.01	0.21±0.02	0.21±0.00
16:3n-1	0.05±0.00	0.05±0.00	0.05±0.00	0.09±0.05	0.07±0.04	0.07±0.04	0.09±0.06
16:4n-3	0.29±0.00	0.30±0.00	0.30±0.01	0.30±0.01	0.30±0.02	0.31±0.00	0.40±0.14
16:4n-1	0.03±0.00	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.04±0.00	0.04±0.00
18:0	4.46±0.09	4.37±0.00	4.38±0.01	4.64±0.41	4.58±0.30	4.50±0.30	4.65±0.45
18:1n-9	17.95±0.21	17.93±0.00	17.88±0.09	17.33±0.58	17.78±0.60	17.48±0.18	17.03±1.18
18:1n-7	3.09±0.03	3.09±0.00	3.08±0.01	3.16±0.11	3.18±0.07	3.13±0.11	3.20±0.16
18:1n-5	0.25±0.00	0.24±0.00	0.25±0.00	0.26±0.01	0.27±0.02	0.26±0.04	0.25±0.00
18:2n-9	0.07±0.00	0.06±0.00	0.07±0.00	0.06±0.01	0.07±0.00	0.06±0.01	0.07±0.01
18:2n-6	3.72±0.04	3.85±0.00	3.70±0.07	3.75±0.03	3.80±0.01	3.76±0.02	3.57±0.19
18:2n-4	0.19±0.02	0.18±0.00	0.18±0.00	0.20±0.02	0.20±0.02	0.20±0.03	0.21±0.04
18:3n-6	0.20±0.00	0.20±0.00	0.20±0.00	0.21±0.02	0.22±0.02	0.22±0.03	0.21±0.02
18:3n-4	0.16±0.00	0.15±0.00	0.16±0.01	0.15±0.02	0.14±0.01	0.15±0.01	0.15±0.02
18:3n-3	1.18±0.01	1.20±0.00	1.20±0.00	1.14±0.07	1.18±0.03	1.18±0.04	1.10±0.14
18:3n-1	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
18:4n-3	1.26±0.03	1.28±0.00	1.30±0.02	1.21±0.12	1.24±0.04	1.27±0.08	1.20±0.16
18:4n-1	0.13±0.00	0.14±0.00	0.13±0.00	0.12±0.01	0.13±0.00	0.13±0.02	0.14±0.00
20:0	0.28±0.01	0.28±0.00	0.28±0.00	0.26±0.02	0.27±0.02	0.26±0.01	0.26±0.02
20:1n-9	0.58±0.00	0.60±0.00	0.58±0.00	0.57±0.01	0.58±0.01	0.58±0.02	0.53±0.08
20:1n-7	4.57±0.05	4.56±0.00	4.51±0.05	4.23±0.42	4.32±0.42	4.25±0.17	3.98±0.82
20:1n-5	0.39±0.01	0.40±0.00	0.36±0.00	0.36±0.01	0.37±0.01	0.35±0.03	0.38±0.04
20:2n-9	0.06±0.02	0.07±0.00	0.05±0.00	0.07±0.00	0.04±0.01	0.06±0.02	0.06±0.01
20:2n-6	0.39±0.00	0.39±0.00	0.40±0.00	0.36±0.02	0.38±0.03	0.37±0.01	0.36±0.05
20:3n-9	0.06±0.00	0.05±0.00	0.06±0.00	0.05±0.00	0.05±0.01	0.06±0.00	0.05±0.00
20:3n-6	0.05±0.01	0.04±0.00	0.04±0.01	0.09±0.06	0.07±0.02	0.09±0.05	0.07±0.04
20:4n-6	1.22±0.04	1.24±0.00	1.23±0.00	1.34±0.16	1.29±0.07	1.29±0.11	1.28±0.06
20:3n-3	0.21±0.02	0.21±0.00	0.20±0.00	0.21±0.00	0.22±0.01	0.22±0.04	0.19±0.01
20:4n-3	0.68±0.01	0.69±0.00	0.70±0.01	0.69±0.01	0.69±0.00	0.70±0.01	0.67±0.05
20:5n-3	6.37±0.23	6.53±0.00	6.57±0.12	6.66±0.05	6.51±0.25	6.63±0.06	7.14±0.66
22:1n-11	4.68±0.03	4.66±0.00	4.62±0.03	4.07±0.74	4.26±0.71	4.19±0.44	3.89±1.11
22:1n-9	0.67±0.01	0.65±0.00	0.65±0.00	0.61±0.06	0.63±0.07	0.63±0.04	0.59±0.12
22:4n-6	0.20±0.02	0.19±0.00	0.22±0.00	0.22±0.01	0.22±0.01	0.20±0.01	0.21±0.01
22:5n-6	0.55±0.02	0.56±0.00	0.56±0.00	0.53±0.02	0.52±0.02	0.53±0.02	0.53±0.06
22:5n-3	1.60±0.05	1.63±0.00	1.65±0.03	1.69±0.04	1.63±0.03	1.67±0.00	1.73±0.06
22:6n-3	13.39±0.55	13.62±0.00	13.72±0.21	14.27±0.43	13.12±0.12	13.82±0.20	13.92±0.12

Table VI. Main fatty acid and relation (mean±SD) of the experimental diets (g/100g fatty acid)

Fatty acid	DIETS						
	E0	E100	E200	E300	E500	E1000	E1500
SATURATES	28.42±0.62	27.87±0.00	28.01±0.06	28.77±1.27	29.03±1.34	28.58±1.02	29.41±2.64
MONOINSATURATES	38.26±0.34	38.20±0.00	37.99±0.24	36.49±1.92	37.53±1.83	36.91±0.77	35.99±3.11
Σn-3	25.20±0.89	25.65±0.00	25.85±0.40	26.38±0.32	25.10±0.34	26.01±0.32	26.57±0.38
Σn-6	6.66±0.09	6.82±0.00	6.71±0.09	6.82±0.23	6.84±0.06	6.80±0.18	6.60±0.16
Σn-3 HUFA	22.25±0.86	22.68±0.00	22.85±0.37	23.51±0.51	22.17±0.39	23.04±0.23	23.66±0.55
Σn-6 HUFA	2.40±0.05	2.42±0.00	2.45±0.00	2.53±0.20	2.48±0.04	2.48±0.14	2.45±0.02
n-3/n-6	3.78±0.08	3.76±0.00	3.86±0.11	3.87±0.08	3.67±0.02	3.83±0.15	4.03±0.15
DHA/EPA	2.10±0.01	2.08±0.00	2.09±0.01	2.14±0.05	2.02±0.06	2.08±0.01	1.96±0.20
TOTAL HUFA	24.66±0.91	25.10±0.00	25.30±0.37	26.04±0.71	24.64±0.43	25.52±0.09	26.11±0.53

Growth performance and feed utilization

Inclusion of dietary vitamin E did not affect fish growth performance, feed utilization and biometric parameters of juveniles meagre fed the different levels of vitamin E for 72 days. Thus, no significant difference ($P > 0.05$) were found for final weight and length, percent weight gain and SGR, fish survival, feed intake, FCR and PER; moreover no differences in fish condition factor (K) and hepatosomatic and visceral indexes for different levels of vitamin E in the diets (**Table VII**) were neither shown.

Table VII. Fish growth performance, feed utilization and biometric parameters (mean±SD) of juveniles meagre fed different levels of vitamin E for 72 day of experiment.

PARAMETERS	DIETS						
	E0	E100	E200	E300	E500	E1000	E1500
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 length (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 length (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
Weight gain (g)	111.87±5.67	120.54±6.68	112.48±2.46	116.17±5.53	112.25±5.24	117.89±6.17	120.02±8.09
Total Growth (%)	177.66±12.04	191.50±8.74	181.17±6.83	184.07±2.08	176.96±11.28	188.27±7.30	191.62±8.63
Final survival (%)	91.00±9.90	94.67±4.16	100.00±0.00	95.33±1.15	90.67±11.02	98.00±2.83	99.00±1.41
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 (Inicial) ⁵	2.42±0.06	2.42±0.03	2.37±0.01	2.35±0.01	2.41±0.05	2.38±0.06	2.42±0.00
K (Final)	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
HSI ⁶	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 ⁷	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

¹FI, Feed intake (g*fish⁻¹*day⁻¹); ²SGR, specific growth rate; ³FCR, feed conversion ratio; ⁴PER, protein efficiency ratio; ⁵K, condition factor (%); ⁶HSI, Hepatosomatic index (%); ⁷VSI, Viscerosomatic index (%).

Whole body, muscle and liver proximate composition.

Whole body proximate composition was affected by the different vitamin E addition (**Table VIII**), being significantly higher values ($P < 0.05$) of protein observed for fish fed on E0 and E300 diets with regard to other treatment, which were accompanied by lowest moisture content mainly for the E0 diet. The whole body lipid and ash showed no differences ($P > 0.05$) between treatments (**Table VIII**).

Biochemical composition of fish muscle did not showed significant differences either in protein, moisture or ash, while the lipid content was highly affected. Thus the highest ($P < 0.05$) lipid content was observed in meagre fed with no addition of vitamin E (E0) compared with the E200 and E300 diets, by the way the lowest observed values. Moreover, E0 lipid muscle results were similar to those for diets E100, E500, E1000 and E1500 (**Table VIII**). Percent of muscle ash, on the other hand, showed a trend to decrease with the dietary vitamin E increment, but without any relationship with the explained differences in the respective lipid content.

Regarding liver content no significant differences were found between the different groups of feeding fish for moisture and lipid, although same tendency to that showed above for muscle was observed, with lower liver lipid for E200 and E300 diets (**Table VIII**).

Table VIII. Biochemical composition (means \pm SD) of whole body, muscle, and liver in juvenil meagre (% wet weight).

	DIETS						
	E0	E 100	E 200	E 300	E 500	E 1000	E 1500
Whole							
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
Muscle							
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
Ash	1.46 \pm 0.16	1.38 \pm 0.14	1.42 \pm 0.06	1.34 \pm 0.18	1.28 \pm 0.02	1.22 \pm 0.04	1.18 \pm 0.03
Liver							
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

Different superscript letters (a, b, c) showed significant differences between groups ($P < 0.05$).

Whole body, muscle and liver fatty acid composition.

An increment of the dietary vitamin E resulted in a modification of whole body fatty acid profile with a decrease of the saturated fatty acid regarded the increment of the dietary vitamin E, except for the 17:0 and 18:0 fatty acids (significantly higher, $P < 0.05$, in the case of 17:0 fatty acid). The monounsaturated fatty acid showed the same pattern that the above fatty acid, although without significant differences with the exception of certain fatty acids as the 18:1n-5, which resulted significantly lower in the meagre, fed on E1000 diet compared with the other treatment. The groups of fish fed with the lowest amount of vitamin E, E0 and E100 diets, were significantly lower in the 16:3n-1 fatty acid respect to other treatment. On the other hand, there was observed a trend of increment for the n-3, n-3 HUFA, totals HUFA, as well as for the n-3/n-6 and DHA/EPA ratios in E1000 and E1500 feeding fish, but without significant difference. The n-6 HUFA fatty acids were significantly increased in meagre fed on E1000 and E1500 respect to other treatment (**Table IX and X**). The most abundant saturated fatty acid were 16:0, 18:0 and 14:0, the monounsaturated fatty acid 16:1n-7, 20:1n-7, 22:1n-11 and 18:1n-7, while the greater component of the n-3 HUFA were docohexanoic acid (DHA, 22:6n-3), eicosapentanoic acid (EPA, 20:5n-3) and linolenic acid (18:3n-3). The n-6 HUFA fatty acids were present in small amounts being the most abundant the linoleic acid (18:2n-6) and the arachidonic acid (ARA, 20:4n-6).

The muscle fatty acids were also altered by increment of dietary vitamin E; thus showed a decrease of the saturated and monoinsaturated fatty acid with an increment of the dietary vitamin E, but without significant differences. The n-3 fatty acid were higher in the muscle of fish fed on E 1000 and E 1500 diets but without significant differences, being reflected in the eicosapentanoic acid (EPA; 20:5n-3) which was significantly higher in the

muscle of the meagre fed with the E1000 diets respect to other treatment. However, the 16:3n-3 fatty acid was significantly higher ($P < 0.05$) in the fish fed on E0 diets compared with the rest of treatment. The n-3 HUFA and total highly unsaturated fatty acids (HUFA) increased with an increase in the level of the dietary vitamin E but without significant difference. Similar response was found for the n-6 HUFA. The observed n-3/n-6 and DHA/EPA ratios were greater in the meagre fed on E 1000 and E 1500 with respect to other treatment, but again without significant difference (**Table XI and XII**).

Table IX. Whole body fatty acid composition (means±SD) of meagre at the end of the trial (g/100 g fatty acid).

Fatty acids	DIETS						
	E 0	E 100	E 200	E 300	E 500	E 1000	E 1500
14:0	4.12± 0.42	4.38±0.12	4.68± 0.59	4.38± 0.09	4.26± 0.04	4.06±0.19	3.89± 0.05
14:1n-7	0.03± 0.00	0.04±0.00	0.04± 0.00	0.04± 0.00	0.04± 0.00	0.03±0.00	0.04± 0.00
14:1n-5	0.18± 0.01	0.19±0.00	0.20± 0.03	0.19± 0.01	0.18± 0.00	0.17±0.01	0.16± 0.00
15:0	0.62± 0.07	0.64±0.01	0.66± 0.08	0.61± 0.02	0.60± 0.01	0.56±0.02	0.54± 0.00
15:1n-5	0.04± 0.00	0.03±0.00	0.04± 0.00	0.03± 0.00	0.03± 0.00	0.03±0.01	0.03± 0.00
16:OISO	0.11± 0.01	0.12±0.00	0.12± 0.02	0.11± 0.00	0.11± 0.00	0.10±0.00	0.10± 0.00
16:0	22.19± 0.90	23.37±0.01	23.82± 2.65	21.74± 0.51	21.52± 0.25	20.01±0.92	20.13± 0.13
16:1n-7	6.64± 0.12	6.75±0.16	6.76± 0.09	6.77± 0.08	6.79± 0.11	6.74±0.07	6.59± 0.08
16:1n-5	0.28± 0.02	0.30±0.00	0.31± 0.03	0.21± 0.14	0.28± 0.01	0.26±0.01	0.26± 0.01
16:2n-6	0.34± 0.01	0.32±0.01	0.31± 0.05	0.34± 0.01	0.34± 0.02	0.35±0.00	0.35± 0.01
16:2n-4	0.80± 0.09	0.71±0.01	0.66± 0.01	0.65± 0.04	0.65± 0.03	0.64±0.02	0.64± 0.04
17:0	0.18± 0.02 b	0.15±0.01 b	0.47± 0.00 a	0.47± 0.02 a	0.47± 0.02 a	0.44±0.02 a	0.44± 0.00 a
16:3n-4	0.48± 0.03	0.48±0.01	0.04± 0.00	0.05± 0.00	0.05± 0.00	0.03±0.02	0.05± 0.00
16:3n-3	0.23± 0.02	0.23±0.00	0.23± 0.02	0.24± 0.01	0.21± 0.00	0.20± 0.01	0.21± 0.01
16:3n-1	0.03± 0.01 c	0.02±0.00 c	0.21± 0.02 a	0.16± 0.01b	0.17± 0.01 b	0.19± 0.02 bc	0.18± 0.01bc
16:4n-3	0.08± 0.01	0.08±0.02	0.08± 0.05	0.10± 0.03	0.11± 0.01	0.11± 0.01	0.14± 0.00
16:4n-1	0.02± 0.00	0.02±0.00	0.04± 0.01	0.02± 0.01	0.02± 0.00	0.02± 0.01	0.03± 0.00
18:0	5.59± 0.39	6.30±0.07	5.95± 0.77	5.16± 0.09	5.41± 0.15	4.74± 0.16	5.09± 0.08
18:1n-9	21.02± 0.85	21.34±0.13	21.12± 0.73	20.80± 0.51	20.72 ±0.33	20.15± 0.11	19.78± 0.10
18:1n-7	3.38± 0.09	3.49±0.08	3.46± 0.16	3.39± 0.09	3.39±0.07	3.25± 0.04	3.21± 0.00
18:1n-5	0.28± 0.00 a	0.29±0.00 a	0.29± 0.01 a	0.28± 0.01 a	0.27±0.01 ab	0.25± 0.01b	0.26± 0.00 ab
18:2n-9	0.09± 0.02	0.09±0.00	0.09± 0.01	0.09± 0.00	0.09±0.01	0.07± 0.02	0.11± 0.01
18:2n-6	5.54± 0.39	5.05±0.06	5.17± 0.26	5.31± 0.12	5.20±0.25	5.37± 0.11	5.23± 0.07
18:2n-4	0.19± 0.01	0.19±0.00	0.18± 0.01	0.18± 0.01	0.18±0.00	0.18± 0.00	0.19± 0.01
18:3n-6	0.14± 0.02	0.11±0.01	0.15± 0.02	0.13± 0.01	0.14±0.00	0.12± 0.01	0.12± 0.00
18:3n-4	0.10± 0.01 b	0.10±0.01 b	0.14± 0.00 a	0.16± 0.01a	0.15±0.00 a	0.14± 0.02 a	0.16± 0.00 a
18:3n-3	1.05± 0.13	0.93±0.01	0.87± 0.26	1.06± 0.05	1.06±0.04	1.15± 0.02	1.11± 0.03
18:3n-1	0.01± 0.00	0.00±0.00	n.d	n.d	n.d	n.d	n.d
18:4n-3	0.73± 0.18	0.59±0.02	0.56± 0.31	0.78 ± 0.09	0.79±0.04	0.94±0.08	0.95± 0.03
18:4n-1	0.08± 0.03	0.10±0.04	0.06± 0.03	0.07± 0.01	0.09±0.01	0.08± 0.01	0.10± 0.00
20:0	0.31± 0.02	0.33±0.00	0.34± 0.05	0.30± 0.02	0.30±0.01	0.26± 0.03	0.28± 0.00
20:1n-9	0.75± 0.02	0.83±0.10	0.82± 0.04	0.82± 0.02	0.81±0.00	0.78± 0.02	0.78± 0.03
20:1n-7	5.20± 0.30	5.36±0.03	5.42± 0.47	5.20± 0.21	5.06±0.07	4.89± 0.36	4.72± 0.00
20:1n-5	0.32± 0.02	0.33±0.01	0.32± 0.02	0.31± 0.01	0.31±0.01	0.29± 0.01	0.29± 0.00
20:2n-9	0.07± 0.00	0.06±0.01	0.06± 0.01	0.07± 0.00	0.07±0.00	0.05± 0.02	0.07± 0.00
20:2n-6	0.38± 0.02	0.39±0.01	0.35± 0.00	0.38± 0.02	0.37±0.01	0.35± 0.01	0.34± 0.00
20:3n-9	0.02± 0.00	0.02±0.00	0.02± 0.00	0.02± 0.00	0.03±0.00	0.02± 0.01	0.03± 0.00
20:3n-6	0.11± 0.00a	0.10±0.00 ab	0.09± 0.02b	0.10± 0.00ab	0.11±0.00 a	0.11±0.00a	0.11± 0.00a
20:4n-6	0.96± 0.00 ab	0.81±0.03 b	0.71± 0.24 b	0.87± 0.03 ab	0.90±0.02 ab	1.00 ±0.08 a	0.98± ±0.02 a
20:3n-3	0.20± 0.00	0.21±0.01	0.15± 0.03	0.18± 0.01	0.18±0.01	0.17 ±0.04	0.18 ±0.00
20:4n-3	0.49± 0.10	0.41±0.02	0.36± 0.18	0.50± 0.03	0.51±0.02	0.58 ±0.04	0.59 ±0.00
20:5n-3	3.15± 0.87	2.52±0.10	2.42± 1.42	3.42± 0.45	3.56±0.12	4.30 ±0.55	4.52 ±0.13
22:1n-11	5.07± 0.15	5.35±0.01	5.58± 0.63	5.31± 0.19	5.07±0.17	4.91 ±0.53	4.76 ±0.01
22:1n-9	0.80± 0.03	0.85±0.02	0.85± 0.09	0.81± 0.03	0.80±0.01	0.75 ±0.04	0.76 ±0.00
22:4n-6	0.17± 0.01	0.15±0.00	0.16± 0.01	0.16± 0.00	0.16±0.00	0.17 ±0.01	0.17 ±0.00
22:5n-6	0.36± 0.01	0.29±0.01	0.25± 0.13	0.35± 0.03	0.37±0.02	0.55 ±0.23	0.43 ±0.00
22:5n-3	0.98± 0.21	0.82±0.02	0.77± 0.44	1.09± 0.13	1.15±0.04	1.42 ±0.21	1.45 ±0.01
22:6n-3	6.10± 1.57	4.77±0.38	4.65± 2.88	6.58± 1.04	6.93±0.44	8.99 ±1.54	9.48 ±0.29

Value in a row with different superscripts are significant differences (P <0.05).

Table X. Main fatty acid and relations of whole body (means±SD) of meagre at the end of the trial (g/100 g fatty acid)

Fatty acids	DIETS						
	E0	E100	E200	E300	E500	E1000	E1500
SATURATES	33.12± 1.79	35.29±0.09	36.03± 4.16	32.77± 0.73	32.66±0.31	30.18 ±1.34	30.46 ±0.26
MONOINSATURATES	43.99± 1.59	45.15±0.48	45.19± 2.12	44.17± 1.23	43.77±0.54	42.50 ±1.21	41.64 ±0.22
Σn-3	13.01± 3.04	10.57±0.57	10.09± 5.55	13.96± 1.80	14.52±0.60	17.86 ±2.38	18.62 ±0.46
Σn-6	8.01± 0.39	7.20±0.02	7.19± 0.69	7.64± 0.18	7.59±0.26	8.01 ±0.21	7.73 ±0.08
Σn-3 HUFA	10.92± 2.75	8.74±0.53	8.35± 4.96	11.77± 1.64	12.34±0.57	15.47 ±2.30	16.21 ±0.41
Σn-6 HUFA	1.99± 0.02ab	1.73±0.05 b	1.56± 0.41b	1.86± 0.06ab	1.91±0.01ab	2.17 ±0.33 a	2.03 ±0.02 a
n-3/n-6	1.62± 0.30	1.47±0.08	1.37± 0.64	1.82± 0.20	1.92±0.10	2.23 ±0.24	2.41 ±0.03
DHA/EPA	1.94± 0.04	1.89±0.07	1.90± 0.08	1.92± 0.07	1.94±0.09	2.08 ±0.09	2.10 ±0.00
TOTAL HUFA	12.91±2.74	10.47±0.57	9.91± 5.37	13.63± 1.69	14.24±0.59	17.64 ±2.63	18.25 ±0.43

The fatty acid in livers showed difference respect to the whole body and muscle fatty acid, with saturated fatty acid were greater values found in the intermediate diets (E300 and E500) with respect to other treatments, but without significant differences. The monounsaturated fatty acid showed higher amount in meagre fed with E0, E300, E500 and E1000 diets but without significant differences. For the 22:1n-11 fatty acid, significantly higher content in fish fed the E0, E200, E300 and E500 diets was detected compared with other treatments. Moreover, the content for the 16:1n-5 fatty acid was significantly higher in livers of those fish fed on E300 and E500 diets respect to other treatment. The 20:1n-9 fatty acid was significantly lower in the meagre fed with E1500 diets compared with the rest of treatment. Significantly higher content for the 18:4n-1 and 20:2n-9 fatty acids were observed in livers from E300, E500 and E1.000 diets than other treatments. Similar decreasing response were observed for the n-3 HUFA and totals HUFA fatty acid were significantly lower ($P<0.05$) for the E300 and E500 diets compared with the other treatment. Similar decreasing response were observed for the n-3, n-6 and n-6 HUFA fatty acid which were also lower in the E300 and E500, but in this case without significant differences (Table XIII and XIV).

Table XI. Muscle fatty acid composition (means \pm SD) of meagre at the end of the trial (g/100 g fatty acid).

Fatty acids	DIETS						
	E 0	E 100	E 200	E 300	E 500	E 1000	E 1500
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
14:1n-7	0.02 \pm 0.01	0.01 \pm 0.01	0.02 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.01	0.02 \pm 0.00	0.02 \pm 0.01
14:1n-5	0.13 \pm 0.02	0.12 \pm 0.02	0.13 \pm 0.01	0.12 \pm 0.01	0.14 \pm 0.01	0.12 \pm 0.00	0.12 \pm 0.01
15:0	0.58 \pm 0.03	0.53 \pm 0.04	0.53 \pm 0.03	0.52 \pm 0.04	0.55 \pm 0.02	0.48 \pm 0.00	0.47 \pm 0.06
15:1n-5	0.01 \pm 0.00	0.02 \pm 0.01	0.01 \pm 0.01	0.01 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01
16:OISO	0.10 \pm 0.01	0.09 \pm 0.01	0.09 \pm 0.01	0.09 \pm 0.01	0.09 \pm 0.00	0.08 \pm 0.00	0.08 \pm 0.01
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
16:1n-7	4.55 \pm 0.46	4.75 \pm 0.31	4.71 \pm 0.03	4.31 \pm 0.13	4.87 \pm 0.63	4.90 \pm 0.01	4.60 \pm 0.67
16:1n-5	0.30 \pm 0.01	0.29 \pm 0.02	0.27 \pm 0.04	0.27 \pm 0.03	0.27 \pm 0.02	0.23 \pm 0.00	0.23 \pm 0.04
16:2n-6	0.18 \pm 0.04	0.20 \pm 0.02	0.21 \pm 0.00	0.19 \pm 0.03	0.21 \pm 0.04	0.25 \pm 0.00	0.21 \pm 0.02
16:2n-4	0.86 \pm 0.08	0.76 \pm 0.05	0.79 \pm 0.03	0.84 \pm 0.10	0.83 \pm 0.08	0.71 \pm 0.02	0.80 \pm 0.05
17:0	0.10 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.03	0.11 \pm 0.02	0.14 \pm 0.01	0.12 \pm 0.00
16:3n-4	0.43 \pm 0.02	0.42 \pm 0.02	0.42 \pm 0.01	0.40 \pm 0.03	0.43 \pm 0.02	0.40 \pm 0.00	0.39 \pm 0.04
16:3n-3	0.24 \pm 0.02 a	0.21 \pm 0.02 ab	0.22 \pm 0.02 ab	0.22 \pm 0.02ab	0.22 \pm 0.01 ab	0.18 \pm 0.00 b	0.19 \pm 0.02 ab
16:3n-1	0.66 \pm 0.18	0.49 \pm 0.10	0.54 \pm 0.03	0.57 \pm 0.12	0.51 \pm 0.16	0.38 \pm 0.01	0.50 \pm 0.01
16:4n-3	0.27 \pm 0.19	0.20 \pm 0.13	0.23 \pm 0.13	0.27 \pm 0.15	0.19 \pm 0.14	0.21 \pm 0.00	0.16 \pm 0.14
16:4n-1	0.12 \pm 0.02	0.10 \pm 0.02	0.11 \pm 0.00	0.12 \pm 0.02	0.11 \pm 0.02	0.09 \pm 0.00	0.11 \pm 0.01
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
18:1n-7	3.60 \pm 0.17	3.25 \pm 0.19	3.32 \pm 0.11	3.40 \pm 0.20	3.36 \pm 0.08	3.06 \pm 0.04	3.12 \pm 0.34
18:1n-5	0.24 \pm 0.01	0.24 \pm 0.02	0.25 \pm 0.01	0.23 \pm 0.02	0.24 \pm 0.02	0.22 \pm 0.01	0.22 \pm 0.02
18:2n-9	0.06 \pm 0.00	0.07 \pm 0.01	0.07 \pm 0.00	0.07 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.00	0.08 \pm 0.02
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
18:2n-4	0.16 \pm 0.01	0.15 \pm 0.00	0.16 \pm 0.01	0.16 \pm 0.01	0.16 \pm 0.02	0.16 \pm 0.00	0.16 \pm 0.02
18:3n-6	0.19 \pm 0.09	0.15 \pm 0.06	0.16 \pm 0.06	0.18 \pm 0.07	0.15 \pm 0.06	0.15 \pm 0.01	0.15 \pm 0.05
18:3n-4	0.13 \pm 0.00	0.13 \pm 0.00	0.14 \pm 0.01	0.13 \pm 0.01	0.13 \pm 0.01	0.13 \pm 0.00	0.13 \pm 0.01
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	0.0.1647 \pm 0.16	0.70 \pm 0.00	0.57 \pm 0.13
18:4n-1	0.14 \pm 0.01	0.11 \pm 0.02	0.11 \pm 0.01	0.12 \pm 0.00	0.15 \pm 0.08	0.10 \pm 0.01	0.10 \pm 0.00
20:0	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
20:1n-7	4.15 \pm 0.31	3.97 \pm 0.20	4.07 \pm 0.18	3.74 \pm 0.37	4.23 \pm 0.29	3.75 \pm 0.03	3.66 \pm 0.13
20:1n-5	0.28 \pm 0.02	0.27 \pm 0.01	0.27 \pm 0.01	0.26 \pm 0.01	0.27 \pm 0.01	0.25 \pm 0.01	0.25 \pm 0.03
20:2n-9	0.07 \pm 0.03	0.05 \pm 0.01	0.06 \pm 0.02	0.05 \pm 0.01	0.05 \pm 0.01	0.06 \pm 0.02	0.05 \pm 0.01
20:2n-6	0.41 \pm 0.02 a	0.38 \pm 0.00 ab	0.39 \pm 0.01 ab	0.39 \pm 0.01 ab	0.39 \pm 0.00 ab	0.37 \pm 0.01 ab	0.36 \pm 0.01 b
20:3n-9	n.d	0.02 \pm 0.00	n.d	n.d	0.02 \pm 0.00	0.02 \pm 0.00	n.d
20:3n-6	0.10 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.00	0.12 \pm 0.00
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
20:3n-3	0.14 \pm 0.02	0.16 \pm 0.02	0.16 \pm 0.01	0.15 \pm 0.01	0.15 \pm 0.03	0.15 \pm 0.00	0.14 \pm 0.01
20:4n-3	0.30 \pm 0.07	0.42 \pm 0.09	0.42 \pm 0.07	0.37 \pm 0.09	0.38 \pm 0.10	0.54 \pm 0.01	0.46 \pm 0.05
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
22:1n-11	3.24 \pm 0.52	3.34 \pm 0.19	3.41 \pm 0.18	2.91 \pm 0.33	3.63 \pm 0.46	3.26 \pm 0.04	3.09 \pm 0.05
22:1n-9	0.61 \pm 0.13	0.61 \pm 0.04	0.57 \pm 0.02	0.52 \pm 0.03	0.60 \pm 0.04	0.56 \pm 0.01	0.56 \pm 0.11
22:4n-6	0.16 \pm 0.03	0.18 \pm 0.02	0.18 \pm 0.02	0.17 \pm 0.03	0.17 \pm 0.02	0.19 \pm 0.00	0.19 \pm 0.02
22:5n-6	0.44 \pm 0.08	0.60 \pm 0.10	0.60 \pm 0.08	0.63 \pm 0.15	0.53 \pm 0.11	0.69 \pm 0.01	0.67 \pm 0.16
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	11.80 \pm 5.01	9.15 \pm 2.19	15.68 \pm 0.30	14.72 \pm 6.17

Different letters in the same row are significant differences between groups (P <0.05).

Table XII. Main fatty acid and relations of muscle (means \pm SD) of meagre at the end of the trial (g/100 g fatty acid)

Fatty acids	DIETS						
	E 0	E 100	E 200	E 300	E 500	E 1000	E 1500
SATURATES	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
MONOINSATURATES	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
Σ n-3	10.79 \pm 3.47	18.60 \pm 4.74	17.54 \pm 3.20	18.43 \pm 6.85	15.32 \pm 3.36	24.79 \pm 0.28	22.69 \pm 7.51
Σ n-6	7.04 \pm 0.18	7.16 \pm 0.25	7.44 \pm 0.18	7.47 \pm 0.20	7.41 \pm 0.31	7.59 \pm 0.11	7.51 \pm 0.65
Σ n-3 HUFA	9.32 \pm 3.34	16.92 \pm 4.50	15.84 \pm 3.12	16.83 \pm 6.81	13.66 \pm 3.05	22.80 \pm 0.31	20.99 \pm 7.48
Σ n-6 HUFA	2.32 \pm 0.18	2.66 \pm 0.26	2.71 \pm 0.24	2.76 \pm 0.40	2.46 \pm 0.28	2.84 \pm 0.00	2.88 \pm 0.44
n-3/n-6	1.32 \pm 0.45	2.35 \pm 0.56	2.12 \pm 0.37	2.24 \pm 0.86	1.83 \pm 0.36	3.00 \pm 0.09	2.76 \pm 0.76
DHA/EPA	2.90 \pm 0.40	3.22 \pm 0.31	3.29 \pm 0.19	3.40 \pm 0.20	3.05 \pm 0.41	3.21 \pm 0.06	3.34 \pm 0.74
TOTAL HUFA	11.64 \pm 3.52	19.59 \pm 4.74	18.56 \pm 3.36	19.59 \pm 7.20	16.12 \pm 3.30	25.64 \pm 0.31	23.87 \pm 7.92
AI ¹	0.79 \pm 0.07	0.63 \pm 0.09	0.64 \pm 0.07	0.64 \pm 0.11	0.68 \pm 0.08	0.53 \pm 0.01	0.57 \pm 0.12
TI ²	0.84 \pm 0.22	0.49 \pm 0.13	0.51 \pm 0.10	0.54 \pm 0.22	0.59 \pm 0.16	0.33 \pm 0.00	0.40 \pm 0.16

Different letters in the same row are significant differences between groups (P <0.05).

¹Atherogenic index (AI) = [C12:0 + ((4*C14:0)+C16:0)/(n-3 HUFA+n-6 HUFA+ MONOINSATURATES)]

²Trombogenic index (TI) = [C14:0 + C16:0 + C18:0] / [(0.5*(C18:1n-9+C18:1n-7+C18:1n-5) + (0.5 * sum of other monoenes) (0.5* n-6 HUFA) + (3*n-3 HUFA) + (n-3 HUFA/ n-6 HUFA).

Muscle thiobarbituric acid reactive substances (TBARS)

The muscle TBARS results showed significantly greater value in the meagre fed the lowest dietary vitamin E (E0 and E100 diets), compared with those fed with the higher vitamin addition (E 500, E1000 and E 1500 diets). Moreover, similar values were observed between the diets: E100-E300, E200-E500 and E300-E1500. TBARS concentrations in muscle of juvenile meagre fed diets containing graded levels of vitamin E could be plotted to tentatively determinate the optimum dietary vitamin E level, in similar way as stated for the red drum (*Sciaenops ocellatus*) livers by Peng, L., and Gatlin III, D.M., (2009). In the present case, the optimum level of vitamin E estimated by broke-line regression analysis of the meagre muscle lipid peroxidation (TBARS) were close to 509.32 mg/kg provided in the form of DL- α -tocopherol acetate (Table XV and Figure 12).

Table XIII. Fatty acid composition (means \pm SD) of meagre liver at the end of the trial (g/100 g fatty acid).

Fatty acids	DIETS						
	E 0	E 100	E 200	E 300	E 500	E 1000	E 1500
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
14:1n-7	0.04 \pm 0.00	0.04 \pm 0.00	0.03 \pm 0.01	0.04 \pm 0.00	0.04 \pm 0.00	0.04 \pm 0.00	0.04 \pm 0.00
14:1n-5	0.15 \pm 0.01	0.13 \pm 0.00	0.15 \pm 0.01	0.15 \pm 0.01	0.14 \pm 0.00	0.14 \pm 0.01	0.13 \pm 0.02
15:0	0.48 \pm 0.02	0.43 \pm 0.03	0.47 \pm 0.01	0.51 \pm 0.04	0.47 \pm 0.02	0.45 \pm 0.02	0.44 \pm 0.07
15:1n-5	0.03 \pm 0.00	0.03 \pm 0.00	0.02 \pm 0.01	0.03 \pm 0.00	0.03 \pm 0.00	0.03 \pm 0.00	0.03 \pm 0.00
16:OISO	0.11 \pm 0.00	0.10 \pm 0.00	0.11 \pm 0.00	0.11 \pm 0.00	0.11 \pm 0.00	0.10 \pm 0.00	0.10 \pm 0.01
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
16:1n-7	8.12 \pm 0.01	8.16 \pm 0.25	8.01 \pm 0.43	8.26 \pm 0.31	8.25 \pm 0.16	8.68 \pm 0.06	8.57 \pm 0.44
16:1n-5	0.14 \pm 0.00 ab	0.13 \pm 0.00b	0.14 \pm 0.00 ab	0.15 \pm 0.00 a	0.15 \pm 0.00 a	0.14 \pm 0.00 ab	0.13 \pm 0.01 ab
16:2n-6	0.22 \pm 0.00	0.20 \pm 0.00	0.22 \pm 0.01	0.22 \pm 0.01	0.22 \pm 0.01	0.22 \pm 0.00	0.24 \pm 0.04
16:2n-4	0.83 \pm 0.01	0.74 \pm 0.01	0.79 \pm 0.03	0.85 \pm 0.07	0.87 \pm 0.03	0.81 \pm 0.04	0.81 \pm 0.11
17:0	0.59 \pm 0.01	0.57 \pm 0.00	0.60 \pm 0.01	0.60 \pm 0.00	0.59 \pm 0.02	0.58 \pm 0.01	0.35 \pm 0.28
16:3n-4	0.05 \pm 0.00	0.05 \pm 0.00	0.05 \pm 0.00	0.05 \pm 0.00	0.05 \pm 0.00	0.05 \pm 0.00	0.31 \pm 0.37
16:3n-3	0.20 \pm 0.00	0.20 \pm 0.00	0.21 \pm 0.01	0.21 \pm 0.01	0.21 \pm 0.01	0.19 \pm 0.00	0.19 \pm 0.02
16:3n-1	0.04 \pm 0.00	0.04 \pm 0.00	0.03 \pm 0.02	0.04 \pm 0.00	0.04 \pm 0.00	0.03 \pm 0.01	0.04 \pm 0.00
16:4n-3	0.07 \pm 0.00	0.06 \pm 0.01	0.06 \pm 0.00	0.05 \pm 0.00	0.05 \pm 0.00	0.06 \pm 0.02	0.09 \pm 0.02
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
18:1n-7	3.44 \pm 0.04	3.27 \pm 0.04	3.48 \pm 0.00	3.54 \pm 0.03	3.52 \pm 0.06	3.49 \pm 0.03	3.49 \pm 0.10
18:1n-5	0.26 \pm 0.02	0.25 \pm 0.02	0.25 \pm 0.00	0.26 \pm 0.02	0.28 \pm 0.01	0.27 \pm 0.00	0.26 \pm 0.01
18:2n-9	0.13 \pm 0.00	0.05 \pm 0.01	0.05 \pm 0.00	0.14 \pm 0.03	0.16 \pm 0.01	0.11 \pm 0.07	0.18 \pm 0.05
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
18:2n-4	0.19 \pm 0.00	0.19 \pm 0.00	0.19 \pm 0.00	0.19 \pm 0.01	0.19 \pm 0.00	0.20 \pm 0.00	0.20 \pm 0.02
18:3n-6	0.10 \pm 0.00	0.09 \pm 0.00	0.10 \pm 0.01	0.10 \pm 0.01	0.10 \pm 0.00	0.09 \pm 0.00	0.13 \pm 0.06
18:3n-4	0.25 \pm 0.01	0.24 \pm 0.05	0.28 \pm 0.01	0.21 \pm 0.03	0.19 \pm 0.01	0.20 \pm 0.01	0.15 \pm 0.08
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
18:3n-1	n.d	n.d	n.d	0.71 \pm 0.05	0.69 \pm 0.02	0.68 \pm 0.00	0.30 \pm 0.42
18:4n-3	0.74 \pm 0.02	0.68 \pm 0.01	0.77 \pm 0.02	0.09 \pm 0.01	0.08 \pm 0.01	0.08 \pm 0.00	0.44 \pm 0.49
18:4n-1	0.09 \pm 0.01b	0.08 \pm 0.01b	0.08 \pm 0.00b	0.25 \pm 0.01 a	0.25 \pm 0.00 a	0.25 \pm 0.00 a	0.16 \pm 0.10 ab
20:0	0.24 \pm 0.01b	0.24 \pm 0.00b	0.24 \pm 0.01b	0.97 \pm 0.02 a	0.94 \pm 0.03 a	0.88 \pm 0.02 a	0.49 \pm 0.34 ab
20:1n-9	0.95 \pm 0.01b	0.86 \pm 0.00b	0.90 \pm 0.05b	4.58 \pm 0.12 a	4.46 \pm 0.16 a	4.19 \pm 0.03 a	2.28 \pm 1.95 ab
20:1n-7	4.47 \pm 0.05	4.10 \pm 0.00	4.42 \pm 0.1	0.33 \pm 0.01	0.32 \pm 0.01	0.31 \pm 0.00	2.21 \pm 2.73
20:1n-5	0.33 \pm 0.0	0.29 \pm 0.01	0.31 \pm 0.0	0.16 \pm 0.01	0.16 \pm 0.01	0.16 \pm 0.01	0.22 \pm 0.14
20:2n-9	0.14 \pm 0.00b	0.12 \pm 0.06b	0.14 \pm 0.01b	0.43 \pm 0.01 a	0.41 \pm 0.02 a	0.39 \pm 0.01a	0.22 \pm 0.17 ab
20:2n-6	0.42 \pm 0.01	0.38 \pm 0.00	0.42 \pm 0.00	0.03 \pm 0.00	0.03 \pm 0.00	0.03 \pm 0.00	0.21 \pm 0.26
20:3n-9	0.04 \pm 0.02	0.05 \pm 0.00	0.03 \pm 0.00	n.d	n.d	n.d	0.03 \pm 0.00
20:3n-6	0.12 \pm 0.01	0.11 \pm 0.00	0.12 \pm 0.00	0.12 \pm 0.00	0.12 \pm 0.01	0.12 \pm 0.00	0.12 \pm 0.01
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
20:3n-3	0.21 \pm 0.01	0.19 \pm 0.00	0.20 \pm 0.00	0.21 \pm 0.01	0.20 \pm 0.02	0.20 \pm 0.02	0.19 \pm 0.04
20:4n-3	0.73 \pm 0.03	0.70 \pm 0.04	0.80 \pm 0.08	0.71 \pm 0.03	0.73 \pm 0.01	0.69 \pm 0.02	0.65 \pm 0.04
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
22:1n-11	2.86 \pm 0.02a	2.68 \pm 0.00ab	2.87 \pm 0.08a	2.96 \pm 0.10 a	2.96 \pm 0.05 a	2.68 \pm 0.03 ab	2.48 \pm 0.27 b
22:1n-9	0.86 \pm 0.02a	0.83 \pm 0.00a	0.85 \pm 0.05a	0.90 \pm 0.02a	0.90 \pm 0.02 a	0.85 \pm 0.01a	0.75 \pm 0.03 b
22:4n-6	0.33 \pm 0.18	0.59 \pm 0.23	0.34 \pm 0.19	0.20 \pm 0.01	0.21 \pm 0.01	0.33 \pm 0.19	0.42 \pm 0.28
22:5n-6	0.93 \pm 0.72	1.66 \pm 0.10	1.04 \pm 0.83	0.41 \pm 0.01	0.41 \pm 0.02	1.00 \pm 0.84	1.05 \pm 0.87
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

Different superscripts in the same row are significant differences between groups (P < 0.05).

Table XIV. Main fatty acid and relations of liver (means \pm SD) of meagre at the end of the trial (g/100 g fatty acid).

Fatty acids	DIETS						
	E 0	E 100	E 200	E 300	E 500	E 1000	E 1500
SATURATES	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
MONOINSATURATES	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
Σ n-3	17.95 \pm 0.75	18.69 \pm 1.07	18.19 \pm 0.57	15.91 \pm 0.59	15.97 \pm 0.48	16.03 \pm 0.45	18.62 \pm 1.38
Σ n-6	6.80 \pm 1.02	7.48 \pm 0.13	7.21 \pm 0.96	5.93 \pm 0.22	5.90 \pm 0.14	6.29 \pm 0.87	6.45 \pm 0.17
Σ n-3 HUFA	16.04 \pm 0.77ab	16.90 \pm 1.09ab	16.19 \pm 0.53ab	14.65 \pm 0.55b	14.72 \pm 0.4b	14.86 \pm 0.45ab	17.10 \pm 0.76 a
Σ n-6 HUFA	2.61 \pm 0.91	3.58 \pm 0.18	2.81 \pm 0.98	1.63 \pm 0.07	1.60 \pm 0.06	2.30 \pm 0.96	2.61 \pm 0.80
n-3/n-6	2.66 \pm 0.29	2.50 \pm 0.19	2.55 \pm 0.42	2.68 \pm 0.01	2.71 \pm 0.10	2.58 \pm 0.43	2.89 \pm 0.29
DHA/EPA	2.66 \pm 0.08	2.60 \pm 0.09	2.50 \pm 0.02	2.55 \pm 0.06	2.57 \pm 0.13	2.41 \pm 0.02	2.46 \pm 0.24
TOTAL HUFA	18.65 \pm 1.69ab	20.48 \pm 0.91a	19.00 \pm 0.46 ab	16.28 \pm 0.58 b	16.32 \pm 0.55 b	17.15 \pm 0.52 ab	19.71 \pm 0.04 ab

Different superscripts in the same row are significant differences between groups (P < 0.05).

Table XV. Thiobarbituric acid reactive substance (TBARS) in fillet of meagre fed the graded levels of vitamin E.

DIETS	TBARS (mg MDA ⁺ x kg of sample)
E0	1.09±0.18 a
E100	0.98±0.06 ab
E200	0.87±0.26 abc
E300	0.73±0.23 bcd
E500	0.53 ± 0.15cd
E1000	0.48±0.04cd
E1500	0.46±0.00 d

+ MDA: Malonaldehyde

Value are means ± standard deviations of three groups by treatment. Different letters in the same column are significant differences.

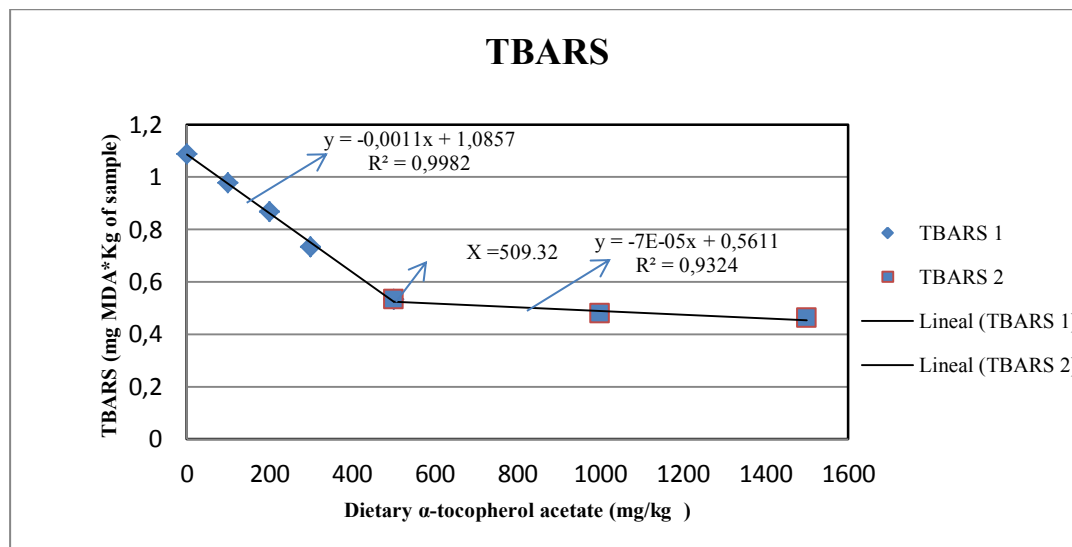


Figure 12. TBARS concentrations in muscle of juvenile meagre fed diets containing graded levels of vitamin E.

Liver histological result

Histological studies of the livers seem to be affected by graded feeding levels of vitamins E in meagre fish during the trial. Thus, significantly higher concentration of granulomes in the meagre fed on E 0, E 1000 and E 1500 diets with regarded to the other treatments. Moreover, a significant greater lipid vacuoles accumulation was observed in those fish fed with E 100, E 200 and E 1000 diets compared with other treatment (**Figure 13**). Non-appreciable liver structure alterations were found for the intermediate levels of vitamin E, diets E 300 and E 500.

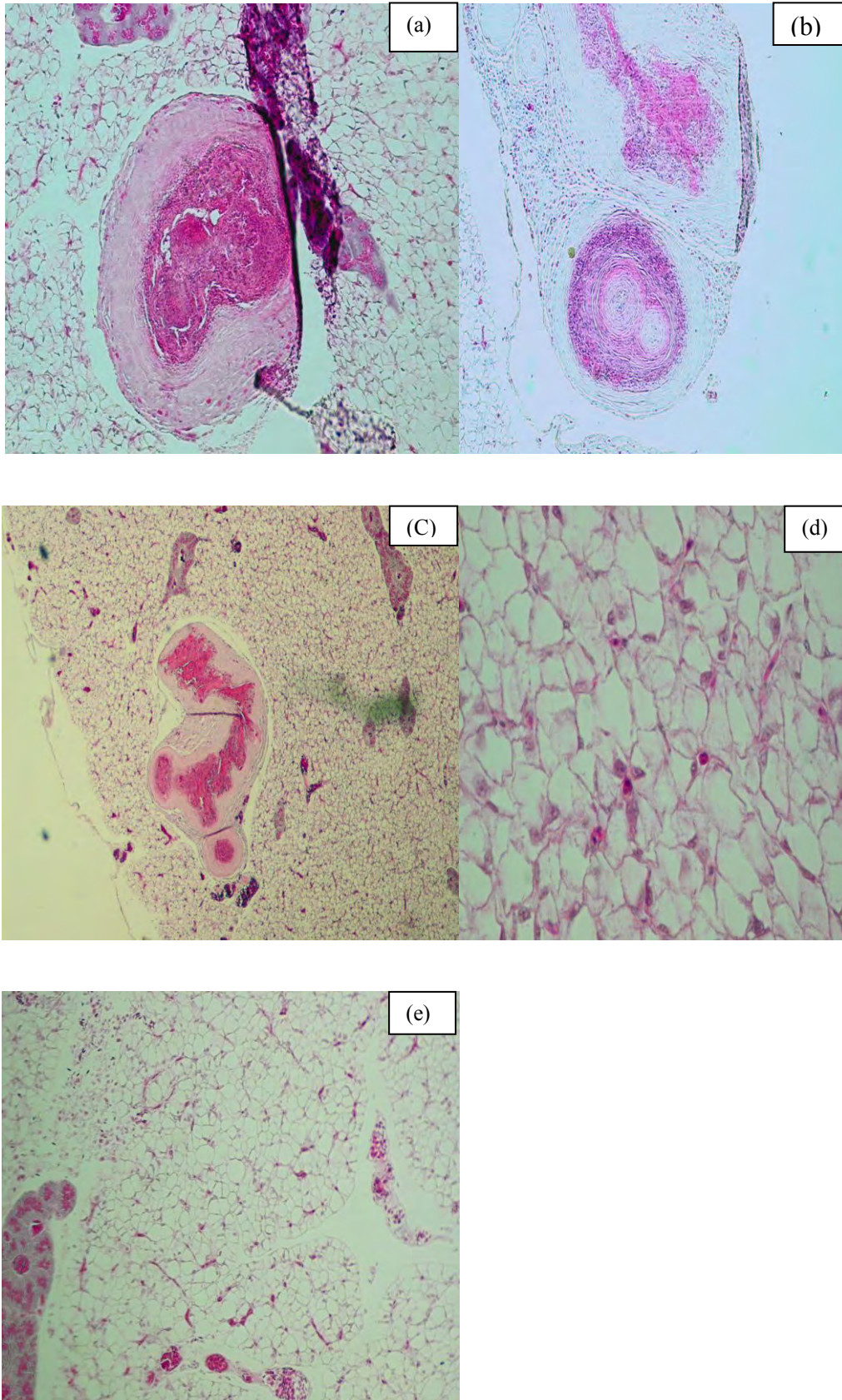


Figure 13. Liver of meagre fed with different levels of vitamin E. (a) and (b) Livers of the diet 1500. Granulomes and migration of the nuclei in hepatocytes (H&E). (c) Diet E1000. Granulomes and migration of the nuclei in hepatocytes (H&E). (d) and (e) Diet E0. Lipid vacuoles accumulation (H&E).

DISCUSSION

Meagre is new specie for the Mediterranean aquaculture and few works exist on its nutritional requirements, and nothing about optimum vitamin levels. In the present work, all the diets formulated on a basal fish meal and fish oil content and different vitamin E levels, were well accepted by the meagre from the beginning of the experiment. This was reflected in the feed intake (1.87 to 2.07 g*fish⁻¹*day⁻¹), specific growth rate (1.41 to 1.49%/day) and feed conversion (0.73 to 0.95) results, which were on the other hand similar between diets (0-1500 mg/kg vitamin E). Poorer results have being reported by Martínez-Llorens, S., *et al.* (2011), with a much lower feed intake (0.8 to 1.6 g*100g fish⁻¹*day⁻¹), lower specific growth rate 0.5 to 1.2%/day and higher feed conversion ratio (1.2 to 5.3), for meagre with an initial mean weight between 79 and 109 g, close to those used in present trial, and fed with 44/25, 43/21, 46/20 and 47/20 (protein/lipid) diets. This difference in the productive performances can be due to a low protein/lipid ratio with low level of protein and excess in the level of lipid in the diets as it was recently shown by Chatzifotis, S., *et al.* (2012) which reported an optimum requirement of dietary protein and lipids of 50% and 17%, respectively, similar to the ratio used in present work. Moreover, present results are better respect to those previous also from Chatzifotis, S., *et al.* (2010), which showed lower fed intake (0.80%) and specific growth rates (0.46%/day) and poor fed conversion ratio (1.38) results, for meagre fish (230g mean weight) fed with a substitution of 45.4% fish protein by vegetable protein. Also, Estévez, A. *et al.* (2011) in a research performed with higher substitutions levels, from 63 to 72%, of fish protein by vegetable protein reported lower SGR and higher FCR, together with a lower feed intake and total weight gain (g). These differences obtained in the productive performance parameters respect to those in current work can be due first to a low percentage of protein in the diets and by the other hand, to that the meagre fish do not accept the vegetable

protein ingredients at high extent. This has been showed in the recent research by Chatzifotis, S., *et al.* (2012) and by this present work with higher protein and fish meal contents in the feed.

Chatzifotis, S., *et al.* (2012) performed a research for juveniles meagre of 25.5g initial weight achieving specific growth rates of the 1.2 to 1.3 %/day and feed conversion ratio (0.9 to 1.2) respect to present trial. The results obtained by these authors are the better productive performance reported for *Argyrosomus regius*, but are however poorer than those found in present work even using higher size of the fish (62.90 ± 12.95 g). This difference observed could be due to the lower culture temperature (19°C) in the first experiment, respect to present one of about 23.5°C. It is also important to note the higher density condition differences between both experiments; thus 1.26 kg/m^3 in the experiment of Chatzifotis, S., *et al.* (2012) respect to that of about 6 kg/m^3 used in the present work. Some authors have shown the important impact of the fish density conditions on a better productive performance, being moreover the number of fish per tank an important parameter affecting growth of the fish (Van der Salm, A., *et al.*, 2004), with higher number of fish accompanied by higher growth rates. So both factors, temperature and density conditions could determine the better growth responses of meagre in present trial.

On the other hand, present experiment was not only focus on growth but have advanced studying different levels of vitamin E (E0 to E1500mg/kg as DL- α -tocopherol acetate) for prevent the oxidative stress effect in fish tissues; thus, the obtained higher growth rates for all the diets, were accompanied with an optimum level close to 500mg/kg vitamin E in the feed, which duplicate the amount of 200-250mg/kg used in the studies by Chatzifotis, S., *et al.* (2010; 2012). Therefore, this could reflect higher requirements of dietary vitamin E for a higher response in meagre fish growth rates.

Considering the impact of the vitamin E in the diets of meagre and the oxidative status of the fillets, another important question will be the level of vitamin C among both trials as the reported synergistic effects between these 2 vitamins, which under certain conditions abstract hydrogen atoms from PUFA at low rates that promoting an initiation of the lipid oxidation (Bowry, V., *et al.*, 1992; Ingold, K., *et al.*, 1993; Hamre, K., 2011); for example high vitamin E accompanied by a low vitamin C concentration, and under these circumstances the vitamin E act as prooxidant (Hamre, K., 2011). In the present work, the level of vitamin C in the diets have been of 5000mg/kg as ascorbic acid (Sigma Aldrich, Spain), and could not be compared with the content in previous works give that they not make mention of the dietary level of vitamin C utilized. In this species give their different relative potential of growth not only the diets, but the environmental culture condition is relevant for the optimum levels of both vitamins in the diet.

The condition factor (K) is a crude measure of the level of energy reserve (Goede, R. and Barton, B., 1990) and indicate the state fish health (Chatzifotis, S., *et al.*, 2010). In this present trial, no significant differences were found for K values between treatments (2.15 to 2.22%), being also observed a slight decrease of this condition factor for the experiment. The results of the condition factor are similar to the obtained by Woolley, L., *et al.* (2010) for *Argyrosomus japonicus* and a little higher that the obtained by previous experiment of meagre, but following the same behavior for the current experiment (Poli, B., *et al.*, 2003; Chatzifotis, S., *et al.*, 2010; Martínez-Llorente, S., *et al.*, 2011), and *Sciaena umbra* (Chatzifotis, S., *et al.*, 2006) for different feeding diets, being *Argyrosomus japonicus* and *Sciaena umbra* other member of the *Sciaenidae* family. Therefore, this higher condition factor shows that the meagre were better fed and with better quality of the feed, which resulted in higher growth in this present work.

Comparisson of feed intake, specific growth rates and protein efficient ratio from current work with that reported by other fish species and similar fish size assayed, highly cultured in the Mediterranean showed effectively the potential of meagre growth respect to the other species: for juveniles of sea bream (*Sparus aurata*) with an initial mean body weight of 68-70 g, achieved lower feed intake (148.4 to 153.6 g*fish⁻¹ or 1.65 to 1.70 g*fish⁻¹*day⁻¹) and lower specific growth rates (0.90 to 1.00) (Aksnes A., *et al.*, 1997); in a research performed by Vergara, J., *et al.* (1999), obtained higher FCR (1.41 to 1.54) for sea bream of an initial average weight of 68-73 g; furthemore, in study performed on sea bass (*Dicentrarchus labrax*) with initial mean weight of 75 g by Montero, D., *et al.* (2005), obtained a lower specific growth rate (0.51 to 0.53) and a higher feed conversion ratio (1.44 to 1.55), respect to that in the present work.

Feed conversion ratio (FCR), specific growth rate and protein efficient ratio compared with some other species of rapid growth have reported in most of the cases poorer results than those in present trial, such as in the study performed by Chatzifotis, S., *et al.* (2006) for brown meagre (*Sciaena umbra*) with a initial weight of 78.8±15.8g, which achieved similar feed intake (1.29 to 1.94%) while lower specific growth rates (0.70 to 0.95%/day), and thus higher feed conversion ratio (1.45 to 2.28) and similar protein efficient ratio (1.05 to 1.40) to the obtained in the present work. Also, in Atlantic salmon (Helland, S. and Grisdale-Helland, B., 1998) with an initial average weight 72 ± 2 g, were obtained a much lower specific growth rate (0.97 to 1.00) and similar protein efficient ratio value (1.24 to 1.34) to the result obtained in this work. In *Scianop ocellatus* showed a lower protein efficient ratio (McGoogan, B. and Gatlilin III, D.M., 1999; Peng, L. *et al.*, 2008; Peng, L. and Gatlin III, D.M., 2009) that the obtained in the current work. Then, for juveniles *Argyrosomus japonicus* with an initial weight of 113.8±2.0 g, Woolley, L., *et al.*

(2010) reported data with lower specific growth rates (0.64 to 1.10), higher feed conversion ratio (1.05 to 1.93) and higher protein efficient ratio (1.12 to 1.95) than in present trial.

With respect to the different dietary levels of vitamin E assayed in this trial with *Argyrosomus regius* no significant differences were observed in final weight, specific growth rate, feed conversion factor, protein efficient ratio and survival in present work. Same responses have been observed in channel catfish (*Ictalurus punctatus*) with two size classes (average weight of 18 and 265g) for four levels (0, 15, 30 and 60 mg/kg) of vitamin E that were added as DL- α -tocopherol acetate (Gaylord, T., *et al.*, 1998). There are also two other researches which confirm no effect of the vitamin E (0, 50 and 500mg/kg as α -tocopherol) in the diet on the growth of the channel catfish (Wilson, R., *et al.*, 1984; Yildirim-Aksoy, M., *et al.*, 2008). Similar results were reported in Atlantic halibut with initial average weight of 312 ± 12.3 g and 189 and 613 mg/kg as α -tocopheryl acetate (Ruff, N., *et al.*, 2002), and also in a research performed by Tocher, D.R., *et al.* (2002 y 2003). A study by Xiao-dong, Z. *et al.* (2007) showed that level of 250, 500 and 1000 mg/kg α -tocopheryl acetate did not affect to the growth of *Sparus macrocephalus* for an initial mean weight 350 ± 12 g. Puangkaew J., *et al.* (2004; 2005) performed a study with different level of vitamin E and highly unsaturated fatty acids, which were observed that level of 0, 100 and 1000 mg/kg vitamin E as α -tocopheryl acetate did not affect to the growth of the rainbow trout (*Oncorhynchus mykiss*) with 100g of initial mean weight. The Atlantic salmon with an mean weight of 62.4g showed no significant difference in the specific growth rate with level of 40, 300 and 1100 mg/kg of vitamin E, and also same was observed in a research performed by Bell, G., *et al.* (2000) with a supplementation of $250 \text{g} \cdot \text{Kg}^{-1}$ as DL- α -tocopherol acetate. In a current research performed by Kashani, Z., *et al.* (2011) on the effect of the vitamin E and highly unsaturated fatty acid on growth, survival and haematocrit of goldfish (*Carassius auratus gibelio*) of an average weight of

0.69±0.12g again no affect on the growth parameters by the supplementation of 0, 50 and 100mg/kg vitamin E was observed. No significant difference were found in specific growth rate and feed conversion rate of sea bass (*Dicentrarchus labrax*) fed diets with 139, 254, 493 and 942 mg/kg of α -tocopherol (Gatta, P.P., *et al.*, 2000). The growth of the turbot (*Scophthalmus maximus*) it was not affected by a supplement of vitamin E (Stéphan, G., *et al.*, 1995; Tocher,D.R., *et al.*, 2002; Ruff, N., *et al.*, 2003; Tocher, D.R., *et al.*, 2003). In a research performed by Lall, S., *et al.* (2008) were showed that level of 300 IU α -tocopherol in the diet no caused effect on the growth performace of the juvenile Atlantic cod (*Gadus morhua*) with mean weight of 54.9g. No were observed also significant different in red drum (*Scianop ocellatus*) with an initial average weight 12.2±0.4g for level of 0, 10, 20 and 40 IU/kg of α -tocopheryl acetate and α -tocopheryl succinate (Peng, L., *et al.*, 2008). On the other hand, sea bream (*Sparus aurata*) were significantly affected in the final weight, specific growth rate and survival with level of 0, 100 and 1000 mg/kg of DL- α -tocopherol acetate and with oxidized oil in the diet (Tocher, D.R., 2002; Tocher, D.R., 2003). In a research performed by Mourente G. *et al.* (2000) for sea bream were observed that dietary vitamin E affect to the growth parameters, whereas that a study performed by Mourente, G., *et al.* (2002) with sea bream fed with oxidized oil growth improve until the 30 days of experiment with the addition of 200mg/kg α - tocopheryl acetate. In a current research performed by Abdel-Haimed, H., *et al.* (2012) were showed that graded levels (0, 20, 40, 60, 100, 140, 180, 220 and 260 mg/kg) of DL- α -tocopheryl acetate in the diet for *Channa punctatus* significantly affected to the growth parameters. Juvenile red drum (*Scianop ocellatus*) with an average weight of 12.2±0.4 were significantly influenced the gain weight and feed efficiency with level of 0, 10, 20, 30, 40, 60 and 80 IU*kg⁻¹ of vitamin E (all-rac- α -tocopheryl acetate) in the diet, where the optimum requirements of vitamin E for this species were of 31 IU/kg. In grouper (*Epinephelus malabaricus*) with a

mean weight 7.80 ± 0.13 g growth improve with an increment of dietary vitamin E (0, 25, 50, 100, 200, 400 and 800 mg/kg of DL- α -tocopheryl acetate) (Lin, Y. and Shiau, S., 2005). In a study performed by Peng, S., *et al.* (2009) on black sea bream (*Acanthopagrus schlegeli*) with a mean weight 18.49 ± 0.20 g and fed on oxidized fish oil, final weight and survival rate improve with α -tocopherol graded level of 0, 150, 250, 450 and 800 mg/kg in the diet. In Korean rockfish (*Sebastes schlegeli*) with an average weight 371 ± 4.3 g were affected the growth by the addition of dietary vitamin E in the diet, although achieved the stability in the weight gain close to a level of 45mg/kg of DL- α -tocopherol acetate in the diet (Bai, C. and Lee, K., 1998). According to the above, there seems no to be pattern for species growth responses to the dietary vitamin E levels between the different species, being the *Argyrosomus regius* one of the higher growth responses and also higher vitamin E requirement.

No significant differences were observed in the hepatosomatic index (1.51 to 1.70 g/cm³) between treatments. Similar values were obtained in previous experiment on meagre (Estévez, A., *et al.*, 2010; Piccolo, G., *et al.*, 2008; Martínez Llorens, S. *et al.*, 2011) in an experiment performed with meagre (94 g mean weight) and also without significant difference. However, higher values of the hepatosomatic index were found in previous study on meagre (Chatzifotis, S., *et al.*, 2006; Chatzifotis, S., *et al.*, 2010; Chatzifotis, S., *et al.*, 2012). Furthermore, the viscerosomatic index were higher than previous experiment performed with meagre (Chatzifotis, S., *et al.*, 2006; Chatzifotis, S., *et al.*, 2010; Martínez-Llorens, S., 2011), due to better growth of the fish and feed utilization in present trial. Different inclusion levels of vitamin E in present experiment showed no significant differences in the viscerosomatic and hepatosomatic index between treatment, but the hepatosomatic index were higher in the E 0, E100, E 1000 and E1500 diet, although without significant differences. As it was explained by Tocher, D.R. *et al.* (2002),

it may be expected that an excess of vitamin E in the diet cause a lengthening of the livers than the liver fed with diets low in vitamin E, because a degeneration in the livers tissue through of a peroxidation of their lipids. This degeneration could be obtained in this current work by feeding the diets E0, E100, E1000 and E1500 diet, by the accumulation of peroxidative lipid. The effect of lipid degeneration in the livers was observed in rainbow trout by a deficient dietary vitamin E (Puangkaew, J., *et al.*, 2005). In Atlantic salmon also were observed lipid degeneration in the liver with depletion of vitamin E and Astaxanthin (Bell, G. *et al.*, 2000).

The whole body protein content in meagre (14.45 and 18.40%) was lower in present work than in the trial by Chatzifotis, S. *et al.* (2010). The proximate composition of the cultured fish is affected by endogenous and exogenous factor. The levels of protein and ash are mostly related to fish size (endogenous factor), while fat depends on endogenous factors, such as the diet (Shearer, K., 1994; Chatzifotis, S., *et al.*, 2010; Chatzifotis, S., *et al.*, 2012). The whole body lipid content obtained this current experiment were around 4.02 to 5.49%, which were lower than those by Chatzifotis, S., *et al.* (2010), which achieved values from 5.76 to 7.41%. In another recent study, similar values for whole body moisture and lipid for about 24 g meagre were found after feeding the fish with 4 diets with 50% of protein and four levels of lipid (12, 14, 17 and 20%) (Chatzifotis, S., *et al.*, 2012); also in the study performed by Martínez-Llorens, S. *et al.* (2011) were achieved similar value in the whole body lipid of meagre fed with diets including different protein/lipid ratio (44/25, 43/21, 46/20 and 47/20). The observed similar results for the body lipid content although feeding meagre with different diets and energy content could be expected, as this species are not able to utilize dietary lipids at high extent, thus no able to accumulate lipids in the body, except in the case of the livers. Furthermore, in this current experiment, were observed higher amount of lipid in the liver of meagre fed with the E0, E500, E1000 and

E1500 and this was associated a greater feed intake. This was also observed in the liver of sea bream according Tocher, D.R. *et al.* (2002).

Muscle lipid (1.12 to 1.89%) and moisture (78.13 to 79.23%) content in this present work were similar to the achieved by Grigorakis, K., *et al.* (2011), but higher lipid and lower moisture that in previous experiment performed for meagre (Poli, B., *et al.*, 2003; Piccolo, G., *et al.*, 2008). In a research performed by Chatzifotis, S., *et al.* (2010), meagre fish showed lower level lipid and moisture that in the present work, while in a previous one carried out with brown meagre by same authors (Chatzifotis, S., *et al.*, 2006), were obtained similar moisture and lipid muscle contents.

On the other hand, the whole body biochemical composition was affected by different levels of vitamin E in the diet, with significantly higher protein in the E0 and E300 diets, accompanied by a reduction in the moisture content and maintaining similar level of lipid. Muscle and liver biochemical composition were also affected by the addition of vitamin E, being the lipid significantly lower in the E200 and E300 diets by which values increased as in the muscle as in the liver, but with more accused shape in the later one. Furthermore, in the fillet a decrease of protein whiles an increase of the lipid and moisture content from 1000 mg/kg of DL- α -tocopherol acetate were observed. These results were reported in a study by Ruff, N., *et al.* (2003) on the turbot fillet quality study, where a decrease of the protein and ash, together with an increased in the moisture and lipid of the fillet from of 1000mg/kg α -tocopheryl acetate in the diet were observed. In a previous study in sea bass by Gatta, P.P., *et al.* (2000), were also observed the same that the above results at a level of 942 mg/kg α -tocopherol. In a research performed on the storage stability of trout fillets fed with diets containing 200 and 5000 mg/kg of α -tocopherol acetate, it was showed that a level of 5000mg/kg α -tocopheryl acetate caused a decrease of the moisture and an increment of lipid in the muscle (Jittinandana, S., *et al.*,

2006). In Atlantic halibut, were also showed similar response with an inclusion levels of α -tocopheryl acetate of 189 and 613mg/kg in the diet (Ruff, N., *et al.*, 2002). At the intermediate level of vitamin E, an increment of the protein, ash and moisture accompanied by a decrease in the muscle were observed in present trial, which was also reported in the above experiment on seabass (Gatta, P.P., *et al.*, 2000). Therefore present results, in agreement with the results by Gatta, P.P., *et al.* (2000), showed that sub-optimum levels of vitamin E until level of 500mg/kg could promote the lipid assimilation and transportation, reducing the accumulation of fat in the site of body storage (Mourente, G., *et al.*, 2007), whereas that levels of 900, 1000 and 1500 mg/kg of dietary vitamin E, may promote an imbalance between the ascorbic acid (vitamin C) and vitamin E and produce a accumulation of lipid, due to an excess of vitamin E and low amount of vitamin C in the diet, do that the tocopheroxyl radicals abstract hydrogen atoms from PUFA at low rates and start up the lipid peroxidation (Bowry, V., *et al.*, 1992; Ingold, K., *et al.*, 1993; Gatta, P.P., *et al.*, 2000; Tocher, D.R., *et al.*, 2002; Hamre, K., 2011). Under this reaction, the vitamin E act as pro-oxidant (Hamre, K., 2011), causing possible damage in the highly unsaturated fatty acid and in the different tissues of the fish.

Whole body fatty acid composition of meagre in the current experiment reflected the composition of the respective diets fatty acid, being higher the content of the monounsaturated fatty acid (41.64 to 45.19%), followed by the saturated fatty acid (30.18 to 36.03%) and the total HUFA (9.91 to 18.25%). In a research performed by Martínez-Llorens, S., *et al.* (2011) on same species, there were obtained lower monounsaturated fatty acid (25.8 to 34.6%), lower saturated fatty acid (24.1 to 28.8%) but similar values of HUFA (9.8 to 20.7). Muscle fatty acid profile in this present work is compound by greater amount of monounsaturated fatty acid (34.29 to 37.98%), saturated fatty acid (30.66 – 41.58%), percentage of n-3 fatty acid between 10.79-24.79% and n-6 fatty acid about a

7.04 to 7.59. In a research performed by Piccolo, G. *et al.* (2008) obtained lower amount of monounsaturated fatty acid (24.79 to 24.91%) and lower saturated fatty acid (23.17 to 26.44), while higher n-6 (14.32 to 20.34%) and lower n-3 (15.74 to 17.35%) in the fillet of meagre that this recent work. However, in other previous study on meagre were achieved lower amount of saturated fatty acid, lower monounsaturated fatty acid and higher amount of n-3 and n-6 fatty acid (Poli, B., *et al.*, 2003; Grigorakis, K., *et al.*, 2011). These difference pointed out in the fatty acids of meagre fillet respect to those obtained in other experiments, may be attributed to the differences in the fatty acid profile of the diet as the diets of the current work were higher in monounsaturated fatty acids than in saturated fatty acid. The range of the atherogenic (0.53 to 0.79) and thrombogenic (0.33 to 0.84) indexes observed in meagre fillet in present trial are some higher than the previously reported by Poli, B., *et al.* (2003), which achieved values of 0.56 to 0.60 and 0.10 to 0.12 for the atherogenic and thrombogenic indexes, respectively. Lower results have been reported by Piccolo, G. *et al.* (2008), with 0.60 to 0.48 and 0.37 to 0.33, and by Grigorakis, K., *et al.* (2011) with 0.39 and 0.26, for the atherogenic and thrombogenic index, respectively. This may be due to that to that the meagre fillet in this work present greater amount of monounsaturated and saturated fatty acid than the above research and this, may indicate cardioprotective problem associated to a defect or excess of dietary vitamin E as were showed in *Scianop ocellatus* (Peng, L. *et al.*, 2008). Liver fatty acid composition of meagre in this work were constituted mainly by the monounsaturated (40.12 to 41.12%), followed by the saturated (28.52 to 30.58%), the n-3 (15.91 to 18.69%) and the n-6 (5.90 to 7.48%) fatty acids; it could be observed a decreasing of the HUFA in those fish feeding the higher vitamin E (E1500 diet) content, probably caused by an excess of the vitamin E while may be a deficient level of vitamin C (Bowry, V., *et al.*, 1992; Ingold, K., *et al.*, 1993; Hamre,

K., 2011). This data cannot be compared with other reports, because there is not previous data on the liver fatty acid profile in meagre.

Reactive oxygen species (ROS) and free radicals at high concentrations can react with a wide variety of biomolecules and in a rather nonspecific manner, producing oxidation in the membrane lipids, proteins, nucleic acid and altered cellular redox status, resulting in tissue pathologies, chemical carcinogenesis and aging (Ames, B., 1989; Mourente, G., *et al.*, 2007). This reaction with the biomolecules cause oxidative stress, which is consequence of the lipid peroxidation. This lipid peroxidation, specifically highly unsaturated fatty acid (HUFA) oxidation, is produced by a reaction of oxidative degradation in the lipids where the free radical obtains an electron from the above fatty acids in the cellular membrane producing peroxidative lipid as primary product. The decomposition of these primary product leads to the formation of aldehydes, ketones, alcohols, hydrocarbons, volatile organic acid and epoxy compounds, known as secondary oxidation products (Shahidi, F. and Zhong, Y., 2005; Zhong, Y. *et al.*, 2008). These primary and secondary products, cause damage in the cellular biomembrane, in the sub-cellular organelles, relatively rich in HUFA, of the different tissues provoked a decrease of the unsaturates fatty acid and increment of the saturates and monoinsaturates fatty acid with a deficient of vitamin E (Watanabe, T. *et al.*, 1970a; Halliwell, B. and Gutterried, J., 1996; Olsen, R., and Henderson, R., 1997; Sargent, J. *et al.*, 1999; Tocher, D.R., *et al.*, 2002; Alves Martins, D., *et al.*, 2007; Peng, S., *et al.*, 2009), which may lead to suppressed growth and affect to the sensory characteristics (Zhong, Y., *et al.*, 2008). This was observed in the meagre liver of this present work at the extreme levels of dietary vitamin E (0, 1000 and 1500mg/kg), as a defect or and excess of this vitamin together with a possible low concentration of ascorbic acid may cause problems of granulome and significant greater lipid vacuoles acumulation to level vitamin E of 100, 200 y 1000mg/kg in the diet,

due to that caused a greater unsaturated fatty acid accumulation and to the reactions between the tocopheroxyl radicals which abstract hydrogen atoms from PUFA as it was mentioned above (Bowry, V. *et al.*, 1992; Ingold, K. *et al.*, 1993 y Hamre, K., 2011), where vitamin E act as prooxidant (Hamre, K., 2011). In the study performed on Atlantic salmon (Bell, G., *et al.*, 2000) it was shown how a deficiency of vitamin E and astaxanthin in the diet caused a stimulation of hepatic fatty acid desaturation and elongation activities promoting hepatic tissues damage such as vacuolated hepatocytes and ceroidosis; same result were also reported in yellowtail (*Seriola quinqueradiata*) livers (Sakai, T., *et al.*, 1998) Also, in a research performed by Bai, S. and Lee, K. (1998), it was showed that a deficiency of vitamin E (0mg/kg) exerts problems of lipidic peroxidation in the livers. Watanabe, T., *et al.* (1977) argued that dietary deficiency of vitamin E in the diet originates some effect on fatty acid composition and that high levels of vitamin E provoke little effect on the fatty acid composition of fish. According to a study performed by Hamre, K., *et al.* (1994) was also obtained vacuoles and necrotic hepatocytes with vitamin E in the diet. Therefore, it could be confirmed that the problems of liver discoloration, ceroid accumulation and necrotic hepatic were obtained with a deficient of vitamin E in the diet (Murray, T., and Adrews, J., 1974; Roem, A., *et al.*, 1990; Hamre, K., 2011). However, intermediate level (300 and 500 mg/kg) dietary vitamin E shown a significant decrease of the highly unsaturated fatty acids with respect to other diet in this work, being the only diets which did not give problems in the livers of lipid vacuoles or granulome. In a study performed in sea bream by Tocher, D R., *et al.* (2002), it was also obtained that at the intermediate tested level (100mg/kg of α -tocopherol), significantly decrease the highly unsaturated acid compared with the other treatments.

Also, the above mention of the effect of the fatty acid on the level the vitamin E were reflected in the whole body and muscle fatty acid, due to that in the whole body fatty

acid with respect to the different level of vitamin E as had shown a tendency decrease of 8.73% for the saturated fatty acid and 5.64% for the monounsaturated fatty acid with an increment of the dietary vitamin E. However, the HUFA increasing by 41.36% with the increment of the vitamin E in the diet, being the 18:3n-3 fatty acid increased a 5.71%, eicosapentanoic acid (EPA) raised a 43.49% and the docohexanoic acid (DHA) up to a 55.40%. In the muscle fatty acid were showed the same that in the whole body fatty acid, where also the saturated fatty acid decrease a 0.76% and a 10.76% the monounsaturated fatty acid, whereas the highly unsaturated fatty acid increment 105.07% with an increase of the dietary vitamin E accompanied with an increment of the 18:3n-3 fatty acid (17.95%), EPA (105.74%) and DHA (140.13%). Therefore, in *Argyrosomus regius* high level of vitamin E cause a high retention of highly unsaturated fatty acid.

The dietary optimum requirement of vitamin E in meagre were of 509.32 mg/kg diet, this was determinate by the broken line regression method using the responses of the TBARS values that were applied by Peng, L. and Gattlin III, D.M., (2009) for red drum, which obtained a dietary minimum requirement of vitamin E around 31 IU/kg-1 in the diet in the form of all-rac- α -tocopheryl acetate. Also, the livers TBARS value of grouper (*Ephineelus malabaricus*) followed the same behavior that the above study and which were determinate the minimum dietary requirement of vitamin E through of the liver TBARS with values from 68 to 115 mg/kg as DL- α -tocopherol acetate, depend on the level of lipid in the diet (Lin, Y. and Shiau, S., 2005). The optimum dietary requirement obtained in meagre had been higher than the obtained for red drum (Peng, L. and Gattlin III, D.M., 2009), 35-60 mg/kg as α -tocopherol acetate in Atlantic salmon (Lall, S., *et al.*, 1988; Hamre, K., *et al.*, 1995), 50 mg/kg as α -tocopherol acetate in rainbow trout (Cowey, C., *et al.*, 1983a), 50 mg/kg as α -tocopherol acetate in channel catfish (Wilson R., *et al.*, 1984), 100 mg/kg as α -tocopherol acetate for common carp (Watanabe T., *et al.*, 1970a), 119

mg/kg as α -tocopherol acetate for yellowtail (Shimeno, S., 1991), 45 mg*kg⁻¹ α -tocopherol acetate for Korean rockfish (Bai, S. and Lee, K., 1998) and 140 and 169 mg*kg as DL- α -tocopherol acetate for *Channa punctatus* (Abdel-Hameid, H., *et al.*, 2012). Therefore, the dietary optimum requirement of vitamin E can be increased by a peroxidation lipid in the tissues exacerbated by factors dietary such as the highly unsaturated fatty acid (Watanabe, T., *et al.*, 1981; Lin, Y. and Shiau, S., 2005; Peng, L. and Gatlin III, D.M., 2009), and also depends on the interaction with other feed component such as the presence or abundance of other antioxidant as the vitamin C and selenium (Sealy, W. and Gatlin, D.M. 2002; Yildrin-Aksoy, M. *et al.*, 2008; Peng, L. and Gatlin III, D.M., 2009), or astaxanthin (Christiansen, R., *et al.*, 1995; Bjerkgeng, B., *et al.*, 1999; Bell, G., *et al.*, 2000; Hamre, K., 2011).

An increase of the fillet quality and lengthen its self life protecting against lipid oxidation by an increment of the vitamin E in the diet have being reported in different works. These results are variable as depends on the flesh fatty acid composition and the storage condition and time (Hamre, K., 2011). In this present work, the muscle thiobarbituric acid reactive substance in the juveniles of meagre decreased with increment of the dietary vitamin E (0.46 to 1.09 mg MDA*kg of sample) after six month freezing, which showed greater lipid peroxidation for 0 and 100 mg*kg vitamin E in the diet. Jittinandana, S., *et al.* (2006), showed in trout that an elevated contents of 5000 mg/kg of vitamin E in the diet produced muscle TBARS values higher than the fillet fish fed with 200mg/kg of vitamin E. In the fillet of the Atlantic halibut, were achieved a dropped of the TBARS value with an increment of the dietary vitamin E (Ruff, N., *et al.*, 2002), this was also observed by other previous experiment (Ruff, N., *et al.*, 2003). Finally, in a research performed by Gatta, P.P., *et al.* (2000), it was observed a dropped of the muscle TBARS value with an increment of the vitamin E in the diet, although the muscle TBARS values

were higher than those obtained in this recent work. These differences in muscle TBARS values between seabass and meagre may be related with the low fat content in the muscle of meagre fish. In a research performed by Stéphan, G., *et al.* (1995), results showed a drop of the muscle TBARS with a supplementation of vitamin E in the diet, after storage of the fillet at -20°C for 6 months in the freezer, thus obtained higher oxidation value than in present meagre fillet after 6 months of freezing at -80°C, which could be due to the fact that meagre have less fat in their fillet or to the different storage condition.

CONCLUSIONS

In this present work, feeding meagre with a 50% protein, 16% lipid, 5000mg/kg⁻¹ of vitamin C and levels from 0 to 1500 mg/kg vitamin E as DL- α -tocopherol acetate, no effect of the vitamin E was determined on the growth and diet productive parameter. Moreover, the obtained growth responses were the best reported by meagre fish during its on-growing phase.

For meagre with a specific growth rate of 1.41 to 1.49%/day the requirements of vitamin E it was determined as 509.32 mg/kg DL- α -tocopherol acetate in the diet. This was determined by the methods of the broken line regression analysis applied to the muscle TBARS, as it was reported by Peng, L. and Gattlin III, D.M., (2009).

Whole body and muscle biochemical composition were affected by the supplementation of vitamin E in the diet, whereas it did not affect that on the livers. An increment of the vitamin E in the diet caused an increment of the muscle and whole body's highly unsaturated fatty acid and a decrease of the saturated and monounsaturated fatty acid. In the livers a decrease of the highly unsaturated fatty acid and increase of the saturated and monounsaturated fatty acid was observed by feeding 300 and 500mg/kg vitamin E, which did not present moreover granulome and lipid vacuoles. On the contrary extreme

levels of 0, 1000 and 1500 mg/kg vitamin E in the diet presented granulomes, while lipid vacuoles were showed when feeding levels of 100, 200 and 1000 mg/kg of vitamin E. Thus, a depletion (0 mg/kg of DL- α -tocopheryl acetate in the diet) or an excess (1000 and 1500 mg/kg of DL- α -tocopheryl acetate in the diet) of the vitamin E content in the diet cause problem of lipid peroxidation in the liver originating lipid vacuole and granulome. However, the muscle lipid peroxidation showed by TBARS was significantly dropped with an increment of the supplementation of the dietary vitamin E.

CONCLUSIONES

Los efectos de una dieta isoproteica (50%) a isocalórica (16%) con un nivel inclusión de 5000 mg/kg de vitamina C (ácido ascorbico) en la dieta y diferentes niveles de inclusión de vitamina E (0, 100, 200, 300, 500, 1000 y 1500 mg/kg) en la forma de DL- α -tocoferol acetato son:

- 1) El crecimiento no se ve afectado para los diferentes niveles de vitamina E en dieta a los valores ensayados., obteniéndose además en el presente estudio los mejores resultados productivos para corvina en fase de engorde, con respecto al resto de estudios publicados.
- 2) Si bien niveles de entre 300 y 500 mg/kg resultarían apropiados durante el engorde de corvina, el óptimo de inclusión de vitamina E estimado para esta especie con una tasa de crecimiento específica de entre 1,41 a 1,49% es de 509,32 mg/kg en la forma de DL- α -tocoferol acetato, el cual fue determinado por el método de regresión de la línea quebrada con los valores de la TBARS del músculo.
- 3) La composición bioquímica del pez entero y del músculo de corvina resultó afectada por la inclusión de vitamina E en la dieta, no así en el caso del hígado en los que no se vio alterada.
- 4) Los perfiles de los ácidos grasos del pez entero, músculo e hígado se vieron afectados por los diferentes niveles de vitamina E en la dieta, encontrándose un aumento de los ácidos grasos insaturados y una disminución de los ácidos grasos saturados y monoinsaturados con el incremento de la vitamina E. Se observó una reducción en el contenido en los ácidos grasos altamente insaturados en los hígados de las corvinas alimentadas con las dietas de 300 y 500 mg/kg de DL- α -tocoferol acetato, en los cuales no se observaron además daños a nivel histológico.

- 5) Los valores de las TBARS disminuyeron con un incremento de la vitamina E en la dieta, por lo tanto, la vitamina E gracias a sus propiedades antioxidantes mejora la calidad del filete.
- 6) Los resultados histológicos del hígado mostraron granulomas en las dietas más extremas, con niveles de 0, 1000 y 1500 mg/kg de vitamina E, y mayor acumulación de vacuolas lipídicas significativamente para los niveles de 100, 200 y 1000mg/kg de vitamina E en la dieta.
- 7) Los resultados del presente estudio muestran que durante el engorde de la corvina, tanto la deficiencia como el exceso de la vitamina E causa peroxidación lipídica.

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