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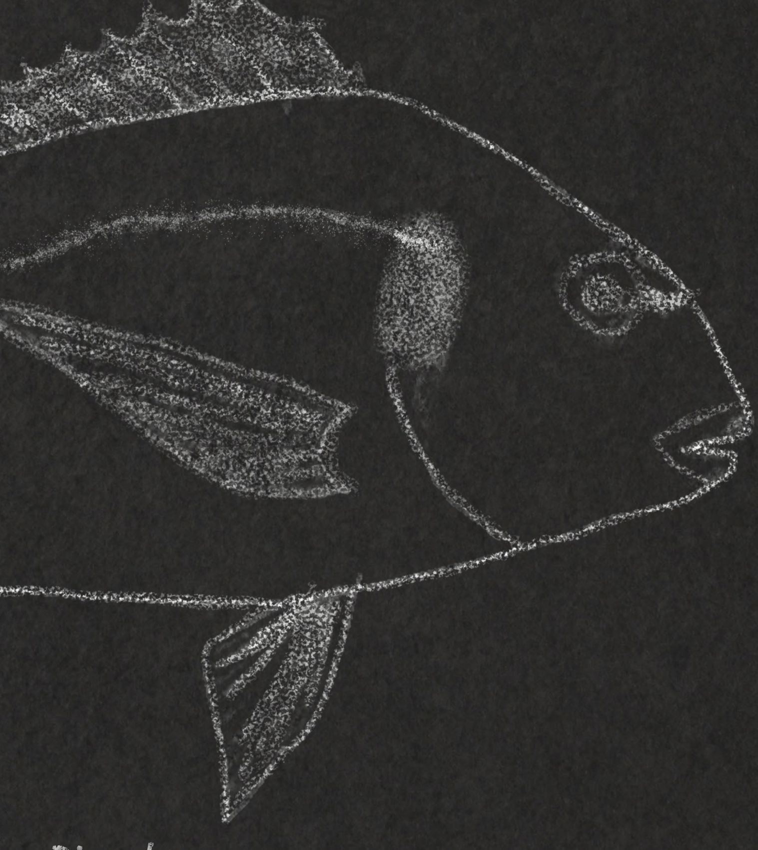
Necesidades de mantenimiento y crecimiento de la Dorada (*Sparus aurata* L.)



Ignacio Jauralde García
Septiembre 2015, Valencia

Directores:
Miguel Jover Cerdá
Silvia Martínez Llorens

Necesidades de mantenimiento y crecimiento de la Dorada (*Sparus aurata* L.)

Maintenance and growth requirements of the Gilthead sea bream (*Sparus aurata* L.)

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Informan:

Que la Tesis Doctoral titulada: Necesidades de mantenimiento y crecimiento de la Dorada (*Sparus aurata* L.) ha sido realizada por el Ingeniero Agrónomo D. Ignacio Jauralde García en el Departamento de Ciencia Animal bajo su dirección y que, una vez revisado y comprobado el trabajo, consideran que reúne los requisitos necesarios para la obtención del grado de Doctor, por lo que autoriza su presentación.

Y para que conste firman el presente informe en Valencia a 25 de 09 de dos mil quince.

Fdo. : Miguel Jover Cerdá

Fdo.: Silvia Martínez Llorens

Resumen

La dorada (*Sparus aurata*) es la especie piscícola que más se produce en los países mediterráneos, sin embargo no ha sido hasta hace relativamente poco cuando se empezaron a estudiar sus necesidades nutritivas reales.

El objetivo de la presente tesis fue desarrollar un modelo factorial que permita estimar las necesidades nutritivas en dorada considerando diversas situaciones de crecimiento para un rango de pesos de entre 20 y 450 gramos, así como diseñar piensos con una composición nutritiva óptima.

Para ello se realizaron 6 experimentos, con doradas de entre 23 g y 433g, a las que se alimento con tasas de alimentación crecientes desde ayunas hasta saciedad aparente, con el fin de determinar las curvas de respuesta, retención de proteína y retención de energía, y de crecimiento ante dosis crecientes de alimento.

Con los resultados obtenidos, se pudieron desarrollar modelos factoriales capaces de determinar las necesidades de crecimiento y con ellas definir los piensos que optimizan la eficiencia de los nutrientes ante diversas situaciones.

Los piensos actuales tienen una proporción de proteína digestible /energía digestible (PD/ED) subestimada, de entre 20 y 25 g MJ^{-1}, lo que produce un engrasamiento paulatino de la dorada. Un ratio de PD/ED de entre 25-30 g MJ^{-1}, parece más adecuado para optimizar las eficiencias y mejorar los índices de conversión alimentarios.

Abstract

The gilthead sea bream (*Sparus aurata*) is the fish specie more produced in the Mediterranean countries, however, the real nutrient requirements have not been studied until recently.

The aim of the present thesis was to develop a multi-factorial model that allow to estimate the nutritional requirements of the gilthead sea bream, considering several growth situations for the weight range between 20 and 450g, as well as to design diets with a optimum nutritive composition.

With that purpose, 6 trials were made, with gilthead sea bream ranged from 23 to 433g, fish were fed with increasing feeding rates from starvation to apparent satiation, with the aim of define the response curves of the protein retention, energy recovery, and growth next to the increasing feeding intakes.

With the results obtained, factorial models able to determine the growing requirements were developed. And with them diets optimizing the nutrient efficiency for several situations were defined.

The actual commercial diets with a ratio of digestible protein/digestible energy between 20 and 25 g MJ^{-1} are underestimated and produce a fat accumulation in the gilthead sea bream. A ratio of DP/DE between 25-30 g MJ^{-1} seems more adequate to optimize efficiencies and for improve the feed conversion ratios.

Resum

L'orada és l'espècie d'aqüicultura més produïda en el països de la mediterrània. No obstant, no fou fins fa ben boc quan es van començar a estudiar les seues necessitats nutritives reals.

L'objectiu d'aquesta tesi ha estat desenvolupar un model factorial que permeta estimar les necessitats nutritives de l'orada, considerant diferents situacions de creixement per a un interval de pesos entre els 20 i els 450 grams, a més de dissenyar pinsos amb una composició nutritiva òptima.

Per tal d'aconseguir aquests objectius, es realitzaren 6 experiments, amb orades d'entre 23 i 433 grams, les quals foren alimentades amb taxes d'alimentació creixents, des del dejuni fins la sacietat aparent, amb la finalitat de determinar les corbes de resposta, de retenció de proteïna i d'energia i de creixements amb dosis creixents d'aliment.

Amb els resultats obtinguts, es pogueren desenvolupar models factorials capaços de determinar les necessitats de creixement, amb les quals es puguen dissenyar els pinsos que optimitzen l'eficiència dels nutrients davant de diverses situacions.

Els pinsos actuals tenen una proporció de proteïna digestible / energia digestible (PD/ED) subestimada, d'entre 20 i 25 g MJ-1, produint un engreixament gradual de l'orada. Una ràtio PD/ED d'entre 25 i 30 g MJ-1 sembla més adequada per optimitzar l'eficiència i millorar els índexs de conversió alimentària.

Agradecimientos

Son muchas las personas a las que tengo que agradecer por la tesis y me temo que escribir unos agradecimientos es la forma más sencilla de ofender a alguien, lo que resulta muy fácil si se tiene una mente tan olvidadiza como la mía. Permítanme pues sintetizar al máximo generalizando, y agradecer por la tesis a familia, amigos, compañeros y colegas. Y en general, por si lo anterior no fuera suficiente, a cualquier persona que crea deba estar incluida en estas líneas.

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Introducción general

Introducción general

1.1 Generalidades

Actualmente en el mundo hay unos 7600 millones de personas, de los cuales 800 millones sufren malnutrición crónica (F.A.O. 2014). Se estima que en el 2050 la población mundial alcanzará los 9600 millones de personas (F.A.O. 2014). La acuicultura se ha planteado en las últimas décadas como una solución necesaria para el abastecimiento de alimento a la población mundial. Continuando con datos de la F.A.O. (2014), actualmente el consumo *per cápita* mundial está en torno a los 19 kilogramos al año, pero se prevé que aumente. Con la creciente demanda de pescado y la producción de los caladeros pesqueros estabilizada al máximo, es necesario para no comprometer el futuro de los recursos naturales, que la acuicultura deje de depender tanto de los peces enteros del medio natural, el balance nutricional de la acuicultura debe ser más favorable, es decir, aumentar la cantidad de pescado que produce la acuicultura en relación a la que está consumiendo.

En términos mundiales el pescado representa el 17% de la proteína ingerida de origen animal. Pero en muchos países puede llegar a representar más del 50% de la proteína animal consumida (F.A.O. 2014). El consumo de pescado proporciona energía, proteínas y una serie de nutrientes esenciales. Asimismo, los alimentos del medio acuático son esenciales como fuente de omega-3, especialmente del ácido eicosapentaenoico (AEP) y ácido docosahexaenoico (ADH). El pescado es una fuente excelente de proteínas, pero lo que hace que sea un alimento verdaderamente único son todos los nutrientes adicionales que contiene en cantidades considerables, tales como el AEP y ADH, el yodo, el selenio, el zinc, el hierro, el calcio, el fósforo y el potasio, así como de vitamina A y D y varias vitaminas del grupo B.

El sector de la acuicultura utiliza aproximadamente el 75% de la producción mundial de aceite de pescado. En la actualidad, no existen buenas fuentes alternativas de AEP y ADH. Se estima que en el momento de su sacrificio el pez conserva el 50% de los ácidos grasos omega 3 que haya consumido durante toda su vida. Pero se calcula que hasta un tercio de las materias primas provienen de desechos y subproductos, y por lo tanto no conllevan una sobre explotación, sino una mejora de utilización de los recursos disponibles. Gracias a los estudios de optimización de los piensos es posible que la acuicultura se convierta en un proveedor neto de ácidos grasos esenciales y valiosos, pero debido fundamentalmente a la gran producción de carpas.

Aunque la acuicultura sea sostenible, en términos globales, y pueda serlo la piscicultura, existen especies que pese a consumir más pescado del que producen su demanda por parte del mercado las cualifica como especies de interés, pero necesitan mejorar su balance nutricional para ser más sostenibles. En el caso de la dorada (*Sparus aurata* L.), los índices de conversión no han mejorado en los últimos años, lo que compromete su sostenibilidad y rentabilidad, debido a los crecientes precios de la harina de pescado.

Afortunadamente se ha avanzado mucho en la sustitución de la proteína por fuentes alternativas a la harina de pescado, y actualmente ya es posible técnicamente producir dorada sin alimentar con piensos hechos harinas de pescado.

Además de la sustitución de los aceites y harinas de pescado, la sostenibilidad de la alimentación es mejorable mediante la optimización de los niveles de nutrientes y las estrategias de alimentación, de forma tal que el aprovechamiento de los nutrientes sea óptimo.

1.2 Situación actual de la acuicultura mundial

Según datos de la F.A.O. (2014) en las últimas décadas ha habido un crecimiento espectacular de la disponibilidad total de pescado, debido principalmente, a la acuicultura, ya que la pesca extractiva se encuentra estabilizada por debajo de las 100 millones de toneladas por año, desde los años 90 (Figura 1).

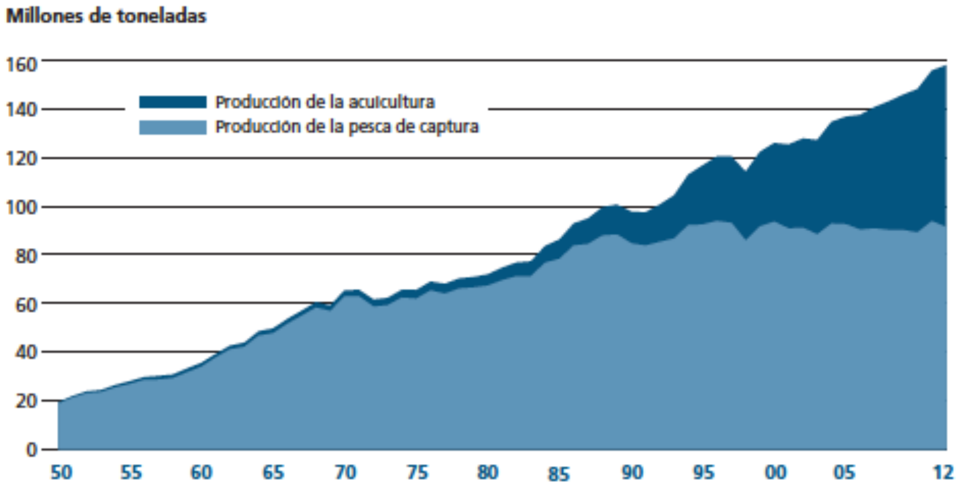

Figura 1.- Producción mundial de la pesca y la acuicultura. F.A.O. (2014)

La producción acuícola mundial, descontadas las algas, se duplico en los últimos años, pasando de los 32,4 millones de toneladas en el año 2000 a las 66,6 millones de toneladas en el 2012. Sin embargo, este aumento de la producción acuícola se debe fundamentalmente al aumento de la acuicultura continental China.

No todos los países aumentan su producción acuícola, en algunos de los países que son principales productores industrializados regionales, la producción de peces con escamas ha disminuido, sobre todo en los Estados Unidos de América, España, Francia, Italia, el Japón y la República de Corea. Se considera que la principal causa de esta disminución en la producción es debida a las importaciones por parte de terceros países, que producen a un menor costo.

1.3 La dorada en la acuicultura

En el 2014 se produjeron en el mundo unas 173 mil toneladas de doradas, un 4,3% menor que en 2013, de las cuales 109mil se producen en la unión europea, es decir el 63% aproximadamente. Esto representa aproximadamente 852 millones de euros anuales, de los cuales 565 corresponden a la Unión Europea (UE) es decir el 66% del valor de la producción de dorada se produce en Europa. Los 565 millones de euros sitúan a la dorada como el segundo producto de valor de la acuicultura en la UE por detrás del Salmon atlántico (779 millones de €).Pero aunque el volumen de producción crece anualmente a un ritmo del 4,8% su valor anual se ve reducido anualmente con un promedio del 4,5% (F.A.O. 2014)..

Esta situación de perdida de valor productivo pese al aumento de la producción no es generalizada entre especies, el salmón aumenta su valor productivo en un 0.7% anual, y la lubina pierde un 7%, pero debido a una perdida en volumen de producción.

Según datos de APROMAR (2015) en España la producción de dorada del año 2014 fue de 16mil toneladas, un 3,4% menos que el año anterior, y muy por debajo de su máximo de casi 24mil toneladas en el año 2008 (Figura 2), Aunque la producción no se ha recuperado de la crisis, los precios parecen estar recuperándose, en el 2014 el precio medio de 5.45 €/kg un 13,8% mayor a la del 2013. Sin embargo esta situación no es achacable enteramente a la crisis económica global, pues especies análogas como la lubina han recuperado e incluso superado los valores productivos anteriores a la crisis.

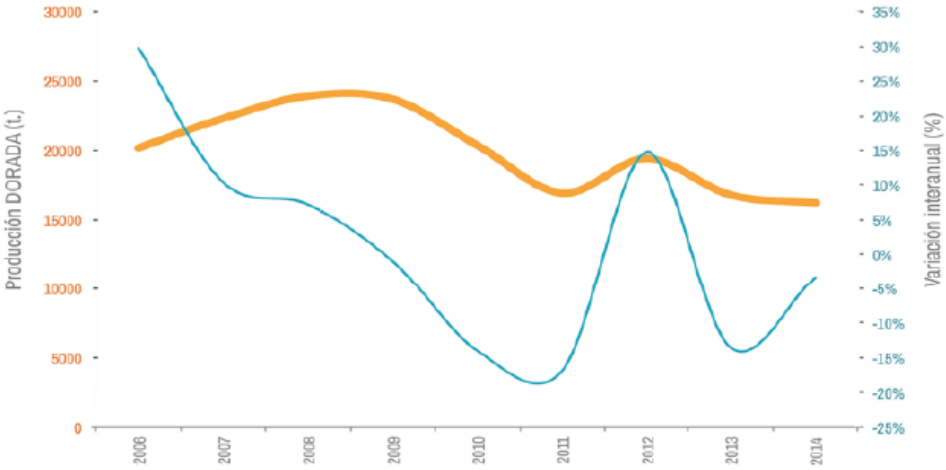

Figura 2.- Producción de dorada en España. APROMAR (2015)

Dentro de los países productores de dorada, la situación de España es bastante característica, es el único país gran productor y consumidor de dorada de la UE que importa más dorada de la que exporta, situación que comparte con Francia, que es un productor mucho más minoritario.

Los precios de la Dorada varían según tallas y según mes de venta. Pero el valor medio de la talla 400-600g, la más común comercialmente, se ha mantenido relativamente estable en los últimos años, entre 4 y 5 €/kg (Figura 3).

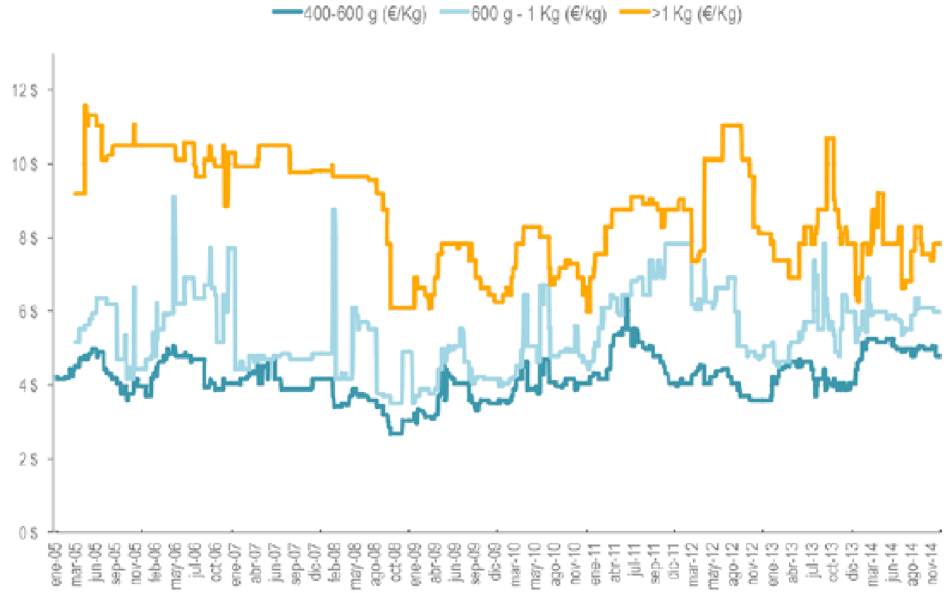

Figura 3.- Variación de precios de la dorada según talla mercamadrid. APROMAR (2015)

La rentabilidad de la dorada estuvo comprometida, con la llegada de la crisis en 2008 y la bajada de los precios de venta, el sector de la dorada y lubina entro en perdidas, de las que hoy en día todavía está intentando salir.

En recientes ponencias APROMAR ha declarado que la situación actual de la dorada está marcada por una perdida de valor productivo probablemente producida por el estancamiento de los parámetros técnicos de producción de dorada tales como el índice de conversión.

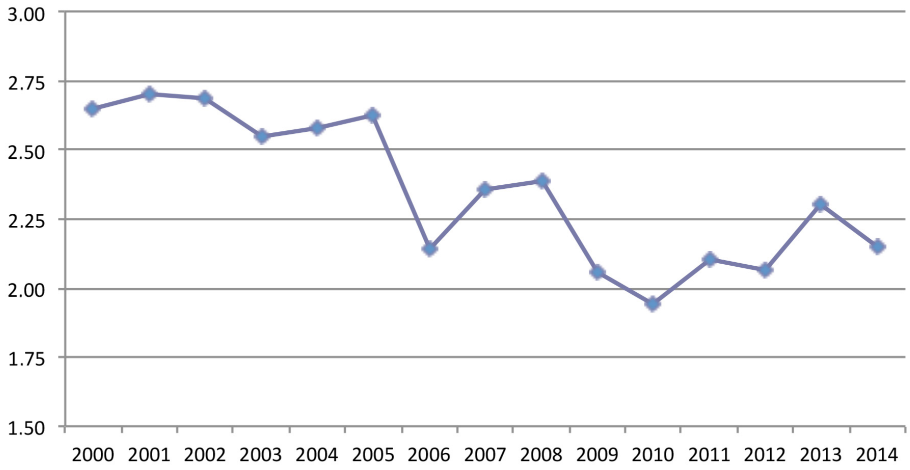

Figura 4.- Índices de Conversión Alimentario (elaboración propia con datos de APROMAR)

En el último informe de STECF (2014) se destaca que en los últimos años la subida de precios de alevines y alimentación ha contribuido de forma notable los resultados de economicos obtenidos (Figura 5).

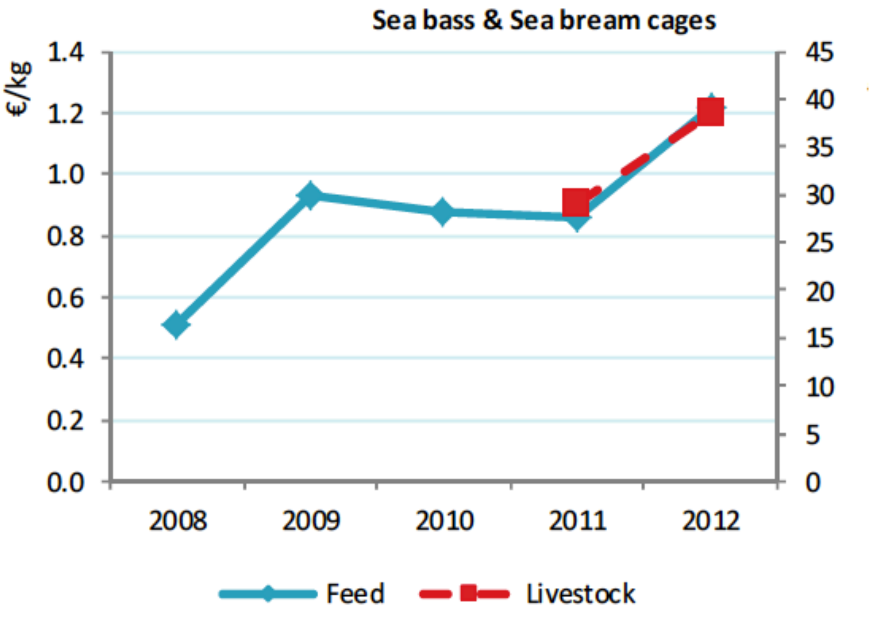

Figura 5.- Costes de alimentación y alevines. STECF (2014)

Pues los costes de producción fueron en 2011 y 2012 superiores a los ingresos (Figura 6).

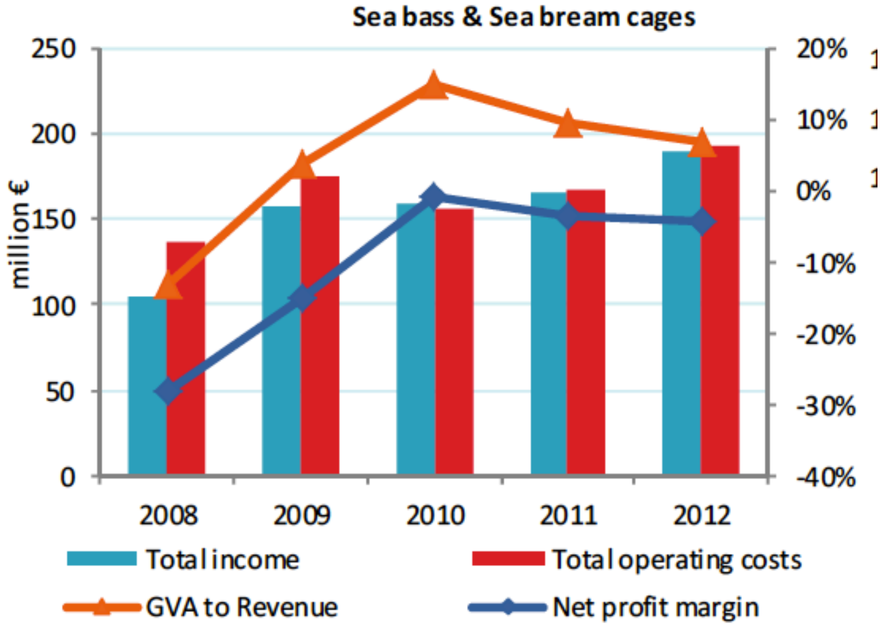

Figura 6.- Ingresos totales, costes de producción y margen de beneficio neto de la dorada y lubina. STECF (2014)

Lo que sí que parece evidente, es que la recuperación del rendimiento productivo y la rentabilidad, pasa necesariamente por una mejora de parámetros técnicos de alimentación y una optimización de la nutrición, pues los datos de la cadena valor de la dorada son preocupantes (Figura 7).

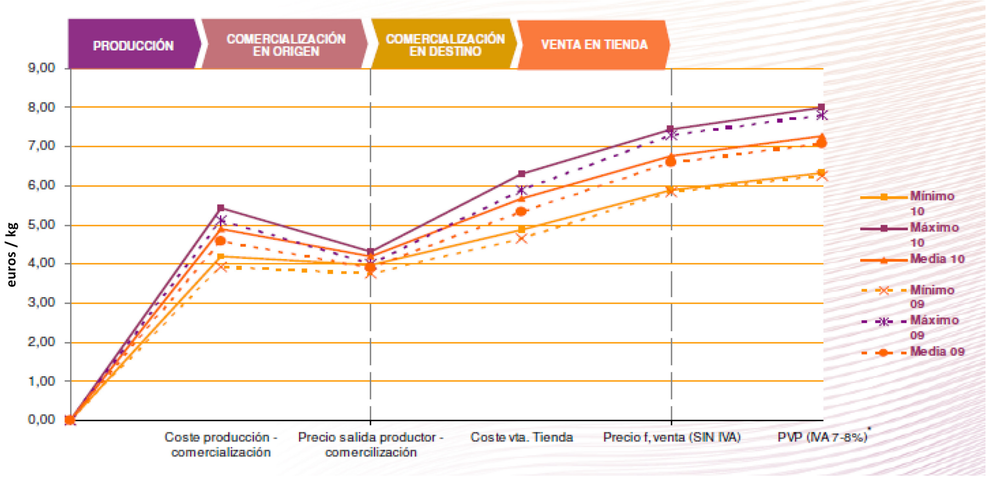

Figura 7.- Cadena de valor de la dorada, campaña 2010 y 2011 (Tragsatec 2012).

En 2014 se utilizaron en España 112.250 toneladas de pienso, un 3,9% más que el año anterior. La gran mayoría del pienso utilizado en acuicultura tiene como destino los peces marinos, el 84%.

Los costes de alimentación pueden suponer hasta aproximadamente el 50% del coste de producción de la dorada, lo que provoca la necesidad de una gran cantidad de dinero circulante para su funcionamiento, por lo que los problemas de caja son una constante habitual en este tipo de empresas. Por ello la reducción del coste de la alimentación es uno de los grandes retos de futuro de la producción de la dorada.

A priori, el pienso más barato no tiene por que ser el más rentable, como han demostrado numerosos estudios (Moñino *et al.*, 2003), pero los costes de financiación del dinero circulante para la obtención de pienso hacen que las empresas ejerzan presión sobre los fabricantes de pienso para conseguir piensos más baratos y competitivos, lo que no suele ser coincidente.

Si se valoran los nutrientes del pienso, la proteína es el nutriente que representa probablemente un mayor coste del pienso, seguido por lípidos y carbohidratos. De las materias primas utilizadas para alcanzar el nivel de nutrientes necesario, la harina de pescado es la principal responsable del precio del pienso, todo por lo que la optimización del nivel proteico y el contenido de harina de pescado en los piensos es fundamental para reducir los ICA.

1.4 La nutrición de la dorada

Durante los últimos años numerosas investigaciones han intentado mejorar la nutrición de la dorada focalizando los esfuerzos en abaratar los piensos, sustituyendo la harina y aceites de pescado por otras fuentes de menor coste, utilizando ingredientes de origen animal (Robaina *et al.*, 1997; Nenegas *et al.*, 1999; Martínez-Llorens *et al.*, 2008), pero fundamentalmente los trabajos han ido dirigidos a sustituir las harinas de pescado por fuentes vegetales, (Pereira and Oliva-Teles, 2002;2004; Gomez-Requeni *et al.*, 2004; De Francesco *et al.*, 2007; Bonaldo *et al.*, 2008; Martínez-Llorens *et al.*, 2009; Sánchez-Lozano *et al.*, 2007, 2009, 2010).

Por la misma razón se ha intentado disminuir el nivel de proteína en los piensos sin perjudicar el crecimiento. En teoría, un aumento de la energía del pienso permitiría ahorrar proteína, haciendo que la proteína del pienso vaya dirigida en mayor proporción a crecimiento en vez de destinarse a energía.

En las tablas 1 y 2 se recopilan los estudios sobre niveles de proteína y energía para dorada pre-engorde y engorde

Luquet and Sabaut (1974) estimaron un óptimo nivel de proteína para juveniles de dorada del 40% revisado posteriormente por (García-Alcázar *et al.*, 1995; Santinha *et al.*, 1999; Vergara *et al.*, 1996) que determinaron valores de entre el 45 y el 51 % de proteína, para pesos desde alevines hasta 146 gramos.

Tabla 1. Niveles de Proteína y Energía Digestible para dorada de hasta menos de 200 gramos

Autor (Peso inicial – Peso final)	**PD (g/Kg)**	**E.D. (MJ/Kg)**	**PD/ED (g / MJ)**	**TCI (%/d)**	**ICA**
Santinha et al. (1999) Saciedad (42 - 145 g)	438	19.4	22.6	1.65	1.56 c
	441	21.2	20.8	1.61	1.26 a
	489	20.3	24.1	1.67	1.41 b
	477	21.2	22.5	1.67	1.26 a
Lupatsch et al. (2001a) Saciedad (25 - 95 g)	374	17.1	21.9	1.19	1.61
	456	18.1	25.2	1.33	1.35
	377	18.2	20.7	1.39	1.27
	547	19.4	28.2	1.41	1.23
Lupatsch et al. (2001a) Saciedad (32 - 110 g)	459	18.9	24.3	1.30	1.26
	421	19.6	21.5	1.33	1.22
	390	20.4	19.1	1.24	1.33
	369	20.6	17.9	1.24	1.23
	339	21.6	15.7	1.19	1.27
Ekmann et al. (2002) (105 - 170 g)	416	19.1	21.8	1.28 b	1.40 b
	410	19.9	20.6	1.37 a	1.20 a
	418	20.4	20.5	1.40 a	1.20 a
	422	21.2	19.9	1.42 a	1.20 a
	427	21.8	19.6	1.55 a	1.15 a
	424	22.8	18.6	1.48 a	1.08 a
Fernandez et al. (2007) (1) Saciedad (2.5 - 30 g)	574	19.0	30.2	2.67 ab	1.37
	486	18.5	26.3	2.76 a	1.22
	401	18.2	22.0	2.53 b	1.35
García-Meilán et al. (2013) (1) (2) Saciedad (70 – 187 g)	297	19.4	15.3	0.94 c	1.38 c
	323	18.8	17.2	1.14 b	1.25 abc
	348	18.7	18.6	1.10 b	1.26 bc
	374	18.7	20.1	1.20 ab	1.16 ab
	399	18.6	21.5	1.22 ab	1.16 ab
	425	18.6	22.9	1.30 a	1.14 ab
	450	18.5	24.3	1.26 a	1.12 a

(1) ED calculada usando: 19.8 kJ/g proteína, 36.0 kJ/g lípidos y 13.3 kJ/g carbohidratos

(2) PD estimada con un 85% digestibilidad proteína

Tabla 2. Niveles de Proteína y Energía Digestible para dorada de hasta más de 200 gramos

Autor (Peso inicial – Peso final)	**PD (g/Kg)**	**E.D. (MJ/Kg)**	**PD/ED (g / MJ)**	**TCI (%/d)**	**ICA**
Vergara et al. (1996) (1) (70 - 400 g)	400	18.6	21.5	0.94 b	1.41-1.54
	398	20.4	19.5	0.98 a	1.41-1.54
	405	21.3	19.0	0.97 a	1.41-1.54
Moñino et al. (2002) (1) Tasa Alimentación (24 - 417 g)	378	15.9	23.8	1.17 b	2.21 c
	395	16.8	23.5	1.17 b	1.78 b
	369	17.4	21.2	1.16 b	1.64 b
	420	23.1	18.2	1.25 a	1.38 a
Bonaldo et al. (2010) (1) (2) Saciedad (73 – 245 g)	439	19.6	22.4	1.54 a	1.33 b
	431	20.8	20.7	1.45 abc	1.35 bc
	425	23.1	18.4	1.49 ab	1.44 c
Bonaldo et al. (2010) (1) (2) 80% saciedad (73 – 224 g)	439	19.6	22.4	1.35 c	1.17 a
	431	20.8	20.7	1.32 bc	1.13 a
	425	23.1	18.4	1.40 abc	1.13 a
Ekmann et al. (2013) Tasa Alimentación (140 - 350 g)	344	19.9	17.3	0.96	1.48 b
	331	21.2	15.6	1.02	1.32 a
	334	21.4	15.6	1.02	1.27 a
	363	19.6	18.5	1.01	1.42 b
	363	20.7	17.5	0.99	1.38 ab
	361	21.6	16.7	1.01	1.29 a
	405	19.7	20.6	1.03	1.38 b
	395	20.6	19.1	1.05	1.29 ab
	395	21.3	18.5	1.04	1.24 a
Mongile et al. (2014) (2) Saciedad (75 – 297 g)	391	17.8	22.0	1.54	1.53 b
	394	18.3	21.5	1.58	1.38 a
	389	18.8	20.7	1.54	1.37 a
	392	19.3	20.3	1.54	1.38 a
	399	19.8	20.1	1.51	1.38 a

(1) ED calculada usando: 19.8 kJ/g proteína, 36.0 kJ/g lípidos y 13.3 kJ/g carbohidratos

(2) PD estimada con un 85% digestibilidad proteína

Respecto a los lípidos los mejores crecimientos se han observado con niveles del 15-17% de lípidos. (Vergara and Jauncey, 1993; Vergara *et al.*, 1996; Bonaldo *et al.*, 2010; Mogile *et al.*, 2014).

Actualmente, los fabricantes de pienso recomiendan piensos de engorde para dorada con unos niveles de proteína que varían entre el 40 y el 47%, con niveles de energía de entre 16 MJ/kg a 22 MJ/kg, con niveles de lípidos que varían entre el 14% y el 21% y con una relación de proteína digestible energía digestible (PD/ED) que varia entre 24 a 20 g MJ^{-1}. Para los peces de menor tamaño se recomiendan mayores niveles de proteína, mientras que para los peces de un mayor tamaño se recomienda una mayor proporción de lípidos en el pienso.

Sin embargo los niveles de proteína y energía en el pienso no corresponden a necesidades especificas del pez, que serían cubiertos o no dependiendo de la cantidad de pienso que sea consumido. Para que el pienso este optimizado, es necesario calcular las necesidades entendidas como gramos diarios de proteína o nitrógeno que necesita el pez o kJ diarios de energía. Debido a casi el infinito número de combinaciones entre diversos pesos, temperaturas y otros factores, en los últimos años se ha desarrollado el estudio de las necesidades nutritivas mediante estudios factoriales, cuyas bases fueron estudiadas por Pfeffer, & Pieper, (1979) y Brett & Groves (1979).

Baker (1986) y Baldwin (1985) apuntaron las ventajas de los métodos factoriales que permitirían extrapolar resultados a varias situaciones y rangos, una vez identificado el efecto de los factores. Posteriormente por Shearer (1995), estudio y comprobó la utilidad de los métodos factoriales como alternativa a estudiar el infinito número de combinaciones posibles.

Lupatsch *et al.*, (1998) realizó el primer estudio factorial de las necesidades nutritivas de la dorada descomponiendo las necesidades totales de proteína digestible (PD) y energía digestible (ED) de la dorada en necesidades de mantenimiento (en función del coeficiente metabólico b) y necesidades netas de crecimiento, función del incremento de proteína o energía y la eficiencia neta de retención (*k*):

$$ED = ED_{man} \text{ x } BW(kg)^{b} + k_{e} \text{ x incremento de energía}$$

$PD= PD_{man} \times BW(kg)^{b} + k_p \times$ incremento de proteína

1.4.1 Coeficientes metabólicos

Los coeficientes metabólicos de la proteína y de la energía se estudian generalmente mediante la función alométrica que relaciona la de perdida de proteína y energía con respecto al peso del pez. La perdida de energía corresponde al gasto metabólico basal y la actividad voluntaria.

El empleo de coeficientes metabólicos para el cálculo de las necesidades de mantenimiento, supone asumir que la proporcionalidad entre el consumo de energía y proteína respecto al peso en ayunas es igual a la proporcionalidad de las necesidades de energía de mantenimiento y proteína de mantenimiento respecto al peso y por lo tanto a las necesidades de mantenimiento son constantes respecto al peso metabólico estimado en ayunas. De esta forma los coeficientes metabólicos se estimaron en Lupatsch *et al.* (1998) fueron:

Peso metabólico de la energía= $kg^{0.83}$
Peso metabólico de la proteína = $kg^{0.70}$

Posteriormente, Lupatsch *et al*. (2003a) revisó el coeficiente metabólico de la energía a 0.82, aunque admitiendo el valor genérico de 0.80 en Lupatsch *et al.* (2003b). Este valor fue aceptado como valor aproximado por Booth *et al.* (2007) en *Pagrus auratus* quien mantuvo el valor de 0.7 para proteína y 0.84 para energía.

1.4.2 Necesidades de Mantenimiento y Eficiencias

Las necesidades de energía de mantenimiento corresponden, habitualmente, a la suma de las necesidades energía para el gasto metabólico más la gastada en la actividad voluntaria. Sin embargo, dada la dificultad para controlar el gasto energético por separado, generalmente se asume que este valor puede aproximarse a la cantidad de energía que necesita consumir el pez para que la energía retenida sea igual a cero. De forma análoga se puede determinar la proteína de mantenimiento.

Lupatsch *et al*. (1998) mediante el estudio de las retenciones en ejemplares de entre 29.7 y 131.1g estimaron las necesidades diarias de mantenimiento denla dorada como:

$ED_{man} = 55.8\ kJ\ kg^{-0.83}$

$PD_{man} = 0.86\ g\ kg^{-0.70}$

La eficiencia neta de la energía se puede definir como la proporción de energía consumida, descontada la energía de mantenimiento, que es finalmente retenida como energía y generalmente calculada como $k_e = ER / (EI - E_{man})$. Donde ER es la energía retenida y EI es la energía ingerida (generalmente energía digestible) y E_{man} la energía de mantenimiento. De forma análoga se puede determinar la eficiencia neta de la proteína.

Las eficiencias netas por encima de mantenimiento obtenidas por Lupatsch *et al*. (1998) fueron constantes e independientes de la ingesta:

$k_e = 0.46$

$k_p = 0.28$

Lupatsch *et al*. (2001) continuaron examinando los requerimientos de la dorada, en este caso mediante un estudio con 15 dietas distintas, de entre 339 a 549 g kg^{-1} de proteína, niveles de energía de entre 10 y 21.57 MJ kg^{-1} y con ratios de Proteína digestible / Energía digestible (PD/ED) de entre 17.9 y 34.3 g MJ^{-1}, mediante las retenciones de energía y proteína en doradas de entre 17 y 109g.

En este trabajo, se demostró la independencia del ratio PD/ED del pienso respecto total de energía consumida entre ciertos rangos, pues por debajo de 16 MJ/kg de energía en el pienso, el consumo de pienso se veía penalizado, mientras que en piensos con relaciones de PD/ED por encima de 16 el pez era capaz de regular su ingesta para igualar la cantidad total de ED ingerida. No ocurría lo mismo con la proteína, pues la ingesta voluntaria no era aumentada o disminuida para conseguir un nivel determinado de ingesta de proteína, como ya habían demostrado otros antes (Boujard & Médale 1994).

Garcia-Meilan *et al.* (2013) corroboraron la capacidad adaptativa de la dorada a distintos niveles de proteína y energía de los piensos, siempre y cuando la energía este por encima de 15 MJ/kg, sin que parezca haber un limite superior de energía o proteína.

La eficiencia parece no depender tampoco de los ratios de PD/ED, la eficiencia neta de la proteína (k_p) solo depende del nivel de proteína consumida. Mientras que la eficiencia neta de la energía (k_e) parece constante e independiente de la ingesta. En (Lupatsch *et al.*, 2001), las eficiencias obtenidas fueron:

k_e= 0.5
k_p= 0.33-0.60

donde se estimó que una k_p = 0.47 era la que mejor eficiencia que producía un crecimiento máximo. En esta ocasión no se determinaron necesidades de mantenimiento.

Posteriormente Lupatsch *et al.* (2003a) estudiaron de nuevo las necesidades nutritivas de la dorada de entre 21.4 y 133g a dos temperaturas 20.5 y 28ºC. Usando piensos con niveles energéticos de entorno a 16 MJ/kg y ratios variables de PD/ED de entre 21 a 28 g/MJ.

Al igual que en los estudios anteriores, el ratio PD/ED no pareció tener influencia en las eficiencias netas de la energía y de la proteína. Pero en este trabajo además se pudo estudiar el efecto de la temperatura sobre las curvas de retención de energía y proteína. En concreto la temperatura no tuvo efecto en las necesidades de PD_{man} ni sobre la eficiencia neta de la proteina (k_p) ni de la energía (k_e). La PD_{man} tuvo un valor constante para todas las temperaturas

PD_{man}= 0.62 (g $kg^{-0.7}$ $día^{-1}$)

En cuanto a la energía digestible de mantenimiento se establecieron distintas necesidades para las dos temperaturas:

ED_{man} (21ºC)= 47.35 kJ $kg^{-0.82}$
ED_{man} (28ºC)= 84.54 g $kg^{-0.82}$

Utilizando la Energía Digestible de mantenimiento a 24ºC obtenida en Lupatsch *et al*. (1998) pudieron establecer la siguiente interpolación:

ED_{man} = 16.6*$e^{0.055*T^a}$ kJ $kg^{-0.82}$

La Eficiencia de la proteína *(k_p)* resultó variable dependiendo de la ingesta de proteína digestible y, como ocurría en el trabajo de Lupatsch *et al*. (2001), se estimó que una k_p=0.47 era la máxima eficiencia posible para no penalizar el crecimiento. La eficiencia de la energía (k_e) fué constante con la temperatura y la ingesta:

k_e= 0.67
k_p=0.33-0.8

1.4.3 Modelos de crecimiento

Para determinar la relación de proteína y energía que debe tener un pienso que cubra exactamente las necesidades nutritivas de proteína y energía, se deben conocer, además de las necesidades de mantenimiento de las doradas, el crecimiento esperado y en consecuencia la energía y proteína que se espera ganar en un determinado tiempo. Para ello se necesita un modelo de crecimiento, y la composición corporal de las doradas en diferentes pesos.

Lupatsch *et al.* (1998, 2003a) en sus estudios factoriales de las necesidades de la dorada estimaron que el crecimiento máximo esperado en función de la temperatura se puede determinar mediante la formula :

$$\Delta \text{Peso}=0.024*\text{Peso}^{0.514}*e^{(0.06*T^{a})}$$

En realidad no hay ninguna razón por la que no se pueda utilizar cualquier otro modelo de crecimiento para predecir el peso final dentro de un modelo factorial. Brett and Grove (1979) indicaron, que las tres variables más importantes en los que debe apoyarse un modelo de crecimiento son, el peso del pez, la temperatura del agua y la ingesta. Sin embargo la mayoría de modelos contemplan la temperatura y el peso pero no la ingesta (Petridis & Rogdakis 1996; Cho & Bureau 1998; Kaushik 1998; Lupatsch *et al.*, 1998, 2003; Mayer *et al.*, 2008; Mayer *et al.*, 2009), asumiendo que la ingesta es tal que produce una repuesta de crecimiento máxima para un determinado peso y temperatura.

Hernández *et al.* (2003) propuso un modelo teórico que involucraba las tres variables, peso, temperatura e ingesta, en forma de funciones. Pero las expresiones sobre la ingesta, por su complejidad, eran finalmente simplificadas en el modelo final haciendo referencia a una proporción sobre una ración estándar. El modelo completo resulta demasiado complejo, pero puede ser resumido de la siguiente forma:

$\Delta Peso = \Gamma f1(w) f2(\theta) f3(r)$

donde Γ hace referencia a todos aquellos parámetros no recogidos en las otras 3 funciones, *f1* es la influencia del peso *f2* de la temperatura y *f3* de la alimentación.

De entre los numerosos tipos de modelos disponibles, parecen destacar aquellos basados en sumatorios de temperaturas como el Coeficiente Térmico de Crecimiento (CTC o *TGC, Thermal Growth Coefficient*) Basados en los modelos de Cho (1992) por que parecen ser independientes del peso y la temperatura y los más acertados dentro de parámetros normales (Jobling 2003; Dumas 2010). Este modelo fue estudiado para dorada por Mayer *et al.* (2008) donde el Peso final (P_f) se calcula en base al peso inicial (P_i), el sumatorio de temperaturas por encima de la temperatura mínima efectiva (ΣT_{ef}) y el número de días.

$Pf = (Pi^{1/3} + CTC * \Sigma T_{ef} * días)^3$

No obstante, sea cual sea el modelo utilizado es conveniente aplicarle parámetros de valoración económica para evaluar el punto de óptimo de crecimiento, como en los trabajos de Martínez-Llorens *et al.* (2007), donde se desarrolla el Índice de Beneficio Económico (IBE) o *Economic Profit Index* (EPI), como una forma de evaluar el valor añadido producido por el engorde de un pez.

$IBE = \Delta Peso \times Precio\ de\ venta - \Delta Peso * ICA * Precio\ del\ pienso$

Donde el ICA es el índice de conversión alimentario o FCR (*Feed conversión Rate*)

1.4.4 Composición corporal

Otro punto clave en el diseño factorial de piensos, es la obtención de la composición corporal en función del peso. Conociendo las variaciones entre las composiciones corporales de dos pesos se pueden estimar la retención de energía y proteína y con ello determinar las necesidades de ingesta para crecimiento neto. Hasta el momento, la evaluación más completa de la composición corporal de las doradas en función del peso ha sido realizada por Lupatsch *et al.* (1998, 2003a) mediante ecuaciones alométricas del tipo $Y=aX^b$ de forma que el nivel de proteína permanecía constante con el peso mientras que lípidos y energía aumentaban en relación inversa con la humedad, resultando las siguientes ecuaciones:

Proteína (mg/g) = 176
Energía (kJ/g) = $4.66*\text{Peso}(g)^{0.139}$
Lípidos (mg/g) = $43.3*\text{Peso}(g)^{0.243}$
Humedad (mg/g) = $777*\text{Peso }(g^{)-0.041}$

Este tipo de ecuaciones pueden linearizarse con facilidad para determinar sus coeficientes y estudiar de su significancia estadística, mediante la formula Log y = Log a + b* Log(x), pues la conveniencia de usar la forma linealizada fue demostrada por White (2011) y por Dumas *et al.* (2010).

Lupatsch *et al.* 1998, y posteriormente ampliado en 2003^a, encontró que

Basándose en los valores de proteína y energía de mantenimiento, en las eficiencias netas de crecimiento, y considerando los crecimientos esperados y las composiciones corporales medias, Lupatsch *et al.* (2003a) pudieron estimar el valor óptimo del ratio proteína digestible / energía digestible (PD/ED) en piensos para doradas en un amplio rango de pesos y temperaturas (Tabla 3).

Tabla 3.- Necesidades nutritivas y ratio PD/ED para doradas a 23ºC. (Lupatsch *et al*., 2003a)

Peso (g)	**10**	**50**	**100**	**300**
ED_{man} (kJ pez^{-1} $día^{-1}$)	1.35	5.04	9.33	22.97
ED_{crec} (kJ pez^{-1} $día^{-1}$)	2.98	8.52	13.40	27.46
ED_{tot} (kJ pez^{-1} $día^{-1}$)	**4.33**	**13.46**	**22.73**	**50.43**
PD_{man} (g pez^{-1} $día^{-1}$)	0.025	0.076	0.124	0.267
PD_{crec} (g pez^{-1} $día^{-1}$)	0.117	0.267	0.361	0.315
PD_{tot} (g pez^{-1} $día^{-1}$)	**0.141**	**0.343**	**0.505**	**0.938**
PD/ED (g MJ^{-1})	**32.5**	**25.4**	**22.6**	**19.0**

1.4.5 Otras especies

La eficacia del modelo factorial para los estudios de las necesidades nutritivas de otras ha sido utilizado por otros autores.

Kaushik, (1998) estableció las bases metodológicas para el estudio bioenergéticos de las necesidades para peces no salmónidos, basándose en modelos de crecimiento según el CTC, composiciones corporales necesidades de mantenimiento, y/o incrementos calóricos de la alimentación, perdidas fecales, y incrementos de nutrientes y energía.

Watanabe *et al*. (2000), estudiaron las necesidades de la *Seriola quinqueradiata*, pero sin utilizar los coeficientes metabólicos de la proteína y energía, sino determinándolo por kilogramos de pez vivo. Obtuvieron una eficiencia bruta de la energía metabolizable del 54-55%. Los resultados de necesidades de energía y proteína obtenidos se resumen en la Tabla 2.

Tabla 4.- Necesidades de la Seriola quinqueratiada. (Watanabe *et al.*, 2000).

Peso (g)	**8**	**63**	**160**	**237**	**280**
Temperatura (ºC)	27.1	27.1	24.6	25	21.7
ED_{tot} (kcal kg^{-1} dia^{-1})	225.3	135.5	124.8	110.5	91.5
PD_{tot} (g kg^{-1} dia^{-1})	21.7	14.8	11.2	10.7	8.2
ED_{man} (kcal kg^{-1} dia^{-1})	39.5	40.8	25.4	35.5	19.0
PD_{man} (g kg^{-1} dia^{-1})	5.4	2.8	1.0	2.9	1.7

Lupatsch *et al.* (2001b; 2003b), estudiaron también las necesidades de energía de la lubina (*Dicentrarchus labrax*) y del mero (*Epinephelus aeneus):*

Dicentrarchus labrax	k_e=0.68
Epinephelus aeneus	k_e=0.69

Dicentrarchus labrax	ED_{man}=45.38 kJ kg$^{-0.8}$
Epinephelus aeneus	ED_{man}=34.05 kJ kg$^{-0.8}$

Dicentrarchus labrax	k_p=0.53
Epinephelus aeneus	k_p=0.56

Azevedo *et al.* (2004a; 2004b, 2005), estudiaron los requerimientos nutritivos de salmónidos mediante aproximaciones factoriales y obtuvieron una eficiencia neta de la proteína:

k_p= 0.51

Booth *et al.* (2007) obtuvieron, en *Pagrus auratus,* coeficientes metabólicos de la proteína de 0.754 pero decidieron usar el valor obtenido por

Lupatsch *et al.* (2003a), además obtuvieron unas necesidades de proteínas de mantenimiento que dependían del ratio PD/ED del alimento siendo mayores con el ratio PD/ED menor.

$PD_{man} = 0.87\text{-}1.45 \text{ g kg}^{-0.7}$

Y unas eficiencias netas de la proteína mayores cuando mayor es el ratio PD/ED

$k_p = 0.29\text{-}0.38$

Además encontraron que un aumento en la relación PD/ED de la dieta producía un menor engrasamiento de los peces y que por debajo de 21-23 g MJ^{-1} de ratio PD/ED la retención de proteína se veía penalizada.

Glencross, (2008), realizó un estudio factorial en *Lates calcarifer*, y encontró energías de mantenimiento variables en función del peso, pero estimó el dato medio diario de mantenimiento en:

$ED_{man} = 42.6 \text{ kJ kg}^{-0.8}$

Y una PD_{man} media diaria de los peces de:

$PD_{man} = 0.45 \text{ g kg}^{-0.7}$

Asimismo, determinó una eficiencia neta de retención de energía digestible dependiente del tamaño de los peces:

Peces pequeños (<1000g) $k_e = 0.61$
Peces grandes (>1000g) $k_e = 0.76$

Para la retención de la proteína utilizó dos curvas distintas en función del tamaño de los peces, con eficiencias variables en función de la ingesta y separadas para peces grandes y pequeños con unas eficiencias medias de retención neta de proteína:

k_p= 0.48-0.49

Finalmente, en función del tamaño de los peces la relación PD /ED óptima fue estimada en 30 g MJ^{-1} para peces pequeños (<1000g) y 20 g MJ^{-1} par peces grandes (>1000g).

CAPÍTULO 2

Justificación y objetivos

Justificación y objetivos

Con el desarrollo de los modelos factoriales se pueden estudiar de una forma más científica las necesidades nutritivas diarias de los peces, expresadas en función de los pesos del pez y no como niveles óptimos en los piensos. En el caso de la Dorada, los trabajos desarrollados por Lupatsch *et al*. (1998; 2001a; 2003a), permiten el estudio de las necesidades para temperaturas entre 21 y 28ºC, y un rango de pesos de entre 10 y 150 gramos aproximadamente., pues si bien los modelos de composición corporal abarcan hasta un peso comercial de 450 gramos, los estudios de retención de energía y proteína se realizaron siempre con peces pequeños de hasta unos 150 gramos de peso final. Teniendo en cuenta que el peso comercial de la dorada ronda los 400 g y que aproximadamente el 80% del pienso es consumido entre 100 y 400 gramos, parece importante determinar las necesidades nutritivas para este rango de pesos.

Además, hay que considerar las situaciones en las que se alimenta la dorada no para máximo crecimiento sino para una óptima utilización del alimento. Por ejemplo, es habitual que durante el primer verano del ciclo de crecimiento de la dorada se intente conseguir un crecimiento máximo, pero el segundo año se priorice mejorar el índice de conversión.

Por todas estas razones el objetivo de la presente tesis fue desarrollar un modelo factorial que permita estimar las necesidades nutritivas en dorada (*Sparus aurata*), considerando diversas situaciones de crecimiento para un rango de pesos de entre 20 y 450 gramos, así como diseñar piensos con una composición nutritiva óptima.

CAPÍTULO 3

Resumen de experimentos

Resumen de experimentos

Con el propósito de estimar el crecimiento y retención de nutrientes de la dorada alimentadas a diferentes niveles de ingesta para todo el ciclo de producción, desde 20 a 450 gramos, se realizaron seis experimentos (Tabla 1).

Los experimentos que componen la presente tesis se pueden resumir en la siguiente tabla:

Tabla 1.- Resumen de experimentos.

	Exp. 1	**Exp. 2**	**Exp. 3**	**Exp. 4**	**Exp. 5**	**Exp.6**
Peso inicial (g)	23.6	38	49.6	110.2	220.1	288.9
Peces / tanque	25	25	30	12	13	12
Replicas	2	2	3	3	2	2
Tasas de alimentación previstas (%)	0 / 1.5 / 3 / 4.5	0 / 0.5 / 1 / 1.6 / 2.2 / 2.8	0 / 1 / 2 / 3	0 / 1 / 2 / 3	0 / 0.5 / 1 / 1.5 / 2	0 / 0.5 / 0.8 / 1.5 / 2.2
Temperatura (ºC)	21.5	22	25	25	22	23
Duración (días)	84	90	83	83	90	64

En todos los experimentos se realizó un control de pesos quincenal y las raciones se recalculaban semanalmente según la biomasa estimada por las previsiones de crecimiento. La ración se dividió en dos tomas y cuando no todo el pienso era consumido, al final del día se pesaba el pienso sobrante para recalcular la tasa de alimentación real.

El pienso utilizado fue el ECOPLUS de DIBAQ (www.dibaq.com) con las siguientes características:

Proteína bruta: 43%

Grasa bruta: 21%

Cenizas: 12%

Humedad: 8%

Energía bruta: 21 MJ kg^{-1}

Ingredientes: Harina de pescado, aceite de pescado, trigo y subproductos, harina de soja, premix vitamínico-mineral, antioxidante y antifúngico.

CAPÍTULO 4

A proposal for modeling the thermal-unit growth coefficient and feed conversion ratio as functions of feeding rate for gilthead sea bream (*Sparus aurata,* L.) in summer conditions.

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A proposal for modeling the thermal-unit growth coefficient and feed conversion ratio as functions of feeding rate for gilthead sea bream (*Sparus aurata,* L.) in summer conditions.

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Abstract

Modeling growth as a function of feeding rate (FR) could be one of the most important tools for fish farms because this knowledge allows growth to be maximized, or the feed conversion rate to be minimized, thereby improving profits. All growth models should include the three principal variables involved in growth: initial body weight, temperature and feeding rate. The thermal-unit growth coefficient (TGC) already includes water temperature variation and initial body weight. Studying variation in TGC for fish fed the same diet but at several feeding rates provides interesting information for modeling. Six different trials were conducted where gilthead sea bream of several different initial weights (24, 38, 50, 110, 220 or 289 g) were fed increasing amounts, and growth and the conversion index response were measured. The TGC response was modeled as a function of FR, and both asymptotic and quadratic responses were examined. The asymptotic model, TGCx1000 = 2.037*(1-exp $^{(-0.8*(FR-0.22))}$), had an adjusted R^2 value of 96.18, whereas the quadratic model, TGCx1000 = -0.381 + 1,715xFR – 0,382xFR^2, $TGC \times 1000 = -0.381026 + 1.71554 \times FR - 0.3821 \times FR^2$ had an adjusted R^2 value of 96.42. Simulations of the feed conversion rate (FCR) and the economical profitability index (EPI) were conducted to provide tools for maximizing efficiency and profitability, and the results suggest that these tools will be useful for future investigations.

Keywords

Sparus aurata, modeling growth, TGC, feed conversion ratio, feeding rate.

Introduction

The feeding rate (FR) of fish is one of the most important factors in commercial fish farming because it may impact both the feed conversion ratio (FCR) and the specific growth rate (SGR). Moreover, knowledge of the “optimum” feeding rate is important for promoting the best growth and feed conversion ratio, which are essential for preventing water quality deterioration as a result of excess feeding and for improving the sustainability of aquaculture (Naylor *et al.,* 2009). Brett & Grove (1979) pointed to the need for modeling fish growth as a function of three major parameters that influence it: feed intake, weight and temperature; however, models predicting growth have usually considered only body weight and temperature but not feeding rate (Petridis & Rogdakis, 1996; Cho & Bureau, 1998; Kaushik, 1998; Lupatsch *et al*., 1998, 2003; Mayer *et al*., 2008 and 2009).

The Von Bertalanffy equation is probably the most-studied and applied equation for describing and predicting growth in ectotherms (Ricker, 1979; Rosa *et al*., 1997; Hernandez-Llamas & Ratkowsky, 2004; De Graaf & Preein, 2005). The specific growth rate (SGR), which is also a special case of the Von Bertalanffy equation (Dumas *et al.,* 2010), is actually the most-used parameter to describe growth, even though it is influenced by body weight and temperature (Petridis *et al*., 1996; Mayer *et al*., 2008). More accurate models have been developed to predict growth as a function of temperature and initial body weight. The thermal-unit growth coefficient (TGC), which is another special case of the Von Bertalanffy equation (Dumas *et al*., 2010), is also widely used (Cho 1992). The TGC parameter is theoretically independent of initial body weight and temperature, and if the parameters are within the regular range of conditions (Jobling, 2003), TGC should provide the most accurate method for studying variation in growth with feeding rate (Dumas *et al.,* 2010). Nevertheless, Dumas *et al.* (2007) have recently reported that the TGC is not independent of initial body weight, and they presented three different values for rainbow trout for three growth periods: the first from 0.2 to 20 g, the second from 20 to 500 g and the third >500 g.

Another option for modeling growth is to use multiple regression models that include body weight and temperature as explanatory variables (Petridis *et al*., 1996; Lupatsch *et al*., 2003; Mayer *et al*., 2008), but in any case feeding rate should be considered. Lupatsch *et al.* (2003) used a multiple regression model to develop a bioenergetic model of protein and energy requirements. Mayer *et al.* (2008) examined five growth models in gilthead sea bream and concluded that models including the "sum of effective temperature" seemed to provide more accurate than those that included the "daily mean temperature".

Libralato & Solidoro (2008) built a bioenergetic model for gilthead sea bream based on energy inputs and losses using previously published data.

A theoretical approach to a complete growth model for gilthead sea bream was developed by Hernandez *et al.* (2003). Their model involved body weight, a thermal effect and a ration effect. The ration effect was simplified because it involved complex expressions that were dependent on body weight and temperature, and the final model was related to the proportion of a normalized ration size.

Some authors have reported on the effect of FR on SGR, first in grass carp *Ctenopharyngodon idella* using linear regression (Cui *et al.*, 1994). Recently, some authors such as Bureau *et al.* (2006) studied nitrogen and energy deposition as function of FR in on-growing rainbow trout, Eroldogan *et al.* (2008) determined the optimum feeding rate in European sea bass fingerlings, and Klaoudatos & Conides (1996) reported a quadratic regression between SGR and FR in gilthead sea bream. Xie *et al.* (1997) established an energy budget for tilapia fingerlings using asymptotic regression, and developing a global model relating SGR and FCR as functions of FR has been developed. Watanabe *et al.* (2000) studied energy and protein requirements by feeding Japanese yellowtail ranging from 8 to 300 g at several feeding rates, and they obtained some linear regression models of SGR as a function of FR for each size class; however, when all data from this study are considered, the response of SGR as a function of FR follows a quadratic response.

Ronyai & Csengeri (2008) studied the relationship between feeding strategy and temperature in pikeperch (*Sander lucioperca*) and obtained a quadratic regression TGC-FR for each temperature; however, no overall model was planned.

Assuming the effect of feeding rate on economic profit, then considering the influence of feeding on growth, as well as its economic impact in aquaculture, justifies economic studies about the proper FR to optimize profit in fish farms. Feeding levels can be established to maximize the growth response, to minimize the FCR, or to maximize profitability. In the first case, the FCR obtained would be high, whereas in the second case, the growth would be low (Brett, 1979). The economically optimal feed level must necessarily consider both growth and the FCR to optimize economic profitability. The economic profit index (EPI) (Martínez-Llorens *et al.*, 2007) as a function of feeding rate provides a simple and powerful approach for determining the most profitable feeding rate. Although this economic estimate needs to consider the price of different diets, fish market prices and the value of fish growth, the first step must be to model both growth and the FCR in relation to the feeding level.

The aim of this work was to develop a model of the TGC and FCR as functions of FR that allows simulation to be made for several body weights of the optimum FR for maximizing EPI response of gilthead sea bream and is the first step in developing a bio-energetic model for this specie that might become a useful tool for other species.

Materials and Methods

Experimental facilities

This study was conducted at the Institute of Animal Science and Technology of the Polytechnic University of Valencia and at the Animal Science Department of Udine Study University. Six trials were carried out with gilthead sea bream (*Sparus aurata*) of different weights (Table 1) obtained from several commercial farms. The fish were placed into 1000 l pens inside 4000 l concrete tanks for trials 1 to 5 and into 500 l tanks for trial 6. For each trial, all fish were weighed and distributed to maintain a uniform density between pens. Throughout the growing period, the re-circulated marine water that they were reared in was permanently aerated in each tank using an air blower. Water parameters such temperature, salinity, ammonia, nitrite and nitrate were monitored weekly and were maintained within adequate levels for fish growth.

Experimental Design

Fish were conditioned to a commercial diet (43% crude protein; 21% crude fat; 12% ash; 8% moisture; and 21 MJ/kg crude energy from Dibaq S.A., Segovia, Spain) for three weeks prior to the experimental period. Different daily rations were tested, from starvation to satiation (Table 1) in each trial. Fish were fed by hand twice a day, at 9:30 and at 16:00, with the higher rations and once at 9:30 in groups with lower rations (<1%). During feedings, observers checked that all of the feed offered was eaten by the fish. For the first meal of the day, the entire ration was proportioned, and, if the fish showed a lack of appetite, feeding was stopped; any remaining ration was proportioned in the second meal. If the fish displayed a lack of appetite during the second meal, then feeding was stopped, the remaining food was weighed and the feeding rate was corrected from a theoretical feeding rate to the actual feeding rate.

Table 1.- Experimental conditions and durations of six feeding trials in gilthead sea bream fed at different feeding rate.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Initial weight (g)	23.6	38	49.6	110.2	220.1	288.9
Fish/pen	25	25	30	12	13	12
Replicates[*]	2	2	3	3	2	2
No. pens	8	12	12	12	10	10
Theoretical feeding rate (%)	0/1.5/3/4.5	0/0.5/1/1.6/2.2/2.8	0/1/2/3	0/1/2/3	0/0.5/1/1.5/2	0/0.5/0.8/1.5/2.2
Water temperature (ºC)[†]	21.5	22	25	25	22	23
Duration (days)	84	90	83	83	90	64

[*] number of pens (experimental units) dedicates to each feeding rate;

[†] Total number of experimental units used on trial;

[‡] Average temperature during the study periods

At the beginning and at the end of the experiments, all of the gilthead sea bream were individually weighed. During the experiment, the fish were weighed every 14 days. The daily rations were adjusted every 7 days to the new biomass value using real weighed values or estimations using the previous growth rate between two weights.

FR, TGC, and FCR were calculated using Equations 1, 2 and 3, respectively, as functions of feed intake (*Feed*), initial body weight (*BWi*), final body weight (*BWf*), expressed in fresh weight number of fish (*n*), and sum of effective temperature ($\sum T_{ef}$) considering the minimum temperature for growth of seabream (12ºC) calculated as $\sum(T^{\underline{o}} - 12)$ (Mayer *et al.*, 2008;2009):

$$FR = \frac{Feed}{\frac{(BWi + BWf)}{2} \times n \times day} \times 100 \qquad \text{(Eq. 1)}$$

$$TGC = \frac{{BW_f}^{1/3} - {BW_i}^{1/3}}{\sum T_{ef}} \qquad \text{(Eq. 2)}$$

$$FCR = \frac{Feed}{(BWf - BWi) \times n}$$

(Eq. 3)

Modeling and Statistical Analysis

Mean data of fish from each pen or tank were used as the experimental units in growth trials for regression analysis. Descriptive statistics are mean ± standard error (SE) if not otherwise reported.

The relationship between TGC and FR was analyzed with an asymptotic model, the Von Bertalanffy model and a quadratic model. All equations were obtained from the results of the regression analysis, and parameters were obtained from the asymptotic curve with the iterative nonlinear least squares algorithm of Levenberg-Marquardt, using Statgraphics for Windows (version 5.1) software.

The expressions used to fit TGC were Equation 4 for the asymptotic model and Equation 5 for the quadratic model.

$$TGC \times 1000 = a \times \left(1 - e^{\left(-b \times \left(FR - c\right)\right)}\right)$$

Asymptotic (Eq. 4)

$$TGC \times 1000 = a + b \times FR + c \times FR^2$$

Quadratic (Eq.5)

The resulting regression equations allowed the feeding level for maintenance (FR *m*) to be obtained by solving the equations for FR with TGC = 0.

FCR is calculated as feed intake divided by the per-body increment, but the FCR must be expressed as a function of FR, initial body weight (BWi) and the sum of temperature. Solving for "feed" from Equation 1 and expressing it as a function of FR (Equation 6), and solving "final body weight" from Equation 2 and expressing it as a function of TGC (Equation 7). Then by substituting feed and final body weight in Equation 3, then FCR can be expressed as a function of BWi, days, $\sum T_{ef}$, FR and TGC (Equation 8).

$$Feed = \frac{\frac{(BWi+BWf)}{2}\times n\times days}{100}\times FR \qquad \text{(Eq. 6)}$$

$$BWf = (\sqrt[3]{BWi} + TGC\times\boldsymbol{\Sigma}(T^{\text{ª}}))^3 \qquad \text{(Eq. 7)}$$

$$FCR = \frac{Feed}{\left(BW_f - BW_i\right)\times n} = \frac{\dfrac{\left(BW_i + \left(\sqrt[3]{BW_i} + TGC\times\sum\left(T^{\text{a}}\right)\right)^3\right)\times days\times FR}{200}}{\left(\left(\sqrt[3]{BW_i} + TGC\times\sum\left(T^{\text{a}}\right)\right)^3 - BW_i\right)}$$

(Eq. 8)

Finally, by substituting TGC in equation 8 for its estimated asymptotic expression (Eq. 4) or quadratic expression (Eq. 5), FCR can be expressed as a function of "initial body weight" (BWi), "feeding rate" (FR) and the "sum of effective temperature" ($\sum T_{ef}$) using an asymptotic model (Equation 9) or a quadratic model (Equation 10).

$$FCR=\frac{\frac{\left(BWi+(\sqrt[3]{BWi}+\left(a\times\left(1-e^{\left(-b\times(FR-c)\right)}\right)\right)\times\boldsymbol{\Sigma}(T^{\underline{a}}))^{3}\right)\times days\times FR}{200}}{\left((\sqrt[3]{BWi}+\left(a\times\left(1-e^{\left(-b\times(FR-c)\right)}\right)\right)\times\boldsymbol{\Sigma}(T^{\underline{a}}))^{3}-BWi\right)} \qquad \text{(Eq. 9)}$$

$$FCR=\frac{\frac{\left(BWi+(\sqrt[3]{BWi}+(a+b\times FR+c\times FR^{2})\times\boldsymbol{\Sigma}(T^{\underline{a}}))^{3}\right)\times days\times FR}{200}}{\left((\sqrt[3]{BWi}+\ (a+b\times FR+c\times FR^{2})\times\boldsymbol{\Sigma}(T^{\underline{a}}))^{3}-BWi\right)} \qquad \text{(Eq. 10)}$$

The economical profit index (EPI) developed by Martinez-Llorens *et al.* (2007) was calculated using Equation 11.

$$EPI=(BWf-BWi)\times Selling\ price-(BWf-BWi)*FCR*Cost\ of\ food$$

(Eq. 11)

In a first step, EPI may be calculated as a function of BWi, FCR, TGC and $\boldsymbol{\Sigma}(T^{\underline{a}})$. By simplifying and replacing final body weight with Equation 7 and considering a selling price of 4.5 €/kg and a cost of food of 0.75 €/Kg, Equation 12 is obtained.

$$EPI=\left(\left(\sqrt[3]{BWi}+TGC\times\boldsymbol{\Sigma}(T^{\underline{a}})\right)^{3}-BWi\right)\times(4.5-FCR\times0.75) \qquad \text{(Eq. 12)}$$

In a second step, FCR from Equation 12 can be substituted for its previous expressions in the asymptotic model (Equation 9) and quadratic model (Equation 10), resulting in the final expressions of EPI as a function of “initial body weight” (BWi), “feeding rate” (FR) and the “sum of effective temperature” ($\sum T_{ef}$).

For final simulation of FCR and EPI, an 84-day growth period and a temperature of 23ºC were used. Optimum daily feeding rates for minimum FCR and maximum EPI (Figure 1) were obtained graphically and algebraically according to the method of Brett *et al.* (1979) and using the mathematical software Derive for Windows (version 4.03).

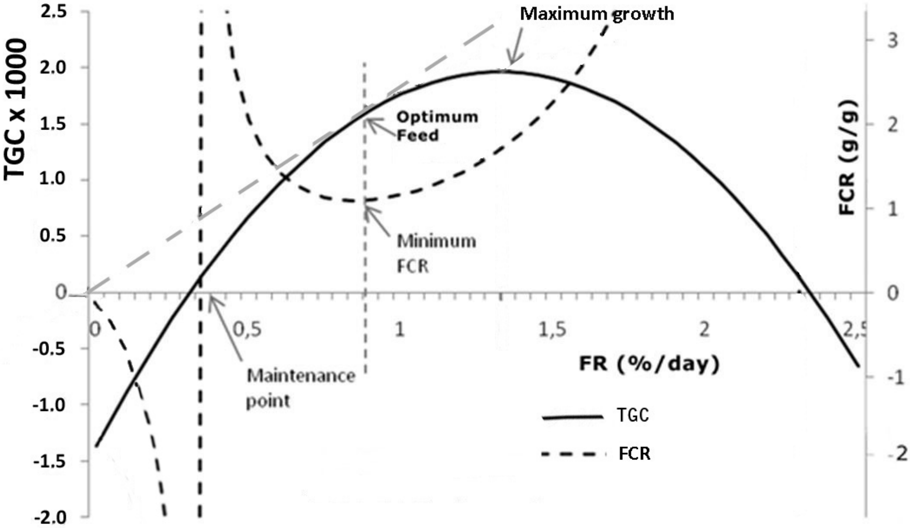

Figure 1.- Methodology for determining critical points in the graphs drawing thermal growth coefficient and feed conversion ratio in function of feeding rate.

Results

The real feeding rate, growth, feed conversion ratio and survival values obtained in six trials are presented in Table 2. Results were not influenced by survival except in Trial 1, in which the starvation of small fish resulted in a lower survival rate (57%) than fed fish.

Table 2.- Feeding rate, growth, feed conversion ratio and survival results for six trials in gilthead sea bream fed at differentfeeding rates.

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Trial	$\sum T^{a}$ (°C)	Feeding rate (%/day)	Initial body weight (g)	Final body weight (g)	Thermal growth coefficient × 1000	Feed conversion ratio (g/g)	Survival (%)
1	798	0.00 ± 0	23.65 ± 0.67	17.3 ± 0.8	−0. 356 ± 0.095	0 ± 0	57.10 ± 4.12
		0.99 ± 0.01	23.4 ± 0.14	45.0 ± 3.7	0. 872 ± 0.132	1.3 ± 0.12	98.15 ± 2.61
		1.89 ± 0.01	22.8 ± 0.7	65.1 ± 6.2	1.487 ± 0.122	1.57 ± 0.08	98.10 ± 2.71
		2.36 ± 0.15	24.7 ± 0.42	72.7 ± 1.6	1.579 ± 0.0167	2.08 ± 0.11	91.85 ± 2.36
2	900	0 ± 0	37.5 ± 1.16	25.2 ± 0.7	−0.464 ± 0.0	0 ± 0	96 ± 0
		0.33 ± 0.02	37.6 ± 0.36	39.7 ± 3.1	0.066 ± 0. 111	−60.14 ± 92.08	94 ± 8.48
		0.62 ± 0.02	38.3 ± 1.1	61.5 ± 2.1	0.642 ± 0.014	1.46 ± 0.03	96 ± 5.65
		0.99 ± 0.01	38.3 ± 2.4	75.7 ± 7.2	0.952 ± 0.068	1.55 ± 0.09	100 ± 0
		1.17 ± 0.01	38.3 ± 0.7	87.9 ± 3.5	1.195 ± 0.041	1.46 ± 0.01	98 ± 2.82
		1.37 ± 0.03	37.9 ± 0.9	94.4 ± 9.5	1.324 ± 0.140	1.56 ± 0.07	100 ± 0
3	1079	0 ± 0	49.4 ± 5.4	37.6 ± 4.4	−0. 296 ± 0.0436	0 ± 0	94.4 ± 4.81
		0.62 ± 0.02	48.4 ± 5.4	68.6 ± 5.8	0.418 ± 0.024	1.80 ± 0.19	97.2 ± 4.81
		1.16 ± 0.02	51.9 ± 3.5	115.5 ± 11.5	1.053 ± 0.074	1.45 ± 0.14	97.2 ± 4.81
		1.62 ± 0.04	48.7 ± 1.3	124.1 ± 13.4	1.234 ± 0.180	1.85 ± 0.33	97.22 ± 4.81
4	1079	0 ± 0	110.7 ± 1.3	92.4 ± 5.0	−0.354 ± 0.072	0 ± 0	84.44 ± 7.70
		0.63 ± 0.01	112.6 ± 4.3	157.6 ± 5.2	0.546 ± 0.028	1.88 ± 0.14	84.44 ± 1.92
		1.18 ± 0.05	110.3 ± 2.1	230.1 ± 18.4	1.321 ± 0.146	1.63 ± 0.20	87.99 ± 6.58
		1.66 ± 0.05	107.3 ± 2.3	241.6 ± 5.0	1.406 ± 0. 048	2.25 ± 0.05	81.33 ± 5.09
5	900	0 ± 0	210.2 ± 5.8	161.1 ± 6.8	−0.560 ± 0.146	0 ± 0	96.15 ± 2.42
		0.30 ± 0.03	221.8 ± 9.1	239.9 ± 24.5	0.175 ± 0.144	5.95 ± 0.68	100 ± 0
		0.55 ± 0.03	226.3 ± 2.1	288 ± 18.0	0.565 ± 0.132	2.45 ± 0.07	100 ± 0
		0.65 ± 0.06	217.9 ± 0.9	285.2 ± 25.5	0.625 ± 0.209	2.70 ± 0.16	92.30 ± 5.43
		0.75 ± 0.10	224.3 ± 1.0	302.5 ± 14.8	0.707 ± 0.112	2.75 ± 0.04	100 ± 0
6	704	0 ± 0	299.2 ± 6.5	267.2 ± 14.2	−0.227 ± 0.0	0 ± 0	91.7 ± 7.2
		0.3 ± 0.0	290.8 ± 14.5	289.0 ± 13.7	−0.008 ± 0.000	17.82 ± 39.07	75 ± 15.3
		0.55 ± 0.07	281.8 ± 0.2	323.0 ± 9.2	0.335 ± 0.097	7.26 ± 5.44	75.3 ± 21.21
		1 ± 0.0	291.2 ± 15.7	373.2 ± 22.0	0.825 ± 0.031	2.51 ± 0.13	100 ± 0
		1.4 ± 0.07	292.4 ± 6.7	422.6 ± 13.6	1.257 ± 0.0	2.32 ± 0.11	88.4 ± 15.3

In most trials, the real feeding rate was lower than the theoretical feeding rate because the fish stopped voluntary ingestion before the entire ration of feed was consumed (in higher rations), but also cause feeding rates where adjusted each 7 days, and feed rate would be lower as fish grew. The growth rate of gilthead sea bream clearly increased in all trials when the feeding rate was raised above starvation, but this increment was reduced when the feeding rate was near the satiation point.

Observed values of TGC and FR in the six trials are represented in Figure 2, in which the asymptotic (Equation 13) and quadratic (Equation 14) curves for TGC-FR are fitted. The asymptotic equation was:

$$\mathrm{TGC}\times 1000 = 2.037\times\left(1\text{-}\mathrm{e}^{\left(\text{-}0{,}8\times(\mathrm{FR}\text{-}0.22)\right)}\right)$$

$$R^2adj = 96.18 \qquad \text{(Eq. 13)}$$

and the quadratic equation was:

$$TGC\times 1000 \;=\; -0.381026 \;+\; 1.71554\times FR - 0.3821\times FR^2$$

$$R^2adj = 96.42 \qquad \text{(Eq. 14)}$$

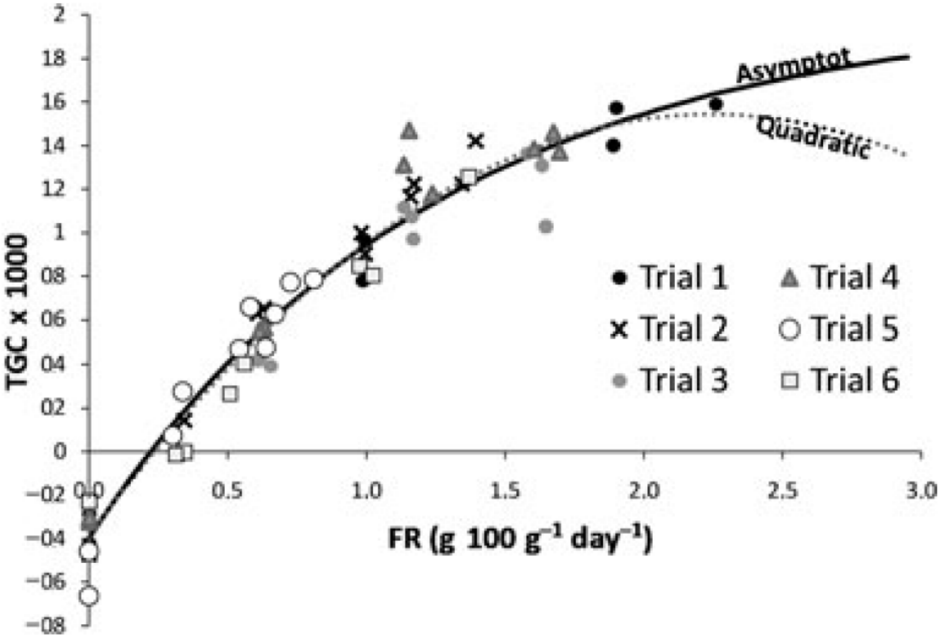


Figure 2.- Thermal growth coefficient response to increasing levels of feeding rate and the response curve of asymptotic and quadratic models considering six feeding trials in gilthead sea bream.

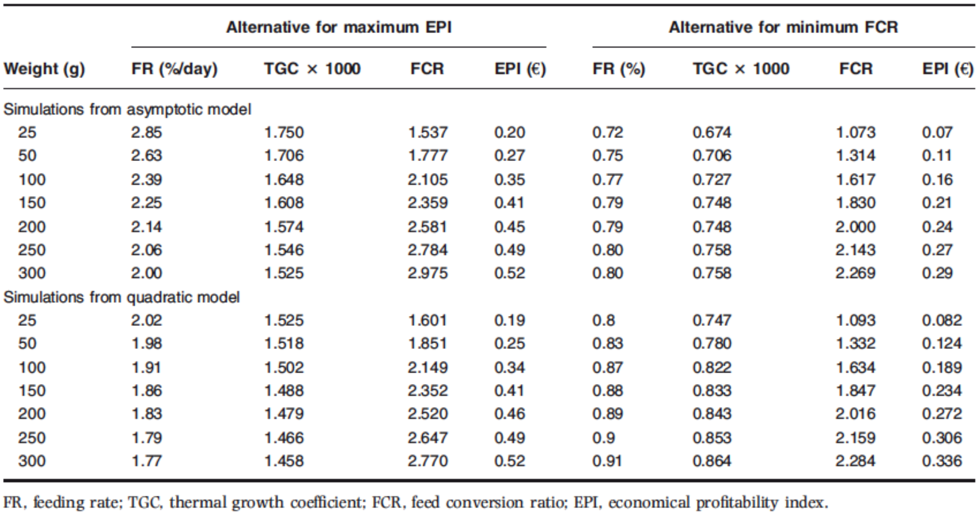
A similar FR for maintenance (FR*m*) was determined from both equations; FR*m* = 0.22 g/100 g/day with the asymptotic curve and FR*m* = 0.23 g/100 g/day with the quadratic curve.

FCR-FR simulation curves (using Equations 9 and 10) for several initial body weights and feeding rates are plotted in Figures 3a and 3b for the asymptotic and quadratic models, respectively. The shape of both curves shows that the biological response of FCR, with a value of zero for starvation, a negative and positive infinite value for maintenance. When feeding rate exceeds the maintenance level, FCR falls quickly to its minimum value, increasing again as FR increases.

In the same way, EPI simulations (using Equation 12 substituting FCR for Equations 9 and 10) have been plotted in Figures 4a and 4b for asymptotic and quadratic models, respectively, for several initial body weights.

The estimated values of FR, TGC, FCR and EPI for gilthead sea bream of several body weights considering the alternatives of “maximum EPI” and “minimum FCR” in both asymptotic and quadratic models are shown in Table 3. Estimated FR values are higher (between two and four times) for maximum EPI than for minimum FCR for all body weights and for both models. Asymptotic FR estimations for maximum EPI are higher than in the quadratic model, but FR estimations for minimum FCR are slightly higher for the quadratic model than for the asymptotic model.

Table 3.- Estimate of feed rate, thermal-unit growth coefficient and feed conversion ratio (FCR) for maximum economic profitability index and minimum FCR using an asymptotic model and a quadratic model (considering 4.5 €/kg as the value of fish and 0.75 €/kg as the cost of the diet (84 days of growth at 23ºC)



	Alternative for maximum EPI				Alternative for minimum FCR			
Weight (g)	FR (%/day)	TGC × 1000	FCR	EPI (€)	FR (%)	TGC × 1000	FCR	EPI (€)
Simulations from asymptotic model								
25	2.85	1.750	1.537	0.20	0.72	0.674	1.073	0.07
50	2.63	1.706	1.777	0.27	0.75	0.706	1.314	0.11
100	2.39	1.648	2.105	0.35	0.77	0.727	1.617	0.16
150	2.25	1.608	2.359	0.41	0.79	0.748	1.830	0.21
200	2.14	1.574	2.581	0.45	0.79	0.748	2.000	0.24
250	2.06	1.546	2.784	0.49	0.80	0.758	2.143	0.27
300	2.00	1.525	2.975	0.52	0.80	0.758	2.269	0.29
Simulations from quadratic model								
25	2.02	1.525	1.601	0.19	0.8	0.747	1.093	0.082
50	1.98	1.518	1.851	0.25	0.83	0.780	1.332	0.124
100	1.91	1.502	2.149	0.34	0.87	0.822	1.634	0.189
150	1.86	1.488	2.352	0.41	0.88	0.833	1.847	0.234
200	1.83	1.479	2.520	0.46	0.89	0.843	2.016	0.272
250	1.79	1.466	2.647	0.49	0.9	0.853	2.159	0.306
300	1.77	1.458	2.770	0.52	0.91	0.864	2.284	0.336

FR, feeding rate; TGC, thermal growth coefficient; FCR, feed conversion ratio; EPI, economical profitability index.

Discussion

TGC values for one species growing under similar farming conditions can be considered constant across a wide range of weight classes (< 20 g, 20-500 g and > 500 g in rainbow trout) and temperatures, as Dumas *et al*. (2007) reported. However, if some farm conditions or diet properties vary, the TGC value could change. Likewise the optimum temperature for maximum growth and feeding rate is different depending on body weight, and TGC model does not resolve this finding. Knowledge about the relationship between TGC and these farm conditions, as well as between TGC and feeding rate (FR) in the current trial, can be fundamental for managing fish farms. The goal of fish farmers should be to optimize the FR to maximize growth or minimize FCR, or, even better, to maximize economic profit.

The two curvilinear TGC-FR models, asymptotic and quadratic, developed with data from several body weights in the six trials, had high adjusted R-squared values (around 96%) and clearly confirmed the effect of feeding level on growth. These models allow for mathematical expressions that relate FR and TGC to be developed for the whole growth cycle. The shapes of both curves were similar in their ascending portions, as Shearer (2000) reported, but small differences between both models occurred in the final portions.

The asymptotic or quadratic shapes of the TGC models place a decay rate of change for the relationship TGC-FR. This effect has been cited by several authors for different fish species (Xie *et al.*, 1997; Eroldoğan *et al.*, 2004;) and can be explained by the fact that the fish species reach their maximum growth potential. Both models, asymptotic and quadratic seem useful and meaning inside the study range, but further considerations to choose between them could be made. In any case, theoretically, growth in fish species should be considered undetermined in relation to time (Dumas *et al.*, 2010). Asymptotic model assumes an exponential decay rate of change for the relationship TGC-FR

$$\frac{dTGC}{dFR} = k \times e^{-b(FR-c)}$$

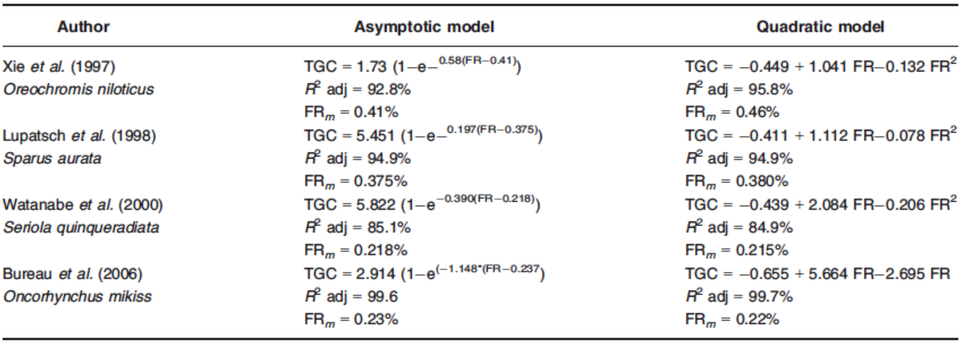
where k = a x b. Moreover, the quadratic model is to assume a linear rate of change, ie

$$\frac{dTGC}{dFR} = b + m \times FR$$

where m = 2 x c. Moreover, unlike the quadratic model, the asymptotic does not assume the existence of a maximum value for growth, although there exists a limiting value that is never reached.

The comparison of asymptotic and quadratic models is difficult in the first place because most authors have determined the growth response to FR when considering only a small range for body weight (Xie *et al.*, 1997; Eroldoğan *et al.*, 2004; Bureau *et al.*, 2006). Nevertheless, Lupatsch *et al.* (1998) and Watanabe *et al.* (2000) reported results considering several body weights, and both asymptotic and quadratic models could have been developed from these results (Table 4 and Figure 5).

Table 4.- Asymptotic and quadratic models for TGC-FR developed in some fish species using data from several authors.



Author	Asymptotic model	Quadratic model
Xie *et al.* (1997) *Oreochromis niloticus*	$TGC = 1.73\ (1-e-^{0.58(FR-0.41)})$ R^2 adj = 92.8% $FR_m = 0.41\%$	$TGC = -0.449 + 1.041\ FR - 0.132\ FR^2$ R^2 adj = 95.8% $FR_m = 0.46\%$
Lupatsch *et al.* (1998) *Sparus aurata*	$TGC = 5.451\ (1-e-^{0.197(FR-0.375)})$ R^2 adj = 94.9% $FR_m = 0.375\%$	$TGC = -0.411 + 1.112\ FR - 0.078\ FR^2$ R^2 adj = 94.9% $FR_m = 0.380\%$
Watanabe *et al.* (2000) *Seriola quinqueradiata*	$TGC = 5.822\ (1-e^{-0.390(FR-0.218)})$ R^2 adj = 85.1% $FR_m = 0.218\%$	$TGC = -0.439 + 2.084\ FR - 0.206\ FR^2$ R^2 adj = 84.9% $FR_m = 0.215\%$
Bureau *et al.* (2006) *Oncorhynchus mikiss*	$TGC = 2.914\ (1-e^{(-1.148^{*}(FR-0.237)})$ R^2 adj = 99.6 $FR_m = 0.23\%$	$TGC = -0.655 + 5.664\ FR - 2.695\ FR$ R^2 adj = 99.7% $FR_m = 0.22\%$

Feeding rate for maintenance (FR*m*) is the feeding rate needed for zero growth. Although FR for maintenance is a concept that is related to temperature, species and diets, and next data are not presented with comparison purpose but just to evaluate the method. In the present study, FR*m* was 0.22 and 0.23 g/100 g/day for the asymptotic and quadratic models, respectively. Most authors do not determine this maintenance point, probably because it is out of the range of the study, but if FR*m* is recalculated from data in Bureau *et al.* (2006) using the present methodology, it results in values of 0.23 for the asymptotic model and 0.22 g/100 g/day for the quadratic model in rainbow trout (Table 4). Similar results were found for Japanese seriola (Watanabe *et al.*, 2000). For gilthead sea bream (Lupastch *et al.*, 1998), FR*m* would be 0.375 g/100 g/day, respectively. However, comparing the maintenance feeding rate between diets, energy and protein of the diet should be similar because the concept of “maintenance” is related to protein and energy intake.

Feeding rate affects both growth and feed conversion, because an increase in FR results in an improvement in growth, as Figure 2 demonstrates, but FCR could deteriorate (Figure 3), as some authors have reported in several species (Bureau *et al*., 2006; Eroldogan *et al*., 2004; Xie *et al*., 1997). In this situation, the FR must optimize both indices (TGC and FCR) to maximize the economic profit index (EPI), as Martínez-Llorens *et al*. (2007) have developed.

The regression of FCR as a function of FR is complicated because at the point of maintenance, fish growth is zero and FCR would be “±infinite,” whereas in the interval from starvation to maintenance, the value of FCR is negative. Because of these problems, Klaoudatos *et al*. (1996) used the inverse of FCR, food efficiency (FE), which has a value of zero at the maintenance point, and they reported a quadratic relationship between FE and FR. FE-FR has a concave shape, and FCR-FR regression presented a convex curve (Ronyai *et al.*, 2008). But both have an unrealistic intervale for starvation to maintenance feeding rate, (zero to negative infinite in FCR-FR model, and negative infinite to zero in Fe-FR). But FCR is probably the most used parameter on farms and researchers to measure efficiency of feed.

In the present study, the FCR values as a function of FR for each weight class have been simulated (Figure 3) following Equations 9 and 10 for the asymptotic and quadratic regressions, respectively, between TGC and FR. First, body weight has an increase effect on FCR, which has been previously reported (Brett *et al*., 1979). Second, the shape of the two models is similar, both having an asymmetric curve with a higher slope in the decreasing section than in the increasing section, and the minimum value of FCR occurs in the proximity of the maintenance point. An alternative for optimizing feeding is to consider the FR that minimizes the FCR, which is rather constant, between 0.70 and 0.80 g/100 g/day for the asymptotic model as well as between 0.80 and 0.9 g/100 g/day for the quadratic model (Table 3); however the growth and EPI values would be low.

If data from other authors are recalculated using the proposed methodology (Table 4), then the shape of the recalculated TGC-FR curve from Bureau *et al.* (2006) (Figure 5) in trout is similar to the present study. Tilapia (Xie *et al*., 1997) and Seriola (Watanabe *et al*., 2000) showed similar tendencies with both asymptotic and quadratic models, but it seems that the maximum growth was not reached. The recalculated data for two models from Lupatsch *et al*. (1998) in gilthead sea bream show closer values but large differences in shape because recalculated curves show a much more linear shape than asymptotic or quadratic curves; the shape of the recalculated curve indicates that maximum growth was not achieved.

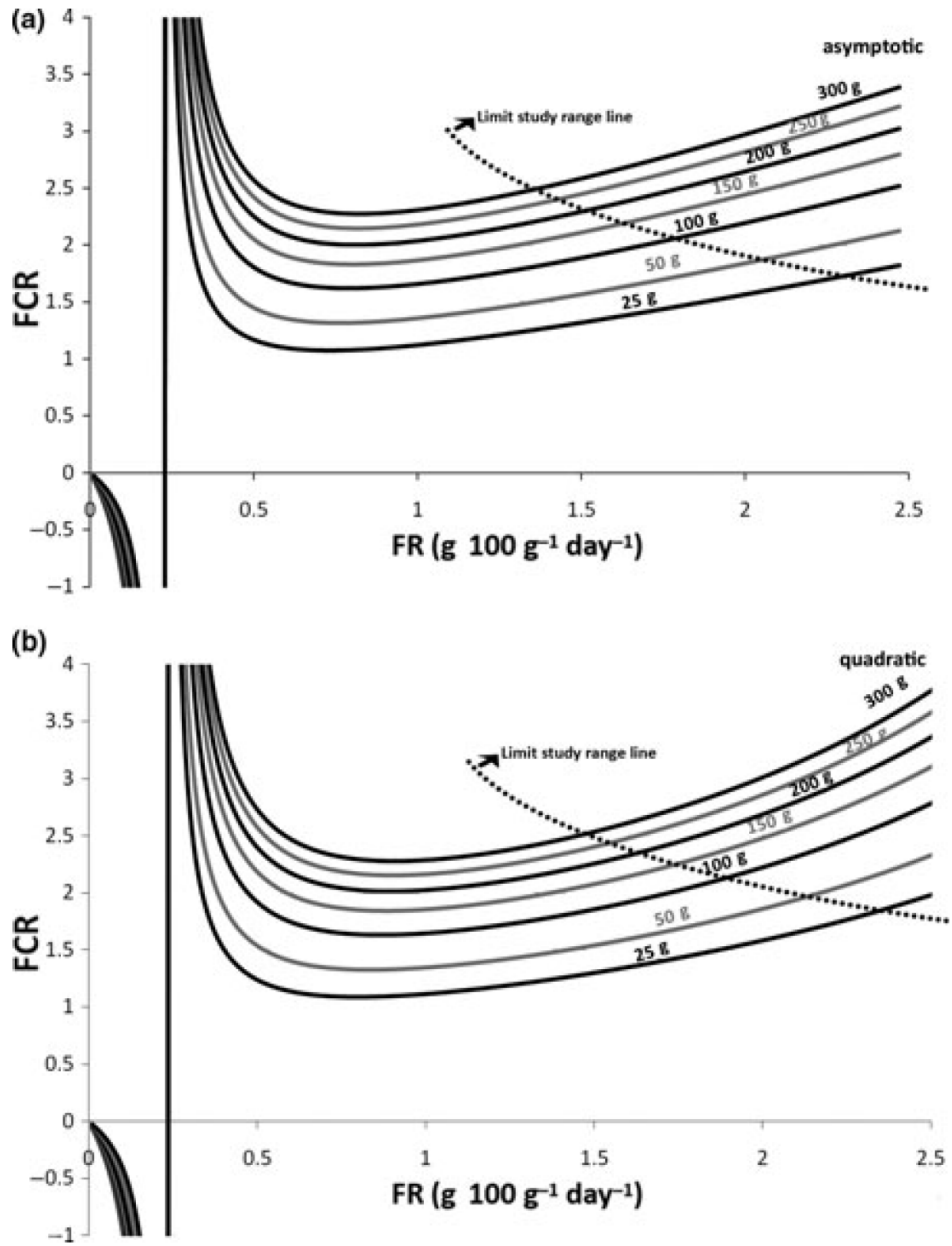


Figure 3.- Feed conversion ratio simulation responses as functions of feeding rate for several initial body weights of gilthead sea bream (84 days at 23ºC) using asymptotic and quadratic models.

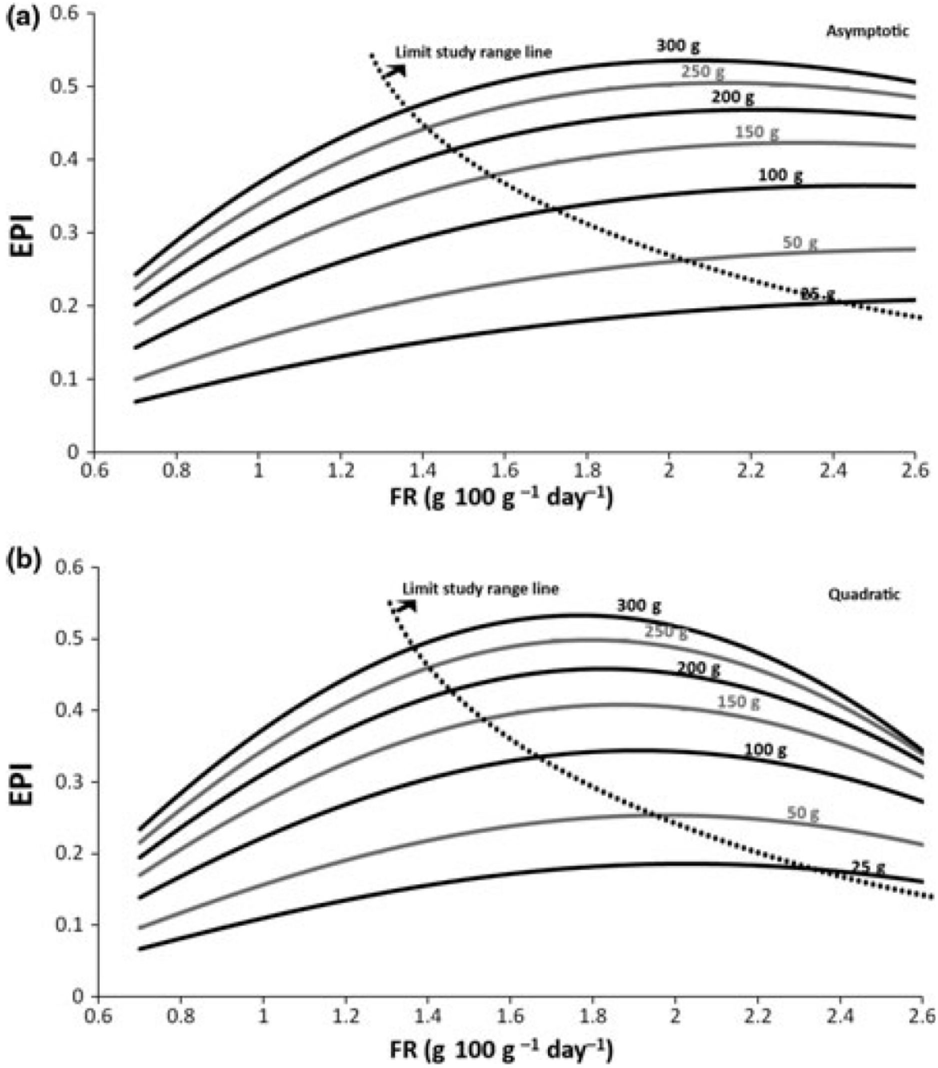


Figure 4.- Economic profitability index simulation responses as functions of feeding rate for several initial body weights of gilthead sea bream (84 days at 23ºC) using asymptotic and quadratic models.

In Table 3, if the FRs for minimizing FCR are selected, then the TGC obtained ranged in the interval from 0.67 to 0.76 for the asymptotic model and from 0.75 to 0.86 for the quadratic model. These values are lower than those obtained in trials (Table 2 and Figure 2), i.e., the FCR would be good, but the growth would be poor. This question was studied by Klaoudatos *et al.* (1996) in gilthead sea bream, who cited an optimum feeding rate of 1.9 g/100 g/day for minimum FCR, but a maximum FR of 3.34 g/100 g/day for maximum growth.

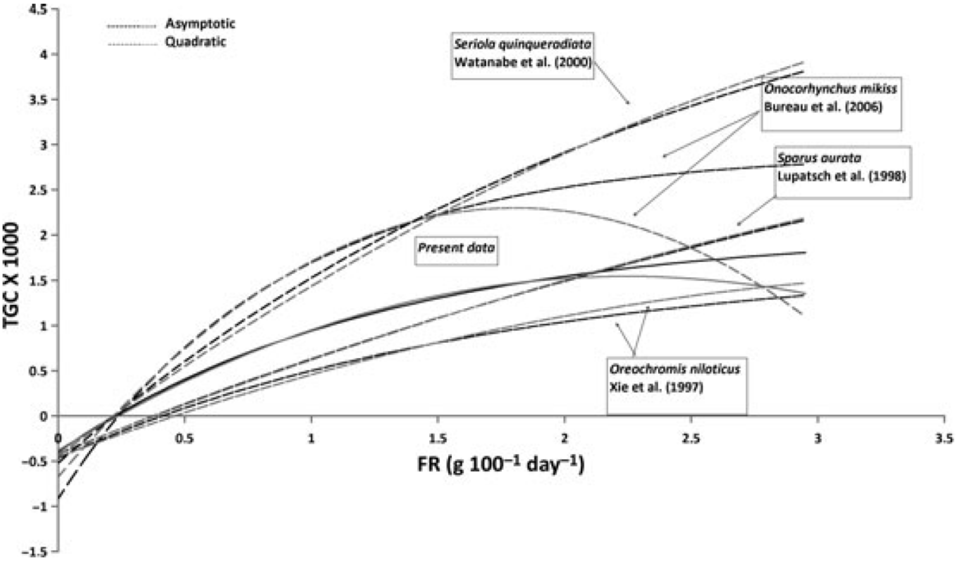


Figure 5.- Comparation of thermal growth coefficient–feeding rate curves obtained in several fish species by some authors.

For this reason, it is convenient to use some index than joins both growth and feed conversion, such as the economic profit index (EPI) developed by Martinez-Llorens *et al.* (2007). EPI simulation graphics (Figure 4) show the added value produced for each fish size in a discrete growth period, considering the cost of fish and the feed price through the comparison of feed cost and profit derived from growth. For each pre-determined body weight, the EPI curve, show a maximum point, ranged between a FR of 1.6 and 2.5 %/d (Table 3), depending of the body weight, which are higher than FRs obtained for minimizing FCR. However, FR values that maximize EPI are out of the range of the study (in Figure 3 and 4 dotted lines are plotted to show the limit of the study) because fish seem to stop voluntary feed intake before they reach that point. Nevertheless, considering only the studied range, to obtain a higher EPI, it would be necessary to increase the FR over the values that optimize FCR, but to reach the maximum EPI, the voluntary ingestion of feed must be increased, probably by increasing the temperature or with another feeding strategy.

Conclusion

Different diets, containing several raw materials or different proportions of protein and energy, might yield different results, but the models developed in this study could be useful tools as a first step toward building a future model that considers the protein and energy intake instead of feed rate. The bioenergetic models developed in last years for gilthead sea bream (Lupatsch *et al.,* 1998, 2003) and rainbow trout (Cho and Bureau 1998; Bureau *et al.,* 2006) allow fit the optimal protein/energy ratio, or better optimize protein and energy intake to maximize efficiency; however, determining the FR for maximum efficiency or profitability is an entirely different question and it must be studied with a different methodology. An interactive study for determining the ratio of digestible protein/digestible energy with the factorial methodology proposed by Lupatsch *et al*. (1998, 2003), together with the study of growth functions for feed intake and its economic consequences, could increase the efficiency of feeding and the profitability of fish farm production, thereby reducing ecological impacts.

Acknowledgments

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CAPÍTULO 5

Protein deposition and energy recovery in gilthead sea bream (*Sparus aurata*): evaluation of nutritional requirements.

Under review in Aquaculture Research

Ignacio Jauralde, Silvia Martínez-Llorens, Ana Tomás & Miguel Jover

Protein deposition and energy recovery in gilthead sea bream (*Sparus aurata*): evaluation of nutritional requirements.

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Abstract

The requirements of energy and protein for gilthead sea bream are studied until commercial weight. Gilthead sea bream with different body weights (from 24 to 422 g) were fed from starvation to apparent satiation, studying protein and energy in the interval of 21-25ºC. Two different models were set up for two different fish sizes, corresponding to fish below and above a weight of 110 g. Energy recovery (ER) was expressed in relation to the digestible energy intake (DEI), $ER= 74.2*(1-e^{(-0.012*(DEI-26.7))})$ for small gilthead sea bream, and $ER = 56.3*(1-e^{(-0.017*(DEI-19.5))})$ for large gilthead sea bream, and protein deposition (PD) was expressed with regard to digestible protein intake (DPI) for small sea bream, $PD= 2.34*(1-e^{(-0.35*(DPI-1.01))})$ and for large sea bream, $PD= 2.18*(1-e^{(-0.3*(DPI-0.86))})$.

Maintenance needs in summer conditions were found to be between 0.86 and 1.01 g $kg^{-0.7}$ day^{-1} of the digestible protein intake and between 19.5 and 26.7 kJ $kg^{-0.82}$

day^{-1} of the digestible energy intake. The response curves to the graded levels of intake of energy and protein should allow the diet formulation to several growth conditions to all sizes until commercial weight.

Introduction

The four main factors with a great influence on growth, in most fish species, including gilthead sea bream (*Sparus aurata L.*), are temperature, feed composition, feed intake, and body weight of the fish (Brett, 1979). For most sea farmers working with offshore systems, the diet usually remains the only factor of choice, as temperature comes with the environment and weight with commercial demands. In this sense, diets should be designed considering the requirements of the fish as well as profitability. Thus, it is necessary to develop several models: for growth, whole body composition, energy recovery and protein deposition response curves and economic profitability index models.

There are several suitable growth models that allow predicting maximum growth with regard to temperature (Lupatsch *et al*., 1998, 2003a; Mayer *et al*., 2008, 2009). A regular practice of sea farmers consists in feeding fish until reaching its growth potential during the first growth phase. Feed efficiency usually is the target of fish farmers after the first year of growth to reach an optimized profitability. The point of maximum feed profitability may not correspond to the amount of feed for maximum growth (Fiogbé & Kestemont, 2003; Martínez-Llorens *et al*., 2009), depending on the cost of production and the price of raw ingredients for diets. Nevertheless, some other models make it possible to estimate growth for a sub-optimal ration size (Hernández *et al*., 2003; Jauralde *et al*., 2013).

Likewise, body composition is crucial in animal nutrition models (NRC, 1998; de Lange *et al*., 2003) and has been widely studied is several fish species

(Shearer, 1994; Jobling, 2001; Dumas *et al*., 2007a; Dumas *et al*., 2010), and particulary for *Sparus aurata* by Lupatsch *et al*. (1998; 2003a), who have also described the nutrient retention and the protein and energy needs in gilthead sea bream below a weight of 100 g. Fournier *et al*. (2002) also studied the protein requirements for maintenance in Sparus aurata of 7.4 g and other 3 species. Nevertheless, there is a lack of data with regard to nutritional requirements when considering that: i) the commercial weight of this species is usually around 400 g; ii) around 80% of the total feed in the cycle is consumed between 100 and 400 g; iii) the cost of feed is around the 40% of the total production cost of gilthead sea bream in the Mediterranean sea; iv) the fact that most of the food is deployed in summer with temperatures between 20 and 25 ºC.

In gilthead sea bream, however, evidence of two growth stanzas has been reported (Mayer *et al*., 2012), as this species changes its growth strategy when reaching a weight of around 117 g. Likewise, Dumas *et al*. (2007b) reported three stanzas in trout growth justified by morphological changes, variations in dynamics of muscular growth, utilization and investment of nutrients in reproduction.

Protein and energy intake have a great influence on the growth speed (Jauralde *et al*., 2013) which are determined by the dietary nutrient level and by feed intake. Diet composition as well as feed intake should be designed to cover the needs of maintenance and growth of the fish (Lupatsch *et al*., 1998; Kaushik, 1998; Azevedo *et al*., 2005; Booth *et al*., 2007; Glencross, 2008; Dumas *et al*., 2010). However, the amount of digestible energy and protein recommended for the same fish body weight and temperature vary in relation to the commercial feed considered, sometimes even if two feeds of the same company are compared. This is because the needs of *Sparus aurata* for growth, expressed in daily g protein or MJ energy over weight are still unknown for varying ranges of weight and temperature, although some studies (Lupatsch *et al*., 1998, 2003a) have approached the needs of *Sparus aurata* weighing less than 100 g and at moderate temperature. In other species, some models consider the body size and water temperature, but not the diet or feeding rate (Björnsson *et al.*, 2007).

On the other hand, the amount of digestible energy or protein that fish need retain for a determined level of growth depends not only on the initial body weight and temperature, but also on the efficiency of retention of the digestible energy intake or digestible protein intake. Most studies consider the net efficiency of retention of the digestible energy as constant independent of intake (Lupatsch *et al.*, 1998, 2001a, 2003a), but in protein, these studies have reported a nonlinear effect, considering an optimum single efficiency, usually for maximum growth. It would then seem possible that fast growth was less efficient than slow growth, as shown by Jauralde *et al.* (2013), and consequently, protein recovery efficiency should depend on the growth rate, measured as protein recovery per day, for example.

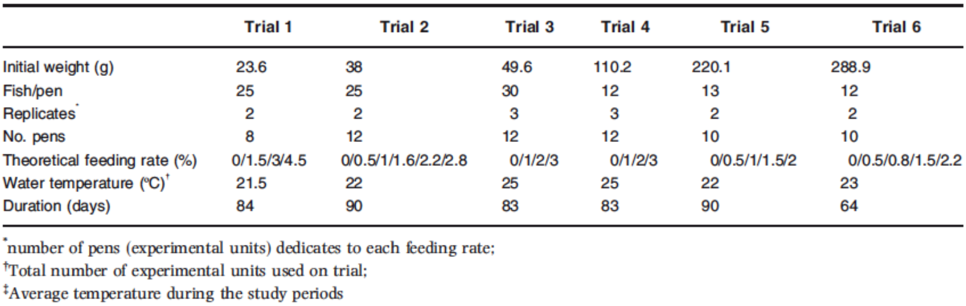
Based on the results reported by Lupatsch *et al.* (1998, 2001a, 2003a, 2003b), the objectives of the present trials were to determine the retentions and efficiencies of digestible protein and energy in gilthead sea bream (*Sparus aurata)*, in an interval of weights from the initial growth period to a commercial weight, 25 g to 450 g, and considering the summer water temperature in the Spanish Mediterranean, before determining nutritional requirements.

Material and methods

Experimental facilities and design

Six trials were conducted with gilthead sea bream (*Sparus aurata*), with varying initial weight (from 23 to 299g). Fish were placed in concrete tanks of 4000 litres, in which 3 pens of 1000 litres were installed to increase the replicates in trials 1 to 5, and in 500 l tanks for trial 6 (Table 1).

Table 1.- Experimental conditions and durations of six feeding trials in gilthead sea bream fed at different feeding rate.



	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Initial weight (g)	23.6	38	49.6	110.2	220.1	288.9
Fish/pen	25	25	30	12	13	12
Replicates*	2	2	3	3	2	2
No. pens	8	12	12	12	10	10
Theoretical feeding rate (%)	0/1.5/3/4.5	0/0.5/1/1.6/2.2/2.8	0/1/2/3	0/1/2/3	0/0.5/1/1.5/2	0/0.5/0.8/1.5/2.2
Water temperature (ºC)†	21.5	22	25	25	22	23
Duration (days)	84	90	83	83	90	64

*number of pens (experimental units) dedicates to each feeding rate;
†Total number of experimental units used on trial;
‡Average temperature during the study periods

A commercial diet (430g kg^{-1} crude protein; 210g kg^{-1} crude fat; 120g kg^{-1} ash; 80g kg^{-1} moisture; and 21 MJ kg^{-1} gross energy from Dibaq S.A., Segovia, Spain) was used at different daily rations, from starvation to satiation in each trial (Jauralde *et al*., 2013). The ingredients of diet in order of inclusion were: fish meal; fish oil; wheat and derivates, soy meal, “premix” mineral and vitamins, antioxidant and antifungal. The energy and lipid in the diet being in agreement with the limits proposed (Vergara *et al*., 1999; Lupatsch *et al*., 2001a; Velázquez *et al*., 2006).

Gilthead sea bream were fed by hand twice a day, (9:30 and at 16:00), with the higher rations, and once (9:30 h) in groups with the lower rations (<1%). During feeding, it was checked that the fish ate all the feed offered. At the first meal of the day, the entire ration was proportioned, but if the fish showed a lack of appetite, feeding was stopped and the remaining ration was proportioned in the second meal. If the fish displayed a lack of appetite during the second meal, then feeding was stopped, the remaining food was weighed and the feeding rate (FR) was corrected from a theoretical FR to the actual FR.

Chemical analysis

The apparent digestibility coefficient of crude protein (ADC_{prot}) and apparent digestibility coefficient of gross energy (ADC_{ener}) were calculated from fecal samples collected in digestibility tanks in triplicate and analyzed using acid insoluble ash as indicator following the method proposed by Atkinson *et al.* (1984).

The proximate body composition of five fish per tank was analyzed when the experiment was initiated, respectively, when it was finalized, and a sample of feed was also taken to analyze the diet composition.

Dry matter was calculated by weight loss after drying for 24 h at 105°C. Crude protein was measured using the Kjeldahl technique and multiplying N by 6.25. Crude lipid was measured after chloroform-methanol extraction (Folch *et al.*, 1957). Ash was calculated from the weight loss after incineration of the samples for 24 h at 550°C in a muffle furnace. Gross energy content was calculated with oxymetric coefficients (23.6 kJ g^{-1} of protein and 39.5 kJ g^{-1} of lipid).

Energy recovery (ER) (Equation 1) and protein deposition (PD) (Equation 2) were calculated using the metabolic coefficient calculated for *Sparus aurata* by Lupatsch *et al.* (2003a):

$$\text{ER (kJ kg}^{-0.82}\text{ day}^{-1}) = ((\text{Final EC-Initial EC})*1000/(\text{days}*\text{GBW}))^{0.82} \quad (1)$$

$$\text{PD (g kg}^{-0.7}\text{ day}^{-1}) = ((\text{Final PC-Initial PC})*1000/(\text{days}*\text{GBW}))^{0.7} \quad (2)$$

where PC and EC are the protein and energy content of the biomass in grams and kJ and GBW are the geometric mean weight of the initial and final biomass in kilograms.

Digestible energy intake (DEI) (Equation 3) and digestible protein intake (DPI) (Equation 4) were calculated as follows:

DEI (kJ per $kg^{-0.82}$per day^{-1}) =

$= (1000*\text{Total Feed Intake}*GE*ADC_{ener}/(GBW*days))^{0.82}$ (3)

DPI (g per $kg^{-0.7}$ per day^{-1}) =

$= (1000*\text{Total Feed Intake}*GP*ADC_{prot}/(GBW*days))^{0.7}$ (4)

where GE is the gross energy of diet in kJ, GP is the gross protein content of the diet, and GBW is the geometric mean from initial and final biomass in kilograms.

Calculations and Statistical Analysis

Mean data of fish from each pen or tank were used as the experimental units for regression analysis. Descriptive statistics are mean ± standard error (SE) if not otherwise reported.

Whole body composition (lipid, energy, protein and dry matter) in relation to body weight was modeled using allometric regression ($y = aBW^b$), where exponents were calculated from their linear version of the equation $LN(y)=LN(a)+b*LN(BW)$ for a better fit as demonstrated by White (2011).

The relation between energy recovery (ER) (Equation 5), or protein deposition (PD), (Equation 6), and digestible ingestion of energy, or protein, was analyzed with Von Bertalanffy's asymptotic model (Rosa *et al.*, 1997; Xie *et al.*, 1997):

$$ER=a*(1-e^{(-b*(DEI-c))}), \quad (5)$$

$$PD=a*(1-e^{(-b*(DPI-c))}) \quad (6)$$

where "a" corresponds to the asymptotic value, "b" is a parameter that determines the curvature, and "c" is the cutting point of the curve with the x axis (maintenance point). The net efficiency of digestible energy (k_e) and net efficiency for digestible protein (k_p) were estimated using equations (7) and (8).

$$k_e = a*(1-e^{(-b*(DEI-c))})/(DEI-c), \quad (7)$$

$$k_p = a*(1-e^{(-b*(DPI-c))})/(DPI-c) \quad (8)$$

All equations were obtained by regression analysis, and parameters from the asymptotic curve were obtained with the iterative nonlinear least squares algorithm of Levenberg-Marquardt, using the Statgraphics for Windows (version 5.1) software package.

Results

Digestibility

The apparent digestibility coefficients (ADC) obtained for dietary energy and protein were 87.7 and 92.8%, respectively.

Whole body composition

The whole body composition of growing fish from 23 to 400 g is presented in Figure 1, and the models resulting from the allometric functions are overplotted together with the functions found by Lupatsch *et al.* (1998).

The linear form of the equations are:

$$\mathrm{LN\ (Energy)} \pm 0.08 = 1{,}782 \pm 0{,}067 + 0.090 \pm 0.014 * \mathrm{LN\ (BW)} \quad (9)$$

$$\mathrm{LN\ (Lipid)} \pm 0.16 = 4{,}215 \pm 0{,}131 + 0.143 \pm 0.027 * \mathrm{LN\ (BW)} \quad (10)$$

$$\mathrm{LN\ (Dry\ matter)} \pm 0.056 = 5{,}484 \pm 0{,}046 + 0.077 \pm 0.009 * \mathrm{LN\ (BW)} \quad (11)$$

$$\mathrm{LN\ (Protein)} \pm 0.045 = 4{,}938 \pm 0{,}036 + 0.042 +/- 0.007 * \mathrm{LN\ (BW)} \quad (12)$$

In all cases intercepts and slopes are significant (P-value<0.0000). As it can be seen the standard error of lipid determination is almost three times the protein standard error and it influence on energy standard error, as consequence of the different feeding rates.

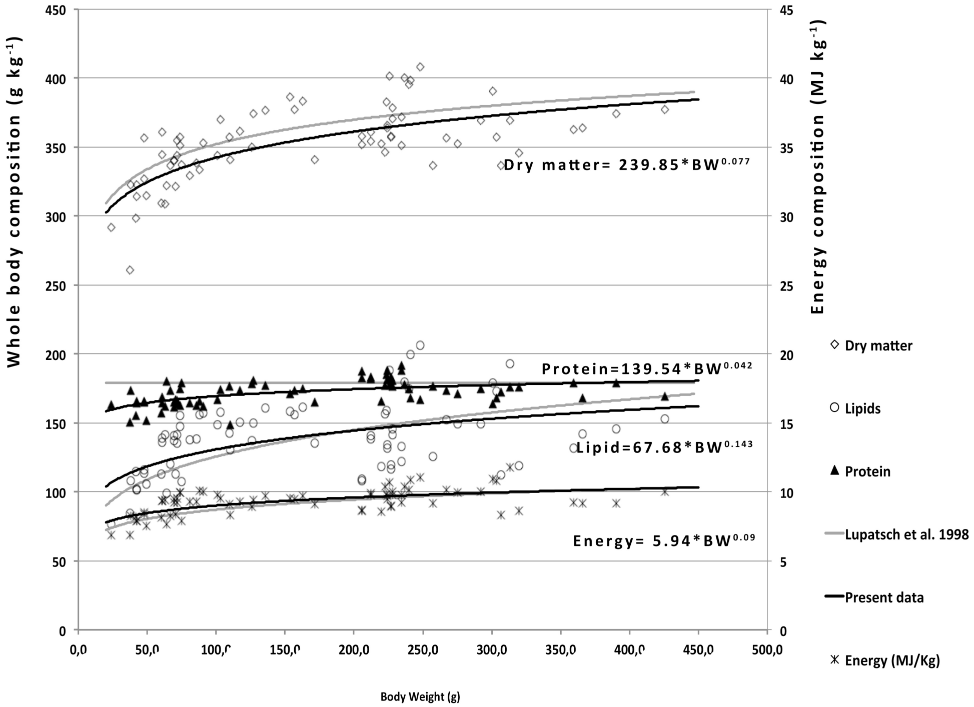


Figure 1.- Whole body composition of gilthead sea bream from 23 to 400 g.

Nutrient intake and retention curves

Mean values of digestible energy intake (DEI), digestible protein intake (DPI), energy recovery (ER) and protein deposition (PD) obtained in the six trials are presented in Table 2. A close relation between nutrient intake and protein deposition or energy recovery was obtained in the six trials, as an increment in retention was observed when ingestion increased.

Table 2.- Protein deposition and energy recovery of sea bream fed at different feeding levels.

Trial	**Feeding Rate** (% day-1)	**Digestible Protein Intake** (g kg$^{-0.7}$ day^{-1})	**Protein Deposition** (g kg$^{-0.7}$ day^{-1})	**Digestible Energy Intake** (kJ kg$^{-0.82}$ day^{-1})	**Energy Recovery** (kJ kg$^{-0.82}$ day^{-1})
Trial 1	0.00	0	-1.05	0	-28.3
	0.99	2.69	1.22	73.4	34.8
	1.89	4.42	1.72	131.3	57.34
	2.36	5.43	1.77	166.9	60.63
SE	±0.04	± 0.05	± 0.04	± 2.0	± 1.7
Trial 2	0	0	-1.23	0	-30.6
	0.33	1.18	-0.35	28.0	-4.6
	0.62	1.89	0.64	48.6	21.0
	0.99	2.67	1.05	72.6	29.6
	1.17	3.04	1.18	84.6	40.6
	1.37	3.44	1.33	97.7	45.9
SE	0.62	± 0.03	± 0.05	±0.9	±3.7
Trial 3	0	0	-0.67	0	-23.4
	0.62	2.14	0.81	59.6	17.9
	1.16	3.49	1.36	105.4	42.8
	1.62	4.54	1.53	143.6	48.46
SE	±0.02	±0.03	±0.06	±1.0	±2.1
Trial 4	0	0	-0.61	0	-24.1
	0.63	2.08	0.76	60.4	23.5
	1.18	3.38	1.31	106.8	45.5
	1.66	4.21	1.29	138.2	46.5
SE	±0.05	±0.04	±0.04	±1.7	±1.8
Trial 5	0	0	-0.70	0	-18.1
	0.30	1.19	0.36	26.9	11.1
	0.55	1.76	0.53	42.8	22.1
	0.55	2.06	0.61	50.2	23.4
	1	2.15	0.69	53.8	26.4
SE	±0.07	±0.08	±0.03	±2.4	±3.7
Trial 6	0	0	-0.56	0	-17.9
	0.3	1.21	-0.13	27.45	-1.3
	0.55	1.76	0.51	42.6	17.3
	1	2.66	0.89	69.0	31.6
	1.4	3.33	1.01	84.8	40.6
SE	±0.07	±0.06	±0.17	±1.7	±6.5

Considering the previous results obtained by Mayer *et al.* (2012), two different equations for ER and PD in small fish from trials 1, 2 and 3 (Equation 13 and 14) and in large fish from trials 4, 5 and 6 (equation 15 and 16) have been developed, which are depicted in Figures 2 and 3.

Small gilthead sea bream (<110 g):

$ER \pm 5.34 = 74.2 \pm 5.387 * (1 - e^{(-0.012 \pm 0.001 * (DEI - 26.7 \pm 1.5))})$ $r^2_{adj} = 97.25$ (13)

$PD \pm 0.19 = 2.34 \pm 0.22 * (1 - e^{(-0.35 \pm 0.05 * (DPI - 1.01 \pm 0.06))})$ $r^2_{adj} = 96.65$

(14)

Large gilthead sea bream (> 110 g):

$ER \pm 5.05 = 56.3 \pm 4.9 * (1 - e^{(-0.017 \pm 0.002 * (DEI - 19.5 \pm 1.41))})$ $r^2_{adj} = 95.62$

(15)

$PD \pm 0.15 = 2.18 \pm 0.38 * (1 - e^{(-0.3 \pm 0.07 * (DPI - 0.86 \pm 0.06))})$ $r^2_{adj} = 95.38$

(16)

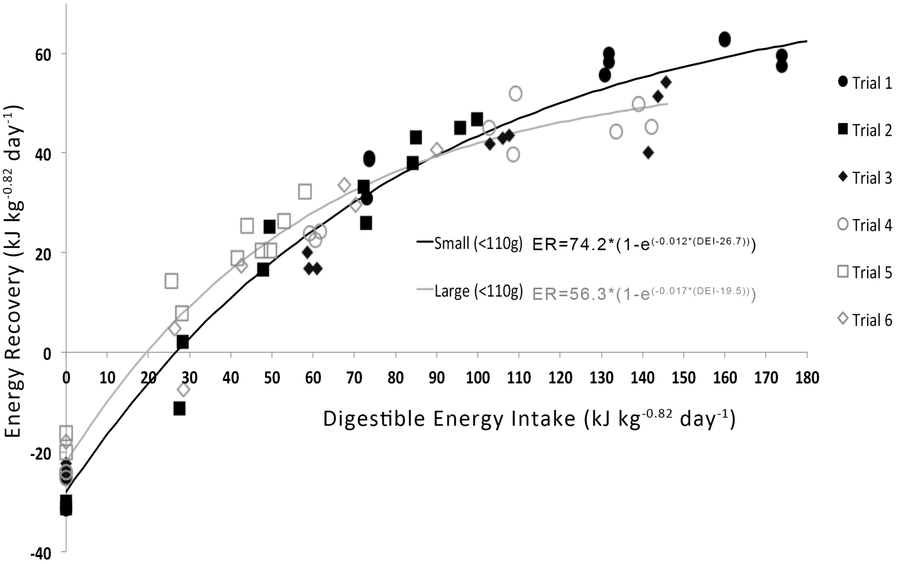


Figure 2.- Energy recovery response curves for different feeding levels of gilthead sea bream below and above 110g of body weight.

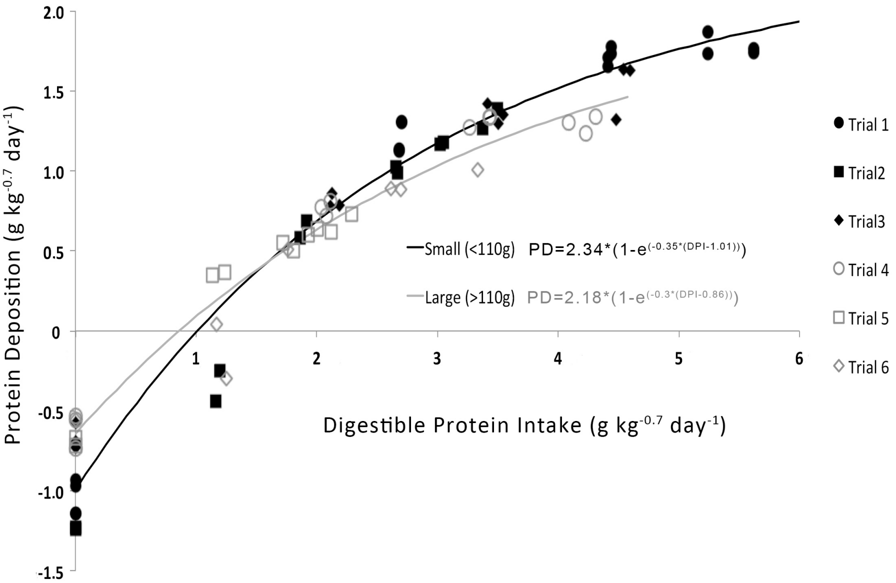


Figure 3.- Protein deposition response curves for different feeding levels of gilthead sea bream below and above 110g of body weight.

Maintenance requirements and efficiency

Maintenance needs were estimated solving equations 13 to 16 for zero energy recovery and protein deposition.Digestible energy for maintenance in small gilthead sea bream was estimated as 26.7 kJ kg$^{-0.82}$ day^{-1} and 19.5 kJ kg$^{-0.82}$ day^{-1} for large gilthead sea bream.Digestible protein for maintenance in small gilthead sea bream was estimated as 1.01 g kg$^{-0.7}$ day^{-1} and 0.86 g kg$^{-0.7}$ day^{-1} for large gilthead sea bream.

The efficiencies of energy (k_e) and protein for growth (k_p) were calculated applying equations 7 and 8 to equations 13 to 16, which are plotted in Figures 4 and 5. The efficiency of energy recovery for growth (k_e) in small gilthead sea bream (<110 g) decreased from k_e=0.88 when the intake was close to maintenance to k_e=0.41 with the highest energy intakes. Likewise, in large gilthead sea bream (> 110 g), the efficiency of energy recovery for growth decreased from k_e=0.90 to k_e=0.39.

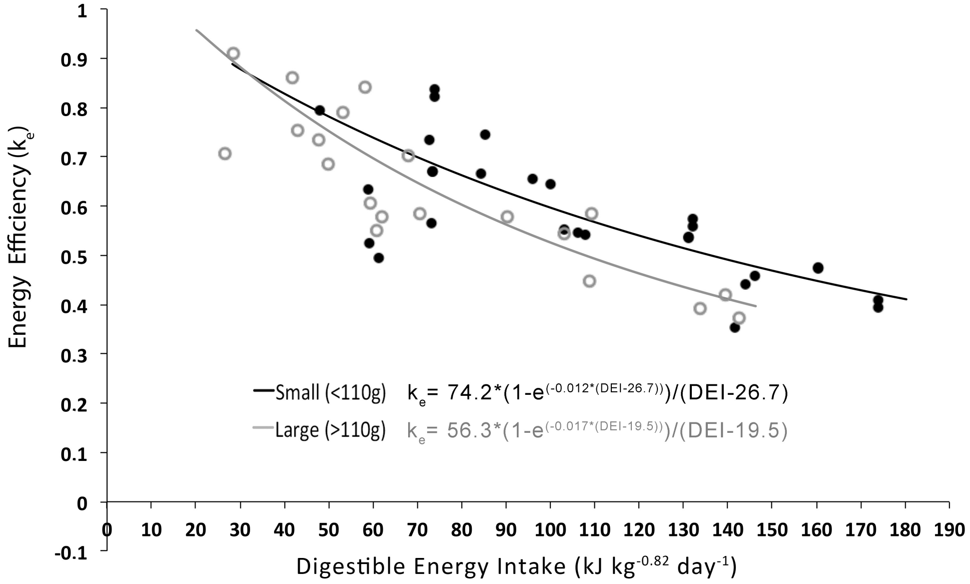


Figure 4.- Energy efficiency of gilthead sea bream fed at different feeding levels and fish size.

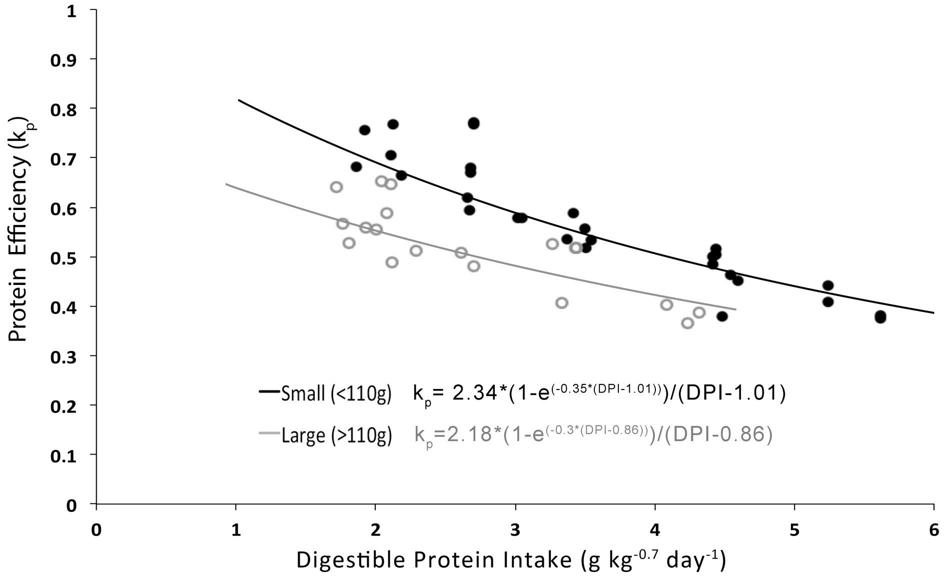


Figure 5.- Protein efficiency of gilthead sea bream fed at different feeding levels and fish size.

The efficiency of protein deposition for growth (k_p) in small gilthead sea bream (<110 g) decreased with the increase of protein intake from k_p=0.8 to k_p=0.4 and in large gilthead sea bream (>110g) from k_p=0.65 to k_p=0.4.

Discussion

Whole body composition

As can be seen, the curves established by Lupatsch *et al.* (1998) and the curves developed in the present work agree closely. Moreover, the body composition curves presented by Lupatsch *et al.* (2003a) also agree, but are not plotted in Figure 1.

In this study, the body protein composition was affected by the body weight but its effect seems to be low, having a significant influence on the lower weights only (Figure 1). The linear regression of the LN(PC) = LN(a)+ b*LN (BW) showed a significance of the slope b and intercept a (P-Value <0.0000). The body protein content varied form 159 to 179 g kg^{-1}, which agrees partially with Lupatsch *et al.* (1998, 2003a) who reported a constant value of 176 ± 8.4 g kg^{-1}. Nevertheless, Lupatsch *et al.* (1998, 2003a) obtained a horizontal line regression for protein body content, whereas, in the current trial, an allometric regression was obtained.

Lipid carcass content increased with body weight, in agreement with Lupatsch *et al.* (1998, 2003a). Obviously, lipid body composition depends on the amount of energy intake, evidencing that fish with a low feeding level have a lower lipid content than fish with a high feeding level. As already reported in other fish species, the lipid content unavoidably affects the energy content of the body (Weatherley & Gill 1987; Azevedo *et al*., 2004a; Azevedo, *et al*., 2004b, Dumas *et al*., 2010), which increases allometrically in relation to body weight.

Dry matter of carcass will also depend on the energy intake (Lupatsch *et al.*, 1998) given the negative relation between moisture and lipids.

The variance of the energy content of the body, lipids and dry matter was high in the present study as different feeding levels result in different levels of energy body content. Dumas *et al.* (2007a) also mentioned this effect in trout, showing that the lipid content may depend on feeding.

Retention curves

The existence of different growth stanzas depending on the fish size in several species (Dumas *et al.*, 2007b in trout other species, and Mayer *et al.*, 2012 in gilthead sea bream) are likely to reflect the changes in growth strategies during fish growth. These changes, in turn, may result in changes in energy recovery and protein deposition.

In previous retention studies, Lupatsch *et al.* (1998, 2001a, 2003a, 2003b) reported a single model for energy recovery and one for protein deposition of all fish in a range between 17 and 93.3 g of initial body weight. Nevertheless, other studies considered differences in energy recovery and protein deposition response curves for different fish sizes, such as Glencross (2008) in *Lates calcarifer*. In the present study best fits were found when the data were examined separately for two different sizes, one for small fish (< 110g) and one for large fish (>110g).

The reason for these changes are not clear, but evidence in growth stanzas indicates changes in growth strategies that can influence the response curves of energy recovery and protein deposition. These small changes in the curves may have a great influence on the feed conversion rate under farm conditions.

On the other hand, the shape of retention curves must be considered. In the case of protein deposition, it seems clear that the curvilinear model instead of the linear model is suitable, and results of the current study agree with Lupatsch *et al.* (2003a) in gilthead sea bream and Glencross (2008) in barramundi. Nevertheless, the case of energy recovery, is somewhat controversial, as Lupatsch *et al.* (2003a) reported a linear model, but Glencross (2008) obtained a curvilinear relation. Also Van Trung *et al.* (2011) in tilapia cited a quadratic model, reporting in the two curvilinear models a reduction of energy recovery efficiency at high levels of energy intake, which agrees with the results obtained in the current work.

Digestible Energy for maintenance

The maintenance energy points (HE_m) in the present study, estimated within the range of temperatures from 22 to 25 ºC, were 26.7 and 19.5 KJ $kg^{-0.82}$ day^{-1} for small, respectively, large gilthead sea bream, which seem lower than the values obtained by Lupatsch *et al.* (1998) at 23-24 ºC, ranging from 55.8 to 61.7 KJ $kg^{-0.83}$ day^{-1}, and by Lupatsch *et al.* (2003b) who estimated 47 KJ $kg^{-0.82}$ day^{-1} for 21ºC and 84 KJ $kg^{-0.82}$ day^{-1} for 27ºC, in both cases with small fish (< 110 g). In other species, results also differ, Glencross (2008) reported a digestible energy maintenance value of 42.6 KJ $kg^{-0.82}$ day^{-1} at 30ºC in barramundi. In trout species Bureau *et al.* (2006) determined, using a factorial approach, a metabolic maintenance energy of 19.2 KJ kg^{-82} day^{-1} and assuming a ratio of ME DE^{-1} of about 0.92–0.96 in fish (calculated by Cho 1992), a HE_m between 20 and 20.86 KJ kg^{-82} day^{-1} could be assumed, although this value is calculated for 8.5ºC, a temperature relatively low even for trout. Nevertheless, Van Trung *et al.* (2011) obtained a maintenance energy demand of 25.9 KJ $kg^{-0.82}$ day^{-1} for tilapia growing at 28 ºC, a value in agreement with that obtained in the current work.

The differences in HE_m between studies could have several reasons. The first was already given by Bureau *et al.* (2006) and was caused by the large standard error related with the estimated resulting from the factorial approach which should be taken with caution. Another hypothesis could be related to the duration of the trials, or with the initial corporal composition, which may influence the metabolism of the fish. According to this, fish consuming a diet under optimal growth conditions for a longer period, or with a lower energy body content, could try to save energy lowering their activity. However, HE_m determination should be further examined.

Digestible Protein for maintenance

The values of digestible protein for maintenance (DP_m) in the present study were 1.01 and 0.86 g $kg^{-0.7}$ day^{-1} for small, respectively, large fish, very close to the results found by Lupatsch *et al.* (1998) for the small fish size, between 0.86 and 0.96 g $kg^{-0.7}$ day^{-1}, but lower than DP_m of 0.62 g $kg^{-0.7}$ day^{-1} found by Lupatsch *et al.* (2003b). Fournier *et al.* (2002) calculate the N for maintenance as 84.7 mg $kg^{-0.75}$ day^{-1} that can be estimated to be in protein 0.53 g $kg^{-0.75}$ day^{-1}. Although DP_m results obtained in the present work might seem high, Booth *et al.* (2007) reported similar values, 0.89-1.45 g $kg^{-0.7}$ day^{-1} in a sparid species (*Pagrus auratus*), but Glencross (2008) cited a DP_m of around 0.45 g $kg^{-0.7}$ day^{-1} in barramundi, demonstrating that protein maintenance needs vary greatly depending on the species and conditions. In fact Fournier *et al.*, (2002) demonstrated that the amino acids profile is relevant, for instance, the maintenance requirements of arginine are close to cero.

Efficiency

Efficiency has also been studied by other authors (Xie *et al.*, 1997; Lupatsch *et al.*, 1998, 2001a, 2001b, 2003a, 2003b; Sá *et al.*, 2006; Bureau *et al.*, 2006; Booth *et al.*, 2007), and there seems to be a general agreement on the shape of the protein deposition curve, as most authors found a nonlinear correlation between ingestion and protein deposition. The efficiency of energy (k_e) and protein

(k_p) in decelerating models, as in the current work, considers that efficiency decreases with the increase of the intake. Hence, there is no single value of k, making it necessary to establish in each case the optimum value of efficiency with respect to ingestion. This model can easily be explained in the case of protein, i.e. when the amount of digestible protein is sufficient to reach maximum growth, the rest of protein can not be destined for body protein deposition. However, in the case of energy, it seems that fish have an unlimited capacity for energy recovery, represented by linear models, which is not in agreement with the current results, and other authors (Glencross, 2008; Van Trung *et al.*, 2011).

In the case of protein efficiencies (Figure 5), a clear difference was observed between the efficiency values of small and large gilthead sea bream, being higher for the entire range of digestible protein in small fish.

With regard to energy retention, most authors found a linear correlation and very few authors found an asymptotic correlation. Bureau *et al.* (2000) concluded that studies involving the rearing of fish under a variety of conditions (water temperature, feeding level, fish size, etc.) had shown that the efficiency of energy utilization was, surprisingly, constant (Cho & Kaushik, 1990; Azevedo *et al.*, 1998; ; Ohta & Watanabe, 1998; Lupatsch *et al.*, 1998, 2003a; Rodehutscord & Pfeffer, 1999; Bureau *et al.*, 2006). Nevertheless, in the present study, best fits were obtained with nonlinear regressions, partially in agreement with Glencross (2008) who found a nonlinear fit in barramundi and also with Booth *et al.* (2007).

In this sense, a constant efficiency of energy retention for gilthead sea bream was reported by Lupatsch *et al.* (1998, 2001a 2003a), but who considered a broken-line regressions for energy retention, with two different slopes below and above maintenance (Lupatsch *et al.*, 1998). In the present work, no trial showed a constant value of net efficiency over maintenance for energy retention, the values obtained for net efficiency show a clear decreasing tendency (Figure 4), which seems more consistent with the asymptotic model, or any other limited growth model than the constant efficiency proposed by Lupatsch *et al.* (1998, 2001a, 2003a). In the present study, the efficiency of retention of digestible energy intake varied from below 0.4 to more than 0.7 with a strong dependence on digestible energy intake (Figure 4), values which agree with those reached by Lupatsch *et al.* (1998), namely 0.54 in efficiency above maintenance, but also with Lupatsch *et al.* (2001a) who obtained a value of 0.5 studying different diet compositions, and with Lupatsch *et al.* (2003a) with an energy efficiency of 0.68.

Energy comes from the deposition of lipids, protein, and carbohydrate, yet the entire digestible lipid intake, exceeding the energy needs, should be stored as lipid. As the digestible protein must be stored as protein deposition or desamined to form acetyl-CoA to be used as energy or deposited as lipid, then the efficiency of use protein as energy source and the energy cost for storing the excess of protein intake as lipid could justify the decrease in efficiency when the protein intake is high. Recently, Ekmann *et al.* (2013) demonstrated in *Sparus aurata* that between 20 and 30% of the deposited lipids can be attributed to dietary protein, as consequence of the *novo* lipogenesis.

Following the scheme proposed by NRC (2011), and presented in Figure 6, this lack of efficiency showed when the energy intake (and also protein) increased, which could be explained by the increase in “heat increment of feeding” (H_iE), which can be separated into three categories: digestion and absorption processes (H_dE), formation and excretion of metabolic waste (H_wE) and transformation and interconversion of the substrates and their retention in tissues (H_rE). H_dE should be proportional to intake and relatively small (NRC 2011). H_wE is also very low in fish as gills efficiently excrete ammonia, but H_rE, could be very high when the digestible protein intake is high, given the elevated energy required to convert protein into lipid. The maximum theorical efficiency of conversion of dietary lipids into body lipids is about 96%, the theorical efficiency of conversion of aminoacids into body protein is around 85%, but the conversion of aminoacids into body lipids is 66%, (NRC, 2011), which means that if the aminoacid intake is not destined to protein deposition, the energy efficiency from aminoacid intake could drop to a minimum of 19% as a consequence of the H_iE increment.

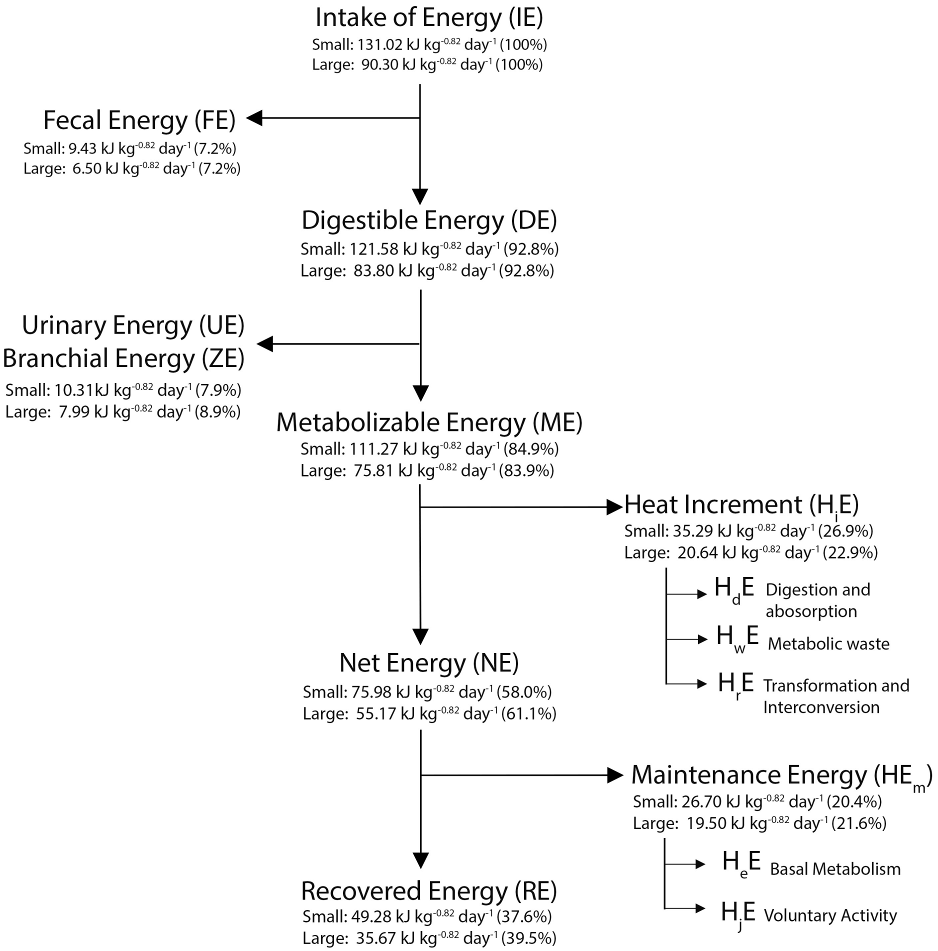


Figure 6.- Schematic representation of energy flows through an animal (based on NRC 1981) and mean values for higher feeding rates and percentages of energy for small (<110g) and large (>110g) gilthead sea bream.

In Figure 6 the mean values of energy flows and their percentages, for the higher feeding rates of each trial of small and large gilthead sea bream, are represented: H_iE is 26.9 % of the energy intake for small fish, and 22.9% for large fish, evidencing that H_iE may represent a remarkable fall in energy efficiency. In the current study, H_iE percentages are even higher than maintenance (HE_m). As Guinea & Fernandez (1997) demonstrated in gilthead sea bream with a mean weight between 30 and 100 g, a rise in the feeding rate lead to an H_iE increase. The reason of could be explained by Company *et al.* (1999), who, in fingerlings of gilthead sea bream, showed that the increase of lipid intake could affect the growth hormone lowering its effect, which explains the decrease of energy deposition efficiency with the increase of the feeding rate.

In the present study, the reduction of protein efficiency at high digestible protein intake (Figure 5) produces a high quantity of aminoacids that needs to be converted into energy or desamined to finish stored as lipid, and consequently a reduction of energy recovery (Figure 4), which could justify the reduction of digestible energy efficiency when the digestible energy increases.

Some other factors might affect energy recovery at high feeding levels, the longer experimental period of the current trials, compared to the 30-day experiment of Lupatsch *et al.* (1998, 2001a, 2003a), could have led to a maximum body fat content, limiting lipid deposition and reduce efficiency in higher feeding rates.

The recommendations for diet formulation of *Sparus aurata*, should be reviewed, for several reasons: until present study needs of *Sparus aurata* were based on studies until 100g of weight and there are evidences a drop on energy and protein net efficiency for growth with the increase of intake. This shows that different growth speed could need different Protein/energy ratio on diet to satisfy the requirements with a optimum efficiency. However, further studies are needed in the quest for the optimum feed composition, maximizing profitability in relation to efficiency and growth.

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CAPÍTULO 6

Defining optimum diets for gilthead sea bream (*Sparus aurata*) using multifactorial approaches.

Under review

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Defining optimum diets for gilthead sea bream (*Sparus aurata*) using multifactorial approaches.

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Abstract

Defining a diet using multifactorial approaches for sea bream involves at least three different models: a growth model, a corporal composition model, and an energy and protein retention model. Diets for sea bream, ranging from 25g to 450g, two temperatures (21ºC an 25ºC) and three Thermal Growth Coefficients (0.00150, 0.00120 and 0.00080) were designed and the limits of the simulations were established. It was found that diets containing a relation of digestible protein over digestible energy between 25 and 30 g MJ^{-1} are preferable.

Keywords

Sparus aurata, modeling , retention, efficiency.

Introduction

In the last years, numerous studies have tried to establish the fish requirements of several fish species using factorial models and other methods (Cho & Kaushik, 1990; Xie *et al.*, 1997; Azevedo *et al.*, 1998; Ohta & Watanabe, 1998; Watanabe *et al.*, 2000, 2001; Lupatsch *et al.*, 1998, 2001a, 2001b, 2003a, 2003b; Kaushik, 1998; Rodehutscord & Pfeffer, 1999; Fournier *et al.*, 2002; Hernández *et al.*, 2003; Azevedo *et al.*, 2005; Bureau *et al.*, 2006; Sá *et al.*, 2006; Booth *et al.*, 2007; Glencross, 2008; Dumas *et al.*, 2010; Van Trung *et al.*, 2011, Jauralde *et al.*, 2015) either for maintenance and for growth, under varying conditions. Shedding light on fish needs is required for the design of diets, optimizing nutrient utilization and profitability. Such designs are usually based on recommendations that have to be considered when designing a particular feed. For example, a diet to maximize efficiency or minimize the feed conversion rate (FCR), even penalizing growth, could improve economic profitability (Martínez-Llorens *et al.*, 2009; Jauralde *et al.*, 2013).

Many factors influence fish requirements: fish body-weight, initial corporal composition, final corporal composition desired, temperature, maintenance needs, efficiency for retention, and many others. Most of these factors are interrelated, and therefore their determination is by no means easy.

Diets are designed for several ranges of body weights and actually most diets are not based on the nutrient requirements, but with “optimum levels” of nutrients that produce a higher growth. Consequently, currently avaible diets increase the lipid composition of sea bream above the desired levels (Grigorakis *et al.*, 2002; Sağlık *et al.*, 2003). In sea bream, the whole body composition is directly related to body weight (Lupatsch *et al.*, 2003b; Jauralde *et al.*, 2015). The proportion of lipids in the body and the MJ/kg of energy increase with body weight. Some authors report a protein content constant with body weight (Lupatsch *et al.,* 2003a; 2003b), while Jauralde *et al.* (2015) found a statistical allometric increment with the body weight. Estimate requirements for growth determine the amount of protein and energy that need to be deposited to increase body weight. The period of time required to increase the targeted final body weight will determine the daily needs of protein deposition and energy recovery. The intake required to reach that daily deposition is related to the efficiency of retention.

To study the suitability of diets, the efficiency of the feeds must be compared. The most common parameter used by fish farmers to compare feed efficiency is the feed conversion rate (FCR), which from a nutritional point of view is not very useful as it does not consider the amount of nutrients. For fish farmers, the FCR is useful to compare expenses, or compare between feeds with a similar amount of nutrients but should not be used to compare between diets with a similar DP/DE ratio but different amounts of nutrients.

Nutritionists commonly use the *net efficiency* for growth, estimated as the proportion of energy or protein retained over the nutrient intake minus the maintenance needs. Some authors found these net efficiencies to be constant with the intake of energy or protein (Cho & Kaushik, 1990; Azevedo *et al.*, 1998; Ohta & Watanabe, 1998; Lupatsch *et al.*, 1998, 2001b, 2003b; Rodehutscord & Pfeffer, 1999; Bureau *et al.*, 2006) while others authors obtained a decreasing net efficiency of nutrient deposition with the increase of intake (Glencross, 2008; Van Trung *et al.*, 2011; Jauralde *et al.*, 2015). In other words, if the fish need to retain a small amount of energy or protein, they are able to do so with more efficiency than when a large amount of energy or protein has to be retained.

Whereas, for fish farmers, net efficiency for growth has a low economic meaning, because their interest focuses on the entire intake, independent of how much of that intake is destined for growth. That efficiency can be studied through *gross efficiency*.

Herein, several diets for various growth conditions based on the needs estimated in previous works studying factorial approaches in sea bream and the role that bodyweight plays in feeding efficiency are developed.

Material and methods

Retention, Maintenance and efficiencies

Data by Jauralde *et al*. (2015) were for fish between 23.3 and 289.9g of initial body weight and temperatures between 21.5ºC and 25ºC, in Jauralde *et al*. (2015). Energy Recovery (ER) equations obtained by Jauralde *et al*. (2015) were used to estimate diets:

Small sea breams (<110g)

$$ER\ (kJ\ kg^{-0.82}\ day^{-1}) = 74.2*(1-e^{(-0.012*(DEI-26.7))}) \qquad (1)$$

Large sea breams (>110g)

$$ER\ (kJ\ kg^{-0.82}\ day^{-1}) = 56.3*(1-e^{(-0.017*(DEI-19.5))}) \qquad (2)$$

These equations reached the implicit maintenance values of 26.7 and 19.5 kJ $kg^{-0.82}$ day^{-1} for small and large sea breams respectively, and a variable net efficiency of energy for growth (k_e) that can be calculated for a determined ER value solving digestible energy intake (DEI) from equations 1 or 2, Thereafter k_e can be calculated applying the equation:

$$k_e = ER/(DEI-E_{man}) \qquad (3)$$

Similary, the values of protein for maintenance (P_{man}) and net efficiency of protein retention for growth (k_p), were obtained by means of Jauralde's *et al.* (2015) equations for protein deposition (PD):

Small sea breams (<110g)

$$PD\ (g\ kg^{-0.7} day^{-1}) = 2.34*(1-e^{(-0.35*(DPI-1.01))}) \quad (4)$$

Large sea breams (>110g)

$$PD\ (g\ kg^{-0.7} day^{-1}) = 2.18*(1-e^{(-0.3*(DPI-0.86))}) \quad (5)$$

A P_{man} of 1.01 and 0.86 were obtained through equations 4 and 5 for large and small sea breams. And the net efficiency of protein for growth (k_p) was calculated from the following equation:

$$k_p = PD/(DPI-P_{man}) \quad (6)$$

Whole body composition

Fish whole body composition equations were obtained applying the equations by Jauralde *et al.* (2015):

Energy Content $EC\ (kJ\ g^{-1}) = 4.66*BW^{0.139}$ (7)

Protein Content $PC\ (g\ kg^{-1}) = 139.54*BW^{0.042}$ (8)

Lipid Content $LC\ (g\ kg^{-1}) = 67.68*BW^{0.143}$ (9)

Dry matter $DM\ (g\ kg^{-1}) = 239.85*BW^{0.077}$ (10)

From the above equations, the total needs of protein and energy were calculated for five consecutive weight ranges: 25g to 50g, 50g to 100g, 100g to 200g, 200g to 300g and 300g to 450g using the following procedure.

Three different levels of growth speed were used to determine requirements: high-speed level, medium-speed level and a low-speed level. To determine a high-speed level and low-speed level, the daily growth was estimated using a TGC value of 0.0015 and 0.0008 based on Jauralde *et al*. (2013) results for maximum Economic Profit Index (EPI), and minimum FCR by means of quadratic simulation. The medium-speed level was fixed at 0.0012, the average value in the interval between the maximum EPI and minimum FCR. In addition, 2 temperatures were studied, 21ºC and 25 ºC, which altogether implies 6 different growth regimens.

Days of growth between two sizes were estimated as follows:

$$\text{days} = (BW_f^{1/3}-BW_i^{1/3})/((T^a-T^a_{min})*TGC) \qquad (11)$$

where T^a_{min} is the minimum temperature for growth, 12ºC in *Sparus aurata.*

The energy increment was estimated in (ΔE) as follows:

$$\Delta E\ (kJ\ fish^{-1}\ day^{-1}) = ((BW_f*EC_f)-(BW_i*EC_i))/days \qquad (12)$$

where E_i and E_f are obtained by working out the energy content of equation 7 with BW_i and BW_f in grams.

And the protein increment in (ΔP) was calculated as follows:

$$\Delta P\ (g\ fish^{-1}\ day^{-1}) = ((BW_f*PC_f/1000)-(BW_i*PC_i/1000))/days \qquad (13)$$

where PC_i and PC_f were obtained by calculating the protein content from equation 8 with BW_i and BW_f in grams.

The geometric body weight (GBW) was calculated in kilograms as follows:

$$\text{GBW (kg)} = (BW_i * BW_f)^{1/2}/1000 \qquad (14)$$

where BW_i and BW_f were introduced in grams.

In case of variable growth efficiency, its value was determined with the equations of table 1 and 2 determining DEI and DPI first determining energy recovery (ER) and protein deposition (PD) as follows:

$$\text{ER (kJ kg}^{-0.82}\text{ day}^{-1}) = (\Delta E/GBW)^{0.82} \qquad (16)$$

$$\text{PD (g kg}^{-0.7}\text{ day}^{-1}) = (\Delta P/GBW)^{0.7} \qquad (15)$$

Once ER and PD have been determined the DEI and DPI can be calculated from the equations 1 and 2, and 4 and 5 and applying Equations 3 and 6 the net efficiencies for growth may be calculated.

The need for maintenance per fish ($E_{man}{}^{f}$ and $P_{man}{}^{f}$) was calculated as follows:

$$E_{man}{}^{f}\text{(kJ fish}^{-1}\text{day}^{-1}) = E_{man} * (GBW)^{0.82} \qquad (17)$$

$$P_{man}{}^{f}\text{(g fish}^{-1}\text{day}^{-1}) = P_{man} * (GBW)^{0.7} \qquad (18)$$

The needs for growth per fish were estimated as follows:

$$E_{growth}\text{ (kJ fish}^{-1}\text{day}^{-1}) = \Delta E * k_e \qquad (19)$$

$$P_{growth}\text{ (g fish}^{-1}\text{day}^{-1}) = \Delta P * k_p \qquad (20)$$

And the total needs:

E_{tot} (kJ fish^{-1}day^{-1}) $=E_{man}{}^{f}+E_{growth}$ (21)

P_{tot} (g fish^{-1}day^{-1}) $=P_{man}{}^{f}+P_{growth}$ (22)

Once the total needs have been calculated, the diet composition can be established. The digestible protein over digestible energy (DP/DE) ratio of the diet is:

DP/DE (g MJ^{-1}) = $1000*P_{tot}/E_{tot}$ (23)

And the gross efficiency for energy and maintenance is calculated as:

Gross $Ef_{protein} = \Delta P/\ P_{tot}$ (24)

Gross $Ef_{energy} = \Delta E/\ E_{tot}$ (25)

After that, an infinite number of combinations of energy and protein may be calculated. Therefore, a digestible energy (DE) of the diet must be arbitrarily determined. In that case, to standardize results and attending to the results found in (Lupatsch *et al.*, 2001b; Velázquez *et al.*, 2006; Company *et al.*, 1999) a diet with a 16 MJ Kg^{-1} is predetermined.

The intake need to cover the requirements can be calculated to satisfy the total energy needs (E_{tot}) as:

Intake (g fish^{-1} day^{-1}) = $E_{tot}/16$ (26)

And the Digestible Protein (DP) of the diet can be calculated as:

$$DP\ (g\ kg^{-1}) = 1000*P_{tot}/Intake \qquad (27)$$

The Feeding rate (FR) corresponding to the previously defined diet to satisfy the needs then is then calculated as:

$$FR\ (g\ 100g^{-1}\ day^{-1}) = 100*Intake/((BW_i+BW_f)/2) \qquad (28)$$

and the estimated Feed Conversion Rate (FCR):

$$FCR=Intake*days/((BW_f-BW_i)) \qquad (29)$$

Results

Six different growth situations were studied: three different TGC (TGC=0.0015, TGC=0.0012 and TGC=0.0008) in combination with 2 temperatures (21ºC and 25ºC)

The protein and energy requirements and the diet ratio DP/DE to cover the needs for the six growth situations (3 TGCs and 2 temperatures) during a growth cycle (from 25 to 450g) are shown in tables 1 and 2. Both, protein and energy needs increase with TGC but, on the other hand, a higher TGC results in a lower DP/DE ratio. DP/DE ratios are higher with the lower temperature of 21ºC, as the increase of energy needs (E_{TOT}) with temperature and TGC is greater than the increase of protein required(P_{TOT}).

The diets to cover these needs based on the estimated P/E ratio, are shown in tables 3 and 4 with the associated Feeding Rate (FR) and FCR considering diets with 16, 18 and 20 MJ kg^{-1}. Dietary protein reduces with higher TGC, higher temperature, and low dietary energy, but the feeding rate increases. FCR decreases with a high dietary energy and low temperature but it is similar in all fish sizes.

Nevertheless, it might be difficult to formulate some of the diets due to the high dietary protein level required.

Table 1.- Daily energy and protein requirements at 21ºC (Jauralde *et al.*, 2015)

BW	TGC	E_{man}	P_{man}	E_{growth}	P_{growth}	E_{TOT}	P_{TOT}	P/E
25-50	0.00150	1.72	0.10	7.14	0.16	8.86	0.26	28.94
	0.00120	1.72	0.10	5.11	0.11	6.83	0.21	30.56
	0.00080	1.72	0.10	2.98	0.07	4.70	0.16	34.69
50-100	0.00150	3.04	0.16	10.96	0.23	14.00	0.39	27.91
	0.00120	3.04	0.16	8.06	0.17	11.11	0.33	29.63
	0.00080	3.04	0.16	4.77	0.10	7.81	0.25	32.52
100-200	0.00150	3.92	0.22	18.84	0.45	22.76	0.66	29.20
	0.00120	3.92	0.22	13.61	0.33	17.53	0.55	31.24
	0.00080	3.92	0.22	7.93	0.20	11.85	0.42	35.37
200-300	0.00150	6.15	0.32	26.00	0.63	32.15	0.95	29.59
	0.00120	6.15	0.32	18.92	0.47	25.07	0.79	31.50
	0.00080	6.15	0.32	11.49	0.29	17.64	0.61	34.45
300-450	0.00150	8.58	0.43	33.67	0.80	42.25	1.23	29.04
	0.00120	8.58	0.43	24.91	0.61	33.49	1.04	30.91
	0.00080	8.58	0.43	14.86	0.35	23.44	0.78	33.27

Cross out data are considered out of range

The units are BW(g), E_{man} (kJ^{-1} $fish^{-1}$ day^{-1}), P_{man} (g $fish^{-1}$ day^{-1}), E_{growth} (kJ^{-1} $fish^{-1}$ day^{-1}), P_{growth} (g $fish^{-1}$ day^{-1}), E_{TOT} (kJ^{-1} $fish^{-1}$ day^{-1}), P_{TOT} (g $fish^{-1}$ day^{-1}), P/E (g MJ^{-1})

Table 2.- Daily energy and protein requirements at 25ºC (Jauralde *et al.*, 2015)

BW	TGC	E_{man}	P_{man}	E_{growth}	P_{growth}	E_{TOT}	P_{TOT}	P/E
25-50	~~0.00150~~	~~1.72~~	~~0.10~~	~~15.32~~	~~0.39~~	~~17.04~~	~~0.48~~	~~28.34~~
	0.00120	1.72	0.10	9.20	0.21	10.92	0.31	28.37
	0.00080	1.72	0.10	4.84	0.11	6.56	0.20	31.01
50-100	~~0.00150~~	~~3.04~~	~~0.16~~	~~20.46~~	~~0.45~~	~~23.50~~	~~0.60~~	~~25.72~~
	0.00120	3.04	0.16	13.72	0.30	16.76	0.45	27.05
	0.00080	3.04	0.16	7.70	0.16	10.75	0.32	29.72
100-200	~~0.00150~~	~~3.92~~	~~0.22~~	~~40.76~~	~~0.81~~	~~44.68~~	~~1.03~~	~~23.11~~
	0.00120	3.92	0.22	24.45	0.56	28.37	0.78	27.60
	0.00080	3.92	0.22	12.93	0.31	16.85	0.53	31.43
200-300	0.00150	6.15	0.32	49.10	1.05	55.26	1.37	24.81
	0.00120	6.15	0.32	32.62	0.77	38.77	1.09	28.05
	0.00080	6.15	0.32	18.29	0.44	24.45	0.76	31.12
300-450	0.00150	8.58	0.43	60.78	1.31	69.36	1.74	25.06
	0.00120	8.58	0.43	41.71	0.95	50.29	1.38	27.44
	0.00080	8.58	0.43	24.00	0.57	32.58	0.99	30.50

Crossed out data are considered out of range

The units are BW(g), E_{man} (kJ^{-1} $fish^{-1}$ day^{-1}), P_{man} (g $fish^{-1}$ day^{-1}), E_{growth} (kJ^{-1} $fish^{-1}$ day^{-1}), P_{growth} (g $fish^{-1}$ day^{-1}), E_{TOT} (kJ^{-1} $fish^{-1}$ day^{-1}), P_{TOT} (g $fish^{-1}$ day^{-1}), P/E (g MJ^{-1})

Table 3.- Dietary protein at 16, 18 and 20 MJ/Kg, Feeding Rate and Feed Conversion Rate at 21 ºC based on Jauralde *et al.* (2015).

BW	TGC	P/E	P_{diet}	FR	FCR	P_{diet}	FR	FCR	P_{diet}	FR	FCR
DE (MJ/kg)				**16**			**18**			**20**	
25-50	0.00150	28.94	463	1.48	1.25	521	1.32	1.11	579	1.18	1.00
	0.00120	30.56	489	1.14	1.20	550	1.01	1.07	611	0.91	0.96
	0.00080	34.69	555	0.78	1.24	624	0.69	1.10	694	0.62	0.99
50-100	0.00150	27.91	447	1.17	1.24	503	1.04	1.10	559	0.94	0.99
	0.00120	29.63	474	0.93	1.23	533	0.83	1.09	593	0.74	0.98
	0.00080	32.52	520	0.65	1.30	585	0.58	1.16	650	0.52	1.04
100-200	0.00150	29.20	467	0.95	1.27	525	0.84	1.13	584	0.76	1.02
	0.00120	31.24	500	0.73	1.22	563	0.65	1.08	625	0.58	0.98
	0.00080	35.37	566	0.49	1.24	637	0.44	1.10	708	0.39	0.99
200-300	0.00150	29.59	474	0.80	1.27	533	0.71	1.13	593	0.64	1.02
	0.00120	31.50	504	0.63	1.22	567	0.56	1.08	630	0.50	0.98
	0.00080	34.45	551	0.44	1.24	620	0.39	1.10	689	0.35	0.99
300-450	0.00150	29.04	465	0.70	1.26	523	0.62	1.12	581	0.56	1.01
	0.00120	30.91	495	0.56	1.25	557	0.50	1.11	619	0.45	1.00
	0.00080	33.27	532	0.39	1.31	599	0.35	1.16	665	0.31	1.05

Crossed out data are considered out of range

The units are BW(g), P/E (g MJ^{-1}), P_{diet} in (g kg^{-1}) and FR (% day^{-1})

Table 4.- Dietary protein at 16, 18 and 20 MJ/Kg, Feeding Rate and Feed Conversion Rate at 25 ºC based on Jauralde *et al.* (2015).

BW	TGC	P/E	P_{diet}	FR	FCR	P_{diet}	FR	FCR	P_{diet}	FR	FCR
DE (MJ/kg)				**16**			**18**			**20**	
25-50	0.00150	~~28.34~~	~~453~~	~~2.84~~	~~1.66~~	~~510~~	~~2.52~~	~~1.48~~	~~566~~	~~2.27~~	~~1.33~~
	0.00120	28.37	454	1.82	1.33	511	1.62	1.18	568	1.46	1.06
	0.00080	31.01	496	1.09	1.20	558	0.97	1.07	620	0.87	0.96
50-100	0.00150	~~25.72~~	~~411~~	~~1.96~~	~~1.44~~	~~462~~	~~1.74~~	~~1.28~~	~~514~~	~~1.57~~	~~1.15~~
	0.00120	27.05	433	1.40	1.29	487	1.24	1.15	541	1.12	1.03
	0.00080	29.72	476	0.90	1.24	536	0.80	1.10	595	0.72	0.99
100-200	0.00150	~~23.11~~	~~370~~	~~1.86~~	~~1.73~~	~~416~~	~~1.65~~	~~1.54~~	~~463~~	~~1.49~~	~~1.38~~
	0.00120	27.60	442	1.18	1.37	497	1.05	1.22	553	0.94	1.10
	0.00080	31.43	503	0.70	1.22	566	0.62	1.08	629	0.56	0.98
200-300	0.00150	24.81	397	1.38	1.50	447	1.23	1.33	496	1.10	1.20
	0.00120	28.05	449	0.97	1.31	505	0.86	1.16	561	0.78	1.05
	0.00080	31.12	498	0.61	1.24	560	0.54	1.10	623	0.49	0.99
300-450	0.00150	25.06	401	1.16	1.44	451	1.03	1.28	501	0.93	1.15
	0.00120	27.44	439	0.84	1.30	494	0.75	1.16	549	0.67	1.04
	0.00080	30.50	488	0.54	1.26	549	0.48	1.12	610	0.43	1.01

Crossed out data are considered out of range

The units are BW(g), P/E (g MJ^{-1}), P_{diet} in (g kg^{-1}) and FR (%)

Discussion

To examine differences with other studies, it is necessary to standardize the data and calculations. For the present species, *Sparus aurata*, recovery data by Lupatsch *et al.* (1998; 2001b; 2003b) can be used to estimate diets applying the same procedures as those applied in the present paper and the following calculations in Materials and Methods.

Several energy recovery (ER) and protein deposition (PD) equations, maintenance requirements and efficiencies can be found in the experiments carried out by Lupatsch, but in the present study the data by Lupatsch *et al.* (2003b) were preferred, as they include a relatively wide range of fish weights (until 150g) and temperatures, and use the information obtained in previous studies. Protein deposition and energy recovery data by Lupatsch *et al.* (2003b) were established for fish between 21.4 and 94.4 g of initial body weight, and a range of temperatures between 21ºC and 28 ºC. The energy net efficiency (k_e) was estimated to be 0.67 from the mean of the slopes of the formulas ER = -32.2+0.68*DEI and ER= -55.8+ 0.66*DEI for 21 and 28ºC, respectively. The E_{man} was estimated to be dependent on temperature related to the formula $ER=16.6*e^{(0.055*T^a)}$ in ($kJ\,kg^{-0.82}\,day^{-1}$). Thus the formula established by Lupatsch *et al.* (2003b) to develop diets can be used as:

Energy Recovery

$$ER\ (kJ\,kg^{-0.82}\,day^{-1}) = -0.67*16.6\ e^{(0.055*T^a)}+0.67*DEI \qquad (30)$$

deduced from Lupatsch *et al.* (2003b) because if ER= a+b*DEI, where b is the efficiency of 0.67 and -a/b= $E_{man,}$ then a= -b*$E_{man.}$.

Similarly, data of protein requirements by Lupatsch *et al.* (2003b) are expressed by the equation:

Protein Deposition

$$\text{PD (g kg}^{-0.7}\text{ day}^{-1}) = 3.16*(1-e^{(-0.159*(DPI-0.62))}) \qquad (31)$$

expressed in (g kg$^{-0.7}$day^{-1}) which has a implicit constant value of P_{man} of 0.62 (g kg$^{-0.7}$day^{-1}) and a variable efficiency for growth k_p= PR/(DPI-P_{man}), but when Lupatsch *et al.* (2003b) calculated diets, a constant efficiency of 0.47 was used. Assumint the value of P_{man} and the constant efficiency is the same as assuming the following equation:

$$\text{PD (g kg}^{-0.7}\text{ day}^{-1}) = -0.2914+0.47*DPI \qquad (32)$$

Resulting in PR_{man}= 0.62 g when solved for PR=0 and a constant efficiency for growth of 0.47. Both options, k_p variable or fixed at 0.47 are examined.

Four different response curves for energy recovery (equations 1,2 and equation 30 particularized for 21ºC and 25ºC) and 4 different response curves for protein deposition (equations 4, 5, 31 and 32) are obtained.

All the response curves for ER have been plotted together in Figure 1, and the response curves for PD have been plotted in Figure 2. The curves are plotted in each case up to their study range.

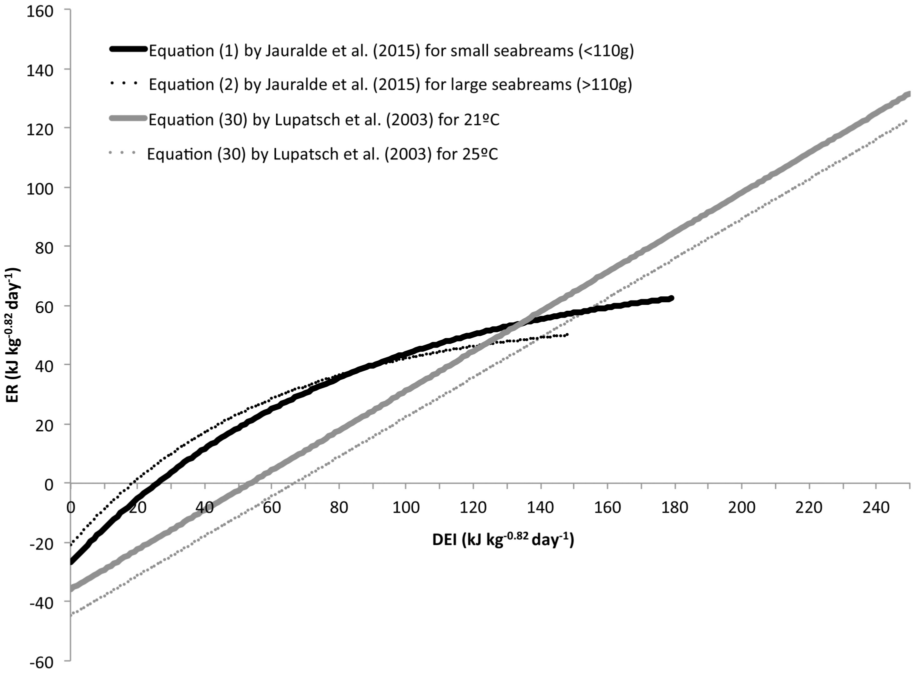

Figure 1.- Energy recovery response curve from two authors and situations.

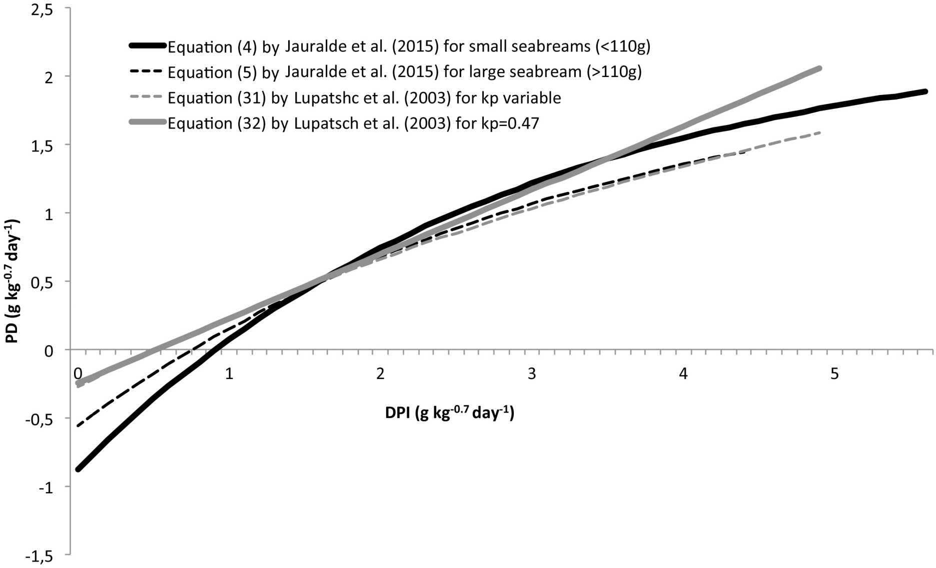

Figure 2.- Protein recovery response curve according to Jauralde *et al.* (2015) and Lupatsch *et al.* (2003) respectively.

Moreover, the whole body composition also reached some differences between Lupatsch *et al*. (2003b) and Jauralde *et al*. (2015). Even if diets could be calculated combining equation requirements according to Jauralde *et al*. (2015) and body composition following Lupatsch *et al*. (2003b), or *vice versa*, in the present study the diet determination by each author using the whole body composition estimated by the same author has been preferred. Used data of body composition by Lupatsch *et al*. (2003b) are shown in the following equations:

Energy Content (kJ g^{-1}) $EC=5.94*BW^{0.09}$ (33)

Protein Content (g kg^{-1}) $PC=176$ (34)

Lipid Content (g kg^{-1}) $LC=43.3*BW^{0.243}$ (35)

Dry matter (g kg^{-1}) $DM=1000-(777*BW^{0.041})$ (36)

With all this equations, nutrient requirements and optimum diets for a fixed value of DE of 16 MJ kg^{-1} were developed following the procedures mentioned, and presented in tables 5, 6, 7 and 8 in which data out of range were crossed out.

Once all the diets have been formulated, similarities and differences can be examined to reach some conclusions. The relation of PD/ED has a huge range of needs related to many factors: the retention equation, body composition, temperature, fish body weight, TGC desired, etc.

Dietary protein decreases with higher temperature, but, contrarily to tables 3 and 4, dietary protein increases with TGC and FCR increases with weight and reduces when the TGC increases.

Table 5.- Energy and protein requirements, diet at 16 MJ/Kg and FR and FCR at 21 ºC based on Lupatsch *et al*. (2003b) with the fixed value of k_p=0.47

BW	**TGC**	**E_{man}**	**P_{man}**	**E_{growth}**	**P_{growth}**	**E_{TOT}**	**P_{TOT}**	**P/E**	**P_{diet}**	**FR**	**FCR**
25-50	0.00150	3.40	0.06	5.81	0.17	9.21	0.23	24.55	393	1.53	1.30
	0.00120	3.40	0.06	4.65	0.13	8.05	0.19	23.96	383	1.34	1.42
	0.00080	3.40	0.06	3.10	0.09	6.50	0.15	22.84	366	1.08	1.71
50-100	0.00150	6.00	0.10	10.15	0.26	16.16	0.36	22.35	358	1.35	1.43
	0.00120	6.00	0.10	8.12	0.21	14.12	0.31	21.82	349	1.18	1.57
	0.00080	6.00	0.10	5.42	0.14	11.42	0.24	20.83	333	0.95	1.90
100-200	0.00150	10.60	0.16	17.75	0.42	28.34	0.58	20.35	326	1.18	1.58
	0.00120	10.60	0.16	14.20	0.34	24.79	0.49	19.88	318	1.03	1.73
	0.00080	10.60	0.16	9.47	0.22	20.06	0.38	19.00	304	0.84	2.10
200-300	~~0.00150~~	~~16.62~~	~~0.23~~	~~27.20~~	~~0.60~~	~~43.83~~	~~0.83~~	~~18.91~~	~~303~~	~~1.10~~	~~1.72~~
	~~0.00120~~	~~16.62~~	~~0.23~~	~~21.76~~	~~0.48~~	~~38.39~~	~~0.71~~	~~18.48~~	~~296~~	~~0.96~~	~~1.88~~
	~~0.00080~~	~~16.62~~	~~0.23~~	~~14.51~~	~~0.32~~	~~31.13~~	~~0.55~~	~~17.67~~	~~283~~	~~0.78~~	~~2.29~~
300-450	~~0.00150~~	~~23.18~~	~~0.31~~	~~37.71~~	~~0.78~~	~~60.89~~	~~1.09~~	~~17.91~~	~~286~~	~~1.01~~	~~1.82~~
	~~0.00120~~	~~23.18~~	~~0.31~~	~~30.17~~	~~0.63~~	~~53.35~~	~~0.93~~	~~17.50~~	~~280~~	~~0.89~~	~~1.99~~
	~~0.00080~~	~~23.18~~	~~0.31~~	~~20.11~~	~~0.42~~	~~43.30~~	~~0.73~~	~~16.75~~	~~268~~	~~0.72~~	~~2.43~~

Crossed out data are considered out of range

The units are BW(g), E_{man} (kJ^{-1} fish^{-1} day^{-1}), P_{man} (g fish^{-1} day^{-1}), E_{growth} (kJ^{-1} fish^{-1} day^{-1}), P_{growth} (g fish^{-1} day^{-1}), E_{TOT} (kJ^{-1} fish^{-1} day^{-1}), P_{TOT} (g fish^{-1} day^{-1}), P/E (g MJ^{-1}), P_{diet} in (g kg^{-1}) and FR (%)

Table 6.- Energy and protein requirements, diet at 16 MJ/Kg and FR and FCR at 25 ºC based on Lupatsch *et al*. (2003b) with the fixed value of k_p=0.47

BW	**TGC**	**E_{man}**	**P_{man}**	**E_{growth}**	**P_{growth}**	**E_{TOT}**	**P_{TOT}**	**P/E**	**P_{diet}**	**FR**	**FCR**
25-50	~~0.00150~~	~~4.24~~	~~0.06~~	~~8.39~~	~~0.24~~	~~12.63~~	~~0.30~~	~~23.75~~	~~380~~	~~2.10~~	~~1.23~~
	~~0.00120~~	~~4.24~~	~~0.06~~	~~6.71~~	~~0.19~~	~~10.95~~	~~0.25~~	~~23.01~~	~~368~~	~~1.82~~	~~1.33~~
	0.00080	4.24	0.06	4.47	0.13	8.71	0.19	21.56	345	1.45	1.59
50-100	~~0.00150~~	~~7.48~~	~~0.10~~	~~14.67~~	~~0.38~~	~~22.15~~	~~0.48~~	~~21.60~~	~~346~~	~~1.85~~	~~1.36~~
	~~0.00120~~	~~7.48~~	~~0.10~~	~~11.73~~	~~0.31~~	~~19.21~~	~~0.40~~	~~20.93~~	~~335~~	~~1.60~~	~~1.47~~
	0.00080	7.48	0.10	7.82	0.20	15.30	0.30	19.63	314	1.28	1.76
100-200	~~0.00150~~	~~13.20~~	~~0.16~~	~~25.64~~	~~0.61~~	~~38.84~~	~~0.76~~	~~19.64~~	~~314~~	~~1.62~~	~~1.50~~
	0.00120	13.20	0.16	20.51	0.48	33.71	0.64	19.04	305	1.40	1.63
	0.00080	13.20	0.16	13.67	0.32	26.88	0.48	17.88	286	1.12	1.95
200-300	~~0.00150~~	~~13.20~~	~~0.16~~	~~25.64~~	~~0.61~~	~~38.84~~	~~0.76~~	~~19.64~~	~~314~~	~~1.62~~	~~1.50~~
	~~0.00120~~	~~13.20~~	~~0.16~~	~~20.51~~	~~0.48~~	~~33.71~~	~~0.64~~	~~19.04~~	~~305~~	~~1.40~~	~~1.63~~
	~~0.00080~~	~~13.20~~	~~0.16~~	~~13.67~~	~~0.32~~	~~26.88~~	~~0.48~~	~~17.88~~	~~286~~	~~1.12~~	~~1.95~~
300-450	~~0.00150~~	~~28.89~~	~~0.31~~	~~54.47~~	~~1.13~~	~~83.36~~	~~1.44~~	~~17.25~~	~~276~~	~~1.39~~	~~1.73~~
	~~0.00120~~	~~28.89~~	~~0.31~~	~~43.58~~	~~0.90~~	~~72.47~~	~~1.21~~	~~16.73~~	~~268~~	~~1.21~~	~~1.88~~
	~~0.00080~~	~~28.89~~	~~0.31~~	~~29.05~~	~~0.60~~	~~57.94~~	~~0.91~~	~~15.72~~	~~251~~	~~0.97~~	~~2.25~~

Crossed out data are considered out of range

The units are BW(g), E_{man} (kJ^{-1} fish^{-1} day^{-1}), P_{man} (g fish^{-1} day^{-1}), E_{growth} (kJ^{-1} fish^{-1} day^{-1}), P_{growth} (g fish^{-1} day^{-1}), E_{TOT} (kJ^{-1} fish^{-1} day^{-1}), P_{TOT} (g fish^{-1} day^{-1}), P/E (g MJ^{-1}), P_{diet} in (g kg^{-1}) and FR (%)

Table 7.- Energy and protein requirements, diet at 16 MJ/Kg and FR and FCR at 21 ºC based on Lupatsch *et al.* (2003b) with variable k_p

BW	TGC	E_{man}	P_{man}	E_{growth}	P_{growth}	E_{TOT}	P_{TOT}	P/E	P_{diet}	FR	FCR
25-50	0.00150	3.40	0.06	5.81	0.22	9.21	0.28	30.75	492	1.53	1.30
	0.00120	3.40	0.06	4.65	0.17	8.05	0.23	28.17	451	1.34	1.42
	0.00080	3.40	0.06	3.10	0.10	6.50	0.16	24.52	392	1.08	1.71
50-100	0.00150	6.00	0.10	10.15	0.33	16.16	0.43	26.63	426	1.35	1.43
	0.00120	6.00	0.10	8.12	0.25	14.12	0.35	24.50	392	1.18	1.57
	0.00080	6.00	0.10	5.42	0.15	11.42	0.25	21.74	348	0.95	1.90
100-200	0.00150	10.60	0.16	17.75	0.50	28.34	0.65	23.09	369	1.18	1.58
	0.00120	10.60	0.16	14.20	0.38	24.79	0.53	21.56	345	1.03	1.73
	0.00080	10.60	0.16	9.47	0.23	20.06	0.39	19.34	309	0.84	2.10
200-300	~~0.00150~~	~~16.62~~	~~0.23~~	~~27.20~~	~~0.68~~	~~43.83~~	~~0.92~~	~~20.89~~	~~334~~	~~1.10~~	~~1.72~~
	~~0.00120~~	~~16.62~~	~~0.23~~	~~21.76~~	~~0.52~~	~~38.39~~	~~0.75~~	~~19.59~~	~~313~~	~~0.96~~	~~1.88~~
	~~0.00080~~	~~16.62~~	~~0.23~~	~~14.51~~	~~0.33~~	~~31.13~~	~~0.57~~	~~18.16~~	~~291~~	~~0.78~~	~~2.29~~
300-450	~~0.00150~~	~~23.18~~	~~0.31~~	~~37.71~~	~~0.87~~	~~60.89~~	~~1.18~~	~~19.42~~	~~311~~	~~1.01~~	~~1.82~~
	~~0.00120~~	~~23.18~~	~~0.31~~	~~30.17~~	~~0.68~~	~~53.35~~	~~0.99~~	~~18.52~~	~~296~~	~~0.89~~	~~1.99~~
	~~0.00080~~	~~23.18~~	~~0.31~~	~~20.11~~	~~0.42~~	~~43.30~~	~~0.73~~	~~16.81~~	~~269~~	~~0.72~~	~~2.43~~

Crossed out data are considered out of range

The units are BW(g), E_{man} (kJ^{-1} fish^{-1} day^{-1}), P_{man} (g fish^{-1} day^{-1}), E_{growth} (kJ^{-1} fish^{-1} day^{-1}), P_{growth} (g fish^{-1} day^{-1}), E_{TOT} (kJ^{-1} fish^{-1} day^{-1}), P_{TOT} (g fish^{-1} day^{-1}), P/E (g MJ^{-1}), P_{diet} in (g kg^{-1}) and FR (%)

Table 8.- Energy and protein requirements, diet at 16 MJ/Kg and FR and FCR at 25 ºC based on Lupatsch *et al.* (2003b) with variable k_p

BW	TGC	E_{man}	P_{man}	E_{growth}	P_{growth}	E_{TOT}	P_{TOT}	P/E	P_{diet}	FR	FCR
25-50	~~0.00150~~	~~4.24~~	~~0.06~~	~~8.39~~	~~0.39~~	~~12.63~~	~~0.45~~	~~35.59~~	~~569~~	~~2.10~~	~~1.23~~
	~~0.00120~~	~~4.24~~	~~0.06~~	~~6.71~~	~~0.28~~	~~10.95~~	~~0.34~~	~~30.61~~	~~490~~	~~1.82~~	~~1.33~~
	0.00080	4.24	0.06	4.47	0.16	8.71	0.22	24.86	398	1.45	1.59
50-100	~~0.00150~~	~~7.48~~	~~0.10~~	~~14.67~~	~~0.54~~	~~22.15~~	~~0.64~~	~~28.78~~	~~460~~	~~1.85~~	~~1.36~~
	~~0.00120~~	~~7.48~~	~~0.10~~	~~11.73~~	~~0.40~~	~~19.21~~	~~0.50~~	~~25.89~~	~~414~~	~~1.60~~	~~1.47~~
	0.00080	7.48	0.10	7.82	0.24	15.30	0.34	21.92	351	1.28	1.76
100-200	~~0.00150~~	~~13.20~~	~~0.16~~	~~25.64~~	~~0.78~~	~~38.84~~	~~0.94~~	~~24.18~~	~~387~~	~~1.62~~	~~1.50~~
	0.00120	13.20	0.16	20.51	0.58	33.71	0.74	22.00	352	1.40	1.63
	0.00080	13.20	0.16	13.67	0.36	26.88	0.52	19.19	307	1.12	1.95
200-300	~~0.00150~~	~~20.72~~	~~0.23~~	~~39.29~~	~~1.05~~	~~60.01~~	~~1.28~~	~~21.33~~	~~341~~	~~1.50~~	~~1.63~~
	~~0.00120~~	~~20.72~~	~~0.23~~	~~31.43~~	~~0.79~~	~~52.15~~	~~1.03~~	~~19.67~~	~~315~~	~~1.30~~	~~1.77~~
	~~0.00080~~	~~20.72~~	~~0.23~~	~~20.96~~	~~0.49~~	~~41.67~~	~~0.72~~	~~17.34~~	~~277~~	~~1.04~~	~~2.12~~
300-450	~~0.00150~~	~~28.89~~	~~0.31~~	~~54.47~~	~~1.35~~	~~83.36~~	~~1.65~~	~~19.84~~	~~317~~	~~1.39~~	~~1.73~~
	~~0.00120~~	~~28.89~~	~~0.31~~	~~43.58~~	~~1.03~~	~~72.47~~	~~1.34~~	~~18.44~~	~~295~~	~~1.21~~	~~1.88~~
	~~0.00080~~	~~28.89~~	~~0.31~~	~~29.05~~	~~0.64~~	~~57.94~~	~~0.95~~	~~16.34~~	~~261~~	~~0.97~~	~~2.25~~

Crossed out data are considered out of range

The units are BW(g), E_{man} (kJ^{-1} fish^{-1} day^{-1}), P_{man} (g fish^{-1} day^{-1}), E_{growth} (kJ^{-1} fish^{-1} day^{-1}), P_{growth} (g fish^{-1} day^{-1}), E_{TOT} (kJ^{-1} fish^{-1} day^{-1}), P_{TOT} (g fish^{-1} day^{-1}), P/E (g MJ^{-1}), P_{diet} in (g kg^{-1}) and FR (%)

Limits of the study

Not all the data calculated should be used to reach conclusions, as some data are out of the study range. Energy and protein retention equations made by Jauralde *et al*. (2015) have a minimum initial body weight of 22.1 g and a maximum final body weight of 435g and the maximum intake of digestible protein (DPI) of 5.7 g $kg^{-0.7}$ day^{-1} for BW_i <110g and 4.5 for BW_i >110g and a maximum of DEI of 180 kJ $kg^{-0.82}$ day^{-1} for BW_i <110g and 140 for BW_i >110g. Calculated data beyond DEI or DPI trial data are crossed out in tables 1, 2, 3 and 4.

Energy and protein retention equations by Lupatsch *et al*. (2003b) have a minimum initial body weight of 21.4g and a maximum initial body weight of 94.4g with a final body weight approximate of 155 g if the equation for maximum growth estimation is used: Body Weight Increment=$0.024*BW^{0.514}*e^{(0.06*T^{a})}$ (Lupatsch *et al*., 2003b). The range of weights of 200 to 300 and 300 to 450g are beyond the trial data, are considered out of the study range and are crossed out in tables 5 to 8.

The maximum intake of digestible protein (DPI) in Lupatsch *et al*. (2003b) was around 5.0 g $kg^{-0.7}$ day^{-1} and the DEI maximum was around 250 kJ $kg^{-0.82}$ $day^{-1.}$ The data that need a higher DEI and DPI than the limits of the study are considered out of the study range and are crossed out in the tables 5 to 8. In both cases, Jauralde *et al*. (2015) and Lupatsch *et al*. (2003b), the whole body composition is studied for the full range needed.

The limits are explored, using the growth model by Jauralde *et al*. (2013) based on TGC, but the same can be done using the growth model developed by Lupatsch *et al*. (2003b). If the growth prediction by Lupatsch *et al*. (2003b) is applied for 100g of BW_i and 25 ºC then the Weight gain (g/fish/day) = $0.024*100^{0.514} * e^{0.060*25}$=1.15 g $fish^{-1}$ day^{-1} and BW_f = 101.15

As a consequence of applying equation 13 with the PC of equation 34, fish should grow 0.2 g of protein per day, and in metabolic weight (apply equations 15 and 31) this means that the fish has to retain 1.62 g $kg^{-0.7}$ day^{-1},and therefore should consume the amount of 5.1 g $kg^{-0.7}$ day^{-1} of digestible protein. This DPI for a fish of 100g is in the limits of the range obtained by Lupatsch. But when the requirements for maximum growth are study for 25g and 50g at 25 ºC, the DPI is 11.50g $kg^{-0.7}$ day^{-1} for 25g and 7.2g $kg^{-0.7}$ day^{-1} for 50g of initial body weight, beyond the study range, see figures 1 and 2. This should be enough to invalidate most of diets recommendations by Lupatsch *et al*. (2003b) but not the ER and PD equations that are still useful to obtain diets recommendations inside of the study range.

Effect of weight.

It is usually assumed that smaller fish need a higher DP/DE ratio in their diet. Lupatsch *et al*. (2003b) showed a range of needs of DP/DE from 19 to 32 g of digestible Protein for MJ of digestible energy, i.e. a decreasing ratio with the increase of weight. This might seem logical, because if body composition changes with weight are examined, the protein composition (PC) stays fairly constant, while the energy composition (EC) increases with fish body weight. However, when the partial efficiency of retention of the digestible energy and protein intake are considered, together with the maintenance needs, the final ratio request of DP/DE might vary greatly. Using the equations by Jauralde *et al.* (2015), the of DP/DE ratio does not decrease as weight increases and remains mostly constant. But when dietary requirements, according to Lupatsch *et al.* (2003), are examined, DP/DE decrease in all cases with weight, as a consequence of the not dependence on energy efficiency with weight or digestible energy intake.

Effect of whole body composition.

When the protein deposition and energy recovery are estimated to determine intake requirements, body composition curves are used, usually estimated using allometric expressions coming from its linear version, (Dumas *et al*., 2007). Curves by Lupatsch *et al*. (2003b) and Jauralde *et al*. (2015) are very close (Figure 3) but some small differences are likely to have a great influence on requirements. The most significant differences are in the lipid content that has a different slope, the equation (35) by Lupatsch *et al*. (2003b) has a higher slope than equation (9) by Jauralde *et al*. (2015); the reason for this difference is probably one of the most important differences to be examined. The energy content (EC) as a consequence of the lipid content (LC) has a great variation for the same body weight, depending greatly on the amount of lipids and energy intake. In fact, LC has a standard error three times greater than PC standard error (Jauralde *et al.,* 2015). The influence of feed ration with lipid composition and its variance, was already mentioned by Shearer (1994).

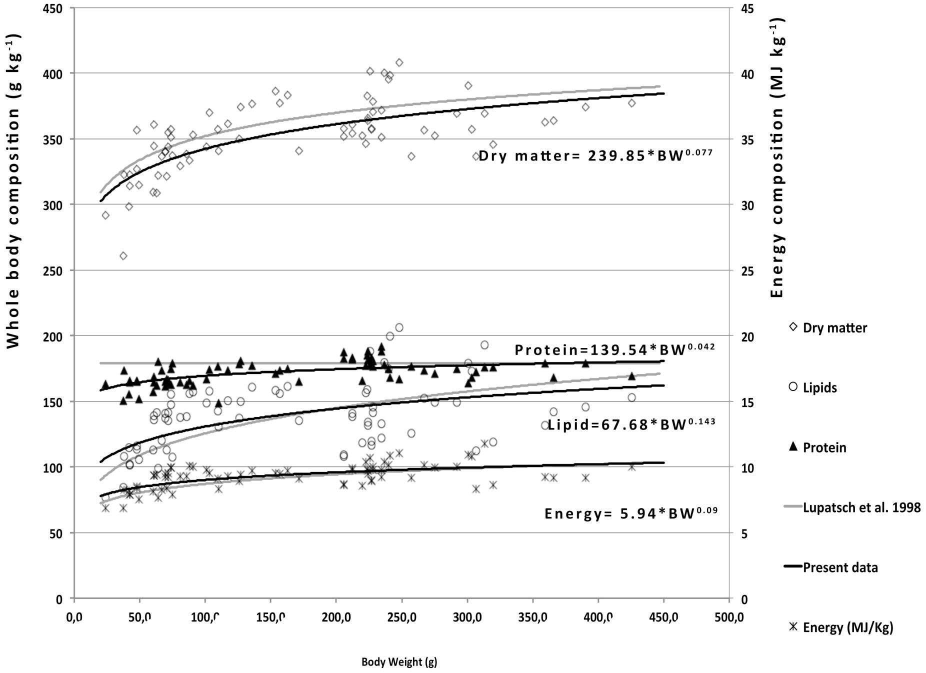

Figure 3.- Whole body composition of gilthead sea bream.

To examine the variability in lipid content, some authors have used the LC:PC ratio as an indicator of the fish condition. In the present study, the LC:PC ratio increases with body weight (P-Value < 0.0000) as previously observed in trout but not in salmon (Azevedo *et al.*, 2005). The main parameter influencing the LC:PC ratio is the feeding rate.

Figure 4 shows the lipid body content of sea bream fed with several feeding rates in relation of body weight, in which fish have been separated into three different groups with regard to the LC:PC ratio. Several curves for LC may be determined, the upper and lower curves show the limits of an acceptable medium lipid content.

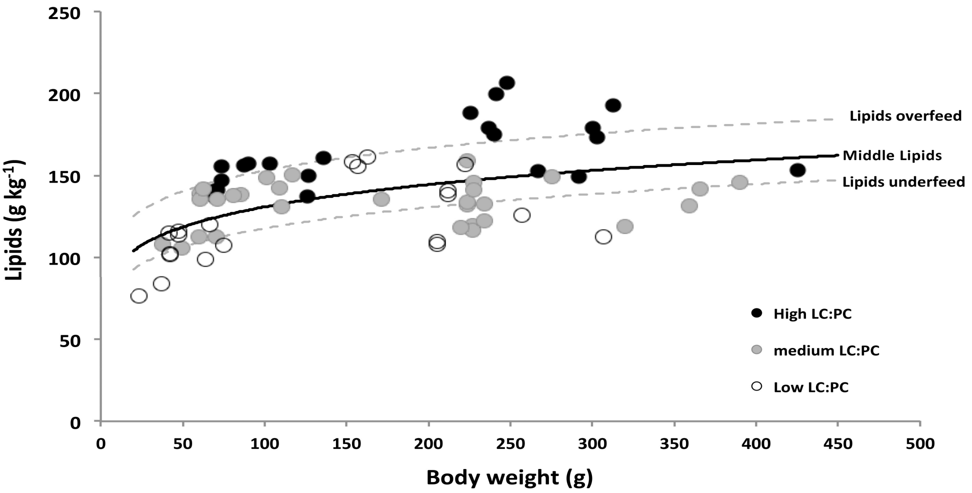

Figure 4.- Lipid content limits related to the LC:PC ratio

Some other small differences may be found in the body content curves but if there are no differences between slopes, they do not represent changes in the growth requirements.

The variability in the lipid content offers a choice with regard to the design of diets. A high-energy intake produces fish with a high lipid content. On the other hand, a low energy intake might result in fish with a low lipid content.

Now, it would be interesting to resolve the next question: what kind of diet might result in an excessively fat fish with a high LC:PC level? The diets which produces a final lipid content considered as over-fat are calculated in relation to the final lipid and energy content, considering equations 37 and 38, and their results are shown in Table 9.

$$LC= 86.19*BW^{0.1245} \quad (37)$$

$$EC_f=(PC*23.6+LC*39.5)/1000 \quad (38)$$

Protein/energy ratios of diets for over-fat are between 19 and 21 g MJ^{-1}

Table 9.- Requirements of energy and protein, diet at 16 MJ/Kg and FR and FCR for a TGC=0.00150 at 21 ºC allowing an increase of the LC:PC ratio together with equations by Jauralde *et al.* (2015).

BW	**TGC**	**E_{man}**	**P_{man}**	**E_{growth}**	**P_{growth}**	**E_{TOT}**	**P_{TOT}**	**P/E**	**P_{diet}**	**FR**	**FCR**
25-50	0.00150	1.72	0.10	10.23	0.16	11.95	0.26	21.46	343	1.99	1.68
50-100	0.00150	3.04	0.16	15.02	0.23	18.06	0.39	21.64	346	1.51	1.60
100-200	0.00150	3.92	0.22	27.29	0.45	31.21	0.66	21.30	341	1.30	1.74
200-300	0.00150	6.15	0.32	41.77	0.63	47.92	0.95	19.86	318	1.20	1.88
300-450	0.00150	8.58	0.43	53.43	0.80	62.01	1.23	19.79	317	1.03	1.85

The units are BW(g), E_{man} (kJ^{-1} fish^{-1} day^{-1}), P_{man} (g fish^{-1} day^{-1}), E_{growth} (kJ^{-1} fish^{-1} day^{-1}), P_{growth} (g fish^{-1} day^{-1}), E_{TOT} (kJ^{-1} fish^{-1} day^{-1}), P_{TOT} (g fish^{-1} day^{-1}), P/E (g MJ^{-1}), P_{diet} in (g kg^{-1}) and FR (%)

These results are of interest as the DP/DE ratio recommended is in agreement with several authors and most commercial diets (Vergara *et al.*, 1999; Santinha *et al.*, 1999, Moñino *et al.*, 2002, Bonaldo *et al.*, 2010, Ekmann *et al.*, 2013, El-Hussseiny *et al.*, 2013; Garcia-Meilán *et al.*, 2013, Mongile *et al.*, 2014). As a consequence of the lower DP/DE, a faster growth can be reached in a limited period of time allowing, or forcing, fish to change their lipid composition from a medium LC:PC state to a high LC:PC state. However, it is impossible to maintain this growth efficiently for a long period, once a high lipid content is reached, the LC:PC ratio cannot be increased indefinitely.

Temperature effect

Temperature is likely to be another factor of great relevance in multifactorial approaches that has not been sufficiently studied. Of course, a higher temperature implies a faster body increment, and higher daily needs for growth. Also, the E_{man} seems to increase with temperature, but not P_{man}, in studies by Lupatsch *et al*. (2001b, 2003b) as well as other authors, which seems logical as a consequence of an increase of the voluntary activity and the basal metabolism. An increase of the slope of the ER or PD response curves should be expected, if an improvement of the FCR is accepted, as reported widely (Brett and Grove, 1979), such an increase indicates a higher efficiency in deposition. However, that slope increment, or an increment in gross efficiency of protein deposition or energy recovery has not been reported, and should be studied for a better understanding.

Maintenance needs

E_{man} was modeled by Lupatsch *et al*. (2003b) for fish with a BW_i between 10 and 300 g but with estimations resulting from studies that reach up to 100g. If the energy of maintenance values in terms of kJ $fish^{-1}$ day^{-1} (E_{man}^{f}) are compared (Figure 5), great differences can be observed when considering the two temperatures and based on the results reached be Lupatsch *et al.* (2003b) and Jauralde *et al.* (2015).

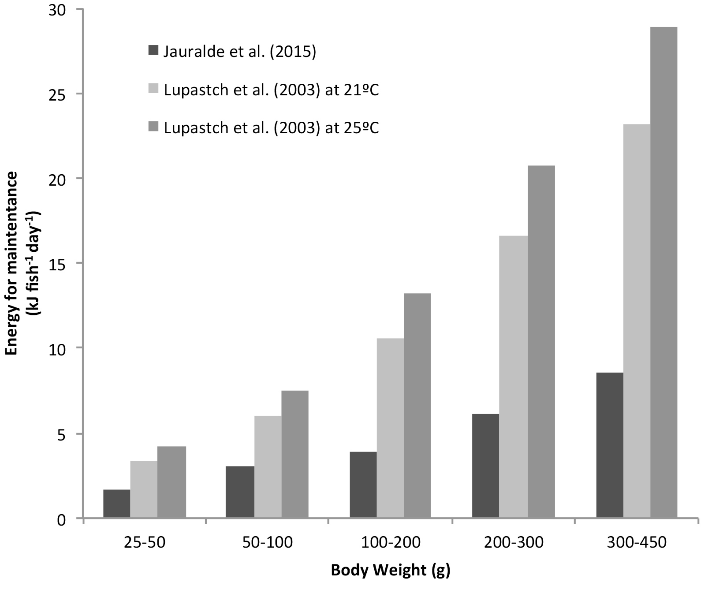

Figure 5.- Energy requirements (kJ $fish^{-1}$ day^{-1}) for maintenance for several body weights and temperatures

Maintenance energy needs from Lupatsch *et al*. (2003) were related to temperature, but much higher than reported by Jauralde *et al*. (2015).

Also, some differences among authors when it comes to protein maintenance requirements in terms of g fish^{-1} day^{-1} (P_{man}^{f}) can be seen in Figure 6. In contrast to the E_{man}^{f} values, the P_{man}^{f} values found by Jauralde *et al*. (2015) are greater than the P_{man}^{f} estimations reached by other authors (Lupatsch *et al*., 2003b; Fournier *et al*., 2002).

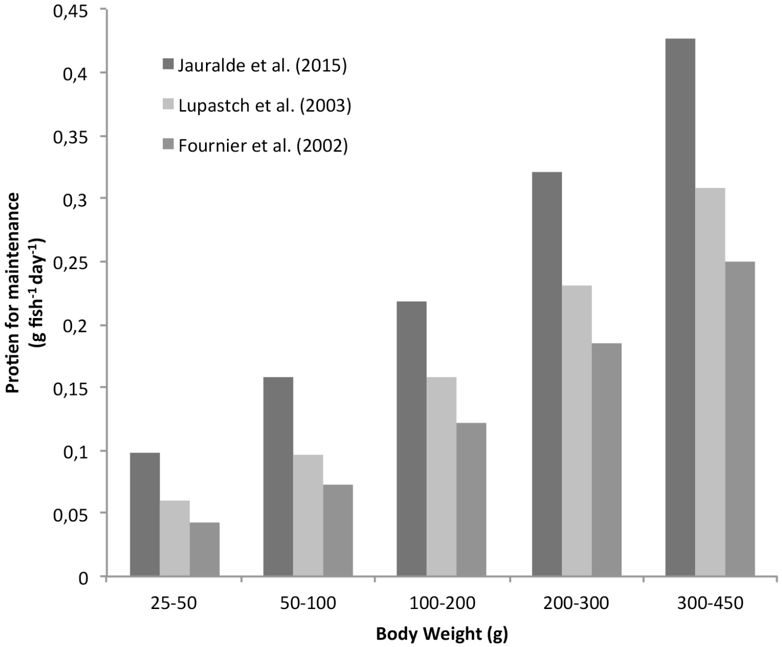

Figure 6.- Protein requirements by fish for maintenance for several body weights.

Theoretically, energy and protein for maintenance in term of metabolic weight (g kg$^{-0.7}$ day^{-1} or kJ kg$^{-0.82}$ day^{-1}) are concepts of nutrition that are constant when compared for sea bream at the same temperature, but the concept of maintenance needs, understood as the requirements destined to basal metabolism and voluntary activity, is calculated as the digestible intake need for growth response equal to zero. Jauralde *et al.* (2015), and as also Glencross (2008) in Barramundi, obtained differences in the E_{man} depending on size and expressed for kilogram of metabolic body weight. An explanation for these differences might be the voluntary activity of a fish under starvation and fish with a maintenance eating point. However, when these maintenance requirements are expressed by fish, the small differences caused by the metabolic exponent determination for Jauralde *et al.* (2015) presented in Figure 5, seems irrelevant compared to the differences among authors. The reasons for the disagreements among authors are not clear, but a possible explanation might be the duration of trials: 24 days in the case of Fournier *et al.* (2002), around 40 days in the case of Lupatsch *et al.* (2003b), and 84 days for Jauralde *et al.* (2015). The maintenance estimation point is strongly influenced by the points closest to it: the point for starvation fish and the point for low feed fish. Fish under starvation or eating little for a long time may suffer changes in several ways: voluntary activity may be affected and fish might try to save energy in this way. Also, the metabolic pathways to obtain energy from protein should be more active, increasing the protein for maintenance needs. Nevertheless, dependence on energy for maintenance with temperature has been widely proved and should be considered, (Guinea and Fernandez 1997). Further studies to determine the maintenance needs in relation to temperature seem to be required.

Effect of net energy efficiency (k_e).

Nutrient needs are often divided into maintenance needs plus needs for growth, and the needs for growth are related to the net efficiency for growth. This division allows the separate study of theorical maintenance requirements as well as growth requirements. Both concepts may be solved by a dose-response curve showing the protein deposition or energy recovery to graded levels of intake. A dose response curve with a linear shape has an implicit constant net efficiency (k_e or k_p) while a non-linear response curve has a variable net efficiency. Lupatsch *et al*. (2003b) found a constant efficiency of the energy for growth, while Jauralde *et al*. (2015) found a decreasing efficiency (Figure 1).

Differences in k_e over different growth situations are plotted in Figure 7. The constant net efficiency value by Lupatsch *et al*. (2003b), 0.67, is similar to the values reached by the equations of Jauralde *et al*. (2015) for 21 ºC, Figure 1 with dependence on the TGC used.

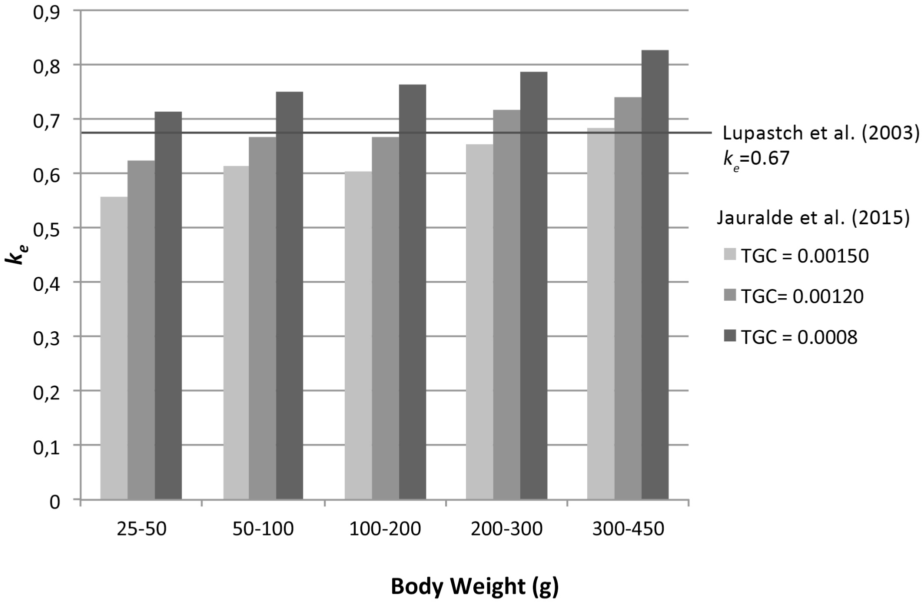

Figure 7.- Net energy efficiency for growth (k_e) for several TGCs and body weights at 21ºC

For diet design, net efficiency must be handled with care and be used together with its maintenance associated, as both concepts are directly related (see equation 3). If the maintenance value and efficiency are taken from different studies, a systematic error might occur. For example, using the E_{man} found by

Lupatsch *et al*. (2003b) together with the efficiencies k_e found by Jauralde *et al*. (2015) will result in an artificial dose-response curve assumption that does not correspond to the results obtained in either study.

Effect of net protein efficiency (k_p).

The protein efficiency for growth proposed by Lupatsch *et al*. (2003b) was a fixed value of 0.47 as a theorical mid-efficiency that could be reached with an ideal amino acid profile without penalizing growth, although these authors also proposed a varying efficiency with the intake. In Figure 8 the fixed k_p value is in agreement with most efficiencies for a fast growth (TGC=0.00150 at 21ºC) estimated, using equations 4 to 6 by Jauralde *et al.* (2015). However, if the needs for a lower TGC are calculated, higher efficiencies may be obtained. Likewise, another set of efficiency estimations for a k_p-variable by Lupatsch *et al*. (2003b) are obtained, applying equation 6 to equation 31.

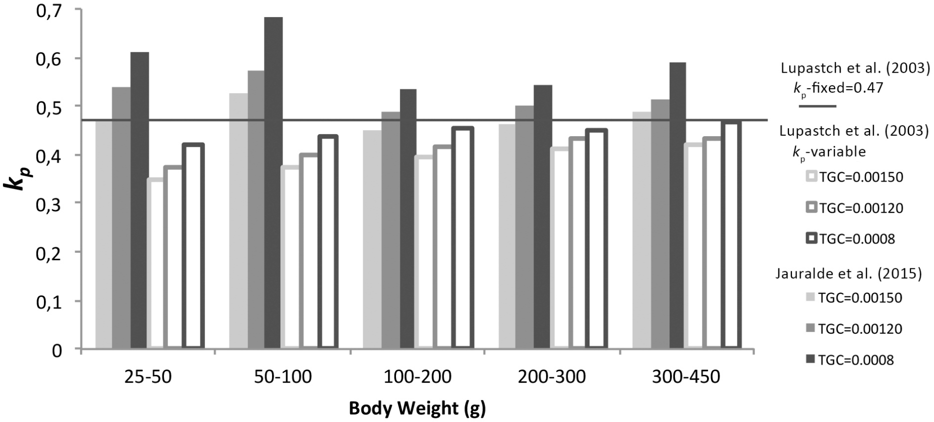

Figure 8.- Net protein efficiency for growth (k_p) for several TGCs and body weights at 21ºC

The equation (32), corresponding to a constant protein efficiency, is a linear response that could be possible, in theory, with ideal protein for each growth level, but has not been achieved yet. Nevertheless, apply a P_{man} and a k_p variable or constant is equivalent to assuming one of the response curves plotted in Figure 2.

As already dicussed for k_e, the split of requirements into maintentance and efficiency for growth is unnecessary when formulating a diet. If an increment of weight is desired, the increment of protein and energy can be determined by fish and day, and the intake to reach that deposition level may be obtained. The gross efficiency will explain in a simplier way the relation between demands and intake independent of whether fish destine a greater or smaller part of the intake for maintenance or growth, both parts are required.

Diets

Currently, most diets for *Sparus aurata* are designed with a DP/DE ratio from 20 to 25 g/kJ, but these recommendations are supported in the latest studies of requirements only if protein efficiency is considered constant (k_p=0.47) (Figure 9c) or likely to occur in fish of 100g not growing with all their potential (figures 9a and 9b), which is the case in the study by Lupatsch *et al.* (2003b). Recommendations of DP/DE are similar for all authors and for BW_i between 25 and 100 g, the values recommended range between 25 and 30 g/kJ (Figure 9c), but if the TGC decreases, the DP/DE ratio calculated applying equations by Jauralde *et al*. (2015) increases and the ratio calculated using the equations by Lupatsch *et al.* (2003b) decreases (Figures 9a and 9b). Azevedo *et al.* (2004) examined different DP/DE ratios in several salmonids and found that, in lake trout and Atlantic salmon, the highest ratio examined (24 g/kJ) reached the highest feed efficiencies. Webster *et al*. (1995) in sunshine bass, also obtained that ratios of DP/DE below 27 g/kJ produce higher body lipids and abdominal lipids. In agreement with

Azevedo *et al.* (2004), Webster *et al.* (1995) and Lupatsch *et al.* (2003b), the results obtained in the present study indicate the usual ratios of DP/DE below 24 g/kJ produce a loss in feed efficiency and a lipid carcass accumulation. Additionally in gilthead sea bream, Company *et al.* (1999) warmed of the risk of adiposity when fed with high energetic diets, García-Meilan *et al.* (2013) found that diets with higher DP/DE ratios achieved higher growth and better FCR than diets with lower DP/DE, but that study reached only until 24 g MJ-1. Ekmann *et al.* (2013) demonstrated that sea bream consumming diets from 15 to 20 g MJ^{-1} accumulated fat up to 22.3% of the body weight.

The greatest causes of differences can be explained by gross efficiency (figures 9g to 9l): the results with k_p-constant of 0.47 seem to over-estimate gross efficiency in comparison with k_p-variable response curves for high demands (Figures 9h and 9i). According to Lupatsch *et al.* (2003b), an increase of demands (increasing TGC or temperature) leads to an increase of either protein gross efficiency and energy gross efficiency, except in protein gross efficiency with a k_p-variable. This increases of gross efficiency considering k_e and k_p constant, can be explained by the proportion of energy or protein destined for maintenance being lower when the intake increases. Therefore, energy gross efficiency seems to be underestimated by Lupatsch, when medium and low growth rates are compared with the energy gross efficiency obtained by the equations of Jauralde *et al.* (2015).

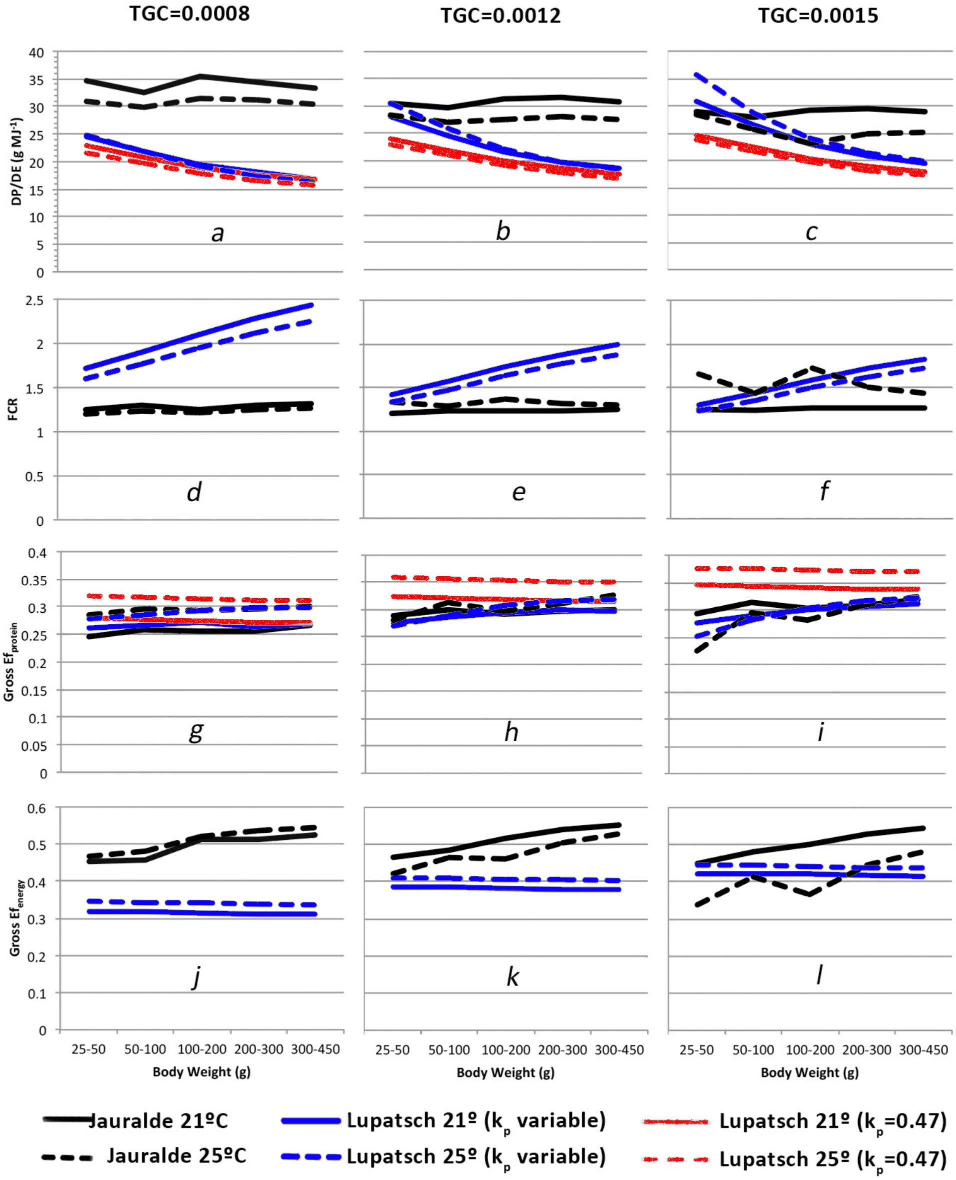

Figure 9.- Different diet designs, FCRs and associated efficiencies for several growth (TGCs), temperatures and based on the results reached by Jauralde *et al.* and Lupatsch *et al*.

According to results achieved by Lupatsch *et al.* (2003b), the best FCRs can be obtained for higher growth, because an increase of TGC carries a decrease of the FCR (Figures 9d, 9e and 9f). However, according to the equations by Jauralde *et al.* (2015), growth does not mean great changes in the FCR if a diet is designed for such a purpose. Obtained FCRs correspond to minimum theorical values of designed diets specially for each TGC, and may be compared among theorical values but not with real values obtained in farms, which are obtained for a single diet and several TGCs. However, it is possible to compare the obtained FCRs with the minimum theorical FCR values obtained for a diet of 20-25 g/MJ as as is the case in current commercial diets, and the diets shown in Table 9. According to the results shown in Table 9, a diet of 20-25 g/MJ will result in an increase of the LC:PC ratio and higher FCR compared to other diets. A decrease of fat with the increase of the DP/DE ratio has already been demonstrated in other species, (Einen and Roem, 1997). In fact, the FCR values of Table 9 are closer to the actual minimum values obtained in fish farms, and the excess of lipid in the carcass of the fish body is a well documented fact. As well, using diets of Table 9, the FCR increases with weight as seems to happen in most fish farms.

Thus, the question should be why have the diets of 20-25 g/MJ been used until today?. If data are examined critically, it is probable that 20-25 g/MJ diet are able to produce higher growth and higher profits in the short or midterm, increasing protein as much as possible and increasing lipids as much as possible but with a higher FCR. That situation does not seem to be desirable and sustainable in the long term, although, the high cost per kilogram of diet with higher levels of protein discourages farmers and feed suppliers to raise the DP/DE ratio. Latest studies support the idea that the energy levels in sea bream diets should be re-evaluated (Company *et al*., 1999; Lupatsch *et al*., 2003b; Velazquez *et al*., 2006; Jauralde *et al.,* 2015). In other species, the “spare” effect of a high energy over protein has also been proved to be incorrect (Azevedo *et al*., 2004). However, the alternate periods of high growth demands (summer) with medium demands (spring and autumn) and with low demands (winter) may justify the increase in the LC:PC ratio composition. An increase of the LC:PC ratio composition could be desirable when facing winter with more storage, but not desirable for sales purposes, taking into account the client's as well as the nutritionist's point of view.

The changes of whole body composition during several seasons and weight should be studied for a better understanding of the real requirements of sea bream under farm conditions. According to the results found in the present study, diets with a DP/DP between 25 and 30 g MJ^{-1} are recommended to optimize efficiencies. A diet of 29 g MJ^{-1} should be recommended for maximum growth at summer temperatures along the entire on-growing cycle of sea bream.

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CAPÍTULO 7

Discusión general

Discusión general

De acuerdo con los resultados de la presente tesis doctoral, parece probable que los diseños actuales de los piensos no estén optimizando la utilización de la proteína y energía. Son varios los estudios que parecen apuntar a que un aumento de la relación PD/ED de los piensos podría ser conveniente para la producción de dorada (Company *et al.*, 1999; Lupatsch *et al.*, 2003b; Velazquez *et al.*, 2006; Ekmann, *et al.*, 2013; García-Meilán, *et al.*, 2013; Jauralde *et al.*, 2015a, 2015b). La baja relación PD/ED contenida en los piensos actuales podría ser responsable de un engrasamiento excesivo de la dorada.

En relación al ratio de PD/ED otros factores a parte de los meramente nutricionales merecen tenerse en cuenta. Una disminución del *Thermal Growth Coeficient* (TGC), requiere un pienso con una relación PD/ED ligeramente más alta, pero rara vez se utilizan pienso para alimentar para que crezcan a una velocidad baja de crecimiento, y sin embargo es habitual que se alimente por debajo de la tasa de alimentación recomendada. Por ello, conviene estudiar los cambios en la composición corporal y otros parámetros de crecimiento que se producen cuando se alimenta con el pienso recomendado a una tasa de alimentación inferior a la recomendada.

Atendiendo a los resultados de la presente tesis, la mejor relación PD/ED, para un TGC de 0.00150 a 21 ºC, varia según el peso del pez entre 27.91 y 29.5 g MJ^{-1}, por lo que se estima que un pienso con una relación PD/ED de 29.04 debería dar buenos resultados para máximo crecimiento en todas las tallas. Atendiendo a las recomendaciones de Lupatsch *et al.* (2001) es recomendable que el pienso tenga al menos 16 MJ kg^{-1} de energía digestible (Company *et al.*, 1999; Lupatsch *et al.*, 2001; Velázquez *et al.*, 2006), por lo que el pienso deberá tener 465 g kg^{-1} de proteína digestible, obteniéndose un ICA de 1.26, pero si la energía fuera de 18 MJ kg^{-1} y la proteína de 525 g kg^{-1} el ICA sería de 1.12.

Con el pienso seleccionado es posible calcular la variación de composición corporal teórica y además se pueden estimar la respuesta a los distintos *Feeding Rates* (FR) que darán lugar a diferentes valores de *Thermal Growth Coeficient* (TGC), *Feed Conversion Ratio* (FCR), y también de *Economic Profit Index* (EPI). En la Tabla 1 se observan dichas predicciones para doradas de 300 gramos a 21ºC alimentadas durante 84 días con el pienso recomendado para alto crecimiento.

Tabla 1.-Parametros de crecimiento y niveles de energía previstos con el consumo de un pienso de PD/ED de 29.04 g MJ^{-1} con una PD de 465 g kg^{-1} y una energía de 16 MJ kg^{-1} y 84 días de crecimiento.

FR	Peso inicial (g)	Peso final (g)	TGC	FCR	Energía Final (MJ kg^{-1})
0.4	300	355	0.00051	2.00	10.64
0.45	300	380	0.00073	1.61	10.33
0.5	300	404	0.00092	1.42	10.06
0.55	300	416	0.00102	1.43	10.05
0.6	300	440	0.00121	1.33	9.81
0.65	300	462	0.00137	1.28	9.60
0.7	300	495	0.00161	1.20	9.22

El peso final debe calcularse de una forma iterativa, de forma que a partir del FR se supone un peso final a partir del cual se calcula la ingesta durante los 84 días, y con la ingesta se calcula la *Digestible Protein Intake* (DPI) en (g kg$^{-0.7}$ día-^{1}) y con ella se estima la retención de proteína. A partir de la retención diaria de proteína se calcula la retención total de proteína que se suma a la proteína inicial y con la proteína final se estima el peso final de nuevo, hasta que los pesos finales estimados y calculados se igualan. Una vez determinado el peso final se obtiene la ingesta ajustada exactamente y se podrá calcular la ingesta y retención de energía. Este cálculo implica que la proteína corporal del pez depende solo del peso del pez y no del nivel de alimentación, como parece probado en Lupatsch *et al.* (2003) y Jauralde *et al*. (2015).

Se puede observar como a medida que aumenta el FR se Incrementa el TGC y disminuye el FCR, pero para obtener un óptimo habría que considerar el coste del pienso.

En Jauralde *et al.* (2013) se propone una forma de estudiar la rentabilidad basándose en la respuesta del TGC (*Thermal Growth coeficient*) en función del FR y del EPI (*Economic Profit Index*) en función de la tasa de alimentación. Estos resultados numéricos obtenidos en Jauralde *et al.* (2013) son solo validos para el pienso que se utilizo en dicho estudio, que tenía una relación PD/ED de 18 g MJ^{-1}. Sin embargo, el procedimiento desarrollado es válido y permite realizar simulaciones basadas en los datos y curvas de respuesta obtenidas para comparar los parámetros de crecimiento y rentabilidad en función del *Feeding rate* (FR) de un pienso.

En la Figura 1, se puede observar la respuesta del TGC de doradas de distintos pesos alimentadas con el pienso recomendado (16MJ kg^{-1} y 465g de proteína kg^{-1}) a diferentes tasas de alimentación (FR).

A menor peso del pez necesita una mayor tasa de ingestión para cubrir sus necesidades, pero, al contrario de lo que pasaba con un pienso no optimizado (Jauralde *et al*., 2013), la respuesta con un pienso optimizado es lineal.

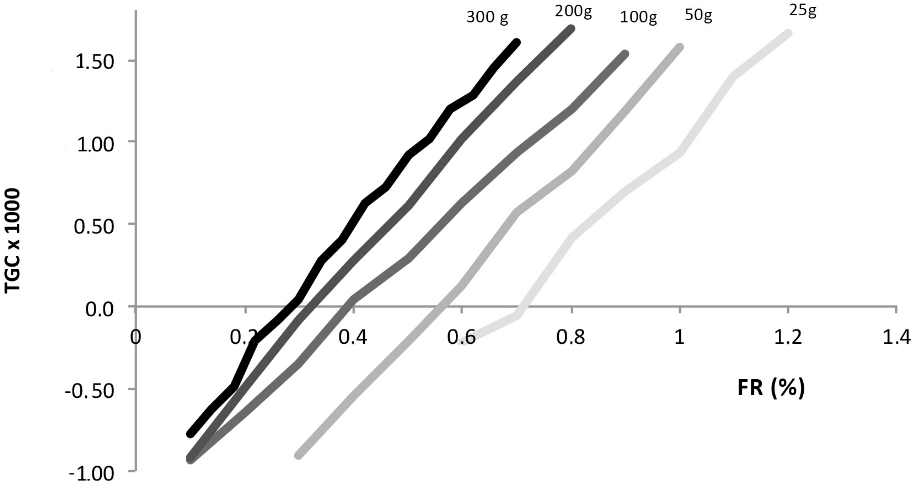

Figura 1.- Thermal Growth Coeficient para distintos FR y pesos de doradas consumiendo un pienso con 465 g kg^{-1} de proteína digestible y 16 MJ kg^{-1} de energía digestible.

Como se vio en Jauralde *et al.* (2013) el *Feed Conversion Ratio* (FCR) también depende el FR, o lo que es lo mismo, la eficiencia del pienso depende de la cantidad de pienso suministrado (Figura 2). Como el pienso está diseñado para optimizar las eficiencias para un máximo crecimiento, el mínimo FCR se da, precisamente, con el máximo TGC previsto para todos los tamaños de la dorada

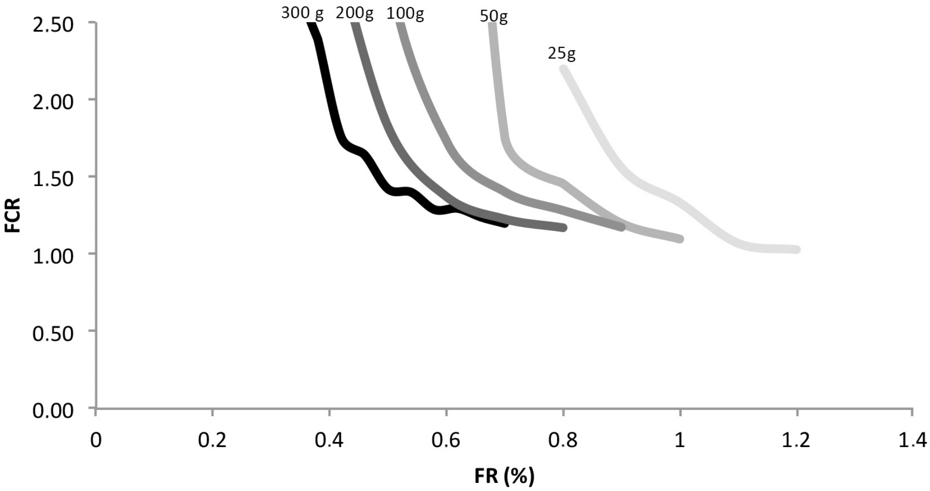

Figura 2.- *Feed Conversion Ratio* para distintos FR y pesos de doradas consumiendo un pienso con 465 g kg^{-1} de proteína digestible y 16 MJ kg^{-1} de energía digestible.

Los valores del *Economic Profit Index* (EPI), calculado mediante la ecuación (1), difieren de los calculados en Jauralde *et al.* (2013) como consecuencia del distinto FCR así como por el cambio en el precio del pienso originado, sobre todo, por el aumento en el nivel de proteína digestible en el pienso, que pasa de 399 (g kg^{-1}) en Jauralde *et al*. (2013) al valor recomendado de 465 (g kg^{-1}). Las estimaciones de la figura (3) se han realizado considerando un precio del pienso de 1 (€ kg^{-1}).

$$\text{EPI } (\text{€ pez}^{-1}) = \Delta\text{Peso(kg)} * (\text{Precio venta}(\text{€ kg}^{-1}) - \text{FCR} * \text{Precio del pienso}(\text{€ kg}^{-1})) \qquad \text{(Eq. 1)}$$

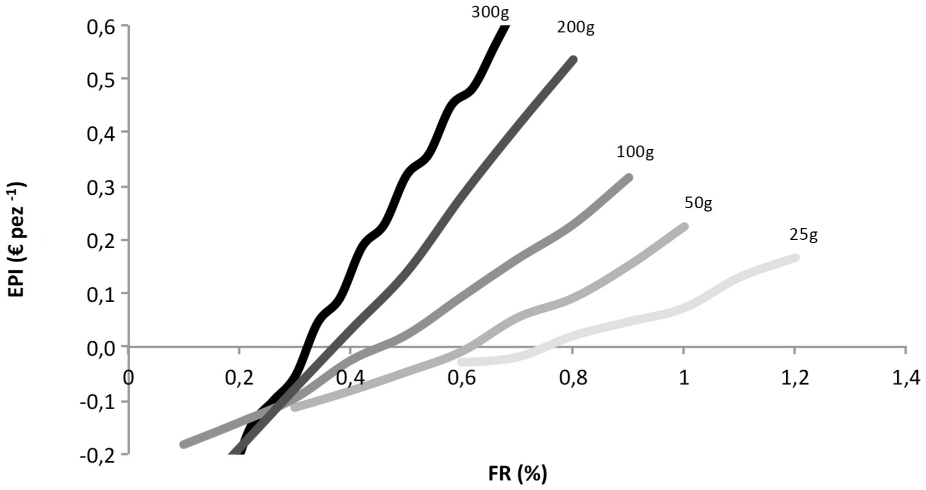

Figura 3.- *Economic Profit Index* para distintos FR y pesos de doradas consumiendo un pienso con 465 g kg-1 de proteína digestible y 16 MJ kg-1 de energía digestible.

Los datos son comparables con los del articulo de Jauralde *et al.* (2013) ya que también fueron calculados para 84 días de crecimiento. Los valores de EPI máximo obtenidos por Jauralde *et al.* (2013) se mejoran ligeramente como consecuencia de la optimización del pienso, pero por la misma razón sufren mayores variaciones ante pequeños cambio de FR.

Según varios autores, (Company *et al.*, 1999; Lupatsch *et al.*, 2001b; Velázquez *et al.*, 2006), el nivel de PD o ED contenida en el pienso no influye en la respuesta de las curvas de retención, y por lo tanto, las figuras 1, 2 y 3, deberían cumplirse. Sin embargo, los resultados no han podido ser refrendados a día de hoy mediante un estudio con un pienso que tenga el ratio PD/ED recomendado. Aunque teóricamente las simulaciones obtenidas a partir de los datos empíricos deberían cumplirse, los estudios factoriales se caracterizan por que un pequeño error en cualquiera de sus factores puede tener como consecuencia grandes cambios en las respuestas previstas (Dumas *et al.*, 2010).

Por ello, es recomendable realizar un proceso iterativo de comprobación de las necesidades. Es decir, el siguiente paso sería repetir los experimentos de la tesis pero con los piensos recomendados.

Por otro lado se han encontrado grandes discrepancias en cuanto a las necesidades de energía y proteína de mantenimiento. En (Jauralde *et al*., 2015b) se apuntaba a la duración del experimento como una probable fuente de variación y debería ser más sistemáticamente estudiado.

El estudio de las necesidades de mantenimiento mediante coeficientes metabólicos lleva implícito que las necesidades dependen del peso metabólico de la energía o de la proteína. Los coeficientes metabólicos deben de igualar los valores de energía y proteína de mantenimiento en función del peso, pero no se calculan en el punto de mantenimiento, pues es muy difícil acertar el punto de alimentación, sino en condiciones de ayunas.

Aunque existen numerosos trabajos donde se estima los coeficientes metabólicos en peces y los valores de los coeficientes están siempre cerca de los evaluados por Lupatsch *et al*. (2003), 0.82 para energía, , y 0.7 para proteína.

En el presente trabajo se han evaluado los coeficientes metabólicos por medio del análisis de la perdida de energía y proteína durante el ayuno (Figura 4) obteniéndose las siguientes ecuaciones:

Perdida de proteína (g pez^{-1} dia^{-1})=0.001768*$\text{Peso}^{0.752}$
Perdida de energía (kJ pez^{-1} dia^{-1})=0.015*$\text{Peso}^{0.752}$

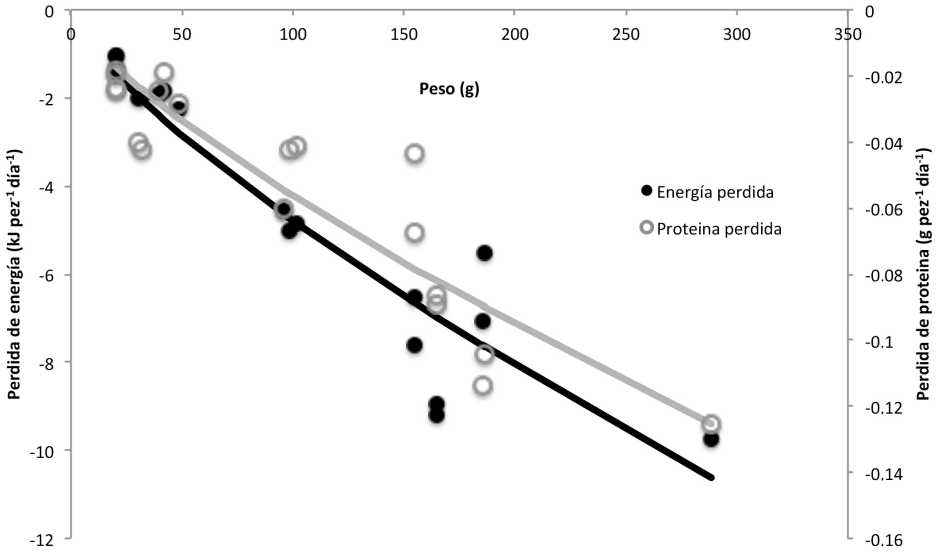

Figura 4.- Modelización de las perdidas diarias de energía y proteína de doradas en ayuno.

El coeficiente metabólico de la proteína está en concordancia con el obtenido por Booth *et al.* (2007) para *Pagrus auratu*s de $kg^{0.754}$. Pero las diferencias con los coeficientes más habituales, pueden estar causadas por la larga duración del ayuno (84 días) además de la gran dispersión de datos que se encuentran en este estudio, y también en otros como Lupastch *et al.* (2003), que podrían causar diferencias en puntos cercanos a las necesidades de mantenimiento. Sin embargo, las parte de la curva que más interesa es la parte de crecimiento, y en condiciones de crecimiento es de esperar que los coeficientes metabólicos averiguados sobre un menor tiempo de ayuno se asemejen más a las verdaderas necesidades de mantenimiento de peces en crecimiento que las realizadas sobre peces con un largo periodo de ayuno. Por ello, en la presente tesis se decidió utilizar los coeficientes metabólicos observados por Lupatsch *et al.* (2003a).

En cuanto a las temperaturas, sería recomendable realizar todos los experimentos a varias temperaturas (sobre todo por debajo de los 20ºC) para poder estudiar más sistemáticamente el efecto de la temperatura en las retenciones. Es especialmente interesante que no se observe un efecto de la temperatura sobre la pendiente de las curvas de retención, según los resultados actuales (Lupatsch *et al*., 2003 y Jauralde *et al*., 2015) lo que implica que la temperatura no afecta a la eficiencia neta de energía, y en el caso de la proteína, al no variar las necesidades de mantenimiento con la temperatura, tampoco cambiaría la eficiencia bruta con la temperatura para un mismo nivel de ingesta.

Así mismo, sería deseable conocer las necesidades nutritivas y de crecimiento en condiciones de temperaturas medias y bajas. Las necesidades de mantenimiento y crecimiento en estas situaciones pueden justificar la necesidad de un engrasamiento durante el verano, o todo lo contrario, conocerlas podría ser la clave para una correcta nutrición durante todo el ciclo de producción.

Existen todavía algunas retenciones que no se han podido comprobar, en doradas de menos de 100 gramos a 25ºC no se han conseguido alcanzar las retenciones de proteína necesarias para crecer con un TGC de 0.0015. Aunque los modelos factoriales se basan en asumir efectos por separado de los factores, extrapolar las eficiencias de retención por encima de las retenciones máximas obtenidas podría tener como consecuencias grandes errores a la hora de diseñar los piensos.

Por otra parte, existen, al menos, tres formas posibles de medir la eficiencia de retención de la proteína y energía, basándose en la curvas de respuesta a la retención: eficiencia neta, eficiencia bruta y eficiencia marginal. La “eficiencia neta”, k_p o k_e tiene la propiedad de ser independiente del mantenimiento, y poder valorar con que eficiencia se están depositando los nutrientes destinados a crecimiento. Pero no es imprescindible conocerla para el diseño de piensos. Así, sería posible valorar las necesidades de pienso para máximo crecimiento mediante la “eficiencia bruta” de la proteína olvidando la división entre necesidades de mantenimiento y necesidades de crecimiento netas, ambas necesidades necesitan ser cubiertas para crecer.

Las “eficiencias brutas” no deben intentar modelizarse directamente, al igual que ocurre con la eficiencia neta, sino a partir de las ecuaciones 2 y 3, ya que son consecuencia directa de las curvas de retención de energía y proteína, puesto que asumir una curva de retención es asumir sus eficiencias asociadas, intentar modelizarlas por separado daría demasiada importancia puntos cercanos a las necesidades de mantenimiento. De las ecuaciones 1 a 4 de Jauralde *et al*. (2015b) se puede deducir fácilmente la ecuación de la eficiencia bruta asociada a la curva de retención como:

$EfB_{prot}= PR/PDI$ (Figura 5) (Eq. 2)

$EfB_{ener}= ER/DEI$ (Figura 6) (Eq. 3)

Se observa en la Figura 5 y 6, que tra un aumento vertiginoso de la eficiencia en el entorno del mantenimiento, la eficiencia llega a un máximo, y se produce una ligera reducción, tanto para las dos clases de tallas consideradas, como para la energía y proteína.

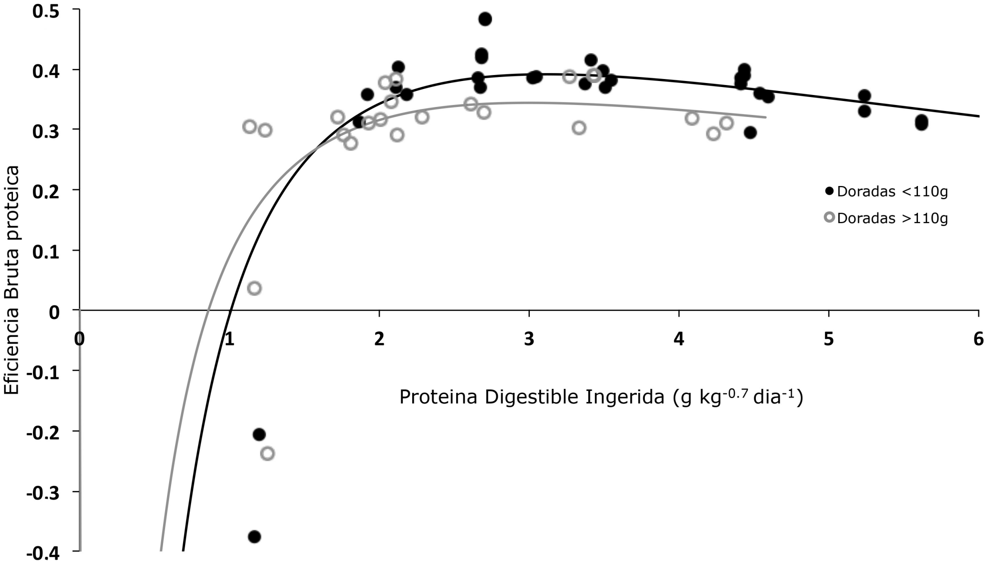

Figura 5.- Eficiencia Bruta de la proteína digestible para doradas de distinto peso

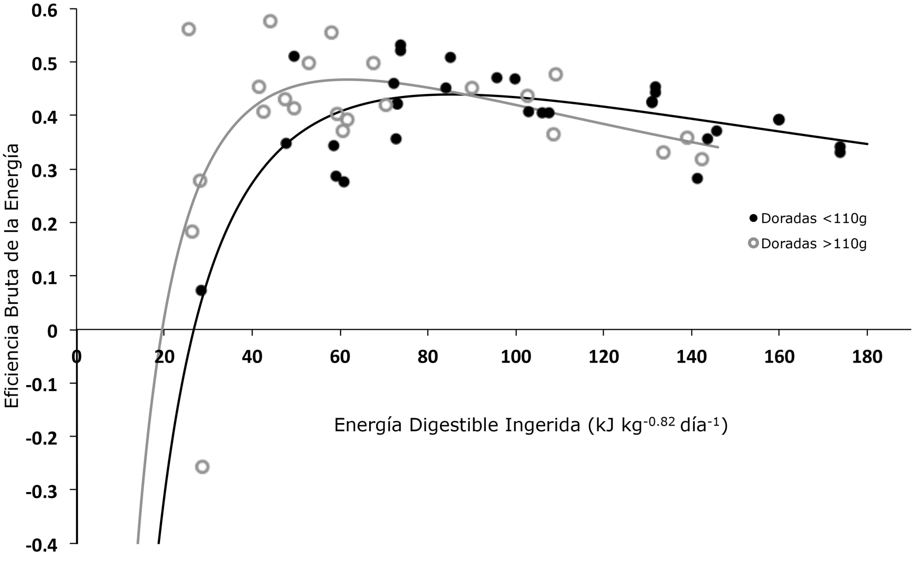

Figura 6.- Eficiencia Bruta de la energía digestible para dorada de distinto peso

Por el contrario, al asumir una respuesta de retención lineal, como la propuesta para la energía en Lupatsch *et al.* (2003) se llega a una eficiencia bruta asintótica (Ecuación 4, Figura 7), por lo que cuanto más se alimenta más eficiente se es.

$$EfBener = (-0.67 * 16.6\ e^{(0.055 * T^{a})} + 0.67 * DEI)/DEI \qquad \text{(Eq. 4)}$$

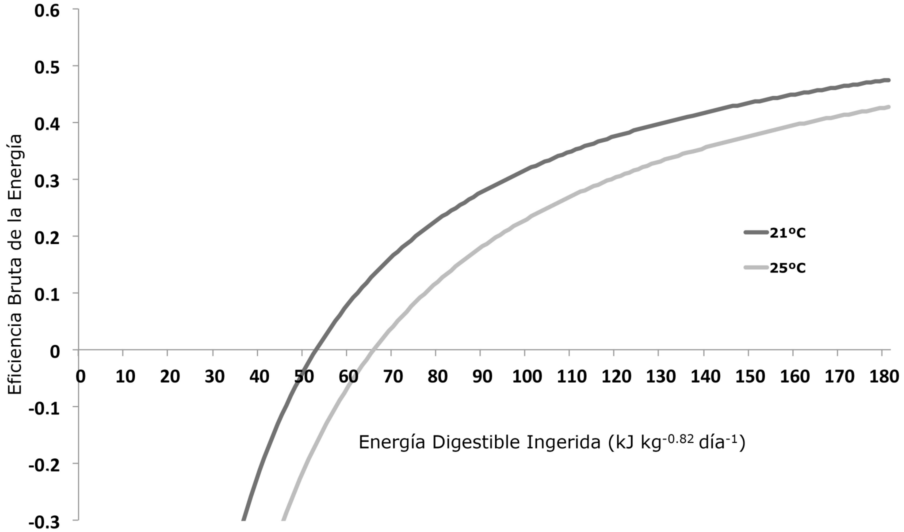

Figura 7.- Eficiencia Bruta de la energía digestible según Lupatsch *et al.* (2003)

Por último cabe la posibilidad de estudiar la "eficiencia marginal", que es la pendiente en cada punto de la función de dosis-respuesta de la energía retenida o proteína retenida y que puede ser estudiada mediante la derivada. Esta pendiente en cada punto determina lo conveniencia de seguir aumentando la dosis para mejorar el crecimiento y el beneficio.

En el caso de una curva de respuesta lineal, como la propuesta para la energía retenida (Lupatsch *et al.*, 2003), la eficiencia marginal es constante e igual a la pendiente. Si se aplican las derivadas a las ecuaciones 1, 2, 4 y 5 de Jauralde *et* *al*., (2015b) se obtienen las ecuaciones 5, 6 7 y 8 del presente capítulo, y las figuras 8 y 9.

(Doradas <110g) $Ef_{Prot}^{Marginal}=0.819*e^{(-0.35*(DPI-1.01))}$ (Eq. 5)

(Doradas >110g) $Ef_{Prot}^{Marginal}=0.654*e^{(-0.3*(DPI-0.86))}$ (Eq. 6)

(Doradas <110g) $Ef_{Ener}^{Marginal}=0.8904*e^{(-0.012*(DEI-26.7))}$ (Eq. 7)

(Doradas >110g) $Ef_{ener}^{Marginal}=0.9571*e^{(-0.017*(DEI-19.5))}$ (Eq. 8)

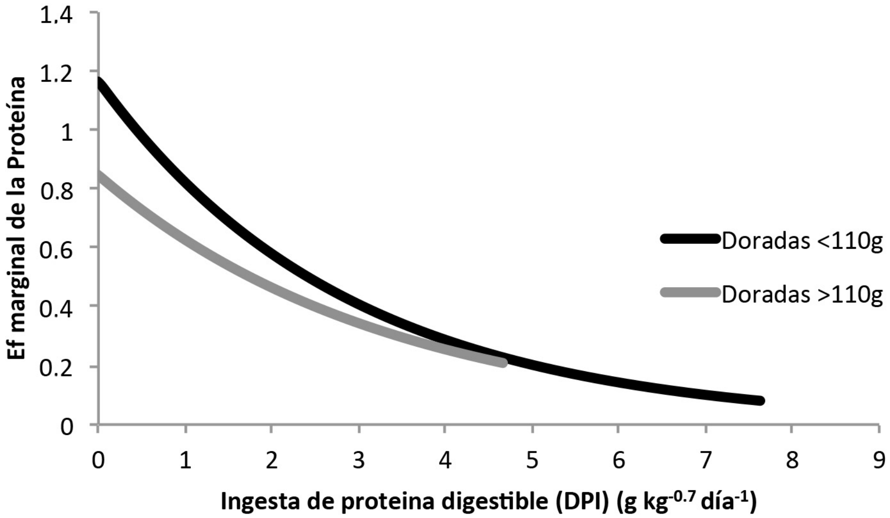

Figura 8.- Eficiencia marginal de la proteína según Jauralde *et al*. (2015b)

Si se asigna un precio a la proteína retenida y un precio a la proteína suministrada se puede analizar en que punto deja de ser conveniente aumentar la alimentación. Suponiendo un precio de venta de la dorada de 4.5 € kg^{-1} y que el 17% del peso del pez es proteína, entonces cada kilo de proteína se paga a 4.5/0.17=26.47 € kg^{-1}. De la misma forma suponiendo un pienso con un 47% de proteína a 1 € kg^{-1}, cada kilo de proteína del pienso sale a 2.12 € kg^{-1}. Por lo tanto será conveniente aumentar la dosis de retención hasta que la pendiente sea menor de 2.12/26.47= 0.08.

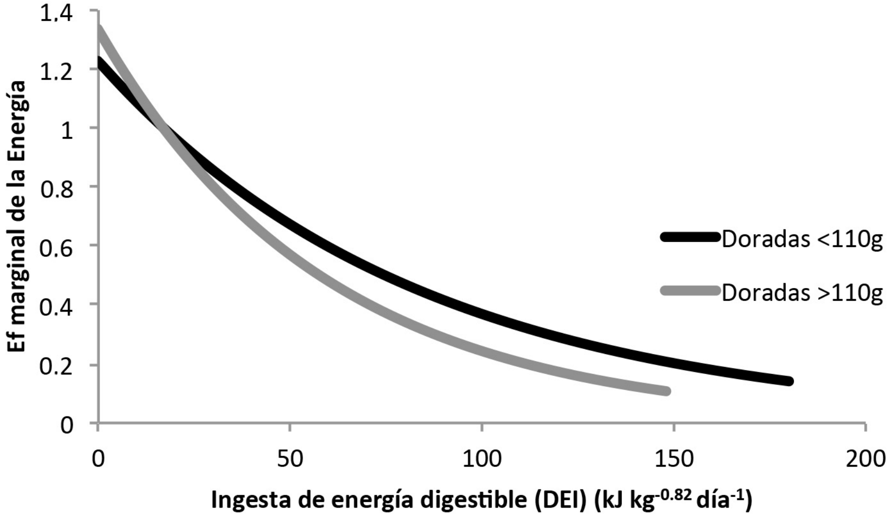

Figura 9.- Eficiencia marginal de la energía según Jauralde *et al*. (2015b)

De forma análoga se puede calcular con la energía, un pienso de 16 MJ kg^{-1} que cueste 1 € kg^{-1} se podría estimar que cada MJ sale a 1/16= 0.0625 € MJ^{-1}. Sin embargo una dorada que contenga 10MJ kg^{-1} de energía a un precio de 4,5 € kg^{-1} cada MJ depositado saldría a 4.5/10 =0.45 € kg^{-1} por lo que conviene aumentar la dosis para aumentar la retención hasta que la pendiente de la curva sea 0.0625/0.45= 0.139

En ambos casos, proteína y energía, en la práctica estas pendientes marginales implican cuanto mayor dosis se proporcione mejor, pues la pendiente que optimizaría el ratio de beneficio se alcanza por encima del rango estudiado. En el caso de la eficacia marginal de la energía, el punto se alcanza aproximadamente en el limite estudiado unos 133 (kJ $kg^{-0.82}$ $día^{-1}$) para doradas de más de 110 gramos y unos 180 (kJ $kg^{-0.82}$ $día^{-1}$) para doradas de menos de 110g. Sin embargo, un encarecimiento de los piensos o una caída del precio de venta de la dorada, podría hacer conveniente que las doradas no se alimentaran a su máximo potencial de retención de energía y proteína.

Para finalizar, sería necesario conciliar los parámetros económicos (EPI y eficiencias marginales) con los parámetros de eficiencia (FCR, eficiencias brutas y netas), pues los parámetros económicos indican que lo más rentable económicamente es aumentar la ingesta de pienso al máximo obteniendo el máximo crecimiento y la máxima rentabilidad, pero solo podrá corresponderse con un mínimo FCR, si los piensos están diseñados para cubrir adecuadamente las necesidades de energía y proteína.

CAPÍTULO 8

Conclusiones

Conclusiones

- Los piensos comerciales actuales para la dorada tienen unos ratios de proteína digestible / energía digestible de entre 20 y 24 g MJ^{-1} y producen un alto engrasamiento del pez y unos elevados índices de conversión alimentaria cuando se utilizan para alcanzar el máximo crecimiento.

- La estimación de las necesidades nutritivas de proteína y energía de la dorada permite, mediante el modelo factorial, diseñar piensos con una relación PD/ED que optimiza las eficiencias para cada situación de crecimiento, determinar las tasas de alimentación diaria ajustadas a las necesidades y reducir los índices de conversión del alimento, que es el índice clave en la mejora de la alimentación y rentabilidad en las granjas.

- Los piensos recomendados presentan una relación PD/ED mayor que los piensos actuales, de entre 25 y 30 g MJ^{-1}, y por lo tanto, para una energía digestible dada, se necesita una mayor inclusión de PD en el pienso, pero se obtienen unos mejores índices de conversión, que podrían compensar el mayor nivel de proteína en la dieta.

- Los modelos desarrollados para estimar las necesidades nutritivas suponen una buena herramienta para el diseño de dietas para la dorada, pero deben completarse ampliando el rango de temperaturas y comprobando las eficiencias reales de los piensos propuestas.

Conclusions

- The actual commercial feeds for the gilthead sea bream have ratios of digestible protein/digestible energy between 20 and 24 g MJ^{-1} and produce a high fat level deposition in the fish and high feed conversion rates when are used to reach the maximum growth potential.

- The estimation of the nutrient requirements of protein and energy allow design diets with a DP/DE ratio optimizing the efficiencies in each growth situation, determine the daily feeding rates fitted to the requirements and, with the factorial models for gilthead sea bream, reduce the feed conversion rates, which is the key index used in farms to improve of feeding.

- The recommended diets have a DP/DE ratio higher than the current diets, between 25 and 30 g MJ^{-1}, as a consequence, for a fixed digestible energy, it is need a higher inclusion of DP in the feed, but better feed conversion are obtained, compensating the higher level in dietary protein.

- The developed models to estimate the nutrient requirements are a suitable tool for the design of diets in gilthead sea bream, but should be completed with wider temperatures range and verify the efficiencies of the estimated diets.

CAPÍTULO 9

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