UNIVERSITAT POLITÈCNICA DE VALÈNCIA

**DEPARTAMENTO DE CIENCIA ANIMAL**

# CONTRIBUCIÓN AL ESTUDIO DE LAS NECESIDADES NUTRITIVAS DE LA CORVINA

(*Argyrosomus regius*, Asso 1801)

Tesis Doctoral Presentada por

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UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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Informan:

Que la Tesis Doctoral Titulada: **CONTRIBUCIÓN AL ESTUDIO DE LAS NECESIDADES NUTRITIVAS DE LA CORVINA** (*Argyrosomus regius*, Asso 1801) ha sido realizada por el Lic. En Biología Don **Jorge Luís Velazco Vargas** 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 autorizan su presentación.

Y para que así conste firman el presente informe en Valencia, Enero de 2014

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Los trabajos de investigación desarrollados en la presente tesis doctoral, se han presentado en:

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# ENGLISH SUMMARY

The meagre is a carnivorous fish that is being produced in the Mediterranean Sea thanks to the high growth rates and meat quality. There is almost no nutritional information related to this species in comparison to sea bass and sea bream production. The objective of this Doctoral thesis was to determinate the nutritive requirement of meagre and study the inclusion of soybean meal as an alternative protein vegetable meal to replace fish meal.

Two groups of fish of 53 and 200 g body weight were used and they were fed with one commercial meal to determinate the protein and energy requirement. In this trial it was applied a factorial model to obtain the maintenance requirements for protein that was 0.0617 g DP 100 g $fish^{-1}$ $day^{-1}$ and for energy 2.74 kJ DE 100 g $fish^{-1}$ $day^{-1}$. The protein requirements for the maximum growth was determinate in 0.64 g DP 100 g $fish^{-1}$ $day^{-1}$ and the energy requirements for the maximum growth was 38.5 kJ DE 100 g $fish^{-1}$ $day^{-1}$. Fish of 52 g of body weight were used to study the effect of digestible protein level (35%, 43%, 49% y 53%). Fish fed with experimental diets of de 43%, 49% y 53% of digestible protein obtained the highest growth (Thermal Growth Coefficient = 2.47, 2.57 y 2.69 x $10^{-3}$, respectively). The optimum digestible protein levels for juvenile meagre were 8 g DP 100 g $fish^{-1}$ $day^{-1}$. There were used fish of 147 g in sea cage to determinate the Protein/Energy relation. In this trial fish were fed with experimental diets of 47/20, 51/28 y 55/17. It was obtained the highest growth and conversion rates for 47/20 diet.

To determinate the soybean meal inclusion in meagre two phases were done. In the first phase, 800 fish of 165g were used during 107 days. They were fed with four isoproteic (50% Crude protein) and isolipidic (17% Crude lipid) with inclusion levels of 0, 15, 30 and 45% of soybean meal. The 15 and 30% diets obtained the best results. And according to the quadratic regression el optimum inclusion level of soybean meal were 27.6%. The relation between the essential amino acids rate in the diet and the corporal essential amino acids rate in fish presented the efficiency in arginine, lysine and treonine, and mainly methionine. In the second phase it was used the same methodology, but 300 fish of 346 g body weight were fed during 26 days. The results showed that meagre obtained the highiest growth rate (Thermal Growth Coefficient = 4.00 x$10^{-3}$) and we recommended the inclusion of soybean meal between 30 and 45%.

According to the results obtained in the different trials, we propose a diet for *Argyrosomus regius* of 47% crude protein and 17% crude lipid and a 30% of soybean meal inclusion, supplemented with methionine and lysine in order to obtain a high growth and diminish the use of fish meal and make more profitable the aquaculture production of this specie in the Mediterranean Sea.

# RESUMEN EN ESPAÑOL

La corvina es una especie carnívora que se ha incorporado a la producción de la acuicultura en el Mar Mediterráneo, por sus altos índices de crecimiento y calidad de la carne. Por la poca información nutricional existente para esta especie, se ha introducido en su producción la experiencia que ya se tiene en la dorada y la lubina. Los objetivos de esta tesis doctoral fue determinar las necesidades nutritivas de la corvina y estudiar la inclusión del turtó de soja como una fuente proteica vegetal alternativa de la harina de pescado.

Para determinar las necesidades de proteína y energía se trabajó con un pienso comercial, se experimentó con dos grupos de peces (53 y 200 g), en donde se aplicó un modelo factorial, obteniendo que las necesidades de mantenimiento para la proteína fue de 0,0617 g PD 100 g $pez^{-1}$ $día^{-1}$ y para la energía de 2,74 kJ ED 100 g $pez^{-1}$ $día^{-1}$. Las necesidades para el máximo crecimiento de la proteína fue de 0,64 g PD 100 g $pez^{-1}$ $día^{-1}$ y de energía 38,5 kJ ED 100 g $pez^{-1}$ $día^{-1}$. Con peces de 52 g, se realizó el estudio del efecto de los niveles de la proteína digestible (35%, 43%, 49% y 53%), los peces alimentados con piensos con niveles de 43%, 49% y 53% de proteína digestible, obtuvieron los mejores crecimientos (Coeficiente térmico de crecimiento = 2,47, 2,57 y 2,69 x $10^{-3}$, respectivamente). El nivel óptimo de proteína digestible ingerida para juveniles de corvina fue de 0,8 g PD 100 g $pez^{-1}$ $día^{-1}$. Para determinar la relación Proteína/Energía se experimentaron con peces de 147 g en jaulas marinas y fueron alimentados con piensos de 47/20, 51/28 y 55/17, obteniendo los mejores crecimientos e índices de conversión con el pienso 47/20.

Para estudiar la inclusión del turtó de soja en la corvina se realizaron dos fases de investigación. En una primera fase se utilizaron 800 peces de 165 g, durante 107 días fueron alimentados con cuatro piensos isoproteíco (50% de proteína bruta) e isolípidico (17% de grasa bruta), con niveles de inclusión del 0, 15, 30 y 45% de turtó de soja. En los piensos del 15 y 30% de turtó de soja se obtuvieron los mejores resultados y de acuerdo a la regresión cuadrática el nivel óptimo de inclusión de turtó de soja fue del 27,6%. La relación entre el porcentaje de aminoácidos esenciales de la dieta y el porcentaje de aminoácidos esenciales a nivel corporal de los peces presentó deficiencias en arginina, lisina, treonina y principalmente de metionina. En la segunda fase se utilizó la misma metodología que en el primer experimento, pero utilizando 300 peces de 346 g de media durante 26 días. Los resultados muestran que la corvina presentó un alto crecimiento (Coeficiente térmico de crecimiento = 4,00 x $10^{-3}$) y se recomienda una inclusión de turtó de soja de entre un 30 – 45%.

En base a los resultados obtenidos, se propone un pienso para *Argyrosomus regius* de 47% de proteína bruta, 17% de grasa bruta y un 30% de inclusión de turtó de soja, suplementado con metionina y lisina, con el fin de obtener altos crecimientos, disminuir el uso de la harina de pescado y hacer más rentable la producción acuícola de esta especie en el Mar Mediterráneo.

# RESUM EN VALENCIÀ

La corbina és una espècie carnívora que s'ha incorporat a la producció de l'aqüicultura al Mar Mediterrani, pels seus alts índex de creixement y qualitat de la carn. Degut a la falta de informació nutricional existent per a aquesta espècie, s'ha introduït en la producció l'experiència que ja es té per a l'orada i el llobarro. Els objectius d'aquesta tesi doctoral van ser determinar les necessitats nutritives de la corbina i estudiar la inclusió del tortó de soia com una font proteica vegetal alternativa a la farina de peix.

Per a determinar les necessitats de proteïna i energia es va treballar amb un pinso comercial, es va experimentar amb dos grups de peixos (53 i 200 g), on es va aplicar un model factorial, obtenint que les necessitats de manteniment per a la proteïna van ser de 0,0617 g PD 100 g peix$^{-1}$ dia$^{-1}$ i per a l'energia de 2,74 kJ ED 100 g peix$^{-1}$ dia$^{-1}$. Les necessitats per al màxim creixement de la proteïna van ser de 0,64 g PD 100 g peix$^{-1}$ dia$^{-1}$ i de energia 38,5 kJ ED 100 g peix$^{-1}$ dia$^{-1}$. Amb peixos de 52 g, es va realitzar l'estudi de l'efecte dels nivells de proteïna digerible (35%, 43%, 49% y 53%), els peixos alimentats amb pinsos en nivells de 43%, 49% y 53% de proteïna digerible, van obtenir els millors creixements (Coeficient Tèrmic de Creixement = 2,47, 2,57 y 2,69 x $10^{-3}$, respectivament). El nivell òptim de proteïna digerible ingerida per juvenils de corbina va ser de 0,8 g PD 100 g peix$^{-1}$ dia$^{-1}$. Per a determinar la relació Proteïna/Energia es va experimentar amb peixos de 147 g en gàbies marines i van ser alimentats amb pinsos de 47/20, 51/28 y 55/17, obtenint els millors creixements e índex de conversió amb el pinso 47/20.

Per a estudiar la inclusió del tortó de soia en la corbina es van realitzar dues fases en la investigació. En una primera fase es van utilitzar 800 peixos de 165 g, durant 107 dies van ser alimentats amb quatre pinsos iso proteics (50% de proteïna bruta) e iso lipídics (17% de greix brut), amb nivells d'inclusió del 0, 15, 30 y 45% de tortó de soia. En els pinsos del 15 i el 30% de tortó de soia es van obtenir els millors resultats i d'acord a la regressió quadràtica el nivell òptim d'inclusió del tortó de soia va ser del 27,6%. La relació entre en percentatge d'aminoàcids essencials de la dieta i el percentatge d'aminoàcids essencials a nivell corporal dels peixos, va presentar deficiències en arginina, lisina, treonina i principalment en metionina. En la segona fase es va utilitzar la mateixa metodologia que en el primer experiment , però utilitzant 300 peixos de 346 g de mitjana durant 26 dies. Els resultats mostren que la corbina presentà un alt creixement (Coeficient Tèrmic de Creixement = 4,00 x $10^{-3}$) i es recomana una inclusió de tortó de soia d´entre 30-45%.

D´ acord amb els resultats obtinguts, es proposa un pinso per a *Argyrosomus regius* de 47% de proteïna bruta, 17% de greix brut i un 30% d'inclusió de tortó de soia, complementant-lo amb metionina i lisina, amb la finalitat d'obtenir alts creixements, disminuir l'ús de la farina de peix i fer mes rendible la producció aqüícola d'aquesta espècie al Mar Mediterrani.

# CONTENIDO

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# ABREVIATURAS

| | |
|---|---|
| AA: | Aminoácidos |
| AAE: | Aminoácidos esenciales |
| AGE: | Ácidos grasos esenciales |
| Atm: | Atmósfera |
| CDA: | Coeficiente de digestibilidad aparente |
| CDE: | Coeficientes de digestibilidad aparente de la energía |
| CDMs: | Coeficientes de digestibilidad aparente para la materia seca |
| CDP: | Coeficientes de digestibilidad aparente para la proteína bruta |
| CEC: | Coeficiente de eficacia en el crecimiento |
| CTC: | Coeficiente térmico de crecimiento |
| EB: | Energía bruta |
| ED: | Energía digestible |
| Ee: | Energía de excreción |
| Eh: | Energía contenida en las heces |
| EM: | Energía metabolizable |
| Em: | Energía de mantenimiento |
| ER: | Energía retenida |
| g: | Gramos |
| GB: | Grasa bruta |
| ICA: | Índice de conversión del Alimento |
| ICE: | Índice de conversión económica del alimento |
| IGV: | Índice de grasa visceral |
| IHS: | Índice hepatosomático |
| InCA: | Incremento calórico del alimento |
| IVS: | Índice viscerosomático |
| kg: | Kilo gramos |
| kJ: | Kilo-Julio |
| MELN: | Materia extractiva libre de nitrógeno |
| MGI: | Modelos gastrointestinales |
| MJ: | Mega-Julio |
| NEmáx: | Necesidades de energía para el crecimiento. |
| NEm: | Necesidades de energía para el mantenimiento. |
| NPmáx: | Necesidades de proteínas para el crecimiento. |
| NPm: | Necesidades de proteínas para el mantenimiento. |
| PB: | Proteína bruta |
| PD: | Proteína digestible |
| P/E: | Relación Proteína/Energía |
| PR: | Proteína retenida |
| rpm: | Revoluciones por minuto |
| TAD: | Tasa de alimentación diaria |
| TCI: | Tasa de crecimiento instantáneo |
| TIEd: | Tasa de ingesta energética digestible |
| TIPd: | Tasa de ingesta proteica digestible |

# ABREVIATURAS

| | |
|---|---|
| tn: | Toneladas |
| VPE: | Valor productivo de la energía |
| VPP: | Valor productivo de la proteína |

# CAPÍTULO 1

# INTRODUCCIÓN GENERAL

## 1.1. PRODUCCIÓN ACUÍCOLA DE LA CORVINA.

Entre las prioridades reconocidas para un desarrollo mantenido de la acuicultura, se encuentra la diversificación de especies. Entre estas especies de interés comercial, la corvina (*Argyrosomus regius*) es la especie que más recientemente se ha incorporado a la producción de la acuicultura Española.

La corvina (*Argyrosomus regius*, Asso 1801) (**Figura 1**), como otros miembros de la Familia Sciaenidae, es objeto de interés internacional de cara a su cría comercial, ya que es una especie altamente fecunda, ampliamente distribuida, con unos precios de mercado medio-altos y con buena aceptación por parte de los consumidores (Monfort, 2010). Además, presenta la ventaja añadida de que se trata de una especie eurihalina, con un amplio rango de tolerancia de salinidad, lo que permite su adaptación a ambientes muy diversos, incluso a la cría terrestre en aguas salobres. También tolera perfectamente la cautividad, como demuestra su presencia en grandes acuarios, y presenta unas elevadas tasas de crecimiento en engorde y unos buenos índices de conversión (Calderón *et al.*, 1997; Pastor *et al.*, 2002).


**Figura 1.** Comercialización de corvinas en un mercado de Valencia durante el mes de octubre de 2012 (Fuente propia).

Aunque, la principal ventaja de *A. regius* es su rápido crecimiento; en la fase larvaria cuadriplica el peso de larva de dorada, y durante el engorde, a los 18 meses puede llegar a alcanzar 1 Kg de peso vivo (Pastor *et al.*, 2007; 2008a, b; Vargas-Chacoff *et al.*, 2007, 2009; Monfort, 2010), bastante superior a los 400 g que alcanza la dorada en este periodo (Cárdenas, 2010).

Por otro lado, el interés en la cría de la corvina no sólo abarca el campo comercial, sino también el de la conservación de los recursos naturales: Las poblaciones mediterráneas de esta especie han sufrido un alarmante retroceso (Quero, 1989), pudiéndose considerar extinguida en áreas como las Baleares, donde antaño era una captura relativamente frecuente, con presencia habitual en los mercados (Mayol *et al.*, 2000; Jiménez *et al.*, 2005). La vulnerabilidad de esta especie, como muchos otros miembros de su familia, es muy grande, a pesar de su elevada fecundidad, ya que su distribución geográfica en aguas costeras, altamente explotadas, y sus agregaciones estuáricas en época reproductiva con producción de sonidos durante éstas, juntamente con su valor de mercado, la hacen objeto de una pesca intensiva y fácil de localizar en tiempo y espacio (Sadovy & Cheung, 2003; Lagardére *et al.*, 2006). Además, la asociación de la puesta a un ambiente estuárico es, en sí misma, una amenaza a la propagación de la especie, debido a la degradación y polución de muchas de estas áreas costeras (Sadovy & Cheung, 2003). Así, la cría en cautividad de la especie es también interesante desde el punto de vista de la repoblación de pesquerías tradicionales sobreexplotadas o extinguidas, como es el caso del Mar Balear y de gran parte del litoral mediterráneo español.

Los primeros estudios de la familia Sciaenidae con stocks provenientes del medio natural se realizaron en Francia. En 1997 tuvieron lugar las primeras producciones comerciales, donde se logró su reproducción en cautividad. Sin embargo,

debido al desconocimiento de la tecnología reproductiva y de cría larvaria empleada por las escasas empresas internacionales que habían conseguido el éxito reproductivo, las empresas españolas dependían del suministro internacional de alevines de la especie.

En 1999 se empezó a trabajar con la corvina en España, con la finalidad de desarrollar técnicas productivas que permitieran diversificar la producción y mejorar la competitividad en el mercado, que en la actualidad se encuentra consolidada, es decir, al igual que otras especies en producción su ciclo biológico está cerrado, es decir, los alevines se logran a partir de reproductores, y durante todo su crecimiento hasta alcanzar la talla comercial son mantenidos en cautividad.

Aunque el empujón a este tipo de producción no vino hasta 2005, cuando se aprobó el proyecto nacional (JACUMAR) de producción de la corvina (PLANACOR) que se ha dedicado a investigar la reproducción en cautividad de la corvina y a realizar pruebas piloto de cría larvaria y pre engorde con piensos comerciales y experimentales.

La producción de corvina en Europa en 2011 ascendió a 3.770 tn, un 4,2% inferior al dato de 2010, cuando se produjeron 3.937 tn. Los principales países productores de corvina de acuicultura son España con 2.880 tn (el 76,4% del total), Francia e Italia (**Figura 2**) (APROMAR, 2012). La producción mediante acuicultura de corvina en España en 2011 ha sido de un 11,4% inferior a la de 2010. Las causas de esta reducción son, por una parte comerciales, y por otra, la prohibición transitoria del cultivo de esta especie en Canarias. Las principales regiones productoras de esta especie son la Comunidad Valenciana (52%) y la Región de Murcia (45%) (APROMAR, 2012).

Sin embargo, la producción en 2012 ha sido de 1.640 tn, una cifra 43% inferior a la de 2011, y casi un 50% menor a las 3.250 tn de 2010. Las causas de esta reducción son por una parte comerciales, dada la complejidad de introducir cantidades relevantes de un nuevo pescado en el mercado; y por otra productivas, por la indefinición del

Gobierno de Canarias sobre la autorización de esta especie que se ha comprobado autóctona en las aguas de las Islas Canarias. Las regiones productoras de esta especie en España son la Región de Murcia (61%) y la Comunidad Valenciana (37%), existiendo una producción menor en Andalucía (2%) (APROMAR, 2013).

La corvina es un pescado muy apreciado en aquellas regiones en las que se ha venido consumiendo tradicionalmente, sin embargo, dada su escasa pesca y su reciente producción mediante acuicultura, es poco conocido en la mayor parte de los mercados. Los principales países que pescan esta especie son Ghana, Mauritania, Egipto y Francia. En 2010 las capturas mundiales de esta especie ascendieron a 5.676 tn, frente a 4.100 procedentes de acuicultura (41,9%). La captura de corvina por parte de la flota de pesca española es prácticamente inexistente (APROMAR, 2012).

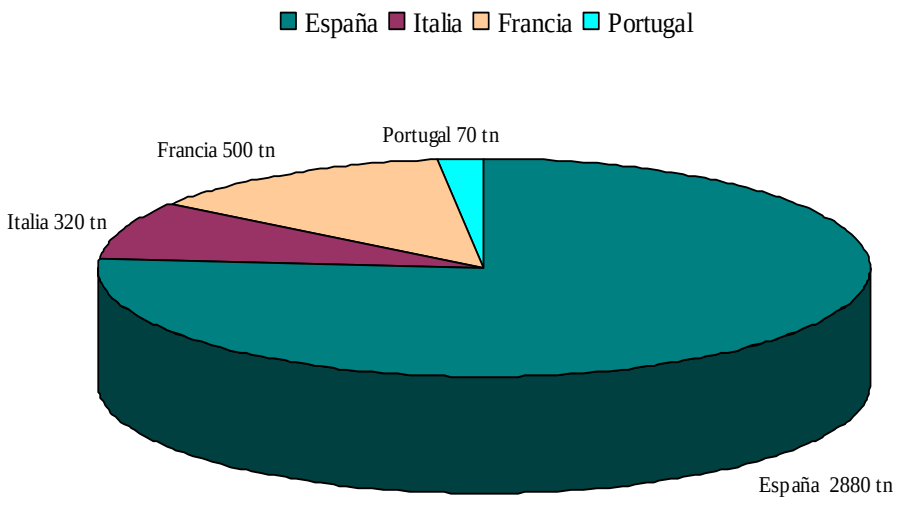


**Figura 2.** Países productores de corvinas en Europa, valores expresados en toneladas (tn) (APROMAR, 2012).

## 1.2. GENERALIDADES DE LA CORVINA.

*A. regius*, pertenece a la familia *Sciaenidae* que comprenden un amplio grupo de 70 géneros y más de 270 especies, distribuida en regiones templadas y tropicales del

mundo. Gran parte de estos géneros se encuentran en el Caribe y el Indo-Pacífico, mientras que en el Mediterráneo se han llegado a determinar 5 especies (Jiménez *et al.*, 2005).

Taxonómicamente la corvina se puede clasificar de la siguiente manera:

- Reino: Metazoa
- Subreino: Eumetazoa
- Rama: Bilateria
- Grado: Coelomata
- Serie: Deuterostomia
- Phylum: Chordata
- Subphylum: Gnathostomata
- Superclase: Peces
- Clase: Actinopterygii
- Subclase: Teleostei
- Superorden: Neognathi
- Orden: Perciformes
- Suborden: Percoidei
- Familia:*Sciaenidae*
- Género: *Argyrosomus*
- Especie: *Argyrosomus regius*
- Nomenclatura: Meagre (inglés), Maigre commun (francés), Corvina (español)

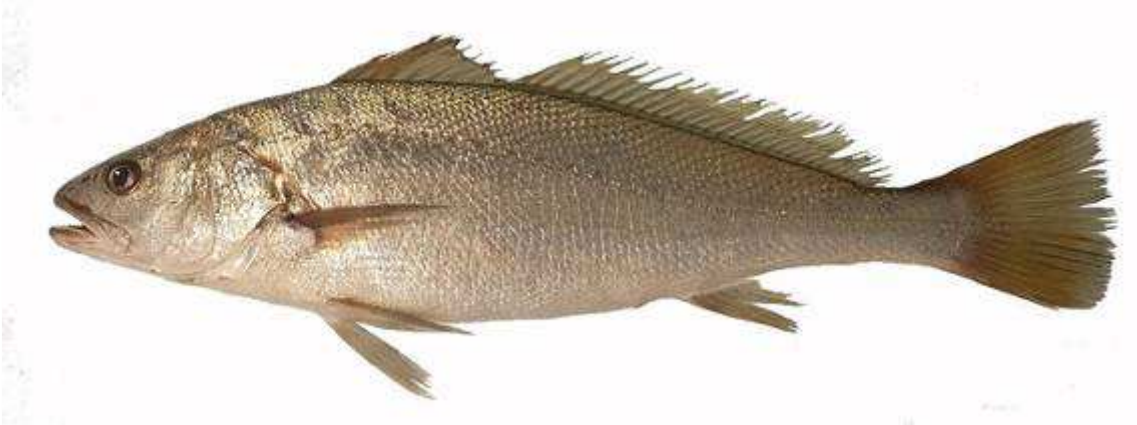

**Figura 3.** *Argyrosomus regius* (wwz.ifremer.fr)

Entre las características morfológicas se puede destacar un cuerpo alargado casi fusiforme y ligeramente comprimido (**Figura 3**), con el dorso de color gris verdoso o azulado y el vientre blanquecino, por todo el cuerpo posee iridiscencias y brillos

dorados y plateados. El interior de la boca es de color amarillo dorado, algo que es particular de esta especie de escianidos y por ello en muchas zonas, sobre todo en Italia, la llaman "boca de oro" (Cárdenas, 2010).

Otra característica resaltante de esta especie son sus otolitos, los cuales, son de gran tamaño, siendo a menudo utilizado como amuleto por los pescadores del sur de la Península Ibérica (Cárdenas, 2010).

*A. regius* se distribuye en el Mar Negro, Mar Mediterráneo y a lo largo de las costas del Atlántico, desde el norte y sur de Noruega y sur del Congo (**Figura 4**). Presentando comportamientos gregarios, viviendo en aguas costeras de la franja del litoral hasta las profundas, con un rango de 15 a 200 metros de profundidad, encontrándose también en estuarios y lagunas costeras (Poli *et al.*, 2003; Lagardére & Mariani, 2006; El-Shelby *et al.*, 2007; González-Quirós *et al.*, 2011). Se han establecido tres principales zonas de puestas para *A. regius*, estas son el Delta del Nilo, la Bahía de Lévrier (Noaudhibou, Mauritania) y el Estuario Gironde, siendo las áreas óptimas para este estadío biológico las costas y estuarios. En cuanto a los periodos de puestas, estos son estimados entre los meses de mayo a julio (Lagardére & Mariani, 2006; González-Quirós *et al.*, 2011; Duncan *et al.*, 2012).

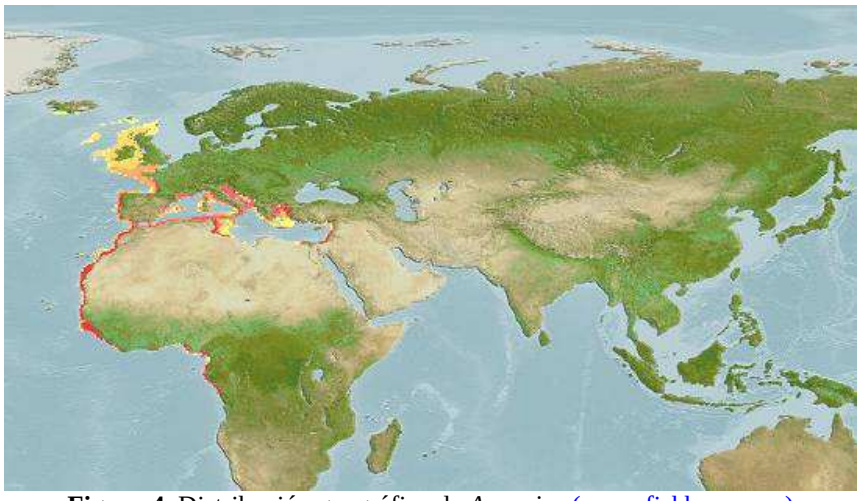

**Figura 4.** Distribución geográfica de *A. regius* (www.fishbase.com)

La talla máxima registrada ha sido de 203 cm y 103 Kg, pero lo más habitual es capturar individuos entre 50 cm y 1 metro de longitud (www.fishbase.org).

## 1.3. ALIMENTACIÓN.

La corvina es una especie muy voraz, que se alimenta de poliquetos, crustáceos, equinodermos y moluscos, además de otras especies de peces más pequeños (cupleidos y mugílidos) (Jiménez *et al.*, 2005) (**Figura 5**). Los juveniles de esta especie presentan una baja diversidad de presas, esencialmente se alimentan de misidáceos y quisquillas (*Crangon crangon*).

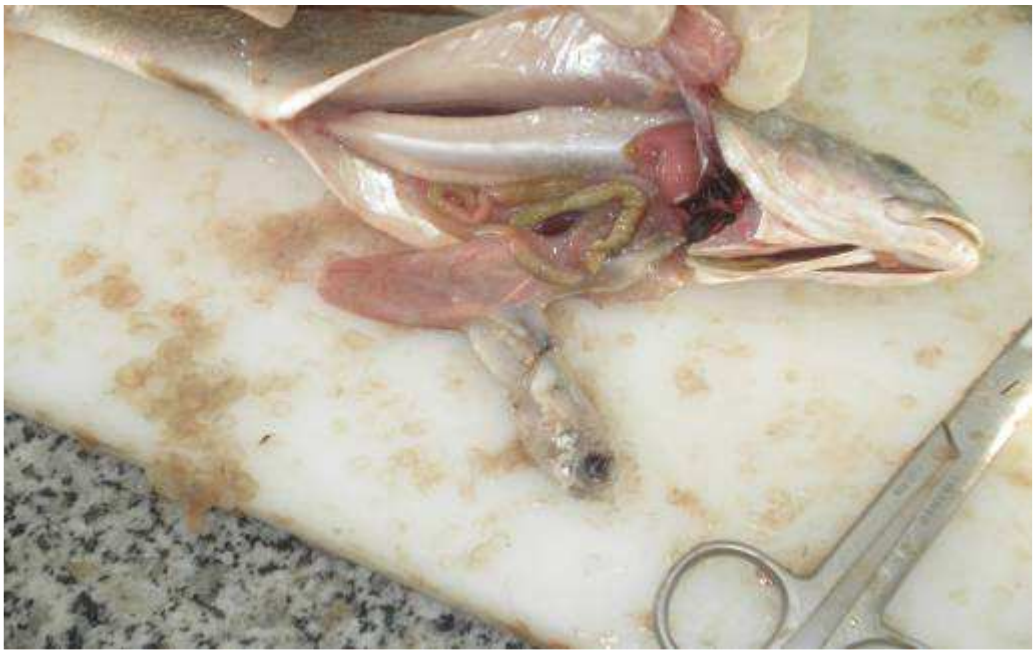

**Figura 5.** Disección de *A. regius*. En el estómago se puede apreciar la presencia de un pez entero (Fuente propia).

Dicho tipo de alimentación justifica la morfología de su tracto digestivo compuesto por un esófago de amplio diámetro y paredes musculosas que acaba en un estómago musculoso presentado en forma de saco y en el que tanto la porción esofágica como la intestinal se insertan en una porción de estómago que se denomina "estómago posterior".

Presenta un mayor grosor en su zona anterior, disminuyendo su diámetro hacia una porción media a partir de la cual vuelve a ensancharse hacia la región anal (Oliva *et al.*, 2005).

Desde mediados de junio a finales de julio dejan los estuarios y se dirigen a lo largo de la costa para alimentarse. Durante el invierno retornan a aguas profundas. Los juveniles se alimentan de peces pequeños y crustáceos mientras que los adultos de peces pelágicos y cefalópodos.

En este sentido, para la producción de la corvina es necesario emplear piensos con alto contenido proteico. Actualmente existe poca información en relación a las necesidades nutricionales de la corvina, siendo necesario para su producción adaptar piensos que han sido diseñados para otras especies comerciales, como la lubina (*Dicentrarchus labrax*) y la dorada (*Sparus aurata*) (Citado en Chatzifotis *et al*., 2012).

Los primeros trabajos de alimentación de corvina con piensos comerciales fueron realizados por Calderón *et al.* (1997), donde alimentaron a corvinas de 180 g de peso medio inicial durante 30 meses alcanzando un peso final de 3.860 g. Para ello emplearon un pienso comercial de dorada suministrado a una tasa de alimentación diaria del 2%.

De acuerdo a lo descrito en esta sección, es fundamental optimizar la alimentación para la producción de *A. regius*, siendo necesario conocer de forma precisa sus necesidades nutritivas y la calidad de los ingredientes que deben componer su dieta especifica.

## 1.4. NECESIDADES NUTRITIVAS.

El principal objetivo de la producción piscícola es el engorde de los peces en el menor tiempo posible y en las condiciones económicas ventajosas. El alimento supone

el mayor de los costes llegando al 40% en muchos casos. El abaratamiento de los piensos o de los costes asociados a la alimentación son claves para rentabilizar la producción y ser competitivos en los mercados.

Los requerimientos mínimos de proteína para la corvina, se sitúan alrededor de un 50% (Martínez-Llorens *et al.*, 2011; Chatzifotis *et al.*, 2012), y superior a los de otros esciánidos (McGoogan & Gatlin III, 1999; Lee *et al.*, 2001; Turano *et al.*, 2002; Pirozzi *et al.*, 2010), mientras que el nivel lipídico de las dietas es inferior al de otras especies carnívoras (aproximadamente un 17%) (Chatzifotis *et al.*, 2010; Martínez-Llorens *et al.*, 2011), posiblemente como consecuencia de su condición de pescado magro (Panagiotidou *et al.*, 2007).

### 1.4.1. NECESIDADES DE ENERGÍA.

La energía no es un nutriente, es el producto final de la absorción de nutrientes que producen energía cuando son oxidados y metabolizados (Cho & Bureau, 1998). Los peces necesitan energía para su actividad diaria y para la reposición y crecimiento de sus tejidos corporales y esta la obtienen a través del alimento mediante la oxidación de la fracción orgánica, constituida por proteínas, grasas y carbohidratos (Sanz, 2009). El valor bruto de energía del alimento depende de su composición química. Los valores promedios de calor por combustión de carbohidratos, proteína y lípidos son de 17,2, 23,6 y 39,5 kJ/g, respectivamente (NRC, 1993).

La energía ingerida no es aprovechada en su totalidad, pues se pierde durante el proceso de digestión y metabolismo, bien como desechos orgánicos, heces y amoniaco, o como calor, siendo el resto retenido en forma de tejidos corporales (**Figura 6**) (Kaushik & Medale, 1994; Guillaume *et al.*, 2004; Sanz, 2009).

En la **Figura 6**, se observa como la energía bruta (EB) sufre pérdidas durante los procesos de digestión mediante las heces, resultando la "energía digestible" (ED). Para determinar la energía digestible es necesario estimar la "digestibilidad" del alimento mediante ensayos de digestibilidad, en los que se cuantifica la cantidad de energía contenida en las heces (Eh). Para ello hay que medir su calor de combustión o bien determinar la digestibilidad de la proteína, lípidos y carbohidratos y aplicarlas sobre los coeficientes oxi-calóricos de la energía bruta.

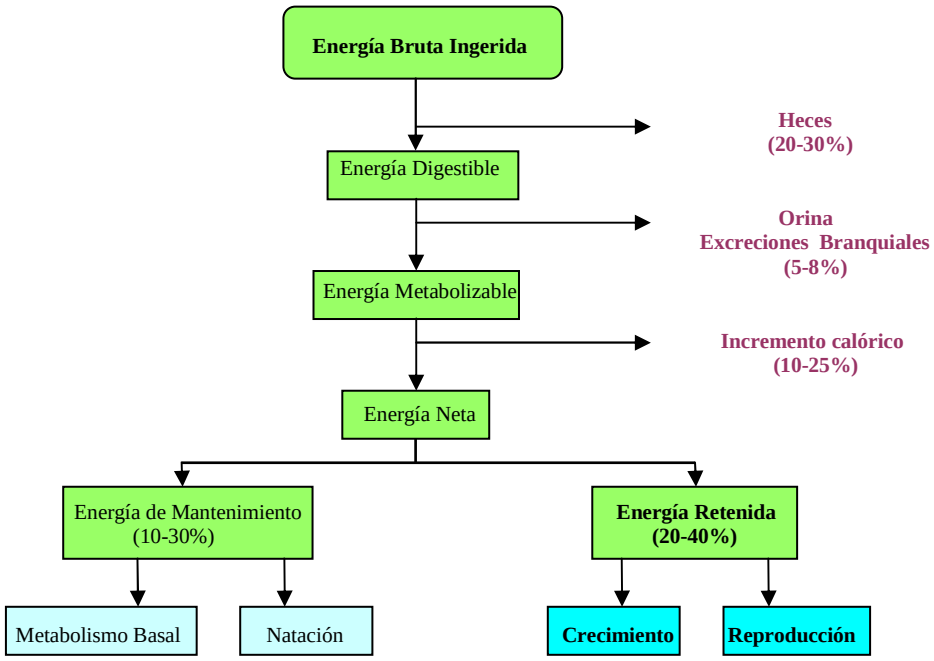


**Figura 6.** Distribución de la energía ingerida en los peces Guillaume *et al.* (2004)

A su vez, una parte de la energía digestible no es utilizable, pues se pierde en forma de los productos de excreción nitrogenada (Ee), amoníaco fundamentalmente y también urea, tanto a través de las branquias como del riñón, de forma que la energía resultante es la "energía metabolizable" (EM). Para determinar la energía excretada es necesario analizar la concentración de amonio y urea en el agua, lo que plantea dificultades técnicas (Sanz, 2009), asociadas al ambiente acuático.

Por otra parte, los procesos digestivos y metabólicos asociados a la ingestión de alimento suponen un gasto de energía, denominada "acción dinámico específica" o "incremento calórico del alimento" (InCA), que habría que restar a la energía metabolizable, para obtener la "energía neta". Los factores que contribuyen al incremento calórico tienen tres orígenes: los procesos de digestión y absorción, los procesos de transformación de los sustratos y su retención como tejidos, los procesos de formación y excreción de los desechos metabólicos. De ellos, el más importante es el gasto asociado a la desaminación de los aminoácidos, por lo que los alimentos ricos en proteína originan unos mayores valores de InCA (Sanz, 2009).

La energía neta es la energía utilizable por los peces para:

- La "energía de mantenimiento" (Em) es la empleada en el metabolismo basal, que incluye los procesos de respiración, circulación, regulación iónica, renovación, etc.; en la actividad involuntaria de reposo o tono muscular, que es muy pequeña en el medio acuático; y en la regulación de la temperatura corporal, nula en los poiquilotermos. Estos menores gastos, hacen que la energía de mantenimiento en los peces resulte de 10 a 20 veces menor que en animales homeotermos.
- Incremento de tejidos o retención, muscular en la fase de crecimiento, o gonadal en la fase de reproducción.

La energía retenida (ER) se obtendría al restar a la energía bruta del alimento todas las pérdidas digestivas y metabólicas, resultando un valor de entre 20 y 40% (Sanz, 2009).

$$ER = EB - [Eh + Ee + InCA + Em]$$

Las necesidades energéticas en acuicultura han sido frecuentemente confundidas con el óptimo contenido energético del alimento, expresado en megajulios por

kilogramo de alimento (MJ/Kg), y en ocasiones con la relación P/E, expresada en gramos de proteína por mega-julio de energía (g/MJ).

Las necesidades energéticas de los peces pueden ser definidas como la cantidad de energía que debe ingerir un pez para optimizar su producción (crecimiento, índice de conversión, rentabilidad, etc.), y deberían ser expresadas en kilojulios de energía por kilogramo de pez y día. Estas necesidades pueden depender del estado fisiológico del pez y de las condiciones ambientales, fundamentalmente de la temperatura que determina el nivel de actividad y el crecimiento (Sanz, 2009).

Las necesidades de energía para el mantenimiento se pueden definir como la cantidad de energía necesaria para mantener las funciones básicas del organismo en condiciones de crecimiento nulo, es decir sin ganancia ni pérdida de peso. En una primera aproximación, las necesidades de mantenimiento se han estimado a partir de la "tasa metabólica basal" mediante el consumo de oxígeno, o de las pérdidas de energía corporal, en periodos de ayuno. En cuanto a las necesidades energéticas de crecimiento de los peces es necesario considerar la energía depositada en forma de crecimiento de los tejidos corporales, es decir la energía retenida, y su eficiencia respecto a la energía digestible ingerida, que se determina como la pendiente de la recta en el tramo de crecimiento (Sanz, 2009).

Las necesidades de energía para el mantenimiento y crecimiento han sido determinadas en la dorada (*Sparus aurata*) (Lupatsch *et al.*, 1998; 2003a), lubina (*Dicentrarchus labrax*) (Lupatsch *et al.*, 2001; 2003b; Peres & Oliva-Teles, 2005), *Argyrosomus japonicus* (Pirozzi *et al.*, 2010), corvinon ocelado (*Sciaenops ocellatus*) (McGoogan & Gatlin III, 1998), mero blanco (*Epinephelus aeneus*) (Lupatsch *et al.*, 2003b; Lupatsch & Kissil, 2005), la trucha arco iris (*Oncorhynchus mykiss*) (Rodehutscord & Pfeffer, 1999; Glencross *et al.*, 2007; 2009), perca plateada (*Bidyanus*

*bidyanus*) (Booth & Allan, 2003), el bacalao del Atlántico (*Gadus morhua*) (Hatlen *et al.*, 2007), la perca gigante (*Lates calcarifer*) (Glencross *et al.*, 2008) y el salmón atlántico (*Salmo salar*) (Helland *et al.*, 2010). En la **Tabla 1** se pueden apreciar los requerimientos de energía establecidos para la dorada y algunos esciánidos.

En *A. regius* Velazco *et al*. (2009), determinaron las necesidades de energía (no digestible) en peces de 50 g (**Tabla 1**), empleando un pienso comercial (Dibaq- Regius) con 47% PB y 19% GB y 5 tasas de alimentación (0, 0,5, 1,75, 3,25, 4,75%), obteniendo que las necesidades de energía para el mantenimiento fueron de 0,06 kJ100 g $pez^{-1}$ $día^{-1}$ y para el máximo crecimiento 0,53 kJ100 g $pez^{-1}$ $día^{-1}$.

**Tabla 1.** Necesidades de energía en *A. regius* y especies de referencia.

| | [A]NEm | [B]NEmáx | Autor |
|---|---|---|---|
| *A. regius* | 0,06 kJ100 g $pez^{-1}$ $día^{-1}$ | 0,53 kJ100 g $pez^{-1}$ $día^{-1}$. | Velazco *et al*. (2009) |
| *S. aurata* | 55,8 kJ ED $kg^{-0,83}$ $pez^{-1}$ $día^{-1}$ | 275 kJ ED $kg^{-0,83}$ $pez^{-1}$ $día^{-1}$ | Lupatsch *et al.* (1998) |
| *A. japonicus* | 44,21 kJ ED $kg^{-0,8}$ $pez^{-1}$ $día^{-1}$ (20 ºC) | 120 kJ ED $kg^{-0,8}$ $pez^{-1}$ $día^{-1}$ (20 ºC) | Pirozzi *et al.* (2010) |
| | 49,59 kJ ED $kg^{-0,8}$ $pez^{-1}$ $día^{-1}$ (26 ºC) | 150 kJ ED $kg^{-0,8}$ $pez^{-1}$ $día^{-1}$ (26 ºC) | |
| *S. ocellatus* | 92 kJ ED kg $pez^{-1}$ $día^{-1}$ (3,4 g) | 958 kJ ED kg $pez^{-1}$ $día^{-1}$ (3,4 g) | McGoogan & Gatlin III (1998) |
| | 97 kJ ED kg $pez^{-1}$ $día^{-1}$ (5,5 g) | 985 kJ ED kg $pez^{-1}$ $día^{-1}$ (5,5 g) | |

[A]NEm: Necesidades de energía para el mantenimiento. [B]NEmáx: Necesidades de energía para el máximo crecimiento.

En la dorada Lupatsch *et al*. (1998), con peces de 30 y 92 g, con tasas de alimentación de 0, 1, 2 y 3%, a temperaturas de 23-24 ºC. Mediante un método factorial, obtuvieron que las necesidades de energía para mantenimiento eran de 55,8 kJ ED $kg^{-0,83}$ $pez^{-1}$ $día^{-1}$ y las necesidades para el máximo crecimiento de 275 kJ ED $kg^{-0,83}$ $pez^{-1}$ $día^{-1}$.

En *Argyrosomus japonicus* Pirozzi *et al.* (2010), trabajaron con peces de 40 y 129 g, con dos temperaturas 20 y 26 ºC, para la alimentación utilizaron una dieta comercial con un 45,5% PB y 18,7 % GB, con tasas de alimentación del 1 al 4%. Los resultados de este trabajo establecen que las necesidades de energía para el mantenimiento aumenta de acuerdo a la temperatura, obteniendo valores de 44,21 kJ ED

kg $^{-0,8}$ pez$^{-1}$ día$^{-1}$ con 20 ºC y 49,59 kJ ED kg $^{-0,8}$ pez$^{-1}$ día$^{-1}$ con 26 ºC, igualmente las necesidades para el máximo crecimiento fueron de 120 kJ ED kg $^{-0,8}$ pez$^{-1}$ día$^{-1}$ con 20 ºC y 150 kJ ED kg $^{-0,8}$ pez$^{-1}$ día$^{-1}$ para 26 ºC.

En corvinón ocelado (*Sciaenops ocellatus*) McGoogan & Gatlin III (1998), estudiaron las necesidades de energía para esta especie con dos grupos de peces, un primer grupo de 3,4 g, en los que probaron tasas de alimentación de 0,5, 1, 2, 4, 6 y 8%. Un segundo grupo de 5,5 g de peso inicial, con tasas de alimentación de 0,75, 1,5, 3, 5, 5,5, 6, 6,5 y 7%. Se obtuvo que las necesidades de energía para el mantenimiento en alevines de esta especie fueron las más altas: 3,4 g (92 kJ ED kg pez$^{-1}$ día$^{-1}$) y 5,5 g (97 kJ ED kg pez$^{-1}$ día$^{-1}$). E igualmente las necesidades de energía para maximizar el crecimiento resultaron muy altas si se compara con dorada y *A. japonicus*, obteniendo 958 kJ ED kg pez$^{-1}$ día$^{-1}$ en peces de 3,4 g y 985 kJ ED kg pez$^{-1}$ día$^{-1}$ en peces de 5,5 g.

### 1.4.2. NECESIDADES PROTEICAS

Una ingesta proteica inadecuada conlleva una reducción o cese del crecimiento y por lo tanto, una pérdida de peso a consecuencia de la utilización de proteína de tejidos menos vitales para el mantenimiento de aquellos más vitales (Wilson, 2002). En caso contrario, un aporte excesivo de proteínas o la inclusión de proteínas inadecuadas en el pienso implica el que sólo una parte de la misma podrá ser utilizada en la generación de nuevas proteínas y el resto catabolizada para dar lugar a carbohidratos o grasas, para producir energía (Tibbetts *et al.*, 2000; Sá *et al.*, 2008; Zhang *et al.*, 2010). De esta forma cualquier desequilibrio, tanto cuantitativo como cualitativo, sobre las necesidades proteicas específicas supone un gasto energético extra necesario para el metabolismo del mismo. El exceso de proteína ingerida puede ser almacenado en los tejidos en forma

de ácidos grasos o glucógeno, dependiendo del destino y estructura de la proteína (Sanz, 2009).

El nivel óptimo de proteína en los piensos para peces está influenciado por diferentes factores: la especie, calidad de la proteína (digestibilidad, perfil de aminoácidos, procesado), relación P/E, estado fisiológico del pez (tamaño, edad, reproducción), parámetros ambientales (temperatura del agua, época del año, etc.), diferencias genéticas y nivel de ingesta de alimento (Cho *et al.*, 1985; NRC, 1993). Mediante el ajuste de la dieta a todos estos factores se puede optimizar el uso de este macro-nutriente que afecta mayoritariamente a la economía de la producción (Sá *et al.*, 2008; Kim *et al.*, 2010; Zhang *et al.*, 2010). Una disminución en la tasa de alimentación incrementa el contenido óptimo de proteína en la dieta para una máxima retención de la misma. Así mismo la intensificación de los sistemas productivos tiende a influir negativamente en la utilización proteica, no sólo por las pérdidas de alimento, sino también por una peor utilización del mismo; la digestibilidad de la proteína bruta disminuye un 3% cuando aumenta un 1% la ración diaria en la trucha arco iris (NRC, 1993).

La energía de la dieta es también un factor determinante de los requerimientos de proteína de los peces, siendo importante por tanto encontrar la relación de P/E que maximice el crecimiento para cada especie y condiciones específicas de producción (McGoogan & Gatlin III, 1999; 2000).

En relación al tamaño de los animales, es comúnmente aceptado que peces pequeños precisan un mayor aporte proteico para un máximo crecimiento que peces mayores (Cho *et al.*, 1985; NRC, 1993) y los requerimientos de proteína como proporción de la dieta disminuyen según el pez llega a su maduración sexual NRC (1993). Este fenómeno está asociado con una disminución en síntesis proteica corporal a

medida que el pez se desarrolla, con una tasa de crecimiento menor en los peces adultos (Sanz, 2009).

Martínez-Llorens *et al.* (2011), trabajaron con juveniles de 94 g de *A. regius* alimentados con cuatro piensos comerciales con proporciones distintas de proteína y lípidos (44/25, 43/21, 46/20 y 47/20%), durante un periodo de 173 días, llegaron a la conclusión de que el mejor crecimiento y eficiencia nutritiva correspondía al pienso con un 47% de proteína bruta (PB) y un 20% de grasa bruta (GB). Este pienso obtuvo como resultado una tasa de crecimiento instantáneo (TCI) de 1,2% día$^{-1}$, tasa de alimentación (TAD) igual a 0,8% día$^{-1}$ y un índice de conversión del alimento (ICA) de 1,2. También en *A. regius* Chatzifotis *et al.* (2012), durante tres meses experimentales a una temperatura de 19 ºC, en peces de 23 g emplearon piensos con cuatro niveles de proteínas (40, 45, 50 y 54% de PB), todos con un mismo nivel de lípidos (17% GB); obtuvieron que el nivel óptimo para juveniles de *A. regius* fue del 50% de PB, los resultados indicaron que para este nivel de proteína los peces alcanzaron un peso final de 66 g, con un TCI de 1,1% día$^{-1}$ y 1 de ICA. Mientras que en corvinón ocelado, el nivel óptimo de proteína se sitúa entre un 40% (Serrano *et al*., 1992) y un 44% (Thoman *et al*., 1999; Turano *et al*., 2002).

La comprensión de cómo los nutrientes se utilizan es un paso esencial hacia el desarrollo de modelos bioenergéticos que predicen las respuestas de crecimiento, requerimientos de alimentación y las pérdidas de nutrientes en el medio ambiente (Pirozzi *et al.*, 2010). El concepto de necesidades para el mantenimiento ha demostrado ser útil para la nutrición animal ya que permite la adecuación de los costes de producción y de mantenimiento basado en la suposición de que los dos son añadidos.

Las necesidades de proteína se han determinado en la dorada (Lupatsch *et al.*, 1998), lubina (Peres & Oliva-Teles, 2005), *A. japonicus* (Pirozzi *et al.*, 2010), corvinon

ocelado (McGoogan & Gatlin III, 1998), trucha arco iris (Glencross *et al.*, 2007; 2009), perca plateada (Booth & Allan, 2003), el mero blanco (Lupatsch & Kissil, 2005), el bacalao del Atlántico (Hatlen *et al.*, 2007), la perca gigante (Glencross *et al.*, 2008) y el salmón atlántico (Helland *et al.*, 2010). En la **Tabla 2** se puede apreciar las necesidades de proteína establecidas para la dorada y algunos esciánidos.

En *A. regius* Velazco *et al*. (2009), determinaron las necesidades de proteína (no digestible) en peces de 50 g (**Tabla 2**), empleando un pienso comercial (Dibaq- Regius) con 47% PB y 19% GB y 5 tasas de alimentación (0, 0,5, 1,75, 3,25, 4,75%), obteniendo que las necesidades de proteína para el mantenimiento fueron de 0,48 g P 100 g $pez^{-1}$ $día^{-1}$ y para el máximo crecimiento 1,07 g P 100 g $pez^{-1}$ $día^{-1}$.

**Tabla 2.** Necesidades de proteínas en *A. regius* y especies de referencias.

| | [A]NPm | [B]NPmáx | Autor |
|---|---|---|---|
| *A. regius* | 0,48 g P 100 g $pez^{-1}$ $día^{-1}$ | 1,07 g P 100 g $pez^{-1}$ $día^{-1}$ | Velazco *et al*. (2009) |
| *S. aurata* | 0,86 g PD $kg^{-0,70}$ $pez^{-1}$ $día^{-1}$ | 5,5 g PD $kg^{-0,70}$ $pez^{-1}$ $día^{-1}$ | Lupatsch *et al*. (1998) |
| *S. aurata* | 0,77 g PD kg $pez^{-1}$ $día^{-1}$ | 3,37 g PD kg $pez^{-1}$ $día^{-1}$ | Jauralde *et al*. (2013) |
| *A. japonicus* | 0,47 g PD $kg^{-0,70}$ $pez^{-1}$ $día^{-1}$ | 2,6 g PD $kg^{-0,70}$ $pez^{-1}$ $día^{-1}$ | Pirozzi *et al*. (2010) |
| *S. ocellatus* | 0,5 a 2,2 g PD kg $pez^{-1}$ $día^{-1}$ | 20 a 25 g PD kg $pez^{-1}$ $día^{-1}$ | McGoogan & Gatlin III (1998) |

[A]NPm: Necesidades de proteínas para el Mantenimiento. [B]NPmáx: Necesidades de proteínas para el máximo crecimiento.

En la dorada Lupatsch *et al.* (1998), obtuvieron que las necesidades de proteína para el mantenimiento fueron de 0,86 g PD $kg^{-0,70}$ $pez^{-1}$ $día^{-1}$ y las necesidades para el máximo crecimiento fueron de 5,5 g PD $kg^{-0,70}$ $pez^{-1}$ $día^{-1}$. También en doradas Jauralde *et al.* (2013), establecen un modelo de crecimiento en función de las tasas de alimentación, obteniendo que las necesidades para el mantenimiento son de 0,77 g PD kg $pez^{-1}$ $día^{-1}$ y las necesidades para el máximo crecimiento de 3,37 g PD kg $pez^{-1}$ $día^{-1}$, de acuerdo a un modelo de crecimiento asintótico.

En *A. japonicus*, Pirozzi *et al.* (2010), obtuvieron que las necesidades de proteína no fueron afectadas por la temperatura y que para el mantenimiento fueron de 0,47 g PD $kg^{-0,7}$ $día^{-1}$, y para el máximo crecimiento fueron de 2,6 g PD $kg^{-0,7}$ $día^{-1}$.

McGoogan & Gatlin III (1998) en el corvinon ocelado determinaron las necesidades de proteína para el mantenimiento en 0,5 a 2,5 g PD $kg^{-1}$ pez $día^{-1}$, y las necesidades de proteínas para maximizar el crecimiento fueron de 20 a 25 PD $kg^{-1}$ pez $día^{-1}$.

La mayor parte de los desechos en acuicultura son nitrogenados y provienen de la alimentación, pues la proteína que no es utilizada para crecimiento muscular es empleada para obtener energía, y el exceso es reconvertido también en lípidos corporales, para lo cual los aminoácidos deben ser desaminados, y el amoníaco excretado, ya que su acumulación en el organismo resulta tóxica (Tibbetts *et al.*, 2000; Sanz, 2009; Zhang *et al.*, 2010). Las tasas de excreción de amonio, mantienen una dependencia lineal respecto el aumento de proteína en la dieta. En la dorada la excreción de amonio fue determinada entre 300,79 y 506,23 mg $N\text{-}NH_4^+$ kg $pez^{-1}$ $día^{-1}$ para peces de 13 g y entre 296,07 y 708,99 mg $N\text{-}NH_4^+$ kg $pez^{-1}$ $día^{-1}$ para peces de 29 g. Respecto a la excreción máxima por periodo se obtuvieron valores de 28,54 mg $N\text{-}NH_4^+$ kg $pez^{-1}$ $h^{-1}$ para doradas de 13 g y 37,52 mg $N\text{-}NH_4^+$ kg $pez^{-1}$ $h^{-1}$ para doradas de 29 g con la dieta 60/9,1 de 15:30 a 20:00 h (Martínez, 2002).

## 1.5. HARINA DE PESCADO Y FUENTES PROTEICAS ALTERNATIVAS.

La harina de pescado es una excelente fuente de proteína para la formulación de piensos para peces, y actualmente es incorporada en un 25% en los piensos comerciales para las especies de peces carnívoros. Presenta un alto contenido en proteína, 65-75% PB, y su perfil de aminoácidos esenciales (AAE) es el más adecuado para la alimentación de los peces, presentando niveles altos de lisina y metionina (Zaldivar *et al.*, 2002) además de treonina y triptófano. Existen varios tipos de harinas de pescado, procedentes de pescado blanco, azul (cupleidos) y subproductos de la industria conservera. Estos productos se obtienen a partir de la pesca industrial, que se dedica a

capturar especies pelágicas pequeñas, especialmente cupleidos (sardinas, arenques), lanzones (género *Ammodytes*) y capelin (*Mallotus villosus*), de bajo valor comercial.

En los últimos años la acuicultura a nivel mundial viene experimentando grandes crecimientos, trayendo como consecuencia incrementos de la demanda de harina de pescado (Murray *et al.*, 2010). Esta materia prima también es empleada en otras actividades ganaderas (Francis *et al.*, 2001; Tacon & Metian, 2008), por lo que las consecuencias han sido:

- Incrementos del precio de harina de pescado.
- Disminución de las poblaciones de peces de forraje, aumento de sus precios en los mercados y menor accesibilidad de estas especies para el consumo humano.
- Disminución de la disposición de harinas y aceite de pescado y derivados.
- Aumento mundial de la energía de procesamiento, gastos de transporte.
- Presión sobre los fabricantes de alimentos para la sustitución de materias primas, con el fin de hacerlas rentables.
- Presión de la sociedad civil y los minoristas para mejorar la sostenibilidad global de uso de los recursos pesqueros dentro del sector de la acuicultura.
- Reducción de la inclusión de la harina y aceite de pescado en los piensos para la acuicultura y engorde de otros animales.
- Mayor tendencia a producir especies marinas de hábitos herbívoros y omnívoros.

Por lo antes expuesto, es necesario diseñar piensos con fuentes proteicas alternativas a las harinas de pescado, que permitan mitigar dichos impactos, como las harinas de origen animal, tales como harina de carne y hueso, harina de aves, harina de sangre y hemoglobina, harina de krill en especies carnívoras. También se han empleado

harinas de organismos unicelulares tales como levaduras, algas, bacterias y hongos (Oliva–Teles & Gonçalves, 2001; Aas *et al*., 2006).

Las fuentes proteicas de origen vegetal son muy numerosas y más económicas, por lo tanto, los fabricantes tratan de incluirlas en los piensos para la acuicultura, y que además de un cierto poder aglutinante, asociado o no a la presencia de sustancias digestibles. Los turtós son co-productos de la extracción de las grasas, con niveles de proteína que suelen oscilar entre un 30-50% de PB (Guillaume *et al.*, 2004). El turtó de soja es una de las fuentes proteicas más utilizadas en piensos para peces, debido a que posee un buen nivel de proteínas (40-50% CP), alta disponibilidad en el mercado, por ser un recurso económico con proteínas y energía altamente digestible y un buen perfil de contenido de aminoácidos (Wang *et al.*, 2006).

También el turtó de soja se ha experimentado en especies comerciales tales como la cobia (*Rachycentron canadum*), en juveniles de esta especie se obtuvo un nivel óptimo de inclusión del turtó de soja de 16,9% (Chou *et al.*, 2004), en la seriola (*Seriola dumerili*), Tomás *et al.* (2005) recomiendan para el máximo crecimiento una inclusión de un 20 a 30%, similares resultados fueron determinados para la dorada con un 20,5% (Martínez-Llorens *et al.*, 2009) y 30,5% cuando la dieta era enriquecida con aminoácidos sintéticos (Martínez-Llorens *et al.*, 2007). No hay referencias bibliográficas de experimentos de inclusión del turtó de soja en corvinas, pero esta fuente proteica, ya ha sido probada en otras especies de la familia Scianidae como es el caso de *Nibea miichthioides*, en la que se recomienda un nivel máximo del 10%, puesto que a mayor inclusión disminuye el crecimiento de los peces (Wang *et al.*, 2006) y el corvinón ocelado (*Sciaenops ocellatus*) en los estudios realizados por McGoogan & Gatlin (1997) obtienen buenos resultados con una inclusión de turtó de soja del 66%,

mientras que en esta misma especie, Reigh & Ellis (1992) obtienen que al incluir turtó de soja hasta un 70%, los crecimiento no se vieron afectado negativamente.

Cuando la sustitución de harina de pescado se realiza por una mezcla de fuentes proteicas los niveles de sustitución alcanzados pueden ser mejores, ya que las deficiencias aminoacidicas de una fuente proteica pueden completarse con otras que forme parte de la mezcla. En este sentido Estévez *et al.* (2010), en *A. regius* experimentaron con una mezcla de proteínas vegetales (turtó de soja, gluten de maíz, turtó de girasol y concentrado de proteína de soja), los peces fueron alimentados con cuatro dietas experimentales con dos niveles de inclusión (42 y 52%, que representaban el 31,5 y el 38% de la proteína total de las dietas) de la mezcla de proteína vegetal con o sin proteínas hidrolizadas, observaron que el crecimiento de los peces se redujo significativamente por la inclusión de la proteína vegetal, aunque el crecimiento de los peces alimentados con dietas con un 42% de inclusión de proteína vegetal en la dieta, obtuvo un crecimiento similar para los peces alimentados con dieta control.

## 1.6. LÍPIDOS.

El aporte de lípidos en la alimentación de los peces, al igual que en los mamíferos, es fundamental para satisfacer los requerimientos de ácidos grasos esenciales (AGE), no sintetizables por el organismo y necesarios para el metabolismo celular (síntesis de prostaglandinas y compuestos similares), así como para el mantenimiento de la integridad de las estructuras de membrana. Además los lípidos son vectores de vitaminas liposolubles y pigmentos carotenoides en el momento de la absorción intestinal y son fundamentales en el suministro de energía (Guillaume *et al.*, 2004).

El aumento de la proporción de lípidos reduce el nivel de proteína en la dieta, incrementando la energía metabolizable, reteniendo eficazmente la energía y mejorando la conversión de la proteína.

Hay algunos trabajos en el que se ha estudiado la influencia del nivel de lípidos en el crecimiento de la corvina, como el de Chatzifotis *et al*. (2010), donde probaron en juveniles de *A. regius* de 230 g de peso inicial, durante un periodo de 110 días, tres piensos experimentales con un 43% de proteína y tres porcentajes diferentes de lípidos 13, 17 y 21%. Determinaron que el pienso con un 17% de lípidos (TCI 0,46% día$^{-1}$; ICA 1,38) el crecimiento fue superior. Martínez-Llorens *et al.* (2011), con piensos comerciales, obtuvieron que el mejor crecimiento correspondía al pienso con un 47% de proteína bruta y un 20% de grasa bruta, en este trabajo para la dieta en cuestión el TCI (1,2% día$^{-1}$) y el ICA (1,2) son mejores que los presentados por Chatzifotis *et al*. (2010). También Chatzifotis *et al*. (2012), en *A. regius* de 22 g alimentados con cuatro piensos isoproteicos (50% PB) y niveles de 12, 14, 17 y 20% de GB, no obtuvieron diferencias significativas en cuanto al peso final y tasa de crecimiento, pero los peces alimentados con el 17 y 20% de lípidos presentaron un índice de conversión significativamente más bajo y un mayor contenidos de lípidos corporales. Y de este modo, concluyeron que en la corvina, el contenido óptimo de lípidos en el pienso está entre el 17 y 20%.

## 1.7. DIGESTIBILIDAD.

La digestibilidad es el porcentaje de alimento absorbido en el tracto digestivo y depende de la especie, el nivel energético del alimento, la composición nutritiva del pienso, las materias primas del pienso, administración del alimento y factores ambientales (Álvarez-González *et al.*, 2001; Zhang *et al.*, 2010).

La digestibilidad constituye una excelente medida de calidad para cuantificar el valor nutricional de las materias primas utilizadas en la alimentación acuícola, ya que no basta que los elementos nutricionales se encuentren en altos porcentajes en los alimentos, sino que deben ser digeribles para que puedan ser asimilados por los peces (Cho, 1987). La digestibilidad tiene una influencia directa sobre el impacto en el medio ambiente y en el costo de las dietas (Irvin & Tabrett, 2005; Luo *et al.*, 2008; Masagounder *et al.*, 2009).

La eficiencia de la digestibilidad puede ser evaluada midiendo bien sea la biodisponibilidad (la fracción del nutriente ingerido que está disponible para su utilización en las funciones fisiológicas normales y reservas) o bioaccesibilidad (la fracción que se libera de la matriz del alimento y está disponible para absorción intestinal). Los métodos *in vivo* proporcionan datos directos de biodisponibilidad y se han utilizado para una gran variedad de nutrientes, mientras que los métodos *in vitro* simulan la digestión y los procesos absorción (por biodisponibilidad) o sólo el proceso de digestión (por bioaccesibilidad) y la respuesta medida es la concentración de un nutriente de algún tipo de extracto final (Parada & Aguilera, 2007; Hamdan *et al.*, 2009; Morales & Moyano, 2010).

Para determinar la digestibilidad *in vivo*, pueden aplicarse métodos de forma directa o indirecta. El método directo requiere el conocer todo el alimento ingerido y todas las heces producidas durante una o más comidas. El método indirecto aplica un marcador, generalmente el indicador utilizado es óxido de crómico ($Cr_2O_3$) (Austreng 1978; Li *et al.*, 2004; Masagounder *et al.*, 2009) u óxido de itrio ($Y_2O_3$) (Gaylord *et al.*, 2009), ó el método de las cenizas insolubles en ácido, en el cual no se emplea marcador externo (Atkinson *et al.*, 1984).

Los peces, en general digieren las proteínas con unos CDA que sobrepasan el 90% y la digestibilidad de la proteína de una fuente dada varía poco de una especie de pez a otra. Mientras que en los lípidos el CDA es mayor de un 95%, independientemente de su origen, animal o vegetal.

Recientemente Feij *et al.* (2012), han realizado un trabajo en el que determinan la digestibilidad de *A. regius*, con dietas con diferentes fuentes proteicas vegetales obteniendo que la digestibilidad de la proteína de los ingredientes experimentales fue alta (> 91%) para el de gluten de maíz, concentrado proteico de soja, concentrado proteico de guisante, gluten de trigo y moderada (84 - 88%) para el turtó de soja, harina de colza y harina de girasol. La digestibilidad de energía para turtó de soja, gluten de maíz, concentrado proteico de soja, concentrado proteico de guisante, gluten de trigo osciló entre el 83 y el 87%, mientras que las de harina de colza y harina de girasol fueron relativamente menores, de 59 y 46%, respectivamente.

Los métodos *in vitro* se han utilizado para determinar la digestibilidad de diferentes proteínas en las dietas (Bassompierre *et al.*, 1997; Rungruangsak-Torrissen *et al.*, 2002). Una vez que se demuestra su correlación con los método *in vivo*, los ensayo *in vitro* adquieren muchas ventajas, ya que son rápidos, seguros, y no tienen las restricciones éticas que afectan a los ensayos realizados con animales vivos. En este sentido, los ensayos *in vitro* son ampliamente utilizados en la evaluación de los alimentos para humanos y piensos para los animales terrestres (Boissen & Eggum 1991; Hamdan *et al.*, 2009; Morales & Moyano, 2010).

Los métodos que permiten simular el proceso de la digestión gastrointestinal en condiciones de laboratorio se conocen como modelos gastrointestinales (MGI), los cuales, tratan de reproducir las condiciones fisiológicas en la boca, estómago, y el intestino durante la masticación, la digestión, y a veces la absorción. En general, los

MGI se dividen en dos grandes categorías: los modelos estáticos, donde el productos de la digestión permanecen en gran parte inmóvil y no imitar procesos físicos tales como cortes, mezclas, hidratación y así sucesivamente; mientras que los modelos dinámicos incluyen procesos físico, mecánicos y cambios temporales en las condiciones luminales para imitar condiciones *in vivo* (Hamdan *et al.*, 2009). Los MGI también resultan de gran interés porque pueden predecir las excreciones de N y P a los sistemas de producción en excreciones solubles, desechos fecales y pienso no consumido, por lo que puede considerarse para los efectos de impactos ambientales dentro de la acuicultura (Talbot & Hole, 1994; Morales & Moyano, 2010).

En *A. regius* Hamdan *et al.* (2009), optimizaron un modelo gastrointestinal aplicable a la evaluación de bioaccesibilidad de los piensos, en este se desarrolla una hidrólisis en dos pasos, usando secuencialmente un reactor cerrado para la digestión ácida y otro reactor con una membrana semi-permeable para la digestión alcalina. En este sistema, la eliminación continua de productos de la digestión durante la hidrólisis de sustratos representa un modelo más práctico y realista, en donde las membranas del borde del epitelio eliminan los productos de la digestión, imitando así las condiciones *in vivo*. Otros estudios han evaluado diferentes pruebas *in vitro* para determinar la digestibilidad de la proteína o la calidad nutricional de los ingredientes de piensos acuícola (Bassompierre *et al.*, 1997; Tonheim *et al.*, 2007), pero pocos han correlacionado sus resultados con los obtenidos *in vivo*. Rungruangsak-Torrissen *et al.* (2002) con tres especies, la trucha arco iris (*Oncorhynchus mykiss*), lubina (*Dicentrarchus labrax*) y el salmón atlántico (*Salmo salar*), han encontraron una buena correlación entre los datos de la hidrólisis de las proteínas de los diferentes piensos utilizados con los resultados obtenidos en los ensayos de digestibilidad *in vivo*. También Tibbets *et al.* (2011) el bacalao del Atlántico (*Gadus morhua*), sugieren que existe una

buena correlación entre los valores de la hidrólisis de las proteínas medido con el pH-staten diferentes fuentes de proteínas y sus CDA para determinados valores de proteína.

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# CAPÍTULO 2

# JUSTIFICACIÓN Y OBJETIVOS

La producción acuícola mediterránea de especies como la dorada y la lubina está totalmente consolidada, y dado que en los últimos años ha tendido a saturar los mercados, su precio ha bajado considerablemente, pasando en la dorada de precios en 1999 de 7 euros g a 5 euros $kg^{-1}$ en 2011, con lo cual los beneficios obtenidos cada vez son más pequeños.

Como alternativa surge la diversificación de especies, en dos vertientes, una en la que se ofrecen nuevas presentaciones del pescado, como filetes, platos precocinados, conservas, y una segunda vertiente en las que se busca variar las especies producidas en acuicultura, y por tanto, la oferta al consumidor.

Dentro de la búsqueda de nuevas especies se ha investigado la seriola (*Seriola dumerili*), el mero (*Epinephelus sp*), la anguila (*Anguilla anguilla*), el atún rojo (*Thumus thynnus*), pero en ellas se han encontrando bloqueos en algún punto de su ciclo productivo. También se ha investigado el dentón (*Dentex dentex*), el sargo picudo (*Diplodus puntazzo*) y el pargo (*Pagrus pagrus*) entre otros, pero en este caso el problema es que son espáridos, que compiten directamente con la dorada.

La corvina, sin embargo, es una especie de elevada fecundidad y ampliamente distribuida, con precios de mercado medios (12 euros $kg^{-1}$), con buena aceptación por parte de los consumidores en determinadas zonas de España, que además, tolera perfectamente la cautividad, como demuestra su presencia en grandes acuarios, donde alcanza elevadas tasas de crecimiento en engorde y buenos índices de conversión (Calderón *et al.*, 1997; Pastor *et al.*, 2002).

A la corvina se le confiere la categoría de producto de alta calidad, debido a sus características organolépticas, es muy baja en grasa mesentérica y muscular, admite largos periodos de conservación en condiciones de refrigeración (Poli *et al.*, 2003). La

calidad de la carne es excelente y apreciada en las zonas costeras del Mediterráneo, y muy utilizada dentro de la alta cocina.

Por otro lado, el interés en la cría de la corvina no sólo reside en el ámbito comercial, pues también comprende el de la conservación de los recursos naturales. Las poblaciones mediterráneas de esta especie han sufrido un alarmante retroceso (Quero, 1989), pudiéndose considerar extinguida en algunas áreas como las islas Baleares (este de España), donde antaño era una captura relativamente frecuente y un producto habitual en los mercados (Mayol *et al.*, 2000).

La producción de la corvina ha ido aumentando a lo largo de los últimos años mostrando una producción significativa en 2006, coincidiendo con el establecimiento de la producción de alevines en el 2005. Según APROMAR (2012), la producción de la corvina en 2011 fue de 2.880 tn y se ha situado a la Comunidad Valenciana como máxima productora de España con 1.510 tn, seguida por Murcia con 1.300 tn. España se mantiene como el máximo productor de corvina.

A pesar del incremento en la producción a escala comercial de esta especie, previo al desarrollo experimental de la presente tesis doctoral existían poca información sobre su crecimiento y piensos específicos para optimizar el crecimiento y el aprovechamiento nutritivo, destacando sólo los trabajos de Calderón *et al.* (1997), Pastor *et al.* (2002), Pastor *et al.* (2007) y El-Shebly *et al.* (2007). Las necesidades nutritivas no están establecidas, y el diseño de pienso para corvina se realizaba en función de las necesidades de otras especies como la dorada y la lubina. Por ello los objetivos planteados en esta tesis doctoral son:

- ✓ Determinar las necesidades de proteína y energía de la corvina, como un aporte al conocimiento de la alimentación de esta especie, en subjuveniles y juveniles, para optimizar el crecimiento y aprovechamiento nutritivo.

- ✓ Estudiar el nivel óptimo de proteína, para el crecimiento en subjuveniles y juveniles de la corvina, producida en tanques y en jaulas con piensos extrusinados.
- ✓ Estudiar la posibilidad de sustitución de la harina de pescado por fuentes proteicas vegetales alternativas, concretamente la inclusión del turtó de soja en piensos para juveniles de corvinas.
- ✓ Diseñar un pienso para su producción a escala comercial.

# CAPÍTULO 3

# PLAN EXPERIMENTAL

Dada la importancia de desarrollar estudios de crecimientos y nutrición en *A. regius*, en la presente tesis se diseñaron diferentes experimentos con corvinas de diferentes tamaños, con el fin de conocer con más precisión diferentes aspectos de alimentación de esta especie, como determinar las necesidades de energía y proteína, el nivel óptimo de proteína en el pienso y por último, y dado la importancia actual de sustituir la harina de pescado por fuentes proteicas vegetales, determinar el nivel de inclusión de turtó de soja en piensos para corvinas.

Para una mejor comprensión de estos ensayos en la **Figura 7**, se muestra un resumen de los experimentos, así como también los parámetros evaluados en cada una de las pruebas.

Estos experimentos se dividen en tres grandes bloques:

1. Estimación de las necesidades de proteína y energía para corvina; las cuales se desarrolla en el **Capítulo 4**, siguiendo un diseño factorial con la metodología descrita en Lupatsch *et al*. (1998). Las pruebas se desarrollaron en jaulas flotantes dentro de tanques de 4000 litros, en sistemas de recirculación.
2. Determinación de los niveles de proteína digestible y la relación de Proteína/Energía de los piensos (**Capítulo 5**). Estos experimentos fueron de dosis-respuesta, se realizaron en tanques de 1700 litros y en jaulas marinas, respectivamente.
3. La inclusión del turtó de soja en los piensos experimentales para la corvina (**Capítulo 6**), como una fuente proteica vegetal alternativa a la harina de pescado. Estas pruebas se desarrollaron en tanques de 4000 litros, evaluando la eficiencia nutritiva de esta materia prima.

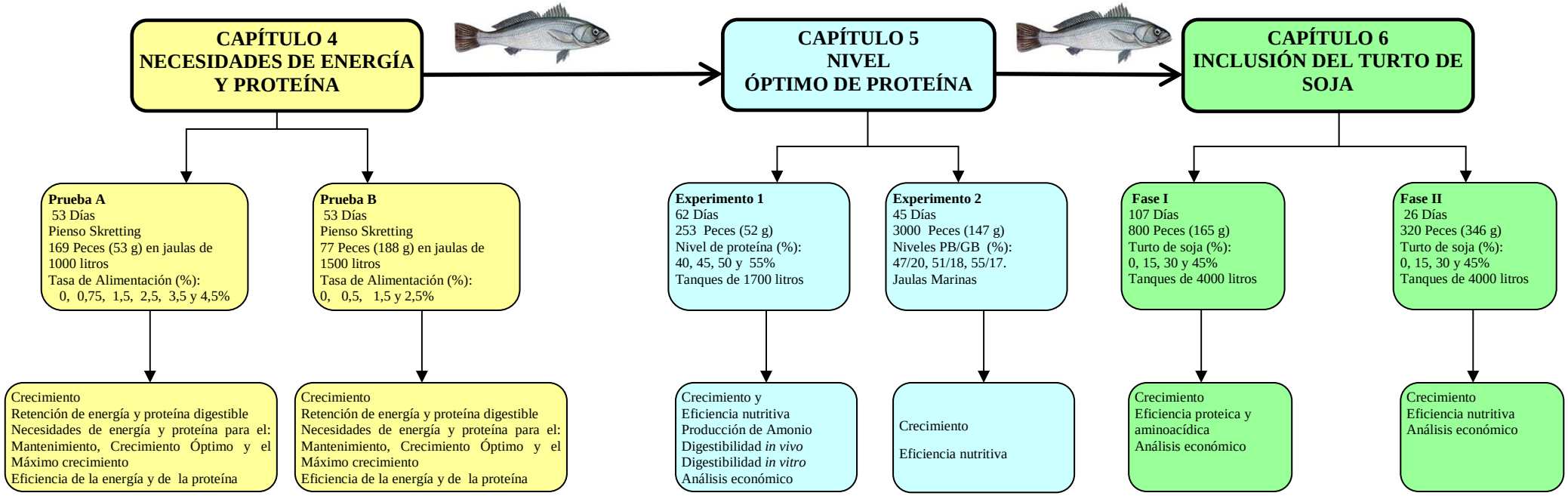


**Figura 7.** Resumen de los experimentos de crecimiento y nutrición en *Argyrosomus regius*.

# CAPÍTULO 4

# PROTEIN AND ENERGY REQUIREMENTS

## INTRODUCTION

Currently, new species are being introduced into aquaculture production necessitating a new approach to energy and protein requirements to reach their maximum growth potential. The meagre (*Argyrosomus regius*) could be a suitable candidate species for the diversification of aquaculture in the Mediterranean region (El-Shebly *et al*., 2007; Estévez *et al*., 2010; Chatzifotis *et al*., 2010; Duncan *et al*., 2012; Velazco-Vargas *et al*., 2013) given its high growth rate and appreciated flesh quality. As body and fillet traits of meagre have shown a very high dressing content with a negligible amount of mesenteric and muscular fat in comparison with other cultured fish, this species becomes even more interesting for industrial processing and human consumption (Poli *et al*., 2003).

Ongrowing techniques were based on the rearing of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), but the huge growth rate of the meagre enables them to reach 1 kg in ten months. However, usual commercial weight ranges from 1.5 to 3 kg (Jiménez *et al*., 2005; Roo *et al*., 2010; Martínez-Llorens *et al*., 2011), although Chatzifotis *et al*. (2012) obtained a specific growth rate (SGR) ranging from 0.7 to 1.3% $day^{-1}$. Yet, if these data are referred to thermal growth coefficient (TGC), these values would be ranging from 1.3 x $10^{-3}$ to 1.98 x $10^{-3}$, thus giving the best results with the highest dietary protein level (50% CP). Nevertheless, information on its growth rates and nutrient requirements is scarce.

The meagre is a carnivorous species, and in its natural environment it feeds on Mysidacea, Decapoda and Teleostei (Chatzifotis *et al*., 2012). The dietary protein level needed for maximum growth of carnivorous species under culture conditions has been reported to vary from 40% to 55% (Alvarez *et al*., 2001). Protein is the most expensive component in balanced fish feeds and also the most important factor affecting the

growth performance of cultured species (Lee *et al*., 2000; Sá *et al*., 2008; Zhang *et al*., 2010). Generally, the increase of dietary protein can lead to improved fish production and broodstock, especially for carnivorous fish (Lee *et al*., 2002; Chong *et al*., 2004; El-Sayed & Kawanna, 2008; Zhang *et al*., 2010). However, excess protein supplied in feeds will be metabolised as an energy source and will increase production of nitrogenous waste material that is excreted in water, which may be detrimental to fish growth and culture environment (Tibbetts *et al*., 2000; Sá *et al*., 2008; Zhang *et al*., 2010). Dietary lipid, as a non-protein energy source, may also influence growth and protein utilisation of fish. Increasing the dietary energy level has been suggested as a strategy to save protein and limit ammonia production for several fish species (Lee *et al*., 2002).

Dietary nutrient requirements in fish are estimated empirically by feeding graded levels of a specific nutrient (dose), in a basal diet containing a deficient level of this nutrient, and then growth, feed intake, body nutrient stores or other variables are measured (response) (Shearer, 2000). The dose-response relationship is then examined using one or more methods, and the nutrient requirement is estimated from the level that produces the maximum response (Shearer, 2000).

Factorial models have been used to estimate energy and protein requirements in growing fish which can be quantified by adding the amounts of energy and protein retained as growth plus the amount of the same nutrients simultaneously lost from the body. The actual requirement for dietary gross energy and protein has to take the partial efficiency of utilisation of these nutrients for maintenance and growth into account. The importance of the factorial approach is that protein and energy requirements are not expressed as a percentage of the diet but in terms of absolute daily feed intake per unit of weight and weight gain (Lupatsch *et al*., 1998). Factorial models also allow using

commercial feed in experiments and a lower number of experimental units than with dose-response trials. Usually, both the digestible protein (DP) intake and digestible energy (DE) intake are used to estimate maintenance requirements and the efficiency of DP and DE retention. Trials of this kind have been carried out in *S. aurata* (Lupatsch *et al.*, 1998; Lupatsch *et al*., 2003), *D. labrax* (Lupatsch *et al*., 2001; Lupatsch *et al*., 2003; Peres & Oliva-Teles, 2005), *Epinephelus aeneus* (Lupatsch *et al*., 2003; Lupatsch & Kissil, 2005), *Argyrosomus japonicus* (Pirozzi *et al*., 2010), *Sciaenops ocellatus* (McGoogan & Gatlin, 1998), *Oncorhynchus mykiss* (Rodehutscord & Pfeffer, 1999; Glencross *et al*., 2007; Glencross 2009), *Bidyanus bidyanus* (Booth & Allan, 2003), *Gadus morhua* (Hatlen *et al*., 2007), *Lates calcarifer* (Glencross, 2008) and *Salmo salar* (Helland *et al*., 2010).

In fish, patterns of protein deposition with increasing levels of DP intake vary considerably among species, diets, experimental conditions (initial weight and temperature), and responses have been described as linear (Lupatsch *et al*., 1998; Fournier *et al*., 2002; Lupatsch & Kissil, 2005; Peres & Oliva-Teles, 2005) or curvilinear (Bureau *et al*., 2006; Pirozzi *et al*., 2010; Jauralde *et al*., 2013).

The objectives of this study were to determine the protein and energy requirements, the protein and energy retention efficiencies and maintenance requirements of juvenile *Argyrosomus regius*.

## MATERIALS AND METHODS

### Experimental setup

The trial was conducted in eight octagonal concrete tanks (4000 L). Inside a marine water recirculation system (65 $m^3$ of capacity) with a rotary mechanic filter and

a gravity biofilter of around 6 $m^3$ capacity at the aquaculture laboratory of the Animal Science Department at Polytechnic University of Valencia (Valencia, Spain). All tanks were equipped with three cages of 1000 litres or two cages of 1500 litres (**Table 3**), aeration, and water was heated by a heat pump installed in the system. The equipment used to control water parameters: were an oxy-meter (OxyGuard, Handy Polaris V 1.26), a refractometer with a 0 - 100 g $L^{-1}$ range (Zuzi, A67410) and a kit applying the colorimetric method to determine nitrate, ammonia and nitrite concentrations. The kits were obtained from AquaMerck (Merck KGaA, Darmstadt, Germany). During the trial, the water temperature (19 ± 1ºC) and dissolved oxygen (7.36 ± 0.4 mg $L^{-1}$) were measured on a daily basis. Salinity (27 ± 1 g $L^{-1}$), pH (7.3 ± 0.5), $NH_4^+$ (0.0 mg $L^{-1}$), $NO_2^-$ (0.22 ± 0.2 mg $L^{-1}$) and $NO_3^-$ (46.1 ± 3.7 mg $L^{-1}$) were measured three times a week. The photoperiod was natural and all tanks had similar light conditions.

**Fish, experimental design and feeding**

Two trials were conducted at Polytechnic University of Valencia. Fish of the first trial were supplied by IRTA (San Carles de la Rápita, Tarragona, Spain), having an initial body weight of 53 g. Fish of the second trial having an initial body weight of approximately 188 g were supplied by IFAPA Center "El Toruño" (Santa María Port, Cádiz, Spain). All fish were acclimatized to the experimental conditions along a 30 day period, and were fed a commercial diet (47% crude protein (CP), 20% crude lipid (CL), 5.8% ash and 1.5% crude fibre (CF), Skretting, Burgos Spain). The two trials (A and B) are summarized in **Table 3**.

All fish were weighed approximately every 4 weeks. Prior to weighing, fish were anaesthetised with 30 mg $L^{-1}$ of clove oil (Guinama ®, Valencia, Spain) containing 87% of eugenol. The fish had been in a 24 hour fast before weighing.

**Table 3.** Experimental design.

| | Trial A | Trial B |
|---|---|---|
| Initial weight | 53 g | 188 g |
| Total of fish specimens | 169 | 77 |
| Experimental system | 12 cages of 1000 litres in 4 tanks of 4000 l, recirculating saltwater system | 8 cages of 1500 litres in 4 tanks of 4000 l, recirculating saltwater system |
| Feeding Rate | 0, 0.75, 1.5, 2.5, 3.5 and 4.5% | 0, 0.5, 1.5 and 2.5% |
| Feeding | 1-2 times daily (hand feeding) | 1-2 times daily (hand feeding) |
| Replicates | 2 | 2 |
| Duration | 53 days | 53 days |

At the beginning of both trials 5 fish were sacrificed per trial and frozen to determine their initial corporal composition, and 10 fish per cage were randomly collected and immediately frozen for corporal analysis at the end of the trials.

The diet used in both trials and during the digestibility analysis period was a commercial diet (Skreting, Burgos Spain). Chemical analyses were performed at the Food Laboratory of Polytechnic University of Valencia, and diet composition is described in **Table 4**.

**Table 4.** Diet composition and proximate analysis.

| | Diet |
|---|---|
| Nutrient contents (% dry matter basis) | |
| Dry matter (%) | 93 |
| Crude protein (%) | 46.06 |
| Crude lipid (%) | 19.46 |
| Ash (%) | 6.35 |
| Crude fibre (%) | 1.5 |
| [A]NFE (%) | 26.63 |
| [B]GE (MJ $kg^{-1}$) | 24.26 |
| CP/GE (g $MJ^{-1}$) | 18.98 |
| Digestible nutrient contents (% dry matter basis) | |
| [C]$ADC_{Ms}$ (%) | 64.6 |
| [D]$ADC_P$ (%) | 84.85 |
| [E]$ADC_E$ (%) | 84.94 |
| [F]DP (%) | 39.08 |
| [G]DE (MJ $kg^{-1}$) | 20.60 |

[A]NFE calculated = 100-%CP-%CL-%Ash-%CF. [B]GE = Gross energy: Determined by direct combustion in an adiabatic bomb calorimeter. [C]$ADC_{Ms}$ = Apparent digestibility coefficients for dry matter. [D]$ADC_P$ = Apparent digestibility coefficients crude protein. [E]$ADC_E$ = Apparent digestibility coefficients energy. [F]DP = (Crude Protein feed x Coefficient Digestible Protein)/100. [G]DE = (Energy feed x Coefficient Digestible Energy)/100. The ingredients used in the commercial diet were mainly fish meal (290 g $kg^{-1}$), soybean meal (150 g $kg^{-1}$), corn gluten(111 g $kg^{-1}$), wheat gluten (140 g $kg^{-1}$), pea meal (80 g $kg^{-1}$), wheat (50 g $kg^{-1}$), fish oil (130 g $kg^{-1}$), soybean oil (30 g $kg^{-1}$), Antioxidant BHT, vitamins A (5000 Ul), vitamins D3 (750 Ul), vitamins E (150 mg $kg^{-1}$).

In all treatments (**Table 3**), fish were fed from Monday through Friday twice a day, and just once on Saturday. During feeding, observers checked that all of the feed offered was eaten by the fish, ensuring equal distribution of the pellets among the fish. For the first meal of the day, the entire ration was given, and, if the fish showed a lack

of appetite, feeding was stopped; any remaining ration was given 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 FR was corrected from the theoretical FR to the actual FR.

The apparent digestibility experiment was carried out in the same tanks at the end of the growth experiment. Two parallel trials were made: one with fish weighing 55 g and another one when the fish weight was 120 g. All fish were fed to satiety and faecal collection took place 15 h later (09:00). Extraction was made by stripping (applying pressure on the ventral region from the pelvic fins to the anus). Wet faecal content was collected and dried at 60 ºC for 48 h before analysis (CP, energy and AIA were used to calculate the apparent digestibility coefficient, ADC).

Digestibility coefficients of energy and protein were determined by faecal analysis with the following formula:

ADC (%) = 100 x [1 - (marker in diet/marker in faeces) x (N in faeces/N in diet)]

Where N is the nutrient.

**Chemical analysis**

Composition of diet, fish carcasses and faeces were analysed following AOAC (1990) procedures: dry matter (105 ºC to constant weight), ash (incinerated at 550 ºC to constant weight), CP (N x 6.25) by the Kjeldahl method after acid digestion (Kjeltec 2300 Auto Analyser; Tecator, Höganas, Sweden), CL extracted with diethyl ether (Soxtec 1043 extraction unit; Tecator). Energy of feed and faeces were determined by direct combustion in an adiabatic bomb calorimeter (Parr Model 1108 oxygen combustion bomb; IL, USA). All analyses were performed in triplicate except faecal

analysis, which was performed in duplicate. The acid insoluble ash (AIA) content of feeds and faeces was estimated by the method suggested by Atkinson *et al*., (1984).

**Statistical analysis**

Growth data and feed utilisation were treated using analysis of variance (ANOVA Factorial, initial weight was used as a covariate) (Snedecor & Cochran, 1971), the Newman–Keuls test was used to assess multiple comparison tests, confidence interval was set at 95% (Stat graphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

Quadratic regression analyses were applied, where the thermal-unit growth coefficient (TGC) was a function of feed intake using the expression $Y= a + b(X) + c(X)^2$. Optimum feed intake was obtained by deriving this equation and equalising it to zero.

The equation used to describe the respective responses was: $y= a[1-e^{-b(x-c)}]$, where y is protein retention or energy retention; x is the digestible protein (DP) intake or digestible energy (DE) intake; a = plateau value for the curve; b = constant characterizing the steepness of the curve; c = DP intake or DE intake at y = 0 and by definition represents the intake for maintenance. The DP or DE intake for maximum retention is defined by the point on the abscissa representing 95% of the value of the upper asymptote on the ordinate.

Retention efficiency (Gross and Net efficiency) can be defined as:

Gross Efficiency= y/x

Net Efficiency = y/(x-c),

Where y is the protein or energy retention, and x is the DP intake or DE intake developing

Gross Efficiency = $a[1-e^{-b(x-c)}]/x$

Net Efficiency = $a[1-e^{-b(x-c)}]/(x-c)$

The maximum efficiency point can be calculated in two ways, graphically as the tangent point between the retention curve and a tangent line crossing the point of origin of the coordinates, or algebraically as the maximum point of the efficiency curve.

**RESULTS**

In both trials, after the growth period of 53 days the final body weight and the TGC varied according to the different feeding rates. **Table 5** shows the statistical results of growth and nutritional parameters of the two trials.

All the fish in starvation lost weight, resulting in the lowest body weight in both trials, 38 and 111 g in trial A and B, respectively. Inside non-starvation rates, in trial A, the final weight of fish fed 0.75% was the lowest (57 g), and the meagre fed the highest rate (4.5%) obtained the highest final body weight (81 g), although there were no significant differences from 1.5 to 4.5% rates. Similarly, in trial B, the meagre fed 1.5 and 2.5% were significantly heavier (295 g and 339 g, respectively) than those fed at 0.5% (248 g). As **Figure 8** shows, similar results were observed with regard to the TGC values; the fish starved presented TGC negative (-0.93 in trial A, and -0.98 in trial B). In trial A, fish fed 0.75% obtained the lowest TGC ($0.27 \times 10^{-3}$), and there were also no significant differences observed among the fish fed at rates of 2.5, 3.5 and 4.5% (TGC of $1.25 \times 10^{-3}$, $1.69 \times 10^{-3}$ and $1.4 \times 10^{-3}$, respectively). Concerning TGC in trial B, there were also significant differences among feeding rates and the TGC values increased significantly as the feeding rates increased.

In the two trials, the feed intake (FI) was significantly different and this FI increased as the designed feeding rate increased, as shown in **Table 5**.

**Table 5.** Growth and parameters of the two experiments of *Argyrosomus regius* fed at the different feeding rates. Corporal analysis of *A. regius* fed at different feeding rates at the end of the trial.

| | Trial A | | | | | | | | Trial B | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Feeding Rate (%) | | | | | | | | Feeding Rate (%) | | | | | |
| | Initial | 0 | 0.75 | 1.5 | 2.5 | 3.5 | 4.5 | SEM | Initial | 0 | 0.5 | 1.5 | 2.5 | SEM |
| Parameter | | | | | | | | | | | | | | |
| Initial weight (g) | | 55 | 52 | 52 | 53 | 54 | 54 | 2 | | $140^{a}$ | $209^{b}$ | $209^{b}$ | $197^{b}$ | 9 |
| Final weight (g) | | $38^{a}$ | $57^{b}$ | $70^{bc}$ | $77^{bc}$ | $88^{c}$ | $81^{c}$ | 4 | | $111^{a}$ | $248^{b}$ | $295^{c}$ | $339^{c}$ | 7.92 |
| [A]TGC x $10^{-3}$ | | $-0.93^{a}$ | $0.27^{b}$ | $0.91^{c}$ | $1.25^{c}$ | $1.69^{c}$ | $1.4^{c}$ | 0.15 | | $-0.98^{a}$ | $0.95^{b}$ | $1.88^{c}$ | $2.74^{d}$ | 0.15 |
| [B]FI (% day$^{-1}$) | | $0^{a}$ | $0.50^{b}$ | $1.05^{c}$ | $1.72^{d}$ | $2.22^{e}$ | $2.75^{f}$ | 0.10 | | $0^{a}$ | $0.38^{b}$ | $1.03^{c}$ | $1.88^{d}$ | 0.06 |
| [C]DP intake (g DP 100 g fish$^{-1}$day$^{-1}$) | | $0^{a}$ | $0.19^{b}$ | $0.41^{c}$ | $0.67^{d}$ | $0.86^{e}$ | $1.07^{f}$ | 0.04 | | $0^{a}$ | $0.15^{b}$ | $0.40^{c}$ | $0.73^{d}$ | 0.02 |
| [D]Protein retention (g 100 g fish$^{-1}$day$^{-1}$) | | $-0.07^{a}$ | $0.074^{b}$ | $0.13^{c}$ | $0.17^{c}$ | $0.18^{c}$ | $0.17^{c}$ | 0.01 | | $-0.07^{a}$ | $0.09^{b}$ | $0.15^{b}$ | $0.18^{b}$ | 0.02 |
| [E]DE intake (kJ DE 100 g fish$^{-1}$ days $^{-1}$) | | $0^{a}$ | $10.33^{b}$ | $21.71^{c}$ | $35.85^{d}$ | $45.79^{e}$ | $56.82^{f}$ | 2.13 | | $0^{a}$ | $7.74^{b}$ | $21.06^{c}$ | $38.72^{d}$ | 1.27 |
| [F]Energy retention (kJ 100 g fish$^{-1}$ days $^{-1}$) | | $-1.98^{a}$ | $3.55^{b}$ | $6.31^{c}$ | $7.82^{cd}$ | $9.22^{d}$ | $8.40^{cd}$ | 0.47 | | $-2.75^{a}$ | $4.02^{b}$ | $8.02^{b}$ | $9.96^{b}$ | 0.92 |
| Carcass composition | | | | | | | | | | | | | | |
| Moisture (%) | 80.06 | $82.83^{a}$ | $77.50^{b}$ | $75.52^{c}$ | $75.23^{c}$ | $74.06^{c}$ | $74.35^{c}$ | 0.40 | 79.35 | $83.54^{a}$ | $76.10^{b}$ | $74.29^{b}$ | $71.59^{b}$ | 1.09 |
| Protein (%) | 11.91 | $11.76^{a}$ | $14.82^{b}$ | $15.77^{c}$ | $16.02^{d}$ | $16.30^{e}$ | $16.10^{de}$ | 0.05 | 13.24 | $11.76^{a}$ | $15.57^{b}$ | $16.24^{b}$ | $16.36^{b}$ | 0.57 |
| Lipid (%) | 1.95 | $1.96^{a}$ | $4.21^{b}$ | $5.46^{c}$ | $5.80^{c}$ | $6.60^{c}$ | $6.24^{c}$ | 0.34 | 1.96 | $0.76^{a}$ | $3.89^{b}$ | $6.42^{b}$ | $7.69^{b}$ | 0.76 |
| Ash (%) | 4.34 | $4.63^{a}$ | $3.38^{b}$ | $3.29^{b}$ | $3.09^{b}$ | $3.33^{b}$ | $3.29^{b}$ | 0.22 | 4.68 | $4.39^{a}$ | $4.03^{b}$ | $3.13^{b}$ | $3.88^{b}$ | 0.22 |
| [G]GE (MJ Kg$^{-1}$) | 3.62 | $3.59^{a}$ | $5.22^{b}$ | $5.94^{c}$ | $6.13^{c}$ | $6.52^{c}$ | $6.33^{c}$ | 0.14 | 3.94 | $3.11^{a}$ | $5.27^{b}$ | $6.43^{b}$ | $6.97^{b}$ | 0.40 |

Means of duplicate groups. Data in the same row not sharing a common superscript letter are significantly different ($P<0.05$). SEM: pooled standard error of the mean. Initial weight was considered as covariable to final weight, TGC, DP intake, Protein retention, DE intake and Energy retention. [A]Thermal Growth Coefficient TGC= 1000 x [Final weight (g) $^{1/3}$− Initial weight (g)$^{1/3}$] /(T°− minimum T° to feed x days); Minimum T° to feed = 12 ºC. [B]Feed intake (% day$^{-1}$) FI = 100 x feed consumption (g)/ average biomass (g) x days. [C]Digestible Protein intake = Protein intake / [days x average biomass (g)] x 100. [D]Protein retention = Protein retention / [days x average biomass (g)] x 100. [E]Digestible Energy intake = Energy intake / [days x average biomass (g)] x 100. [F]Energy retention = Energy retention / [days x average biomass (g)] x 100. [G]GE: Gross energy: Calculated using: 23.9 kJ g$^{-1}$ proteins. 39.8 kJ g$^{-1}$ lipids and 17.6 kJ g$^{-1}$ carbohydrates.

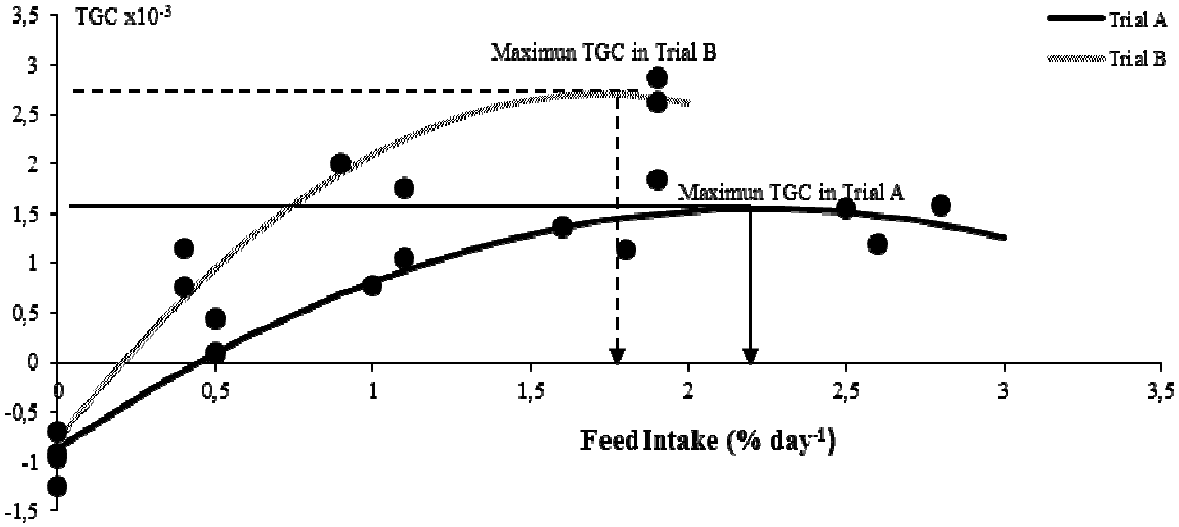


**Figure 8.** Thermal growth coefficient (TGC) response to increasing levels of feed intake (FI) and the response curve of quadratic models considering the trial A and trial B in meagre.

With the aim to determine the FI for maximizing fish growth, a second-order polynomial regression analysis was carried out, and the equation that describes the relationship between TGC and the FI is shown as follows:

Trial A

$$TGC = -0.487641 \times 10^{-3} (FI)^2 + 2.17334 \times 10^{-3} (FI) - 0.87187 \times 10^{-3}$$

$R^2 = 95\%$ (**Equation 1**)

Trial B

$$TGC = -1.17882 \times 10^{-3} (FI)^2 + 4.05533 \times 10^{-3} (FI) -0.78587 \times 10^{-3}$$

$R^2 = 94\%$ (**Equation 2**)

Optimum daily FI for maximum TGC (2.2% $day^{-1}$ in trial A, 1.73% $day^{-1}$ in trial B) was obtained by deriving these equations and equalising them to zero.

The DP intake and DE intake increased significantly as feeding rates increased (**Table 5**). The highest value in trial A was 4.5% (1.07 g DP 100 g fish$^{-1}$ day$^{-1}$ and 56.8 KJ DE 100 g fish$^{-1}$ day$^{-1}$, respectively), and in trial B the rate was 2.5% (0.73 g DP 100 g fish$^{-1}$ day$^{-1}$ and 38.72 KJ DE 100 g fish$^{-1}$ day$^{-1}$, respectively).

In trial A, protein retention (**Table 5**) was significantly higher at the feeding rates of 1.5, 2.5, 3.5 and 4.5%, than in the fish feed at 0.75% (0.074 g Prot 100 g fish$^{-1}$ day$^{-1}$); the energy retention of the 0.75% (3.55 KJ 100 g fish$^{-1}$ day$^{-1}$) feeding rate was the lowest, and the 3.5% (9.22 KJ 100 g fish$^{-1}$ day$^{-1}$) feeding rate showed higher energy retention than fish fed at the 1.5% (6.31 KJ 100 g fish$^{-1}$ day$^{-1}$) feeding rate. In trial B, no significant differences were observed in protein retention and energy retention. **Table 5** also shows the statistical results of body composition. The percentage of moisture and ash in the starved fish were the highest. In trial A, the moisture, lipid and energy contents were significantly higher at the rates of 1.5, 2.5, 3.5 and 4.5%, than in the fish fed at 0.75% (77.5%, 4.21% and 5.22%, respectively); the percentage of ash did not show significant differences with regard to the feeding rates, while the protein content was significantly higher at the rate of 3.5% (16.3%), and also higher at 4.5% (16.10%) and 2.5% (16.02%) but lower in fish fed at 0.75% (14.82%). Trial B did not show significant differences in the statistical results of body composition.

To determine the daily DP and DE requirement of *A. regius*, it was necessary to combine the data of trial A and B to obtain the response curve of retention (**Figure 9** and **Figure 10**). The DP and DE intake to maximise retention and to determine maintenance was developed according to the asymptotic regression model (**Equations 3** and **4**) relating protein retention with DP intake and Energy retention with DE intake.

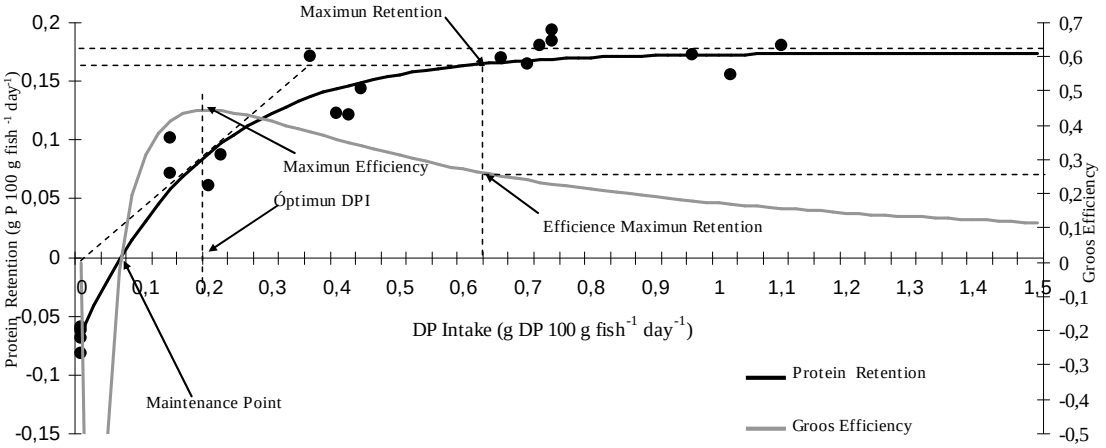


**Figure 9.** Effect of digestible protein intake (g DP 100 g fish$^{-1}$ day$^{-1}$) on protein retention (g Prot 100 g fish$^{-1}$ day$^{-1}$). Maximun Retention: Point of the retention curve having the retention value of 95% of plateu point. Efficience Maximum retention: Point of the gross efficiency curve for the digestible protein intake for maximum retention. Maximum Efficiency: Maximum point of the efficiency response curve. Optimun DPI: Digestible protein intake which gives the maximum gross efficiency coinciding with the tangent line from the origin of coordinates to the retention curve. Maintenance Point: Digestible protein intake point that gives a retention equal to zero.

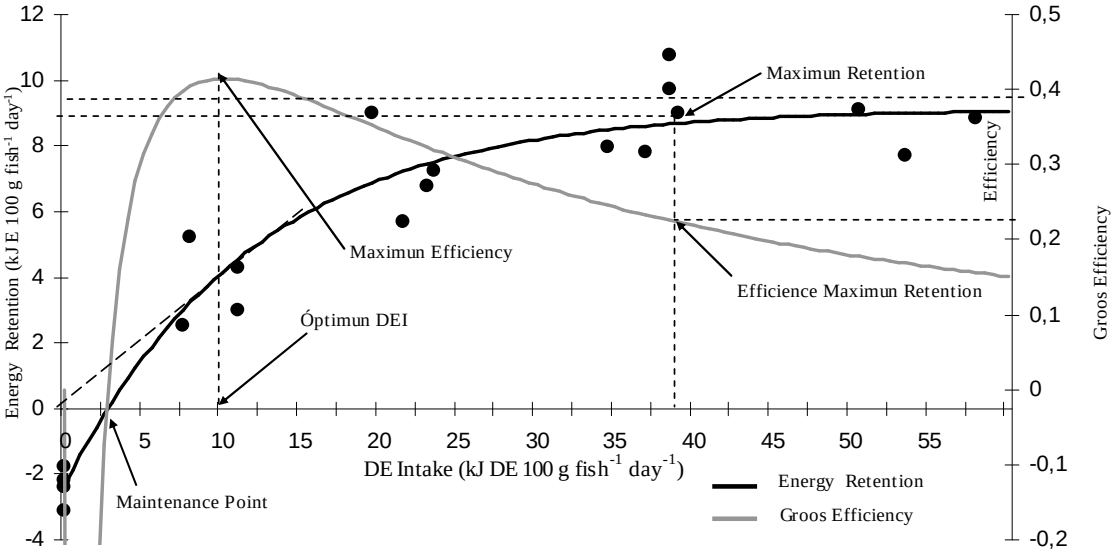


**Figure 10.** Effect of digestible energy intake (kJ DE 100 g fish$^{-1}$ day$^{-1}$) on energy retention (kJ 100 g fish$^{-1}$ day$^{-1}$). Maximun Retention: Point of the retention curve having the retention value of 95% of plateu point. Efficience Maximum Retention: Point of the gross efficiency curve for the digestible energy intake for maximum retention. Maximum Efficiency: Maximum point of the efficiency response curve. Optimun DEI: Digestible energy intake which gives the maximum gross efficiency coinciding with the tangent line from the origin of coordinates to the retention curve. Maintenance Point: Digestible energy intake point that gives a retention equal to zero.

The asymptotic equations were:

$$\text{Protein retention}=0.17[1-\exp^{-5.16(\text{DP intake}-0.06)}]$$

$R^2$= 96% (**Equation 3**)

$$\text{Energy retention}= 9.13[1-\exp^{(-0.083(\text{DE intake}-2.74)}]$$

$R^2$=93% (**Equation 4**)

According to the regression models (**Equation 3** and **4**) and their plots (**Figure 9** and **10**), the points for maintenance, maximum efficiency and maximum retention were determined and are presented in **Tables 6** and **7** for both protein and energy retention. Net efficiency is included. The requirement of DP intake for maintenance was obtained for protein retention 0 (y=0), the DP intake for the maintenance point was 0.0617 g DP 100 g fish$^{-1}$ day$^{-1}$. The maximum retention point was calculated at 95% of the asymptotic value. The DP intake to maximise retention was 0.64 g DP 100 g fish$^{-1}$ day$^{-1}$ and its associated retention at that point was 0.17 g Prot 100 g fish$^{-1}$ day$^{-1}$ obtaining a efficiency of protein retention of 0.25. The point of maximum efficiency was calculated graphically or algebraically as the maximum of the curve and was reached with a level of digestible protein intake of 0.24 g DP 100 g fish$^{-1}$ day$^{-1}$, this intake produced a protein retention of 0.088 g Prot 100 g fish$^{-1}$ days$^{-1}$, with a maximum efficiency of 0.44.

**Table 6**. Protein retention key points

| | DP Intake (g DP 100 g fish$^{-1}$day$^{-1}$) | Protein Retention (g 100 g fish$^{-1}$day$^{-1}$) | Gross Efficiency | Net Efficiency |
|---|---|---|---|---|
| Maintenance | 0.0617 | 0 | 0 | - |
| [A]Max. Retention | 0.64 | 0.17 | 0.25 | 0.29 |
| [B]Max. Efficiency | 0.24 | 0.088 | 0.44 | 0.49 |

[A]Max. Retention: Maximum retention. [B]Max. Efficiency: Maximum efficiency.

**Table 7.** Energy retention key points

| | DE Intake (kJ DE 100 g fish$^{-1}$day$^{-1}$) | Energy Retention (kJ 100 g fish$^{-1}$day$^{-1}$) | Gross Efficiency | Net Efficiency |
|---|---|---|---|---|
| Maintenance | 2.74 | 0 | 0 | - |
| [A]Max. Retention | 38.5 | 8.67 | 0.22 | 0.24 |
| [B]Max. Efficiency | 10 | 4.14 | 0.41 | 0.57 |

[A]Max. Retention: Maximum retention. [B]Max. Efficiency: Maximum efficiency.

The DE intake for the maintenance point was 2.74 kJ DE 100 g fish$^{-1}$ days$^{-1}$. The maximum retention point was obtained with an DE intake of 38.5 kJ DE 100 g fish$^{-1}$ days$^{-1}$ and its corresponding retention at that intake was 8.67 kJ 100 g fish$^{-1}$ days$^{-1}$ obtaining an energy retention efficiency of 0.22. The point of maximum efficiency was obtained with a DE intake of 10 kJ DE 100 g fish$^{-1}$ days$^{-1}$ DE producing an energy retention of 4.14 kJ 100 g fish$^{-1}$ days$^{-1}$ and with a maximum efficiency of 0.41.

## DISCUSSION

One of the goals of aquaculture production is an overall cost-effectiveness with a minimum of waste outputs. To achieve this aim, it is important to optimise the feeding strategies evaluating the effect of diet ration level over the fish growth. In this sense, the curvilinear response allows identifying the optimal level ration, that maximises fish growth and feeding efficiency (Pirozzi *et al*., 2010). Results of this trial showed that the feeding rate was higher in fingerlings than juveniles. These data agreed with expected results as small fish have a higher metabolic activity, i.e. they need a higher feeding rate and higher protein and energy intake; most of this energy (requirement) spent on growth, muscle development and a higher activity level than juvenile meagres. The maintenance requirement depends on fish activity which is inversely proportional to fish size. Thus, the energy and protein intake for maintenance tend to be higher in fingerlings than juveniles. There were significant differences between the calculated and the observed feed intake, which were great in trial A. In the case of fish fed 4.5 % (feed rate calculated), an FI of 2.75% day$^{-1}$ was registered.

The 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 (Dumas *et al*., 2007; Jauralde *et al*., 2013). In meagre, the TGC increased in agreement with the feeding rate. Polynomial regression showed that optimum daily FI for maximum TGC ($1.55 \times 10^{-3}$) was obtained with the highest FI, such as 2.2% $day^{-1}$ FI in trial A, and 1.73% $day^{-1}$ ($2.70 \times 10^{-3}$) in trial B. Under the same experimental conditions (facilities, temperature, photoperiod) fingerlings showed a higher growth than juveniles, which was not observed in the present study. The main reason of this could be attributed to the fact that the fish in the present experiment have different origins and hence came from different batches; fish in trial B being of a better batch and more adapted to experimental conditions than fish in trial A. Fish batch quality should be taken into account to improve fish growth. This opens the door to extensive aquaculture research, namely genetic improvement, which together with feed optimisation could lead to high growth and high feed efficiency (Knibb, 2000).

In mulloway (*Argyrosomus japonicus*), Pirozzi *et al*. (2010) found that that in fish of 40 g, the effect of the ration on weight, protein and energy retention varied significantly depending on the temperature. Likewise, in fish of 127 g, the ration level, but not the temperature, affected the weight, protein, and energy retention. The TGC was recalculated using the weight data presented herein. The small mulloway presented a TGC of $1.79 \times 10^{-3}$ (20 ºC); these results were higher than the TGC obtained in the present experiments for meagre fingerlings and the TGC in the large mulloway was $1.19 \times 10^{-3}$ (20 ºC), lower than the TGC obtained in meagre juveniles.

The graded levels of DP and DE intake also produce increasing graded retentions, as expected and in agreement with Watanabe *et al*. (2000a) and Pirozzi *et al*.

(2010), and in case of fish under starvation conditions these values were negative. Protein retention was high at all the feeding rates, but it decreased at a rate below 1.5%, and the FI for this feeding rate was 1.05% $day^{-1}$. Energy retention increased, only in Trial A, when fish growth also increased.

Protein is the basic component of all animal tissues and constitutes about 65-75 % of the dry matter in fish tissue, and dietary protein provides essential and non-essential amino acids to synthesise body protein and energy for maintenance (Arshad *et al*., 2010). In general, fish require a higher level of dietary protein than terrestrial farmed vertebrates (Kaushik & Seiliez, 2010) and protein requirements vary between species, with carnivorous fish generally having higher dietary protein requirements than omnivorous and herbivorous species (Gunasekera *et al*., 2000). Chatzifotis *et al*. (2012) proved the best dietary protein level for meagre to be 50% as it is a carnivorous species with a high protein requirement, comparable to the other species commonly cultured in the Mediterranean Sea, such as European sea bass or gilthead sea bream. For these two species, there are dietary protein requirements in a range of 45–55 %. Meagre juveniles seems to have similar lipid requirements as other Mediterranean species, and dietary lipid level excess should be avoided since the increase from 17 % to 21 % resulted in higher fat accumulation and impaired growth performance (Chatzifotis *et al*., 2010; Chatzifotis *et al*., 2012). Also, Martínez-Llorens *et al*. (2011) found that the fish fed a commercial diet with 47 % crude protein and 20 % crude lipid showed the best results in meagre growth. These studies determined the nutritional profile of *A. regius*, based on percentage levels, hence it is necessary to be more specific in their nutritional requirements. The benefits of the factorial approach are that requirements are not expressed as a percentage of the diet but rather in terms of absolute daily feed

requirements per unit of weight and weight gain (Lupatsch *et al*., 1998; Booth *et al*., 2010).

When making a comparison with other species it is necessary to re-evaluate the results shown in **Tables 6** and **7**. In factorial method studies, researchers use the metabolic body weight of each species; in gilthead seabream, Lupatsch *et al*. (1998) determined the protein requirement ($kg^{-0.7}$) and energy requirement ($kg^{-0.8}$). Similar results were reported by Pirozzi *et al*. (2010) for *A. japonicus*. In this study, these values were used as the theoretical metabolic body weight for *A. regius*. In this sense, the protein necessity for maintenance in meagre is 0.71 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ and the energy necessity for maintenance is 15.61 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$, while protein requirements for maximum growth are 3.68 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ and the energy necessity for the maximum growth is 139.94 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$.

The maintenance requirement for DP has been recorded between 0.45 and 0.96 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ (**Table 8**), while the DE maintenance requirements for fish have been shown to range from 32 to 77 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ (**Table 8**) and vary depending on temperature, species, and fish size (Pirozzi *et al*., 2010). In meagre, the protein necessity for maintenance is among this range but the energy necessity for maintenance is below this range. The explanation of this fact is the method used in the present experiment. The results allowed the development of an asymptotic equation, while in the trial described in **Table 8**, responses were linear equations given higher maintenance points. It should be noted the asymptotic equations have greater accuracy and have been applied to describe and predict growth in ectotherms (Rosa *et al*., 1997; Hernandez-Llamas & Ratkowsky, 2004; De Graaf & Prein, 2005). In seabream, Jauralde *et al*. (2013) found that the feeding rates for maintenance were similar when obtained with asymptotic or quadratic curves.

**Table 8.** Maintenance protein and energy requirements estimated for several fish species.

| | Maintenance Protein Requirements | Maintenance Energy Requirements | Temperature | References |
|---|---|---|---|---|
| Meagre (*Argyrosomus regius*) | 0.71 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ | 15.61 kJ DE $kg^{-0.83}$ $fish^{-1}$ $day^{-1}$ | 19 ºC | Present study |
| Gilthead seabream (*Sparus aurata*) | 0.86 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ | 55.8 kJ DE $kg^{-0.83}$ $fish^{-1}$ $day^{-1}$ | 23–24 ºC | Lupatsch *et al*. (1998) |
| European seabass (*Dicentrarchus labrax*) | 0.66 g DP $kg^{-0.69}$ $fish^{-1}$ $day^{-1}$ | 43.6 kJ DE $kg^{-0.79}$ $fish^{-1}$ $day^{-1}$ | 19–26 ºC | Lupatsch *et al.* (2001) |
| European seabass (*Dicentrarchus labrax*) | 0.87 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ | 50.9 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ | 25 ºC | Peres & Oliva-Teles (2005) |
| White grouper (*Epinephelus aeneus*) | | 34.05 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ | 19–27 ºC | Lupatsch *et al*. (2003) |
| Mulloway (*Argyrosomus japonicus*) | 0.47 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ | 44.21–49.59 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ | 20-26 ºC | Pirozzi *et al*. (2010) |
| Red drum (*Sciaenops ocellatus*) | 0.5 - 2.2 g DP kg $fish^{-1}$ $day^{-1}$ | 58 to 97 kJ DE kg $fish^{-1}$ $day^{-1}$ | 25 ºC | MacGoogan & Gatlin III (1998) |
| Yellowtail (*Seriola quinqueradiata*) | 2.7 – 3.1 g DP kg $fish^{-1}$ $day^{-1}$ | 62.7 kJ DE kg $fish^{-1}$ $day^{-1}$ | 22–27 ºC | Watanabe *et al*. (2000b) |

In Mediterranean species such as gilthead sea bream, Lupatsch *et al*. (1998) reported the necessity for protein maintenance at 0.86 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ and the necessity for energy maintenance at 55.8 kJ DE $kg^{-0.83}$ $fish^{-1}$ $day^{-1}$, while the protein necessity for the maximum retention was 5.5 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$ and the energy necessity for the maximum retention was 275 kJ DE $kg^{-0.83}$ $fish^{-1}$ $day^{-1}$. Comparatively, the necessities for gilthead sea bream are higher than those obtained for *A. regius*. Jauralde *et al*. (2013) also established a growth model according to the feed intake rate. These researchers determined the necessity for maintenance at 2.3 g Kg $fish^{-1}$ $day^{-1}$, and the necessity for the maximum growth at 19 g Kg $fish^{-1}$ $day^{-1}$, according to the asymptotic growth model.

In *D. labrax*, the requirement for digestible protein for maintenance was calculated at 0.66 g DP $kg^{0.69}$ $fish^{-1}$ $day^{-1}$ (Lupatsch *et al*., 2001) and 0.87 g DP $kg^{0.7}$ $fish^{-1}$ $day^{-1}$ (Peres & Oliva-Teles, 2005), for digestible energy 43.6 kJ DE $kg^{0.79}$ $fish^{-1}$ $day^{-1}$ (Lupatsch *et al*., 2001) and 50.9 kJ DE $kg^{0.8}$ $fish^{-1}$ $day^{-1}$ (Peres & Oliva-Teles, 2005). Peres & Oliva-Teles (2005) recalculated maximum retention using the data presented in this paper, they obtained the protein requirement for the máximum retention at 3.58 g DP $kg^{0.7}$ $fish^{-1}$ $day^{-1}$ and for energy it was 105.83 kJ DE $kg^{0.8}$ $fish^{-1}$ $day^{-1}$. According to these data the protein necessity for the maintenance of meagre would be between the established range for European sea bass and similar to the protein requirements for maximum retention, but the energy necessities for maximum retention are higher in meagre than in European sea bass.

The nutritional requirements of other species of the Sciaenidae family have have been well studied, in this sense the studies made by Pirozzi *et al.* (2010) in *A. japonicus* are noteworthy. They indicated that the protein necessity was not affected by the temperature and maintenance was at 0.47 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$; similarly, the

necessities for maximum retention were determinated at 2.6 g DP $kg^{-0.7}$ $fish^{-1}$ $day^{-1}$. Energy requirements for maintenance increased according to temperature, and we obtained values of 44.21 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ (20 ºC) and 49.59 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ (26 ºC). On the contrary, the necessities for maximum retention were 120 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ (20 ºC) and 150 kJ DE $kg^{-0.8}$ $fish^{-1}$ $day^{-1}$ (26 ºC). Comparing the results at 20 ºC with those obtained in *A. regius*, at 19 ºC, it can be observed that the necessities in meagre are higher than mulloway except for the energy requirement for maintenance.

In red drum (*Sciaenops ocellatus*), another Scianidae, the protein maintenance requirement in fish weighing between 3.4 and 5.5 g, were estimated at 0.5 and 2.2 g DP kg $fish^{-1}$ $day^{-1}$, and the energy requirements for maintenance ranged from 92 to 97 kJ ED kg $fish^{-1}$ $day^{-1}$. The protein requirements for maximum retention ranged from 20 to 25 g DP kg $fish^{-1}$ $day^{-1}$, whereas energy requirements for maximum retention were 914 to 985 kJ ED kg $fish^{-1}$ $day^{-1}$ (McGoogan & Gatlin III, 1998). In general, these results show that necessities of the red drum are higher than *A. regius*, but these are conditionated by the initial red drum body weight, corresponding to fish with higher requirements.

Besides, in yellowtail (*Seriola quinqueradiata*), a high requirement species due to its fast growth, the DP and DE requirements for maintenance of body weight were 3.1 g DP kg $fish^{-1}$ $day^{-1}$ and 129 kJ ED kg $fish^{-1}$ $day^{-1}$, for fish with the initial body weight of 31 and 94; while the requirements for maximum retention were 22.5 g DP kg $fish^{-1}$ $day^{-1}$ and 862-1147 kJ ED kg $fish^{-1}$ $day^{-1}$ (Watanabe *et al*., 2000b). Protein and energy requirements for maintenance and maximum retention are higher in yellowtail than in meagre.

Retention efficiency has been included in the **Tables 6** and **7** to allow comparisons with other research trials. Protein retention efficiency was determined at

0.52 (Lupatsch *et al*., 2001) and 0.64 (Peres & Oliva-Teles, 2005) in *D. labrax*, 0.54 for *E. aeneus* (Lupatsch & Kissil, 2005), 0.49–0.51 for *L. calcarifer* (Glencross, 2008), 0.58 for *A. japonicus* (Pirozzi *et al*., 2010). Many factors affect the efficiency by which dietary protein is utilised for maintenance and growth, including the quantity and quality of the dietary protein (i.e. amino acid profile), protein digestibility, body weight and age of fish, feed intake and numerous environmental factors (Arshad *et al*., 2010). Differences in protein retention efficiencies could be caused by the amino acid dietary profile (Sandberg *et al*., 2005; Sánchez-Lozano *et al*., 2009; 2011; Pirozzi *et al*., 2010). Besides, Kaushik & Seiliez (2010) affirmed that the lack of control of the amino acid catabolism is affected by dietary protein levels, which is indeed considered to be one major reason for the high protein requirements of fish. The high obligatory nitrogen losses incurred in the conversion of nitrogen from indispensable amino acids to dispensable amino acids in the liver and the slow rate of catabolism of indispensable amino acids may be the main explanation for this.

The energy retention efficiency results of the present study agree with Bureau *et al*. (2006) reported for fish species within the range of 0.4–0.7. These results were also similar to those reported for *D. labrax* (Lupatsch *et al*., 2001), *O. mykiss* (Azevedo *et al*., 1998) and *A. japonicus* (Pirozzi *et al*., 2010).

According to Hillestad & Johnsen (1994), McGoogan & Gatlin III (1998) and Lee *et al*. (2002), the inclusion of inadequate quantities of protein and energy can cause a reduction in growth and an excessive quantity of energy can also reduce feed consumption, which would also lead to growth reduction. Besides, protein is more expensive than lipid and carbohydrate and fish use it for tissue synthesis and growth. Also, a decrease in DP/DE ratios has indeed shown to be extremely efficient in improving protein utilisation and decreasing nitrogen loses in most farmed fish (Lee *et*

*al*., 2002). Kaushik & Seiliez (2010) also indicated an optimisation of the ratio between digestible protein and digestible energy through dietary digestible protein level reduction with or without a concomitant increase in the dietary non-protein digestible energy supply.

Concerning body composition, a loss of energy and protein as well as an increase in ash content were reported only in fish in starvation, as a natural consequence of muscle decreases. The body composition at the remaining feeding rates was similar, and only fish fed 0.75% in Trial A obtained the lowest value. It seems that 0.75% was too low for feeding fingerlings, as mentioned before a higher feeding rate is required. At the same time, in Trial A, the crude protein was higher in fish fed 3.5%, even higher than those fish fed 4.5%. Although, the crude protein content of fish is kept relatively constant along life stages and is only slightly affected by dietary factors (provided the dietary essential amino acid is adequate); lipid content of fish is variable depending on energy intake and growth (Shearer, 1994; Bureau *et al*., 2006). In general, the CP content was 16% and 7% for lipids. These results demonstrate the excellent meat quality of the meagre; its low fat content being its main characteristic, representing an important parameter of quality for the consumer (Poli *et al*., 2003).

## CONCLUSION

The optimum TGC was obtained with the highest feed intake, i.e. 2.2% in trial A and 1.73% in trial B. The DP intake for maintenance and maximum growth was recorded at 0.0617 g and 0.64 g DP 100 g fish$^{-1}$ day$^{-1}$, respectively. The optimum intake of digestible protein was 0.24 g DP 100 g fish$^{-1}$ day$^{-1}$ for maximum protein efficiency retention. The DE intake requirements for maintenance and growth maximisation were 2.74 kJ 100 g fish$^{-1}$ day$^{-1}$ and 38.5 kJ 100 g fish$^{-1}$ day$^{-1}$, respectively, and the optimum

point for maximum energy efficiency was 10 kJ 100 g $fish^{-1}$ $days^{-1}$. The retention efficiency of protein and energy in *Argyrosomus regius* tends to be within the range reported for other fish species.

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# CAPÍTULO 5

# OPTIMUM PROTEIN LEVEL

## INTRODUCTION

The manipulation of diet formulations with respect to ingredient costs, nutrient profile and digestibility, as well as the adaptation of feeding regimes designed for site-specific farming conditions can result in significant reductions in pollution loading and cost (Turano *et al.*, 2002).

*Argyrosomus regius* is a species with higher growth rates than most common Mediterranean-cultured species, such as gilthead sea bream and European sea bass (Estevez *et al.*, 2010). Diets formulated for sea bass or gilthead sea bream are currently used for meagre. The best growth performance in meagre has been observed at a dietary lipid level of 17% (Chatzifotis *et al.*, 2010). However, the knowledge about nutritional requirements and the most appropriate ingredients and feed formulations for this species is scarce.

Protein is the basic component of all animal tissues and constitutes about 65–75% of the dry matter in fish tissues and dietary protein provides essential and nonessential amino acids to synthesize body protein and energy for maintenance (Arshad *et al.*, 2010). An increase in digestible protein in diets can lead to improved fish production, especially for carnivorous fish (Lee *et al.*, 2002; Chong *et al.*, 2004; El - Sayed & Kawanna, 2008; Zhang *et al.*, 2010). However, excess protein supplied in feeds will be metabolized as an energy source and will increase production of nitrogenous waste material that is excreted in water, which may be detrimental to fish growth and the culture environment (Tibbetts *et al.*, 2000; Zhang *et al.*, 2010). It is then essential to determine the optimum digestible protein level in diets for fish growth as this is the main factor in determining feed cost (Lee *et al.*, 2000).

Protein amino acid composition and digestibility govern the quantity and rate of absorption of each amino acid, and hence, protein utilization in the body. Therefore, in

attempting to estimate protein quality and one of the most important factors to evaluate is digestibility (Bassompierre *et al*., 1997). The digestion efficiency of digestion can be evaluated by measuring either bioavailability (the fraction of ingested nutrient available for utilization in normal physiological functions and for storage) or bioaccessibility (the fraction that is released from the food matrix and is available for intestinal absorption). *In vivo* methods provide direct data of bioavailability and have been employed for a large variety of nutrients. *In vitro* methods simulate either the digestion and absorption processes (for bioavailability) or only the digestion process (for bioaccessibility) and the response measured is the concentration of a nutrient in some kind of final extract (Handam *et al*., 2009). The gastrointestinal models (GIM) operates using fish enzyme extracts and can be used to estimate the bioaccessibility of some major nutrients, like protein or carbohydrates, present in the feeds, as well as the resulting bioavailability of amino acids, oligosaccharides or even mineral elements (Morales & Moyano, 2010). *In vitro* digestion is being used to predict the quality of experimental feeds. Many *in vitro* methods have been developed and tested for measuring digestibility of different dietary proteins (Bassompierre *et al*., 1997; Rungruangsak-Torrissen *et al*., 2002). Once their correlation to the *in vivo* response is demonstrated, those assays have a number of advantages since they are rapid, safe, and do not have the ethical restrictions affecting assays carried out with live animals. Thus, *in vitro* assays are now routinely used to assess digestibility of ingredients used in feeds for terrestrial animals (Morales & Moyano, 2010). Methods that simulate the gastrointestinal digestion process under laboratory conditions are known as gastrointestinal models (GIMs).

The main objective of this study was to determine the optimum digestible protein level in diets for juvenile meagre (*Argyrosomus regius*) and the results obtained were also used to evaluate the diets from an economic point of view.

A secondary aim was to test whether the results on the nutritional utilization of protein obtained by *in vivo* assay could be correlated to those obtained by a gastro-intestinal model (GIM) previously used in the assessment of protein bioavailability (Hamdan *et al.*, 2009; Morales & Moyano, 2010; Perera *et al.*, 2010). The presence of such a correlation would then support the use of the GIM as an additional tool within the framework of fish nutrition studies.

## MATERIALS AND METHODS

### Experiment 1.

**Determination of the optimum digestible protein level for juvenile meagre** (*Argyrosomus regius*)

Two different experiments were designed to meet these objectives. The first consisted of a feeding study aimed at assessing the nutritional utilization of protein by the meagre in diets containing different levels of crude protein, while in the second, *in vitro* bioavailability assays were performed on the same diets using enzyme extracts obtained from fish used in the first experiment.

### Production system

The trial was conducted in 12 tanks (1700 l) at the aquaculture laboratory of Animal Science Department at the Polytechnic University of Valencia, (Valencia, Spain). The tanks were set up in a seawater recirculation system (65 $m^3$ capacity) with a rotary mechanic filter and a gravity biofilter of around 6 $m^3$ capacity. All tanks were equipped with aeration and the water was heated by a heat pump installed in the system. The water parameters were controlled by an oxy-meter (OxyGuard, Handy Polaris V

1.26), a refractometer with 0 - 100 g $l^{-1}$ range (Zuzi, A67410) and a kit using the colorimetric method to measure nitrate, ammonia and nitrite concentrations (AquaMerck, Merck KGaA, Darmstadt, Germany). The water temperature (24±1ºC) and dissolved oxygen (8±0.4 mg $l^{-1}$) were measured daily throughout the trial and the salinity (34±1 g $l^{-1}$), pH (7.3±0.5), $NH_4^+$ (0.0 mg $l^{-1}$), $NO_2^-$ (0.34±0.2 mg $l^{-1}$) and $NO_3^-$ (46.1±3.7 mg $l^{-1}$) were measured three times a week. The photoperiod was natural and all tanks had similar light conditions.

**Fish and experimental design**

The fish were transported to the facilities at the Polytechnic University of Valencia from a commercial hatchery localized in Castellón, Spain. Meagre juveniles were acclimated to the experimental conditions for 30 days and fed a commercial diet (Skretting, Spain) 47% of crude protein (CP), 20% of crude lipid (CL), 5.8 % ash and 1.5% crude fibre (CF).

A group of 253 fish of 52 g mean weight were distributed in 12 tanks; three replicates per treatment were randomly assigned. The experiment finished when fish tripled their initial weight. All fish were weighed every 20-21 days, approximately. The fish were first anaesthetised with 30 mg $L^{-1}$ of clove oil (Guinama ®, Valencia, Spain) containing 87% of eugenol. The fish were not fed for 24 hours before weighing.

The trial lasted 62 days (from September 2009 to November 2009). 5 fish per tank, at the beginning of the trial, and 10 fish per tank, at the end of the trial, were euthanized by thermo shock in a melting ice bath, and were stored at -30 ºC to determine proximate body composition.

**Diets and feeding**

Four experimental isolipidic diets (17% CL) were designed with four level crude protein (40%, 45%, 50% and 55% CP), using commercial ingredients (**Table 9**). Diets were designed by digestible protein content (35%, 43%, 49% and 53% DP). Additionally, all experimental diets also contained 1% chromic oxide as an inert marker. Diets were prepared by cooking-extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). Processing conditions were as follows: 100 rpm speed screw, 110 ºC temperature, 30-40 atm pressure and 3 and 6 mm diameter pellets, according to fish size.

**Table 9.** Formulation and proximate composition of the experimental diets

| | Diet | | | |
|---|---|---|---|---|
| | 35% | 43% | 49% | 53% |
| Ingredients (g $kg^{-1}$) | | | | |
| Fish meal, herring (5-02-000) | 495.7 | 577 | 658.9 | 740.2 |
| Wheat (4-05-268) | 379.4 | 304.1 | 227.3 | 152 |
| Fish oil (7-08-048) | 106.6 | 100.6 | 95.4 | 89.3 |
| [A]Vitamin–mineral–AA mix | 18.3 | 18.3 | 18.4 | 18.4 |
| Chromic oxide | 10 | 10 | 10 | 10 |
| Nutrient contents (% dry matter basis) | | | | |
| Dry matter (%) | 91.77 | 92.41 | 91.90 | 92.11 |
| Crude protein (%) | 39.74 | 44.69 | 50.43 | 54.50 |
| Crude lipid (%) | 17.75 | 17.48 | 17.78 | 17.73 |
| Ash (%) | 9.03 | 10.38 | 11.17 | 12.62 |
| Crude fibre (%) | 1.7 | 1.4 | 1.1 | 0.8 |
| [B]NFE (%) | 31.78 | 26.05 | 19.52 | 14.35 |
| [C]GE (MJ $kg^{-1}$) | 22.2 | 22.2 | 22.6 | 22.6 |
| CP/GE (g $MJ^{-1}$) | 1.79 | 2.01 | 2.23 | 2.41 |
| Digestible nutrient contents (% dry matter basis) | | | | |
| [D]DP (%) | 34.6 | 43.2 | 48.6 | 53.3 |
| [E]DE ( MJ $kg^{-1}$) | 18.1 | 20.9 | 21.6 | 21.2 |
| DP/DE ratio (g $MJ^{-1}$) | 19.1 | 20.7 | 22.5 | 25.1 |
| Cost of diet (€ $Kg^{-1}$) | 0.96 | 1.06 | 1.16 | 1.25 |

[A]Vitamin mineral and amino acids mix (values are g $kg^{-1}$): Premix: 5; Choline, 2; DL-α-tocopherol, 1; ascorbic acid, 1; (PO4)2Ca3, 1. Premix composition: retinol acetate, 1,000,000 IU $kg^{-1}$; calcipherol, 500 IU $kg^{-1}$; DL-α-tocopherol, 10; menadione sodium bisulphite, 0.8; thiamin hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride,15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides, 12; Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Trcp, 0.7; except 1000 g (Dibaq-Diproteg, S. A., Segovia, Spain). [B]NFE calculated: 100 - %CP - %CL - %Ash - %CF. [C]GE: Gross energy: Calculated using: 23.9 kJ $g^{-1}$ proteins, 39.8 kJ $g^{-1}$ lipids and 17.6 kJ $g^{-1}$ carbohydrates. [D]DP = (Crude Protein feed x Coefficient Digestible Protein)/100. [E]DE = (Energy feed x Coefficient Digestible Energy)/100.

During the trial, the fish were fed the experimental diets by hand twice daily (08:30 and 17:00) to apparent satiation.

**Apparent digestibility coefficient**

Apparent digestibility coefficients for dry matter, protein and energy in experimental diets were determined using four cylindro-conical faeces collection tanks (137 L) with one settling column (Tomás *et al.*, 2005). Five fish per tank of 70–180 g were fed ad libitum once a day in the morning (8:00) everyday and faecal collection took place 24 h later. Wet faecal content was collected, centrifuged and dried at 60 ºC for 24 h prior to analysis. (CP, energy and chromic oxide were used to calculate apparent digestibility coefficients, ADCs).

Digestibility coefficients of energy and protein were determined by faeces analysis with the following formula:

ADC (%) = 100 x [1 - (marker in diet/marker in faeces) x (N in faeces/N in diet)]

Where N is the nutrient.

**Chemical analysis**

Composition of the diets (**Table 9**), fish carcass and faeces were analysed following AOAC (1990) procedures: Dry matter (105 ºC to constant weight), ash (incinerated at 550 ºC to constant weight), CP (N x 6.25) by the Kjeldahl method after acid digestion (Kjeltec 2300 Auto Analyser; Tecator, Höganas, Sweden), CL extracted with diethyl ether (Soxtec 1043 extraction unit; Tecator). All analyses were performed in triplicate except for faecal samples, which were performed in duplicate. The chromic

oxide content of feeds and faeces was analysed by atomic absorption spectrophotometry (Perkin Elmer 3300, Perkin Elmer, Boston, MA, USA).

**Ammonia excretion**

The ammonia excretion was used as an indirect evaluation of the metabolic use of protein in diets containing 35 and 49% DP. Assays were carried out in quadruplicate as detailed in McGoogan & Gatlin III (1999) by placing 4 fish in tanks (137 L) and taking samples of water at different times (0, 1, 3, 5, 7, 9, 11 and 13 hours) after feeding.

**Economic analysis**

The Economic Conversion Ratio [ECR (€ $kg^{-1}$ fish) = feed conversion ratio (kg diet $kg^{-1}$fish)*price of diet (€ $kg^{-1}$ diet)] was used to evaluate the diets from an economic point of view. The price of each feed was determined by multiplying the respective contributions of each feed ingredient by their respective costs per kg and adding the values obtained for all the ingredients in each of the formulated diets. The prices of the raw materials were obtained from FAO GLOBEFISH, Instituto Técnico y de Gestión Ganadero, S.A and "Mercados Agroalimentarios" (Official FOB price as at January 2010): fish meal=1.38 € $kg^{-1}$; defatted soybean meal= 0.321 € $kg^{-1}$; wheat meal= 0.154 € $kg^{-1}$; fish oil= 0.780 € $kg^{-1}$; vit-min mix= 7.50 € $kg^{-1}$.

**In vitro assessment of protein bioavailability in diets for juvenile meagre**

**Preparation of enzyme extracts**

The digestive enzyme extracts used in the assays were obtained from 36 fish sampled at the end of experiment 1 (6 fish from each nutritional treatment). Prior to sampling, fish were starved for 6 h and then sacrificed by immersion in ice-cold water

containing several drops of clove oil. After dissection of the organs (stomach or proximal intestine including pyloric caeca), crude extracts were prepared by manual homogenization in distilled water (1:10, w/v) followed by centrifugation (12,000 rpm, 3 °C, 15 min). Supernatants were stored at -20 °C until used in the assays. Acid protease activity in stomach extracts was measured using the Anson method (1938) using substrate haemoglobin (5 g $l^{-1}$) in 100 mmol $l^{-1}$ glycine-HCl buffer (pH 2.0). Total alkaline protease activity in the extracts from intestine was evaluated using the Kunitz method as modified by Walter (1984) using substrate casein (5 g $l^{-1}$) in 50mmol $l^{-1}$ Tris-HCl buffer (pH 9.0). Results obtained from these assays (6.500 Units/fish and 16.500 Units/fish for acid and alkaline protease activities, respectively) were used as a reference to design the experiments aimed to determine N bioavailability for the different diets used in experiment 1.

**Development of the in vitro assay**

The in vitro assay involved a two-step hydrolysis designed to simulate stomach and intestinal digestion in the GIM. Details of the operation are described in Hamdan *et al.* (2009) and Morales & Moyano (2010). In short, the acid phase of the digestion was simulated in a closed chamber; after inclusion of substrate (the feed was 2% of fish body weight) HCl was added to achieve the desired pH (2.0), this being followed by the addition of crude enzymatic extract from fish stomach. After this time, the reaction mixture was transferred to a semi-permeable membrane reactor formed by an inner reaction chamber separated from an outer chamber by a dialysis membrane of 1,000 Da MWCO (SpectraPor 6, Spectrum Medical Industries, Los Angeles, CA, USA). Crude extract from pyloric caeca providing alkaline proteases was added to the inner chamber and the pH was gradually raised to 8.0 by pumping 100 mmol $l^{-1}$ borate buffer.

The alkaline hydrolysis was maintained for 180 min. Amino acids released during this digestion phase passed across the membrane and were continuously removed from the outer chamber by the continuous flow of buffer controlled by a peristaltic pump (0.5 ml $min^{-1}$). Different sampling points were established during alkaline hydrolysis to measure the release of amino acids. These were determined in dialysates using the *ortho*-phthaldehyde method (Church *et al.*, 1983).

**Experiment 2**.

**Effect of protein/energy ratio on the diet of meagre.**

**Production system**

The trial lasted 45 days and was conducted in three sea cages (8 m of diameter), divided into two sub-cages (**Figure 11**). The test was carried out in the Port of Sagunto (Valencia, Spain) with an average temperature of 16.5 °C.

**Figure 11.** Sea cages wed in the trial.

**Fish and experimental design**

A group of 3000 fish were transported to the sea cages from a commercial hatchery localized in France. In each sub-cages were installed 500 fish with an initial weight of 147 g.

**Diets and feeding**

Three experimental diets were designed with different P/E ratio (47/20, 51/18.5 and 55/17 CP/CL), using commercial ingredients (**Table 10**). Diets were prepared by cooking-extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). Processing conditions were as follows: 100 rpm speed screw, 110 ºC temperature, 40-50 atm pressure and 4 mm diameter pellets, according to fish size.

During the trial, the fish were fed the experimental diets by hand twice daily (09:00 and 17:00), and just once on Saturday (09:00) to restricted feeding rate of 2.5% $day^{-1}$.

**Table 10**. Formulation and proximate composition of the experimental diets P/E ratio

| | Diet | | |
|---|---|---|---|
| | 47/20 | 51/18 | 55/17 |
| Ingredients (g $kg^{-1}$) | | | |
| Fish meal, herring (5-02-000) | 636 | 699 | 761 |
| Wheat (4-05-268) | 156 | 113 | 70 |
| Maltodextrin | 50 | 50 | 50 |
| Fish oil (7-08-048) | 138 | 118 | 99 |
| [A]Vitamin–mineral–AA mix | 20 | 20 | 20 |
| Nutrient contents (% dry matter basis) | | | |
| Dry matter (%) | 91.9 | 91.94 | 91.4 |
| Crude protein (%) | 47.01 | 51.05 | 55.01 |
| Crude lipid (%) | 19.94 | 18.47 | 17.08 |
| [B]NFE (%) | 19.01 | 15.605 | 12.19 |
| Crude fibre (%) | 0.84 | 0.683 | 0.52 |
| Ash (%) | 12.13 | 11.25 | 10.3 |
| [C]GE (MJ $kg^{-1}$) | 18.6 | 18.7 | 18.6 |
| CP/GE (g $MJ^{-1}$) | 25.2 | 27.3 | 29.6 |

[A]Vitamin mineral and amino acids mix (values are g $kg^{-1}$): Premix: 5; Choline, 2; DL-α-tocopherol, 1; ascorbic acid, 1; (PO4)2Ca3, 1.Premix composition: retinol acetate, 1,000,000 IU $kg^{-1}$; calcipherol, 500 IU $kg^{-1}$; DL-α-tocopherol, 10; menadione sodium bisulphite, 0.8; thiamin hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride,15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides, 12; Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Trcp, 0.7; except 1000 g (Dibaq-Diproteg, S. A., Segovia, Spain). [B]NFE calculated: 100 - %CP - %CL - %Ash - %CF. [C]GE: Gross energy: Calculated using: 23.9 kJ $g^{-1}$ proteins, 39.8 kJ $g^{-1}$ lipids and 17.6 kJ $g^{-1}$ carbohydrates.

**Statistical analysis**

Growth data and nutritional parameters were treated using multifactor analysis of variance (ANOVA), introducing the initial live weight as a covariate (Snedecor & Cochran, 1971). The Newman-Keuls test was used to assess specific differences among diets at significance levels of $P<0.05$ significance levels (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA). Assays in experiment 2 were carried out in quintuplicate. Cumulated values of amino acid in the dialysates were plotted against time and fitted to straight lines, the slope of which representing the rate of amino acid released during the time of hydrolysis. Progressive liberations of amino acids were subjected to ANOVA, using time as the covariate.

**RESULTS**

**Experiment 1.**

**Determination of the optimum digestible protein level for juvenile meagre**

The meagre did not reveal problems of adaptation or stress during the experiment. No significant differences in survival, which ranged from 93% to 95%, were observed between dietary treatments at the end of the feeding trial (**Table 11**).

Digestible protein level in diets had an effect on growth with fish fed on a diet including 53% DP reaching a significantly greater final weight (191.8 g) than those fed on 35% DP diet (**Table 11**). Fish fed on diets 43% ($2.47 \times 10^{-3}$), 49% ($2.57 \times 10^{-3}$) and 53% DP (and $2.69 \times 10^{-3}$) showed significantly higher values of TGC than fish fed on the 35% DP diet ( $2.14 \times 10^{-3}$). In contrast, FCR was significantly higher in fish fed on diet 35% and 43% DP than in those fed on the 53% DP diet. No significant differences were observed among dietary treatments in the FI, but there is some differences in digestible protein intake (DPI) being 35% diets that showed the lowest DPI (0.67 g DPI

100 g fish$^{-1}$ day$^{-1}$) and 53% the highest (0.94 g DPI 100 g fish$^{-1}$ day$^{-1}$) (**Table 11**). PER was diminished according digestible protein level was increasing, fish fed 35% diet presented significantly PER (3 g fish g DP intake$^{-1}$) than fish fed diet 53% (1.98 g fish g DP intake$^{-1}$).

**Table 11.** Effect of dietary protein level on growth and feed utilisation of meagre *Argyrosomus regius.*

| | Diet | | | | |
|---|---|---|---|---|---|
| | 35% | 43% | 49% | 53% | SEM |
| Parameter | | | | | |
| Initial weight (g) | 53.3 | 50.7 | 51.5 | 52.8 | 9.26 |
| Survival (%) | 94.2 | 95.5 | 93.4 | 95.5 | 2.76 |
| Final weight (g) | 150.8$^{a}$ | 173.6$^{ab}$ | 179.2$^{ab}$ | 191.8$^{b}$ | 7.86 |
| $^{A}$TGC x 10$^{-3}$ | 2.14$^{a}$ | 2.47$^{b}$ | 2.57$^{b}$ | 2.69$^{b}$ | 0.09 |
| $^{B}$FI (g 100 g$^{-1}$ fish day$^{-1}$) | 1.95 | 1.89 | 1.76 | 1.78 | 0.04 |
| $^{C}$DPI (g DP 100 g$^{-1}$ fish day$^{-1}$) | 0.67$^{a}$ | 0.81$^{b}$ | 0.86$^{b}$ | 0.94$^{c}$ | 0.02 |
| $^{C}$FCR | 1.24$^{a}$ | 1.08$^{a}$ | 0.99$^{ab}$ | 0.97$^{b}$ | 0.05 |
| $^{E}$PER | 2.20 | 2.27 | 2.19 | 2.05 | 0.1 |

Data in the same row with different superscripts differ at P<0.05. Initial weight was considerer as covariable for final weight and TGC. $^{A}$Thermal Growth Coefficient TGC= 1000 x [Final weight (g) $^{1/3}$– Initial weight (g)$^{1/3}$] /(T°− minimum T° to feed x days); Minimum T° to feed = 12 ºC. $^{B}$ Feed intake (% day$^{-1}$) FI= 100 x feed consumption (g)/ average biomass (g) x days. $^{C}$Digestible Protein intake = Protein intake / [days x average biomass (g)] x 100. $^{D}$ Feed conversion ratio FCR= feed offered (g)/ Weight gain (g). $^{E}$Protein efficiency ratio PER= Weight gain (g)/protein offered (g).

Whole body composition and crude and digestible protein efficiency (CPE and DPE, respectively) did no present differences (**Table 12**).

The apparent digestibility coefficients (ADCs) for dry matter, crude protein and energy were significantly affected by crude protein level in diets as is shown in **Table 13**. Meagre fed on the 35% DP diet presented significantly lower values for these parameters (69.7, 86.6 and 81.8%, respectively), while no significant differences were observed among the rest of diets.

**Table 12.** Effect of dietary protein level on biometric parameters and body composition of *Argyrosomus regius* the end of the trial (day 62).

| | | Diet | | | | |
|---|---|---|---|---|---|---|
| | Initial | 35% | 43% | 49% | 53% | SEM |
| Parameter | | | | | | |
| [A]CF | 1.32 | 1.24 | 1.25 | 1.35 | 1.35 | 0.04 |
| [B]VSI (%) | 6.54 | 9.81 | 9.25 | 9.7 | 9.69 | 0.55 |
| [C]HSI (%) | 2.52 | 1.76 | 1.71 | 1.66 | 1.45 | 0.1 |
| [D]MF (%) | 0.00 | 3.03 | 3.43 | 3.35 | 3.37 | 0.14 |
| [E]DR (%) | 70.15 | 68.49 | 69.36 | 67.78 | 70.24 | 0.81 |
| Moisture (g $kg^{-1}$) | 730.3 | 713.0 | 701.4 | 704.6 | 710.2 | 0.55 |
| Crude Protein (g $kg^{-1}$) | 161.0 | 177.5 | 182.7 | 181.6 | 182.0 | 0.21 |
| Crude Lipid (g $kg^{-1}$) | 64.0 | 77.5 | 81.1 | 81.6 | 76.2 | 0.43 |
| Ash (g $kg^{-1}$) | 37.0 | 28.7 | 35.8 | 32.6 | 29.8 | 0.28 |
| [F]CPE (%) | | 41.3 | 43.0 | 41.7 | 39.2 | 1.68 |
| [G]GEE (%) | | 31.1 | 36.7 | 39.7 | 38.7 | 2.22 |
| [H]DPE (%) | | 47.6 | 45.0 | 42.9 | 40.21 | 1.88 |

Data in the same row with different superscripts differ at P<0.05. [A] Condition factor CF = 100 x total weight (g)/total length$^3$ (cm). [B] Viscerosomatic index (%) VSI = 100 x visceral weight (g)/ fish weight (g) [C] Hepatosomatic index (%) HIS = 100 x liver weight (g) / fish weight (g). [D] Mesenteric fat (%) MF = 100 x mesenteric fat weight (g)/fish weight (g). [E] Dressout percentage (%) DR = 100 x [total fish weight-visceral-weight head weight (g)]/fish weight (g). [F] Crude protein efficiency (%) CPE = Fish protein gain (g) x 100/ crude protein intake (g). [G] Gross energy efficiency (%) GEE = Fish energy gain (kJ) x 100/energy intake (kJ). [H] Digestible protein efficiency (%) DPE = Fish protein gain (g) x 100/ digestible protein intake (g)

**Table 13.** Apparent digestibility coefficients (ADCs) of the diets containing different protein levels for juvenile *Argyrosomus regius*.

| | Diet | | | | |
|---|---|---|---|---|---|
| | 35% | 45% | 49% | 53% | SEM |
| [A]$ADC_{Ms}$ | 69.74[a] | 80.89[b] | 81.91[b] | 78.98[b] | 2.03 |
| [B]$ADC_P$ | 86.56[a] | 96.08[b] | 97.13[b] | 96.99[b] | 0.97 |
| [C]$ADC_E$ | 81.88[a] | 94.22[b] | 95.45[b] | 93.92[b] | 0.91 |

Digestibility coefficients were determined by faeces analysis with the following formula: ADC (%) = 100 x [1 - (marker in diet/marker in faeces) x (N in faeces/N in diet)]; where N is the nutrient. [A]$ADC_{Ms}$, Apparent digestibility coefficients for dry matter. [B]$ADC_P$, Apparent digestibility coefficients for protein. [C]$ADC_E$, Apparent digestibility coefficients for energy. Values are presented as mean ± SD ($n$ = 2); means with different superscript letters in the same column differ significantly ($P$ <0.05).

The ammonia excretion (**Figure 12**) during the period analysed was lower in fish fed with 35% DP (393 mg N-$NH_4^+$/kg fish 13 h) when compared to that measured in fish fed on the 49% DP diet (466 mg N-$NH_4^+$/kg fish 13 h). The maximum excretion

in fish fed on 35% DP diet was measured at the 1st hour (73 mg N-$NH_4^+$/kg fish h) while that of fish fed the 49% diet was measured at the 3rd hour (94 mg N-$NH_4^+$/kg fish h).

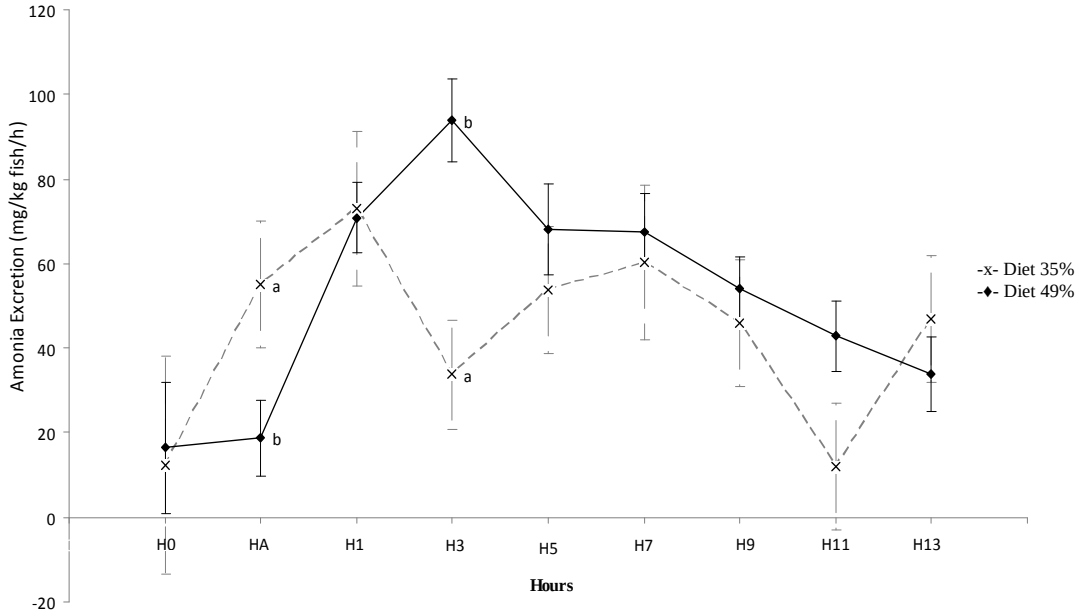


**Figure 12.** Variation of ammonia excretion with time in meagre fed with 40% and 50% diets.(n=4). Newman-keuls test (P<0.05). H (time of feeding); H0 (taking of first sample).

The cost of the diets increased linearly with their protein content (**Figure 17**), but no significant differences were found in the economic conversion ratio (ECR). However, a quadratic regression was obtained between ECR and DPI to minimized ECR was 0.8 g DPI 100 g $fish^{-1}$ $day^{-1}$.

**In vitro assessment of protein bioavailability in diets for juvenile meagre**

Changes in the concentration of soluble protein within the reaction chamber of the GIM during alkaline phase of the hydrolysis are detailed in **Figure 13**. A different pattern of protein solubilisation was observed for the diet including 53% DP which showed a significantly lower initial value and a steady state during the course of the reaction.

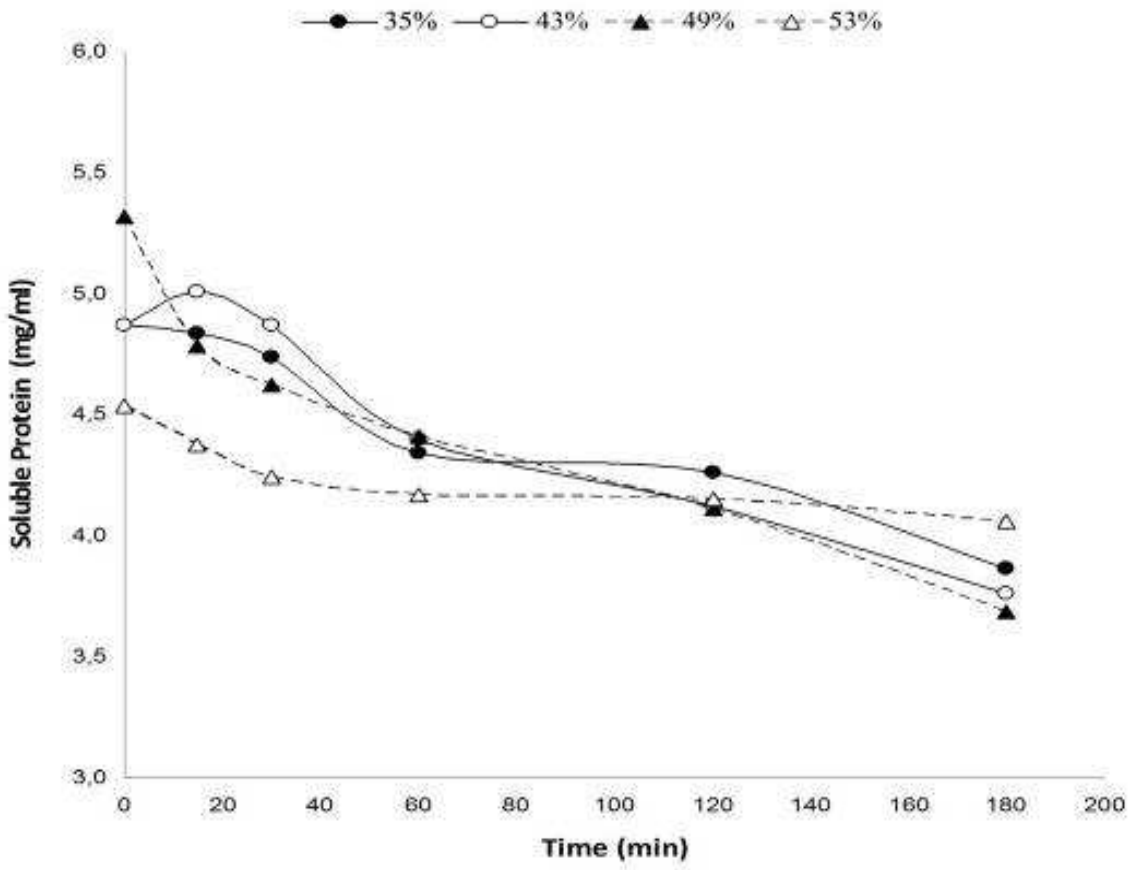


**Figure 13.** Concentration of soluble protein (expressed as mg/ml) within the reaction chamber of the GIM during the alkaline phase of the hydrolysis

The total amount of amino acids released after the acid and alkaline hydrolysis performed by meagre proteases is detailed in **Figure 14**. Significant differences were found between the final amount of amino acids released from diets including 53% DP and those including 43% or 35% DP. The slopes of the lines represented the rate of amino acid release by enzyme hydrolysis and the comparison of these slopes revealed significant differences between the rate of amino acid release from the diet including 49% DP and the group formed by diets 43% and 53% DP. Significant differences were also found between the values obtained with all the diets and the low value obtained.

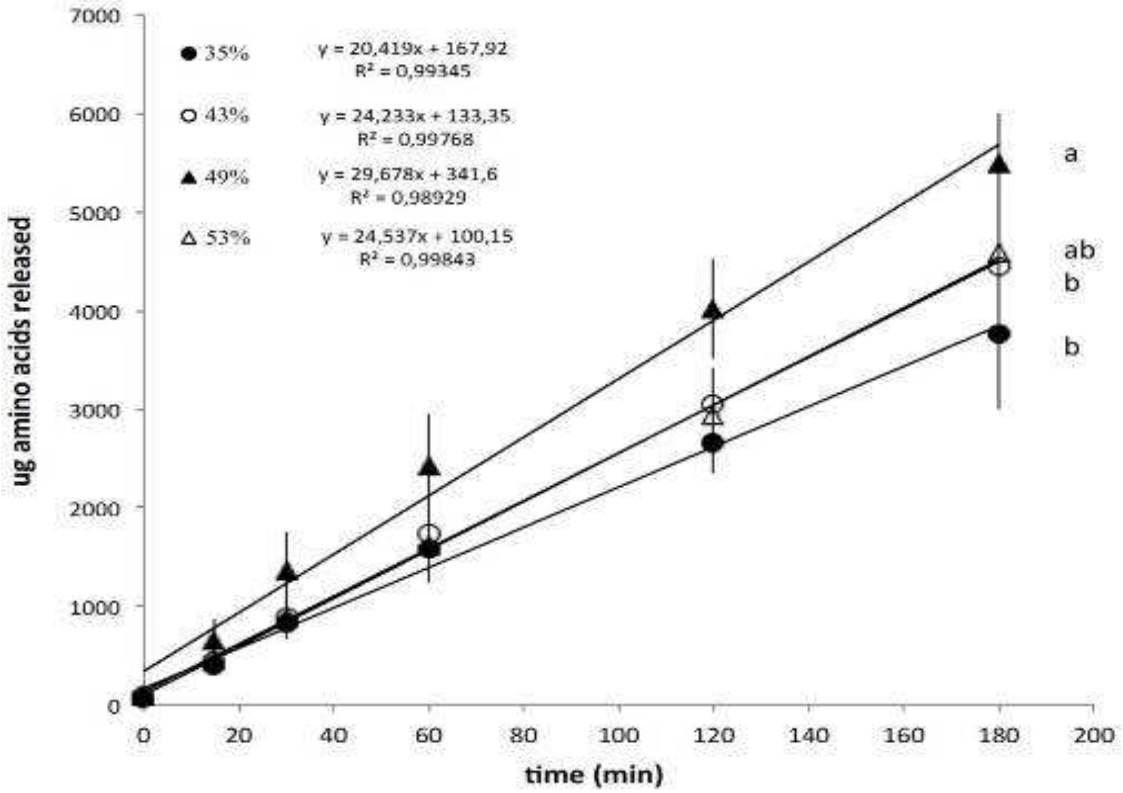


**Figure 14.** Total amount of amino acids released after acid and alkaline hydrolysis performed by meagre proteases (expressed as μg of amino acids).

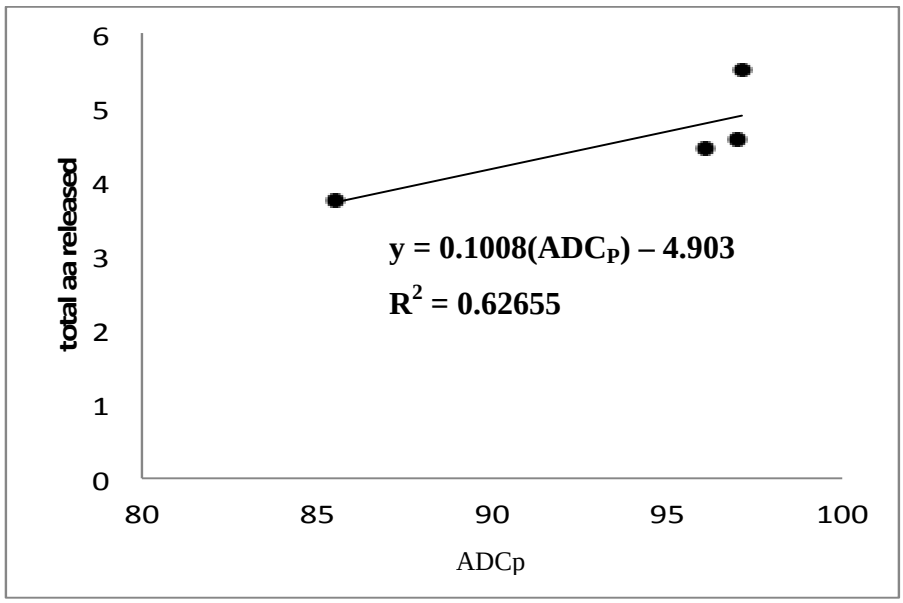


**Figure 15.** Correlation between values of ADC for protein obtained in Experiment 1 for the different diets and total amino acid release measured in the GIM.

The correlation between values of ADC for crude protein obtained for the different diets (**Table 13**) and total amino acid release measured in the GIM is shown in **Figure 15.**

**Experiment 2**

**Effect of protein/energy ratio on the diet of meagre.**

At the end of trial, no significant differences in the analyzed parameters were reported (**Table 14**), but although no significant differences were found, there was a slight improvement in nutritional parameter, possibly due to an increased protein efficiency.

**Table 14.** Effect of the protein/energy ratio on growth of meagre *Argyrosomus regius.*

| | Diet | | |
|---|---|---|---|
| | 47/20 | 51/18 | 55/17 |
| Parameter | | | |
| Initial weight (g) | 147 | 147 | 147 |
| Survival (%) | 90.3 | 90.8 | 90.5 |
| Final weight (g) | 230 | 213 | 211 |
| [A]TGC x $10^{-3}$ | 3.85 | 3.15 | 3.06 |
| [B]SGR % día$^{-1}$ | 1.35 | 1.22 | 1.09 |
| [C]FCR | 1.96 | 2.39 | 2.54 |
| [D]PER | 1.17 | 0.89 | 0.76 |

Data in the same row with different superscripts differ at P<0.05. [A]Thermal Growth Coefficient TGC= 1000 x [Final weight (g) $^{1/3}$− Initial weight (g)$^{1/3}$] /(T°− minimum T° to feed x days); Minimum T° to feed = 12 ºC. [B]Specific Growth Rate SGR= 100x ln (Final Weigth/Initial Weigth)/days. [C] Feed conversion ratio FCR= feed offered (g)/ Weight gain (g). [D]Protein efficiency ratio PER= Weight gain (g)/protein offered (g).

**DISCUSSION**

**Determination of the optimum digestible protein level for juvenile meagre.**

Improved fish growth according to digestible protein dietary is showed in present experiment as in others carnivorous fish species (Lee *et al*., 2002). The results of the present trial showed the optimum digestible protein content in diets for *Argyrosomus regius* was 43% and the optimal digestible protein intake was 0.80 g DP/100 g fish and day, for the specific fish size and digestible energy dietary assayed.

According to these results, there was a tendency to increase growth as dietary protein level increased. Only fish fed 35% DP presented significant differences in

comparison with other treatments. However, increase digestible protein dietary level above 43% may produce protein wasting. Other species of high growth such as *Seriola dumerili* (Tomás *et al*., 2008) also showed the same pattern in similar tests. This entails greater environmental pollution due to increased ammonia excretion and an increase in diets cost. This CP level (43%) coincides with the minimum ECR recommended obtained by quadratic regression.

Meagre presented high growth and therefore it need high protein requirements. Comparing with others fast-growing species, *Seriola quinqueradiata* (Watanabe *et al*., 2000) and *Seriola dumerili* (Tomas *et al*., 2008) presented similar digestible protein intake requirement for maximum growth (0.8 g 100 fish$^{-1}$ day$^{-1}$) that those obtained in present study with meagre. However, digestible protein requirement of *Seriola lalandi* (Booth *et al*., 2010) resulted higher (between 1.99 and 3.55 g fish $^{-1}$, in 100 and 250 g fish weight respectively) that the optimum digestible protein intake obtained in present trial.

The high levels of protein in the experimental diets were based on previous results obtained with *Argyrosomus regius* and other carnivorous Sciaenids, emphasizing the studies of Serrano *et al*. (1992), McGoogan & Gatlin (1999), Thoman *et al*. (1999), Chatzifotis *et al*. (2010) and Pirozzi *et al*. (2010). In the present trial the SGR ranged from 1.75-2.10% day$^{-1}$, this being significantly lower in the diet including 35% DP. These growth rates were much higher than those obtained in other studies with this species. In a test of the effect of lipid levels on the growth of *Argyrosomus regius*, using diets with a high percentage of protein and at temperatures above 22 °C, the highest SGR obtained was 0.46 (Chatzifotis *et al*., 2010). In another trial, with different levels of plant proteins on the on-growing of *Argyrosomus regius* and under a temperature of about 22.9 °C, the SGR ranged from 1.31-1.82% day$^{-1}$, this being significantly higher at

the highest protein levels (Estevez *et al.*, 2010). In *Sciaenops ocellatus* reared at 28 °C the values were similar to those obtained in this experiment (1.86-2.16% day$^{-1}$), and followed the pattern of higher SGR with higher crude protein levels (44%) (Thoman *et al.*, 1999).

The average value of TGC obtained with diets with 43, 49 and 53% DP was 2.6 x10$^{-3}$ (**Figure 16**), this being higher than those obtained by Chatzifotis *et al.* (2012) in this species (1.9 x10$^{-3}$) and also higher in comparison with other marine species such as gilthead sea bream (1.72 x10$^{-3}$) (Mayer *et al.*, 2008); *Sciaenops ocellatus* (2.23 x10$^{-3}$) (Thoman *et al.*, 1999), *Diplodus sargus* (0.89 x10$^{-3}$) (Sá *et al.*, 2008) and *Sparus macrocephalus* (1.65 x10$^{-3}$) (Zhang *et al.*, 2010). This rapid growth approves its consolidation as a viable alternative for their production in marine sea cages.

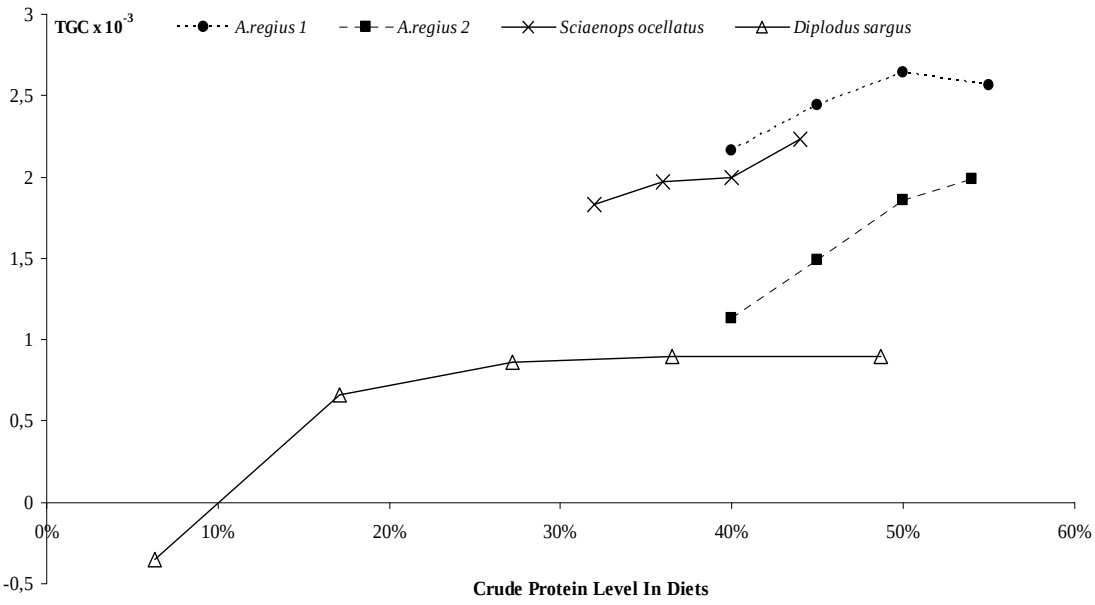


**Figure 16.** TGC of *A. regius* 1 in the present trial, compared, *A. regius* 2 (Chatzifotis *et al.*, 2011), *S. ocellatus* (Thoman *et al.*, 1999) and *D. sargus* (Sá *et al.*, 2008).

Feed intake showed no significant differences, however 35% diet showed the lowest DE, but the reduction of digestible energy did not increase the feed consumption, which was offered of apparent satiation as it has been suggested daily feed intake is

negatively correlated with the dietary DE (Lee *et al*., 2002). This being the case of red drum fed with different levels of protein and energy, which did not significantly affect feed intake (Thoman *et al*., 1999).

The FCR was better in fish fed 49% and 53% diets. According to Chatzifotis *et al*. (2012), the FCR for the two assayed diets containing the highest protein levels were similar to those in 49 and 53% diets, but higher than 35 and 43% diets. Likewise, in this same species, but with different levels of plant proteins, Estevez *et al*. (2010) found that the FCR fluctuated between 1.33 and 1.88. However, the worst value obtained in meagre fed with 35% diet (1.24) was lower than those obtained by the authors mentioned above.

Digestibility data are important to the fish nutritionist because the nutrients contain in poorly digested ingredients are less available to support growth and metabolism than those in better-digested ingredients of similar compositions (Li *et al*., 2004; Zhang *et al*., 2010). The apparent digestibility coefficients (ACDs) of the diets containing different protein levels for juvenile *Argyrosomus regius*, was significantly higher in diets from 43 to 53%, reaching values up to $ACD_P$ (97.6%). Results obtained in the present work may largely could to explain by the use of high levels of fish meal. This protein source, which is generally incorporated at levels between 30% and 60% in feeds for carnivorous marine fish (Wang *et al*., 2006) has a high protein content, excellent amino acid profile, good nutrient digestibility and no antinutritional compounds (Gatlin III *et al*., 2007). These results may be due to a lower fishmeal (496 g $kg^{-1}$) content and higher wheat content (380 g $kg^{-1}$) that reduced digestibility and growth. Estévez *et al*. (2010) showed that plant proteins can be used in diets for meagre in amounts as high as 315 g $kg^{-1}$ of total protein without affecting growth or feed utilization. These protein digestibility values are higher than those obtained in other fish

such as *Sparus macrocephalus* 86 - 93% by Zhang *et al*. (2010); in *Gadus morhua* 86 – 88% (Gridale-Helland *et al*., 2008); in *Seriola dumerili* 78 – 82% (Takakuwa *et al*., 2006) and in *Labeo rohita* 85% (Singh *et al*., 2005). However, in present study, faeces were recollected by settlement in column method and leaching of nutrients from faeces collected could be occur, resulting higher ADCs than in others methods as stripping (Vandenberg & De La Nouè, 2001). These results can be in an overestimation of the apparent digestibility coefficients.

The lower growth joined with the poorest ACD for protein obtained in diet 35% (TGC 2.14 x $10^{-3}$ and 86%) could be due to the high wheat content in 35% diet (**Table 9**) as according to Martínez-Llorens *et al.* (2012) wheat forms complexes with the proteins that produce intestinal viscosity and diminish the digestion of amino acids in carnivorous fish. High carbohydrate and fibre content have also been noted and it is known that high-fibre diets lead to low protein digestibility as the decreased gut transit time causes incomplete digestion and absorption (Jobling, 1981; Martínez-Llorens *et al*., 2012). Furthermore, both gross and digestible energy content of 35% diet shows a reduction as a result of the high fibre content, and similar results have been seen in others experiments (Lee, 2002; McGoogan & Reigh, 1996).

From these results the optimal protein level for growth in the meagre may be said to be close to 43%, somewhat lower than the 49% value established by Chatzifotis *et al*. (2012) in juveniles of 23 g. In carnivorous fish, the increase in dietary protein has been often associated with higher growth rates since this component provides the essential amino acid building blocks for protein synthesis (McGoogan & Gatlin, 1999). According to Serrano *et al*. (1992) the optimum crude protein level to maximize growth in *Sciaenops ocellatus* was 40% but Thoman *et al*. (1999) and Turano *et al*. (2002) recommended 44% of crude protein to optimize growth. In other marine species, the

optimum protein level for carnivorous marine fish, such as black sea bream (*Sparus macrocephalus*) would be 41.4% (Zhang *et al.*, 2010), in Atlantic cod (*Gadus morhua*) 54 % CP (Hatlen *et al.*, 2007); in *Epinephelus coioides* 48% CP (Luo *et al.*, 2004); in Pike Perch (*Sander lucioperca*) 54.9% CP (Schulz *et al.*, 2007); and in Malabar grouper (*Epinephelus malabaricus*) 55% CP (Tuan *et al.*, 2007).

High levels of protein in marine carnivores generate high growth rates, but it is very important to determine the optimal values for fish species, since an excess of dietary amino acids may not be absorbed from the gastrointestinal tract, or are metabolically derived to deamination, this reducing feed efficiency and increasing ammonia production (McGoogan & Gatlin III, 1999). In this trial, ammonia production was only assessed in two diets (35% and 49% DP) and the results showed a lower ammonia excretion for the lower dietary protein level. Based on the results obtained with respect to ammonia excretion, growth parameters, *in vivo* digestibility and retention, the optimum dietary protein level would be between 43 and 49% DP. The use of a higher protein level such as 53% DP produced adverse effects, for example, higher ammonia excretion in comparison to the lower protein levels (43 – 49%) and there was no evidence of any increase on fish growth. Protein is the main source of feed cost and the high production of ammonia not only affects diet cost but also fish health, because ammonia is the most toxic factor in aquaculture (McGoogan & Gatlin III, 1999). According to Webb & Gatlin III (2003) *S. ocellatus* obtained a similar ammonia excretion to meagre with a 43% DP (82.4 mg N- N-$NH_4^+$/kg fish h). Although, the total ammonia excretion in meagre over 13 hours was higher in a diet of 35% DP (393 mg N-$NH_4^+$/kg fish) and in diet 49% DP (466 mg N-$NH_4^+$/kg fish) in relation to sea bass (233 mg N-$NH_4^+$/kg fish day) fed with 44% DP (Tulli *et al.*, 2007).

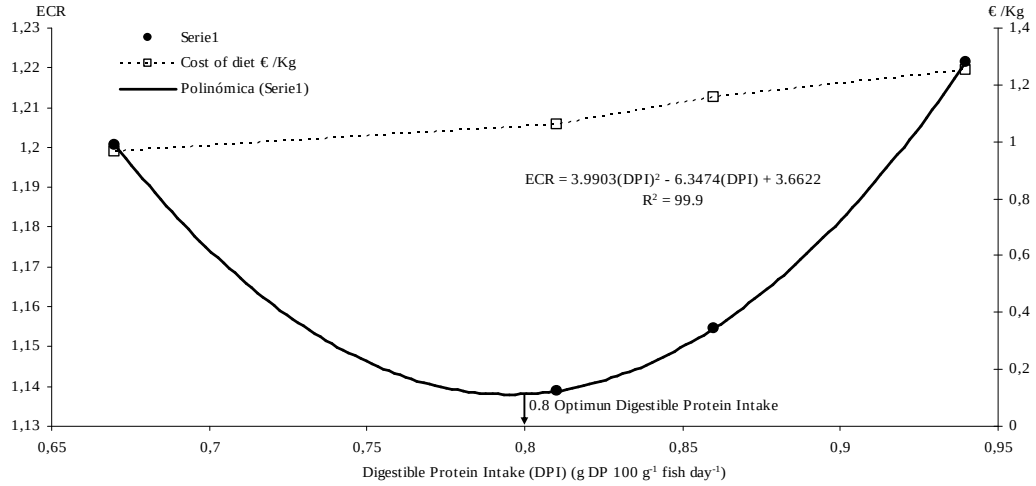


**Figure 17.** Quadratic regression optimum digestible protein level for ECR depending on dietary protein level %. Estadistical differences were not found in ECR. Calculated from price of ingredients: Fish meal=1.38 € $kg^{-1}$; Soybean meal=0.32 € $kg^{-1}$; Wheat= 0.154 € $kg^{-1}$; Fish oil=0.78 € $kg^{-1}$; Vit–Min–AA Mix=7.5 € $kg^{-1}$. ECR (€ $kg^{-1}$ fish) = feed conversion ratio (kg diet $kg^{-1}$fish)*price of diet (€ $kg^{-1}$ diet).

The results show that the protein levels for fish fed diets significantly influence the ECR, with the best values being obtained for diets of 43 and 49%. However, in accordance with the polynomial regression, the optimal level for the ECR was 47% crude protein (**Figure 17**). This finding is of great interest, as there were no significant differences in final weight of meagre fed up to 43% diet. This 47% protein diet is recommended because of the necessity to optimize feed costs during all aspects of culture and on account of its potential for nutrient requirements. According to Thoman *et al.* (1999) dietary protein is the high cost nutrient in aquaculture feed and the incorrect use of the same may cause water quality problems in culture systems. The optimization of dietary protein levels makes it possible to adapt feed costs and nitrogen loads to the production medium. On the basis of these criteria, researchers and producers are interested in optimizing protein content in diets for different species used in aquaculture in order to reduce production costs. With respect to protein retention it is also necessary to consider the digestibility of feed ingredients and the protein content of the diet in addition to the amino acid balances and the relation between dietary energy and protein ratios.

**In vitro assessment of protein bioavailability in diets for juvenile meagre**

*In vitro* digestibility assays are increasingly used in nutritional studies in fish (Haard *et al*., 1996; Bassompierre *et al*., 1997; Moyano & Savoie, 2001; Hamdan *et al*., 2009; Morales & Moyano, 2010; Sáenz *et al*., 2011) since they are rapid, safe, and do not have the ethical restrictions of *in vivo* methods. However, their use is still constrained by the need to correlate the results obtained with those assays and data obtained *in vivo*. In the present work, the bioaccessibility of protein in the experimental diets by digestive enzymes of the meagre and its further release of amino acids was estimated under pH conditions resembling those existing in the fish gut and the values were correlated to those obtained after *in vivo* protein digestibility assays.

As the susceptibility of proteins to enzymatic hydrolysis by digestive proteases depends on a number of factors such as their solubility, structural complexity and amino acid composition (Sáenz *et al*., 2011), protein solubilization was the first parameter assessed in the present work. Results showed that the solubilization of protein in the diet including 53% CP was significantly lower during the first hour than that observed in the rest of diets (**Figure 13**). This should be related to its greater content of fish meal, a raw source composed by muscle proteins (actin, myosin) or subproducts of their degradation, as well as by collagen, all of which showing a great variability in its composition (Morales & Moyano, 2010). When taking into account that the bioaccessibility of a given substrate to the action of digestive proteases is the preliminary step required for hydrolysis (Alarcón *et al*., 2002), this reduced solubility during the initial stages of the assay resulted in a comparatively lower release of amino acids at the end of the experiment. However, significant differences were obtained between the amount of amino acids released from feeds containing 35 and 49% DP, while values measured for the 43% DP diet were intermediate. In this way, in spite of

the very low number of data, a clear correlation between the values of amino acid bioavailability and ADC for protein was obtained (**Figure 15**). Although a number of studies have evaluated different *in vitro* assays aimed at testing protein digestibility or nutritional quality for aquafeed ingredients (Dimes *et al*., 1994; Bassompierre *et al*., 1997; Bassompierre *et al*., 1998; Tonheim *et al*., 2007), very few of these have attempted to correlate their results to those obtained *in vivo*. Rungruangsak-Torrissen *et al*. (2002) found a good correlation between data of protein hydrolysis of different fish meals by fish enzymes with results obtained with in vivo digestibility assays performed in mink. Tibbets *et al*. (2011) also suggested good correlations between values of protein hydrolysis measured with pH-stat in different protein sources and their ADCs for protein values determined in cod. However, these correlations were only found when considering a wide range of different plant and animal proteins with quite different ADC for protein, but not when the diets assayed showed such small variations in protein content or *in vivo* digestibility values as those determined in the present study. The absence of a total coincidence with values of protein digestibility determined *in vivo*, which showed the higher value in the case of the diet with the higher protein content, may be explained taking into account that the *in vitro* assay only simulated the hydrolysis of protein, but not further intestinal absorption of amino acids. This may be a very important factor determining the final balance of digested protein. Furthermore, the time used for the assay (240 min) is far from the total time available for complete digestion in fish and this may also affect the final result. In spite of these limitations, there may be seen to be a reasonable correlation between both types of data and the refining of this technique could well prove to be a very interesting tool within nutritional studies, and particularly in new fish species with a limited availability of specimens, like the meagre.

## CONCLUSION

In summary, in accordance to fish size assayed, the requirement for meagre was 0.8 g DP/100g fish and day in diets containing 20.9 MJ $kg^{-1}$ of DE, obtained with this DPI the best ECR.

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# CAPÍTULO 6

# INCLUSION LEVEL OF DEFATTED SOYBEAN MEAL

## INTRODUCTION

The species belonging to the Scianidae family and selected to the present experiment is *Argyrosomus regius* known as Meagre, is a good candidate for the diversification on commercial aquaculture in Mediterranean and Eastern Atlantic for its good flesh and growth rate (El-Shebly *et al*., 2007; Roo *et al*., 2010; Duncan *et al*., 2012).

The meagre produced in floating cages has shown good management (Jiménez *et al*., 2005) and high growth rate, reaching 1 kg in 10-13 months (Calderón *et al*., 1997; Roo *et al*., 2010). Limited information about the optimal feeding nutritional requirements of meagre is available, it only exists a recent study of dietary lipid requirements (Chatzifotis *et al*., 2010). Likewise the effects of different levels of plant proteins on the ongrowing of meagre have been studied very recently (Estévez *et al*., 2010).

Increase of aquaculture production around the world depends upon the development of sustainable protein sources to replace fish meal in aquafeeds. Fish meal is generally incorporated at levels between 30% and 60% in feeds for carnivorous marine fish (Wang *et al*., 2006a). Aquaculture production demands more and more alternative proteins to substitute fish meal. These meals should not have good amino acids profiles, but also lower prices than fish meal to reduce the production cost. The alternative meals should be highly digestible protein sources of plant and/or animal origin that support similar fish performance and concurrently have no adverse effects upon the environment (Murray *et al*., 2010).

Defatted soybean meal (standard toasted and solvent-extracted, SB) is the most used vegetable meal in aquafeeds, because is a widely available, economical protein source with relatively high digestible protein and energy contents and good amino acid

profile (Wang *et al*., 2006b). The use of defatted soybean protein as a dietary protein has been examined for many commercial important marine fish species such as cobia (*Rachycentron canadum*) (Zhou *et al*., 2005), Mediterranean yellowtail (*Seriola dumerili*) (Tomás *et al*., 2005), European sea bass (*Dicentrarchus labrax*) (Tibaldi *et al*., 2006), Sharpsnout seabream (*Diplodus puntazzo*) (Hernández *et al*., 2007) and gilthead sea bream (*Sparus aurata*) (Martínez-Llorens *et al*., 2009).

Soybean meal has a different acceptance in other carnivorous sciaenid species, both qualitatively and quantitatively, but there is no information available on meagre. In the Sciaenidae family SB meal has been tested in different species, *Nibea miichthioides* has a limited ability to utilize SB as a protein source in practical feeds (Wang *et al*., 2006b) and *Sciaenops ocellatus* gained much weight with diets containing 50% of protein from soybean meal (McGoogan & Gatlin, 1997; Reigh & Ellis, 1992). These results indicate a considerable variation in the ability of different species of the same family to utilize SB protein as an alternative to fish protein in the diet.

The aim of this trial was to determinate the optimum inclusion level of deffated soybean meal (SB) in experimental diets for meagre (*A. regius*), to maximize growth, feed efficiency parameters and amino acid retention and relate it with economic analysis.

## MATERIALS AND METHODS

### Experimental setup

The trial was conducted in 8 octagonal concrete tanks (4000 L) inside a recirculated seawater system at the aquaculture laboratory of Animal Science Department at the Polytechnic University of Valencia, (Valencia, Spain). The tanks

were set up in a marine water recirculation system (65 $m^3$ of capacity) with a rotary mechanic filter and a gravity biofilter of around 6 $m^3$ capacity. All tanks were equipped with aeration and water was heated by a heat pump installed in the system. The equipments used to control water parameters were an oxy-meter (OxyGuard, Handy Polaris V 1.26), a refractometer with 0 - 100 g $L^{-1}$ range (Zuzi, A67410) and a kit using the colorimetric method to determinate nitrate, ammonia and nitrite concentrations. The kits were obtained from AquaMerck (Merck KGaA, Darmstadt, Germany). During the trial, the water temperature (23±1ºC) and dissolved oxygen (7±0.5 mg $L^{-1}$) were measured daily. Salinity (33±1 g $L^{-1}$), pH (7.3±0.5), $NH_4^+$ (0.0 mg $L^{-1}$), $NO_2^-$ (0.34±0.2 mg $L^{-1}$) and $NO_3^-$ (46.1±3.7 mg $L^{-1}$) were measured three times a week. Photoperiod was natural throughout the experimental period, and all tanks had similar lighting conditions.

**Fish and experimental design**

The fish were transported to the experimental facilities of Polytechnic University of Valencia from a commercial hatchery localized in France. The fish were acclimated to the experimental conditions and fed a commercial diet (47% of crude protein (CP), 20% of crude lipid (CL), 5.8% Ash and 1.5% crude fibre (CF), Skretting, Spain.

The trial was divided into two phases: phase (I), the experiment lasted 107 days (from December 2009 to March 2010). A group of 800 fish, 165 g in mean weight, were distributed in 8 tanks; two replicates per treatment were randomly selected. The experiment finished when fish doubled the initial weight. All fish were weighed every 5-6 weeks, approximately. Previously, fish were anaesthetised with 30 mg $L^{-1}$ of clove oil (Guinama®, Valencia, Spain) containing 87% of eugenol. The fish were not fed for 24 hours before weighing.

At the beginning 16 fish per tank and the end 10 fish per tank, were slaughtered by a thermo shock in a melting ice bath, to determine body composition and biometric parameters and were stored at -30 ºC to determine proximate and amino acid body composition.

The phase (II), lasted 26 days (from April 2010 to May 2010). This experiment was developed using the same methodology of the Phase I, with an initial weight of 345 g fish and mean temperature of 21.3 °C.

**Diets and feeding**

Four isoproteic (50% CP) and isolipidic diets (17% CL) were formulated using commercial ingredients (**Table 15**), in which defatted SB was included at 0, 15, 30 and 45% (**Table 16**). Diets were prepared by cooking-extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45, St. Etienne, France). Processing conditions were as follows: 100 rpm speed screw, 110 ºC temperature, 30-40 atm pressure and 3 and 6 mm diameter pellets, according to fish size. Each experimental diet was tested in duplicate tanks. Fish were fed by hand twice a day to apparent satiation from Monday to Saturday. Pellets were distributed slowly, allowing all fish to eat.

**Table 15.** Proximate composition of ingredients used in experimental diets

| | Ingredients | | |
|---|---|---|---|
| | Fish meal, herring | Wheat | Soybean meal |
| International Feed Nº | (5-02-000) | (4-05-268) | (5-04-604) |
| Nutrient contents (% dry matter basis) | | | |
| Dry matter (%) | 91.9 | 87.7 | 89.35 |
| Crude protein (% DM) | 72.4 | 10.6 | 45.28 |
| Crude lipid (% DM) | 9.6 | 1.5 | 1.61 |
| Crude fibre (% DM) | 0.3 | 4.2 | 5.6 |
| Ash (% DM) | 15.8 | 1.6 | 7.7 |
| [A]NFE (% DM) | 1.9 | 82.1 | 39.81 |

[A]NFE was calculated = 100 - %CP - %CL - %Ash - %CF.

**Table 16.** Formula and proximate composition of the experimental diets.

| | Diet | | | |
|---|---|---|---|---|
| | 0 | 15 | 30 | 45 |
| Ingredients (g $kg^{-1}$) | | | | |
| Fish meal, herring (5-02-000) | 660 | 576 | 493 | 407 |
| Soybean meal (5-04-604) | 0 | 150 | 300 | 450 |
| Wheat (4-05-268) | 216 | 144 | 70 | 0 |
| Fish oil (7-08-048) | 104 | 110 | 117 | 123 |
| [A]Vitamin–mineral Mix | 20 | 20 | 20 | 20 |
| Nutrient contents (% dry matter basis) | | | | |
| Dry matter | 91.9 | 91.31 | 90.09 | 90.41 |
| Crude protein (%CP) | 50.43 | 50.13 | 50 | 50.98 |
| Crude lipid (%CL) | 17.78 | 17.94 | 18.09 | 17.42 |
| Ash (%) | 11.17 | 11.22 | 10.95 | 10.55 |
| Crude fibre (%CF) | 1.11 | 1.62 | 2.12 | 2.64 |
| Calculated values | | | | |
| [B]NFE | 19.51 | 19.09 | 18.84 | 18.41 |
| [C]GE (MJ $kg^{-1}$) | 22.57 | 22.48 | 22.34 | 22.36 |
| CP/GE (g $MJ^{-1}$) | 22.35 | 22.3 | 22.38 | 22.8 |
| Essential amino acid content calculated (g $100^{-1}$g) | | | | |
| Arginine | 4.19 | 4.13 | 4.07 | 4.00 |
| Histidine | 1.82 | 1.75 | 1.68 | 1.61 |
| Isoleucine | 2.43 | 2.44 | 2.45 | 2.46 |
| Leucine | 3.90 | 3.92 | 3.94 | 3.95 |
| Lysine | 3.00 | 3.00 | 3.01 | 3.00 |
| Methionine | 1.10 | 1.04 | 0.99 | 0.92 |
| Phenylalanine | 3.38 | 3.29 | 3.21 | 3.11 |
| Threonine | 2.45 | 2.39 | 2.33 | 2.27 |
| Valine | 2.66 | 2.65 | 2.64 | 2.62 |
| Non essential amino acid content calculated (g $100^{-1}$ g) | | | | |
| Alanine | 3.03 | 2.94 | 2.84 | 2.74 |
| Aspartate | 4.31 | 4.59 | 4.87 | 5.14 |
| Cystine | 0.73 | 0.72 | 0.71 | 0.69 |
| Glutamine | 6.66 | 7.09 | 7.51 | 7.93 |
| Glycine | 3.65 | 3.48 | 3.31 | 3.13 |
| Proline | 4.93 | 4.60 | 4.27 | 3.92 |
| Serine | 2.09 | 2.14 | 2.20 | 2.25 |
| Tyrosine | 2.41 | 2.28 | 2.16 | 2.03 |
| EAA/NEAA | 0.90 | 0.88 | 0.87 | 0.86 |

[A]Vitamin mineral and amino acids mix (values are g $kg^{-1}$): Premix: 5; Choline, 2; DL-α-tocopherol, 1; ascorbic acid, 1; (PO4)2Ca3, 1.Premix composition: retinol acetate, 1,000,000 IU $kg^{-1}$; calcipherol, 500 IU $kg^{-1}$; DL-α-tocopherol, 10; menadione sodium bisulphite, 0.8; thiamin hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride,15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides, 12; Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Trcp, 0.7; except 1000 g (Dibaq-Diproteg, S. A., Segovia, Spain). [B]NFE calculated =100-%CP-%CL-%Ash-%CF. [C]GE: Gross energy Calculated using= 23.9 kJ $g^{-1}$ proteins. 39.8 kJ $g^{-1}$ lipids and 17.6 kJ $g^{-1}$ carbohydrates.

**Proximate composition and amino acid analysis**

Chemical analyses of the dietary ingredients were determined prior to diet formulation. Diets and their ingredients as well as the whole fish were analysed according to AOAC (1990) procedures: dry matter (105 ºC to constant weight), ash (incinerated at 550 ºC to constant weight), crude protein (N x 6.25) by the Kjeldahl method after an acid digestion (Kjeltec 2300 Auto Analyser, Tecator Höganas, Sweden), crude lipid extracted with methyl-ether (Soxtec 1043 extraction unit, Tecator) and crude fibre by acid and basic digestion (Fibertec System M., 1020 Hot Estractor, Tecator). All analyses were performed in triplicate.

The amino acid content in diets and whole body of phase I, were determined after acid hydrolysis with HCL 6N at 110 ºC for 23 h. as previously described Bosch *et al*. (2006), through a Waters (Milford, MA, USA) HPLC system consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717, Waters), a fluorescence detector (Mod. 474, Waters) and a temperature control module. Aminobutyric acid was added as internal standard after hydrolysation. The amino acids were derivatised with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and separated with a C-18 reverse-phase column Waters Acc. Tag (150 mm x 3.9 mm). Methionine and Cystine were determined separately as methionine sulphone and cysteic acid respectively after performic acid oxidation followed by acid hydrolysis.

**Economic analysis**

The price of each diet was determined by multiplying the respective contributions of each feed ingredient by their respective costs per kg and summing the values obtained for all the ingredients in each of the formulated diets. The used raw material prices were the average prices in FAO GLOBEFISH (January 2010), Instituto

Técnico y de Gestión Ganadero, S.A and "Mercados Agroalimentarios" (Official FOB prices). The price of each ingredient (January 2011) was: Fish meal=1.38 € $kg^{-1}$; Defatted soybean meal= 0.321 € $kg^{-1}$; Wheat meal= 0.154 € $kg^{-1}$; Fish oil= 0.780 € $kg^{-1}$; Vit-Min Mix= 7.50 € $kg^{-1}$.

The Economic Conversion Ratio (ECR) was used to evaluate the diets from an economic point of view and it was calculated following the expression:

ECR (€ $kg^{-1}$ fish) = feed conversion ratio (kg diet $kg^{-1}$fish) x price of diet (€ $kg^{-1}$diet)]

**Statistical analysis**

Growth data and nutritive parameters were treated using multifactor analysis of variance (ANOVA), introducing the initial live weight as covariate (Snedecor & Cochran, 1971). Newman-Keuls test was used to assess specific differences among diets at a significance levels of $P < 0.05$ significance levels (Statgraphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

Quadratic regression analyses were applied, where specific growth rate (SGR) and feed conversion ratio (FCR) were a function of soybean meal (SB) dietary level using the expression:

$$Y=a + b(SB) + c(SB)^2$$

Optimum soybean meal dietary level was obtained by deriving this equation and equalising to zero. All experiments were carried out according to the rules or protocols of the Animal Welfare Commission at the Polytechnic University of Valencia.

**RESULTS**

The composition of test diets including dry matter, CP, CL, ash and gross energy (GE) was similar (**Table 16**). The essential amino acid (EAA) profiles of the diets were

variable (**Table 16**), Arg, His, Met, Phe and Thr decreased according to SB increased in the diets and the opposite trend was observed in the Iso and Leu content. The Lys content was similar in all diet. In relation to non essential amino acids (NEAA), the dietary content of Asp and Glu were increased according to dietary level of SB. The relation EAA/NEAA also diminished with dietary soybean meal level until 0.86.

No significant difference was observed in final survival (that it was around 84% ± 10.61). The meagre did not present adaptation problems nor exhibit stress behaviour.

At the end of the trial, in the first phase a significant effect of soybean meal inclusion was observed on fish growth (**Table 17**). Meagre fed diets 15 and 30 obtained the highest final weight (380 and 385 g, respectively). The final weight of fish fed diet 45 was also higher (360 g), than fish fed diet 0 (333 g). Likewise, fish fed diet 15 and 30 obtained significantly higher Thermal Growth Coefficient (TGC) (3.10 x $10^{-3}$ and 3.15 x $10^{-3}$, respectively) than fish fed 45 diet (2.87 x $10^{-3}$) and fish fed the 0 diet that obtained the lowest TGC (2.56 x $10^{-3}$).

**Table 17.** Main performances of meagre fed increasing levels of dietary soybean meal

| | Diet in phase I | | | | | Diet in phase II | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | SEM | 0 | 15 | 30 | 45 | SEM |
| Parameter | | | | | | | | | | |
| Inicial weight (g) | 164 | 166 | 166 | 165 | 3.84 | 355 | 334.7 | 340.9 | 353.7 | 6.17 |
| Final weight (g) | 333$^{c}$ | 380$^{a}$ | 385$^{a}$ | 360$^{b}$ | 1.2 | 518.8 | 529.02 | 533.05 | 526.78 | 15.71 |
| $^{A}$TGC x $10^{-3}$ | 2.56$^{c}$ | 3.10$^{a}$ | 3.15$^{a}$ | 2.87$^{b}$ | 0.144 | 3.4 | 4 | 3.9 | 3.5 | 0 |
| $^{B}$FI (% day$^{-1}$) | 0.79 | 0.90 | 0.95 | 0.89 | 0.03 | 1.31 | 1.34 | 1.41 | 1.27 | 0.07 |
| $^{C}$FCR | 1.30 | 1.34 | 1.43 | 1.45 | 0.05 | 0.91$^{b}$ | 0.79$^{a}$ | 0.84$^{ab}$ | 0.85$^{ab}$ | 0.02 |
| $^{D}$PER | 1.53 | 1.49 | 1.40 | 1.36 | 0.05 | 2.17$^{a}$ | 2.52$^{b}$ | 2.39$^{ab}$ | 2.29$^{ab}$ | 0.05 |

Means of duplicate groups. Data on the same row not sharing a common superscript letter are significantly different different ($P < 0.05$). SEM: Pooled standard error of the mean. Initial weight was considered as covariable for final weight and TGC. $^{A}$Thermal Growth Coefficient TGC= 1000 x [Final weight (g) $^{1/3}$− Initial weight (g)$^{1/3}$] /(T°− minimum T° to feed x days); Minimum T° to feed = 12 ºC. $^{B}$ Feed intake (% day$^{-1}$) FI= 100 x feed consumption (g)/ average biomass (g) x days $^{C}$Feed conversion ratio FCR = feed offered (g)/ Weight gain (g). $^{D}$Protein eficiency ratio PER = Weight gain (g)/protein offered (g).

Regarding nutritional parameters, the daily feed intake, the feed conversion rate (FCR) and the protein efficiency (PER) ratio were not different for all the diets (**Table 17**).

The second phase (day 26) showed higher growth than in the first phase, and no significant differences were observed in live weight and CTC among treatments (**Table 17**), but in the FCR and PER were significanttly better for fish fed 15% soybean meal (0.79 and 2.52, respectively).

With the aim to determinate the SB dietary levels that maximizing the fish growth, a second-order polynomial regression analysis was assessed and the equation that describes the relationship between TGC and the dietary SB level is expressed in **Figure 18** based on the above polynomial equation, the point maximum of this quadratic curve is the dietary SB level to maximize the TGC (26.4% SB). Likewise, **Figure 18** shows the second-polynomial regression between FCR and dietary level SB and the SB level that obtain the minimum FCR resulted 27.6% SB.

Biometric parameters and body composition were not affected by experimental diets (**Table 18**). Significant differences were not observed in whole body composition. The energy retention (GEE) resulted similar in all tested diets with values between 24 and 25.7%. Neither, significant differences were observed among diets in the efficiency of protein (CPE), between 27.6 and 30.6%.

Biometric parameters and body composition (**Table 18**), were not affected by the experimental diets, except the hepatosomatic index (IHS) in Diet 45 (1.57%), during the phase II.

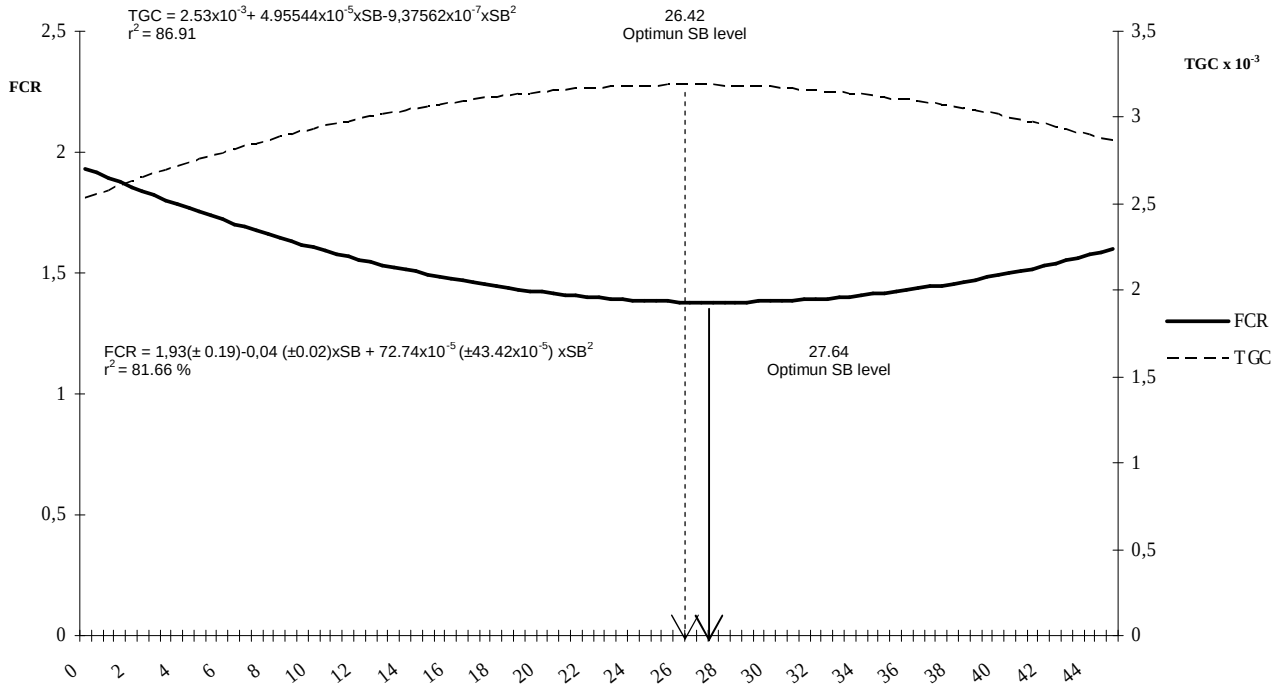


**Figure 18.** Optimum dietary soybean meal level for TGC and FCR depending on dietary soybean meal obtaining by quadratic regression.

**Table 18.** Biometric indices and proximate composition (expressed as percentage of wet weight) of *A. regius* fed increasing levels of soybean meal.

| | Diet of phase I | | | | | Diet of phase II | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | SEM | 0 | 15 | 30 | 45 | SEM |
| Parameter | | | | | | | | | | |
| [A]CF | 1.11 | 1.18 | 1.17 | 1.16 | 0.02 | 1.35 | 1.38 | 1.31 | 1.31 | 0.38 |
| [B]VSI (%) | 6.12 | 5.77 | 5.49 | 6.02 | 0.24 | 8.8 | 8.37 | 8.73 | 8.28 | 0.39 |
| [C]HSI (%) | 1.96 | 1.6 | 1.58 | 1.61 | 0.11 | 2.43[b] | 2.12[b] | 2.47[b] | 1.57[a] | 0.16 |
| [D]MF (%) | 2.43 | 2.53 | 2.33 | 2.48 | 0.16 | 1.43 | 1.64 | 1.45 | 1.4 | 0.16 |
| [E]DP (%) | 70.33 | 70.9 | 70.56 | 69.17 | 0.84 | 89.04 | 87.88 | 89.1 | 87.89 | 1.06 |
| [F]MI (%) | 58.76 | 60.75 | 58.39 | 59.39 | 1.44 | 60.74 | 60.31 | 62.4 | 60.01 | 1.43 |
| Moisture (%) | 74.05 | 72.62 | 71.8 | 71.9 | 0.55 | 70.67 | 71 | 71.59 | 70.83 | 0.47 |
| Crude Protein (% ww) | 17.37 | 17.95 | 18.6 | 17.37 | 0.37 | 17.68 | 18.49 | 17.46 | 18.44 | 0.6 |
| Crude Lipid (% ww) | 6.5 | 6.63 | 6.81 | 7.64 | 0.25 | 7.73 | 7.53 | 6.75 | 7.66 | 0.46 |
| Ash (% ww) | 2.45 | 2.65 | 3.12 | 2.57 | 0.19 | 2.63 | 2.72 | 2.61 | 2.78 | 0.16 |
| [G]CPE (%) | 29.7 | 30.4 | 30.6 | 27.6 | 1.83 | 34.94 | 48. 31 | 39.26 | 42.27 | 3.51 |
| [H]GEE (%) | 24.0 | 24.8 | 25.4 | 25.7 | 1.33 | - | - | - | - | - |

The data are the mean (n=10) ± SEM. Data in the same row with different superscripts differ at $P < 0.05$. [A]Condition factor CF = 100 x total weight (g)/total length$^3$ (cm). [B]Viscerosomatic index (%) VSI = 100 x visceral weight (g)/ Fish weight (g). [C]Hepatosomatic index (%) HIS = 100 x liver weight (g) / Fish weight (g). [D]Mesenteric fat (%) MF = 100 x mesenteric fat weight (g)/fish weight (g). [E]Dressout percentage (%) DP = 100 x [total fish weight (g)-visceral weight (g)-head weight (g)]/ fish weight (g). [F]Meat index (%) MI = 100 x meat weight (g)/fish weight (g). [G]Crude protein efficiency (%) CPE = Fish protein gain (g) x 100/ protein intake (g). [H]Gross energy efficiency (%) GEE = Fish energy gain (kJ) x 100/energy intake (kJ).

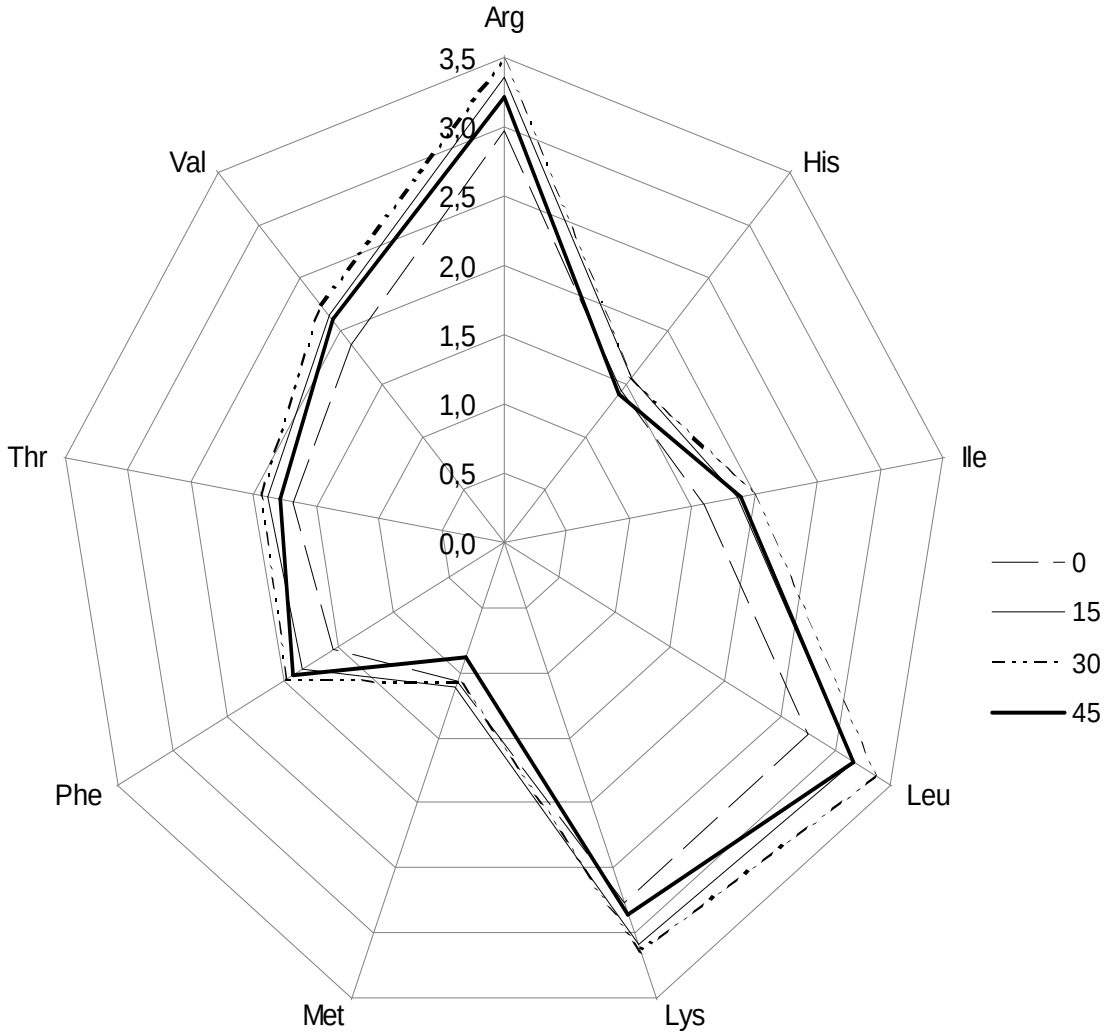


**Figure 19.** Ingestion of essential amino acids (EAA) in each experimental diet expressed as g per 100 $g^{-1}$ of fish and day. Each value is the mean of duplicate groups. Different superscripts indicated differ at $P < 0.05$.

The ingestion of essential amino acids (expressed as g AA x $100g^{-1}$ of fish and day) did not showed significant differences with the diets (**Figure 19**). Overall, the Met intake was the lowest (from 0.8 to 1.24 g AA x $100g^{-1}$ of fish and day) followed the His intake (from 1.9 to 2.1g AA x $100g^{-1}$ of fish and day) and the Lys and Leu intake were higher than the others amino acids intake (upper to 3g AA x $100g^{-1}$ of fish and day in

the four experimental diets). **Figure 20** is showed the retention efficiency (%) of essential amino acids of fish fed with the experimental diets at the end of the experiment. The His was the amino acids with the lowest retention efficiency (25% of average) and the Thr presented the highest retention (around of 35%) following by Arg (34%) and Lys and Met (32%). There were no significant differences of amino acids efficiency retention among diets. Ratio between dietary EAA level of experimental diets and EAA in the carcass was calculated (expressed as $\%EAA_{diet}/\%EAA_{fish}$) and the results are showed in **Figure 22** The His presented the upper value of this ratio (upper to 130%), and Arg, Lys, Met and Thr presented the ratio below to 100%. No differences were observed in ratio $\%EAA_{diet}/\%EAA_{fish}$ in relation to diets, with a exception of Met that presented the lowest ratio in diet 30 and 45 and the highest in the 0 diet.

Regarding to economic analyses of the diets, the cost of diets was reduced with the increase of soybean meal in diets (**Table 19**). Phase I significant differences were showed in the economic conversion ratio (ECR), that was higher in control diet (diet 0) than in the others diets.

**Table 19.** Global results of economic parameters at the end of the experiment

| | Diet | | | | |
|---|---|---|---|---|---|
| | S0 | S15 | S30 | S45 | SEM |
| Parameter | | | | | |
| [A]Cost of diet (€ Kg $^{-1}$) | 1.18 | 1.10 | 1.03 | 0.95 | |
| [B]ECR (€ Kg $^{-1}$) Phase I | 2.31[b] | 1.50[a] | 1.55[a] | 1.47[a] | 0.06 |
| ECR (€ Kg $^{-1}$) Phase II | 1.07 | 0.86 | 0.86 | 0.8 | 0.04 |

Data in the same row with different superscripts differ at $P < 0.05$. [A]Calculated from price of ingredients: Fish meal=1.38 € kg$^{-1}$; Soybean meal=0.32 € kg$^{-1}$; Wheat= 0.154 € kg$^{-1}$; Fish oil=0.78 € kg$^{-1}$; Vit–Min–AA Mix=7.5 € kg$^{-1}$. [B]Economic conversion ratio ECR (€ kg$^{-1}$ fish) = feed conversion ratio (kg diet kg$^{-1}$fish)*price of diet (€ kg$^{-1}$ diet).

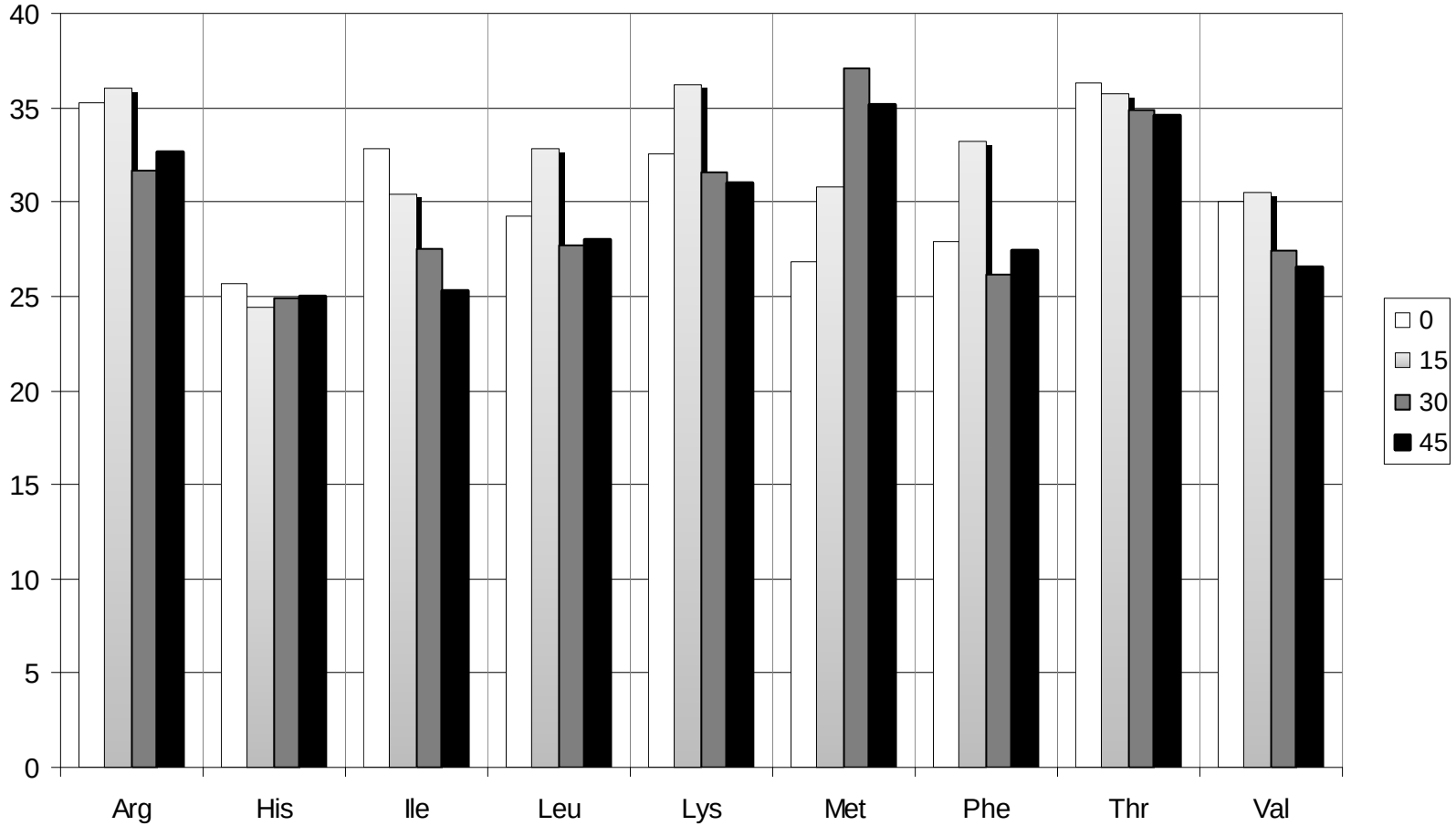


**Figure 20.** Retention efficiency (%) of essential amino acids in *A. regius* fed with the experimental diets at the end of the experiment. Each value is the mean of duplicate groups. Different superscripts indicated differ at $P < 0.05$. Retention of ingested protein (%) = Fish amino acid gain (g)/ ingested amino acids (g) x 100.

## DISCUSSION

The results of the present trial show that the meagre exhibits a high growth, with TGC around 3.00 x10$^{-3}$, greater than other marine species such as gilthead sea bream with TGC average of 1.72 x10$^{-3}$ (Mayer *et al.*, 2008) and others scianids as *Argyrosomus japonicus* (Pirozzi *et al.*, 2009) feed with commercials diets (1.46 x10$^{-3}$). Likewise the TGC in present trial were also higher that TGC recalculated by the growth results obtained by Calderon *et al.* (1997) with *A. regius* feeding with pelletized diets (2.02 x10$^{-3}$), Estevez *et al.* (2010) feeding *A. regius* with experimental diet with extruded commercial diet (1.73 x10$^{-3}$) and by El-Shebly *et al.* (2007) (1.78 x10$^{-3}$) with feed based in tilapia and shrimp.

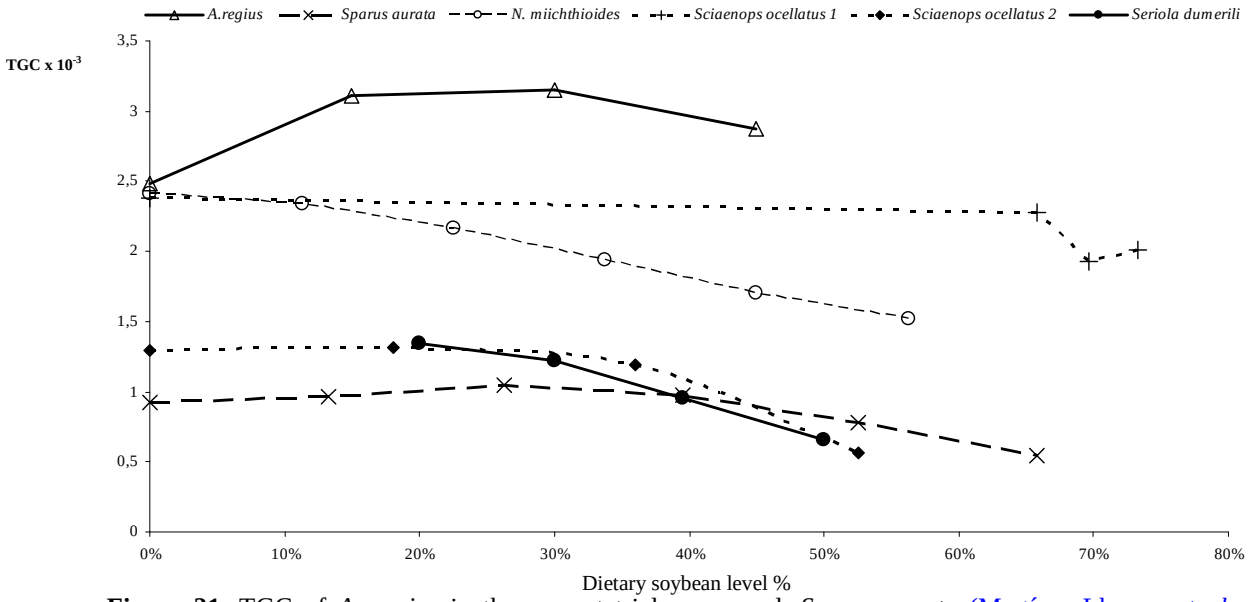


**Figure 21.** TGC of *A. regius* in the present trial, compared, *Sparus aurata* (Martínez-Llorens *et al.*, 2009), *N. miichthioides* (Wang *et al.*, 2006), *Sciaenops ocellatus* 1 (McGoogan & Gatlin III, 1997), *Sciaenops ocellatus* 2 (Reigh & Ellis, 1992) and *Seriola dumerili* (Tomás *et al.*, 2005).

The diets with 15 or 30% of SB obtained the best final weight and TGC (385 g and 3.15 x 10$^{-3}$, respectively) in phase I and 45% (527 g and 3.5 x10$^{-3}$, respectively) in phase II, but the optimum soybean inclusion in diets for *A. regius* was 27% for both, growth and feed conversion. **Figure 21** it is showed the TGC obtained in this

experiment compared with different marine species that were feeding with different levels of plant proteins in diets. Despite de knowledge of meagre nutrition is limited there are a few feeding studies in fish of the scianids family. The studies made by Wang *et al*. (2006b) recommended soybean meal dietary level below 10% (40% of fish meal substitution) in diets for cuneate drum, since exceeding this level produced a detriment in the fish growth. Higher levels of dietary soybean meal were recommended for other scianids as *Sciaenops ocelatus,* for example McGoogan & Gatlin (1997) obtained a good growth results when soybean meal was included at 66% in diets and in the same species, Reigh & Ellis (1992) also observed that the 70% of soybean meal dietary inclusion did not affected negatively to fish growth. Regarding to replaces fish meal with a vegetable protein mixtures, Estevez *et al*. (2010) fed *A. regius* with four experimental diets with two inclusion levels (42 and 52%, those represented the 315 and 38% of total protein of diets) of mixture plant protein (soy cake, corn gluten, soy protein concentrate and sunflower cake) with or without fish protein hidrolysates and observed that the fish growth was significantly reduced by the inclusion of plant protein, although the growth of fish fed diets with 42% of plant protein dietary inclusion obtained similar growth to fish fed control diet. The quadratic regression analysis was recommended by Shearer (2000) because it was used to obtain optimum levels of ingredients or nutrients (Martínez-Llorens *et al*., 2009; Sánchez *et al*., 2007). According to the polynomial regression showed that the dietary soybean meal obtained for a maximum growth was 26.4%. Similar results were reported in other marine species, such as gilthead sea bream that recommended between 20.5% (Martínez-Llorens *et al*., 2009) and 30.5% (Martínez-Llorens *et al*., 2007) of dietary soybean meal level for maximum growth, and likewise Tomás *et al*., (2005) recommended from 20 to 30% of defatted soybean meal dietary inclusion for maximum growth of *Seriola dumerili*. Chou *et al*., (2004) for

juvenile cobia (*Rachycentron canadum*) estimated by quadratic regression a growth optimum at 16.9% replacement of fish meal protein by soybean meal protein.

Although daily feed intake, FCR, and PER do not statistically differ between diets, they do not appear so close. Daily feed intake of diet 0, in particular, is 13% lower than the value recorded for diets 15 and 45, and 17% lower than diet 30. To provide evidence that the may be caused by the nutrient imbalances besides to palatability properties of plant proteins.

In the phase II, we did not observed significant differences between treatments respect to the fish growth. Theses results show that fish with a mean weight of 350 g can accept a diet with a level of inclusion of SB up to 45%. In meagre Estevez *et al*. (2010) evidenced that fish meal can be replacement with a high level of vegetable protein mixture (42%). In red drum, showed that using soybean meal, the level of inclusion in the diet can be up tu 70% of SB (Reigh & Ellis, 1992; McGoogan & Gatlin, 1997). On the other hand, observed that fish in the phase II had a higher growth than those in the phase I. Theses differences could be due to the larger fish have lower protein requirements than the smaller fish, and this condition could explain the high values obtained from the VPP in phase II. Also observed that the highest feed intake was registered during the phase II, coinciding with a mean temperature of 21.3 ° C, which is within the recommended range to reach the best growth in meagre (El-Shebly *et al*., 2007).

In relation to biometric parameters no effect of diet was observed and similar indexes were obtained by Poli *et al.* (2003); 1.04 condition factor, 44% fillets, 6% VSI, but the mesenteric fat obtained in present trial (2.4%) were higher than the obtained by Poli *et al*. (2003), and the cause of this differences probably could be to that meagres in the present experiment not formed gonad. There was not significant effect on the whole

body composition of meagre by the experimental diets and approximately content was 72% of humidity, 17% of CP and 7% of lipids. These results demonstrate the excellent meat quality that presents the meagre, being the main characteristic its lower fat content, representing an important parameter of quality for the consumer (Poli *et al*., 2003).

The detriment of growth in 45 diet could be to several factors as the presence of anti nutritional factors in plant proteins (Francis *et al*., 2001; Gatlin *et al*., 2007), that various effects can also caused the activities reduces of alkaline phosphatase and aminopeptidase in meagre (Estevez *et al*., 2010). In addition, the growth could be affected by the amino acid deficiencies of diets (Gomez-Requeni *et al*., 2004; Peres *et al*., 2003; Refstie *et al*., 2006; Wang *et al*., 2006b). Following this reasoning, the information about amino acids requirements for meagre is not available. Nevertheless, a first approach about the excess or defect of EAA could be done by estimating amino acid retention, which has been carried out in other fish species (Peres & Oliva-Teles, 2009; Sánchez-Lozano *et al*., 2010). In present experiment, no significant effect in amino acid retentions was observed with the different diets, but great differences can be observed among amino acids, Arg, Lys, Met and Thr presented the highest retention and the His the lowest. The amino acid efficiency retention and amino acid intake is closely related and the reason of the high retention is due to the low amino acid intake, if this one is made below its requirements. Ratio between EAA profile of diets and whole body (**Figure 22**) could be a good tool to estimate the deficiencies as Sanchez-Lozano *et al*. (2009, 2010) have shown in the sea bream, so, that if this relation is less than 100% this amino acid would be deficient and if it is greater than 100% it would be in excess. Arg, Lys and Thr were deficient in diets and therefore the efficiency retention of these amino acids was high. Met was significant different among diets and fish fed diet 0 (100% of fish meal) presented the highest relation between $\%EAA_{diet}/\%EAA_{fish}$ and

for reason Met efficiency retention increased with soybean meal dietary level. In summary, it is necessary to determine the amino acids requirement of meagre because, even in the diet with the 100% of fish meal, there are amino acids that could be deficient, such as Arg, Lys and Thr. From an economic point of view, it was clearly improved the ECR when fish meal was substituted for soybean meal in the diet.

Then, if the growth and body indices show that diets 15 and 30% were quite similar, although the cost of the diets is not significantly different, the best diet must be considered the diet 30 Reigh & Ellis (1992) who recommended up to 70% of dietary soybean meal for a growth of *Sciaenops ocelatus*, but observed that the 35.5% soybean level (50% of dietary protein from soybean meal) was the most cost-effective diet. The soybean meal level for minimum ECR (optimum ECR) in diets for *Seriola dumerili* (Tomás *et al*., 2005) resulted around of 20.5% and similar results (Martínez-Llorens *et al*., 2007) were obtained for gilthead sea bream (22%).

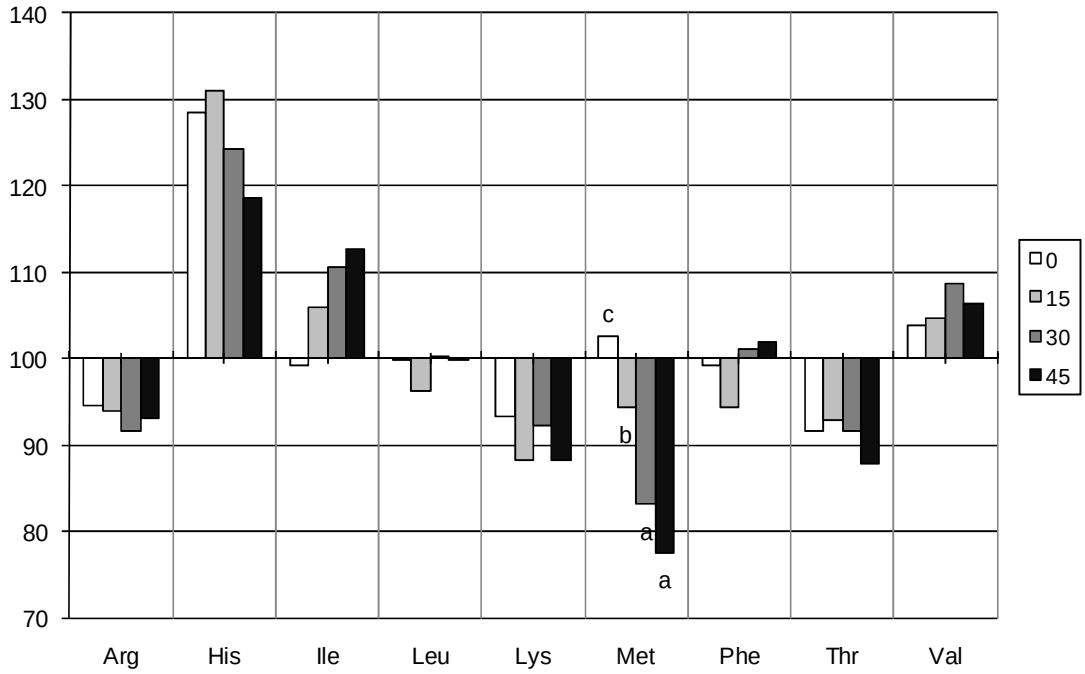


**Figure 22.** Ratio between essential amino acids profile of experimental diets and whole body fish expressed as g per 100 g-1 of protein. Each value is the mean of duplicate groups. Different superscripts indicated differ at $P < 0.05$.

## CONCLUSION

The results obtained in present trial showed that 30% and 45% SB (depending on the size) inclusion could be an excellent plant meal to substitute fish meal dietary, because no effects on growth and feed efficiency parameters were detected and in addition improve the profitability of diets.

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# CAPÍTULO 7

# DISCUSIÓN GENERAL

En el año 2010, a pesar de que ya habían sido publicado algunos trabajos en *A. regius*: Calderón *et al.* (1997), Pastor *et al.* (2002; 2007), Quéméner *et al.* (2002), Poli *et al.* (2003), Piccolo *et al.* (2006; 2008), El-Shebly *et al.* (2007), Hernández *et al.* (2009), algunos autores como Roo *et al.* (2010), Estévez *et al.* (2010), Chatzifotis *et al.* (2010) y Cárdenas (2010) aún manifestaban la necesidad de seguir estudiando esta especie debido a la poca información disponible sobre su nutrición.

Recientemente, se han publicado otros trabajos de gran interés en el tema como lo son Martínez-Llorens *et al.* (2011), Chatzifotis *et al.* (2012), Feij *et al.* (2012) y Sáenz *et al.* (2013), que junto con los experimentos desarrollados en la presente tesis doctoral ayudan a completar la información que ya habia sobre esta especie.

En el presente capítulo se van a abordar los aspectos más importantes de todos estos estudios, lo cual dará una vía para el mayor entendimiento en cuanto a la nutrición y alimentación de la corvina.

## 7.1. CRECIMIENTO Y PARÁMETROS NUTRITIVOS DE LA CORVINA.

En primer lugar, es conveniente comentar la importancia en cuanto a las condiciones de manejo y la calidad del agua, ya que *A. regius* es una especie que tiene la capacidad de adaptarse a diferentes sistemas de producción (Martínez-Llorens *et al.*, 2011). Dada esta condición versátil, los experimentos desarrollados con esta especie en las instalaciones de la Universidat Politècnica de València, se adecuaron a las condiciones de un sistema de recirculación. De esta manera, se siguieron las recomendaciones para los crecimientos óptimos y para reducir el estrés en los peces, adecuando las concentraciones de oxígeno disuelto (superior a 5.0 mg/L, Aquafarmer, 2004), las concentraciones de $NH_3$-N, que fueron inferiores a 0.05 mg/L (Timmons *et al.*, 2002), los niveles de $NO_2$-N recomendados para sistemas acuícolas que fueron

menores de 1,0 mg/L (Pillay & Kutty, 2005) y los nitratos ($NO_3$-N), cuyas concentraciones estuvieron por debajo de los 10 mg/L (Pillay & Kutty, 2005).

La temperatura fue un factor importante a considerar ya que el crecimiento aumenta con la temperatura del agua hasta un máximo específico de la especie después del cual disminuye rápidamente (Collett *et al.*, 2008). Aunque el intervalo de temperatura estimada para el crecimiento en *A. regius* es de 17 a 21 ºC (El-Shebly *et al*, 2007; Estévez *et al*, 2010), con un rango aceptable de 14 a 23 °C (Martínez-Llorens *et al.*, 2011), en los experimentos en tanques de la presente tesis doctoral el rango de temperatura fue de 19 a 24 °C, el experimento en jaulas marinas (**CAPÍTULO 5 Experimento 2**) se realizó a 16,5 ºC, siendo el crecimiento favorable (TCI de 1,35% día$^{-1}$ e ICA de 1,96, para el pienso experimental del 47 PB y 20 GB, y sin diferencias con el resto). Por el contrario, en el estudio realizado por Estévez *et al.* (2010), en dos periodos de temperaturas (fría: 15,6 ºC y cálida: 20 ºC), se obtuvo a baja temperaturas un TCI negativo (-0,44% día$^{-1}$) confirmando en este caso que la corvina crece mejor a temperaturas altas.

Una vez especificadas las condiciones, se puede decir que en general el crecimiento en la mayoría de los experimentos desarrollados con corvinas en las instalaciones de la Universidad Politécnica de Valencia resultaron buenos (**Tabla 20**), confirmando de esta manera que realmente la corvina es una buena candidata para la diversificación de la acuicultura en el Mar Mediterráneo, según lo venían planteando diferentes autores Quéméner *et al.* (2002), Poli *et al.* (2003), El-Shebly *et al.* (2007), Estévez *et al.* (2010), Chatzifotis *et al.* (2010), Roo *et al.* (2010), Chatzifotis *et al.* (2012) y Duncan *et al*. (2012).

**Tabla 20.** Crecimiento general de los experimentos realizados en el Laboratorio de la UPV.

| | Peso Inicial (g) | [A]CTC x $10^{-3}$ | [B]TAD (% $day^{-1}$) | [C]ICA | Tº |
|---|---|---|---|---|---|
| **CAPÍTULO 4** | | | | | |
| **Prueba A:** | | | | | |
| (Necesidades de | 53 | **0,27** | 0,5 | 2,25 | |
| Energía y Proteína) | | **1,69** | 2,22 | 2,3 | |
| | | | | | 19 ± 1 ºC |
| **Prueba B:** | | | | | |
| (Necesidades de | 188 | **0,95** | 0,38 | 1,31 | |
| Energía y Proteína) | | **2,74** | 1,88 | 2,25 | |
| **CAPÍTULO 5** | 52 | **2,14** | 1,95 | 1,24 | |
| **Experimento A** | | **2,69** | 1,78 | 0,97 | 24 ±1 ºC |
| Niveles de Proteína | | | | | |
| **CAPÍTULO 6** | 165 | **2,56** | 0,79 | 1,30 | |
| Nivel de inclusión de | | **3,15** | 0,95 | 1,43 | 23 ±1 ºC |
| soja (Fase I) | | | | | |
| Nivel de inclusión de | 346 | **3,4** | 1,31 | 0,91 | 21.3 ºC |
| soja (Fase II) | | **4** | 1,34 | 0,79 | |

[A]Coeficiente térmico de crecimiento CTC= 1000 x [Peso final (g) $^{1/3}$– Peso inicial (g)$^{1/3}$] /(T° – T° minima de alimentación x días); T° Minima de alimentación = 12 ºC. [B]Tasa alimentación diaria (% día$^{-1}$) TAD = 100 x Ingesta (g)/ Biomasa promedio (g) x días.; [C]Índice conversión de alimento ICA = Ingesta (g)/ Incremento de biomasa (g)

Hay que tener en cuenta que la procedencia de los peces varió entre experimentos, pudiendo incidir en los resultados y no tener una uniformidad total de los mismos. En la **Tabla 20** se puede apreciar que los CTC correspondientes a los **CAPÍTULOS 4, 5, 6** son superiores a los obtenidos por Estévez *et al.* (2010) (1,73 x $10^{-3}$), Martínez-Llorens *et al.* (2011) (1,46 x $10^{-3}$) y Chatzifotis *et al.* (2012) (1,9 x $10^{-3}$). Asimismo, hay que destacar que el crecimiento (CTC) obtenido en estas pruebas fue superior al de otras especies de la familia Scianidae, como *A. japonicus* (1,46 x $10^{-3}$) (Pirozzi *et al.*, 2009) o (1,79 x$10^{-3}$) (Pirozzi *et al.*, 2010), *S. ocellatus* (2,23 x $10^{-3}$) (Thoman *et al.*, 1999), *N. miichthioides* (2,2 x $10^{-3}$) (Wang *et al.*, 2006) y en otras especies del mediterráneo tales como *Sparus aurata* (1.72 x$10^{-3}$) (Mayer *et al.* 2008) o (1,1 x $10^{-3}$) (Martínez-Llorens *et al.*, 2009), *Diplodus sargus* (0,89 x $10^{-3}$) (Sá *et al.*,

2008), *Seriola dumerili* (1,3 x $10^{-3}$) (Tomás *et al.*, 2005), aunque muy similares a los obtenidos para el salmón, *Salmo salar* (3,41 x $10^{-3}$) (Helland *et al.*, 2010).

Los índices de conversión también fueron excelentes en los **CAPÍTULOS 5 y 6**, confirmando los obtenidos en otros trabajos como los de Chatzifotis *et al.* (2010) (ICA mínimo: 1,38; ICA máximo: 1,61), Chatzifotis *et al.* (2012) (ICA mínimo: 0,9; ICA máximo: 1,6), y Martínez-Llorens *et al.* (2011) (ICA mínimo: 1,2; ICA máximo: 5,3). Sin embargo, los ICA en los estudios de necesidades de energía y proteína, resultaron altos, de acuerdo a lo que normalmente se estima para esta especie, y son similares a los resultados obtenidos por Velazco *et al.* (2009); esto puede deberse a que en este tipo de estudio al tener tasas de alimentación muy altas, en muchos casos puede darse una sobrealimentación que puede generar dichos resultados.

Las raciones adecuadas de piensos nutricionalmente balanceados son muy importantes para el óptimo crecimiento, supervivencia, desarrollo inmunitario y para la obtención de excelentes propiedades organolépticas en los peces, es por ello que se recomienda determinar las tasas óptimas de alimentación en las especies acuícolas, con mayor énfasis en aquellas que se producen a escala comercial, dado que permiten incrementar la producción disminuyendo así los costes económicos y los impactos ambientales (Okorie *et al.*, 2013).

La tasa de alimentación diaria para maximizar el crecimiento en la corvina fue determinada en el **CAPÍTULOS 4,** fueron obtenidas en función del tamaño de los peces (**Tabla 21**) y comparada con las obtenidas por Velazco *et al.* (2009) y García (2012). Resultaron mayores en los experimentos de peces de menor tamaño, debido a que tienen mayores necesidades proteicas para un máximo crecimiento que los peces mayores (Cho *et al.*, 1985; NRC, 1993).

**Tabla 21.** Tasas de alimentación diaria para el máximo crecimiento

| Peso | Pienso | [A]TAD Máx. | Autor |
|---|---|---|---|
| 50 g | Regius | 2,61% día$^{-1}$ | Velazco *et al.* (2009) |
| 53 g | Skreting | 2,2% día$^{-1}$ | Presente estudio |
| 62 g | Skreting | 1,6 % día$^{-1}$ | García (2012) |
| 200 g | Skreting | 1,73% día$^{-1}$ | Presente estudio |

[A]TAD Máx: Tasa de alimentación diaria para el maximo crecimiento.

En dorada (Jauralde *et al.*, 2013), establecieron un modelo de crecimiento en función de las tasas de alimentación, obteniendo que las necesidades para el mantenimiento eran de 0,23 g 100 g$^{-1}$ día$^{-1}$ y las necesidades para el máximo crecimiento de 1,9 g 100 g$^{-1}$ día$^{-1}$, de acuerdo a un modelo de crecimiento asintótico, que como vemos resultan ligeramente inferiores a las de esta especie, lo cual resulta lógico si tenemos en cuenta las diferencias de crecimiento presentadas anteriormente.

### 7.2. APORTE A LAS NECESIDADES NUTRITIVAS.

Las necesidades de proteína y energía que debe ingerir el pez para optimizar su producción (crecimiento, índice de conversión, rentabilidad, etc.) deben ser expresadas en gramos de proteína por kg de pez y día, y kJ de energía por kg de pez y día. De acuerdo con Sanz (2009), estas necesidades pueden depender del estado fisiológico del pez y de las condiciones ambiéntales, fundamentalmente de la temperatura que determina el nivel de actividad y el crecimiento. El método factorial fue de gran utilidad, porque ofreció varias ventajas a la hora de estimar las necesidades de proteína y energía, como por ejemplo, que se emplearon menores cantidades de unidades experimentales que con pruebas de tipo dosis-respuesta, y otra muy importante es que se puede emplear piensos comerciales.

Para comparar los resultados obtenidos en corvinas con otras especies fue necesario recalcular nuestros resultados correspondientes a las **Tablas 6** y **7** del

**CAPÍTULO 4**, dado que en muchos trabajos se emplea el peso metabólico. En dorada se estimó en la proteína $Kg^{-0,7}$ y $Kg^{-0,8}$ para la energía (Lupatsch *et al*., 1998), y resultados similares fueron obtenidos para *A. japonicus* (Pirozzi *et al*., 2010). En el presente trabajo se utilizaron estos valores, como peso metabólico teórico para *A. regius*.

Las necesidades para el mantenimiento en corvinas se estimaron en 0,71 g PD $kg^{-0,7}$ $pez^{-1}$ $día^{-1}$ y 15,61 kJ ED $kg^{-0,8}$ $pez^{-1}$ $día^{-1}$, mientras que las necesidades de proteína para el máximo crecimiento fueron 3,68 g PD $kg^{-0,7}$ $pez^{-1}$ $día^{-1}$ y las necesidades de energía para el máximo crecimiento 139,94 kJ ED $kg^{-0,8}$ $pez^{-1}$ $día^{-1}$.

En la **Figura 23 A** se relaciona las necesidades de proteína de mantenimiento de *A. regius* con otras especies. Se puede observar que las necesidades de proteína de mantenimiento para la corvina están dentro del rango establecido para estas especies, siendo en general, menores que en las especies producidas en el Mar Mediterráneo y mayores que en las especies de la familia Scianidae.

La misma tendencia se observa en la **Figura 23 B**, donde se relacionan las necesidades de proteína para el máximo crecimiento, sólo que *S. ocellatus* muestra los mayores valores para maximizar el crecimiento, aunque estos datos corresponden a alevines de 4 g, por lo que las necesidades tienden a ser mayores. Los datos correspondientes a *A. japonicus* son los obtenidos a 20 ºC por Pirozzi *et al.* (2010), estos en general son menores que en la corvina a la misma temperatura, que es la óptima para su producción.

Las necesidades de energía para el mantenimiento (**Figura 24 A**) resultaron más bajas en *A. regius*. Esto puede obedecer al tipo de modelo aplicado, puesto que en las especies comparadas se establecen modelos lineales dando estimaciones más altas, sin embargo, los modelos curvilíneos tienden a ser muy aceptados y recomendados para

estos tipos de estudios factoriales (Pirozzi *et al.*, 2010). En cuanto a las necesidades de energía para el mantenimiento y el máximo crecimiento (**Figura 24 B**), sólo se observa que son mayores que en *A. japonicus* a 20 ºC. A la temperatura óptima de producción de 26 ºC de esta especie (Collett *et al.*, 2008), las necesidades de energía aumentan, tanto para el mantenimiento como para el máximo crecimiento (Pirozzi *et al.*, 2010).

En la **Tabla 22** se observa el resumen de las recomendaciones nutricionales de acuerdo a los resultados obtenidos en el **CAPÍTULO 4,** que se determinaron mediante un modelo bioenergético. También se muestran los resultados de Velazco *et al.* (2009), que fueron recalculados en proteína digestible y energía digestible, utilizando regresiones cuadráticas, las necesidades de proteína para el máximo crecimiento fue de 0,78 g PD 100 g pez$^{-1}$ día$^{-1}$, muy similar a las **Pruebas A y B** del **CAPÍTULO 4** para las tasas de máximo crecimiento y el nivel óptimo de la proteína digestible determinada en el **CAPÍTULO 5**. En relación a las necesidades de energía (**Tabla 22**), tanto para el mantenimiento como para el máximo crecimiento podemos ver que existe la misma tendencia de correlacionarse los resultados obtenidos en el **CAPÍTULO 4** y por Velazco *et al.* (2009).

**Tabla 22.** Recomendaciones nutricionales para *A. regius.*

| | Velazco *et al.* (2009) | **CAPÍTULO 5** Experimento A | **CAPÍTULO 4** Prueba A | **CAPÍTULO 4** Prueba B |
|---|---|---|---|---|
| Peso | 50 g | 52 g | 53 g | 200 g |
| Pienso | Regius | Experimental | Skreting | Skreting |
| [A]NPm (g PD 100 g pez$^{-1}$ día$^{-1}$) | 0,09 | | 0,0617 | 0,0617 |
| [B]NPmáx (g PD 100 g pez$^{-1}$ día$^{-1}$) | 0,78 | 0,8 | 0,86 | 0,73 |
| [C]NEm (kJ ED 100 g pez$^{-1}$ día$^{-1}$) | 2,43 | | 2,74 | 2,74 |
| [D]NEmáx (kJ ED 100 g pez$^{-1}$ día$^{-1}$) | 43,08 | | 45,79 | 38,72 |

[A]NPm: Necesidades de proteína para el mantenimiento. [B]NPmáx: Necesidades de proteína para el máximo crecimiento. [C]NEm: Necesidades de energía para el mantenimiento. [D]NEmáx: Necesidades de energía para el máximo crecimiento.

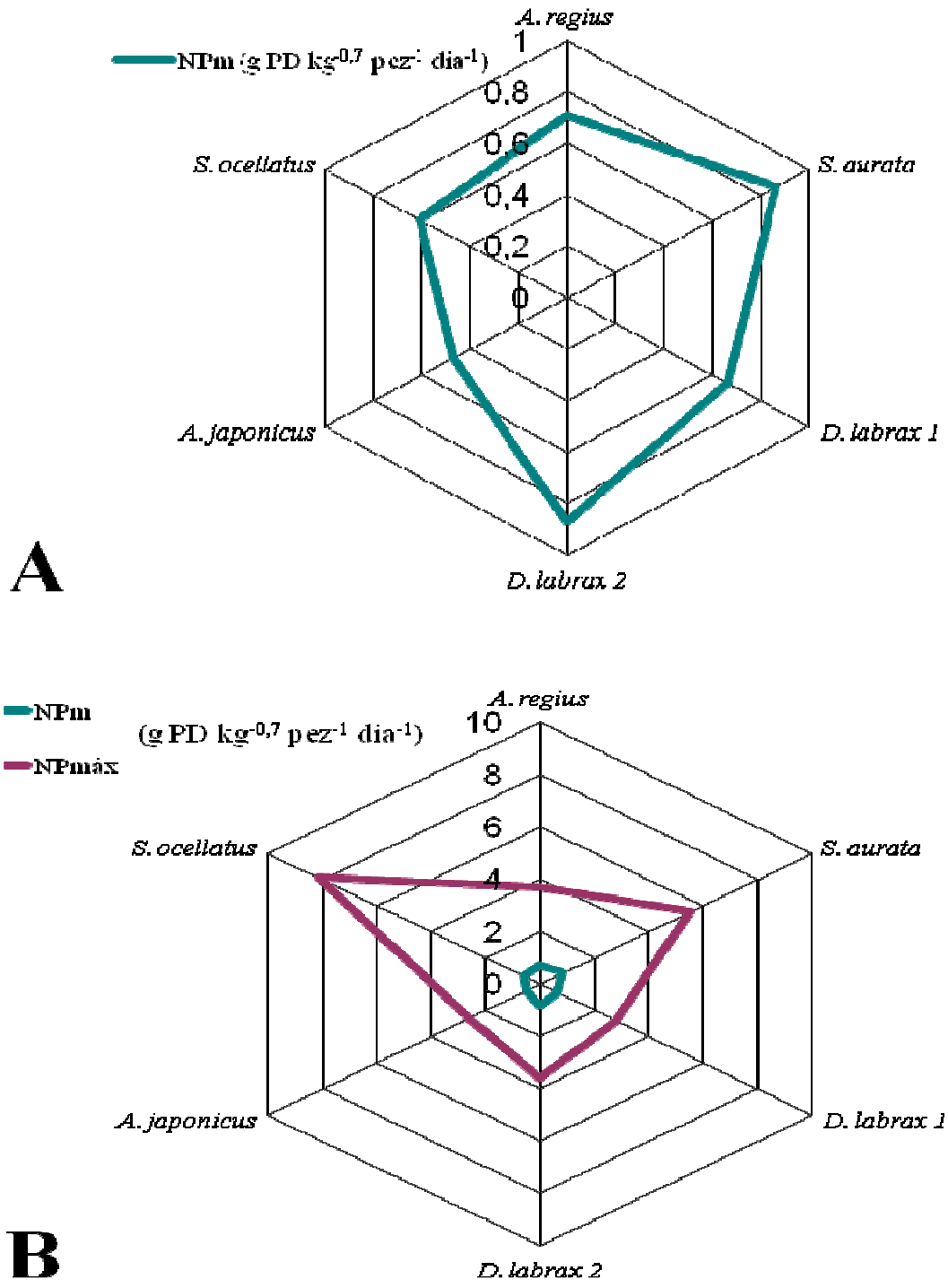
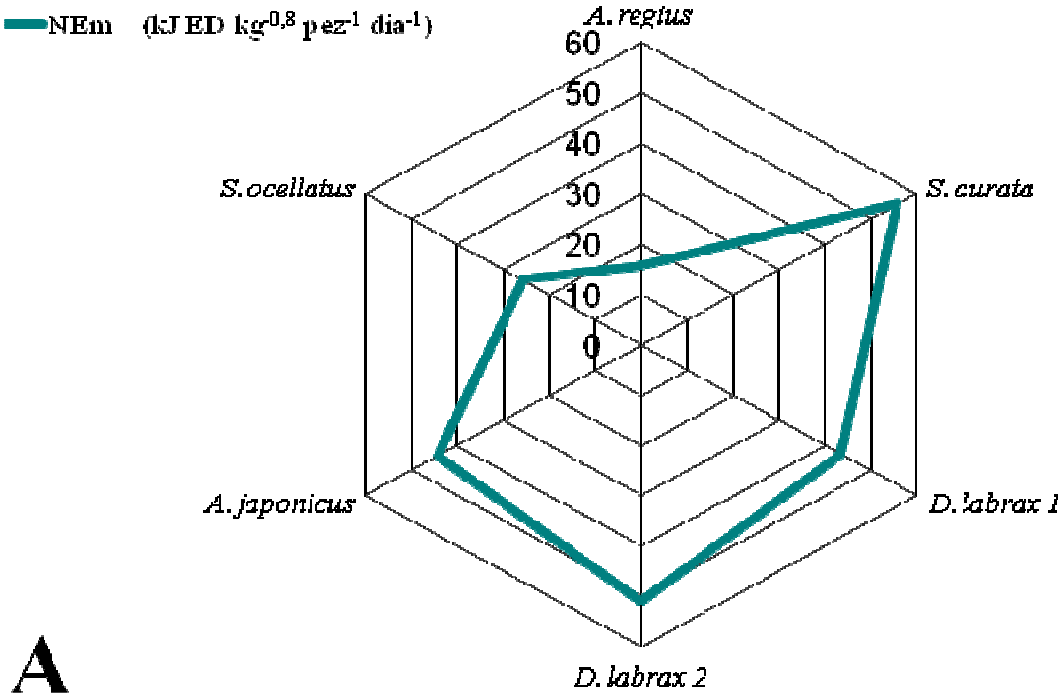



**Figura 23**. En **A** se observan las necesidades de proteína digestible para el mantenimiento (NPm) para *Argyrosomus regius* (Presente estudio) comparativamente con *Sparus aurata* (Lupatsch *et al.*, 1998), *Dicentrarchus labrax* 1 (Lupatsch *et al.*, 2001) *Dicentrarchus labrax* 2 (Peres & Oliva-Teles, 2005), *Argyrosomus japonicus* (Pirozzi *et al.*, 2010), *Sciaenops ocellatus* (McGoogan & Gatlin III, 1998). En **B** se observan las necesidades de proteína digestible para el mantenimiento (NPm) y las necesidades de proteína digestible para el máximo crecimiento (NPmáx) para las mismas especies.



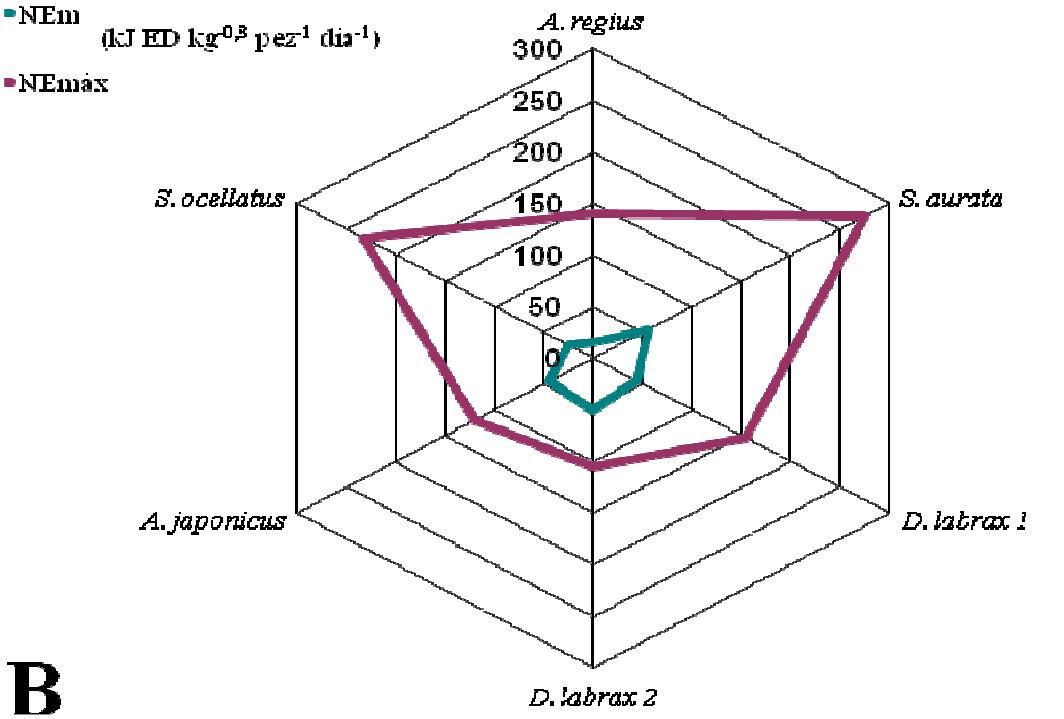




**Figura 24**. En **A** se observan las necesidades de energía digestible para el mantenimiento (NEm) para *Argyrosomus regius* (Presente estudio) comparativamente con *Sparus aurata* (Lupatsch *et al.*, 1998), *Dicentrarchus labrax* 1 (Lupatsch *et al.*, 2001) *Dicentrarchus labrax* 2 (Peres & Oliva-Teles, 2005), *Argyrosomus japonicus* (Pirozzi *et al.*, 2010), *Sciaenops ocellatus* (McGoogan & Gatlin III, 1998). En **B** se observan las necesidades de energía digestible para el mantenimiento (NEm) y las necesidades de energía digestible para el máximo crecimiento (NEmáx) para las mismas especies.

En dorada (Jauralde *et al.*, 2013), también encontraron una correlación para las tasas de mantenimiento, utilizando un modelo de regresión cuadrática y modelo de crecimiento asintótico (0,22 g 100 $g^{-1}$ $día^{-1}$ y 0,23 g 100 $g^{-1}$ $día^{-1}$, respectivamente).

En el **CAPÍTULO 5** se determinó el nivel óptimo de proteína digestible para esta especie (0,8 g PD 100 g $pez^{-1}$ $día^{-1}$) entrando dentro del rango correspondiente a lo establecido para peces carnívoros (Wang *et al.*, 2006; McGoogan & Gatlin, 1999; Serrano *et al.*, 1992; Thoman *et al.*, 1999; Turano *et al.*, 2002). En jaulas marinas, aunque los resultados no dieron diferencias significativas, se observaron mejores índices de conversión en peces alimentados con un pienso de 47/20, que también coincide con Martínez-Llorens *et al.* (2011) con juveniles de 94 g alimentados con piensos comerciales, llegando a la conclusión de que el mejor crecimiento y eficiencia nutritiva correspondía al pienso con un 47/20, es decir un 0,8 g PD 100 g $pez^{-1}$ $día^{-1}$.

El contenido de lípidos en el músculo de la corvina es bastante bajo, estimándose que esta especie no requiere de altos niveles de lípidos en sus dietas (Poli *et al.*, 2003; Piccolo *et al.*, 2006; 2008; Hernández *et al.*, 2009; Chatzifotis *et al.*, 2010; Chatzifotis *et al.*, 2012). En la presente tesis doctoral no se desarrollaron experimentos para determinar las necesidades de lípidos de la corvina, ni para la sustitución de fuentes lípidicas, sin embargo, existen trabajos que nos pueden permitir discernir sobre este tema. Martínez-Llorens *et al.* (2011) y Chatzifotis *et al.* (2010), establecen para juveniles y sub-juveniles que el nivel de lípidos debe estar en torno a un 17 – 20%.

Generalmente, el aumento del nivel de lípidos de la dieta mejora el crecimiento, la eficiencia alimenticia y proteica, ahorrando proteínas que pueden ser catabolizadas y utilizadas como una fuente de energía (Hillestad & Johnsen, 1994; Helland & Grisdale-Helland, 1998; Grisdale-Helland & Helland, 1998; De Silva *et al.*, 2001; Torstensen *et al.*, 2001; Lee *et al.*, 2002; Skalli *et al.*, 2004). Los peces tienen un nivel óptimo de

lípidos en su dieta, un nivel superior puede causar depresión del crecimiento (Pei *et al*., 2004; Du *et al*., 2005; López *et al*., 2006), lo cual ha sido observado en corvina (Chatzifotis *et al*., 2010) y otras muchas especies: *Lates calcarifer* y la lubina blanca (*Atractoscion nobilis)* (Williams *et al*., 2003; López *et al*., 2006), dorada, lubina, cobia (*Rachycentron canadum*), el rodaballo (*Psetta maxima*) y el dentón (*Dentex dentex*) (Péres & Oliva Teles, 1999; Chou *et al*., 2001; Regost *et al*., 2001; Wang *et al*., 2005).

## 7.3. INCLUSIÓN DEL TURTÓ DE SOJA EN LA ALIMENTACIÓN DE LA CORVINA

Para el estudio sobre la inclusión del turtó de soja (**CAPÍTULO 6)** en los piensos para corvinas se llevaron a cabo dos Fases: La Fase I, en esta prueba se obtuvo un mejor crecimiento con los piensos con un 15 ($3{,}10 \times 10^{-3}$) y un 30% ($3{,}15 \times 10^{-3}$) de inclusión de turtó de soja, inclusive mayor que con el pienso control (0%) ($2{,}56 \times 10^{-3}$). Y la Fase II, donde no se encontraron diferencias significativas en el crecimiento, en ninguno de los piensos probados (**Tabla 17**), posiblemente debido al tamaño de los peces.

Por lo tanto, para esta especie la inclusión de fuentes proteicas vegetales formaría parte de su dieta, y el nivel óptimo para el máximo crecimiento sería de un 26,42% de turtó de soja en el caso de peces pequeños. A pesar de éste nivel razonable de inclusión, un nivel mayor perjudicaría el crecimiento de la corvina ya que las dietas serían deficientes en arginina, lisina, treonina y principalmente en metionina, lo que se observa teniendo en cuenta la relación entre el porcentaje de AAE de la dieta y el porcentaje de AAE a nivel corporal de los peces (**Figura 22**). Por lo que sería necesaria una suplementación adicional de estos aminoácidos en el pienso para mejorar los parámetros de crecimiento.

En peces de pesos mayores, las necesidades de proteína disminuyen, y por ende, las de AAE, de ahí que no haya diferencias en el crecimiento con una dieta con un 45% de turtó de soja.

Los resultados de crecimiento de esta fase, muestran el potencial de crecimiento de esta especie, con un CTC alrededor de 4,00 $x10^{-3}$, mayor que en la Fase I y que en el trabajo de Calderón *et al*. (1997) (2,02 $x10^{-3}$), o el de Estévez *et al.* (2010) con una dieta experimental comercial (1,73 $x10^{-3}$), así como también en otras especies marinas, como la dorada (1,72 $x10^{-3}$) (Mayer *et al.*, 2008) y otros esciánidos (1,46 $x10^{-3}$) como *Argyrosomus japonicus* (Pirozzi *et al.*, 2009) alimentados con dietas comerciales.

De acuerdo a los resultados obtenidos en ambas fases, se puede recomendar una inclusión del turtó de soja en las dietas de entre un 30-45%.

Esta fuente proteica vegetal, también ha sido estudiada en especies comerciales tales como en juveniles de la cobia (*Rachycentron canadum*), con un nivel de inclusión máximo de 16,9 % (Chou *et al.*, 2004) ó en la seriola (Tomás *et al.*, 2005) donde se recomienda una inclusión de un 20 a un 30%, muy similares a los de la dorada, de un 20,5% (Martínez-Llorens *et al.*, 2007) y 30,5% cuando la dieta era enriquecida con aminoácidos sintéticos (Martínez-Llorens *et al.*, 2009).

No hay referencias bibliográficas de experimentos de inclusión del turtó de soja en corvinas, pero esta fuente proteica, ya ha sido probada en otras especies de la familia Scianidae como es el caso de *Nibea miichthioides*, en la que se recomienda un nivel máximo del 10%, puesto que a mayor inclusión disminuye el crecimiento de los peces (Wang *et al.*, 2006) y el corvinón ocelado (*Sciaenops ocelatus*), en los estudios realizados por McGoogan & Gatlin (1997), donde obtienen buenos resultados con una inclusión de turtó de soja del 66%. En el estudio realizado en *A. regius* por Estévez *et al.* (2010), experimentaron con una mezcla de proteínas vegetales, observaron que el

crecimiento de los peces se redujo significativamente por la inclusión de proteína vegetal, aunque el crecimiento de los peces alimentados con una dieta que contenía un 42% de inclusión de proteína vegetal, no se obtuvieron diferencias de crecimiento respecto al pienso control.

Recientemente Feij *et al.* (2013) determinaron la digestibilidad de diferentes fuentes vegetales en corvina. Estos resultados son de gran interés para los estudios de la presente tesis doctoral, dado que en los experimentos de inclusión de turtó de soja no se realizó digestibilidad. En este trabajo se puede comprobar que el turtó de soja, posee buenos coeficientes de digestibilidad y esto indudablemente confirma una vez más que es una fuente proteica vegetal alternativa con un gran potencial para su inclusión en la dieta de corvinas, porque genera muy buenos crecimientos y disminución en el coste de los piensos.

Sin embargo, los estudios de digestibilidad, por ser de corta duración, no contemplan las alteraciones que pueden causar sobre el tracto digestivo, los cuales pueden afectar a la absorción de nutrientes. Dichos cambios a nivel intestinal pueden además reducir la actividad de secreción de las enzimas del borde del cepillo de los enterocitos y además alterar las proteínas transportadoras de AA y de péptidos (Krogdahl *et al.*, 2003). Por lo tanto, la digestibilidad real podría ser inferior, ya que en estas pruebas el corto periodo de ingesta de los piensos puede que sea insuficiente para que produzcan dichas alteraciones tan severas.

En relación a los índices biométricos y a la composición corporal (**Tabla 18**), no se vieron afectados por las dietas experimentales en ninguna de las dos fases. No se observaron diferencias significativas en la composición de todo el cuerpo a excepción del índice hepatosomático (IHS) en la fase II, donde en la dieta S45 (1,57 %) el peso del hígado llega a reducirse un 30 por ciento respecto al resto de las muestras.

En cuanto a los análisis económicos de las dietas (**Tabla 19**), el coste de las dietas se redujo con el aumento de turtó de soja, en la Fase I con la dieta control se obtuvo un ICE estadísticamente más alto, sin embargo en la Fase II, no se encontraron diferencias significativas entre los diferentes niveles de inclusión, aunque se puede apreciar la misma tendencia a disminuir con el aumento de nivel de inclusión y sobre todo, con el tamaño de los peces.

### 7.4. DISEÑO DE UN PIENSO PARA *Argyrosomus regius*.

En general en el desarrollo de los experimentos, los resultados obtenidos se pueden considerar de gran interés, tomando en consideración los crecimientos, las necesidades de energía y de proteína, las tasas de alimentación, los niveles de proteína y lípidos, así como también la inclusión de fuentes proteicas vegetales para reducir los costes de producción. Por ello podríamos estimar que un pienso comercial para la alimentación de corvina debería tener: 47% de proteína, 17% de lípidos y con una inclusión de entre un 30% y un 45% de turtó de soja, suplementado con metionina y lisina (**Tabla 23**).

Este pienso debería ser suministrado a corvinas de entre 50 y 200 g con una tasa de alimentación diaria de un 2,2% día$^{-1}$, mientras que en peces con más de 200 g la tasa de alimentación diaria debería ser de 1,73% día$^{-1}$, lo cual supliría a las corvinas de sus requerimientos diarios y del nivel óptimo de proteína digestible estimada en 0,8 g PD 100 g pez$^{-1}$ día$^{-1}$ y de un 45,79 kJ ED 100 g pez$^{-1}$ día$^{-1}$.

**Tabla 23.** Diseño de pienso para *A. regius.*

| | Pienso | |
|---|---|---|
| | % Materia Seca | % Materia Húmeda |
| Ingredientes (g $kg^{-1}$) | | |
| Harina de pescado | 388 | 426,14 |
| Turtó de soja | 300 | 333 |
| Trigo | 103 | 113,6 |
| Maltodextrina | 50 | 50 |
| Aceite de pescado | 132 | 132 |
| Metionina | 12 | 12 |
| Lisina | 5 | 5 |
| Mezcla vitamínico-mineral | 10 | 10 |
| Composición nutritiva teórica (% en Materia Seca) | | |
| Proteína Bruta | 47 | |
| Grasa Bruta | 17 | |
| [A]MELN | 23 | |
| Fibra Bruta | 2 | |
| Cenizas | 9 | |
| [B]E (MJ $kg^{-1}$) | 18,24 | |
| [C]PB/E | 21,92 | |

[A]Materia extractiva libre de nitrógeno calculado = 100 - %PB - %GB - %Cenizas - %FB. [B]Energía.
[C]Relación proteína bruta/ energía.

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# CONCLUSIONES

En vista de los resultados de la presente Tesis Doctoral, se concluye que:

- ✓ Las necesidades de proteína para el mantenimiento en la corvina es de 0,0617 g PD 100 g $pez^{-1}$ $día^{-1}$, y las necesidades de energía para el mantenimiento 2,74 kJ ED 100 g $pez^{-1}$ $día^{-1}$.

- ✓ Las necesidades de proteína para la máxima retención en la corvina son de 0,64 g PD 100 g $pez^{-1}$ $día^{-1}$, y las necesidades de energía para máxima retención son 38,5 kJ ED 100 g $pez^{-1}$ $día^{-1}$.

- ✓ La tasa óptima de ingesta de proteína digestible para la corvina es de 0,8 g PD 100 g $pez^{-1}$ $día^{-1}$, que es equivalente a un nivel del 43% PD en piensos.

- ✓ En corvinas de 167 gramos, se puede incluir hasta un 30% de turtó de soja en la dieta sin tener efectos negativos sobre el crecimiento y sobre los parámetros de eficiencia alimenticia, siendo el nivel óptimo de inclusión para el máximo crecimiento de un 27%.

- ✓ En corvinas de 350 gramos esta inclusión puede ser de hasta un 45% sin efectos negativos en el crecimiento.