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Efecto de los ácidos grasos de cadena larga en el crecimiento y metabolismo del abulón azul (*Haliotis fulgens*)

TESIS

que para obtener el grado de

DOCTOR EN CIENCIAS

presenta

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RESUMEN

Con el propósito de aportar mayor conocimiento sobre el efecto y la importancia nutricional de los ácidos grasos insaturados de cadena larga en el crecimiento, composición de ácidos grasos y metabolismo de abulón azul (*Haliotis fulgens*) se desarrollo este estudio. El estudio comprendió tres experimentos cuyos resultados se integraron en 4 artículos científicos. El **primer experimento** (**ARTÍCULO 1**) se realizó en una granja comercial de abulón en el cual se evaluó el efecto del uso de alimento balanceado, macroalga y la combinación de ambos alimentos, en la sobrevivencia, crecimiento y composición de ácidos grasos en músculo de juveniles de *H. fulgens*. en un período de 329 días. Los resultados mostraron la mayor sobrevivencia y las mayores tasas de crecimiento con el uso de la dieta mixta. Los perfiles de ácidos grasos en los alimentos y en los abulones sometidos a los tres tratamientos de alimentación sugirieron que el abulón azul podría tener la capacidad de sintetizar ácidos grasos insaturados de cadena larga a partir de precursores de cadena más corta. En el **segundo experimento** (**ARTÍCULO 2**) se estudió el efecto de alimentación balanceada con cuatro tipos de aceites (oliva, maíz, linaza e hígado de bacalao), tres niveles de inclusión (1.5, 3 y 5%) sobre el crecimiento y la composición de ácidos grasos en tejido muscular de juveniles de abulón azul. Los resultados de crecimiento no mostraron diferencias con relación al tipo de aceite, sin embargo se determinaron diferencias significativas con relación al nivel de inclusión en la dieta. Los tratamientos con 5% de aceite presentaron las menores tasas de crecimiento en tanto que las dietas con un nivel de aceite de 1.5% se asociaron con los mayores crecimientos. Abulones juveniles con un consumo de 2, 0.2, 0.2 y 0.2 μg/g de peso vivo/día de 18:2n-6, 18:3n-3, 20:4n-6 y 22:6n-3, respectivamente, presentaron una ganancia en peso del 120% con relación al inicio del experimento después de 75 días de alimentación balanceada. Los tratamientos con un aporte alto de 18:2n-6 y 18:3n-3 mostraron la capacidad de *H. fulgens* para sintetizar 20:4n-6, 22;4n-6, 20:5n-3 y 22:5n-3. En el **tercer experimento** se evaluó la depositación y metabolismo de ácidos grasos a través de inanición y alimentación restringida en lípidos, el experimento comprendió dos bioensayos. En el **primer bioensayo** (**ARTÍCULO 3**) se realizó un estudio de alimentación con dos niveles de lípidos (0.14 y 5%) durante 50 días e inanición hasta los 90 días. Los resultados no mostraron una reducción en el contenido de lípidos totales de músculo a los 50 días de alimentación restringida en lípidos o 70 días de inanición. En abulones en inanición no se detectó la presencia de PUFA n-3, la relación n-3/n-6 mostró una tendencia a incrementarse con relación al tiempo, esto se asoció con la conservación de los HUFA n-3 y a la disminución en los PUFA n-6. A los 90 días de inanición se observó una disminución en los contenidos totales de ácidos grasos saturados, monoinsaturados y PUFA, en tanto que los HUFA n-3 y n-6 se conservaron. Los abulones sometidos a alimentación restringida en lípidos mostraron una disminución en los contenidos de PUFA n-6, HUFA n-3 y n-6, así como la relación n-3/n-6 disminuyó con relación al inicio del bioensayo. En el **segundo**

bioensayo (**ARTÍCULO 4**) se estudió el efecto de inanición y alimentación con dos niveles de lípidos (0.12 y 3%)sobre los contenidos de ácidos grasos en lípidos polares y neutros en el músculo de *H. fulgens*, y en lípidos totales del contenido digestivo, durante 60 días. Los resultados de crecimiento en peso y longitud no mostraron diferencias significativas entre los abulones sometidos a alimentación mientras que los organismos en inanición presentaron un 19% de pérdida en peso. Los abulones en inanición no presentaron disminución en el contenido de lípidos totales en músculo, así mismo, mostraron una alta conservación en los ácidos grasos de lípidos polares y neutros con relación al inicio del ensayo. En abulones sometidos a alimentación los lípidos polares en músculo también presentaron la tendencia a conservarse al termino del ensayo; sin embargo, se determinó una apreciable reducción en el contenido de los ácidos grasos presentes en lípidos neutros. El contenido de lípidos totales y ácidos grasos en el contenido digestivo en abulones alimentados fueron mayores que los presentes en los alimentos. En los abulones sometidos a alimentación los perfiles de los ácidos grasos de los contenidos digestivos no mostraron una relación definida con respecto a los alimentos correspondientes; ni el perfil de los organismos en inanición con relación al del inicio del bioensayo.

"EFECTO DE LOS ÁCIDOS GRASOS DE CADENA LARGA EN EL CRECIMIENTO Y METABOLISMO DEL ABULÓN AZUL (*Haliotis fulgens*)"

Tesis que para obtener el grado de

DOCTOR EN CIENCIAS

presenta:

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La ciencia empieza y acaba siempre en la naturaleza, en una realidad externa que se postula. La base de la ciencia es que el hombre tiene la curiosidad de conocer o entender la naruraleza.

Arturo Rosenblueth
"El Método Científico"

Dedicatoria

A mi familia:

Beatriz Alicia,

Eduardo Alberto,

Rebeca Alejandra

y

Elena del Carmen.

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Í N D I C E

1. INTRODUCCIÓN

Los lípidos son un grupo heterogéneo de sustancias, presentes en tejidos animales y vegetales, que comparten la propiedad de ser solubles en solventes orgánicos como benceno, hexano y cloroformo además de ser insolubles en agua. Los lípidos actúan como fuente y reserva de energía, como componentes de membranas biológicas y en reacciones metabólicas como acarreadores de electrones y sustratos (McDonald, 1995).

En general, los lípidos pueden ser clasificados en dos grupos de acuerdo a sus propiedades químicas: no polares o neutros y polares. Los lípidos no polares comprenden a los triacilgliceroles, diacilgliceroles, ceras, esteroles y terpenos, mientras que los lípidos polares están integrados por los fosfolípidos y glicolípidos. Los lípidos son componentes esenciales de todas las células, al aportar los ácidos grasos necesarios en el mantenimiento e integridad de membranas celulares. Participan en el transporte lipídico, como es el caso de los fosfolípidos los cuales favorecen la emulsificación de lípidos; también son precursores de hormonas, como en el caso del ácido araquidónico, 20:4n-6, el cual es precursor de prostaglandinas (Nelson y Cox, 2000).

En el caso de los animales marinos los lípidos presentan también importancia en aspectos como: mantenimiento de estructura corporal, siendo el caso de la presencia de ácidos grasos poliinstaurados ("polyunsaturated fatty

acids", PUFA) n-3 en biomembranas que les permite mantener fluidez a bajas temperaturas (Glémet *et al.*, 1997; Farkas et al., 2001); adaptación a cambios de salinidad a través de la síntesis de prostanglandinas (Bell y Sargent, 2003); y en características físicas como la flotación, debido a la baja densidad de los lípidos y al cambio de esta con la temperatura (Nelson y Cox, 2000).

En nutrición animal los lípidos tienen dos funciones principales, una, el ser la mayor forma de energía corporal almacenada a través de las reservas de triglicéridos, y la otra, el formar una parte estructural importante de los componentes de la membrana celular a través de los fosfolípidos en complejos de lipoproteínas (McDonald *et al.*, 1995).

La alimentación balanceada ha demostrado ser de suma importancia dentro de la nutrición animal para conseguir mejores tasas de crecimiento a menor costo con un mínimo de desperdicio del alimento, o sea, de mejor digestibilidad, hecho que no excluye a las especies marinas. Una alimentación balanceada al aportar los nutrientes necesarios en cantidad y calidad, dentro de los cuales podemos mencionar a los lípidos. En organismos marinos también se favorecen la maduración gonadal y el desarrollo larval.

Los lípidos de la dieta deberán de suministrar los PUFA esenciales n-6 y/o n-3 requeridos para propósitos estructurales o fisiológicos que el organismo no puede sintetizar. Ya que los animales acuáticos no tienen la capacidad de

sintetizar *de novo* ácidos grasos con configuración n-6 ó n-3 (Figura 1), su contenido y balance en la dieta son de suma importancia para cubrir los requerimientos esenciales (Bell, 1998; Sargent *et al.*, 2002).

Estudios de nutrición en abulón han mostrado que el contenido y composición de los lípidos, juegan un papel esencial en la nutrición del organismo (Uki *et al.*, 1985; Uki *et al.*, 1986; Mai *et al.*, 1995; Floreto *et al.* 1996; Nelson *et al.* ,2002). Con base en estudios con alimentación balanceada se recomienda que el alimento contenga de 3-5% de lípidos, a efecto de favorecer las mayores tasas de crecimiento del organismo (Uki *et al.*, 1985; Uki y Watanabe, 1992; Mai *et al.*, 1995; Bautista y Millamena, 2000). Sin embargo, se reporta que alimento comercial japonés con 1.5% de lípidos totales, provenientes solo de pescado, permite obtener altas tasas de crecimiento, (Fleming *et al.*, 1996). En el caso del abulón los lípidos de la dieta deben de cubrir los requerimientos de PUFA n-3 y n-6 del organismo, más que considerarlos como un aporte energético. Con base en estudios en *H. discus hannai*, se estiman que los requerimientos de ácidos grasos altamente insaturados ("highly unsaturated fatty acids", HUFA) como 20:5n-3 y 22:6n-3 se cubren con un aporte en la dieta de 20% de n-3 y 2% de n-6 (Uki *et al.*, 1986; Fleming *et al.*, 1996).

En animales se considera que los lípidos suministrados a través de la dieta probablemente tienen una mayor influencia sobre el metabolismo de los

lípidos corporales que el efecto del consumo de otro nutriente, como proteínas o carbohidratos, sobre su contraparte presente en el organismo. Esto es debido a la baja especificidad de las interacciones enzima-sustrato en las rutas metabólicas a través de las cuales los lípidos son metabolizados (Sargent *et al.*, 1993). Un ejemplo evidente de la baja especificidad de sustrato se presenta en las insaturasas de ácidos grasos, como es el caso de la insaturasa Δ6 que puede tener como sustratos a 16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 24:5n-3 y 24:4n-6 (Figura 2). Esta situación tiene como efecto que la conversión de PUFA como 18:2n-6 a 18:3n-6 y 18:3n-3 a 18:4n-3, pueda competir con la conversión de HUFA como 20:5n-3 a 22:6n-3 y 20:4n-6 a 22:5n-6, ya que la insaturasa Δ6 participa en dichas conversiones. La competencia entre ácidos grasos de las familias n-3 y n-6 ocurre al nivel de insaturación y elongación de la cadena. Con la insaturasa Δ6 se tiene que 18:3n-3 es mejor sustrato que 18:2n-6, donde la abundancia de 18:3n-3 puede reducir en forma efectiva la formación de 20:4n-6 a partir de 18:2n-6. En general la competencia entre 18:2n-6 y 18:3n-3 por la insaturasa Δ6 reduce la formación y acumulación de ácidos grasos insaturados n-9. Sin embargo, en animales con deficiencia en ácidos grasos esenciales, la competencia no se presenta y ácidos grasos como 18:0 ó 18:1n-9 pueden ser utilizados como sustratos para reacciones de insaturación y elongación (Sargent *et al.*, 1995; Cook, 1996).

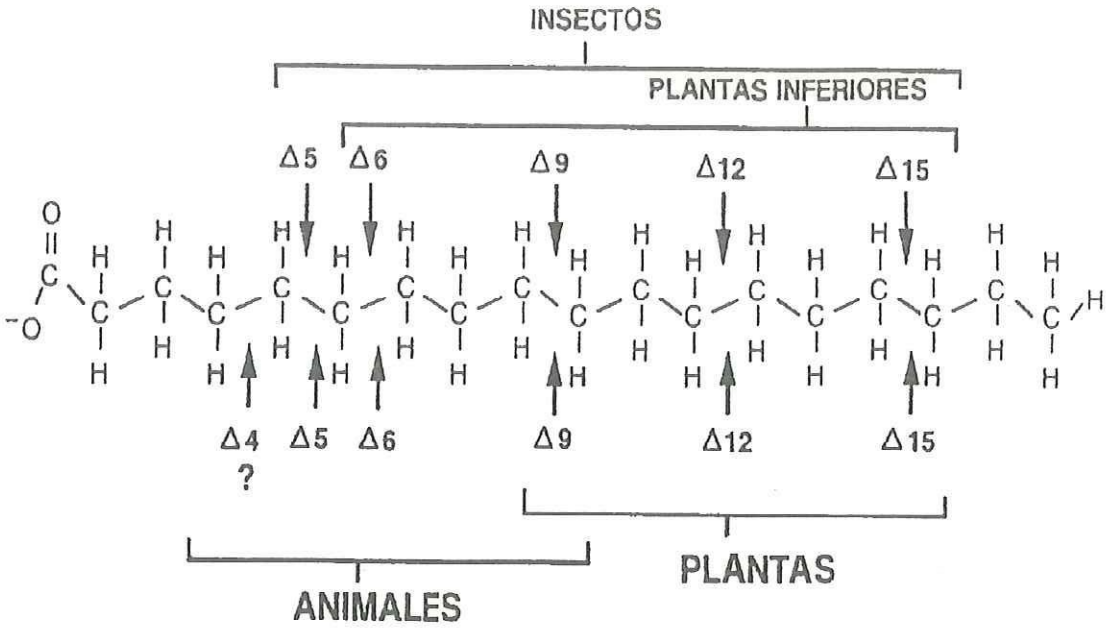

Figura 1. Posiciones de insaturación en la cadena de un ácido graso C_{18} por insaturasas de animales, plantas, insectos y plantas inferiores (Cook, 1996).

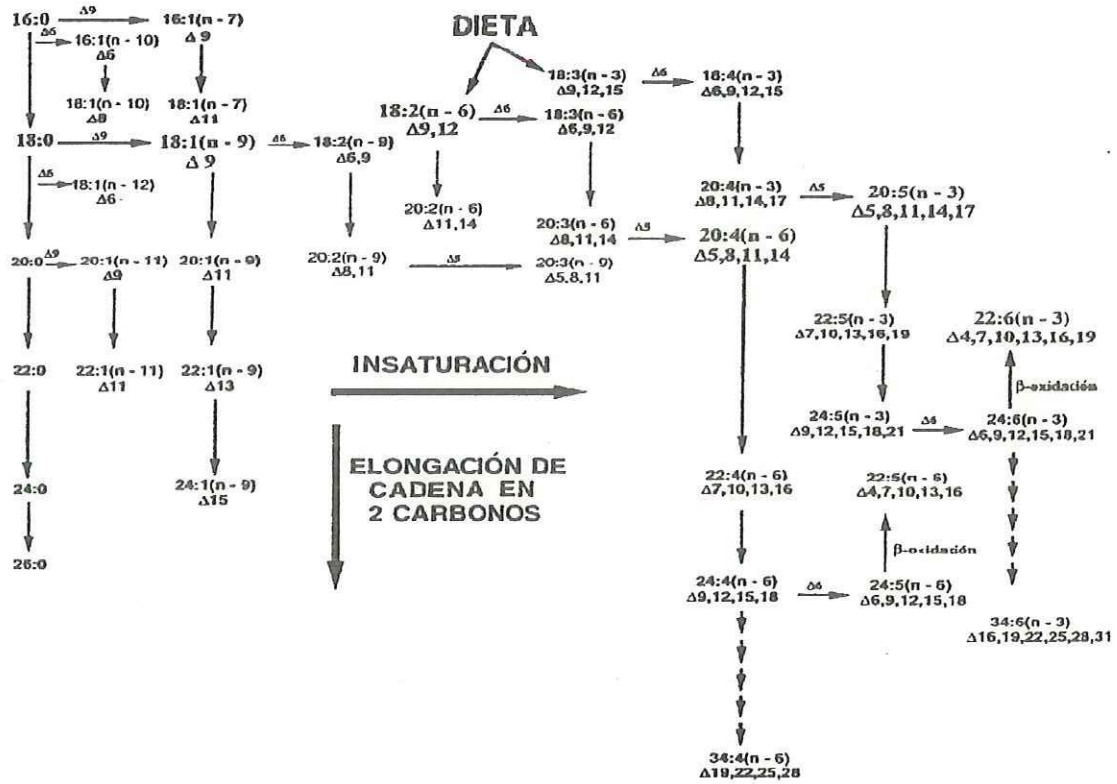

Figura 2. Rutas mayores de biosíntesis de ácidos grasos en tejidos animales por insaturación y elongación de cadenas (Cook, 1996).

A partir de estudios en mamíferos se ha determinado que la preferencia por sustrato de la insaturasa Δ6 muestra el siguiente orden: 18:3n-3 > 18:2n-6 >> 18:1n-9, donde existe competencia entre estos posibles sustratos (Cook, 1996). Estudios en peces han mostrado que 18:3n-3 es más efectivo en inhibir la elongación e insaturación de 18:2n-6 con respecto al efecto de 18:2n-6 sobre 18:3n-3 (Sargent *et al.* 1995; Caballero *et al.*, 2002). En trucha arcoiris (*Salmo gairneri*) se ha demostrado que la conversión de 20:5n-3 a 22;6n-3 esta sujeta a control por el nivel de PUFA n-3 presentes en la dieta (Buzzi *et al.*, 1996).

El aporte de ácidos grasos n-3 y n-6 y su balance en la dieta son de gran importancia para cubrir los requerimientos del organismo y favorecer su crecimiento. En peces como *S. gairneri* y *Oncorhynchus kistch* se ha encontrado que si la dieta presenta un elevado contenido de ácidos grasos n-3 y/o n-6 con relación a los requerimientos de los organismos, se inhibe el crecimiento (Watanabe y Takeguchi, 1989). Se sugiere que esta inhibición es una respuesta metabólica al exceso de ácidos grasos n-3 y/o n-6 en la dieta, condición inusual para el organismo (Yu y Sinhuber, 1976).

Investigaciones sobre la respuesta bioquímica del abulón bajo estrés nutricional es limitada (Carefoot *et al.*, 1993; Watanabe *et al.*, 1993, 1994; Takami *et al.*, 1995; Nelson *et al.*, 2002). En condiciones de inanición se presenta una disminución del crecimiento, se reducen la tasa metabólica y las reservas de energía. La movilización de las reservas de energía, incluidos los

lípidos, origina cambios en la composición bioquímica de tejidos y sus constituyentes (Carefoot *et al.*, 1993). Cuando se utiliza alimentación restringida en el estudio de la dinámica metabólica de los lípidos, los cambios que se presentan en la composición bioquímica de tejidos y la movilización de reservas del organismo permiten evaluar el efecto diferencial del contenido y tipo de lípidos sobre la depositación y composición de tejidos (Navarro y Gutiérrez, 1995).

Con el propósito de aportar mayor conocimiento sobre el efecto y la importancia nutricional de los ácidos grasos insaturados de cadena larga en el crecimiento, composición de ácidos grasos y metabolismo de abulón azul (*Haliotis fulgens*) se desarrollo este estudio.

2. PROBLEMA DE ESTUDIO E HIPÓTESIS DE TRABAJO

El problema de estudio que se estableció investigar comprendió los siguientes cuestionamientos: ¿Cuál es el efecto de los ácidos grasos insaturados de la dieta y su nivel de inclusión sobre el crecimiento? ¿Cuál es la capacidad de síntesis de HUFA a partir de PUFA y el patrón de conservación de HUFA en *H. fulgens* ?

La hipótesis de trabajo propuesta para abordar el problema de estudio fue la siguiente: El abulón azul presenta un bajo requerimiento de ácidos grasos insaturados y tiene la capacidad de sintetizar HUFA n-3 y n-6 a partir de PUFA C_{18}, los cuales son altamente conservados

Para probar la hipótesis de trabajo se plantearon los siguientes pasos:

1) Evaluar el efecto de alimentación con diferentes fuentes y niveles de PUFA n-3 y n-6 sobre crecimiento y composición de ácidos grasos en tejido muscular (experimentos 1 y 2).
2) Evaluar la capacidad del abulón azul para realizar la bioconversión de PUFA C_{18} a HUFA (experimento 2).
3) Evaluar las características de la depositación y metabolismo de ácidos grasos en tejido muscular mediante inanición y alimentación restringida en lípidos.(experimento 3).

3. OBJETIVOS

Objetivo general

Mediante un enfoque nutricional, evaluar el efecto de los ácidos grasos insaturados n-3 y n-6 de la dieta sobre el crecimiento, perfil de ácidos grasos en tejidos y metabolismo de ácidos grasos.en *H. fulgens*.

Objetivos específicos

a) Determinar el efecto de diferentes fuentes y niveles de ácidos grasos en la dieta sobre el crecimiento y perfil de ácidos grasos en tejido muscular.
b) Evaluar la capacidad de *H. fulgens* para sintetizar HUFA n-3 y n-6 a partir de precursores PUFA.
c) Evaluar las características de la depositación y metabolismo de ácidos grasos en tejido muscular mediante inanición y alimentación restringida en lípidos.

4. DISCUSIÓN GENERAL

Como una primera aproximación al problema de estudio se realizó el **PRIMER EXPERIMENTO** de nutrición en una granja comercial de abulón (**ARTÍCULO 1**). El objetivo fue el evaluar en condiciones de cultivo comercial el efecto de tres tratamientos de alimentación: alimento balanceado, *Macrocystis pyrifera* (alga) y la combinación de ambos alimentos, en la sobrevivencia, crecimiento y composición de ácidos grasos en músculo de juveniles de *H. fulgens*. El estudio abarcó un período de 329 días. Aún cuando no fue posible evaluar el consumo de alimento, se observó que los abulones alimentados con la dieta mixta presentaron la mayor sobrevivencia y las mayores tasas de crecimiento en peso y longitud, las ganancias en peso y longitud fueron de 7915 y 345% respectivamente. No obstante que no se midió el consumo ni se controló el crecimiento de diatomeas, así como alimento asociado a las macroalgas, los perfiles de composición de ácidos grasos en los alimentos y en los abulones sometidos a los tres tratamientos de alimentación sugieren que el abulón azul podría sintetizar ácidos grasos insaturados de cadena larga a partir de precursores de cadena más corta (Figura 3). En *M. pyrifera* no se determinó la presencia de 22:4n-6 y 22:5n-3, los cuales si fueron detectados en los abulones alimentados con esta alga. De igual forma, en el alimento balanceado no se detectó la presencia de 20:3n-6, 20:4n-3 y 22:4n-6, los cuales estuvieron presentes en los abulones sometidos a la dieta balanceada. En el tratamiento con alimentación mixta *H. fulgens* mostró la presencia de 22:4n-6 aun cuando

este ácido graso no se encontró en la dieta mixta. La presencia de estos ácidos grasos en el abulón azul se podrían relacionar con la capacidad que muestran los eucariontes para realizar reacciones de elongación de cadenas de ácidos grasos (Stryer, 1995). En el caso de 22:4n-6, ausente en los alimentos y presente en los abulones de todos los tratamientos, se determinó una correlación significativa entre los niveles de 22:4n-6 en músculo y 20:4n-6 en las dietas ($r = 0.99$, $P = 9.3x10^{-5}$), lo cual sugeriría que 22:4n-6 podría ser producto de la elongación de 20:4n-6. Así mismo, la ausencia en la dieta balanceada de 20:3n-6 y su presencia en organismos alimentados con esta apuntaría que 18:2n-6 podría ser precursor de 20:3n-6.

Con base en los resultados obtenidos en el 1^{r} experimento y a la necesidad de contar con información sobre los requerimientos de ácidos grasos insaturados n-3 y n-6 en el abulón azul se realizó el **SEGUNDO EXPERIMENTO** de nutrición (**ARTICULO 2**). En este experimento se estudió el efecto de alimentación balanceada con cuatro tipos de aceites (oliva, maíz, linaza e hígado de bacalao), tres niveles de inclusión (1.5, 3 y 5%) en un 5% de lípidos totales (uso de tripalmitina para completar los lípidos totales) sobre el crecimiento y la composición de ácidos grasos en tejido muscular. El diseño experimental se estableció con el objetivo principal de evaluar el efecto de dosis-respuesta de *H. fulgens* ante un gradiente en la dieta de 18:1n-9, 18:2n-6 y 18:3n-3 a partir de los

ALIMENTOS

Macrocystis pyrifera

PUFA: 18:2n-6, 18:3n-3, 18:4n-3, 20:3n-6

HUFA: 20:4n-6, 20:4n-3, 20:5n-3, 22:6n-3

Alimento balanceado

PUFA: 18:2n-6, 18:3n-3, 18:4n-3

HUFA: 20:4n-6, 20:5n-3, 22:5n-3, 22:6n-3

ABULÓN

Alimentado con *M. pyrifera*

PUFA: 18:2n-6,

HUFA: 20:4n-6, 20:4n-3, 20:5n-3, **22:4n-6**, **22:5n-3**, 22:6n-3

Alimentado con alimento balanceado

PUFA: 18:2n-6, 18:3n-3, 18:4n-3, **20:3n-6**

HUFA: 20:4n-6, **20:4n-3**, 20:5n-3, **22:4n-6**, 22:5n3, 22:6n-3

Alimentado con *M. pyrifera* y alimento balanceado

PUFA: 18:2n-6, 18:3n-3, 18:4n-3, 20:3n-6

HUFA: 20:4n-6, 20:4n-3, 20:5n-3, **22:4n-6**, 22:5n-3, 22:6n-3

Figura 3. PUFA y HUFA detectados en alimentos y músculo de *H. fulgens* alimentado con *M. pyrifera* (alga), alimento balanceado y dieta mixta durante 329 días en condiciones de cultivo comercial. Los ácidos grasos marcados con negritas en el abulón no se detectaron en los alimentos.

aceites vegetales, y de HUFA n-3 a partir del aceite de pescado. Los resultados de crecimiento de este experimento no mostraron diferencias con relación al tipo de aceite, sin embargo se determinaron diferencias significativas con relación al nivel de inclusión en la dieta. Los tratamientos con 5% de aceite presentaron las menores tasas de crecimiento, lo cual no fue atribuible al consumo de alimento ya que no se encontraron diferencias significativas ($P>0.05$) entre las tasas de consumo de los tratamientos evaluados. Este efecto inhibitorio del crecimiento se atribuyó a un exceso de ácidos grasos n-3 y/o n-6 con relación a los requerimientos del organismo, situación que ha sido reportada en especies acuáticas (Yu y Sinhuber, 1976, 1979; González-Felix *et al.*, 2002). A partir de regresiones gausianas se determinó que todas las dietas con un nivel de aceite de 1.5% se asociaron con los mayores crecimientos en peso ($y = 7.31+ 3.38\ e^{[-0.5(x-1.42/1.14)^2]}$, $R^2 = 0.824$, $P=0.01$) y en longitud ($y = 54.76 + 21.26\ e^{[-0.5(x-1.22/1.22)^2]}$, $R^2 = 0.876$, $P=0.03$). Estos resultados contrastan con la recomendación de la inclusión de un nivel 5% de lípidos en dietas balanceadas para abulón para favorecer un máximo crecimiento (Uki *et al.*, 1985; Hahn, 1989; Uki *y* Watanabe, 1992; Mai *et al.*, 1995; Bautista- Teruel *et al.*, 2000), la cual no considera el posible efecto de un exceso de ácidos grasos n-3 y/o n-6. De igual forma, con base en datos de crecimiento, se reporta que una dieta con 5% de lípidos deberá de contener 1% de HUFA n-3 (20:5n-3 +22:6n-3) para cubrir los requerimientos del abulón (Uki *et al.*, 1986). Sin embargo, si se analizan los datos de crecimiento en peso reportados por Uki *et al.* (1986) para sustentar su recomendación, no se encuentran diferencias significativas ($P>0.05$) entre los

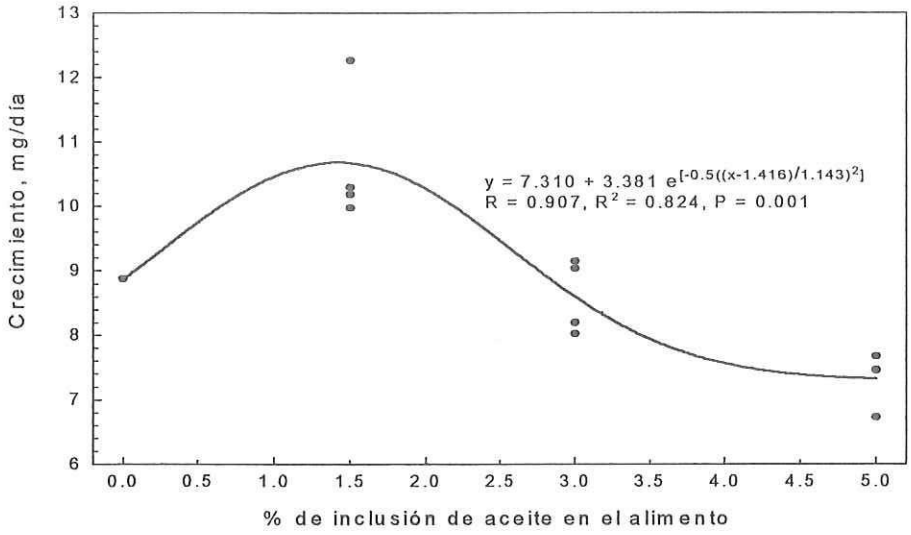

Figura 4. Efecto del % de aceite en alimentación balanceada sobre el crecimiento en peso (mg/día) de juveniles de *H. fulgens* alimentados durante 75 días.

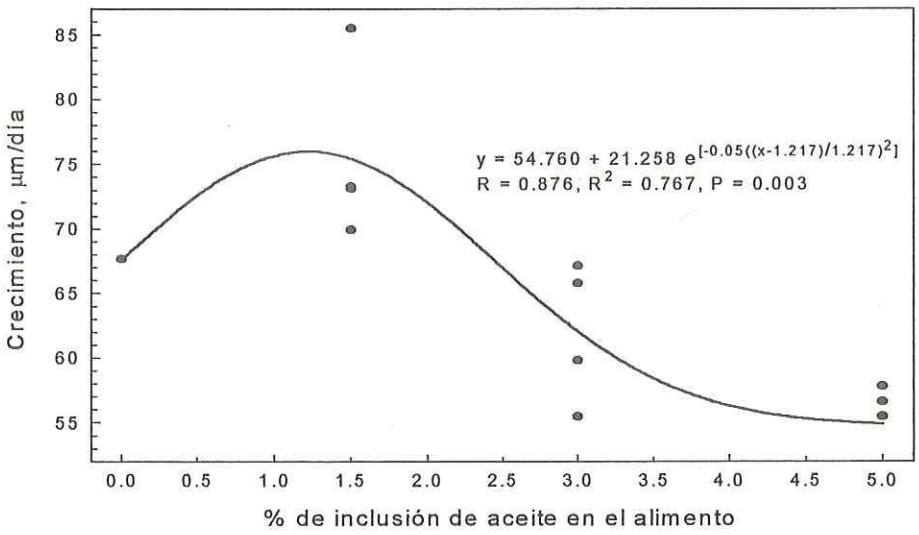

Figura 5. Efecto del % de aceite en alimentación balanceada sobre el crecimiento en longitud (µm/día) de juveniles *H. fulgens* alimentados durante 75 días.

tratamientos con 18:3n-3 y 18:2n-6, HUFA n-3 y mezcla de aceites de soya y pescado. Por lo tanto hay que tomar con reserva dicha recomendación.

En este 2º experimento no se pudo establecer una relación significativa entre crecimiento con un determinado tipo o nivel de ácido graso o con una relación n-3/n-6 suministrados en las dietas. Sin embargo, los resultados mostraron que los abulones alimentados durante 75 días con la dieta de referencia (0.25% de lípidos totales) presentaron una ganancia en peso del 120% con relación al inicio del experimento, con un consumo de 2, 0.2, 0.2 y 0.2 μg/g de peso vivo/día de 18:2n-6, 18:3n-3, 20:4n-6 y 22:6n-3, respectivamente. En tanto, los organismos alimentados con la dieta con 1.5% de aceite de maíz (5% de lípidos totales) mostraron una ganancia en peso del 170%, con un consumo de 9.1, 2, 1 y 0.1 μg/g de peso vivo/día de 18:2n-6, 18:3n-3, 20:4n-6 y 22:6n-3, respectivamente.

Los tratamientos con aceite de maíz y linaza con un aporte alto de 18:2n-6 y 18:3n-3, respectivamente, mostraron la capacidad del abulón azul para sintetizar 20:4n-6, 22:4n-6, 20:5n-3 y 22:5n-3. Mediante un análisis de regresión se determinaron regresiones significativas ($P<0.05$) entre 18:2n-6 y 18:3n-3 en el alimento y 18:2n-6, 18:3n-3, 20:4n-6, 22:4n-6, 22:5n-3 y 22:6n-3 en músculo de los abulones (Cuadro 1, Figuras 6-13). A partir de estos resultados se puede afirmar que *H. fulgens* muestra la capacidad para elongar e insaturar 18:2n-6 hasta 22:4n-6, aun cuando no se detectó 18:3n-6 en músculo se estima que

dicha bioconversión se efectúa por un mecanismo similar al reportado por Sargent *et al.* (1995) y Cook (1996).

Cuadro 1. Resultados de regresiones entre contenidos de ácidos grasos insaturados en el alimento y en músculo de abulón (n=13).

Variable Indep.	Variable depend.	Ecuación	R	R^2	P
18:2n-6 en alimento	18:2n-6 en músculo	$y = 1.415 + 0.177x$	0.855	0.731	0.002
18:2n-6 en alimento	20:4n-6 en músculo	$y = 2.34 + 0.30x + 0.008x^2$	0.878	0.771	0.001
18:2n-6 en músculo	20:4n-6 en músculo	$y = 2.119 + 0.518x$	0.732	0.535	0.004
18:2n-6 en músculo	22:4n-6 en músculo	$y = 0.22 + 0.164x$	0.668	0.446	0.013
20:4n-6 en músculo	22:4n-6 en músculo	$y = -0.458 + 0.319x$	0.919	0.845	<0.0001
18:3n-3 en alimento	18:3n-3 en músculo	$y = 0.435 + 0.329 - 0.01x^2$	0.979	0.959	<0.0001
20:5n-3 en músculo	22:5n-3 en músculo	$y = 0.557 + 0.604x$	0.941	0.885	<0.0001
22:5n-3 en músculo	22:6n-3 en músculo	$y = -0.341 + 0.300x$	0.598	0.357	0.031

El efecto de dosis-respuesta del contenido de 18:3n-3 en el alimento con relación al contenido de 18:3n-3 en músculo fue manifiesto, obteniéndose una regresión significativa de 2º orden con altos coeficientes de correlación y determinación ($R=0.979$, $R^2=0.959$). Sin embargo, en el caso de 20:5n-3 y 22:5n-3 en músculo no se encontró correlación o regresión significativas con el 18:3n-3 presente en el alimento o entre 18:3n-3 en músculo con HUFA n-3 presentes en músculo. Solo se determinaron regresiones significativas entre 20:5n-3, 22:5n-3 y 22:6n-3 de músculo. Hay que señalar que no se detectaron los intermediarios 18:4n-3 y 20:4n-3, los cuales se relacionan con la síntesis de HUFA n-3 a partir de 18:3n-3. Esto no se asociaría con una inhibición de la insaturasa Δ6, ya que esta enzima muestra una mayor afinidad de sustrato por PUFA n-3 con relación a los ácidos grasos n-9 ó n-6 (Greene y Seliovonchick,

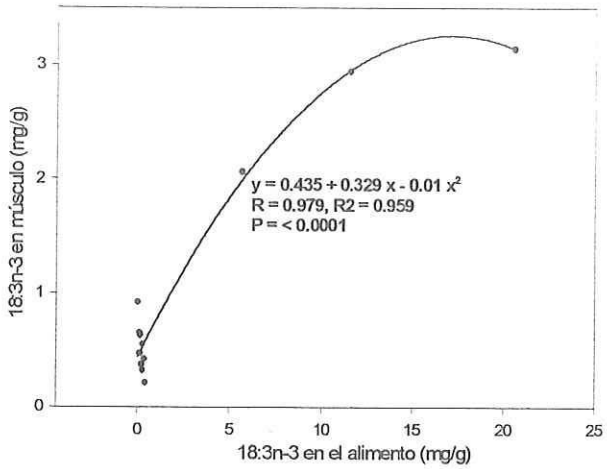

Figura 6 Regresión cuadrática entre 18:3n-3 presente en alimentos balanceados y 18:3n-3 de músculo de juveniles de *H. fulgens* después de 75 días de alimentación.

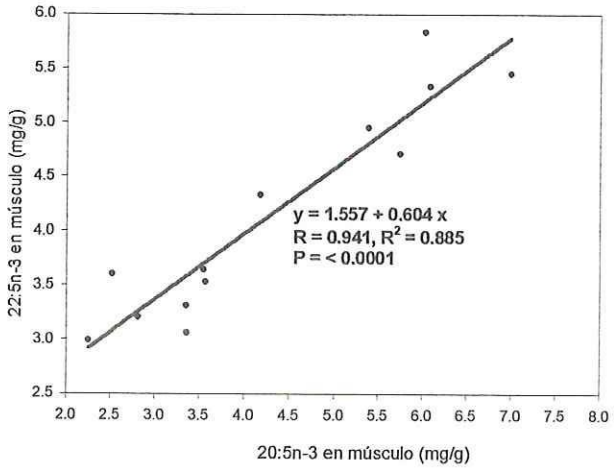

Figura 7. Regresión lineal entre 20:5n-3 y 22:5n-3 presentes en músculo de juveniles de *H. fulgens* después de 75 días de alimentación balanceada .

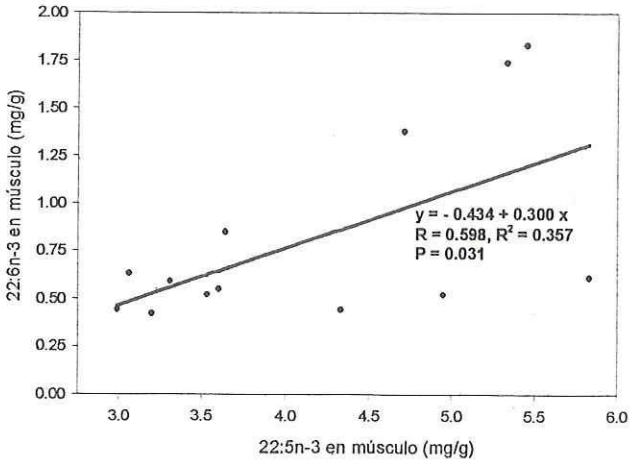

Figura 8. Regresión lineal entre 22:5n-3 y 22:6n-3 presentes en músculo de juveniles de *H. fulgens* después de 75 días de

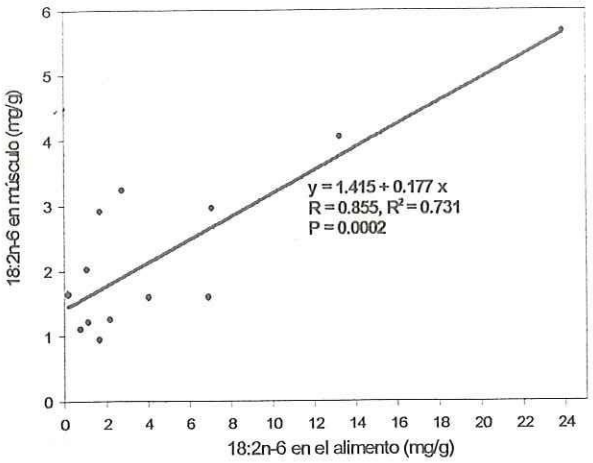

Figura 9. Regresión lineal entre 18:2n-6 presente en alimentos balanceados y 18:2n-6 de músculo de juveniles de *H. fulgens* después de 75 días de alimentación.

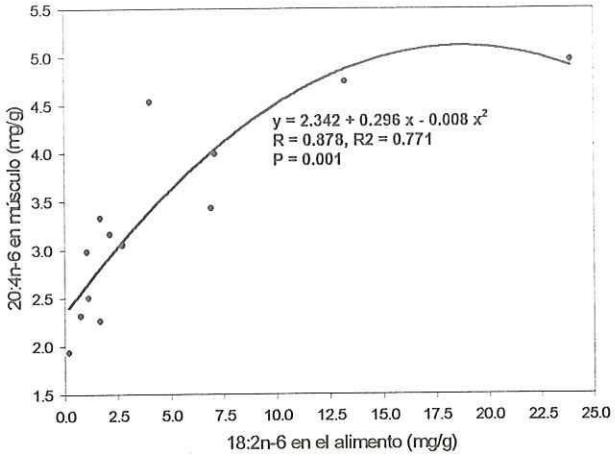

Figura 10. Regresión cuadrática entre 18:2n-6 presente en alimentos balanceados y 20:4n-6 de músculo de juveniles de *Haliotis fulgens* después de 75 días de alimentación.

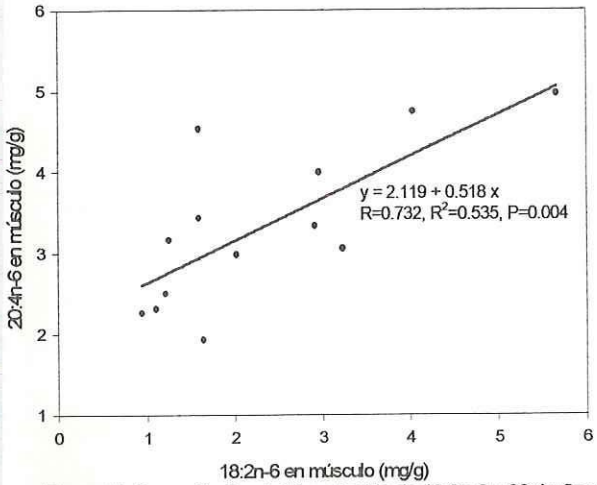

Figura 11. Regresión lineal del contenido de 18:2n-6 y 20:4n-6 en músculo de juveniles de *H. fulgens* sometidos a alimentación balanceada durante 75 días.

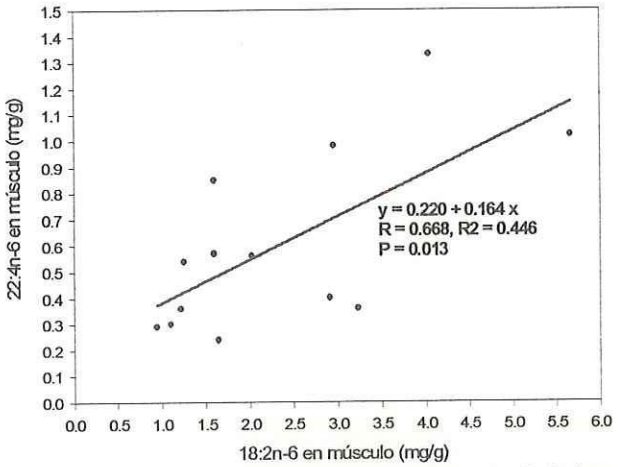

Figura 12. Regresión lineal del contenido de 18:2n-6 y 22:4n-6 en músculo de juveniles de *H. fulgens* sometidos a alimentación balanceada durante 75 días.

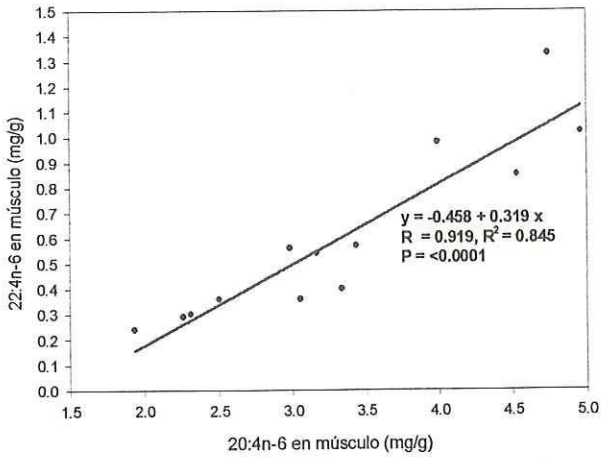

Figura 13. Regresión lineal del contenido de 20:4n-6 y 22:4n-6 en músculo de juveniles de *H. fulgens* sometidos a alimentación balanceada durante 75 días.

1987). Si se considera que el mecanismo de elongación presenta una baja especificidad de sustrato (Mourente y Tocher, 1994; Cook, 1996) y se detectó elongación en la síntesis de HUFA n-6, se intuye que la incapacidad para detectar 18:4n-3 y 20:4n-3 podría estar relacionada con su rápida conversión a un homólogo superior.

Con el objetivo de evaluar las características de la depositación y metabolismo de ácidos grasos a través de inanición y alimentación restringida en lípidos se desarrolló el **TERCER EXPERIMENTO**, el cual se realizó mediante dos bioensayos. En el **primer bioensayo** (**ARTÍCULOS 3**) se realizó un ensayo con alimentación balanceada con dos niveles de lípidos, bajo (0.14% lípidos totales) y alto (1.5% aceite de maíz + 3.5% tripalmitina), durante 50 días e inanición hasta los 90 días. Los resultados no mostraron una reducción en el contenido de los lípidos musculares a los 50 días de alimentación restringida en lípidos o 70 días de inanición, lo cual indica que no son una fuente primaria de energía. Se reporta que los abulones adultos o juveniles presentan la capacidad de sobrevivir por varias semanas en inanición o con una alimentación muy limitada, atribuyéndose esta capacidad a que utilizan primero sus reservas de glucógeno y lípidos y por último las proteínas corporales (Segawa, 1991; Takami *et al.*, 1995). Bajo las condiciones experimentales de este 1^{r} bioensayo, la disminución de los lípidos totales en músculo de *H. fulgens* se presentó después de 70 días de inanición, encontrándose a los 90 días una reducción del 30.8% con relación al inicio del ensayo.

En los abulones bajo inanición no se detectó la presencia de PUFA n-3, sin embargo la relación n-3/n-6 mostró una tendencia a incrementarse en relación al tiempo de inanición, esto se asoció con la conservación de los HUFA n-3 y a la disminución en los PUFA n-6. En *H. fulgens* sometido a inanición se reporta la tendencia a reducirse en músculo los niveles de 18:3n-3 y 18:2n-6 (Nelson *et al.*, 2002). Así mismo, a los 90 días de inanición se observó una disminución en los contenidos totales de ácidos grasos saturados, monoinsaturados y PUFA, en tanto que los HUFA n-3 y n-6 se conservaron. Los HUFA participan en la conformación de la membrana celular a través de los fosfolípidos, con una gran importancia en su integridad estructural y fluidez, por lo cual los HUFA tienden a ser conservados preferentemente sobre otros tipos de ácidos grasos, especialmente en condiciones de falta de alimento e inanición (Navarro y Gutiérrez, 1995).

Los abulones sometidos a alimentación restringida en lípidos mostraron en el perfil de ácidos grasos de músculo un claro efecto de la dieta. Los contenidos de PUFA n-6, HUFA n-3 y n-6, así como la relación n-3/n-6 disminuyeron con relación al inicio del bioensayo, esto denotó una alimentación deficiente en ácidos grasos. Así mismo, la alta acumulación de 18:1n-9 en músculo sugiere una deficiencia de ácidos grasos esenciales en la dieta (Innis, 1991; Lim *et al.*, 1997).

En el **segundo bioensayo** del **TERCER EXPERIMENTO** (**ARTÍCULO 4**) se estudió el efecto de inanición y alimentación restringida en lípidos sobre los contenidos de ácidos grasos en lípidos polares y neutros en el músculo de *H. fulgens*, y en lípidos totales del contenido digestivo. En el bioensayo se evaluaron tres tratamientos experimentales: alimentación balanceada con dos niveles de lípidos, bajo (**LL**, 0.12% lípidos totales) y alto (**HL**, 1.5% aceite de maíz + 1.5% tripalmitina), e inanición por un período de 60 días, tiempo en el que no podrá ser evaluado el crecimiento ya que éste había sido evaluado en el **ARTÍCULO 2**, por lo que los resultados de crecimiento en peso y longitud no mostraron diferencias significativas ($P>0.05$) entre los abulones sometidos a alimentación mientras que los organismos en inanición presentaron un 19% de pérdida en peso. La ganancia en peso en *H. fulgens* alimentado con la dieta **LL** y **HL** fue de 23% y 29%, respectivamente, estos valores podrían considerarse bajos para un ensayo de crecimiento con dietas balanceadas (Viana *et al.*, 1993; Serviere-Zaragoza *et al.*, 2001), sin embargo, el objetivo principal del 2º bioensayo fue el evaluar el efecto de inanición y de alimentación con bajo suministro de lípidos, sobre los perfiles de ácidos grasos en los organismos con relación al tratamiento experimental, en los cuales se encontraron diferencias significativas en músculo y contenido digestivo.

Los tratamientos de inanición y alimentación mostraron un claro efecto en la composición de ácidos grasos en el músculo de *H. fulgens.* Los abulones en inanición aun cuando presentaron una pérdida del 19% en peso no se vieron

afectados en el contenido de lípidos totales, así mismo, presentaron una alta capacidad de conservación en los ácidos grasos de lípidos polares, con un incremento significativo ($P<0.05$) en los HUFA 20:5n-3 y 22:6n-3 con relación al inicio del ensayo. Esto muestra que a los 60 días de inanición *H. fulgens* no utilizó a los lípidos como fuente de energía, y sugiere que como estrategia bioquímica para favorecer la integridad estructural en la membrana celular los lípidos polares son preferentemente conservados, en especial los HUFA n-3, ya que juegan un papel importante a nivel funcional (Zabelinskii *et al.*, 1995). En los abulones sometidos a alimentación los perfiles de ácidos grasos en lípidos polares también presentaron la tendencia a conservarse, lo cual se manifestó al no encontrarse diferencias significativas importantes en los perfiles al inicio y final del experimento.

Abulones en inanición mostraron en lípidos neutros un perfil de ácidos grasos con una alta conservación con relación al inicio del bioensayo, lo cual se atribuiría a la disminución del metabolismo en condiciones de inanición (Segawa, 1991). En contraste, los abulones sometidos a alimentación balanceada mostraron en músculo una apreciable reducción en el contenido de los ácidos grasos presentes en lípidos neutros con relación al inicio del bioensayo, lo cual es atribuible a los requerimientos bioquímicos y de energía asociados con el crecimiento.

El contenido de lípidos totales y ácidos grasos en el contenido digestivo en abulones alimentados fueron mayores que los presentes en los alimentos **HL** y **LL**. Estos incrementos se podrían asociar con una baja digestibilidad de los lípidos (Uki y Watanabe, 1992), sin embargo la posible contribución de material endógeno y actividad microbiana en el sistema digestivo podrían estar también relacionados con esta tendencia (Merchen, 1988). En los abulones sometidos a alimentación los perfiles de los ácidos grasos de los contenidos digestivos no mostraron una relación definida con respecto a los alimentos correspondientes; ni el perfil de los organismos en inanición con relación al del inicio del bioensayo. Para comprender mejor el metabolismo de los lípidos en *H. fulgens* se requiere el estudiar el proceso digestivo y los factores que lo determinan, lo cual está en ciernes.

5. CONCLUSIONES GENERALES

a) *H. fulgens* alimentado con dietas balanceadas con 1.5% de aceite de oliva, maíz, linaza o hígado de bacalao presenta tasas de crecimiento mayores con relación a dietas con un contenido de 5% del respectivo aceite.

b) El consumo de 2, 0.2, 0.2 y 0.2 μg/g de peso vivo/día de 18:2n-6, 18:3n-3, 20:4n-6 y 22:6n-3 durante 75 días no limita el crecimiento de juveniles de *H. fulgens*.

c) *H. fulgens* presenta la capacidad para sintetizar HUFA n-3 y n-6 a partir de precursores PUFA C_{18} y de HUFA de menor longitud de cadena y grado de insaturación.

d) Los lípidos no son una fuente primaria de energía para el abulón azul, aún en condiciones de inanición .

e) *H. fulgens* en inanición disminuye en músculo los contenidos de PUFA C_{18} n-3 y n-6, en tanto que los HUFA n-3 y n-6 muestran una tendencia a conservarse.

f) Juveniles de *H. fulgens* sometidos a alimentación balanceada baja en lípidos y en ácidos grasos n-3 y n-6 durante 60 días muestran en lípidos neutros de músculo un menor contenido de ácidos grasos con relación a organismos mantenidos en inanición.

6. RECOMENDACIONES

Se sugiere el iniciar el estudio del metabolismo digestivo de lípidos del abulón azul a través de la caracterización de lipasas, tasas de depositación de lípidos en tejidos, evaluación de la digestibilidad de lípidos, efecto de la actividad microbiana en sistema digestivo y del aporte endógeno.

Para establecer en forma más precisa los intermediarios y productos asociados con la biosíntesis de ácidos grasos insaturados en *H. fulgens* se recomienda el uso de ácidos grasos n-3 y n-6 marcados, los cuales para evitar su oxidación podrían ser administrados directamente en músculo mediante inyección.

Con base en los hábitos de alimentación del abulón y la alteración que pueden presentar los ácidos grasos insaturados en un medio oxidante, el uso de PUFA y HUFA n-3 y n-6 de alta pureza (>95%) para evaluar requerimientos de ácidos grasos esenciales en abulón azul deberá de considerar la estabilidad de los ácidos grasos y su biodisponibilidad.

7. REFERENCIAS

Bautista-Teruel, M.N., Millamena, O.M., 2000. Diet development and evaluation for juvenile donke'ys ear abalone, *Haliotis asinina* Linn.: lipids levels. J. Shellfish Res. 19, 501-502.

Bell, J.G. 1998. Current aspects of lipid nutrition in fish farming. En: Black, K.D., Pickering, A.D. (Eds.), Biology of Farmed Fish. Sheffield Academic Press, Sheffield, pp. 114-145.

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218, 491-499.

Buzzi, M., Henderson, R.J., Sargent, J.R., 1996. The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus m*ykis*)* fed diets containing fish oil or olive oil. Biochim. Biophys. Acta 1299:235-244.

Caballero, M.J., Obach, Rosleund, G., Montero, D., Gisvold, M., Izquierdo, M.S., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, Oncorhynchus mykiss. Aquaculture 214, 253-271.

Carefoot, T.H., Quian, P.-Y., Taylor, B.E., West, T., Osborne, J.M., 1993. Effect of starvation on energy reserves and metabolism in the Northern abalone, *Haliotis kamtschatkana*. Aquaculture 118, 15-325.

Cook H.W., 1996. Fatty acid desaturation and chain elongation in eukaryotes. En: Vance, D.E., Vance J.E. (Eds.), Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier Science, Amsterdam, pp. 129-152.

Farkas, T., Fodor, E., Kitajka, K., Halver, J.E., 2001. Response of fish membrane to environmental temperature. Aquaculture Research 32, 645-655.

Fleming, A.E., Van Barneveld, R.J. , Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. Aquaculture 140: 5-63.

Floreto, E.A.T., Teshima, S.-i. And Koshio, S., 1996. The effect of seaweed diets on the lipid and fatty acids of the Japanese disc abalone *Haliotis discus hannai.* Fish. Science. 62(4): 582-588.

Glémet, H.C., Gerrits, M.F., Ballantyne, 1997. Membrane lipids of red muscle mitochondria from land-locked and sea-run Arctic char, *Salvelinus alpinus*. Marine Biology 129, 673-679.

González-Felix, M.L., Gatlin III, D.M., Lawrence, A.L., Perez-Velazquez, M., 2002. Effect of various dietary lipid levels on quantitative essential fatty acid requirements of juvenile Pacific white shrimp *Litopeneaus vannamei*. J. World Aquac. Soc. 33, 330-340.

Greene, D.H.S., Seliovonchick, D.P., 1987. Lipid metabolism in fish. Prog. Lipid Res. 26: 53-85.

Henderson, R.J., Tocher, D.R., 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26: 281-347.

Innis, S,.M., 1991. Essential fatty acids in growth and development. Prog. Lipid Res. 30, 39-103.

Lim C., Ako H., Brown C.L., Hahn K., 1997. Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. Aquaculture 151, 143-153.

Mai, K., Mercer, J.P., Donlon, 1995. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. III. Response of abalone to various levels of dietary lipid. Aquaculture 134, 65-80.

Mai, K., Mercer, J.P., Donlon, J., 1996. Comparative studies on the nutrition of two species of abalone *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. V. The role of polyusaturated fatty acids of macroalgae in abalone nutrition. Aquaculture 139: 77-89.

McDonald, P, Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., 1995. Animal Nutrition, 5th ed. Addison Wesley Longman Ltd, UK, 283 pp.

Mourente, G., Tocher, D.R., 1994. In vivo metabolism of [1-^{14}C]linolenic acid (18:3(n-3) and [1-^{14}c]eicosapentaenoic acid in a marine fish: Time-course of the desaturation/elongation pathway. Biochem. Biophys. Acta 1212, 109-118.

Navarro I., Gutiérrez J., 1995. Fasting and starvation. In: Biochemistry and Molecular Biology of Fishes, Vol. 4. En: Hochachka, P.W., Mommsen, T.P. (Eds.). Elsevier Science, Amsterdam, pp. 393-434.

Nelson, D.L., Cox, M.M., 2000. Lehninger Principles of Biochemistry. 3rd ed. Worth Publishers, New York, 1024 pp.

Nelson, M.M., Leighton, D.L., Phleger, C.F., Nichols, D.P, 2002. Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgal diets. Comp. Biochem. Physiol. 131B, 695-712.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. En: Lahlou, B., Vitiello, P. (Eds.), Aquaculture; Fundamental and Applied Research. Coastal and Estuarine Studies 43. American Geophysical Union. Washington, DC, pp. 103-124.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. J. Appl. Ichthyol. 11: 183-198.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. En: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, 3rd ed. Elsevier, USA, pp. 181-257.

Segawa, S., 1991. Oxygen consumption and ammonia excretion by the abalone *Sulculus diversicolor aquatilis* in starved condition. Nippon Suisan Gakkaishi 57, 2001-2006.

Serviere-Zaragoza, E., Mazariegos-Villareal, A., Ponce-Días, G., Montes-Magallón, S., 2001. Growth of juvenile abalone, *Haliotis fulgens* Philippi, fed different diets. J. Shellfish Res. 20, 689-693.

Stryer, L., 1995. Biochemistry, 4th ed., W.H. Freeman, and Co., New York, 1064 pp.

Takami, H., Yamanaka, H., Nakano, H., 1995. Survival and physiological stress of juvenile disk abalone *Haliotis discus discus* during long-term starvation. Fish. Sci. 61, 111-115.

Uki, S., Kemuyama, S., Watanabe, T., 1985. Development of semipurified test diets for abalone. Bull. Jpn. Soc. Scient. Fish. 51(11): 1825-1833.

Uki, N., Sugiura, M., Watanabe, T., 1986. Requirement of essential fatty acids in the abalone *Haliotis discus hannai*. Bull. Jpn. Soc. Scient. Fish. 52(6): 1013-1023.

Uki, N. and Watanabe, T., 1992. Review of nutritional requierements of abalone (*Haliotis* spp.) and development of more efficient artificial diets. En: Shepherd, S.A., Tegner, M.J., Guzmán-del Próo, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp. 504-517.

Viana, M.T., Lopez, L.M., Salas, A., 1993. Diet development for juvenile abalone, *Haliotis fulgens*. Evaluation of two artificial diets and macroalgae. Aquaculture 117: 149-156.

Watanabe, H., Yamanaka, H., Yamanaka, H., 1993. Changes in the content of extractive components in disk abalone fed with marine algae and starved. Nippon Suisan Gakkaishi 59, 2031-2036.

Watanabe, H., Yamanaka, H., Yamanaka, H., 1994. Daily variations of glycolitic metabolites in the muscle of juvenile disk abalone fed with marine algae and starved. Nippon Suisan Gakkaishi 60, 91-94.

Watanabe, T., Takeguchi, M., 1989. Implications of marine oils and lipids in aquaculture. In: Ackman, R.G. (Ed.), Marine Biogenic Lipids, Fats and Oils, Vol. 2. CRC Press Inc., Boca Raton, FL, pp. 457-479.

Yu, T.C., Sinhuber, R.O., 1976. Growth response of rainbow trout (*Salmo gairdneri*) to dietary ω 3 and ω 6 fatty acids. Aquaculture 8, 309-317.

Yu, T.C., Sinhuber, R.O., 1979. Effect of dietary ω 3 and ω 6 fatty acids on growth and feed conversion efficiency of coho salmon (*Oncorhynchus kisutch*). Aquaculture 16, 31-38.

Zabelinskii S.A., Brovtsyna, N.B., Chevotareva M.A., Gorbunova, O.B., Krivchenko A.I., 1995. Comparative investigation of lipid and fatty acid composition of fish gills and mammalian lungs. A model of the membrane lipid composition areas. Comp. Biochem. Physiol. 111B, 127-140.

8. ARTÍCULOS

Artículo 1: The effect of seaweed and balanced diets on growth and fatty acid incorporation in green abalone, Haliotis fulgens, under commercial culture conditions. **Enviado a *Ciencias Marinas*, enero 2003**.

Artículo 2: Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*). **Aceptado en Aquaculture, marzo 2003**.

Artículo 3: Effect of starvation and the absence of dietary lipid on the fatty acid composition of muscle tissue of the juvenile green abalone (*Haliotis fulgens*). **Enviado a Aquaculture Research, noviembre 2002.**

Artículo 4: Effect of starvation and dietary lipid on the fatty acid composition of muscle tissue and digestive content of the juvenile green abalone (*Haliotis fulgens*). **En preparación para su envío a Aquaculture**.

ARTÍCULO 1

"The effect of seaweed and balanced diets on growth and fatty acid incorporation in green abalone, Haliotis fulgens, under commercial culture conditions"[1]

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Short Title: The effect of diets on the fatty acid composition of abalone

[1] **Enviado a Ciencias Marinas, enero 2003.**

"The effect of seaweed (Macrosystis pyrifera) and balanced diets on growth and fatty acid incorporation in green abalone, Haliotis fulgens, under commercial culture conditions"

Abstract

The effect of a balanced diet (**BD**), seaweed (**SW**), and mixture of both (**BD+SW**) on growth, survival rate, and fatty acid content in the tissue from juvenile abalone Haliotis fulgens grown under commercial culture conditions were analyzed over a 329-day period. Survival and growth rate in length and weight was different among the diets investigated ($P<0.05$), being significantly higher for the mixed diet (**BD+SW**) followed by **SW** and **BD**. Even if feed intake could not been determined in this primary work, and therefore in can not be assume that growth was the result of diets more than feed ingested, the importance of this work was to show the significant impact on the fatty acid profiles shown in the muscle from the diets studied, suggesting that treatments contribute significantly on the chemical composition without being masked by the possible contribution of diatoms naturally grown under commercial conditions. The polyunsaturated fatty acid (PUFA) 22:4n-6 was not detected in any of diets, whereas the 20:4n-3 was only present in the **SW** diet and 22:5n-3 only in **BD** diet. However, after the feeding experiment all these PUFAs were present in abalone tissue. The possible synthesis of PUFAs from dietary n-3 and n-6 fatty acids of shorter hydrocarbon chain is discussed.

Keyords: Haliotis fulgens, commercial culture, balanced diet, Macrocystis pyrifera, fatty acids

"Efecto de la macroalga (Macrocystis pyrifera) y dietas blanceadas sobre el crecimiento e incorporación de ácidos grasos en el abulón azul Haliotis fulgens, bajo condiciones de cultivo comercial"

Resumen

El efecto de macroalgas (**SW**), una dieta balanceada (**BD**) y la mezcla de ambas (**SW+BD**) fue analizada con el crecimiento, tasa de sobrevivencia y contenido de ácidos grasos en el tejido de juveniles de abulón azul Haliotis fulgens, bajo condiciones comerciales durante un período de 329 días. La tasa de sobrevivencia y crecimiento mostró diferencias significativas entre los tratamientos estudiados ($P<0.05$), donde la mezcla fue significativamente mayor (**BD+SW**) seguida por **SW** y **BD**. Aún cuando la tasa de consumo no pudo ser determinada por tratarse de condiciones comerciales en este primer experimento, y por lo tanto no se puede asumir que el crecimiento sea el resultado del alimento consumido, la importancia en la contribución de este trabajo fue el observar que los tratamientos tuvieron un impacto sobre los perfiles de ácidos grasos contenidos en el tejido de los abulones, lo cual sugiere que los tratamientos contribuyeron significativamente sobre la composición química sin enmascarar la posible contribución de las diatomeas que crecen de manera natural bajo condiciones comerciales. El ácido graso poliinsaturado (PUFA) 22:4n-6 no fue detectado en ninguna de las dietas mientras que el 20:4n-3 estuvo presente solo en la **SW** y el 22:5n-3 en la **BD**. Sin embargo, al finalizar el experimento todos esos PUFAs estuvieron presentes en el tejido de abulón. Se discute la posible síntesis de los PUFAs a través de los ácidos grasos n-3 y n-6 de cadena más corta presentes en la dieta.

Palabras clave: Haliotis fulgens, cultivo comercial, dietas balanceadas, Macrocystis pyrifera, ácidos grasos.

Introduction

Abalone nutrition research has been done so far under strict experimental conditions. However, sometimes it is observed that new diets behave different under commercial conditions. This happen when aspects like temperature, light, abalone manipulation and the presence of natural food are not taken in consideration, resulting in better or poorer results (Viana et al., 1996). In fact, in several experimental reports has been shown that seaweed performed significantly poorer than balanced diets (Viana et al., 1993a; Alarcón, 2000), whereas under commercial conditions seaweed still in use (Fleming et al., 1996), due to their apparent performance since no data are available to support that seaweed is more nutritive than balanced diets. This has lead to question several aspects like the role that diatoms may play in the general abalone nutrition, by growing attached to the seaweeds and ponds, being available for abalone to grasp them to complete their nutritional requirements. Thus, any contribution to learn the role that seaweed plays under commercial scale will help to know their nutrition requirements and possible substitution.

The goal of any abalone producer the same as happen with most of the aquatic organisms and livestock it is to growth them under balanced diets to optimize their growth efficiency, unless their natural food is cheaper and available. Therefore, any balanced diet

should contain all necessary nutrients to meet their nutrition requirements, usually formulated with a variety of ingredients available in the market (Jobling, 2001a). Therefore the studies on nutrition are essential to know their needs in order to offer efficient diets to be able to sustain and predict the production.

Recently, several reports on abalone nutrition have resulted in the modification of diets in order to improve the feed conversion efficiency. In this way, protein level has change from 40 to 27% in the diet. Cellulose that earlier reports explained that abalone showed a negative growth effect with their inclusion (Uki and Watanabe, 1992) today it has been proved that can be efficiently used by the abalone (Monje and Viana, 1998; Erasmus et al., 1997). However, lipids may be the ingredients less studied, probably since they are recommended in no more that 5% in the diet, quantity that will be naturally incorporated when fish and soybean meals are used as protein sources. Then, the balanced of fatty acids will be depending on the type and level of protein source used. Nevertheless, it has been shown that abalone exhibit a higher growth when n-3 and n-6 polyunsaturated fatty acids (PUFAs) , such as 18:3n-3, 18:2n-6, 20:4n-6 and 20:5n-3 are present in the diet (Uki et al., 1986; Floreto et al., 1996; Mai et al., 1996).

Besides growth, tissue composition is an important factor to study since the overall performance of diets, including feed composition, feeding frequency and management will be manifested (Carter et al., 2001; Jobling, 2001b). Even under commercial conditions tissue composition will show the presence of natural foods besides the formulated diets.

Therefore, the aim of the present work is to evaluate the performance of a balanced diet compared to seaweed and the combination of these two, on the growth, survival and later on, the effect of fatty acid composition in the diets and tissue incorporation in the juvenile abalone Haliotis fulgens.

Materials and methods

Balanced diet (BD) preparation and Seaweed (SW) composition

The **BD** formulation was based on the constituents and total protein content as recommended by Viana et al. (1996) and Mai et al. (1995) (Table 1). Vitamin and mineral mixtures were those recommended by Hahn (1989). Silage was made as described by Viana et al. (1993b). The lipid content in the **BD** was the result of the corresponding lipid concentration present in the ingredients used. Butyl-hydroxy toluene was added to the formulation to prevent lipid oxidation. All ingredients were mixed with a blender (Robot Coupe® R10) until a homogeneous paste was obtained. The diet was then flattened into 2-mm thick sheets using a pasta maker (Rossito Bisanti®). Pieces of 2 x 2 cm were then cut and dried (45°C for 24h) and stored in plastic containers at room temperature until their use. Diet was made every second week.

Fresh seaweed, Macrocystis pyrifera, from coastal areas close to Ensenada (Ejido Erendira, Baja California, Mexico) was used as seaweed diet (**SW**). Prior to feeding, the seaweed was transported to the abalone farm and left in a large pond where it was washed thoroughly with seawater. Fresh seaweed samples were stored in sealed containers at –25°C to proximate and fatty acid analyses.

Experimental Procedure

The study was carried out in a commercial abalone farm (BC Abalone, S.A. de C.V., Ejido Erendira, Baja California, Mexico). Three month old H. fulgens with an average length of 5.9±0.06 mm and average weight of 24.2±1.5 mg were held in flow-through (20L min^{-1}) and aerated seawater. The water temperature varied between 13.1 and 21.1°C during winter and summer, respectively. The animals were confined in six 850L rectangular fiberglass tanks filled with open seawater flow to contain 300L in each tank (two replicates per treatment) with 2000 abalone per tank. Abalone were fed with **BD** in satiation, and the **SW** was given ad libitum once a week by introducing fresh fronds in sufficient amount to cover the pond surface. The mixed diet (**SW+BD**) was also given to satiation. The **BD** was offered for 12h overnight every night, and the remaining balanced diet was siphoned by the system or removed daily.

Abalone growth was evaluated as the total growth for the experimental period both in length and body weight. In the same way, the abalone growth rate was evaluated in μm day^{-1} and mg day^{-1}. Whole-body weight was measured with an electronic balanced (±0.001g) and shell length with an electronic digital caliper (±0.05mm) at 0, 141, 234 and 329 days. Percentage of abalone survival (%S) was calculated as follows:

$$\%S = (\text{final abalone number/original abalone number})\ 100 \qquad (1)$$

To prevent injury under abalone handling, $MgSO_4$ (4%) or 2-phenoxyethanol (1mL L^{-1}) were used as anesthetics (White et al., 1996). To evaluate the effect of diets on the chemical composition in the tissue, forty abalone per treatment were collected at the end of the experiment.

Proximate analysis

Diets and muscle tissue of abalone were analyzed. Dry weight was calculated after drying the sample to constant weight at 100°C. Total nitrogen was determined by the Kjeldhal method (AOAC, 1995), and crude protein was calculated (N x 6.25). Crude lipids were determined by extraction using chloroform-methanol-water (1:1:0.9, v/v) following the Bligh and Dyer extraction method (1959). Ash was determined gravimetrically heating the sample at 550°C for 18h.

Fatty Acid Analysis

Aliquots of the lipid extract were first refluxed during 3min in a 0.5M KOH solution in methanol prior methylation, achieved by refluxing again (3min) in 14% boron trifluoride in methanol (BF_3-MeOH) (Metcalfe et al., 1966). Fatty acid methyl esters (FAMEs) were analyzed in a Hewlett Packard 5890II gas chromatograph equipped with a flame ionization detector (260°C). FAMEs were separated with a capillary column (Omegawax 320 by Supelco Inc.; 30m x 0.32mm, film thickens 0.25μm) using hydrogen as carrier gas. The initial oven temperature was 140°C. Five minutes after sample injection (1μL) the temperature was increased at a rate of 4°C min^{-1} until 240°C was reached, temperature that was held for an additional 10 min. Fatty acids were identified by using

commercial standards, both 37 Component FAME Mix (Supelco Inc.; GLC 87 , Nu-Chek Prep) and marine oils (PUFA1 and PUFA3, Supelco Inc.), performed under similar conditions. An internal standard (23:0) was used to calculate the concentration for each fatty acid using the software package HP ChemStation rev. A.06 for Windows.

Statistical Analysis

Survival, growth rates and fatty acid profiles among treatments were analyzed using a one-way ANOVA, followed by a SNK multiple comparisons (Zar, 1999). For percentage values data was transformed to Arc sen. Statistical analysis was performed using the statistical package SAS 6.08 (Cary, NC, USA).

Results

The overall performance from the different diet treatments resulted in significant differences where the mixed diet (**BD+SW**) showed a higher growth rate in length and weight, followed by the **SW** diet and **BD** (Table 2). However survival rates were higher for the mixed diet followed by the **BD** treatment and the lowest for the **SW**.

Despite the differences in growth rate observed in abalone fed the experimental diets, the proximate composition of abalone muscle was quite similar (Table 2), being slightly higher lipids concentration for the mixed diet. Among the fatty acids profiles

differences were observed both in the diet composition as well as in the tissue after the experimental feeding period (Table 3).

In the present work diet treatment **BD** showed the highest content of 15:0, 16:0, 16:1n-7, 16:3n-6, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 18:3, 20:1n-9, 22:1n-9 and 22:6n-3. Whereas seaweed showed the highest content of 16:2n-6, 17:1n-7, 18:4n-3, 20:4n-6, and 20:5n-3. The abalone tissue from the **BD** treatment showed the highest content of 16:3n-6, and 18:2n-6 than that observed in abalone fed with the other diets. Additionally, the PUFAs 20:3n-6, 20:4n-3 and 22:4n-6 were present in the abalone muscle from the **BD** treatment whereas these fatty acids were not detected in the corresponding diet. Thus, the presence of 22:4n-6 in the abalone tissues where this fatty acid is absent in the diets with a significant correlation between levels of 22:4n-6 and 20:4n-6 ($r = 0.99$, $P = 9.3 \times 10^{-5}$). Moreover, **SW** treatment showed the highest content of 20:5n-3 and the lowest content of 18:2n-6. Likewise, 22:4n-6 and 22:5n-3 were present in abalone muscle from the **SW** treatment whereas this fatty acids were not detected in the seaweed. Abalone fed with mixed diet showed the highest content in 13 of the 32 fatty acid reported.

Discussion

Figures obtained for survival (Table 2) seems to be low, but not to far from figures found under commercial conditions at this organism size during 329 days (Preece and Mladenov, 1999; Neori et al., 1998). The **BD** diet resulted in the lower growth in length and weight, whereas the mixed diet performed better. In a similar work done under lab

scale with similar treatments and diet (Alarcón, 2000), different results were observed, where the mixed diet and **BD** diet performed equally well whereas **SW** resulted in the lowest growth with almost no survival after 45 days. Even if here the **BD** diet did not achieve a good growth, results showed a positive effect when present together with the seaweed. Moreover, the feed intake was not evaluated and therefore the differences in growth cannot be attributed to the diet quality itself rather than availability of feed plus environmental conditions (Jobling, 2001b). Unfortunately the estimation of feed intake under commercial scale was difficult where water is siphoned from the tanks under an open flow system, besides the 12 hours feed exposure in tanks that lead a high dry matter loss of pellets. Moreover, abalone has a negative phototaxis (Leighton, 2000) and as a result, when light is present, abalone gathers in groups concentrating in the darker areas of the tank. Here it was possible to observed that abalone from the **BD** treatment were hiding whereas in the presence of seaweed abalone where all over the tanks. Therefore it is suggested to do more studies to improve the commercial systems to contain balanced diets where feed will be available in equal way to all organisms in the tanks.

Among the fatty acids profiles differences were observed both in the diet composition as well as in the tissue after the experimental feeding period (Table 3). Even if it is unknown feed ingested, it is possible to suggest that abalone were feeding their corresponding treatments rather than grazing food naturally grown on seaweeds and ponds (diatoms), due to the differences in the fatty acid profiles obtained in tissue, otherwise, abalone would be incorporating the high amount of lipids found in diatoms (up to 20%), where more than 70% of the reported fatty acids were different among treatments.

The abalone tissue from the **BD** treatment showed the highest content of 16:3n-6, and 18:2n-6 than that observed in abalone fed with the other diets. Additionally, the PUFAs 20:3n-6, 20:4n-3 and 22:4n-6 were present in the abalone muscle from the **BD** treatment whereas these fatty acids were not detected in the corresponding diet. Thus, the presence of 22:4n-6 in the abalone tissues where this fatty acid is absent in the diets suggests that 22:4n-6 comes from the elongation of 20:4n-6. Moreover, **SW** treatment showed the highest content of 20:5n-3 and the lowest content of 18:2n-6. Likewise, 22:4n-6 and 22:5n-3 were present in abalone muscle from the **SW** treatment whereas this fatty acids were not detected in the seaweed. Abalone fed with mixed diet showed the highest content in 13 of the 32 fatty acid reported. The effect of dietary treatment on fatty acids content in abalone tissue is difficult to explain, because the PUFAs n-3 and n-6, required for normal growth and development of abalone (Floreto et al., 1996; Mai et al., 1996) are metabolized by the same enzyme systems of sequential desaturation and elongation that result in long chain of the n-3 and n-6 series (Bell, 1998). Therefore, in dietary studies it is important to consider the influence that one type of fatty acid can have on the metabolism of the other. However, fatty acid profiles lead us to suggest that H. fulgens can synthesize long-chain polyunsaturated fatty acids from short-chained fatty acids. Studies on H. discus hannai established that this specie is able to convert 18:2n-6 and 18:3n-3 into 22:4n-6 and 22:5n-3 (Uki et al., 1986). Moreover, it has been determined that H. laevigata and H. rubra are capable of producing C20 polyunsaturated fatty acids from C18 (Dunstan et al., 1996).

Thus, it is suggested to study the effect of specific lipids and fatty acids on growth and tissue deposition under strict control with no presence of natural food and measuring the feed intake under lab conditions where a strict control can be obtained. However, more

work is necessary to establish the importance of seaweeds under commercial conditions in order to understand their role in abalone production to improve their production system.

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Literature cited

Alarcón, A.B. (2000). Gracilaria pacifica fertilizada como alimento para abulones cultivados. Tesis para obtener el grado de Maestro en Ciencias de la Universidad Autónoma de Baja California. 63 pp.

AOAC (1995). Official Methods of Analysis of AOAC. 16th. Ed., vol. I, Arlington, Virginia.

Bell, J.G. (1998). Current aspects on lipid nutrition in fish farming. In: K.D. Black and A.D. Pickering (eds.), Biology of Farmed Fish. Sheffield Academic Press, Sheffield, pp. 114-1145.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 912-917.

Carter, C., Houlihan, D., Kiessling, A., Médale, F. and Jobling, M. (2001). Physiological Effects of Feeding. In: D. Houlihan, T. Boujard and M. Jobling (eds), Food intake in fish. Blackwell Science, Ltd, Oxford, pp. 297-331.

Dunstan, G.A., Baillie, H.J., Barrett, S.M. and Volkman, J.K. (1996). Effect of diet on the lipid composition of wild and cultured abalone. Aquaculture, 140: 115-127.

Erasmus, J.H., Cook, P.A. and Coyne, V.E. (1997). The role of bacteria in the digestion of seaweed by the abalone Haliotis midae. Aquaculture, 155:377-386.

Fleming, A.E., Van Barneveld, R.J., Hone, W.O. (1996). The development of artificial diets for abalone: A review and future directions. Aquaculture, 140:5-53.

Floreto, E.A.T., Teshima, S.-I. and Koshio, S. (1996). The effect of seaweed diets on the lipid and fatty acids of the Japanese disc abalone Haliotis discus hannai. Fish. Sci., 62(4): 582-588.

Hahn, K.O. (1989). Nutrition and growth of abalone. In: K.O. Hahn (ed.), CRC Handbook of Culture of Abalone and other Gastropods. CRC Press, Boca Raton, FL, pp. 135-156.

Jobling, M. (2001a). Feed Composition and Analysis. In: D. Houlihan, T. Boujard and M. Jobling (eds), Food Intake in Fish. Blackwell Science, Ltd, Oxford, pp. 1-24.

Jobling, M. (2001b). Nutrient Partitioning and the Influence of Feed Composition on Body Composition. In: D. Houlihan, T. Boujard and M. Jobling (eds), Food Intake in Fish. Blackwell Science, Ltd, Oxford, pp. 354-375.

Leighton, D.L. (2000). The Biology and Culture of the California Abalones. Dorrance Publ. Co., Inc., Pittsburgh, PA, pp.19-20, 199.

Mai, K., Mercer, J.P. and Donlon, J. 1995. Comparative studies on the nutrition of abalone, Haliotis tuberculata L. and Haliotis discus hannai Ino. IV. Optimum dietary protein level for growth. Aquaculture, 136: 165-180.

Mai, K., Mercer, J.P. and Donlon, J. (1996). Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata L. and Haliotis discus hannai Ino. V. The role of polyusaturated fatty acids of macroalgae in abalone nutrition. Aquaculture, 139: 77-89.

Metcalfe, L.D., Schmitz, A.A. and Pelka, J.R. (1966). Rapid preparation of fatty acids acid esters from lipids for gas chromatographic analysis. Anal. Chem., 38: 514-515.

Monje, H. and Viana, M.T. (1998). The effect of cellulose on the growth and cellulolytic activity of abalone Haliotis fulgens when used as a ingredient in formulated artificial diets. Journal of Shellfish Research, 17(3): 667-671.

Neori, A., Ragg, N.L.C. and Shpigel, M. (1998). The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Performance and

nitrogen partitioning within an abalone (Haliotis tuberculata) and macroalgae culture system. Aquacultural Engineering, 17: 215-239.

Preece, M.A. and Mladenov, P.V. (1999). Growth and mortality of the New Zealand abalone Haliotis iris Martyn 1784 cultured in offshore structures and fed artificial diets. Aquaculture Research, 30: 865-877.

Uki, N., Sugiura, M. and Watanabe, T. (1986). Requirement of essential fatty acids in the abalone Haliotis discus hannai. Bull. Jap. Soc. Sci. Fish., 52: 1013-1023.

Viana, M.T., López, L.M. and Salas, A. (1993a). Diet development for juvenile abalone, Haliotis fulgens, evaluation of two artificial diets and macroalgae. Aquaculture, 117:149-156.

Viana, M.T., Nava, C. and Solana-Sansores, R. (1993b). Acid fish silages. Effect of preheating and addition of phosphoric and citric acids on the biochemical quality. Ciencias Marinas, 19(4): 415-433.

Viana, M.T., López, L.M., García-Esquivel, Z. y Méndez, E. (1996). The use of silage made from fish and abalone viscera as an ingredient in abalone feed. Aquaculture, 140: 87-98.

White H.I., Hecht, T. and Potgieter, B. (1996). The effect of four anesthetics on Haliotis midae and their suitability for application in commercial abalone culture. Aquaculture, 140: 145-151.

Zar, J.H. (1999). Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ, pp. 195-202.

Table 1. Ingredient composition in percentage (dry basis) of the balanced diet and seaweed (Macrocystis pyrifera) used.

Ingredients (g/100g diet)	Balanced diet	Seaweed
Fishmeal[a]	30.00	
Corn starch	14.66	
Kelp meal[b]	10.00	
Corn flour[c]	10.00	
Gelatin (50 blooms)	10.00	
Soybean meal[d]	8.00	
Cellulose[e]	5.00	
Modified starch[f]	5.00	
Mineral mixture[g]	4.00	
Vitamin mixture[h]	1.50	
Fish silage[i]	1.40	
Stay-C[j]	0.20	
Choline chloride	0.10	
Sodium benzoate	0.10	
BHT[k]	0.04	
Composition (%)		
Crude protein	30.8±0.7	9.6±0.5
Ash	12.6±0.1	37.5±0.5
Total lipid	6.3±0.1	3.1±0.1

[a] 64% protein. [b] Made from Macrocystis pyrifera. [c] Corn flour (Maseca®). [d] 39% protein, 21% lipid. [e] α-cellulose (Alphacel). [f] Modified corn starch (Clearjel®). [g] ICN vitamin diet fortification. [h] ICN salt mixture #5 Briggs. [i] Acid fish silage from tuna viscera. [j] Ascorbyl polyphosphate (Roche). [k] Butylatedhydroxy toluene.

Table 2. Growth performance and survival of juvenile abalone Haliotis fulgens fed with a balanced diet (**BD**), seaweed (**SW**) and a mixture of both (**BD+SW**) under commercial conditions during 329 days and their proximate tissue composition. Different super indices letters indicate statistical differences between treatments.

	TREATMENTS		
	BD	**SW**	**BD+SW**
Initial Weight (mg)	26.25 ± 2.05^{a}	22.45 ± 1.05^{a}	23.90 ± 0.30^{a}
Final weight (mg)	984.50 ± 25.50^{b}	1057.00 ± 41.00^{b}	1915.50 ± 86.50^{a}
Total growth in weight (mg)	958.25	1034.55	1891.6
Initial shell length (mm)	6.13 ± 0.11^{a}	5.69 ± 0.09^{b}	5.89 ± 0.14^{ab}
Final length (mm)	20.69 ± 0.21^{c}	21.58 ± 0.23^{b}	26.21 ± 0.26^{a}
Total growth in length (mm)	14.56	15.89	20.32
Growth			
($\mu m\ day^{-1}$)	44.26 ± 0.65^{c}	48.31 ± 0.72^{b}	61.77 ± 0.78^{a}
($mg\ day^{-1}$)	2.91 ± 0.07^{b}	3.15 ± 0.13^{b}	5.75 ± 0.26^{a}
Survival (%)	48.88 ± 3.68^{b}	36.97 ± 5.08^{c}	53.70 ± 0.65^{a}
PROXIMATE TISSUE COMPOSITION %			
Crude Protein	76.3	75.5	76.7
Total lipids	4.7	4.9	5.4
Ash	8.2	6.9	7.2

Table 3. Fatty acid composition (mg fatty acid/g dry weight) of seaweed (SW), balanced diet (BD), and muscle of Haliotis fulgens fed during 329 days with the corresponding diets (SW, BD and SW + BD).

Fatty acid	Diets		Abalone muscle fed with		
	Balanced Diet (BD)	Seaweed (SW)	Balanced diet (BD)	Seaweed (SW)	Mixed diet (BD+SW)
14:0	3.31±0.01	3.33±0.03	1.40±0.00^{c}	1.43±0.01^{b}	1.56±0.01^{a}
15:0	0.31±0.01^{a}	0.06 ±0.00^{b}	0.23±0.00^{b}	0.28±0.01^{ab}	0.30±0.01^{a}
16:0	14.37±0.03^{a}	5.32±0.06^{b}	8.13±0.02^{c}	8.33±0.03^{b}	9.57±0.03^{a}
16:1n-9	nd	0.24 ±0.01	0.25±0.01^{b}	0.24±0.01^{b}	0.31±0.00^{a}
16:1n-7	3.22 ±0.01^{a}	0.22 ±0.01^{b}	0.35±0.00^{c}	0.37±0.00^{b}	0.40±0.01^{a}
16:2n-6	0.15±0.00^{b}	0.24 ±0.01^{a}	0.09±0.01	0.09±0.01	0.11±0.01
16:3n-6	0.46 ±0.0^{a}	0.34±0.00^{b}	2.68±0.01^{a}	2.13±0.02^{b}	1.12±0.01^{c}
17:0	0.65±0.01	nd	0.51±0.02^{c}	0.74±0.00^{b}	0.85±0.01^{a}
17:1n-7	0.29±0.01^{b}	0.40±0.00^{a}	0.27±0.01^{cb}	0.36 ±0.00^{a}	0.36±0.01^{a}
16:4n-1	0.08±0.01	nd	2.15±0.02^{c}	3.04±0.05^{b}	3.66±0.04^{a}
18:0	3.87±0.00^{a}	0.43±0.00^{b}	3.00±0.01^{c}	3.06±0.00^{b}	3.44±0.01^{a}
18:1n-9	11.06±0.03^{a}	4.95±0.03^{b}	2.50±0.03^{c}	2.67±0.03^{b}	2.96±0.03^{a}
18:1n-7	2.11±0.01	tr	2.95±0.04^{b}	3.14±0.04^{ab}	3.36±0.05^{a}
18:2n-6	14.22±0.04^{a}	1.78±0.01^{b}	2.95±0.01^{a}	1.89±0.01^{c}	2.34±0.00^{b}
18:3n-6	tr	0.23±0.00	nd	nd	nd
18:3n-3	1.41±0.01^{a}	0.97±0.01^{b}	0.67±0.00^{a}	0.60±0.00^{b}	0.67 ±0.01^{a}
18:3	0.57±0.01^{a}	0.46±0.00^{b}	0.96±0.00^{a}	0.75±0.02^{b}	0.95±0.00^{a}
18:4n-3	0.10±0.01^{b}	2.04±0.02^{a}	nd	nd	nd
18:4n-1	nd	0.05 ±0.00	0.17±0.00^{b}	0.17±0.01^{b}	0.20±0.01^{a}
20:0	nd	0.27±0.00	0.14±0.01^{b}	0.16±0.01^{b}	0.19±0.01^{a}
20:1n-11	0.12±0.00	tr	1.27±0.01^{b}	1.20±0.01^{c}	1.35±0.01^{a}
20:1n-9	1.00±0.01^{a}	0.10 ±0.00^{b}	0.30±0.02	0.30±0.01	0.32±0.00
20:1n-7	0.12±0.01	nd	0.14±0.02	0.11±0.01	0.12±0.01
20:3n-6	nd	0.23 ±0.00	0.24±0.01^{a}	0.16±0.01^{b}	0.25±0.01^{a}
20:4n-6	0.27 ±0.02^{b}	5.37±0.05^{a}	2.55±0.01^{c}	3.14±0.01^{b}	3.67±0.00^{a}
20:4n-3	nd	0.11±0.00	0.08±0.01	0.09±0.00.	0.09±0.01
20:5n-3	0.36±0.01^{b}	2.17±0.02^{a}	2.36±0.01^{c}	3.45±0.00^{a}	3.21±0.00^{b}
22:0	0.11±0.01	tr	0.24±0.03	0.20±0.02	0.27 ±0.03
22:1n-9	0.29±0.01^{a}	0.07±0.00^{b}	0.12 ±0.02	0.10±0.00	0.14±0.01
22:4n-6	nd	nd	0.32 ±0.00^{c}	0.65±0.00^{b}	0.71±0.01^{a}
22:5n-3	0.09±0.01	nd	3.22±0.01^{c}	4.23±0.01^{a}	4.59±0.01^{a}
22:6n-3	0.50±0.30^{a}	0.06±0.01^{b}	0.59±0.07	0.45±0.01	0.68±0.03

Mean ± standard error
nd: no detected
tr :trace (<0.05mg/g).

ARTÍCULO 2

Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*) [1].

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Abstract

Isocaloric formulated diets containing four different sources of triglycerides (olive, corn, linseed and cod liver oils) at three levels (1.5, 3.0 and 5.0%, total added dietary lipid = 5.0%) and a crude protein content of 37.5 to 39.9% were fed to juvenile green abalone (*Haliotis fulgens*). Growth and fatty acid composition of the muscle tissue were compared to that of a reference diet that contained no added lipids (0.25% total lipids). After 75 days of culture in a flow-through system, no significant differences in growth were found among oil types. Responses to different dietary levels of lipid were significantly different but not to sources of oils. Maximum growth was achieved at a 1.5% inclusion of oil sources. It appears that abalone

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have a great capacity to synthesize lipid from carbohydrate sources. Results also suggest that abalone are capable of desaturation and elongation of 18 C polyunsaturated fatty acids (PUFAs) of the n-3 and n-6 family to 20:5n-3 and 20:4n-6. Synthesis of 22:6n-3 from 20:5n-3 is not reflected in the results. The lack of a growth response to the different levels of HUFA provided by the different oils included in the experimental diets may be due to an insufficient duration of the experiment to achieve an essential fatty acid deficiency. The relationship between fatty acid profiles of tissue and the diets fed to the abalone suggest that metabolic activity of the gut microflora is not a source of essential fatty acids.

Keywords: Fatty acid requirement, abalone, nutrition, edible oil, growth.

1. Introduction

Lipids are regarded as the most important energy source in animal tissues, generally stored as triglycerides, in depot organs or adipose tissue. Lipid nutrition in mollusks remains largely undefined relative to dietary levels, as an energy source, and essential fatty acids.

The polyunsaturated fatty acids (PUFA) of the linoleic (n-6) and linolenic (n-3) families have been recognized as important nutrients for growth and reproduction in fish (Sargent et al., 1999; Izquierdo et al., 2000), crustaceans (Shiau, 1998; Sheen and Wu, 1999; Jeffs et al., 2002) and mollusks (Caers et al., 2000; Navarro and Villanueva, 2000; Nelson et al., 2002). All terrestrial and aquatic organisms are able to synthesize unsaturated fatty acids of the n-9 family *de novo* (Cook, 1996). However, fatty acids from n-3 and/or n-6 series are synthesized *de novo*

only by photosynthetic organisms and insects. Some aquatic species can elongate and desaturate dietary 18:2n-6 or 18:3n-3 to satisfy or partially contribute to their nutritional requirements for highly unsaturated fatty acids (HUFAs) like 20:4n-6, 20:5n-3 and 22:6n-3, and this biosynthetic ability varies from species to species (Sargent et al., 1995; Buzzi et al., 1996).

For some species of mollusks, the nutritional importance of 20:5n-3 and 22:6n-3 has generally been established (Caers et al., 2000; Navarro and Villanueva, 2000). However, for gastropods like abalone, studies devoted to essential fatty acid requirements are lacking (Hanna and Sinclair, 1996). Uki et al. (1986) reported that in a diet containing 5% lipids, a 1% level of n-3 PUFAs should be present as 20:5n-3 and 22:6n-3. Several investigations report total dietary lipid without including a fatty acid profile. In general, the lipid level of balanced diets developed for abalone ranges from 1.5 to 5% (Fleming et al., 1996) with a maximum growth achieved at levels between 3-5% (Uki and Watanabe, 1992; Mai et al., 1995). Mai et al., (1996) reported that 18:2n-6, 18:3n-3, 20:4n-6 and/or 20:5n-3 are important for growth. However, except for the investigation of Uki et al. (1986) no evaluation of a growth response relative to fatty acid content at a constant level of lipid has been conducted.

In the abalone, as well as in most of aquatic organisms, the function of both n-3 and n-6 PUFAs in the tissue appears to be primarily structural; therefore, their use as an energy source is limited. It has also been observed that adult invertebrates are not able to oxidize these PUFAs efficiently due to a lack of proper enzymes and transport proteins for the peroxisomal β-oxidation (Floreto et al., 1996).

As a preliminary qualitative determination of requirements of fatty acids at different levels, other lipids can be used as a filler to avoid interference with the experimental fatty acids under investigation. Palmitic acid (16:0) is the final product of fatty acid synthesis in animal tissues, and is the most abundant saturated fatty acid in plankton and fish. It is a biosynthetic precursor of long chain saturated fatty acids and the *de novo* synthesis of unsaturated n-9 fatty acids (Sargent, 1976; Holland, 1978). Tripalmitin is a triacylglycerol of palmitic acid (16:0), and is generally used as filler in balanced diets to study essential fatty acids requirements.

Aquaculture of the green abalone (*Haliotis fulgens*) is becoming an increasingly important enterprise in Mexico. Information about the FA requirements for growth and reproduction of this species is unavailable. Thus, the goal of the present work is to determine the effect of qualitative fatty acid differences in dietary lipid on growth and fatty acid composition of juvenile green abalone, *H. fulgens*.

2. Materials and methods

2.1 Diet preparation

A basal diet was used in the formulation of 11 diets that served as treatments (Table 1). Test diets contained 1.5, 3 or 5% of either olive oil (OL), corn oil (CO), linseed oil (LI) or cod liver oil (CL). Tripalmitin was added to achieve a total triacylglycerol level of 5%. A reference diet (RD) contained no lipid supplement, and starch was included as a replacement. Fish protein concentrate and chicken egg albumin were ingredients that served as principal

sources of dietary protein. Dietary vitamin and mineral mixtures were those recommended by Hahn (1989). Silage was produced as described by Viana et al. (1993) and the liquid fraction was separated by centrifugation. BHT and α-tocopherol were added to prevent oxidation of lipids. All ingredients were blended with 50% water until a completely homogeneous dough-like mixture was obtained. The diets were then rolled flat to a thickness of 2 mm and 10 mm x 5 mm pieces were cut and stored in sealed plastic bags at –25 °C until required.

2.2 Experimental conditions

Three hundred ninety juvenile green abalone *H. fulgens* with a mean shell length of 19.67±0.12 mm and a mean weight of 0.91±0.01 g were obtained from a commercial abalone farm, BC-Abalone in Ejido Eréndira B.C. The abalone were held in a flow-through tank system where filtered, aerated water of 34‰ salinity flowed at a rate of 300 ml min^{-1}. Temperature was maintained at 21.73±0.06°C throughout the feeding trials. Abalone were acclimated to laboratory conditions and fed a mixed diet composed of *M. pyrifera* and a balanced formulated diet used in commercial culture for 21 days prior to the initiation of the feeding experiments.

Each experimental unit consisted of a 3.8-l plastic container with 10 abalone. There were three replicates per treatment. The abalone were held under the same conditions described previously for the acclimation period and were fed the appropriate experimental diet (dry weight) at a rate of 2% of their wet weight daily during the evening. Any uneaten food was collected the following morning, dried and weighed. Feed intake (FI) was calculated as reported by López and Viana (1995) and the feed conversion efficiency (FCE) as stated by Uki and Watanabe (1992):

$$FI = [\, G\,(\, S/100\,)\,] - R \qquad (1)$$

where G represents the amount of feed offered, S is the amount of feed recovered from the control containers without abalone, and R is the uneaten feed remaining in the containers with the experimental abalone. Mean daily rate of feed intake for each treatment was then calculated and expressed as a percentage of body weight of the abalone.

$$\textbf{FCE = wet weight gain (g)/dry weight feed consumed (g)} \qquad (2)$$

The experiment was terminated after 75 days and growth of abalone was expressed as daily growth rate in length (μm/day) and weight (mg/day) and as percent weight gain as follows:

$$\textbf{\% Weight gain = [(final weight – initial weight)/initial weight] x 100} \qquad (3)$$

Whole-body weight was determined using an electronic balance (± 0.001 g) and shell length was measured with an electronic digital caliper (± 0.05 mm) at 0 and 75 days. Nine abalone per treatment were randomly selected to obtain muscle tissue for fatty acid analyses.

2.3 Chemical analysis

Proximate analysis of each experimental diet was conducted in triplicate. Percent dry weight of a sample of each diet was calculated after drying to constant weight at 100°C. Total nitrogen was determined by the Kjeldahl method (AOAC, 1995), and multiplied by 6.25 to estimate crude protein content. The gross energy content of each diet was determined by direct combustion in an adiabatic calorimeter Parr 1281. Total lipid in diets and muscle was determined by extraction using chloroform-methanol (2:1, v/v) following the extraction method of Folch et al. (1957). Ash content was determined by weight after heating the sample at 550°C for 18 h.

Aliquots of the lipid extracts from diets and muscle tissue of abalone were initially refluxed for 3 min in a 0.5M KOH solution in methanol and then followed by methylation of fatty acids through additional refluxing (3 min) in 14% borontrifluoride in methanol (BF_3-MeOH) (Metcalfe et al., 1966). Fatty acid methyl esters (FAMEs) were analyzed in a Hewlett Packard 5890II gas chromatograph equipped with a flame ionization detector (260°C). FAMEs were separated with a capillary column (Omegawax™ 320 by Supelco Inc.; 30 m x 0.32 mm, film thickness 0.25 μm) using hydrogen as the carrier gas. The initial oven temperature was 140°C. Five minutes after injection of the sample (1 μl), the temperature was increased to 240°C at a rate of 4°C/min. This temperature was maintained for an additional 10 min. Fatty acids were identified by comparison with the retention times of standards (37 Component FAME Mix, Supelco Inc.; GLC 87 , Nu-Chek Prep) and well-characterized profiles of samples of marine oils (PUFA1 and PUFA3, Supelco Inc.). The concentration for each fatty acid was calculated from the corresponding area in the chromatogram with a help of an internal standard (23:0) using the software package HP ChemStation rev. A.06 for Windows.

2.4 Statistical Analysis

To determine whether growth, expressed as final body weight (log transformed), shell length, daily feed intake, FCE and caloric intake (g/abalone) were significantly different among diets, a factorial vs reference analysis was conducted. The factorial was constructed of four triglyceride sources and three levels (1.5, 3 and 5) and a reference diet. The effect of level was analyzed using orthogonal contrasts to estimate the regression equation and to determine the optimum level. Data expressed as a percentage were arcsin square root transformed for

analysis. A correlation analysis was used to identify whether a possible relationship existed between dietary oil and level vs growth. All the statistical analyses were performed using SAS-GLM procedures (SAS 8.2, 2001).

3. Results

The ingredient and proximate composition of the experimental diets containing three levels of olive oil, corn oil, linseed oil, cod liver oil and a reference diet are presented in Table 1. The dietary crude protein content among diets ranged from 37.5 to 39.9%. Total lipid analyses indicated that diets formulated to contain 5% lipid actually contained levels ranging from 4.9 to 5.2%. For the reference diet without lipid supplementation, the level of lipid originating from those ingredients common to all diets was 0.25%. Gross energy values of the experimental diets ranged from 4.61 to 4.77 kcal/g. The reference diet contained the lowest level of gross energy (4.39 kcal/g), reflecting the lack of a lipid addition.

The growth response of juvenile abalone fed the experimental diets for the 75-day feeding trial is presented in Table 2. No significant differences in growth were observed between oil types; however, significant differences in growth ($P<0.01$) were observed among oil levels. The polynomial regression shows that all diets that contain an oil level 1.5% are associated with significant increases in growth measured by either length ($y = 71.79 + 2.19x-1.50x^2$, $R^2 = 0.4505$, $P<0.01$) or weight ($y = 9.85 + 0.36x-0.179x^2$, $R^2 = 0.4505$, $P<0.01$).

Moreover, no significant differences ($P>0.05$) in feed intake and energy intake were found among *H. fulgens* fed the experimental diets. Significant differences ($P<0.01$) in FCE

were observed among oil levels, and a polynomial regression indicated that a maximum FCE was achieved at a 1.5% level of dietary oil ($y = 0.6958 + 0.147x-0.369x^2$, $R^2 = 0.4505$, $P<0.01$).

The fatty acid compositions of the experimental and reference diets are presented in Table 3. Levels of the principal dietary unsaturated fatty acids in the diets reflect the supplemented quantity and fatty acid composition of the oil source. The fatty acid composition of the reference diet suggested a vegetable origin. The fatty acid content of total lipid of *H. fulgens* (Table 4) was influenced by the fatty acid composition of the diets. The muscle of abalone fed diets containing olive oil had high levels of 18:1n-9 whereas abalone fed diets containing corn oil or linseed oil, had high levels of either 18:2n-6, or 18:3n-3, respectively. The contents of these fatty acids were appreciably lower in abalone fed the other diets. The muscle tissue content of 22:6n-3 was high for abalone fed cod liver oil. The abalone fed diets containing olive oil resulted in levels of 18:1n-9 and 20:1n-9 that were higher than those observed in the other treatments. The muscle of *H. fulgens* fed diets containing corn oil rich in 18:2n-6 contained levels of 18:2n-6, 20:2n-6, 20:4n-6 and 22:4n-6 that were higher than those of abalone in the other treatments. Abalone fed the reference diet had high levels of 18:2n-6 that exceeded those fed diets containing linseed or cod liver. Highly unsaturated fatty acids of the n-3 and n-6 families were present in the muscle tissue of abalone fed diets that did not contain these fatty acids. The lipid levels within the muscle tissue were consistent among abalone representing the different dietary treatments, from 52.9 to 66.7 mg/g.

4. Discussion

The experiment lasted for 75 days, presumably sufficient time to demonstrate any differences in response to dietary treatment. The growth increment in weight of 169.0% exceeds that recommended by D'Abramo and Castell (1997). One of the interesting results of this experiment was the growth retardation associated with diets supplemented with 5% oil without any corresponding reduction in feed consumption. Either the absence of tripalmitin or a correspondingly high level of oil resulted in comparatively less growth. However, as previously reported some type of muscle like rat heart as well as chondrichthian lacks the capacity to utilize FFA. As a result, anaerobic metabolism using carbohydrates and ketonic bodies becomes more important, and FFA inhibits the utilization of carbohydrates (Moyes and West, 1995). A similar condition may exist in abalone tissue. Moreover, Watanabe and Takeuchi (1989) showed that a high concentration of essential FA (18:3n-3) and a mixture of 20:5n-3 and 22:6n-3, which is four times higher that that required by the trout, resulted in poor growth. Therefore, it is suggested that in the present work, the FA present in the oil level at 5% were too high and may have contributed to the poor growth response.

Mai et al. (1995) observed maximum weight gain of *H. discus hannai* at dietary lipid levels of 3-7% whereas the response of *H. tuberculata* was better at 3% lipid content. Those results are similar to the present work considering that P:E ratio was not similar for all diets in that experiment (from 54 to 62). However, the amount of food consumed by abalone is apparently based upon satisfaction of an energy requirement, with an optimum P:E ratio of 100 (mg protein:cal present) (Gomez-Montes et al., in press). Therefore, previously observed differences in growth could be due to differences in feed intake rather than levels of oil. As stated previously, the lack of differences in feed intake in the present study suggest that differences in growth are the result of differences in nutrient utilization.

Even without a source of dietary lipid, the level of lipid in the muscle tissue of abalone in the reference diet remained similar to that of abalone fed the experimental diets containing supplemented lipid. This observation suggests that the muscle tissue lipid of the abalone fed the reference diet was synthesized from carbohydrate.

However, the fatty acid profile of the constituent lipid does change and is reflective of the dietary lipid. The 18:1n-9 and 20:1n-9 accumulation can be associated to the high content of 18:1n-9 in the diets containing olive oil with their capacity to elongate to 20:ln-9 and to incorporate those fatty acids into the muscle. Moreover, no desaturation products derived from the 18:1n-9, such as the 18:2n-9 or 20:2n-9 were detected, probably due to competition for further unsaturation of 18-carbon unsaturated fatty acids that is known to be in the order of n-3 > n-6 > n-9 (Henderson and Tocher, 1987). The accumulation of 18:1n-9 and 20:1n-9 has been suggested to be an indicator of essential fatty acids deficiency (Deering et al., 1997). However, since no differences in growth were observed among the different oils used in the present work, the accumulation is probably the result of a concentration effect of 18:1n-9 in the diet more than a deficiency of essential fatty acids. Amounts of 20:4n-6, 20:5n-3, and 22:6n-3 in the muscle tissue of the abalone fed the reference diet without lipid suggest that these fatty acids are preferentially conserved.

Abalone fed diets containing either 18:2n-6 or 18:3n-3 had greater amounts of 20:5n-3 and 20:4n-6 than those of abalone fed the reference diet, suggesting that the abalone have at least a limited capacity to synthesize 20:4n-6 from 18:2n-6 and 20:5n-3 from 18:3n-3. Similar results have been reported in *H. laevigata* fed with a diet balanced to a high level of 18:2n-6

(Dunstan et al., 1996) and in a study of *H. discus hannai* where the capacity to synthesize 20:4n-6 and 22:4n-6 from 18:2n-6 was suggested (Uki et al., 1986). Even if the elongation from 18:2n-6 to a higher homologous fatty acid is reduced due to the competition with n-3 fatty acids for the Δ6 and Δ5 desaturases (Sargent, et al., 1995), the higher 18:2n-6 content in diets still contributes to the accumulation of n-6 HUFAs in the abalone muscle. Based upon examination of the levels of 22:6n-3 in the muscle tissue of abalone fed the reference diet versus those of abalone fed the experimental diets without a direct source of 22:6n-3, there is no evidence of synthesis of 22:6n-3 from 20:5n-3.

Abalone fed diets containing linseed oil showed a direct effect on the 18:3n-3 content in the muscle related to their content in the diet, similar to that observed by Uki et al. (1986) and Floreto et al. (1996). However, the increase in dietary 18:3n-3 did not yield a corresponding increase in growth. The synthesis of 20:5n-3 from 18:3n-3 creates competition for desaturation and elongation between fatty acids of the n-3 and n-6 families (Cook, 1996). However, it is known that 18:3n-3 is a better substrate than 18:2n-6 to desaturate, whereas the conversion from 18:3n-3 to a longer chain fatty acid is influenced by the dietary n-3 to n-6 ratio (Sargent et al., 1995). In the present study, this condition is evidenced by the high content of 20:5n-3 and 22:5n-3 in the muscle of abalone fed diets containing linseed oil.

The muscle tissue of abalone fed diets containing cod liver oil had higher levels of 20:5n-3 and 22:6n-3, reflecting the higher levels of these fatty acids in cod liver oil. Marine oils, rich in 20:5n-3 and 22:6n-3 are regarded as a better growth promoters than vegetable oils in species of shrimp like *Peneaus japonicus*, *P. monodon* and *P. vannamei* (Deering et al., 1997; Lim et al., 1997), as well as marine fish (Watanabe and Takeguchi, 1989; Hertrampf and

Piedad-Pascual, 2000); however, in the present work, the growth enhancing effect of these fatty acids could not be observed. In fish, the relationship between dietary n-3 and n-6 fatty acids has been observed to influence growth (Sargent et al., 1993), whereas no reports are available for abalone. No evidence of this ratio effect was found due to the lack of differences in growth rates with different dietary fatty acid profiles at a particular dietary level.

Abalone can efficiently digest lipids to a certain level with the 5% level considered optimum (Maguire et al., 1993; Wee et al., 1994). However, in the present work, growth achieved with diets with less oil content in combination with tripalmitin to reach a 5% total lipid was better than that achieved with the addition of an oil exclusively. Growth of abalone fed the reference diet with 0.25% oil was similar to that achieved with diets containing 1.5 and 3.0 % oil level, but less than that of the diets containing 5.0 % oil. The comparable growth achieved with the reference diet containing no supplemented lipid suggests that essential fatty acid deficiencies had yet to be manifested. Levels of 20:5n-3 and 20:4n-6 in the muscle tissue of abalone fed the reference diet were either lower or the same level as those of abalone that did not have sources of either 18:2n-6 or 18:3n-3.

The results of this research suggest that abalone have a capacity to desaturate and elongate 18:2n-6 and 18:3n-3 into 20:4n-6 and 20:5n-3 respectively, and store them. Whether this synthesis is sufficient to satisfy the essential fatty acid requirements is unknown. Without a direct source or precursor for 20:5n-3 and 20:4n-6, levels of these fatty acids declined in the composition of the lipid of the muscle tissue of abalone fed the reference diet without lipid. However, an adverse effect on growth had yet to be detected after 75 days. The ability to synthesize 22:6n-3 from 20:5n-3 is not reflected in the levels of 22:6n-3 in the muscle tissue.

For all experimental diets except those that directly provided 22:6n-3, the levels of 22:6n-3 in the muscle tissue are essentially equivalent to that of abalone fed the reference diet. The source of the HUFA in the muscle tissue could be attributed to metabolic activity of the gut flora rather than to the abalone's ability to synthesize. The results of several investigations have ascribed an important digestive function activities to the gut microflora (Harris, 1993; Erasmus et al., 1997; Bisset, 1998; Enriquez et al., 2001). However, the contribution of gut microflora is not suspected because bacteria are not known for containing PUFA and HUFA, and PUFA and HUFA levels in the muscle tissue closely reflect the quality and quantity available through the different oils added to the experimental diets. Given the results of this study, it appears that abalone at the initiation of the experiment must have had very high levels of essential fatty acids such that adverse effects on growth could not be observed within the confines of the duration of the experiment. Any future attempt to determine qualitative and quantitative essential fatty acid requirements of green abalone will require that experimental organisms be placed on a lipid free diet to reduce levels of essential fatty acids as much as possible before initiating feeding of experimental diets. This type of experiment will also allow the determination of whether the rate of synthesis of HUFAs from PUFAs is sufficient to satisfy requirements or a direct source of HUFAs is also needed.

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References

AOAC, 1995. Official Methods of Analysis of AOAC. 16th. edn., vol. 1. Association of Official Analytical Chemists, Arlington, VA, USA.

Bissett, A., Burke, C., Dunstan, G.A., Maguirre, G.B., 1998. Bacterial colonization of a formulated abalone diet during extended immersion. J. Shellfish. Res. 17, 995-1002.

Buzzi, M., Henderson, R.J., Sargent, J.R., 1996. The desaturation and elongation of linolenic acid and eicosapentaenoic acid by hepatocytes and liver microsomes from rainbow trout (*Oncorhynchus mykis)* fed diets containing fish oil or olive oil. Biochim. Biophys. Acta 1299, 235-244.

Caers, M., Coutteau, P., Sorgeloos, P., 2000. Incorporation of different fatty acids, supplied as emulsions or liposomes, in the polar and neutral lipids of *Crassostrea gigas* spat. Aquaculture 186, 157-171.

Cook, H.W., 1996. Fatty acid desaturation and chain elongation in eukaryotes. In: Vance D.E., Vance J.E. (Eds.), Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier Science, Amsterdam, pp. 129-152.

D'Abramo, L.R., Castell, J.D., 1997. Research methodology. In: D'Abramo, L.R., Conkin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. Advances in World Aquaculture. World Aquaculture Society, Baton Rouge, LA, pp. 3-25.

Deering, M.J., Fielder, D.R., Hewitt, D.R., 1997. Growth and fatty acid composition of juvenile leader prawns, *Peneaus monodon*, fed different lipids. Aquaculture 151, 131-141.

Dunstan, G.A., Baillie, H.J., Barrett, S.M., Volkman, J.K., 1996. Effect of diet on the lipid composition of wild and cultured abalone. Aquaculture 140, 115-127.

Enríquez, A., Viana, M.T., Vásquez, C., Shimada, A., 2001. Digestion of cellulose by stomach homogenates of green abalone (*Haliotis fulgens*). J. Shellfish Res. 20, 297-300.

Erasmus, J.H., Cook, P.A., Coyne, V.E., 1997. The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. Aquaculture 155, 377-386.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., 1996. The development of artificial diets for abalone: a review and future directions. Aquaculture 140, 5-63.

Floreto, E.A.T., Teshima, S., Koshio, S., 1996. The effects of seaweed diets on the lipid and fatty acids of the Japanese disc abalone *Haliotis discus hannai*. Fisheries Sci. 62, 582-588.

Folch, J., Lee, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 22, 477-509.

Gómez-Montes, L., García-Esquivel, Z., D'Abramo, L.R.D., Shimada, A., Vásquez-Peláez, C., Viana, M.T., 2003. Effect of dietary protein-energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*. Aquaculture, in press.

Hahn, K.O., 1989. Nutrition and growth of abalone. In: Hahn, K.O. (Ed.), CRC Handbook of Culture of Abalone and other Gastropods. CRC Press, Boca Raton, FL, pp. 135-156.

Hanna, P.J., Sinclair, A.J., 1996. Artificial food for abalone aquaculture: A choice of lipids? Austasia Aquacult. 10(4), 50-52.

Harris, J., 1993. The presence, nature and role of gut microflora in aquatic invertebrates: a synthesis. Microb. Ecol. 25, 195-231.

Henderson, R.J., Tocher, D.R., 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26, 281-347.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingrediets for Aquaculture Feeds. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 281-290.

Holland, D.L., 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: Malins, D.C., Sargent, R. (Eds.), Biochemical & Biophysical Perspectives in Marine Biology, Vol. 4. Academic Press, London, pp. 85-123.

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernández-Cruz, C.M., 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiol. Biochem. 22, 97-107.

Jeffs, A.G., Phleger, C.H., Nelson, M.M., Mooney, B.D., Nichols, P.D., 2002. Marked depletion of polar lipid and non-essential fatty acids following settlement by post-larvae of the spiny lobster *Jasus verreauxi*. Comp. Biochem. Physiol. 131A, 305-311.

Lim, C., Ako, H., Brown, C.L., Hahn, K., 1997. Growth response and fatty acid composition of juvenile *Peneaus vannamei* fed different sources of dietary lipid. Aquaculture 151, 143-153.

Lopez, L.M., Viana, M.T., 1995. Determination of the quality of food elaborated from unheated and heated fish silages for abalone juveniles of *Haliotis fulgens*. Ciencias Marinas 21, 331-342.

Maguirre, G.B., Wee, K.L., Hindrum, S.M., 1993. Digestibility studies. The "ins" and "outs" of abalone guts Austasia Aquacult. 7(1), 42-49.

Mai, K., Mercer, J.P., Donlon, 1995. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. III. Response of abalone to various levels of dietary lipid. Aquaculture 134, 65-80.

Mai, K., Mercer, J.P., Donlon, 1996. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. V. The role of polyunsaturated fatty acids of macroalgae in abalone nutrition. Aquaculture 139, 77-89.

Metcalfe, L.D., Schmitz, A.A., Pelka, J.R., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38, 514-515.

Moyes, C.D., West, T.G., 1995. Exercise metabolism of fish. In : Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes, Vol. 4, Metabolic Biochemistry. Elsevier Science B.V., Amsterdam, pp. 367-392.

Navarro, J.C., Villanueva, R., 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. Aquaculture 183, 161-177.

Nelson, M.M., Leighton, D.L., Phleger, C.F., Nichols, D.P, 2002. Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgal diets. Comp. Biochem. Physiol. 131B, 695-712.

Sargent, J.R., 1976. The structure, metabolism and function of lipids in marine organisms. In: Malins, D.C., Sargent, R. (Eds.), Biochemical & Biophysical Perspectives in Marine Biology, Vol. 3. Academic Press, London, pp. 149-212.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. In: Lahlou, B., Vitiello, P. (Eds.), Aquaculture; Fundamental and Applied Research. Coastal and Estuarine Studies 43. American Geophysical Union. Washington, DC, pp. 103-124.

Sargent, J.R., Bell, J.G., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. J. Appl. Ichtyol. 11, 183-198.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191-199.

Sheen, S.-S., Wu, S.W., 1999. The effects of dietary lipid levels on the growth response of juvenile mud crab. Aquaculture 175, 143-153.

Shiau, S.-Y., 1998. Nutrient requirements of penaeid shrimps. Aquaculture 164, 77-93.

Uki, N., Watanabe, T., 1992. Review of the nutritional requirements of abalone *Haliotis* spp and development of more efficient diets. In: Sheperd, S.A., Tegner, M.J., Guzmán del

Proó, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp. 504-517.

Uki, N., Sugiura, M., Watanabe, T., 1986. Requirement of essential fatty acids in the abalone *Haliotis discus hannai*. Bull. Jpn. Soc. Sci. Fish. 52, 1013-1023.

Viana, M.T., Nava, C., Solana-Sansores, R., 1993. Acid fish silages. Effect of preheating and addition of phosphoric and citric acids on the biochemical quality. Ciencias Marinas 19, 415-433.

Watanabe, T., Takeguchi, M., 1989. Implications of marine oils and lipids in aquaculture. In: Ackman, R.G. (Ed.), Marine Biogenic Lipids, Fats and Oils, Vol. 2. CRC Press Inc., Boca Raton, FL, pp. 457-479.

Wee, K.L., Maguirre, G.B., Hindrum, S., 1994. Methodology for digestibility studies with abalone.2. Comparison of markers in an artificial diet for blacklip abalone (*Haliotis rubra*) and greenlip abalone (*H. laevigata*). In: Chou, L.M., Munro, A.D., Lam, T.J., Chen, T.W., Cheong, L.K.K., Ding, J.K., Hooi, K.K., Khoo, H.W., Ophang, V.P.E., Shim, K.F., Tan, C.H. (Eds.), The Third Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines, pp. 152-55.

Table 1. Ingredient and chemical composition of diets used to feed green juveniles abalone with different oil types and levels and a reference diet (RD) with no lipids added.

	TREATMENTS												
	OL5	OL3	OL1.5	CO5	CO3	CO1.5	LI5	LI3.0	LI1.5	CL5.0	CL3.0	CL1.5	RD[2]
Composition	%	%	%	%	%	%	%	%	%	%	%	%	%
Common ingredients[1]	95	95	95	95	95	95	95	95	95	95	95	95	100
Tripalmitin	-	2.0	3.5	-	2.0	3.5	-	2.0	3.5	-	2.0	3.5	-
Olive oil	5.0	3.0	1.5	-	-	-	-	-	-	-	-	-	-
Corn oil	-	-	-	5.0	3.0	1.5	-	-	-	-	-	-	-
Linseed oil	-	-	-	-	-	-	5.0	3.0	1.5	-	-	-	-
Cod liver oil	-	-	-	-	-	-	-	-	-	5.0	3.0	1.5	-
Proximate composition %													
Crude protein	38.30	38.08	38.71	38.08	38.08	38.91	38.38	37.77	39.19	37.45	38.53	39.87	37.74
Total lipid	5.22	5.10	5.17	5.15	4.87	5.00	5.23	5.04	5.00	4.91	5.02	5.03	0.25
Ash	5.39	5.62	5.19	5.80	5.58	5.33	5.69	5.33	5.98	4.75	5.66	5.83	5.27
Gross energy[3]	4.725	4.766	4.765	4.754	4.631	4.629	4.677	4.633	4.607	4.684	4.664	4.708	4.388
Ratio protein/energy[4]	81.06	79.90	81.24	80.10	82.23	84.06	82.06	81.52	85.07	79.95	82.81	84.69	86.01

[1]Common ingredients; Corn starch, 24.08; Modified corn starch (Clearjel®), 5.0; Fish protein concentrate (97% protein), 18.0; Chicken egg albumin, 18.0; Soybean defatted flour (ICN), 10.0; Alphacel cellulose, 8.0; Gelatin (50 bloom), 5.0; Mineral mixture (ICN salt mixture #5 Briggs), 4.0; Vitamins mixture (ICN diet fortification), 1.5; Acid fish silage from tuna viscera (liquid phase), 1.0; Stay-C (Ascorbyl polyphosphate, Roche), 0.2; Choline chloride 0.10; Sodium benzoate, 0.10; Butylatedhydroxytoluene (BHT), 0.01; α-tocopherol, 0.01.

[2]For the Reference Diet (RD) 5% corn starch was added additionally instead of lipids to adjust a 100% composition

[3] kcal/g dry weight

[4] mg crude protein/kcal gross energy/g dry weight

Table 2. Biological indices of juvenile green abalone measured before and after feeding the different treatments with various oil types and levels and a reference diet.

	TREATMENTS												
	OL5	OL3	OL1.5	CO5	CO3	CO1.5	LI5	LI3.0	LI1.5	CL5.0	CL3.0	CL1.5	RD
Initial weight (mg)	550.3±0.9	576.3±10.6	550.3±0.3	557.3±4.8	546.0±5.7	540.7±12.6	565.3±5.9	565.7±6.0	558.3±6.9	560.3±6.4	575.4±11.5	567.5±10.7	553.3±9.4
Final weight (mg)	1054.4±18.3	1195.2±24.6	1332.7±61.4	1109.3±29.9	1236.6±90.5	1459.3±71.7	1129.9±22.8	1248.4±43.1	1308.3±34.5	1136.0±50.5	1182.3±4.3	1334.4±102.2	1216±126.8
Growth rate (mg/day)	6.73±0.38^{b}	8.20±0.33^{ab}	10.30±0.82^{ab}	7.46±0.40^{b}	9.15±1.21^{ab}	12.26±0.95^{a}	7.47±0.31^{b}	9.04±0.58^{ab}	9.98±0.46^{ab}	7.68 ± 0.67^{b}	8.03±0.06^{a}	10.19±1.36^{ab}	8.88±1.69
Weight gain (%)	91.61±5.25^{b}	107.38±4.27^{ab}	139.13±11.02^{ab}	101.58±5.43^{b}	126.49±16.58^{ab}	169.90±13.26^{a}	99.88 ±4.03^{b}	120.68±7.62^{ab}	134.34±6.18^{ab}	102.77±13.26^{b}	105.48±0.75^{a}	135.13±18.00^{ab}	119.77±22.91
Initial length (mm)	16.72±0.09	16.91±0.13	16.77±0.15	16.60±0.06	16.67±0.07	16.56±0.11	16.82±0.03	16.77±0.26	16.91±0.12	16.77±01.0	16.83±0.23	16.89±0.05	16.67±0.16
Final length (mm)	20.39±0.10	21.40±0.07	22.27±0.32	20.85±0.26	21.70±0.60	22.97±0.36	21.16±0.12	21.70±0.24	22.38±0.26	20.93±0.34	22.06±0.21	22.14±0.50	21.74±0.77
Growth rate (μm/day)	48.95±1.39^{b}	59.79±0.97^{b}	73.26±4.23^{ab}	56.60±3.55^{b}	67.11±8.06^{ab}	85.51±4.73^{a}	57.81±1.62^{b}	65.76±3.21^{ab}	73.09±3.49^{ab}	55.46±4.52^{b}	69.74±2.79^{ab}	69.94±6.68^{ab}	67.66±10.27
Feed intake (% w wt)[1]	1.06±0.07	1.07±0.07	1.16±0.14	1.15±0.07	1.14±0.07	1.24±0.07	1.03±0.07	1.16±0.07	1.08±0.07	1.20±0.07	1.19±0.09	1.15±0.07	1.11±0.07
FCE	0.62±0.07	0.82±0.07	0.93±0.07	0.83±0.07	0.93±0.07	1.10±0.07	0.82±0.07	0.91±0.07	1.01±0.07	0.74±0.07	0.87±0.09	1.13±0.07	0.94±0.07
Energy intake (cal/g w wt)	55.00±3.03	50.99±3.72	55.29±3.13	54.83±3.30	52.83±3.66	57.60±3.84	48.34±2.84	53.72±2.98	49.76±3.53	56.03±3.15	55.45±5.50	56.06±7.92	48.66±3.05

[1] Mean ± SE.

[2] Wet weight

MMeans in the same row with the different superscript are significantly different ($P < 0.05$).

Table 3. Fatty acid content (mg fatty acid/g dry weight) in total lipid of diets containing olive oil (OL), corn oil (CO), linseed oil (LI), cod liver oil (CL) in three levels (1.5, 3.0, 5.0%) and a reference diet with no oil added (RD).

Fatty acid	OL5	OL3	OL1.5	CO5	CO3	CO1.5	LI5	LI3.0	LI1.5	CL5.0	CL3.0	CL1.5	RD
14:0	0.03	0.23	0.38	0.06	0.23	0.36	0.06	0.26	0.37	2.45	1.65	1.10	0.02
14:1n-9	0.17	0.15	0.16	0.18	0.17	0.04	0.18	0.17	0.17	0.27	0.24	0.24	0.06
15:0 aiso	0.35	0.30	0.33	0.34	0.34	0.35	0.39	0.33	0.34	0.35	0.39	0.34	0.13
15:0	-	-	-	-	-	-	-	-	-	0.08	0.09	0.07	-
16:0	6.03	19.57	31.14	5.30	19.05	30.47	3.09	19.33	30.47	6.37	21.59	32.25	0.25
16:1n-7	0.40	0.24	0.18	0.08	0.06	0.02	0.09	0.06	0.05	4.17	2.49	1.27	0.02
16:2n-4	-	-	-	-	-	-	-	-	-	0.28	0.16	0.09	-
17:0	0.85	0.78	0.85	0.80	0.80	0.80	0.95	0.85	0.85	0.90	0.72	0.58	0.16
16:3n-4	-	-	-	-	-	-	-	-	-	0.24	0.14	0.07	-
17:1n-7	0.06	0.05	-	-	-	-	-	-	-	0.14	0.09	0.06	0.03
16:4n-1	-	-	-	-	-	-	-	-	-	0.37	0.21	0.12	-
18:0	2.81	2.63	2.46	2.51	2.30	2.28	3.70	3.08	2.55	1.87	2.01	2.09	0.14
18:1n-9	32.66	18.88	9.82	11.40	6.48	3.43	9.98	5.78	2.89	7.49	4.51	2.36	0.08
18:1n-7	1.34	0.77	0.41	0.56	0.39	0.18	0.63	0.38	0.17	1.55	0.91	0.53	0.01
18:1n-5	0.09	tr	-	0.15	0.14	0.07	0.15	0.09	0.05	0.10	0.07	0.02	0.01
18:2n-6	2.75	1.68	1.05	23.88	13.23	7.09	6.92	4.04	2.15	1.65	1.12	0.75	0.19
18:2n-4	-	-	-	-	-	-	-	-	-	0.10	-	-	-
18:3n-6	-	-	-	-	-	-	-	-	-	0.08	-	-	-
18:3n-4	-	-	-	-	-	-	-	-	-	0.08	-	-	-
18:3n-3	0.28	0.17	0.11	0.38	0.23	0.14	20.56	11.54	5.65	0.44	0.28	0.16	0.02
18:4n-3	0.13	0.13	0.13	0.15	0.13	0.13	0.17	0.12	0.10	0.88	0.58	0.34	0.04
18:4n-1	-	-	-	-	-	-	-	-	-	0.12	0.04	-	-
20:0	0.17	0.11	0.06	0.19	0.12	0.07	0.08	0.05	-	0.43	0.24	0.12	-
20:1n-11	-	-	-	-	-	-	-	-	-	0.36	0.20	0.11	-
20:1n-9	0.13	0.08	0.03	0.10	0.06	0.02	0.08	0.07	-	2.11	1.23	0.62	-
20:1n-7	-	-	-	-	-	-	-	-	-	0.13	0.08	0.02	-
20:2n-6	0.09	0.08	0.10	0.11	0.08	0.05	0.09	0.09	0.08	0.15	0.09	0.12	0.03
20:4n-6	0.11	0.10	0.12	0.10	0.10	0.11	0.09	0.11	0.11	0.35	0.23	0.15	0.03
20:3n-3	0.02	0.03	-	0.05	0.04	0.02	0.07	0.08	0.05	-	-	-	tr
20:4n-3	-	-	-	-	-	-	-	-	-	0.33	0.19	0.12	-
20:5n-3	-	-	-	-	-	-	-	-	-	3.92	2.39	1.22	0.01
22:0	-	0.03	0.06	0.06	0.06	0.06	0.06	0.07	0.06	0.35	0.19	0.12	-
22:1n-11	-	-	-	-	-	-	-	-	-	1.17	0.69	0.34	-
22:1n-9	0.07	0.07	0.07	0.09	0.08	0.08	0.1 0	0.08	0.08	0.31	0.22	0.12	0.03
21:5n-3	-	-	-	-	-	-	-	-	-	0.21	0.12	0.06	-
22:5n-6	-	-	-	-	-	-	-	-	-	0.08	-	-	-
22:5n-3	-	-	-	-	-	-	-	-	-	1.12	0.61	0.35	-
22:6n-3	0.31	0.21	0.16	0.07	0.06	0.01	0.11	0.09	0.07	3.68	2.32	1.18	0.02
Total lipid mg/g	52.15	50.97	51.71	51.49	48.65	49.97	52.31	50.44	50.00	49.09	50.25	50.35	2.46

tr : trace (< 0.01 mg/g); - : not detected

Table 4. Fatty acid content (mg fatty acid/g dry weight) in total lipid of muscle of *Haliotis fulgens* after fed for 75 days experimental diets containing olive oil (OL), corn oil (CO), linseed oil (LI), cod liver oil (CL) with three levels (5.0, 3.0 and 1.5 %) and a reference diet with no oil added (RD).

Fatty acid	Diet												
	OL5	OL3	OL1.5	CO5	CO3	CO1.5	LI5	LI3.0	LI1.5	CL5.0	CL3.0	CL1.5	RD
14:0	0.84	1.63	1.89	1.05	1.39	1.88	1.50	2.38	2.12	1.96	2.13	2.02	2.22
15:0	0.40	0.46	0.48	0.42	0.39	0.41	0.55	0.64	0.54	0.46	0.45	0.44	0.37
16:0	9.61	10.95	9.02	10.15	10.49	9.02	10.39	12.20	10.12	10.41	9.88	9.26	7.88
16:1n-7	0.56	0.61	0.54	0.38	0.41	0.38	0.49	0.36	0.44	0.48	0.47	0.36	0.39
16:3n-6	0.32	0.36	0.34	0.36	0.34	0.35	0.38	0.46	0.46	0.40	0.37	0.39	0.40
17:0	0.37	0.42	0.35	0.45	0.39	0.38	0.53	0.56	0.50	0.45	0.43	0.44	0.36
17:1n7	-	-	-	-	-	-	-	-	-	-	-	0.25	0.28
16:4	5.62	5.27	3.80	6.22	5.46	4.83	6.94	7.52	6.27	5.77	5.18	5.20	4.23
18:0	3.27	3.65	2.89	3.75	3.39	3.18	4.10	4.45	3.85	3.58	3.56	3.41	3.18
18:1n-9	6.69	6.84	3.86	3.21	3.14	2.61	3.18	3.69	2.88	3.60	3.12	2.57	2.17
18:1n-7	2.95	3.37	3.36	2.18	2.42	2.93	2.48	3.38	3.40	4.31	4.46	4.18	4.68
18:1n-5	-	-	-	-	-	-	-	-	-	0.08	0.12	0.17	0.41
18:2n-6	3.23	2.91	2.02	5.66	4.04	2.96	1.59	1.59	1.25	0.94	1.21	1.10	1.64
18:2n-4	-	-	-	-	-	-	-	-	-	0.18	0.16	0.10	-
18:3n-3	0.55	0.63	0.65	0.42	0.37	0.47	3.14	2.94	2.06	0.21	0.32	0.47	0.92
20:0	-	-	-	-	-	-	-	-	-	0.10	0.12	0.12	-
20:1n-11	2.19	2.55	2.17	2.41	2.25	2.24	2.71	3.21	2.83	3.24	3.21	3.17	2.93
20:1n-9	1.12	1.02	0.54	0.65	0.64	0.37	0.71	0.51	0.32	0.62	0.54	0.20	0.16
20:1n-7	-	-	-	0.39	0.32	0.25	-	-	-	0.17	0.38	0.13	0.29
20:2n-6	0.32	0.37	0.25	1.47	1.22	0.63	0.72	0.53	0.31	0.14	0.19	0.14	-
20:3n-6	0.56	0.67	0.47	0.84	0.60	0.54	0.67	0.61	0.54	0.20	0.34	0.42	0.73
20:4n-6	3.05	3.33	2.98	4.96	4.74	3.99	3.43	4.53	3.16	2.26	2.50	2.31	1.93
20:5n-3	3.55	3.57	3.35	2.52	2.25	2.81	4.17	6.02	5.38	6.99	6.08	5.74	3.36
22:2	6.48	6.54	5.61	4.45	4.15	3.36	5.58	5.91	4.96	4.68	4.71	4.24	3.27
21:5n-3	0.65	0.64	0.44	2.10	1.72	1.49	1.37	1.43	1.19	0.33	0.62	0.63	0.62
22:4n-6	0.36	0.40	0.56	1.02	1.33	0.98	0.57	0.85	0.54	0.29	0.36	0.30	0.24
22:5n-6	-	-	-	-	-	-	0.40	0.43	0.40	0.08	0.08	0.08	0.12
22:5n-3	3.64	3.53	3.31	3.60	2.99	3.20	4.33	5.83	4.95	5.45	5.33	4.71	3.06
22:6n-3	0.85	0.52	0.59	0.55	0.44	0.42	0.44	0.61	0.52	1.83	1.74	1.38	0.63
Total lipid mg/g	60.73	65.60	64.22	62.27	57.68	52.85	63.79	66.65	61.79	65.60	64.01	58.72	53.78

- : not detected

ARTÍCULO 3

Effect of starvation and the absence of dietary lipid on the fatty acid composition of muscle tissue of the juvenile green abalone (*Haliotis fulgens*)[1]

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Short title: Effect of balanced diets and starvation on the fatty acid profile of green abalones

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Lipids play an important role in abalone nutrition, particularly the polyunsaturated n-3 and n-6 fatty acids (Uki, Sigiura & Watanabe, 1986; Dunstan, Baillie, Barrett & Volkman 1996; Bautista-Teurel, Millamena & Fermin, 2001). Recent observations in our lab suggest that abalone synthesize lipid from carbohydrate and have some ability to biosynthesize 20:5 n-3 and 20:4 n-6 from 18:3 n-3 and 18:2 n-6. A capacity to synthesize lipid from carbohydrate allows the abalone to be subject to restricted feeding or long starvation periods without loss of their fat body reserves, (Carefoot, Quian, Taylor, West & Osborne 1993; Takami, Yamakawa & Nakano 1995). A recent study conducted by us and several other studies conducted with different abalone species (*H. discus hannai*, *Haliotis laevigata*, *H. rubra* and *H. fulgens*) have shown that the quality and quantity of dietary lipid affect growth rate and fatty acid composition of muscle (Uki *et al.*, 1986; Dunstan *et al.*, 1996; Floreto, Teshima & Koshio 1996; Nelson, Leighton, Phleger & Nichols 2002).

H. fulgens has been the focus of several nutritional studies mainly with the objective of gaining information to promote its culture. Nevertheless, the information about green abalone's lipid requirements and their association with parameters such as growth rate, reproduction, and metabolism is still limited. In the present work, the fatty acid profile of muscle tissue of green abalone, *H. fulgens,* was evaluated under conditions of starvation, and feeding either a diet without lipid (0.14% total lipids, diet 1), or a diet containing 1.5% corn oil and 3.5% tripalmitin (4.95% total lipid content, diet 2). For 30 days, 57 juvenile *H. fulgens* (2.98 ± 0.03 g ; 28.02 ± 0.22 mm) obtained from a commercial farm BC Abalone (Erendira BC, Mexico) were held in a flow-through, sand filtered water system (300 ml min^{-1}) at constant temperature (21.73 ± 0.06 °C) and fed a standard, nutritionally balance diet. Thereafter, the three dietary treatments began. Each treatment was conducted in triplicate in 3.8 L plastic containers with 6 abalone per container (54 total abalone). An additional three experimental abalone were sacrificed at the start of the experiment for lipid analysis. The experimental diets (Table 1) were prepared as described previously by Viana *et al.* (1999), but the feed was cut and stored at –25 °C in sealed plastic bags until fed. After 50 days, muscle samples from three abalone from all treatments were collected for lipid extraction and fatty acid analysis individually processed. Also, at 70 and 90 days, additional muscle samples were obtained from the starvation dietary treatment. Lipid in

diets and abalone muscle was extracted according to the method of Folch, Lees & Stanley (1957). Analysis of fatty acid methyl esters (FAMEs) was performed according to Christie (1982) using a Hewlett Packard 6890II gas chromatograph equipped with a flame ionization detector with a capillary column (OmegawaxÔ 320 by Supelco Inc.; 30 m x 0.32 mm, film thickens 0.25 mm). Fatty acids were identified by comparison with standards from marine oils (PUFA1 and PUFA3, Supelco Inc.) FAME mixtures. The respective concentration of each identified fatty acid was determined using an internal standard (19:0) and the software package HP ChemStation rev. A.06 for windows.

The total lipid content and fatty acid composition of diets are presented in Table 2. The standard diet served as a good source of n-3 and n-6 fatty acids to fulfill the abalone requirements (Uki et al. 1986). The fatty acid composition of each of the experimental diets is presented in Table 2. The levels of both n-3 and n-6 fatty acids in diet 1 were lower than those in diet 2. However, the n-3/n-6 ratio of diet 1 (0.30) was higher than that of diet 2 (0.02) due to the higher content of 18:2n-6 originating from the corn oil ingredient.

At the beginning of the experiment, the principal fatty acids of the muscle tissue were 16:0, 18:0, 16:2n-6, 16:4n-1, 22:2, 20:5n-3, and 22:5n-3, combined with a high n-3 HUFA content (Table 3). At the end of the experiment, the muscle tissue of abalone fed the diet without added lipids showed increases in the levels of both 18:1n-9 and 16:3n-6 (Table 3). The concentration of most of the other fatty acids decreased, particularly the n-3 and n-6 HUFA and n-6 PUFA. The observed accumulation of 18:1n-9 in muscle may be an indicator of essential fatty acid deficiency, probably due to the lack of biosynthesis to polienoic forms. A similar condition has been reported for *Peneaus vanammei* (Lim, Ako, Brown & Hahn, 1997). Moreover, the presence of fatty acids from n-3 and n-6 families reduces the activity of the 9 desaturase (Cook, 1996), thereby not permitting the accumulation of 18:1n-9. However, in the present work, muscle tissue of abalone fed the diet with a low lipid content contained a higher level of 18:1n-9 compared to the level present at the initiation of the experiment. This observation may be the result of high variation within samples and more research is needed for this explanation to be confirmed. In fact, the fatty acid profiles of the muscle tissue of abalones starved for 50 and 70 days were quite similar to those of fed abalone except for n-3 PUFA. This observation suggests

that under the culture conditions of this experiment, lipids are not used as an energy source during the first 70 days of starvation (Carefoot et al., 1993). However, at 90 days of starvation, total lipid content of the muscle tissue decreased as reflected in decreases in the content of saturated (SFA), monosaturated (MFA), and polyunsaturated (PUFA) fatty acids. In contrast, levels of highly unsaturated (HUFA) remained essentially unchanged. The HUFAs, the main lipid constituents of cell membranes as components of phospholipids, appear to be preferentially conserved to meet the need of physiological maintenance in abalones (Sargent, Bell, Bell, Henderson & Tocher, 1993; Zabelinskii, Chebotareva, Kostkin & Krivchenko, 1999). The tendency to conserve HUFAs has been reported in starved fish where SFA and MFA are preferentially mobilized and HUFA (like 20:5n-3 and 22:6n-3) levels remain constant (Navarro & Gutiérrez, 1995).

The differences between the fatty acid profiles of abalone at the beginning of the experiment and of those fed the experimental diets reflect a clear dietary influence, particularly after 50 days. After 70 days, the fatty acid profile of the starved abalone was significantly different from that of abalone fed the diet with added lipid. At 70 days of starvation, the level of total lipid had begun to decrease, declining by approximately 23 % within the next 20 days (90 days). The initial (pre-experiment) levels of HUFAs did not change throughout the total length of the starvation period. HUFA content is highly conserved even after a 90–day starvation period.

More studies are needed to explain fully the fatty acid requirements and metabolism of this species. It appears that future study of essential fatty acid requirements will require the feeding of a no lipid diet for approximately 50 days under the conditions of this experiment. This conditioning period will sufficiently reduce the tissue levels of presumed essential fatty acids to a baseline to observe responses to qualitative and quantitative changes in dietary n-3 and n-6 PUFA and HUFA provided in experimental diets.

References

Bautista-Teurel M.N., Millamena O.M. & Fermin A.C. (2001) Reproductive performance of hatchery-bred donkey's ear abalone, *Haliotis asinina*, Linne, fed natural and artificial

diets. Aquaculture Research **32** (Suppl. 1), 249-254.

Carefoot T.H., Quian P.-Y., Taylor B.E., West T. & Osborne J. (1993) Effect of starvation on energy reserves and metabolism in the Northern abalone, *Haliotis kamtschatkana*. Aquaculture **118**, 315-325.

Christie W.W. (1982) Lipid Analysis: Isolation, Separation and Structural Analysis of Lipids, 2nd edn. Pergamon Press, Oxford.

Cook H.W. (1996) Fatty acid desaturation and chain elongation in eukaryotes. In: *Biochemistry of Lipids, Lipoproteins and Membranes* (ed. by D.E. Vance & J.E. Vance), pp. 129-152. Elsevier Science, Amsterdam.

Dunstan G.A., Baillie H.J., Barrett S.M. & Volkman J.K. (1996) Effect of diet on the lipid composition of wild and cultured abalone. Aquaculture **140**, 115-127.

Floreto E.A.T., Teshima S. & Koshio S. (1996) The effects of seaweed diets on the lipid and fatty acids of the Japanese disc abalone *Haliotis discus hannai*. Fisheries Science **62,** 582-588.

Folch J., Lees M & Stanley S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry **226**, 497-509.

Lim C., Ako H., Brown C.L. & Hahn K. (1997). Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. Aquaculture **151**, 143-153.

Navarro I. & Gutiérrez J (1995) Fasting and starvation. In: Biochemistry and Molecular Biology of Fishes, vol. 4. (ed. by P.W. Hochachka & T.P. Mommsen), pp. 393-434. Elsevier Science, Amsterdan.

Nelson M.M., Leighton D.L., Phleger C.F. & Nichols P.D. (2002) Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgal diets. Comparative Biochemistry and Physiology **131B**, 695-712.

Sargent J.R., Bell J.G., Bell, M.V., Henderson R.J. & Tocher D.R. (1993) The metabolism of phospholipids and polyunsaturated fatty acids in fish. In: Aquaculture: Fundamental and Applied Research (ed. by B. Lahlou & P.Vitiello), Coastal and Estuarine Studies 43, pp. 103-124. American Geophysical Union, Washington, DC.

Takami H., Yamakawa H. & Nakano H. (1995) Survival and physiological stress of

juvenile disk abalone *Haliotis discus hannai* during long-term starvation. Fisheries Science **61**, 111-115.

Uki N., Sugiura M. & Watanabe T.(1986) Requirement of essential fatty acids in the abalone *Haliotis discus hannai*. Bulletin of the Japanese Society of Scientific Fisheries **52**, 1013-1023.

Viana M.T., Guzmán J.M. & Escobar R. (1999) Effect of heated and unheated fish silage as a protein source in diets for abalone *Haliotis fulgens*. Journal of the World Aquaculture Society **30**, 481-489.

Zabelinskii S.A., Chevotareva M.A., Kostkin V.B. & Krivchenko A.I. (1999) Phospholipids and their fatty acids in mitochodria, synaptosomes and myelin from the liver and brain of trout and rat: a new view on the role of fatty acids in membranes. Comparative Biochemistry and Physiology **124B**, 187-193.

Table 1 Ingredient composition of a standard diet fed during the acclimatization period and each of the experimental diets fed to abalone *Haliotis fulgens*.

Ingredients (g/100g diet)	Standard	Experimental	
		1	2
Corn starch	4.34	29.08	24.08
Fish protein concentrate[a]		18.00	18.00
Albumin chicken egg[b]		18.00	18.00
Soybean flour defatted[c]		10.00	10.00
Cellulose[d]		8.00	8.00
Modified starch[e]	10.00	5.00	5.00
Gelatine (50 blooms)	6.00	5.00	5.00
Lipids[f]		0.00	5.00
Mineral mixture[g]	2.00	4.00	4.00
Vitamin mixture[h]	1.30	1.50	1.50
Fish silage: liquid phase[i]	2.00	1.00	1.00
Stay-C[j]	0.40	0.20	0.20
Choline chloride	0.11	0.10	0.10
Sodium benzoate	0.23	0.10	0.10
BHT[k]	0.09	0.01	0.01
α-tocopherol		0.01	0.01
Fish meal[l]	35.00		
Kelp meal[m]	15.00		
Corn meal[n]	12.00		
Soybean meal[o]	10.00		
Methionine	0.23		
Proximal composition (%)			
Crude protein	36.47	33.93	35.26
Total lipid	7.09	0.14	4.95
Ash	20.79	5.73	5.25
Gross energy (kcal/g)	4.15	4.38	4.64

[a] 97% protein. [b] Freeze dried egg white. [c] ICN. [d] α-cellulose (Alphacel). [e] Modified corn starch (Clearjel®). [f] Corn oil 1.5%+ 3.5% tripalmitin. [g] ICN salt mixture #5 Briggs. [h] ICN vitamin diet fortification. [i] Acid fish silage from tuna viscera. [j] Ascorbyl polyphosphate (Roche). [k] Butylatedhydroxy toluene. [l] 64% protein, 10% lipid. [m] Made from Macrocystis pyrifera. [n] 8% protein, 3.9% lipid (Maseca®, Mexico). [o] 39% protein, 21% lipids.

Table 2 Fatty acid and total lipid contents (mg/g dry weight) of the standard and experimental diets (mean ± SE, n = 3)

Fatty acid	Diet		
	Standard	1	2
14:0	2.15±0.01	-	0.31±0.00
16:0	14.57±0.06	0.36±0.01	33.92±0.02
17:0	0.39±0.00	-	0.06±0.00
18:0	4.78±0.02	0.11±0.00	1.87±0.02
20:0	0.31±0.00	-	0.08±0.00
22:0	0.09±0.01	-	0.08±0.00
Σ SFA[a]	22.29±0.10	0.47±0.01	36.32±0.04
16:1n-7	2.35±0.02	0.05±0.00	-
18:1n-9	12.35±0.05	0.20±0.00	4.13±0.00
18:1n-7	2.54±0.01	0.02±0.01	0.20±0.01
20:1n-9	1.29±0.01	-	0.04±0.00
22:1n-9	0.40±0.02	-	0.05±0.00
Σ MFA[b]	18.93±0.11	0.27±0.02	4.42±0.01
16:3n-6	0.15±0.03	0.06±0.01	0.07±0.0
16:4n-1	0.17±0.01	0.03±0.00	-
18:2n-6	13.14±0.17	0.34±0.02	8.11±0.01
18:2n-4	0.08±0.00	-	0.07±0.01
18:3n-3	1.76±0.02	0.02±0.01	0.13±0.00
Σ PUFA[c]	15.3±0.23	0.45±0.04	8.38±0.02
20:4n-6	0.52±0.01	0.03±0.00	-
20:5n-3	3.12±0.05	0.03±0.00	-
22:5n-3	0.55±0.01	-	-
22:6n-3	4.58±0.08	0.08±0.01	0.07±0.00
Σ HUFA[d]	8.77±0.15	0.14±0.06	0.07±0.00
Σ n-3	10.01±0.16	0.13±0.02	0.20±0.00
Σ n-6	13.81±0.21	0.43 ±0.03	8.18±0.01
Ratio n-3/n-6	0.72	0.30	0.02
Total lipid	70.89±1.49	1.38±0.84	49.46±1.14

- : no detected

[a]SFA: saturated fatty acids

[b]MUFA: monounsaturated fatty acids

[c]PUFA: polyunsaturated fatty acids, $< C_{20} \geq 2$ double bonds + $\geq C_{20} \leq 2$ double bonds

[d]HUFA: highly unsaturated fatty acids, $\geq C_{20} \geq 3$ double bonds.

Table 3 Fatty acid and total lipid contents (mg /g dry weight) in muscle of juvenile abalone *Haliotis fulgens* at start of the experiment, fed the experimental diets and starved (mean ±SE, n = 3)

Fatty acid	Start of the experiment	Treatment				
		Diet		Starvation		
		1	2	50 days	70 days	90 days
14:0	0.59±0.04	0.13±0.04	0.67±0.35	-	-	-
16:0	9.85±1.14	7.32±0.16	8.36±3.10	8.45±0.09	8.20±0.49	3.13±0.31
17:0	0.67±0.03	0.09±0.02	0.25±0.13	0.54±0.02	0.59±0.07	0.42±0.04
18:0	4.24±0.09	2.39±0.07	2.31±0.09	6.18±1.24	5.04±0.42	3.87±0.30
Σ SFA	15.35±1.3	9.93±0.29	11.59±3.67	15.17±1.35	13.83±0.98	7.42±0.65
16:1n-7	0.64±0.13	0.07±0.00	0.41±0.12	0.61±0.03	0.39±0.15	-
17:1n-7	2.75±0.48	-	-	1.38±0.63	0.70±0.09	1.38±0.57
18:1n-9	3.13±0.04	41.98±0.97	14.57±9.08	4.72±0.48	4.53±0.17	0.61±0.13
18:1n-7	2.68±0.06	1.87±0.10	1.79±0.62	1.21±0.09	1.44±0.34	1.26±0.27
20:1n-11	1.97±0.21	0.25±0.06	-	2.07±0.19	1.85±0.26	1.19±0.29
20:1n-9	0.49±0.02	0.17±0.01	1.06±0.63	0.91±0.03	0.73±0.08	0.08±0.08
Σ MFA	11.66±0.94	44.34±1.14	17.83±10.45	10.9±1.45	9.64±1.09	4.52±1.34
16:2n-6	4.79±0.95	-	1.16±0.87	3.28±0.04	2.51±0.10	1.58±0.13
16:3n-6	-	0.36±0.03	0.21±0.04	0.19±0.19	0.28±0.02	-
16:2n-4	0.46±0.03	-	-	1.15±0.33	0.80±0.13	-
16:4n-1	7.03±0.20	0.35±0.05	1.90±1.41	4.29±1.23	5.78±0.69	3.88±1.43
18:2n-6	1.27±0.34	2.86±0.03	1.59±0.52	1.22±0.03	1.09±0.08	0.64±0.02
18:3n-3	0.39±0.01	0.33±0.19	0.22±0.05	-	-	-
18:4n-3	0.09±0.01	-	-	-	-	-
20:2n-6	0.18±0.07	tr	2.71±2.56	0.25±0.25	0.34±0.06	0.24±0.12
22:2	5.22±0.08	0.41±0.09	1.99±1.28	6.08±0.73	5.06±0.60	4.18±0.86
Σ PUFA	19.43±1.69	4.31±0.39	9.78±6.73	16.46±2.80	15.86±1.68	10.52±2.56
20:3n-6	0.25±0.02	0.03±0.01	0.18±0.05	-	-	-
20:4n-6	1.81±0.23	0.26±0.06	1.49±0.96	2.14±0.04	2.23±0.28	2.46±0.47
20:5n-3	3.22±0.57	0.46±0.09	2.22±1.56	3.13±0.08	3.43±0.72	3.84±0.72
21:5n-3	0.89±0.08	0.04±0.01	0.39±0.23	0.79±0.09	0.74±0.03	0.43±0.13
22:4n-6	0.53±0.01	0.06±0.02	0.29±0.14	0.83±0.32	0.58±0.02	0.39±0.14
22:5n-3	3.54±0.53	0.44±0.09	2.06±1.53	4.38±0.10	4.67±0.60	3.62±0.73
22:6n-3	1.12±0.08	0.30±0.03	0.95±0.40	1.09±0.12	1.31±0.15	1.07±0.14
Σ HUFA	11.36±1.52	1.59±0.31	7.58±4.87	12.36±0.75	12.96±1.8	11.81±2.33
PUFA n-3	0.48±0.02	0.33±0.19	0.22±0.05	0	0	0
HUFA n-3	8.77±1.26	1.24±0.22	5.62±3.72	9.39±0.39	10.15±1.50	8.96±1.72
PUFA n-6	6.49±1.29	3.22±0.06	5.85±3.99	4.94±0.51	4.22±0.26	2.46±0.27
HUFA n-6	2.34±0.26	0.32±0.09	1.78±1.15	2.97±0.36	2.81±0.30	2.85±0.61
Ratio n-3/n-6	1.05	0.44	0.77	1.19	1.53	1.74
Total lipid	64.55±5.12	61.73±3.82	63.93±0.59	64.57±2.16	60.47±3.45	44.67±1.65

- : no detected, tr; trace (< 0.01 mg/g).

ARTÍCULO 4

Effect of starvation and dietary lipid on the fatty acid composition of muscle tissue and digestive content of the juvenile green abalone (*Haliotis fulgens*)[1]

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[1] **En preparación para su envío a Aquaculture**

1. Introduction

The starvation or restricted feeding it is not an unusual state in the marine invertebrates, where now and then and for long period of times food is scarce or not available follow-on with a reduction of their metabolism in order to spare the energy reserves until food is available again. The knowledge of this process it is importance not only to understand their role into the marine environment, but also to understand their nutrition physiology and further on their requirements. The starvation has deep effects in their overall physiological condition like health and muscle quality as food. The glycogen and lipids has been considered as the primary reserve energy in some mollusk species being the glycogen the first one to be used (Carefoot et al., 1993).

Within lipids, are the triglicerides used before than phospholipids under starvation, both being part to the reserve and structural lipids, respectively. Being the fatty acids 14:0, 16:1, 18:1 and 20:1 the ones that mobilized first. The retention of certain group of fatty acids over the others is not only regulated by an energetic need, but to maintain the structural integrity and fluidity of cell membranes. In starved hake (*Merluccius capensis*) a relative increase of instauration in lipids has been found. In *Fugu vermicularis porphyreus* it has been reported that the levels of 20:5n-3 and 22:6n-3 were maintained or increased uniformly during early starvation (Navarro and Gutierrez, 1995).

It has been demonstrated that abalone can endure long starvation periods before showing body reserves depletion (Carefoot *et al.*, 1993). Abalone juveniles and adults

are able to survive for several weeks under starvation or food restriction regime by using all carbohydrates and lipids and at last the protein (Roberts *et al.*, 2001). In the starved abalone *H. discus hannai* it was observed glycogen and triglycerol depletion during the first 30 days being the principal reserve nutrients found in muscle and digestive gland. Further on, an increment in protease activity in muscle was associated to tissue protein utilization as energy source. However, the time tolerance to starvation depends on abalone size, where larger organisms are more able to resist starvation (Takami *et al.*, 1995). Moreover, it has been observed that oxygen requirements decrease proportionally to the starvation period (Segawa, 1991).

Thus, the aim of the present work was to study the starvation, lipid restricted feeding effect under the total lipids, both polar and no polar, in the green abalone *H. fulgens*.

2. Materials and methods

2.1 Diet preparation

Two experimental diets were formulated to undertake this experiment (Table 1) to be compared to a starved group. Diet **LL** contained lipid free ingredients (lipid restricted diet) using starch as a replacement. Diet **HL** was balanced with 1.5% of corn oil and tripalmitin to achieve a total triacylglycerol level of 3%. Dietary vitamin and mineral mixtures were those recommended by Hahn (1989). Silage was produced as described by Viana et al. (1993) using the liquid fraction with no lipids, separated by centrifugation.

α-tocopherol were added to prevent lipid oxidation. The seaweed meal, fish protein concentrate, chicken egg albumin, corn starch, modified starch, soybean meal and silage ingredients were defatted three times with hot methanol (1:1 w/v) before incorporation into the diet. All ingredients were blended with 50% water until a completely homogeneous dough-like mixture was obtained. The diets were then rolled flat to a thickness of 2 mm and 10 mm x 5 mm pieces were cut and stored in sealed plastic bags at –25°C during the experiment. A batch of feed samples were stored at -80°C for fatty acid analysis.

2.2 Experimental conditions

Thirty-nine juvenile green abalone *H. fulgens* with a mean shell length of 35.84±0.25 mm and mean weight of 6.22±0.15 g were obtained from a commercial abalone farm, BC-Abalone in Ejido Eréndira B.C. Abalone were held in a flow-through system with filtered, aerated seawater (34 ppm salinity and flow rate of 300 ml min^{-1}). Temperature was controlled at 20.45±0.04°C throughout the feeding trials. Abalone were acclimated to laboratory conditions with a standard balanced diet (Table 1) during 56 days.

Each experimental unit consisted of a 3.8 l plastic container with 4 abalone. Three replicates per treatment were used plus 3 more abalone that were killed at the start of the experiment for lipid analysis. Abalone were held under the same conditions described previously for the acclimation period. Those abalone consuming diet LL and HL were fed daily during the evening at a rate of 2% feed dry weight related to their wet

body weight. The third group, starved (S) did not receive any food and buckets from all treatments were cleaned daily to avoid diatoms growth. Any uneaten food was collected the following morning, dried and weighed from the experimental and control buckets (to calculate feed stability). Feed intake was calculated as reported by López and Viana (1995) and the feed conversion efficiency (FCE) as stated by Uki and Watanabe (1992). Whole-body weight was determined using an electronic balance (± 0.001 g) and shell length was measured with an electronic digital caliper (± 0.05 mm) at 0 and 60 days. Growth of abalone was expressed as daily growth rate in length (μm/day) and weight (mg/day) and as percent weight gain. After 60 days, six abalone per treatment were randomly selected to obtain muscle tissue and digestive content for fatty acid analyses. Abalone were rapidly slaughter to separate muscle from the digestive system and frozen with liquid nitrogen, prior were freeze-dried and stored in glass vials under nitrogen atmosphere at -80° C.

2.3 Chemical analysis

Dry weight of each diet was calculated as the weight of triplicate samples (4-5g) after drying to constant weight at 100°C. Mean total nitrogen content was determined from samples analyzed by the micro-Kjeldahl method (AOAC, 1995), and percent crude protein was then calculated as %N x 6.25. Mean total lipid content in diets, muscle and digestive content was determined by extraction using chloroform-methanol (2:1, v/v) following the method of Folch et al. (1957). Mean ash content was determined by

heating samples of each diet to 550^{0}C for 18 h. The gross energy content of each diet was determined by direct combustion in an adiabatic calorimeter Parr 1281.

Muscle samples for lipid extraction and fatty acid analysis were individually processed. The digestive content of two abalone from each experimental unit were pooled to gain weight for analysis. Lipid from muscle was extracted into polar and neutral classes by silica cartridges (Resprep™, Restek Corp. Bellefonte, PA), using with chloroform and methanol as eluting solvents as described by Juaneda and Rocquelin (1985).

Fatty acid methyl esters (FAME) were prepared from diets, muscle tissue and digestive content of abalone according to Christie (1982). FAME were analyzed in a Hewlett Packard 5890II gas chromatograph equipped with a flame ionization detector and a capillary column (Omegawax™ 320 by Supelco/Sigma-Aldrich; 30 m x 0.32 mm, film thickness 0.25 μm) using hydrogen as the carrier gas. Fatty acids were identified by comparing the retention times from standards (37 Component FAME Mix, Supelco/Sigma-Aldrich; GLC 87 ,GLC 96, Nu-Chek Prep) and well-characterized profiles samples of marine oils (PUFA1 and PUFA3, Supelco/Sigma-Aldrich). Each fatty acid concentration was calculated from the corresponding chromatogram area by using an internal standard (19:0) and a software package HP ChemStation rev. A.06 for Windows.

2.4 Statistical Analysis

Data from biological indices and fatty acid contents were treated with one-way analysis of variance and means compared using the Student-Newman-Keuls multiple comparisons. A pair comparison was treated by a Student's *t*-test to see possible differences between fatty acid contents in cases were only two treatments were compared. Differences were reported as statistically significant with a $P<0.05$. All the statistical analyses were performed using the SPSS 11.0.1 for windows program (SPSS Inc., 2001).

3. Results

Proximate composition and energy content of the standard and experimental diets are presented in Table 1. Both experimental diets (**LL** and **HL**) were balanced to be isonitrogenous and isocaloric. Protein content being 39.0 and 38.7 %, with a gross energy values of 3.93 and 4.04 kcal/g with a protein energy ratio of 99.4 and 95.9 mg crude protein/kcal /g dry weight for diets **LL** and **HL** respectively.

The fatty acid compositions of the standard and experimental diets are presented in Table 2. The standard diet resulted in higher polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA) n-3 and n-6 contents compared to diet **LL** and **HL**. The levels of both n-3 and n-6 fatty acids in diet 1 were lower than those in diet **HL**. However, the n-3/n-6 ratio of diet 1 (0.41) higher than that observed in diet 2 (0.06), due to the higher content of 18:2n-6 in the diet **HL**.

The growth response of juvenile abalone fed the experimental diets and starved for the 60-day trial is presented in Table 3. No significant differences ($P>0.05$) in growth were observed between *H. fulgens* fed the diet LL and diet HL, whereas the starved group lost 18.8 % in weight. Moreover, no significant differences ($P>0.05$) were observed in feed intake, FCE and energy intake between abalone fed the experimental diets.

The total lipid levels within the muscle tissue and digestive content of abalone under experimental groups are presented in Table 3, were significant differences ($P<0.05$) are observed among lipid contents in muscle, being the starved abalone the group with the highest level. The total lipid in muscle of starved abalone was 21.5% higher than the initial ones but starved abalone decreased 18.8% in total body wet weight in comparison the start of the trial. In contrast, the lipid level in digestive content of starved abalone was significantly lower ($P<0.05$) than those fed experimental diets.

The fatty acid composition from the polar lipid fraction of abalone muscle is presented in Table 5. At the start of the feeding trial the main fatty acids were 16:0, 16:4, 18:0, 18:1n-9, 20:4n-6, 20:5n-3, 22:2 and 22:6n-3. The HUFA 20:3n-6 and 21:5n-3 were no detected, and 22:5n-3 was trace, whereas at the end of the experiment, the starved abalone resulted in a significant increase ($P<0.05$) of 18:1n-9, 18:3n-3, 20:1n-9, 20:3n-9, 20:5n-3, 22:2n-6 and 22:6n-3. Moreover, abalone fed diet LL showed the highest content of 22:1n-7 and 22:5n-3, while abalone fed diet 2 had the highest content of 22:2n-

6. No significant differences (P>0.05) in n-3/n-6 ratio were observed among *H. fulgens* at the start of the trial and the end of the 3 experimental treatments.

The fatty acid composition of the neutral lipid fraction in abalone muscle, both at the start and at the end of the experiment is presented in Table 6. The contents of 16:0, 18:0, 18:1n-7, 18:2n-6, 20:4n-6 and 20:5n-3 were significantly higher (P<0.05) at the start than those of the abalone at the end of the trial. Fifteen of the fatty acids found at the beginning were no detected and 4 were at trace level at the end in abalone fed diet LL. In a similar way, 11 fatty acids from the start were in trace amounts or no detected at the end of the experiment in abalone fed diet HL. In contrast, the fatty acid composition of abalone in starvation (S) at the end of the experiment showed a high conservative pattern, where no significant differences (P>0.05) were observed in contents of 14 from the 27 fatty acids observed in the initial samples.

The fatty acid composition from the digestive contents of abalone is presented in Table 7. At the beginning of the experiment, the contents of 16:1n-7, 18:0, 18:3n-3 and 22:1n-11 were significantly higher (P<0.05) than those observed in abalone fed the LL, HL and S. The abalone fed diet LH showed that content of 14:0 and 18:1n-7 in the digestive content were significantly higher (P<0.05) than the levels found at the start of the experimental samples, plus abalone fed diet HL and starved (S). The levels of 20:2n-6 and 20;4n-6 in the digestive content of abalone fed diet HL were significantly higher (P<0.05) than those observed in LL and S. The contents of 18:4n-3, 20:3n-6, 20:3n-3, 22;5n-3 and the ratio n3/n6 in the digestive content of starved abalone (S) were

significantly higher than those observed in abalone fed standard and experimental diets LL and HL.

4. Discussion

The aim of the present work was to evaluate the effect of lipids under starvation and restricted lipid feed regime, and therefore the length of the experiment was not enough to register over 100% growth suggested as appropriate to show significant differences (D'Abramo and Castell, 1997). However in a previous study was determined that juveniles from *Haliotis fulgens* fed a diet containing 0.15% total lipids with a similar diet composition resulted in an equal weight gain after than that observed in a group fed a diet containing 1.5% corn oil and 3.5% tripalmitin with a 120% overall growth increment (Durazo-Beltrán et al., in press). Thus, in the present work it was observed that even if growth was similar after 60 days between both fed groups, LL and HL (29 and 23%, respectively), whereas the starved group (S) showed a decrease in weight (19%), significant differences were observed in the fatty acids in muscle. In this way, the S group showed a capacity to preserve in general their polar fatty acids even with an increment in 20:5n-3 and 22:6n-3 content. This could show a physiological strategy to maintain their cellular membrane integrity sparing the phospholipids (polar fraction) by using other source of fuel as energy, where the HUFA were predominantly conserved, specially the n-3, because they play an important role in the cell membrane (Tocher, 1995; Zabelinskii *et al.*, 1995). In starved fish it has been reported that saturated fatty

acids and mono unsaturated fatty acids are mainly mobilized in relation to the HUFA (Koven et al, 1989; Navarro and Gutierrez, 1995). In the present work fed abalone (LL and HL) also showed the same tendency to preserve the polar fatty acids failing to show any significant difference in the ratio n-3/n6 ratio between the start and the end of the experiment.

The high 16:4 content in muscle from all treatments could be associated with the unsaturated fatty acid regulation in polar lipids. This strategy to preserve and accumulate unsaturated fatty acids to regulate the instauration degree in polar fatty acids has been reported before in marine organisms like *Pecten maximus* (Soudant *et al.*, 1996), *Sparus aurata* (Ibeas *et al*, 1996), and *Peneaus monodon* (Deering *et al.*, 1997).

In the present work, the starved abalone failed to show the lipids as an energetic source when these tend to be preserved, in relation to the initial level of fatty acids, probably due to the lower metabolism condition (Segawa, 1991). At the contrary, abalone fed with balanced diets showed a clear effect on the feed regime and growth, in this way, abalone fed diet LL and HL showed a significant reduction in the content of 16:0 and 20:5n-3, showing that neutral lipids can be used to cover their biochemical and energetic requirements.

The total lipid amount in the digestive content of fed abalone was higher than that found in the diets itself. In a similar way, the fatty acid content in the digestive content of fed abalone was higher than that found in the diets. This fact of apparent total lipids

accumulation could be due to the result of a low digestibility of lipids in the abalone as demonstrated that low lipid level in the diet does not affect the growth response at short term (Durazo et al., in press). It is important to state that abalone were sampled at the middle of the day whereas food is offered during the night and therefore digestion could be taken for several hours before. Also, the presence of tissue debris or microbial activity should be considered as well (Merchen, 1988).

The starved group also showed a high lipid content even after 60 days starvation, even if this was significant lower than that figure reported at the beginning of the experiment, the total lipid content decrease from 11.41 to 8.19%

According to the fatty acids profiles found in the digestive content of fed abalone no clear pattern can be drown from the different treatments compared to their diets neither the starved group compared to the initial fatty acid profile in the digestive content.

References

AOAC, 1995. Official Methods of Analysis of AOAC. 16th. edn. Association of Official Analytical Chemists, Arlington, VA.

Carefoot, T.H., Quian, P.-Y., Taylor, B.E., West, T., Osborne, J.M., 1993. Effect of starvation on energy reserves and metabolism in the Northern abalone, *Haliotis kamtschatkana*. Aquaculture 118, 15-325.

Christie W.W., 1982. Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids, Pergamon Press, Oxford, 207 pp.

D'Abramo, L.R., Castell, J.D., 1997. Research methodology. In: D'Abramo, L.R., Conkin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. Advances in World Aquaculture. World Aquaculture Society, Baton Rouge, LA, pp. 3-25.

Deering, M.J., Fielder, D.R., Hewitt, D.R., 1997. Growth and fatty acid composition of juvenile leader prawns, fed different lipids. Aquaculture 151, 131-141.

Durazo-Beltrán, E., D'Abramo, L.R., Toro-Vazquez, J.F., Vásquez-Peláez, C., Viana, M.T., in press. Effect of triacylglycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*). Aquaculture, in press.

Folch, J., Lee, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 22, 477-509.

Hahn, K.O., 1989. Nutrition and growth of abalone. In: Hahn, K.O. (Ed.), CRC Handbook of Culture of Abalone and other Gastropods. CRC Press, Boca Raton, FL, pp. 135-156.

Ibeas,C., Cejas, J., Gómez, T., Jerez, S., Lorenzo, A., 1996. Influence of dietary n-3 highly unsaturated faty acid levels on juvenile gilthead seabream (*Sparus aurata*) grwth and tissue fatty acid composition. Aquaculture 142, 221-235.

Juaneda, P., Rocquelin, G., 1985. Rapid and convenient separation of phospholipids and non phosphorus lipids from rat heart using silica cartridges. Lipids 30, 40-41.

Koven, W.M., Kissil, G.W., Tandler, A., 1989. Lipid and n-3 requirement of *Sparus aurata* larvae during starvation and feeding. Aquaculture 79, 185-191.

Lopez, L.M., Viana, M.T., 1995. Determination of the quality of food elaborated from unheated and heated fish silages for abalone juveniles of *Haliotis fulgens*. Ciencias Marinas 21, 331-342.

Merchen, N.R., 1988. Digestion, absorption and excretion in ruminants. In: Church, D.C. (Ed.), The Ruminant Animal. Digestive Physiology and Nutrition. Waveland Press, Inc., Prospect Heights, Illinois, pp. 172-201.

Navarro, I., Gutiérrez, J., 1995. Fasting and starvation. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes, Vol. 4, Metabolic Biochemistry. Elsevier Science B.V., Amsterdam, pp. 393-434.

Roberts, R.D., Lapworth, C., Barker, R.J., 2001. Effect of starvation on the growth and survival of post-larval abalone (*Haliotis iris*). Aquaculture 200, 323-338.

Segawa, S., 1991. Oxygen consumption and ammonia excretion by the abalone *Sulculus diversicolor aquatilis* in starved condition. Nippon Suisan Gakkaishi 57, 2001-2006.

Soudant, P., Marty, Y., Moal, J., Robert, R., Quéré, C., Le Coz, J.R., Samain, J.F., 1996. Effect of food fatty acid and sterol quality on *Pecten maximus* gonad composition and reproduction process. Aquaculture 143, 361-378.

Takami, H., Yamanaka, H., Nakano, H., 1995. Survival and physiological stress of juvenile disk abalone *Haliotis discus discus* during long-term starvation. Fish. Sci. 61, 111-115.

Tocher, D.R., 1995. Glycerophospholipid metabolism. In : Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes, Vol. 4, Metabolic Biochemistry. Elsevier Science B.V., Amsterdam, pp. 119-157.

Uki, N. and Watanabe, T., 1992. Review of the nutritional requirements of abalone *Haliotis* spp and development of more efficient diets. In: Sheperd, S.A., Tegner, M.J., Guzmán del Proó, S.A. (Eds.), Abalone of the World: Biology, Fisheries and Culture. Fishing News Books, Oxford, pp. 504-517.

Viana, M.T., Nava, C., Solana-Sansores, R., 1993. Acid fish silages. Effect of preheating and addition of phosphoric and citric acids on the biochemical quality. Ciencias Marinas 19, 415-433.

Zabelinskii S.A., Brovtsyna, N.B., Chevotareva M.A., Gorbunova, O.B., Krivchenko A.I., 1995. Comparative investigation of lipid and fatty acid composition of fish gills and mammalian lungs. A model of the membrane lipid composition areas. Comp. Biochem. and Physiol. 111B, 127-140.

Table 1. Ingredient content (g/100 g), proximate composition and energy content of the standard and experimental diets.

Ingredients	Standard	Diet LL	Diet HL
Kelp meal[a]	15.00	24.00	26.00
Albumin chicken egg		12.50	12.50
Fish protein concentrate[b]		12.50	12.50
Corn starch	4.34	14.05	11.59
Modified starch[c]	10.00	14.05	11.50
Soybean meal defatted		6.00	6.00
Cellulose		5.00	5.00
Gelatine	6.00	5.00	5.00
Mineral mixture[f]	2.00	4.00	4.00
Lipids[g]			3.00
Vitamin mixture[h]	1.30	1.50	1.50
Fish silage: liquid phase[i]	2.00	1.00	1.00
Stay-C[j]	0.40	0.20	0.20
Choline chloride	0.11	0.10	0.10
Sodium benzoate	0.23	0.10	0.10
α-tocopherol			0.01
Fish meal[k]	35.00		
Corn meal[l]	12.00		
Soybean meal[m]	10.00		
Methionine	0.23		
BHT[n]	0.09		
Proximate composition %			
Crude protein	36.47	39.05	38.74
Total lipid	7.09	0.12	3.04
Ash	20.79	14.75	15.44
Gross energy (kcal/g dry weight)	4.15	3.93	4.04
Ratio protein/energy[o]	87.81	99.41	95.87

[a] Meal of *Macrocystis pyrifera*. [b] 97% protein. [c] Modified corn starch (Clearjel®). [d] 97% protein ICN. [e] α-cellulose (Alphacel).
[f] ICN salt mixture #5 Briggs. [g] 1.5% corn oil + 1.5% tripalmitin.
[h] ICN vitamin diet fortification. [i] Acid fish silage from tuna viscera.
[j] Ascorbyl polyphosphate (Roche). [k] 64% protein, 10% lipid.
[l] 8% protein, 3.9% lipid (Maseca™, Mexico). [m] 39% protein, 21% lipid.
[n] Butylatedhydroxy toluene.
[o] mg crude protein/kcal gross energy/g dry weight.

Table 2. Fatty acid and total lipid contents (mg/g dry weight) of the standard and experimental diets (mean ± SE, n = 3) .

Fatty acid	Standard diet	Diet LL	Diet HL
14:0	1.24 ± 0.02	0.07 ± 0.00	0.28 ± 0.01
16:0	14.55 ± 0.07	0.29 ± 0.06	16.21 ± 0.02
16:1n-7	2.35 ± 0.01	0.01± 0.00	nd
17:0	0.39 ± 0.00	nd	nd
17:1n-7	0.17 ± 0.01	nd	nd
16:4	0.17 ± 0.01	nd	nd
18:0	4.78 ± 0.04	0.05 ± 0.00	1.09 ± 0.02
18:1n-9	12.33 ± 0.09	0.19 ± 0.00	4.06 ± 0.01
18:1n-7	2.54 ± 0.04	0.02± 0.00	0.27± 0.01
18:2n-6	13.11 ± 0.06	0.32 ± 0.01	7.39 ± 0.02
18:2n-4	0.08 ± 0.00	tr	0.06± 0.00
18:3n-6	0.08 ± 0.01	tr	nd
18:3n-3	1.76 ± 0.01	0.06 ± 0.00	0.25 ± 0.00
18:4n-3	0.56 ± 0.01	0.03 ± 0.00	0.09 ± 0.01
20:0	0.31 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
20:1n-11	0.16 ± 0.00	nd	0.02±0.01
20:1n-9	1.29 ± 0.00	tr	nd
20:1n-7	0.14 ± 0.02	0.01 ± 0.00	nd
20:2n-6	0.12 ± 0.01	nd	nd
20:4n-6	0.52 ± 0.01	tr	nd
20:4n-3	0.20 ± 0.00	0.03 ± 0.00	0.07 ± 0.00
20:5n-3	3.11 ± 0.03	tr	0.04±0.00
22:1n-11	1.41 ± 0.01	nd	nd
22:1n-9	0.41 ± 0.02	nd	nd
21:5n-3	0.10 ± 0.00	nd	nd
22:5n-6	0.10 ± 0.00	nd	nd
22:5n-3	0.55 ± 0.00	0.01 ± 0.00	tr
22:6n-3	4.57 ± 0.04	nd	nd
n-3/n-6	0.72 ± 0.01	0.40 ± 0.02	0.06 ± 0.00
Total lipid	70.94 ± 0.15	1.20 ± 0.40	30.41 ± 1.60

nd: no detected; tr: trace (<0.01 mg/g)

Table 3. Biological indices measured in juveniles of green abalone (*Haliotis fulgens*) before and after fed diets with two lipid levels and starvation during 60 days (mean ± SE, n =12)

	Treatments		
	Diet LL	Diet HL	Starvation
Initial weight (g)	6.42 ± 0.30	6.33 ± 0.23	5.91 ± 0.22
Final weight (g)	8.28 ± 0.59[a]	8.24 ± 0.38[a]	4.97 ± 0.19[b]
Growth rate (mg/day)	30.97 ± 5.72[a]	31.74 ± 4.13[a]	-15.59 ± 0.41[b]
Weight gain (%)	28.90 ± 5.26[a]	22.99 ± 2.39[a]	-18.80 ± 0.47[b]
Initial length (mm)	35.74 ± 0.49	36.14 ± 0.40	35.64± 0.43
Final length (mm)	37.51 ± 0.90	38.54 ± 0.56	
Growth rate (μm/day)	29.53 ± 8.00	40.00 ± 11.52	
Feed intake (% w wt)[1]	0.59 ± 0.04	0.65 ± 0.03	
FCE	0.85 ± 0.09	0.73 ± 0.05	
Energy intake (cal/g w wt)	23.26 ± 10.04	26.62 ± 7.73	

[1] wet weight

Means in the same row with different superscript are significantly different ($P < 0.05$).

Table 4. Total lipid contents (g/100 g dry weight) in muscle and digestive content of *H. fulgens* at start of the experiment, fed the experimental diets and starved during 60 days (mean ± SE, n = 3).

Treatment	Muscle	Digestive content
Start of the experiment	5.21 ± 0.06^{b}	11.41 ± 0.99^{a}
Diet LL	4.79 ± 0.22^{b}	10.25 ± 0.63^{a}
Diet HL	5.26 ± 0.14^{b}	10.72 ± 0.43^{a}
Starvation	6.33 ± 0.31^{a}	8.19 ± 0.33^{b}

Means in the same column with different superscript are significantly different ($P < 0.05$).

Table 5. Fatty acid composition (mg fatty acid/g dry weight) of the polar lipid from muscle of *Haliotis fulgens* at start of the experiment, fed the experimental diets and starved during 60 days (mean ± SE, n = 6).

Fatty acid	Start of the experiment	Diet LL	Diet HL	Starvation
14:0	0.32 ± 0.09	0.49 ± 0.11	0.34 ± 0.03	0.55 ± 0.04
15:0	0.17 ± 0.07^{bc}	0.13 ± 0.03^{c}	0.13 ± 0.05^{c}	0.32 ± 0.05^{ab}
16:0	4.62 ± 1.52^{bc}	3.33 ± 0.50^{c}	3.87 ± 0.47^{c}	6.71 ± 0.26^{ab}
16:1n-7	0.25 ± 0.09	0.28 ± 0.02	0.62 ± 0.17	0.43 ± 0.05
17:0	0.13 ± 0.04^{ab}	0.14 ± 0.04^{b}	0.16 ± 0.02^{ab}	0.27 ± 0.02^{a}
16:3n-4	tr	nd	nd	0.35 ± 0.07
17:1n-7	0.13 ± 0.09	0.20 ± 0.04	0.23 ± 0.05	0.37 ± 0.07
16:4	5.53 ± 3.93	9.70 ± 1.00	12.16 ± 1.56	11.80 ± 0.44
18:0	1.15 ± 0.75	2.31 ± 0.24	2.33 ± 0.36	2.63 ± 0.12
18:1n-9	0.94 ± 0.24^{b}	0.89 ± 0.12^{b}	1.11 ± 0.09^{b}	2.29 ± 0.16^{a}
18:1n-7	0.79 ± 0.21^{ab}	1.40 ± 0.21^{a}	0.87 ± 0.13^{b}	1.32 ± 0.05^{a}
18:2n-6	0.60 ± 0.25	0.75 ± 0.11	0.91± 0.11	0.87 ± 0.04
18:3n-3	0.05 ± 0.03^{b}	0.14 ± 0.04^{ab}	0.06 ± 0.03^{b}	0.17 ± 0.01^{a}
18:4n-3	nd	0.08 ± 0.03	0.12 ± 0.03	0.03 ± 0.02
20:1n-11	tr	1.22 ± 0.27	1.40 ± 0.21	nd
20:1n-9	0.78 ± 0.45^{b}	0.29 ± 0.21^{b}	0.25 ± 0.04^{b}	1.76 ± 0.05^{a}
20:1n-7	0.01 ± 0.00	0.14 ± 0.03	0.26 ± 0.03	0.24 ± 0.05
20:2n-6	tr	0.05 ± 0.02^{b}	0.26 ± 0.04^{a}	nd
20:3n-9	tr	0.06 ± 0.03^{b}	0.04 ± 0.03^{b}	0.14 ± 0.01^{a}
20:3n-6	nd	0.08 ± 0.02	0.06 ± 0.03	0.03 ± 0.01
20:4n-6	0.98 ± 0.60	1.94 ± 0.37	1.46 ± 0.52	2.11 ± 0.16
20:5n-3	0.95 ± 0.44^{b}	1.45 ± 0.29^{b}	0.95 ± 0.21^{b}	2.66 ± 0.16^{a}
22:1n-11	nd	0.61 ± 0.56	0.12 ± 0.04	nd
22:1n-7	0.09 ± 0.06^{b}	2.64 ± 0.37^{a}	1.00 ± 0.63^{ab}	0.08 ± 0.04^{b}
22:2	1.38 ± 0.97^{a}	0.39 ± 0.06^{b}	2.09 ± 0.76^{a}	3.02 ± 0.08^{a}
22:2n-6	0.08 ± 0.06^{b}	0.06 ± 0.02^{b}	nd	0.26 ± 0.01^{a}
21:5n-3	nd	0.26 ± 0.07	0.40 ± 0.15	0.08 ± 0.03
22:4n-6	nd	0.69 ± 0.31	0.60 ± 0.08	nd
22:5n-6	0.28 ± 0.16	tr	nd	0.62 ± 0.04
22:5n-3	tr	3.07 ± 0.40^{a}	2.04 ± 0.32^{b}	0.06 ± 0.04^{c}
22:6n-3	1.54 ± 0.84^{b}	0.99 ± 0.44^{b}	0.21± 0.02^{b}	3.51 ± 0.21^{a}
24:1n-9	0.37 ± 0.18^{a}	0.09 ± 0.03^{b}	nd	0.42 ± 0.02^{a}
n-3/n-6	1.38 ± 0.09	1.69 ± 0.20	1.44 ± 0.38	1.99 ± 0.04

nd: no detected; tr: trace (<0.01 mg/g).

Means in the same row with different superscript are significantly different ($P < 0.05$).

Table 6. Fatty acid composition (mg fatty acid/g dry weight) of the neutral lipid from muscle of *Haliotis fulgens* at start of the experiment, fed the experimental diets and starved during 60 days (mean ± SE, n = 6).

Fatty acid	Start of the experiment	Diet LL	Diet HL	Starvation
14:0	0.11 ± 0.06	nd	0.07 ± 0.06	nd
16:0	2.11 ± 1.02[a]	0.34 ± 0.06[b]	0.46 ± 0.17[b]	1.46 ± 0.19[ab]
16:1n-7	0.13 ± 0.04	nd	0.19 ± 0.16	0.08 ± 0.04
17:0	0.13 ± 0.07	nd	tr	0.10 ± 0.02
16:4	3.15 ± 1.91	0.27 ±0.10	0.99 ± 0.60	0.53 ± 0.27
18:0	2.37 ± 1.15[a]	0.17 ± 0.04[b]	0.24 ± 0.07[b]	0.82 ± 0.09[b]
18:1n-9	0.54 ± 0.18	0.28 ± 0.06	0.33 ± 0.08	0.46 ± 0.05
18:1n-7	0.59 ± 0.36[a]	0.07 ± 0.03[b]	0.05 ± 0.04[b]	0.12 ± 0.03[b]
18:2n-6	0.58 ± 0.26[a]	0.07± 0.01[b]	0.12 ± 0.05[b]	0.11 ± 0.02[b]
18:3n-3	0.14 ± 0.08	nd	nd	nd
18:4n-3	tr	0.06 ± 0.02	0.08 ± 0.02	0.05 ± 0.01
20:1n-11	nd	tr	tr	0.04 ± 0.03
20:1n-9	0.87 ± 0.50	tr	nd	0.15 ± 0.04
20:1n-7	0.07 ± 0.04	nd	tr	0.10 ± 0.02
20:3n-9	tr	nd	nd	0.02 ± 0.01
20:3n-6	0.07 ± 0.04	nd	tr	nd
20:4n-6	1.89 ± 0.95[a]	0.03 ± 0.01[b]	0.10 ± 0.09[b]	0.11 ± 0.01[b]
20:5n-3	1.74 ± 0.96[a]	tr	tr	0.09 ± 0.02[b]
22:1n-11	0.04 ± 0.02	tr	nd	nd
22:1n-7	0.06 ± 0.01	nd	tr	0.11 ± 0.05
22:2	tr	nd	0.13 ± 0.12	0.19 ± 0.05
22:2n-6	0.09 ± 0.05	nd	nd	0.06 ± 0.02
21:5n-3	0.04 ± 0.03	nd	0.07 ± 0.04	nd
22:5n-6	0.30 ± 0.16	tr	tr	0.07 ± 0.02
22:5n-3	nd	0.89 ± 0.64	0.10 ± 0.09	0.14 ± 0.05
22:6n-3	2.17 ± 1.26	0.26 ± 0.22	0.38 ± 0.22	0.61 ± 0.44
24:1n-9	0.41 ± 0.22	0.05 ± 0.04	0.05 ± 0.03	0.54 ± 0.50
n-3/n-6	1.09 ± 0.46	11.61 ± 7.17	2.30± 1.04	1.26 ± 0.18

nd: no detected; tr: trace (<0.01 mg/g).

Means in the same row with different superscript are significantly different ($P < 0.05$).

Table 7. Fatty acid composition (mg fatty acid/g dry weight) in total lipid from digestive content of *Haliotis fulgens* at start of the experiment, fed the experimental diets and starved during 60 days (mean ± SE, n = 3).

Fatty acid	Start of the experiment	Diet LL	Diet HL	Starvation
14:0	2.32 ± 0.05^{b}	5.51 ± 0.77^{a}	2.69 ± 0.06^{b}	2.51 ± 0.06^{b}
16:0	18.52 ± 0.51^{a}	16.99 ± 1.28^{a}	13.23 ± 0.35^{b}	10.80 ± 0.19^{c}
16:1n-7	1.78 ± 0.05^{a}	0.39 ± 0.07^{b}	0.48 ± 0.02^{b}	0.44 ± 0.03^{b}
17:0	0.68 ± 0.02^{a}	0.45 ± 0.06^{b}	0.32 ± 0.02^{b}	0.75 ± 0.02^{a}
16:3n-3	0.15 ± 0.07^{b}	tr	nd	0.58 ± 0.04^{a}
17:1n-7	0.44 ± 0.02	0.38 ± 0.04	tr	tr
16:4	7.00 ± 0.09	9.87 ± 3.09	6.05 ± 0.69	11.29 ± 0.65
18:0	6.69 ± 0.21^{a}	4.19 ± 0.43^{b}	3.96 ± 0.09^{b}	4.08 ± 0.08^{b}
18:1n-9	14.75 ± 0.43^{a}	4.19 ± 0.43^{b}	12.89 ± 1.14^{a}	3.13 ± 0.22^{b}
18:1n-7	4.67 ± 0.06^{b}	12.11 ± 1.50^{a}	4.26 ± 0.56^{b}	5.60 ± 0.21^{b}
18:2n-6	21.94 ± 0.58^{a}	3.46 ± 0.59^{b}	22.13 ± 1.84^{a}	1.96 ± 0.11^{b}
18:3n-3	2.70 ± 0.33^{a}	1.34 ± 0.16^{b}	1.07 ± 0.02^{b}	0.17 ± 0.02^{b}
18:4n-3	0.09 ± 0.05^{b}	tr	0.08 ± 0.01^{b}	2.86 ± 0.09^{a}
20:1n-11	2.76 ± 0.06^{ab}	3.39 ± 0.08^{a}	2.49 ± 0.20^{b}	0.98 ± 0.13^{c}
20:1n-9	1.94 ± 0.04^{a}	0.71 ± 0.01^{b}	2.01 ± 0.02^{a}	0.60 ± 0.06^{b}
20:1n-7	0.31 ± 0.01^{b}	0.64 ± 0.08^{a}	0.40 ± 0.07^{ab}	0.34 ± 0.04^{b}
20:2n-6	0.57 ± 0.09^{b}	0.43 ± 0.10^{b}	3.10 ± 0.25^{a}	0.25 ± 0.02^{b}
20:3n-6	0.12 ± 0.06^{c}	0.20 ± 0.02^{c}	0.62 ± 0.07^{b}	4.11 ± 0.05^{a}
20:4n-6	2.47 ± 0.06^{b}	2.63 ± 0.51^{b}	4.50 ± 0.39^{a}	0.45 ± 0.08^{c}
20:3n-3	nd	0.33 ± 0.04^{b}	tr	4.96 ± 0.06^{a}
20:5n-3	2.72 ± 0.31^{ab}	3.40 ± 0.52^{a}	1.53 ± 0.16^{b}	nd
22:1n-11	0.51 ± 0.03^{a}	0.24 ± 0.07^{b}	0.22 ± 0.02^{b}	0.19 ± 0.02^{b}
22:1n-9	0.22 ± 0.05	nd	nd	0.13 ± 0.01
22:2	3.35 ± 0.76	3.51 ± 0.26	4.20 ± 0.51	4.50 ± 0.20
21:5n-3	0.49 ± 0.05^{c}	0.30 ± 0.02^{c}	1.41 ± 0.13^{a}	0.84 ± 0.03^{b}
22:5n-6	0.14 ± 0.07	0.09 ± 0.05	tr	nd
22:5n-3	1.75 ± 0.05^{b}	1.75 ± 0.14^{b}	1.32 ± 0.10^{b}	4.95 ± 0.30^{a}
22:6n-3	3.21 ± 0.32^{a}	0.55 ± 0.10^{b}	0.81 ± 0.44^{b}	2.00 ± 0.54^{ab}
n-3/n-6	0.44 ± 0.02^{c}	1.15 ± 0.07^{b}	0.21 ± 0.04^{d}	2.42 ± 0.10^{a}

nd: no detected; tr: trace (<0.01 mg/g).

Means in the same row with different superscript are significantly different ($P < 0.05$).