DIETAS COMPUESTAS PARA LA CRÍA DE JUVENILES DE TENCA (*Tinca tinca* L.): PROTEÍNA, NECESIDADES Y ALTERNATIVAS PARA UNA ACUICULTURA SOSTENIBLE.

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Departamento de Producción Animal

Álvaro González Rodríguez

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Memoria de Tesis Doctoral presentada por Álvaro González Rodríguez y dirigida por Jesús Domingo Celada Valladares y José Manuel Carral Llamazares para acceder al grado de Doctor

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"La ciencia es una sola luz, e iluminar con ella cualquier parte es iluminar con ella el mundo entero"

(Isaac Asimov)

ABREVIATURAS

FM	Harina de pescado
FEM	Harina de plumas
IC	Índice de conversión del alimento
K	Factor de condición
LT	Longitud total
NRC	National Research Council
OPP	Organización de productores piscicultores
P	Peso
PBM	Harina de subproductos de pollo
PPC	Concentrado proteico de guisante
SPC	Concentrado proteico de soja
TCE	Tasa de crecimiento específico
VPP	Valor productivo de la proteína

1. INTRODUCCIÓN Y JUSTIFICACIÓN DE LA UNIDAD TEMÁTICA DE LAS PUBLICACIONES

Durante las últimas décadas, el consumo mundial de productos acuáticos ha experimentado un fuerte crecimiento, superando los 136 millones de toneladas en 2012 (FAO 2014). Considerando el previsible incremento de la demanda y el estancamiento de la pesca desde mediados de la década de los 80, la acuicultura constituye la opción más viable para solventar un desabastecimiento a largo plazo. De esta manera, el sector acuícola ha crecido espectacularmente durante los últimos años, pasando de 35 millones de toneladas en 2001 a más de 66 millones de toneladas en 2012, lo que ha permitido igualar el volumen de especies acuáticas animales destinado a alimentación humana procedente de la pesca (FAO 2014).

Dentro de la acuicultura, la producción en aguas continentales supone el 57% del total mundial (excluyendo plantas acuáticas), destacando los ciprínidos con un 40%. Entre las especies que engloba la familia Ciprinidae, la tenca (*Tinca tinca* L. 1758) presenta un gran potencial para la acuicultura (Wang et al. 2006) por su fuerte demanda y el elevado precio que alcanza en el mercado. En nuestro país, es el ciprínido más apreciado y se cría principalmente en Extremadura y en las provincias de Segovia, Zamora y Salamanca.

La tenca ha sido cultivada tradicionalmente en sistemas extensivos en Centroeuropa desde la Edad Media y en España, al menos, desde el siglo XVI, mediante el mantenimiento de individuos de ambos sexos en charcas de poca profundidad y con vegetación, donde tiene lugar la puesta y posterior crecimiento de juveniles. En la actualidad, los sistemas de cultivo siguen siendo generalmente de tipo extensivo y, en el mejor de los casos, semiextensivo. En tales circunstancias, las diferentes etapas del proceso productivo están sujetas a factores ambientales de difícil control, con rendimientos bajos e impredecibles. Esto ha conducido al desarrollo durante los últimos años de líneas de investigación para la intensificación y mejora de las distintas fases del proceso productivo.

En anteriores investigaciones, nuestro grupo ha desarrollado técnicas para el control de la reproducción, transferidas con éxito a centros de producción, que abarcan desarrollo gonadal de los reproductores, inducción de la ovulación, obtención de gametos y fecundación e incubación artificial (Carral et al. 2003, Rodríguez 2003, Rodríguez et al. 2004, Aguilera et al. 2005, Rodríguez et al. 2005, Carral et al. 2006, Rodríguez et al. 2008), permitiendo la obtención de alevines vesiculados (comúnmente llamadas larvas) bajo condiciones controladas.

Tras lograr una aceptable eficiencia en la producción de alevines vesiculados, el siguiente paso ha de abordar la mejora de las tasas de supervivencia y de crecimiento. Dentro de ello, la alimentación constituye un factor que influye decisivamente sobre los resultados desde el inicio de la alimentación externa. El alimento no solo debe cubrir los requerímientos nutritivos, además debe adecuarse a los diferentes estados de desarrollo. Con la eclosión se inicia la etapa conocida como larvaria. La ingestión de alimento comienza pasados aproximadamente 5 días, cuando empieza el proceso de cría, que se ha llegado a extender hasta dos meses en condiciones de experimentación (Celada et al. 2008), alcanzando por tanto el estado postlarvario. En consecuencia, este periodo podría denominarse etapa larvaria-postlarvaria, a la que seguiría posteriormente la cría de juveniles.

En hábitats naturales, la alimentación de la tenca es la propia de un depredador, como revelan estudios del contenido del tracto digestivo con la presencia de crustáceos planctónicos y otros invertebrados (Steffens 1995, Pyka 1996,1997), lo que significa una dieta rica en proteína animal. La inexistencia de dietas compuestas específicas para la tenca ha conducido a la búsqueda de soluciones aplicables a corto plazo a problemas prácticos de la cría, orientadas a una adecuada utilización de alimentos disponibles en el mercado.

Durante la etapa larvaria-postlarvaria, el aporte de alimento vivo ha permitido obtener tasas de supervivencia y de crecimiento aceptables tras la reabsorción del vitelo. En este sentido, se han obtenido resultados aceptables proporcionando diferentes especies de zooplancton recolectadas del medio natural (Šestáková et al. 1989, Hamáčková et al. 1995, 1998, Kamler et al. 1995) o nauplios vivos de *Artemia* (Wolnicki y Myszkowski 1998, Fleig et al. 2001, Wolnicki et al. 2003a, Celada et al. 2007, 2008). Posteriormente, se comprobó la posibilidad de sustituir nauplios vivos por quistes decapsulados de *Artemia* de diferentes calidades y sometidas a procesos de conservación, reduciendo los costes y mejorando el crecimiento (García et al. 2010, Celada et al. 2013).

Durante la etapa juvenil, la escasez de conocimientos sobre las necesidades nutritivas ha obligado a usar piensos comerciales formulados para otras especies acuáticas en los ensayos realizados bajo condiciones controladas (Quirós y Alvariño 1998, Wolnicki y Myszkowski 1998, Quirós et al. 2003, Rennert et al. 2003, Kamler et al. 2006, Wolnicki et al. 2003b, 2006, Mareš et al. 2007, Celada et al. 2007, 2009, García et al. 2010, Myszkowski et al. 2010, Zakęś et al. 2010). Considerando en conjunto los mencionados estudios, los resultados no han sido satisfactorios. Posteriormente, Celada et al. (2009) evidenciaron que la suplementación con nauplios de *Artemia* de una dieta seca formulada para salmónidos mejora en gran medida la supervivencia y el crecimiento y previene la aparición de deformidades. Recientemente, García et al. (2010, 2014) pusieron de manifiesto que el suplemento de nauplios vivos puede ser sustituido por quistes decapsulados de *Artemia*, reduciendo los costes y mejorando el crecimiento.

En el marco del proyecto del Plan Nacional de I+D+i ref. AGL2010-16554 "Alimentación de la tenca (*Tinca tinca* L.) durante las etapas larvaria, postlarvaria y juvenil en condiciones controladas", se formuló y elaboró una dieta práctica para juveniles con la que se consiguieron tasas de supervivencia próximas al 100% y un buen crecimiento (García et al. 2013). Partiendo de esta base, el objetivo para lograr la intensificación de la cría de tenca es desarrollar piensos específicos para la etapa juvenil. Considerando esta premisa, el primer propósito de esta Tesis Doctoral consiste en precisar la formulación y fabricación de una dieta práctica específica para juveniles de tenca que garantice resultados aceptables, en términos de supervivencia y de crecimiento, bajo condiciones controladas. En este sentido, es prioritario establecer el nivel proteico óptimo, ya que su contenido determina en gran medida el coste de una dieta. En consecuencia, el segundo objetivo de esta Tesis consiste en determinar los niveles óptimos de proteína de la dieta, usando harina de pescado como fuente proteica.

La harina de pescado es el principal ingrediente proteico usado en acuicultura (Tacon y Metian 2008). Sin embargo, la insostenible presión de la pesca sobre las poblaciones salvajes, así como los elevados precios derivados de su creciente demanda, hacen inviable mantener sus actuales niveles de inclusión en las dietas (Hannesson 2003, Tacon y Metian 2008, Naylor et al. 2009, FAO 2009). Bajo estas consideraciones, el tercer propósito de esta Tesis consiste en evaluar las posibilidades de sustitución de harina de pescado por fuentes alternativas de proteína.

La Universidad de León concedió una beca de Investigación a fin de desarrollar los mencionados objetivos en el marco del proyecto del Plan Nacional de I+D+i ref. AGL2010-16554 "Alimentación de la tenca (*Tinca tinca* L.) durante las etapas larvaria, postlarvaria y juvenil en condiciones controladas". La Tesis Doctoral, que incluye parte de los resultados obtenidos, se centra en la etapa juvenil y se presenta en la modalidad de Compendio de Publicaciones. Su contenido es el siguiente:

- Publicación I: Formulación y fabricación de una dieta práctica base y evaluación de posibilidades de sustitución de proteína de harina de pescado por proteína de harina de pluma.
- Publicación II: Determinación de niveles óptimos de proteína de origen animal (harina de pescado) en la dieta.
- Publicación III: Valoración de posibilidades de sustitución de proteína de harina de pescado por proteína de harina de subproductos de pollo.
- Publicación IV: Estudio de posibilidades de sustitución de proteína de harina de pescado por proteína de concentrado proteico de guisante.
- Publicación V: Evaluación de posibilidades de sustitución de proteína de harina de pescado por proteína de concentrado proteico de soja.

Las cinco publicaciones que sustentan esta Tesis se relacionan en el apartado 2.

2. LISTA DE PUBLICACIONES

I. Autores: Á. González-Rodríguez, J. D. Celada, J. M. Carral, M. Sáez-Royuela, J. B. Fuertes (2014).

Título: Evaluation of a practical diet for juvenile tench (*Tinca tinca* L.) and substitution possibilities of fish meal by feather meal.

Revista: Animal Feed Science and Technology 187, 61– 67.

Factor de impacto (JRC 2013): 2,086.

II. Autores: Á. González-Rodríguez, J. D. Celada, J. M. Carral, M. Sáez-Royuela, J. B. Fuertes (2014).

Título: Effects of varying protein level in practical diets on survival, growth, feed utilization and body composition of juvenile tench (*Tinca tinca* L.).

Revista: Aquaculture International 22 (5), 1723-1735.

Factor de impacto (JRC 2013): 0,960.

III. Autores: Á. González-Rodríguez, J. D. Celada, J. M. Carral, M. Sáez-Royuela, V. García, J. B. Fuertes (2104).

Título: Evaluation of poultry by-product meal as partial replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.).

Revista: Aquaculture Research. Doi: 10.1111/are.12622. **Factor de**

impacto (JRC 2013): 1,320.

IV. Autores: Á. González-Rodríguez, J. D. Celada, J. M. Carral, M. Sáez-Royuela, J. B. Fuertes (2015).

Título: Evaluation of pea protein concentrate as partial replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.).

Revista: Aquaculture Research. Doi: 10.1111/are.12732 **Factor de**

impacto (JRC 2013): 1,320.

V. Autores: Á. González-Rodríguez, J. D. Celada, J. M. Carral, M. Sáez-Royuela, V. García, J. B. Fuertes (2014).

Título: Evaluation of soy protein concentrate as replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.).

Revista: Turkish Journal of Fisheries and Aquatic Sciences 14, 807-815.

Factor de impacto (JRC 2013): 0,384.

3. REVISIÓN BIBLIOGRÁFICA

3.1. PRODUCCIÓN DE CIPRINIDOS

En las últimas decadas, el crecimiento de la acuicultura ha permitido equiparar el volumen de productos destinados a consumo humano procedentes de la pesca (FAO 2014). Entre los peces de agua dulce cultivados, el grupo de los ciprínidos, con aproximadamente 40 especies, es el de mayor importancia y genera actualmente más de 25 millones de toneladas anuales, lo que supone algo más del 70% de la acuicultura continental y en torno al 40% de la mundial. La producción de ciprínidos tiene particular importancia en China, donde se obtuvieron 18 millones de toneladas en 2012. Respecto a las especies, la carpa plateada, *Hypophthalmichthys molitrix* y la carpa común, *Cyprinus carpio* ocupan los dos primeros puestos en producción a nivel mundial, aportando en conjunto 8 millones de toneladas.

En Europa, la producción de ciprínidos tiene lugar fundamentalmente en la región Central y Oriental, superando las 242.000 t en 2012 (FAO 2014), en su mayoría procedentes de la cría de la carpa común. En España, el interés por el cultivo de ciprínidos para consumo humano se centra en la tenca. Según datos de la FAO (2014), la producción mundial de tenca fue de 1100 t en 2012, encabezando la lista de productores Francia (500 t), seguida de la República Checa (166 t) y Alemania (161 t). En nuestro país, las particulares características de su explotación y mercado dificultan enormemente obtener datos fiables de su producción, que en 2012 se cifró en tan sólo 4t (FAO 2014), cantidad claramente insuficiente para satisfacer la demanda.

3.2. TENCA

La tenca (*Tinca tinca* L. 1758) es un pez de agua dulce que pertenece a la familia de los ciprínidos (Cyprinidae). Es una especie autóctona de Europa y Siberia, pero en la actualidad está presente en todos los continentes (Freyhoff y Kottelat 2008). En la península Ibérica, se considera que fue introducida por los romanos (Cerdá y Mulet 1992), y se cría principalmente, aunque no de forma exclusiva, en las comunidades de Extremadura y Castilla y León. Su distribución en España está más asociada a las repoblaciones realizadas en cuerpos de agua cerrados del oeste peninsular que a su desarrollo natural en ríos, donde su presencia es más bien escasa.

Foto 1. Tenca adulta.

3.2.1. Ciclo vital y características biológicas de interés productivo

La tenca es una especie demersal que habita en aguas poco profundas, con abundante vegetación y fondos limosos o fangosos como charcas, lagos y embalses; en los ríos selecciona zonas con escasa velocidad de corriente y fondos blandos. Es considerado un pez de agua caliente que presenta tolerancia a bajos niveles de oxígeno (Coad 1999) y rangos de pH entre 6,5 y 8, siendo letales valores por debajo de 4,5 y por encima de 10,8 (Lukowick y Proske 1980).

En hábitats naturales, es una especie carnívora (Kennedy y Fitzmaurice 1970) y su dieta se basa principalmente en zooplacnton (cladóceros, copépodos y ostrácodos), crustáceos bentónicos (anfípodos y decápodos), insectos bentónicos (quironómidos, efímeros, odonatos, hemípteros e hirudíneos) y moluscos (gasterópodos y pequeños bivalvos) (Michel y Oberdorff 1995, Rowe et al. 2008).

Respecto a la reproducción, aunque con un año de vida puede producir gametos fértiles (Rodríguez et al. 2004), se considera que alcanza la madurez sexual entre los dos y los cuatro años. La reproducción se inicia a finales de primavera, cuando la temperatura del agua se encuentra por encima de los 18-20ºC y se prolonga durante el verano, periodo en el que pueden tener lugar hasta 3 ó 4 oviposiciones (Morawska 1984). El número de huevos producido depende del tamaño de la hembra y varía entre 140.000 y 230.000 huevos/kg de peso vivo (Linhart y Billard 1995). Para la eyección es necesaria la presencia de vegetación sumergida donde los huevos permanecen adheridos para su posterior fecundación y desarrollo embrionario. Los huevos eclosionan a los 100 grados-día (Kennedy y Fitzmaurice 1970, Pérez-Regadera 1987), obteniéndose larvas de unos 4 mm de longitud, transparentes y con una vesícula vitelina muy alargada. Los alevines recién nacidos permanecen adheridos a la vegetación durante los primeros momentos de vida. Tras reabsorber la vesícula vitelina, su alimentación se basa en microplancton y, posteriormente, cuando alcanzan una talla aproximada de 15 mm, comienzan a alimentarse de presas de mayor tamaño como zooplancton y otros pequeños invertebrados (Kennedy y Fitzmaurice 1970). En condiciones naturales, tras el primer verano (en el mes de octubre), los juveniles pueden alcanzar una talla entre 2,5 y 10 cm (OPP 2006).

3.3. SITUACIÓN ACTUAL DEL CULTIVO DE TENCA

Dependiendo del grado de intensificación, los sistemas de cría se pueden clasificar en extensivos, semiextensivos e intensivos.

- **Extensivo.** Basado exclusivamente en la repoblación periódica con alevines en cuerpos de agua cerrados y en la recolección de los animales cuando han alcanzado la talla comercial. Se caracteriza por una producción muy variable y de bajo rendimiento. La cosecha normalmente se realiza a finales de verano-principios de otoño, cuando el volumen de agua es menor y los peces están más concentrados, lo que facilita su captura. Esta práctica productiva tradicional no requiere de importantes inversiones económicas, pero presenta numerosos inconvenientes derivados de la dependencia de las condiciones meteorológicas y la imposibilidad de control de enfermedades y depredadores.

Foto 2: Estanque de un sistema de producción extensivo.

- **Semiextensivo**. La fase reproductiva y la cría posterior se separan, utilizándose dos tipos de estanques en el proceso. Para la reproducción, se utilizan estanques con macrovegetación sumergida (*Myrophyllum* sp, *Potamogeton* sp o *Ceratophyllum* sp, etc.) sobre la que los reproductores realizan la freza. En ocasiones, se realiza un aporte de alimento suplementario a la alimentación natural.

- **Intensivo.** Su objetivo es lograr una elevada producción en el menor espacio y tiempo posibles, mediante el control de los factores con influencia en la reproducción y posterior engorde. Respecto a la reproducción, se han desarrollado técnicas eficientes que garantizan la obtención de larvas de tenca bajo condiciones controladas. Sin embargo, la cría posterior de estos animales se encuentra limitada por la escasa información sobre requerimientos nutricionales y sobre una alimentación adecuada. Por ello, su cría bajo condiciones intensivas no es posible y únicamente la investigación podría aportar conocimientos que la hagan posible.

3.4. ESTADO ACTUAL DE LA INVESTIGACIÓN EN EL CULTIVO DE TENCA: Etapas larvaria-postlarvaria y juvenil

3.4.1. Reproducción

El desarrollo de técnicas para el control de la reproducción es clave para la intensificación de los procesos de cría de tenca. Por ello, gran parte de las investigaciones desarrolladas por nuestro grupo han incidido en la fase reproductiva permitiendo obtener resultados, transferidos con éxito a centros de producción, que abarcan desarrollo gonadal de los reproductores, inducción de la ovulación, obtención de gametos, fecundación e incubación artificial y recogida de alevines vesiculados (Carral et al. 2003, Rodríguez 2003, Rodríguez et al. 2004, Aguilera et al. 2005, Rodríguez et al. 2005, Carral et al. 2006, Rodríguez et al. 2008).

Una vez garantizada la producción de larvas en condiciones controladas, los esfuerzos de investigación han de encaminarse a los factores que determinan su supervivencia y crecimiento. A continuación se expone un breve resumen de los niveles actuales de conocimientos en los diferentes aspectos estudiados:

3.4.2. Cría larvaria-postlarvaria

Las investigaciones realizadas durante esta etapa han determinado un rango de temperatura óptima que oscila entre 22ºC y 28ºC (Korwin-Kossakowski y Jezierska 1984, Peñaz et al. 1989, Aguilera 2004).

En ensayos específicos sobre los efectos de la densidad, se han probado concentraciones iniciales entre 20 y 320 animales por litro (Celada et al. 2007a). Entre 20 y 160 animales por litro no se detectaron diferencias significativas de supervivencia ni de crecimiento.

En cuanto a la alimentación, el empleo de piensos secos como único alimento resulta insatisfactorio en términos de supervivencia y crecimiento, siendo necesario el aporte de alimento natural después de la reabsorción del vitelo. De esta forma, la utilización de diferentes especies de zooplancton recolectadas de su hábitat natural (Šestáková et al 1989, Hamáčková et al. 1995, 1998, Kamler et al. 1995) ha permitido aceptables resultados. Sin embargo, la dificultad para cultivar este zooplancton ha conducido a la búsqueda de alternativas más sencillas. Los resultados han demostrado que los nauplios vivos de *Artemia* pueden ser utilizados desde el primer día de alimentación externa, obteniéndose altos porcentajes de supervivencia y buenas tasas de crecimiento en experimentos con una duración entre 20 y 60 días (Wolnicki y Mizkowski 1998, Fleig et al. 2001, Wolnicki et al. 2003a, Celada et al. 2007, 2008). Recientemente, García et al. (2011) han evidenciado que los nauplios vivos pueden ser sustituidos por quistes decapsulados de *Artemia*, mejorando incluso los valores de crecimiento. Esto ha permitido establecer un protocolo de alimentación durante la etapa larvaria-postlarvaria de la tenca basado en el uso de quistes decapsulados, de diferentes calidades y/o sometidos a procesos de conservación (Celada et al. 2013, García et al. 2014).

3.4.3. Cría de juveniles

3.4.3.1. Abastecimiento de agua, temperatura y fotoperiodo

No es necesario aportar grandes volúmenes de agua comparado con otras formas de acuicultura. Sin embargo, es evidente la necesidad de disponer de agua de buena calidad y en cantidad suficiente para mantener los parámetros químicos dentro de rangos tolerables por los animales.

El crecimiento de los peces teleósteos depende directamente de la temperatura y del fotoperíodo (Thorpe et al. 1989, Imsland et al. 1995). Por ello, ambos parámetros son manipulados habitualmente con objeto de mejorar el rendimiento, la rentabilidad y la sostenibilidad de las prácticas de cultivo.

Las investigaciones realizadas han determinado un rango de temperatura óptima que oscila entre 22ºC y 30ºC (Backiel 1986). Puesto que temperaturas elevadas pueden afectar negativamente a la calidad del agua, en los ensayos realizados por nuestro equipo se ha mantenido un rango térmico de 22-26ºC que coincide, aproximadamente, con el de la época de crecimiento en condiciones naturales.

En cuanto al fotoperiodo, existe una gran variabilidad interespecífica en la receptividad de los peces a los cambios de la luz. Así, la mayoría de las especies muestran diferente actividad de alimentación en relación con el ciclo diario de alternancia de luz/oscuridad (Boujard 2004). En la tenca, la escasa información disponible sugiere la existencia de diferencias en la actividad de alimentación dependiendo de la edad (Pyka 1997). Se considera que las tencas adultas tienen hábitos estrictamente nocturnos (Siegmund 1969, Herrero et al. 2003), pero durante las primeras etapas de vida (menos de 100 días) muestran una intensa actividad en horas diurnas (Pyka 1997). Los estudios realizados por Carral et al. (2013) aportan la primera información acerca de los efectos del fotoperiodo en la etapa juvenil. Las altas tasas de supervivencia obtenidas con los fotoperiodos probados apoyan la idea de que los juveniles de tenca son capaces de adaptarse a condiciones extremas de luz (oscuridad o luz continua); pero estas condiciones afectaron negativamente al crecimiento. El mayor crecimiento y el índice de conversión del alimento más bajo fue observado con el periodo natural extendido (16 h luz/8 h oscuridad), que coincide con el de la estación de crecimiento de la tenca.

3.4.3.2. Densidad

Las pruebas realizadas bajo condiciones controladas han utilizado densidades iniciales entre 0,13 g/L y 4,08 g/L (Quirós y Alvariño 1998, Wonicki y Myzkowski 1998, Wolnicki et al. 2003b, Quirós et al. 2003, Kamler et al. 2006, Wolnicki et al. 2006, Mareš et al. 2007, Celada et al. 2007a, 2009, Zakęś et al. 2010, Myszkowski 2010, García et al. 2010, 2013). En experimentos específicos sobre el efecto de la densidad, se evidenció que el crecimiento individual fue mayor con bajas densidades (0,18 g/L), mientras que la biomasa obtenida por unidad de volumen fue muy superior con densidades más altas (1, 3 o 4 g/L) (Celada et al. 2007b). Ambos factores han de combinarse en función de las circunstancias y teniendo en cuenta que cuanto más alta es la densidad mayores son el trabajo de limpieza y el riesgo frente a cualquier eventualidad.

3.4.3.3. Alimentación

La inexistencia de dietas específicas ha forzado al uso de piensos comerciales formulados para otras especies de peces (carpa, trucha, dorada y anguila) que, aportados como único alimento, han determinado bajas tasas de supervivencia, reducidos índices de crecimiento y altos porcentajes de deformidades corporales (Quirós y Alvariño 1998, Quirós et al. 2003, Rennert el at. 2003, Kamler et al. 2006, Wolnicki et al. 2003b, 2006, Mareš et al. 2007, Celada et al. 2009, Myszkowski et al. 2010). Se ha demostrado que la adición de alimento natural a los mencionados piensos comerciales, como *Daphnia* sp. (Quirós y Alvariño 1998, Quirós et al. 2003) o larvas de quironómidos congelados (Wolnicki et al. 2003b) mejora los resultados, alcanzándose valores máximos de crecimiento mediante la suplementación con nauplios vivos (Celada et al. 2007a, 2009) o quistes decapsulados de *Artemia* (García et al. 2010, 2014).

Respecto a la incidencia de deformidades corporales, Kamler et al. (2006), Wolnicki et al. (2006) y Myszkowski et al. (2010) han sugerido una posible relación entre el uso de dietas comerciales para otras especies y altos valores del coeficiente de condición (1,3-1,4). De este modo, los juveniles con rápido crecimiento tendrían mayor probabilidad de presentar deformidades corporales (Rennert et al. 2003, Kamler et al. 2006, Myszkowski et al. 2010). Para evitar este problema, Kamler et al. (2006) recomiendan dosis diarias por debajo de la saciedad (hasta el 2,5% de la biomasa). No obstante, los bajos porcentajes de animales con deformidades obtenidos cuando se administra alimento natural junto a dietas no específicas para tenca, independientemente del factor de condición alcanzado, apoya la idea propuesta por García et al. (2010) sobre su efecto en la prevención de la aparición de deformidades.

Tras establecer posibles protocolos de alimentación durante la etapa juvenil, García et al. (2013) propusieron una dieta práctica para juveniles en la que se incluyó una premezcla vitamínico-mineral formulada para trucha, que permitió resultados aceptables. Además, aportaron los primeros datos sobre los requerimientos proteicos para esta etapa.

Referente a la frecuencia y a la tasa de alimentación para juveniles bajo condiciones controladas, en los ensayos realizados hasta ahora se ha aportado alimento entre 1 y 5 veces al día, en cantidades que oscilaron entre 0,7% y 7% del peso vivo por día (Quirós et al. 2003, Kamler et al. 2006, Wolnicki et al. 2006, Celada et al. 2007, Mareš et al. 2007, Zakęś et al. 2010, Myszkowski et al. 2010, García et al. 2010, 2013).

3.4.4. Perspectivas de futuro

Actualmente, la principal limitación para aumentar la producción de la tenca es el déficit de juveniles con fines de engorde y repoblación. Por ello, es indispensable desarrollar técnicas de cultivo para juveniles dentro de su primer año de vida bajo condiciones controladas, centrándose principalmente en la alimentación como un factor esencial. Hasta el presente, no existe una dieta formulada específicamente para estos animales en todos sus componentes y se dispone de escasa información acerca de los niveles adecuados de macronutrientes de la dieta. Así, la presente Tesis Doctoral aborda la formulación y fabricación de una dieta específica, el estudio del contenido proteico de la dieta y la evaluación de diferentes fuentes alternativas de proteína.

4. OBJETIVOS

La finalidad de esta Tesis Doctoral es la formulación y fabricación de una dieta práctica base específica para juveniles, el estudio del contenido proteico de la dieta y la evaluación de posibilidades de sustitución de harina de pescado por fuentes alternativas de proteína en juveniles de tenca. Concretamente los objetivos son:

1. Formulación y fabricación de una dieta práctica específica para juveniles de tenca (Publicación I).

2. Determinar niveles óptimos de proteína de harina de pescado en la dieta (Publicación II).

3. Evaluar posibilidades de sustitución de proteína de pescado por proteína de pluma (Publicación I).

4. Evaluar posibilidades de sustitución de proteína de pescado por proteína de subproductos de pollo (Publicación III).

5. Evaluar posibilidades de sustitución de proteína de pescado por proteína de concentrado proteico de guisante (Publicación IV).

6. Evaluar posibilidades de sustitución de proteína de pescado por proteína de concentrado proteico de soja (Publicación V).

Estos objetivos se encuentran en el marco del proyecto del Plan Nacional de I+D+i ref. AGL2010-16554 "Alimentación de la tenca (*Tinca tinca* L.) durante las etapas larvaria, postlarvaria y juvenil en condiciones controladas". Además, están perfectamente adecuados a las prioridades del VI Plan Nacional de I+D+i, Área de Ciencias y Tecnologías Agroalimentarias y Medioambientales. Para ello, la Universidad de León concedió una Beca de Investigación.

5. MATERIAL Y MÉTODOS GENERALES

5.1. ANIMALES, INSTALACIONES Y PROCEDIMIENTO BÁSICO DE EXPERIMENTACIÓN

Los estudios que componen esta Tesis se llevaron a cabo en las instalaciones y laboratorios de acuicultura del Departamento de Producción Animal de la Universidad de León, ubicado en la Facultad de Veterinaria. Todos los procedimientos fueron aprobados por el Comité de Ética de la Universidad de León.

La especie utilizada fue *Tinca tinca* L. (1758). Durante los meses de mayo y junio, reproductores procedentes de piscifactoría, eran trasladados al laboratorio, donde mediante técnicas de reproducción artificial (Rodríguez et al. 2004) se obtenían los alevines vesiculados. El quinto día después de la eclosión de los huevos, los alevines eran trasladados a unos tanques circulares de fibra de vidrio situados en un patio exterior, con una capacidad de 2.500 L. Durante dos semanas, los animales se alimentaban con quistes decapsulados de *Artemia* (Celada et al. 2013) y, posteriormente, con una combinación de una dieta seca comercial para carpa (ALLER AQUA) y quistes decapsulados de *Artemia* hasta la edad de 5-6 meses, cuando comenzaban los experimentos.

Foto 3. Juvenil de tenca.

Los ensayos se realizaron durante 5 años consecutivos. En total, se llevaron a cabo 5 experimentos: 1 de 120 días y 4 de 90 días. En todos los casos, los juveniles procedían de al menos 3 hembras y 3 machos, y fueron distribuidos al azar entre los diferentes tratamientos. Al comienzo de las pruebas, se tomaban muestras de los juveniles que eran medidos y pesados con calibre y balanza de precisión, respectivamente.

Un pozo artesiano proporcionaba el agua utilizada en los estudios. Sus parámetros de calidad se analizaron periódicamente en el laboratorio de la Oficina Municipal del Consumidor del Ayuntamiento de León, y dichos análisis se complementaron con mediciones efectuadas en nuestro laboratorio. Los valores de las características físicoquímicas más relevantes del agua a la entrada de las instalaciones de experimentación fueron:

1. pH = 8,1

2. Dureza total = 5,3ºdH (calcio 33,1 mg/L)

3. Sólidos totales en suspensión = 35,2-39,6 mg/L

4. Sólidos disueltos totales = 112,2-115,7 mg/L

Cada dos días, se efectuaban mediciones de oxígeno disuelto, de amonio y de nitritos del agua donde se encontraban los animales. Las medidas de oxígeno en los tanques se realizaron con un oxímetro HACH HQ30d y los valores oscilaron entre 5,7 y 8,2 mg/L. Amonio y nitritos eran medidos con un espectofotómetro HACH DR 2800 a partir de muestras de agua tomadas del interior de los tanques. Los valores siempre fueron: amonio < 0,15 mg/L y nitritos < 0,02 mg/L.

Las instalaciones de experimentación se encuentran localizadas en tres recintos de la Facultad de Veterinaria. La recepción y tratamiento del agua se realizaba en depósitos de fibrocemento. Para el mantenimiento de los animales, se utilizaban tanques de fibra de vidrio aglomerada con poliéster (0,5 x 0,25 x 0,25 m) con una capacidad de 25 litros, situados en el interior de un invernadero. En los experimentos II, III y V, se distribuyeron 30 animales en cada tanque. En los experimentos I y IV, se distribuyeron 25 g de biomasa en cada tanque (1 g/L), para obtener las réplicas correspondientes a los diferentes tratamientos.

El régimen de circulación del agua fue en sistema abierto y cada tanque tenía su propia entrada de agua con un flujo continuo de 0,25 L/min y una salida provista con un filtro de malla de 250 µm (para evitar la pérdida de alimento durante los experimentos), además de una ligera aireación. La temperatura se mantuvo en 24°C a lo largo de todos los estudios utilizando resistencias blindadas de 3200 W ubicadas en los tanques de abastecimiento de agua y conectadas a sus correspondientes termostatos. Dicha temperatura se registraba diariamente con termómetros máxima-mínima en los tanques que contenían los animales. Además, los animales fueron mantenidos con un fotoperiodo conseguido con temporizadores conectados a fluorescentes (16 horas luz/ 8 horas oscuridad).

Foto 4. Vista general de los tanques.

Foto 5. Vista de un tanque.

La limpieza de los fondos de los tanques, mediante sifonado, y la de los filtros, con agua a presión, se realizaba cada dos días. Tras la finalización de cada prueba, los tanques se limpiaban, desinfectaban con lejía y se dejaban en seco.

5.2. DIETAS Y ALIMENTACIÓN

Se formuló una dieta práctica base tomando como referencia la propuesta anteriormente por este grupo, que incluía un complejo vitamínico-mineral para trucha arco iris (García et al. 2013). Procesos de experimentación posteriores aportaron conocimientos que han permitido mejorar dicha dieta en la formulación de sus ingredientes. También, se llegó a la formulación de una premezcla de vitaminas y minerales mejor adaptada para juveniles de tenca. Esta dieta base fue utilizada como referencia para determinar el nivel de proteína óptimo (estudio II) y realizar sustituciones de la proteína de pescado por fuentes alternativas de proteína en los estudios I, III, IV y V. Las dietas eran casi isoproteicas e isoenergéticas en cada ensayo.

Foto 6. Dieta práctica.

Para la fabricación de las dietas, se utilizó un molino rotatorio BRABENDER para la molienda y una mezcladora STEPHAN UMC5 para mezclar los ingredientes. La mezcla resultante, con aproximadamente un 15-20% de humedad, era sometida a un proceso de extrusión en una extrusora BRABENDER KE19/25D. Los pellets obtenidos, con un diámetro de 1 mm, se secaban en una campana de flujo laminar durante 24 h a una temperatura aproximada de 30 ºC y después recubiertos con aceite de hígado de bacalao. Una vez finalizado el proceso, las dietas se almacenaban a 3-4 ºC hasta su aporte a los animales.

Foto 7. Molino BRABENDER.

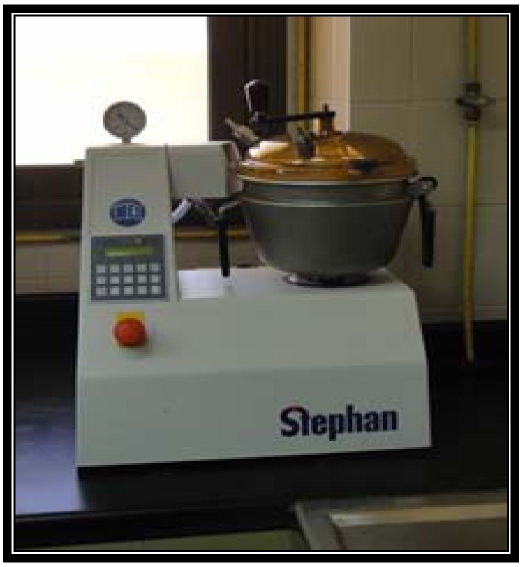

Foto 8. Mezcladora STEPHAN UMC5.

Foto 9. Extrusora BRABENDER KE19/25D.

La frecuencia de alimentación fue 4 veces al día, en cantidad suficiente para que fuera ligeramente en exceso (3% del peso vivo, valor ajustado durante las pruebas tomando como base los datos de biomasa registrados cada 30 días). Las dietas fueron suministradas manualmente (experimentos I, III, IV y V) o con comederos automáticos “Automated Microdiet Dispenser AMD^{TM}” (experimento II).

Foto 10. Vista general de los comederos automáticos.

5.2.1. Análisis químicos de dietas y animales

Una vez fabricado cada pienso, se tomaba una muestra para analizar su composición. Al comienzo y al final de los estudios II, III, IV y V, se tomó una muestra de juveniles de los distintos tratamientos para ser analizados posteriormente. Los peces se mantuvieron en ayunas durante 14 horas antes de tomar las muestras, que eran almacenadas a -30ºC hasta el análisis. Todos los análisis se realizaron por duplicado.

El contenido de macronutrientes de dietas y animales fue analizado según las normas de la Organización Internacional de Normalización:

1. Humedad: ISO R-1442 (ISO 1979).
2. Proteína: ISO R-937 (ISO 1978).
3. Lípidos: ISO R-1443 (ISO 1973).
4. Cenizas: ISO R-936 (ISO 1998).
5. Energía: ISO 9831 (ISO 1998b).
6. El contenido de hidratos de carbono se obtuvo por diferencia, restando el contenido de humedad, proteínas, lípidos y cenizas del peso total.

Para analizar los aminoácidos esenciales y los aminoácidos no esenciales, se realizó una cromatografía líquida de alta eficacia (HPLC) usando el método de AccQTag de Waters. Los aminoácidos fueron derivatizados con el reactivo 6-aminoquinolil-N-hydrosyccinimidyl-carbamato (AQC) por el método de Cohen y Michaud (1993) y Cohen y De Antonis (1994), y fueron detectados por el detector de absorbancia Waters 2487 Dual λ Absorbance Detector a 254 nm. La cuantificación se llevó a cabo con el software Empower Pro 2.0.

5.3. RECOGIDA DE DATOS Y ANÁLISIS ESTADÍSTICO

Cada 30 días, se realizaron muestreos intermedios tomando una muestra representativa de animales de cada réplica (entre 20-50% del total de animales de cada tanque) para registrar longitud total (LT) y peso (P) de cada individuo. Para facilitar el manejo y minimizar los daños de los juveniles, se anestesiaban con metasulfato de tricaína MS-222 (Ortoquímica S.L, Barcelona, España).

Al final de cada experimento, se cuantificaron los resultados mediante el registro de los siguientes parámetros:

1. Longitud total (LT).
2. Peso (P).
3. Número de supervivientes.

4. Número de animales con deformidades externamente visibles.

Con los resultados obtenidos de los análisis y experimentos se calcularon los siguientes índices:

1. Porcentaje de supervivencia.

2. Tasa de crecimiento específico (TCE) = expresa el crecimiento diario en forma de porcentaje según la fórmula: (Ln Peso final (g) – Ln Peso inicial (g)) 100/ días.

3. Índice de conversión del alimento (IC) = Alimento suministrado (g)/ (Peso final (g) – Peso inicial (g)).

4. Factor de condición (K) = Peso (mg)/ Longitud total (mm)^3.

5. Valor productivo de la proteína (VPP) = 100 (((Peso final (g) x concentración final de proteína corporal (g)) – (Peso inicial (g) x concentración inicial de proteína corporal (g)))/ (concentración de proteína en la dieta (g) x total de comida aportada (g)).

6. Porcentaje de peces deformes. Al final de cada experimento, se observaban uno por uno todos los peces usando una lupa para detectar deformidades externamente visibles.

La longitud total de cada individuo se midió con un calibre digital Sylvac (±0,01 mm) y el registro del peso se realizó con una balanza de precisión COBOS M-150-SX (±0,001 g), previa eliminación del agua retenida con papel absorbente.

Diariamente, se inspeccionaban cuidadosamente los tanques para verificar el correcto mantenimiento de las condiciones de experimentación, así como para retirar y anotar los animales muertos.

Todas las dietas fueron probadas por triplicado (tres tanques por tratamiento), y la unidad experimental era cada tanque. Para la realización de los estudios estadísticos, se utilizó el programa SPSS (SPSS Inc. Chicago, IIL., USA). Las comparaciones entre los tratamientos de los diferentes experimentos fueron realizadas mediante un análisis de varianza (ANOVA). La comparación de medias se llevó a cabo por el método Duncan (experimentos II, III, IV y V) o por el método Tukey (experimento I), y el nivel de significación establecido fue siempre $P < 0,05$. Los porcentajes fueron transformados al arcoseno previamente a los análisis estadísticos. El valor de las medias en cada tratamiento se acompaña de ± E.E.M. (error estándar de la media).

Foto 11. Registro del peso y medición de longitud total.

6. SECUENCIA DE EXPERIMENTACIÓN

6.1. ESTUDIO I: EVALUACIÓN DE UNA DIETA PRÁCTICA PARA JUVENILES DE TENCA Y POSIBILIDADES DE SUSTITUCIÓN DE HARINA DE PESCADO POR HARINA DE PLUMA

Evaluation of a practical diet for juvenile tench (*Tinca tinca* L.) and substitution possibilities of fish meal by feather meal.

Á. González-Rodríguez, J.D. Celada, J.M. Carral, M. Sáez-Royuela, J.B. Fuertes 2013

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6.1.1. Planteamiento experimental

Uno de los mayores obstáculos para la intensificación de la cría de la tenca es el déficit de juveniles destinados al engorde y la repoblación. Por ello, es indispensable desarrollar técnicas para su cría bajo condiciones controladas, centrándose en la alimentación como un factor esencial.

La acuicultura es altamente dependiente de las capturas para suministrar harina de pescado (FM), el ingrediente proteico más importante en alimentos para animales acuáticos (Tacon y Metian 2008). Así, la creciente demanda de harina de pescado hace insostenible la presión pesquera sobre las poblaciones salvajes (Hanneson 2003, Naylor et al. 2009) y provoca un incremento de su precio (Tacon y Metian 2008, FAO 2009). Por ello, se plantea la necesidad de buscar fuentes alternativas de proteínas (Naylor et al. 2009, Hardy 2010). Dentro de las fuentes proteicas de origen animal permitidas por la legislación se encuentra la harina de pluma (FEM), un ingrediente barato cuya incorporación en piensos formulados para animales acuáticos es creciente (Poppi et al. 2011). Los efectos de su inclusión en la dieta se ha estudiado en diferentes especies piscícolas, como salmón real, *Oncorhynchus tshawytscha* (Fowler 1990), lenguado arenero, *Paralichthys olivaceus* (Kikuchi et al. 1994), labeo roho, *Labeo rohita* (Hasan et al. 1997) y carpa común, *C. carpio* (Jahan et al. 2001), mostrando variaciones según la especie, con niveles de inclusión óptimos que oscilan entre 104 y 150 g FEM/kg de dieta. El objetivo de este estudio fue la formulación y fabricación de una dieta práctica base para juveniles *T. tinca* y a partir de ella se evaluaron posibilidades de sustitución de proteína de pescado por proteína de pluma. Para ello, se diseñó la siguiente prueba:

Experimento I: Se formuló una dieta práctica base tomando como referencia la propuesta anteriormente por este grupo, que incluía un complejo vitamínico-mineral para trucha arco iris (García et al. 2013). Procesos de experimentación posteriores aportaron conocimientos que han permitido mejorar dicha dieta en la formulación de sus ingredientes. También, se llegó a la formulación de una premezcla de vitaminas y minerales mejor adaptada para juveniles de tenca. Sobre esta dieta base, con un contenido proteico del 50%, se realizaron sustituciones de proteína de pescado por proteína de pluma del 25% y 35%, que correspondían a 148 y 210 g FEM/kg de dieta, respectivamente. El nivel máximo de sustitución de proteína de pescado por proteína de pluma fue elegido teniendo en cuenta las recomendaciones de Yu (2008). La harina de pluma fue proporcionada por la empresa COREN (Santa Cruz de Arrabaldo, Orense) y se obtuvo mediante cocción bajo presión de plumas de aves de corral, que posteriormente eran molidas y secadas. La composición proximal y el perfil de aminoácidos de las harinas se muestran en la tabla 1. La formulación y composición proximal de las dietas se presentan en la tabla 2 y su perfil de aminoácidos en la tabla 3.

Tabla 1. (Experimento I) Composición proximal y perfil de aminoácidos de la harina de pescado (FM) y la harina de pluma (FEM) (g/kg).

	FM	FEM
Composición		
Humedad	79,9	53,3
Proteína bruta	680,0	761,0
Lípidos	90,1	158,7
Cenizas	150,0	3,4
Aminoácidos esenciales		
Arginina	113,8	62,5
Histidina	12,7	42,1
Isoleucina	25,6	26,5
Leucina	44,2	26,8
Lisina	44,0	11,2
Metionina	19,7	7,2
Fenilalanina	19,3	24,9
Treonina	36,3	29,5
Triptófano	0,1	0,1
Valina	30,1	55,8
Aminoácidos no esenciales		
Alanina	38,2	34,1
Aspartato	51,3	89,8
Cisteína	1,1	12,1
Glutamato	79,9	93,3
Glicina	24,0	20,3
Prolina	24,7	64,5
Serina	36,9	86,4
Tirosina	14,0	8,7

Tabla 2. (Experimento I) Formulación y composición proximal de las dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de pluma (g/kg).

	Nivel de sustitución (%)		
	0 (dieta base)	25	35
Ingredientes			
Harina de pescado [a]	662	490	422,6
Harina de pluma [b]	0	148	210
Harina de maíz [c]	121	147	153,4
Aceite de hígado de bacalao [d]	20	20	20
Quistes de *Artemia* decapsulados [e]	100	100	100
Fosfato dicálcico [f]	10	10	10
Cloruro de colina [f]	2	2	2
L-ascorbil-2-monofosfato-Na [f]	10	10	10
Lecitina de soja [g]	5	3	2
Carboximetilcelulosa [h]	30	30	30
Premezcla de minerales [i]	20	20	20
Premezcla de vitaminas [j]	20	20	20
Composición			
Humedad	74,0	70,0	68,0
Proteína bruta	502,2	501,7	502,3
Lípidos	108,6	113,1	114,2
Cenizas	170,4	150,2	143,4
Energía bruta (MJ/kg)	19,0	19,1	19,4

[a] BioMar Iberia / ProAqua Nutrición S.A., Dueñas (Palencia), España.
[b] COREN, Santa Cruz de Arrabaldo (Orense), España.
[c] ADPAN, Siero (Asturias), España.
[d] ACOFARMA, Terrassa (Barcelona), España.
[e] INVE Aquaculture Nutrition, High HUFA 430µ, Hoogyeld 91, Dendermonde, Belgyum.
[f] NUTRAL S.A, Colmenar Viejo (Madrid), España.
[g] BIOVER NV/SA, Brujas (Bélgica).
[h] HELM IBERICA S.A., Alcobendas (Madrid), España.
[i] (mg/kg dieta): $MgSO_4$-$7H_2O$, 500; $ZnSO_4$-$7H_2O$, 25; $MnSO_4$-H_2O, 13; $CuSO_4$-$5H_2O$, 3; $CoSO_4$, 0,1; $FeSO_4$-$7H_2O$, 150; KI, 2,5; Na_2SeO_3, 0,3.
[j] (mg/kg dieta): Mioinositol, 500; tiamina, 5; riboflavina, 8; niacina, 50; piridoxina, 15; ácido pantoténico, 50; biotina, 1,5; ácido fólico, 35; cianocobalamina, 0,05; retinol, 10; α-tocoferol, 300; colecalciferol, 0,0625; naftoquinonas, 50; ethoxiquina, 700.

Tabla 3. (Experimento I) Perfil de aminoácidos de las dietas prácticas con diferentes niveles de sustitución de la proteína de pescado por proteína de pluma (g/kg dieta).

	Nivel de sustitución (%)		
	0	25	35
Aminoácidos esenciales			
Arginina	88,3	79,9	72,1
Histidina	11,6	14,8	16,1
Isoleucina	19,0	18,6	18,3
Leucina	35,0	34,1	33,6
Lisina	39,8	34,7	31,7
Metionina	13,2	10,9	10,1
Fenilalanina	15,0	15,4	15,2
Treonina	22,0	21,4	20,9
Triptófano	0,2	0,2	0,2
Valina	25,2	27,9	28,2
Aminoácidos no esenciales			
Alanina	31,5	30,4	29,6
Aspartato	40,5	45,0	45,8
Cisteína	1,1	2,7	3,5
Glutamato	63,3	63,7	62,4
Glicina	16,2	14,9	14,2
Prolina	18,3	23,5	25,3
Serina	24,3	30,7	32,1
Tirosina	10,1	9,0	8,4

Se utilizaron 932 juveniles de tenca de 5 meses de edad (27,45 ± 0,47 mm LT y 0,308 ± 0.011 g P (n = 120)), que fueron distribuidos aleatoriamente en 9 tanques de fibra de vidrio con una capacidad de 25 litros de agua. En cada tanque se alojaron 25 g de tencas para obtener las réplicas correspondientes a los diferentes tratamientos (densidad = 1 g/l). El número medio de animales por tanque fue 103 ± 1,6 (media ± error estándar de la media). Cada dieta fue probada por triplicado (tres tanques por tratamiento) durante 120 días. Las dietas fueron suministradas manualmente 4 veces al día. Cada 30 días, se realizaron medidas de longitud y peso sobre 20 juveniles de cada réplica (aproximadamente un 20% del total). En el muestreo final (120 días), se realizaron las medidas mencionadas sobre 40 animales de cada réplica, se procedió al recuento de supervivientes y a la observación individualizada de todos los juveniles con una lupa con objeto de detectar deformidades externamente visibles.

6.1.2. Resultados

Los juveniles ingirieron todas las dietas prácticas desde el inicio del experimento. Los valores de supervivencia, crecimiento y porcentaje de peces deformes al final del experimento se muestran en la tabla 4. El porcentaje de supervivencia varió entre 96% y 97%, sin diferencias significativas entre tratamientos. El mayor crecimiento (59,3 mm LT, 2,6 g P y 1,74 %/día TCE) correspondió a los juveniles alimentados con la dieta base (0% de sustitución) y fue significativamente mayor que el de los alimentados con las dietas con 25% y 35% de sustitución de proteína de pescado por proteína de pluma (148 y 210 g FEM/kg dieta, respectivamente), siendo las diferencias significativas a partir del día 60.

El índice de conversión del alimento (IC) no presentó diferencias significativas los primeros 90 días (Tabla 5), pero al final (120 días) fue significativamente menor en los peces alimentados con la dieta base que en los alimentados con las dietas que incluían harina de pluma (1,36 vs 1,47; Tabla 4). El factor de condición (K) osciló entre 1,22 (dieta base) y 1,29 (35% de sustitución). El porcentaje de peces deformes con las dietas que incluían harina de pluma (media: 15%) fue significativamente mayor que con la dieta base (3%). Las deformidades afectaron a la columna vertebral y al pedúnculo caudal.

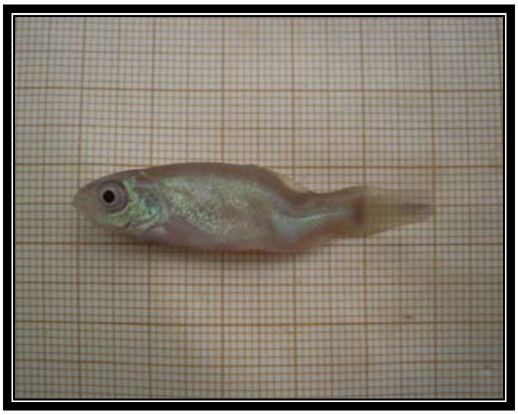

Foto 11. Juvenil con deformidad en columna vertebral.

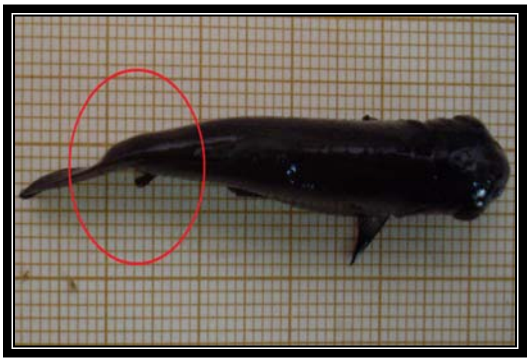

Foto 12. Juvenil con deformidad en pedúnculo caudal.

El perfil de aminoácidos de las dietas prácticas se presenta en la tabla 3. No se encontraron diferencias en el contenido de isoleucina, leucina, fenilalanina, treonina, triptófano y valina. La inclusión de harina de pluma dio lugar a un mayor contenido de histidina, pero los contenidos de arginina, lisina y metionina fueron significativamente más bajos que los presentes en la dieta base. Con el 25% de sustitución de proteína de pescado, el contenido de los mencionados aminoácidos se redujo 9,5% (arginina), 12,9% (lisina) y 17,8% (metionina).

Tabla 4. (Experimento I) Valores finales de supervivencia, crecimiento y porcentaje de peces deformes de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de pluma durante 120 días.

	Nivel de sustitución (%)			EEM[a]	*P* valor
	0	25	35		
Supervivencia (%)	97	97	96	0,129	0,061
Longitud total (mm)	59,3[a]	56,3[b]	54,4[c]	0,252	<0,001
Peso (g)	2,6[a]	2,2[b]	2,1[b]	0,033	<0,001
TCE	1,74[a]	1,62[b]	1,58[b]	0,012	<0,001
Alimento consumido	3,07[a]	2,76[b]	2,56[c]	0,013	<0,001
IC	1,36[a]	1,48[b]	1,46[b]	0,024	0,032
K	1,22[a]	1,24[a]	1,29[b]	0,009	0,002
Peces deformes (%)	3[a]	16[b]	14[b]	0,330	<0,001

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).
[a] Error estándar de la media agrupado.

Tabla 5. (Experimento I) Longitud total, peso e índice de conversión (IC) de los juveniles de tenca alimentados con diferente niveles de sustitución de proteína de pescado por proteína de pluma durante los primeros 90 días.

		Nivel de sustitución (%)			EEM [a]	*P* valor
Día		0	25	35		
30	Longitud total (mm)	34,97	35,20	34,46	0,162	0,162
	Peso (g)	0,48	0,51	0,47	0,008	0,101
	IC	0,88	0,78	0,97	0,053	0,370
60	Longitud total (mm)	$44,73^a$	$43,11^b$	$40,78^b$	0,300	<0,001
	Peso (g)	$1,05^a$	$0,94^b$	$0,82^b$	0,018	<0,001
	IC	0,90	0,97	1,05	0,031	0,145
90	Longitud total (mm)	$53,89^a$	$50,96^b$	$49,59^c$	0,291	<0,001
	Peso (g)	$1,90^a$	$1,62^b$	$1,50^b$	0,030	<0,001
	IC	0,95	1,06	1,10	0,031	0,105

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).
[a] Error estándar de la media agrupado.

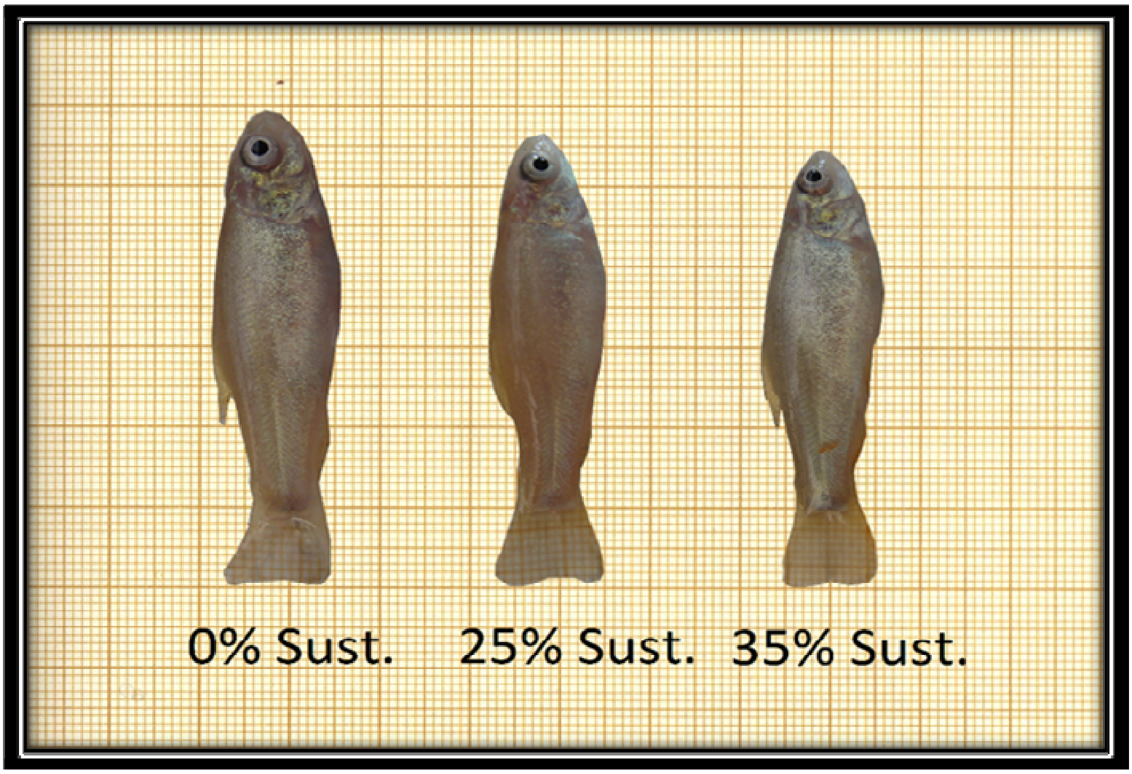

Foto 13. Juveniles alimentados con dietas con diferente nivel de sustitución de proteína de pescado por proteína de harina de pluma al final del experimento I.

6.1.3. Discusión

La mayoría de los estudios sobre intensificación de la cría de juveniles de tenca se han llevado a cabo con peces entre 3 y 7 meses de edad, y las tasas de crecimiento variaron entre 0,70 y 1,98, siendo mayores cuando las dietas secas formuladas para otras especies fueron suplementadas con alimento natural (García et al. 2010). En el estudio I, juveniles de 5 meses fueron alimentados con una dieta práctica base como único alimento y la tasa de crecimiento específico fue 1,74. Por otro lado, el índice de conversión de los peces alimentados con la dieta base fue 1,36, en el rango de los estimados (entre 0,8 y 1,5) para otras especies cuya cría intensiva está consolidada (Hardy y Barrows 2002), e inferior a los registrados en trabajos previos sobre cría intensiva de juveniles de tenca, cuyo IC osciló entre 1,75 y 4,15 (Rennert et al. 2003, Mareš et al. 2007). Por tanto, es posible utilizar con éxito la dieta extrusionada propuesta sin adición de suplemento natural.

El crecimiento de los peces alimentados con la dieta del 25% de sustitución de proteína de pescado por proteína de pluma fue significativamente menor que el de los alimentados con la dieta base (0% de sustitución). En diferentes especies piscícolas, se ha evidenciado que la deficiencia de determinados aminoácidos esenciales provoca una reducción del crecimiento. Este es el caso de la arginina para la carpa común, *C. carpio* (NRC 2011), y de arginina y lisina para el salmón real, *O. tshawytscha* (NRC 1993), y la carpa mrigal, *Cirrhinus mrigala* (Ahmed y Khan 2004a,b). En este sentido, la dieta con un nivel de sustitución del 25% presentó contenidos de arginina, lisina y metionina significativamente menores que la dieta base (Tabla 3). Los contenidos de arginina y lisina fueron 79,9 y 34,7 g/kg de dieta, respectivamente. A pesar de que estas cantidades fueron superiores a los requerimientos de las especies anteriormente mencionadas, el crecimiento de los juveniles de tenca fue afectado negativamente. Considerando los aminoácidos esenciales, únicamente la metionina (10,9 g/kg de dieta) estuvo por debajo de los requerimientos para salmón real, *O. tshawytscha* (NRC 1993), carpa común, *C. carpio* (NRC 1993), y trucha arco iris, *Oncorhinchus mykiss* (Bae et al. 2011).

6.2. ESTUDIO II: EFECTOS DE VARIOS NIVELES DE PROTEÍNA EN DIETAS PRÁCTICAS SOBRE SUPERVIVENCIA, CRECIMIENTO, UTILIZACIÓN DEL ALIMENTO Y COMPOSICIÓN CORPORAL

Effects of varying protein level in practical diets on survival, growth, feed utilization and body composition of juvenile tench (*Tinca tinca* L.)

Á. González-Rodríguez, J.D. Celada, J.M. Carral, M. Sáez-Royuela, J.B. Fuertes 2014

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6.2.1. Planteamiento experimental

En la búsqueda de piensos piscícolas comerciales eficientes, de bajo coste y respetuosos con el medioambiente, la proteína es considerada el componente más importante. El coste de una dieta seca generalmente aumenta a medida que lo hace su contenido proteico. Además, si la cantidad de proteína aportada está por encima del requerimiento de los peces, ésta es metabolizada y utilizada con fines energéticos generando un aumento de residuos nitrogenados excretados debido al exceso de proteína suministrada (Hardy y Gatlin 2002). En consecuencia, por razones económicas y medioambientales, la determinación del nivel mínimo de proteína en dieta que permita un crecimiento óptimo es un factor clave en la alimentación de animales acuáticos. Los requerimientos de proteína de juveniles han sido estudiados en varias especies de peces carnívoros de agua dulce y los contenidos óptimos varían entre 36% y 54,9% (Webster et al.1997, Gunasekera et al. 2000, Portz et al. 2001, Martínez-Palacios et al. 2007, Schulz et al. 2007a, NRC 2011). Teniendo en cuenta que el nivel óptimo de proteína no se ha establecido para juveniles de tenca, el objetivo del estudio II fue evaluar los efectos de dietas prácticas con diferentes contenidos proteicos sobre supervivencia, crecimiento, utilización del alimento y composición corporal.

Experimento II. Se fabricaron seis dietas prácticas con diferente contenido proteico (40%, 44%, 48%, 52%, 56% y 60%). Los distintos niveles de proteína se obtuvieron mediante aumento de la cantidad de harina de pescado y reducción de la harina de maíz. La formulación y la composición proximal de las dietas se muestran en la tabla 6 y el perfil de aminoácidos en la tabla 7. Como referencia, otro grupo de animales (3 réplicas) se alimentó con un pienso comercial para carpa (ALLER AQUA, producido por Aller Aqua A/S Allervej, Christiansfeld, Denmark. Composición: humedad 71,4 g/kg, proteína bruta 638 g/kg, lípidos 121,2 g/kg, carbohidratos 44,9 g/kg, cenizas 124,5 g/kg, energía bruta 20,9 MJ/kg, diámetro de pellet 0,9-1,6 mm).

Tabla 6. (Experimento II) Formulación y composición proximal de las dietas prácticas con diferente contenido proteico (g/kg).

	Contenido proteico (%)					
	40	44	48	52	56	60
Ingredientes						
Harina de pescado [a]	506	566	626	686	746	806
Harina de maiz [b]	295	235	180	130	75	25
Aceite de hígado de bacalao [c]	40	40	35	25	20	10
Quistes de *Artemia* decapsulados [d]	100	100	100	100	100	100
Carboximetilcelulosa [e]	30	30	30	30	30	30
L-ascorbil-2-monofosfato-Na [f]	5	5	5	5	5	5
Fosfato dicálcico [f]	10	10	10	10	10	10
Cloruro de colina [f]	3	3	3	3	3	3
Cloruro de sodio [g]	1	1	1	1	1	1
Premezcla vitamínico-mineral [h]	10	10	10	10	10	10
Composición						
Humedad	79,1	75,9	74,1	75,0	74,3	73,0
Proteína bruta	402,8	440,8	481,6	520,7	559,6	601,9
Lípidos	115,0	118,0	116,1	109,5	106,6	101,0
Carbohidratos	289,8	243,0	198,0	156,8	112,5	70,3
Cenizas	113,3	122,3	130,2	138,0	147,0	153,8
Energía bruta (MJ/kg)	19,2	19,3	19,5	19,4	19,5	19,5

[a] BioMar Iberia/ProAqua Nutrición S.A., Dueñas (Palencia), España.
[b] Adpan Europa S.L., Siero (Asturias), Spain.
[c] ACOFARMA, Terrassa (Barcelona), España.
[d] INVE Aquaculture Nutrition, High HUFA 430μ, Hoogyeld 91, Dendermonde, Belgium.
[e] HELM IBERICA S.A., Alcobendas (Madrid), España.
[f] NUTRAL S.A, Colmenar Viejo (Madrid), España.
[g] Unión Salinera de España S.A., Madrid, Spain.
[h] (mg/kg premezcla): inositol, 50000; tiamina, 500; riboflavina, 800; niacina, 5000; piridoxina, 1500; ácido pantoténico, 5000; biotina, 150; ácido fólico, 3500; cianocobalamina, 5; retinol, 2400; α-tocoferol, 30000; colecalciferol, 6,25; naftoquinonas, 5000; etoxiquina, 70000; $MgSO_4$-$7H_2O$, 300000; $ZnSO_4$-$7H_2O$, 11000; $MnSO_4$-H_2O, 4000; $CuSO_4$-$5H_2O$, 1180; $CoSO_4$, 26; $FeSO_4$-$7H_2O$, 77400; KI, 340; Na_2SeO_3, 68.

Tabla 7. (Experimento II) Perfil de aminoácidos de las dietas prácticas con diferente contenido proteico (g/kg dieta).

	Contenido proteico (%)					
	40	44	48	52	56	60
Aminoácidos esenciales						
Arginina	55,70[a]	64,48[b]	71,00[c]	79,20[d]	86,78[e]	94,57[f]
Histidina	6,38[a]	7,55[b]	8,82[c]	9,56[d]	10,74[e]	12,23[f]
Isoleucina	19,09[a]	21,08[b]	23,69[c]	25,07[d]	26,67[e]	27,77[f]
Leucina	34,33[a]	37,12[a]	41,28[b]	44,71[c]	47,71[c]	51,51[d]
Lisina	37,04[a]	40,53[b]	43,46[c]	46,76[d]	49,85[e]	51,54[f]
Metionina	9,21[a]	9,89[b]	10,65[c]	11,32[d]	12,25[e]	13,02[f]
Fenilalanina	15,76[a]	18,55[b]	20,20[c]	21,70[d]	23,21[e]	25,32[f]
Treonina	20,13[a]	22,02[b]	24,06[c]	26,21[d]	27,67[e]	28,07[f]
Triptófano	2,28[a]	2,51[a,b]	2,70[b,c]	2,82[b,c]	2,88[c]	3,05[c]
Valina	22,71[a]	23,20[a]	25,22[b]	26,78[b]	27,73[c]	28,32[c]
Aminoácidos no esenciales						
Alanina	32,15[a]	34,30[b]	36,58[c]	38,81[d]	40,29[e]	41,53[f]
Aspartato	36,15[a]	39,67[b]	43,36[c]	47,19[d]	51,82[e]	55,60[f]
Cisteína	1,30[a]	1,52[a,b]	1,62[b]	1,73[b,c]	1,94[c,d]	2,05[d]
Glutamato	41,05[a]	44,53[b]	47,83[c]	50,93[d]	53,89[e]	57,71[f]
Glicina	13,79[a]	14,88[b]	15,90[c]	17,23[d]	19,39[e]	20,92[f]
Prolina	17,03[a]	18,74[b]	20,25[c]	22,11[d]	23,27[e]	24,54[f]
Serina	24,09[a]	26,38[b]	29,27[c]	31,57[d]	33,84[e]	36,06[f]
Tirosina	11,05[a]	12,66[b]	13,93[c]	14,59[d]	15,23[e]	16,35[f]

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

Se utilizaron 630 juveniles de 6 meses de edad (34,35 ± 0,38 mm LT y 0,411 ± 0,015 g P (n = 120)), que fueron distribuidos aleatoriamente en 21 tanques de fibra de vidrio con una capacidad de 25 litros de agua. En cada tanque se alojaron 30 juveniles para obtener las réplicas correspondientes a los diferentes tratamientos. Cada dieta fue probada por triplicado (tres tanques por tratamiento) durante 90 días. Las dietas fueron suministradas mediante comederos automáticos (Automated Microdiet Dispenser AMD^{TM}) 4 veces al día.

Cada 30 días, se realizaron medidas de longitud y peso sobre 15 juveniles de cada réplica (50% del total). En el muestreo final (90 días), se realizaron las medidas mencionadas sobre todos los animales, se procedió al recuento de supervivientes y a la observación individualizada de todos los juveniles con una lupa con objeto de detectar deformidades externamente visibles.

Al comienzo y al final del estudio, se tomó una muestra de juveniles que habían recibido el mismo tratamiento, para ser analizados posteriormente.

6.2.2. Resultados

Los juveniles ingirieron todas las dietas prácticas desde el inicio del experimento. Los valores finales de supervivencia, crecimiento, utilización del alimento y porcentaje de peces deformes se muestran en la tabla 8. El porcentaje de supervivencia varió entre 96% y 100% sin diferencias entre tratamientos.

En cuanto al crecimiento, el 52% de proteína permitió los valores más altos

(55,49 mm LT, 2,11 g P, 1,80%/día TCE), sin diferencias significativas con el 48%. Con niveles proteicos inferiores (40% o 44%) o superiores (56% o 60%), los valores de crecimiento se redujeron significativamente. Los gráficos 1 y 2 muestran los cambios en peso y longitud, respectivamente, a lo largo de los 90 días de prueba. Considerando los diferentes muestreos, sólo hubo diferencias significativas al final del experimento (día 90). El análisis de regresión de segundo orden, basado en la tasa de crecimiento específico, indica que el nivel de proteína óptimo para un crecimiento máximo podría ser 52,7% (gráfico 3).

El factor de condición (K) osciló entre 1,20 y 1,26 con las dietas prácticas. Los peces alimentados con la dieta seca para carpa tuvieron el factor de condición más alto (1,34). El índice de conversión del alimento (IC) varió entre 1,61 y 1,95, y el menor correspondió al nivel proteico de 52% sin diferencias significativas con el 48%. El valor productivo de la proteína (VPP) varió entre 15,64 y 22,01, siendo el de los peces alimentados con los niveles entre 40% y 52% de proteína (media: 21,28) significativamente mayor que el de los alimentados con niveles más altos (56% y 60%). Al final del experimento, el porcentaje de peces deformes fue bajo con todas las dietas prácticas, oscilando entre 1,1% y 4,4%. Las deformidades afectaron a la columna vertebral y al pedúnculo caudal.

El perfil de aminoácidos de las dietas prácticas se presenta en la tabla 7. Comparando el nivel de 52% de proteína con el 48%, se redujo el contenido de todos los aminoácidos esenciales, que variaron entre 4,25% (valina) y 10,35% (arginina); sin embargo, el crecimiento no fue afectado. Entre 48% y 44% de proteína, la reducción del contenido de aminoácidos esenciales osciló entre 6,74% (lisina) y 14,40% (histidina), y el crecimiento disminuyó significativamente. Con niveles de proteína por encima de 52%, el crecimiento no mejoró a pesar del aumento del contenido de todos los aminoácidos esenciales.

La composición proximal y el perfil de aminoácidos del cuerpo de los juveniles de tenca al inicio y al final del experimento se muestran en la tabla 9. No hubo diferencias significativas en el contenido de humedad, lípidos y cenizas entre los peces alimentados con las dietas prácticas. El contenido proteico de los peces alimentados con los niveles de proteína más altos (52%, 56% y 60%) fue significativamente mayor que el de los alimentados con los niveles de proteína más bajos (40%, 44% y 48%). En cuanto al perfil de aminoácidos en el cuerpo de los juveniles, el contenido de todos los aminoácidos aumentó hasta el nivel de 52% de proteína en dieta, y se mantuvo sin diferencias significativas con niveles proteicos más altos (56% y 60%).

Tabla 8. (Experimento II) Valores finales de supervivencia, crecimiento, utilización del alimento y porcentaje de peces deformes de juveniles alimentados con dietas prácticas con diferentes contenidos proteicos y una dieta seca comercial para carpa durante 90 días.

	Contenido proteico (%)						
	40	44	48	52	56	60	CS
Supervivencia (%)	96,7 ± 0,3	98,9 ± 0,2	96,7 ± 0,3	100 ± 0,0	97,8 ± 0,2	100 ± 0,0	100 ± 0,0
Longitud total (mm)	51,64 ± 0,37^{a}	51,94 ± 0,36^{a}	54,74 ± 0,36^{b,c}	55,49 ± 0,22^{c}	53,83 ± 0,37^{b,d}	53,28 ± 0,26^{d}	52,24 ± 0,21^{a}
Peso (g)	1,70 ± 0,05^{a}	1,73 ± 0,05^{a}	2,03 ± 0,05^{b}	2,11 ± 0,04^{b}	1,99 ± 0,05^{b,c}	1,88 ± 0,03^{c}	1,92 ± 0,03^{b,c}
TCE (%/day)	1,54 ± 0,03^{a}	1,56 ± 0,03^{a}	1,74 ± 0,03^{b,c}	1,80 ± 0,02^{c}	1,72 ± 0,03^{b}	1,67 ± 0,02^{b}	1,70 ± 0,02^{b}
K	1,20 ± 0,01^{a}	1,21 ± 0,01^{a,b}	1,21 ± 0,01^{a,b}	1,23 ± 0,01^{a,b}	1,26 ± 0,01^{c}	1,24 ± 0,01^{b,c}	1,34 ± 0,01^{d}
IC	1,95 ± 0,07^{a}	1,91 ± 0,06^{a}	1,71 ± 0,05^{b,c}	1,61 ± 0,04^{b}	1,79 ± 0,06^{a,c}	1,83 ± 0,04^{a,c}	1,71 ± 0,03^{b,c}
VPP	22,01 ± 0,78^{a}	21,07 ± 0,71^{a}	21,47 ± 0,70^{a}	20,57 ± 0,44^{a}	17,94 ± 0,54^{b}	15,64 ± 0,30^{c}	15,06 ± 0,26^{c}
Peces deformes (%)	4,4 ± 0,1^{a}	3,3 ± 0,2^{b}	2,2 ± 0,2^{c,d}	1,1 ± 0,2^{c}	2,2 ± 0,2^{c,d}	1,1 ± 0,2^{c}	47,8 ± 1,2^{e}

Los valores son media ± error estándar.
Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

Tabla 9. (Experimento II) Composición proximal y perfil de aminoácidos del cuerpo de juveniles alimentados con dietas prácticas con diferentes contenidos proteicos durante 90 días (g/kg).

	Inicial	Contenido proteico (%)					
		40	44	48	52	56	60
Composición							
Humedad	*772,9*	762,9	757,5	753,8	754,3	753,8	752,8
Proteína bruta	*138,9*	150,8^{a}	154,9^{b}	158,1^{c}	159,8^{d}	159,9^{d}	159,7^{d}
Lípidos	*48,3*	57,6	57,4	58,5	55,6	56,3	57,0
Cenizas	*39,9*	28,7	30,2	29,6	30,3	30,0	30,5
Aminoácidos esenciales							
Arginina	*26,58*	20,09^{a}	20,76^{b}	20,91^{b}	21,26^{c}	21,21^{c}	21,28^{c}
Histidina	*5,16*	6,99^{a}	7,15^{b}	7,27^{b}	7,43^{c}	7,42^{c}	7,49^{c}
Isoleucina	*6,27*	8,29^{a}	8,48^{a}	8,63^{b}	8,75^{b}	8,80^{b}	8,77^{b}
Leucina	*10,18*	12,91^{a}	13,22^{b}	13,37^{b}	13,55^{c}	13,59^{c}	13,57^{c}
Lisina	*12,21*	16,98^{a}	17,32^{a}	17,56^{a}	17,87^{b}	17,83^{b}	17,82^{b}
Metionina	*1,47*	2,55^{a}	2,71^{b}	2,82^{c}	2,89^{c}	2,93^{c}	2,91^{c}
Fenilalanina	*7,02*	8,75^{a}	8,98^{a}	9,07^{a}	9,29^{b}	9,30^{b}	9,27^{b}
Treonina	*4,61*	6,86^{a}	6,99^{a}	7,15^{b}	7,25^{b}	7,30^{b}	7,24^{b}
Triptófano	*0,06*	0,61	0,62	0,62	0,63	0,65	0,67
Valina	*5,71*	8,19^{a}	8,30^{b}	8,48^{c}	8,60^{c}	8,59^{c}	8,58^{c}
Aminoácidos no esenciales							
Alanina	*9,08*	10,97^{a}	11,25^{b}	11,43^{c}	11,56^{c}	11,52^{c}	11,50^{c}
Aspartato	*29,85*	10,08^{a}	10,39^{b}	10,46^{b}	10,67^{c}	10,69^{c}	10,63^{c}
Cisteína	*0,49*	0,56	0,56	0,57	0,59	0,62	0,59
Glutamato	*11,26*	10,39^{a}	10,97^{b}	11,06^{c}	11,21^{d}	11,19^{d}	11,11^{d}
Glicina	*4,81*	7,21^{a}	7,46^{b}	7,50^{b}	7,66^{c}	7,64^{c}	7,62^{c}
Prolina	*5,07*	5,79^{a}	5,89^{a}	6,12^{b}	6,24^{b}	6,26^{b}	6,30^{b}
Serina	*4,33*	8,41^{a}	8,73^{b}	8,83^{c}	8,90^{c}	8,94^{c}	8,90^{c}
Tirosina	*3,91*	4,18^{a}	4,44^{b}	4,53^{c}	4,59^{c}	4,60^{c}	4,57^{c}

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

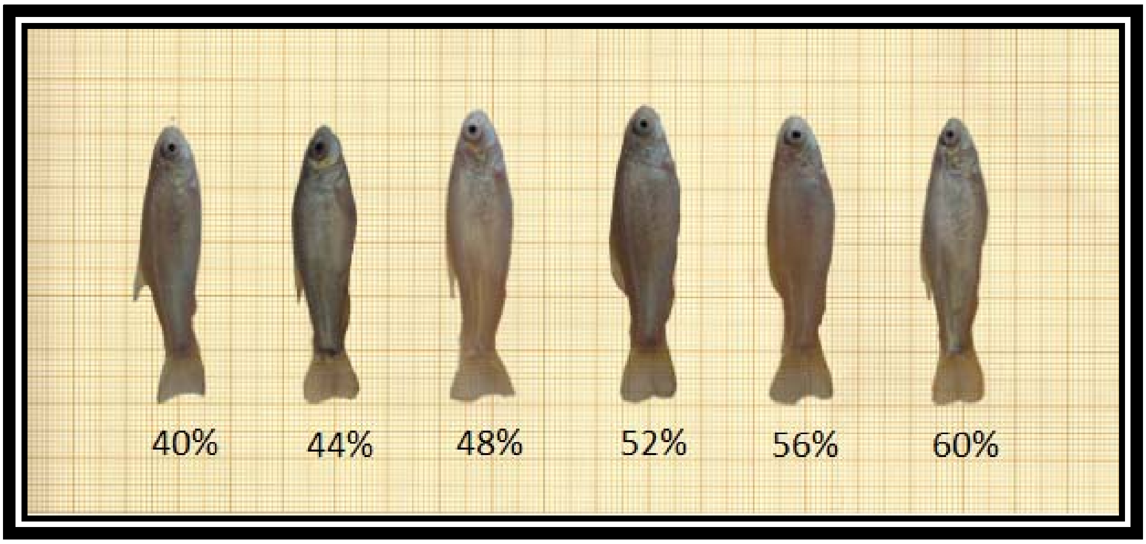

Foto 14. Juveniles alimentados con dietas con diferente contenido proteico al final del experimento II.

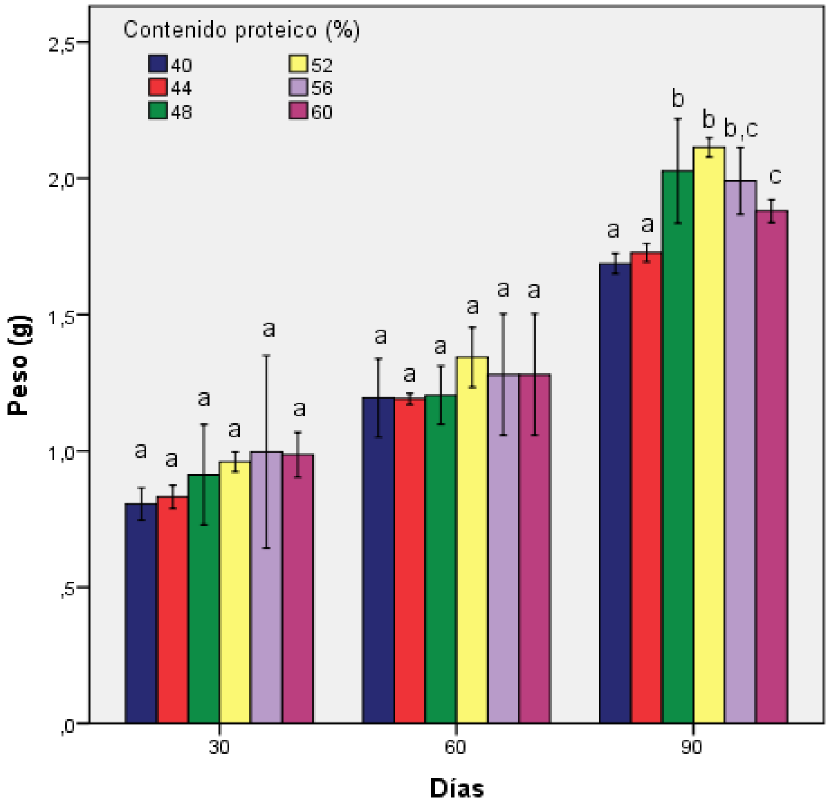

Gráfico 1. (Experimento II) Peso de juveniles alimentados con dietas prácticas con diferentes contenidos proteicos durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas ($P < 0{,}05$).

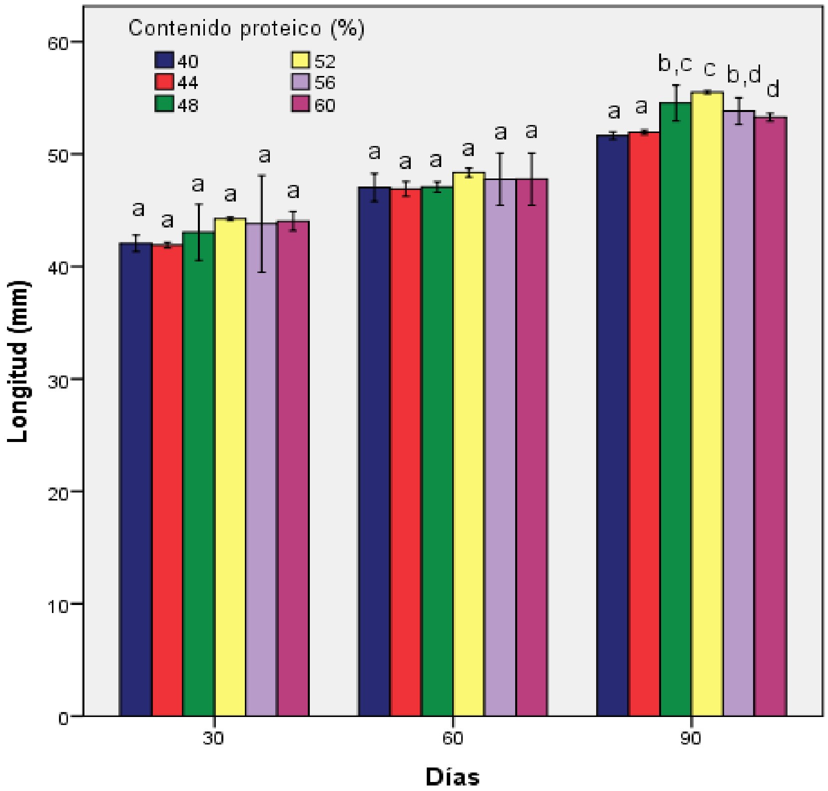

Gráfico 2. (Experimento II) Longitud de juveniles alimentados con dietas prácticas con diferentes contenidos proteicos durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas (*P* < 0,05).

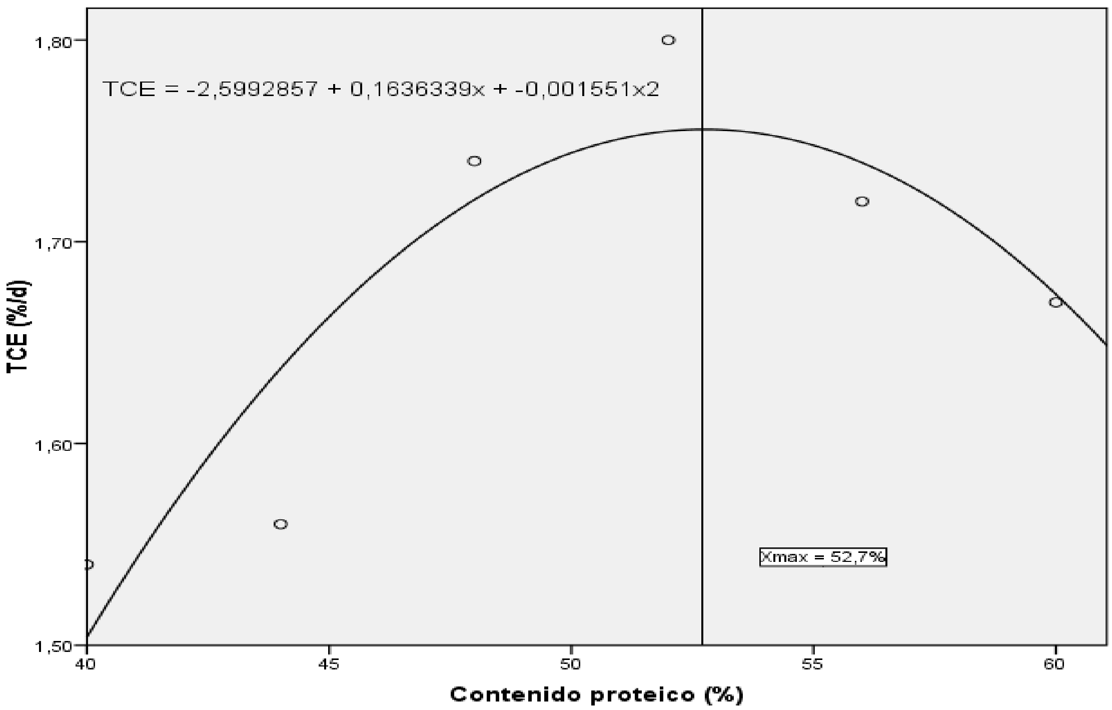

Gráfico 3. (Experimento II) Relación entre el nivel de proteína de la dieta y la TCE de juveniles de tenca basada en análisis de regresión de segundo orden.

6.2.3. Discusión

Los resultados del presente estudio mostraron que un nivel de proteína entre 48% y 52% resulta adecuado para juveniles de tenca. Con niveles superiores al 52% no mejoró el crecimiento, e incluso se evidenció una disminución del mismo (Tabla 8). Esta reducción del crecimiento ha sido observada en otras especies de peces, como tilapia, *Sarotherodon mossambicus* (Jauncey 1982), lubina negra, *Micropterus salmoides* (Portz et al. 2001), y mahasher, *Tor putitora* (Islam y Tanaka 2004), cuando se alimentaron con niveles de proteína superiores al óptimo, y fue relacionada con la utilización de energía disponible para metabolizar los aminoácidos absorbidos en exceso (Jauncey 1982). Cuando la proteína se redujo al 44% o niveles inferiores, se observó una disminución significativa del crecimiento, probablemente debida a un aporte proteico insuficiente para cubrir los requerímientos de un crecimiento óptimo.

Tanto el contenido de proteína como de aminoácidos del cuerpo de los juveniles de tenca aumentaron cuando lo hizo el nivel de proteína en la dieta hasta el 52%, manteniéndose sin variaciones a partir de este nivel. De la misma forma, el valor productivo de la proteína no mejoró por encima de 52%. Tendencias similares también se han observado en otras especies de peces de agua dulce, como tilapia, *S. mossambicus* (Jauncey 1982), trucha común, *Salmo trutta* (Arzel et al. 1995), perca plateada, *Bidyanus bidyanus* (Yang et al. 2002), mahasher, *T. putitora* (Islam y Tanaka 2004), y pez gato asiático de cola roja, *Hemibagrus wyckioides* (Deng et al. 2011). Es sabido que el contenido de proteína corporal puede aumentar hasta que los requerimientos son cubiertos, y con niveles superiores la proteína es metabolizada con fines energéticos, en lugar de ser utilizada para la síntesis de tejidos (Hardy y Gatlin 2002).

El análisis de la dieta con 48% de proteína evidenció que los contenidos de todos los aminoácidos esenciales eran más altos que los requerimientos determinados para los juveniles de otras especies de agua dulce resumidos en el NRC (2011). Por el contrario, la dieta con 44% de proteína presentó un contenido de histidina (7,55 g/kg de dieta) por debajo de los requerimientos para carpa común, *C. carpio* (Nose 1979), y carpa mrigal, *C. mrigala* (Ahmed y Khan 2005), y un contenido de metionina (9,89 g/kg dieta) por debajo de los requerimientos para perca amarilla, *Perca flavescens* (Twibell et al. 2000), y carpa mrigal, *C. mrigala* (Ahmed et al. 2003). Considerando estos datos, se puede plantear la hipótesis de que el contenido de histidina y metionina estaban por debajo de los requerimientos para juveniles de tenca.

Además de la posible deficiencia de aminoácidos, la disminución del crecimiento de los juveniles alimentados con las dietas con 40% y 44% de proteína podría ser también atribuida al contenido de carbohidratos de estas dietas. A medida que se reducía el nivel de proteína aumentaban los hidratos de carbono por la mayor incorporación de harina de maíz en dieta. En juveniles de otras especies de agua dulce como pejerrey mejicano, *Menidia estor* (Martínez-Palacios et al. 2007), y pez gato sol, *Horabagrus brachysoma* (Giri et al. 2011), alimentados con piensos con un alto contenido en carbohidratos, también se ha observado una reducción del crecimiento, que se ha asociado a la correlación negativa entre el alto contenido de carbohidratos y la digestibilidad de la proteína (Hepher 1985), derivada de la disminución de la actividad de la enzima proteasa (Mohanta et al. 2009).

6.3. ESTUDIO III: EVALUACIÓN DE HARINA DE SUBPRODUCTOS DE POLLO COMO SUSTITUTO PARCIAL DE HARINA DE PESCADO

Evaluation of poultry by-product meal as partial replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.).

Á. González-Rodríguez, J.D. Celada, J.M. Carral, M. Sáez-Royuela, V. García, J.B. Fuertes
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6.3.1. Planteamiento experimental

Entre las fuentes proteicas de origen animal, la harina de subproductos de pollo (PBM) es uno de los ingredientes más prometedores como sustituto de la harina pescado (Tacon y Jackson 1985), debido a la similitud de ambas en contenido proteico y perfil de aminoácidos (NRC 2011). Además, la harina de subproductos de pollo presenta un suministro estable y bajo precio (Hu et al. 2008). Este ingrediente se ha probado en dietas para juveniles de varias especies de peces de agua dulce, como tilapia del Nilo, *Oreochromis niloticus* (El-Sayed 1998), pez gato Africano, *Clarias gariepinus* (Abdel-Warith et al. 2001), carpa común, *C. carpio* (Emre et al. 2003), carpín, *Carassius auratus* (Yang et al. 2004), y carpa herbívora, *Ctenopharyngodon idella* (Tabinda y Butt 2012), mostrando que el nivel de inclusión óptimo depende de la especie (entre 170 y 470 g PBM/kg dieta). El objetivo de este estudio fue evaluar los efectos de una sustitución parcial de harina de pescado por harina de subproductos de pollo en dietas prácticas para juveniles de tenca (*T. tinca*) sobre supervivencia, crecimiento y composición corporal.

Experimento III. Tomando como referencia la dieta base propuesta en el estudio I, se probaron varios niveles de sustitución de proteína de pescado por proteína de subproductos de pollo. Los niveles de sustitución se elegieron teniendo en cuenta las recomendaciones de estudios realizados con diferentes especies de peces (Yu 2008). Así, se prepararon ocho dietas prácticas para evaluar diferentes niveles de sustitución de proteína de pescado por proteína de subproductos de pollo: 0% (control), 25%, 31%, 37%, 43%, 49%, 55% y 61%, correspondientes a 0, 184,8, 229,2, 273,5, 317,8, 362,1, 406,5 y 450,8 g PBM/kg dieta, respectivamente. La composición proximal y el perfil de aminoácidos de las harinas se muestran en la tabla 10. La formulación y composición proximal de las dietas se muestran en la tabla 11 y su perfil de aminoácidos en la tabla 12.

Tabla 10. (Experimento III) Composición proximal y perfil de aminoácidos de la harina de pescado (FM) y de la harina de subproductos de pollo (PBM) (g/kg).

	FM	PBM
Composición		
Humedad	79,7	50,3
Proteína bruta	678,1	601,2
Lípidos	90,3	190,8
Carbohidratos	0,0	34,3
Cenizas	151,9	123,4
Aminoácidos esenciales		
Arginina	88,8	82,4
Histidina	13,9	11,0
Isoleucina	35,0	29,2
Leucina	45,3	44,8
Lisina	60,6	38,9
Metionina	19,6	13,0
Fenilalanina	28,1	22,1
Treonina	37,8	28,5
Triptófano	5,7	4,6
Valina	27,6	32,7
Aminoácidos no esenciales		
Alanina	43,2	37,8
Aspartato	62,1	53,0
Glutamato	85,4	55,2
Glicina	27,2	17,6
Prolina	25,8	47,6
Serina	38,3	42,3
Tirosina	21,0	15,4
Cisteína	4,3	6,5

Tabla 11. (Experimento III) Formulación y composición proximal de las dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de subproductos de pollo (g/kg).

	Nivel de sustitución (%)							
	0	25	31	37	43	49	55	61
Ingredientes								
Harina de pescado [a]	655	491,5	452	412,7	373,4	334,1	294,8	255,5
Subproductos de pollo [a]	0	184,8	229,2	273,5	317,8	362,1	406,5	450,8
Harina de maíz [b]	131	114,7	114,8	114,8	114,8	114,8	112,2	108,2
Quistes de *Artemia* decapsulados [c]	100	100	100	100	100	100	100	100
Carboximetilcelulosa [d]	30	30	30	30	30	30	30	30
Aceite de hígado de bacalao [e]	25	25	25	25	25	25	25	25
L-ascorbil-2-monofosfato-Na [f]	5	5	5	5	5	5	5	5
Fosfato dicálcico [f]	10	10	10	10	10	10	10	10
Cloruro de colina [f]	3	3	3	3	3	3	3	3
Lecitina de soja [g]	30	25	20	15	10	5	2,5	1,5
Cloruro de sodio [h]	1	1	1	1	1	1	1	1
Premezcla vitamínico-mineral [i]	10	10	10	10	10	10	10	10
Composición								
Humedad	84,3	85,5	84,8	86,1	85,2	83,9	84,7	83,9
Proteína bruta	499,4	500,1	500,0	499,9	499,8	499,9	500,0	499,8
Lípidos	134,9	149,2	149,6	147,4	148,4	149,1	152,2	155,1
Carbohidratos	122,3	122,0	125,1	128,2	131,0	133,0	134,4	134,7
Cenizas	159,1	143,2	140,5	138,4	135,6	134,1	128,7	126,5
Energía bruta (MJ/kg)	20,3	20,5	20,6	20,6	20,7	20,6	20,7	20,8

[a] Skretting, Cojóbar (Burgos), España.
[b] Adpan Europa S.L., Siero (Asturias), Spain.
[c] INVE Aquaculture Nutrition, High HUFA 430µ, Hoogyeld 91, Dendermonde, Belgium.
[d] HELM IBERICA S.A., Alcobendas(Madrid), España.
[e] ACOFARMA, Terrassa (Barcelona), España.
f NUTRAL S.A, Colmenar Viejo (Madrid), España.
[g] BIOVER NV/SA, Brujas (Bélgica).
[h] Unión Salinera de España S.A., Madrid, Spain.
[i] (mg/kg premezcla): inositol, 50000; tiamina, 500; riboflavina, 800; niacina, 5000; piridoxina, 1500; ácido pantoténico, 5000; biotina, 150; ácido fólico, 3500; cianocobalamina, 5; retinol, 2400; α-tocoferol, 30000; colecalciferol, 6,25; naftoquinonas, 5000; etoxiquina, 70000; $MgSO_4$-$7H_2O$, 300000; $ZnSO_4$-$7H_2O$, 11000; $MnSO_4$-H_2O, 4000; $CuSO_4$-$5H_2O$, 1180; $CoSO_4$, 26; $FeSO_4$-$7H_2O$, 77400; KI, 340; Na_2SeO_3, 68.

Tabla 12. (Experimento III) Perfil de aminoácidos de las dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de subproductos de pollo (g/kg dieta).

	Nivel de sustitución (%)							
	0	25	31	37	43	49	55	61
Aminoácidos esenciales								
Arginina	66,6	67,3	67,4	67,7	67,7	67,9	68,1	68,3
Histidina	9,9	9,7	9,6	9,6	9,5	9,4	9,4	9,3
Isoleucina	25,2	24,9	24,8	24,7	24,6	24,5	24,5	24,4
Leucina	33,2	34,1	34,2	34,5	34,6	34,8	35,0	35,2
Lisina	44,2[a]	41,5[b]	40,8[b,c]	40,2[b,c,d]	39,5[b,c,d]	39,4[b,c,d]	38,7[c,d]	37,6[d]
Metionina	11,1[a]	10,4[b]	9,7[c]	9,3[c]	9,2[c]	8,4[d]	8,3[d,e]	7,8[e]
Fenilalanina	19,7	19,2	19,0	18,9	18,8	18,7	18,6	18,4
Treonina	27,4	26,7	26,5	26,3	26,0	25,8	25,6	25,4
Triptófano	4,3	4,2	4,2	4,2	4,2	4,1	4,1	4,1
Valina	20,4	21,9	22,3	22,7	23,0	23,4	23,8	24,1
Aminoácidos no esenciales								
Alanina	31,7	31,7	31,6	31,6	31,6	31,6	31,5	31,5
Aspartato	46,4	46,1	45,9	45,9	45,7	45,7	45,6	45,5
Glutamato	64,3[a]	60,6[b]	59,6[b,c]	58,8[b,c,d]	57,8[c,d,e]	56,9[c,d,e]	56,0[d,e]	55,1[e]
Glicina	19,5	18,4	18,0	17,8	17,4	17,2	16,9	16,6
Prolina	19,5[a]	24,0[b,c]	25,0[b,c]	26,1[b,c,d]	27,2[b,c,d]	28,4[c,d]	29,6[c,d]	30,6[d]
Serina	28,4[a]	30,0[a,b]	30,3[b,c]	30,7[b,c]	31,0[b,c]	31,4[b,c]	31,8[b,c]	32,2[c]
Tirosina	15,0	14,4	14,2	14,1	13,9	13,8	13,7	13,5
Cisteína	3,0[a]	3,5[a,b]	3,6[a,b]	3,7[a,b]	3,8[b]	4,0[b]	4,1[b]	4,2[b]

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

Se utilizaron 720 juveniles de 5 meses de edad (31,95 ± 0,14 mm LT, 0,396 ± 0,005 g P (n = 120)), que fueron distribuidos en 24 tanques de fibra de vidrio con una capacidad de 25 litros de agua. En cada tanque se alojaron 30 juveniles de tenca para obtener las réplicas correspondientes a los diferentes tratamientos. Cada una de las dietas fue probada por triplicado (tres tanques por tratamiento) durante 90 días. Las dietas fueron suministradas manualmente 4 veces al día.

Cada 30 días, se tomaron 10 juveniles de cada tanque (33% del total), realizando medidas de peso (g) y longitud total (mm). En el muestreo final (90 días), se realizaron las medidas mencionadas sobre todos los animales y se procedió a la observación individualizada de todos los juveniles con una lupa con objeto de detectar deformidades externamente visibles.

Al comienzo y al final del estudio, se tomó una muestra de juveniles que habían recibido el mismo tratamiento, para ser analizados posteriormente.

6.3.2. Resultados

Los juveniles ingirieron todas las dietas prácticas desde el inicio del experimento. Los valores finales de supervivencia, crecimiento y porcentaje de peces deformes se muestran en la tabla 13. La supervivencia fue 100% en todos los tratamientos.

En cuanto al crecimiento, no hubo diferencias significativas entre el 25% de sustitución de proteína de pescado por proteína de subproductos de pollo (184,8 g PBM/kg dieta) y la dieta control (0% de sustitución) (media de los dos tratamientos: 62,28 mm LT, 3,14 g P y 2,27 %/d TCE). Con mayores niveles de sustitución (desde 31% hasta 61%), todos los valores de crecimiento fueron significativamente menores. Los gráficos 8 y 9 muestran los cambios en peso y longitud, respectivamente, a lo largo de los 90 días de prueba. Los juveniles alimentados con la dieta control y 25% de sustitución presentaron un mayor ritmo de crecimiento que los alimentados con las dietas con mayores niveles de sustitución (31% a 61%), siendo las diferencias significativas a partir del día 60.

El factor de condición (K) varió entre 1,24 y 1,29 sin diferencias significativas entre tratamientos. El índice de conversión del alimento (IC) osciló entre 1,25 y 1,99, siendo el IC de los peces alimentados con la dieta control y 25% de sustitución significativamente menor (promedio: 1,25). El valor productivo de la proteína (VPP) varió entre 17,56% y 28,98%, y los peces alimentados con las dieta control y 25% de sustitución mostraron valores significativamente mayores (media: 28,84). El porcentaje de peces con deformidades externamente visibles varió entre 1,1% y 3,3%. Las deformidades afectaron a la columna vertebral y al pedúnculo caudal.

Los perfiles de aminoácidos de las dietas prácticas se presentan en la tabla 14. Teniendo en cuenta los aminoácidos esenciales, no hubo diferencias significativas en el contenido de arginina, histidina, isoleucina, leucina, fenilalanina, treonina, triptófano y valina. Comparando las dietas control y 25% de sustitución, hubo una reducción significativa en el contenido de lisina y metionina (6,1% y 6,3%, respectivamente). Cuando la sustitución de la proteína de pescado aumentó hasta el 31%, hubo una reducción significativa del contenido de metionina (12,6%) en comparación con la dieta control, y el crecimiento se redujo significativamente.

La composición proximal y el perfil de aminoácidos del cuerpo de los juveniles de tenca al inicio y al final del experimento se muestran en la tabla 14. No hubo diferencias significativas en el contenido de humedad y cenizas entre tratamientos. Los peces alimentados con la dieta control y 25% de sustitución tuvieron un contenido lipídico significativamente menor y un contenido proteico significativamente mayor que los alimentados con superiores niveles de sustitución (de 31% a 61%). En cuanto al perfil de aminoácidos, los juveniles alimentados con el 31% o niveles superiores de sustitución tuvieron un contenido de lisina y metionina significativamente menor que los alimentados con la dieta control.

Tabla 13. (Experimento III). Valores finales de supervivencia, crecimiento y porcentaje de peces deformes de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de subproductos de pollo durante 90 días.

	Nivel de sustitución (%)							
	0	25	31	37	43	49	55	61
Supervivencia (%)	100	100	100	100	100	100	100	100
Longitud total (mm)	62,52 ± 0,47[a]	62,04 ± 0,41[a]	59,33 ± 0,33[b]	59,64 ± 0,39[b,c]	58,86 ± 0,34[c]	54,17 ± 0,36[d]	53,32 ± 0,32[d]	51,49 ± 0,40[e]
Peso (g)	3,20 ± 0,08[a]	3,08 ± 0,07[a]	2,62 ± 0,04[b]	2,68 ± 0,06[b]	2,55 ± 0,05[b]	2,02 ± 0,05[c]	1,92 ± 0,04[c]	1,75 ± 0,05[d]
TCE (%/d)	2,29 ± 0,03[a]	2,25 ± 0,03[a]	2,08 ± 0,02[b]	2,10 ± 0,02[b]	2,05 ± 0,02[b]	1,79 ± 0,02[c]	1,74 ± 0,02[c]	1,61 ± 0,03[d]
K	1,29 ± 0,01	1,27 ± 0,01	1,24 ± 0,01	1,25 ± 0,01	1,24 ± 0,01	1,25 ± 0,01	1,26 ± 0,01	1,25 ± 0,01
IC	1,25 ± 0,03[a]	1,25 ± 0,03[a]	1,41 ± 0,03[b]	1,46 ± 0,04[b]	1,40 ± 0,03[b]	1,63 ± 0,04[c]	1,68 ± 0,04[c]	1,99 ± 0,07[d]
VPP (%)	28,98 ± 0,77[a]	28,70 ± 0,74[a]	23,90 ± 0,48[b]	23,53 ± 0,57[b]	24,47 ± 0,57[b]	20,68 ± 0,57[c]	19,64 ± 0,47[c]	17,56 ± 0,66[d]
Peces deformes (%)	1,1 ± 0,2[a]	2,2 ± 0,2[a,b]	1,1 ± 0,2[a]	3,3 ± 0,3[b]	1,1 ± 0,1[a]	2,2 ± 0,2[a,b]	3,3 ± 0,3[b]	2,2 ± 0,2[a,b]

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

Tabla 14. (Experimento III) Composición proximal y perfil de aminoácidos del cuerpo de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de subproductos de pollo durante 90 días (g/kg).

	Inicial	Nivel de sustitución (%)							
		0	25	31	37	43	49	55	61
Composición									
Humedad	*794,1*	743,3	744,4	741,9	738,7	739,4	743,7	741,1	741,0
Proteina bruta	*147,2*	166,6[a]	165,2[a]	159,7[b]	160,2[b]	160,2[b]	156,1[b,c]	154,2[c]	154,3[c]
Lipidos	*32,2*	58,1[a]	59,0[a]	66,7[b]	68,1[b]	68,2[b]	68,1[b]	72,4[c]	71,6[c]
Cenizas	*26,5*	32,0	31,4	31,7	33,0	32,2	32,1	32,3	33,1
Aminoácidos esenciales									
Arginina	*24,3*	21,5	21,6	21,5	21,6	21,7	21,7	21,7	21,8
Histidina	*4,8*	6,1	6,1	6,0	6,0	6,0	5,8	5,7	5,6
Isoleucina	*6,9*	8,2	8,2	8,1	8,1	8,0	8,0	7,9	7,8
Leucina	*9,8*	13,2	13,3	13,3	13,3	13,4	13,4	13,5	13,6
Lisina	*11,6*	15,1[a]	14,8[a,b]	14,4[b,c]	14,3[c,d]	14,2[c,d]	14,2[c,d]	14,2[c,d]	14,0[d]
Metionina	*1,4*	2,6[a]	2,5[a]	2,3[b]	2,3[b]	2,3[b]	2,3[b]	2,2[c]	2,2[c]
Fenilalanina	*6,6*	8,6	8,4	8,4	8,4	8,3	8,3	8,2	8,2
Treonina	*4,5*	7,3	7,2	6,9	6,8	6,7	6,6	6,6	6,5
Triptófano	*0,2*	0,7	0,7	0,6	0,6	0,5	0,5	0,5	0,5
Valina	*5,6*	8,0	8,1	8,1	8,2	8,3	8,3	8,4	8,5
Aminoácidos no esenciales									
Alanina	*8,9*	11,8	11,8	11,9	12,0	12,0	12,0	12,1	12,3
Aspartato	*27,3*	16,1	16,0	16,0	15,8	15,8	15,8	15,7	15,6
Glutamato	*0,5*	0,6[a]	0,6[a]	0,6[a]	0,7[a,b]	0,7[a,b]	0,7[a,b]	0,7[a,b]	0,8[b]
Glicina	*12,8*	11,1[a]	11,0[a,b]	10,8[a,b,c]	10,7[a,b,c]	10,7[a,b,c]	10,6[b,c]	10,5[b,c]	10,4[c]
Prolina	*4,4*	7,4	7,3	7,1	7,0	7,0	6,9	6,9	6,9
Serina	*5,0*	6,5[a]	6,4[a,b]	6,2[a,b,c]	6,1[b,c]	6,1[b,c]	6,0[c]	5,9[c]	5,9[c]
Tirosina	*4,2*	8,3	8,5	8,5	8,6	8,6	8,6	8,7	8,7
Cisteína	*3,6*	3,1	3,0	3,0	3,0	2,9	2,9	2,7	2,6

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

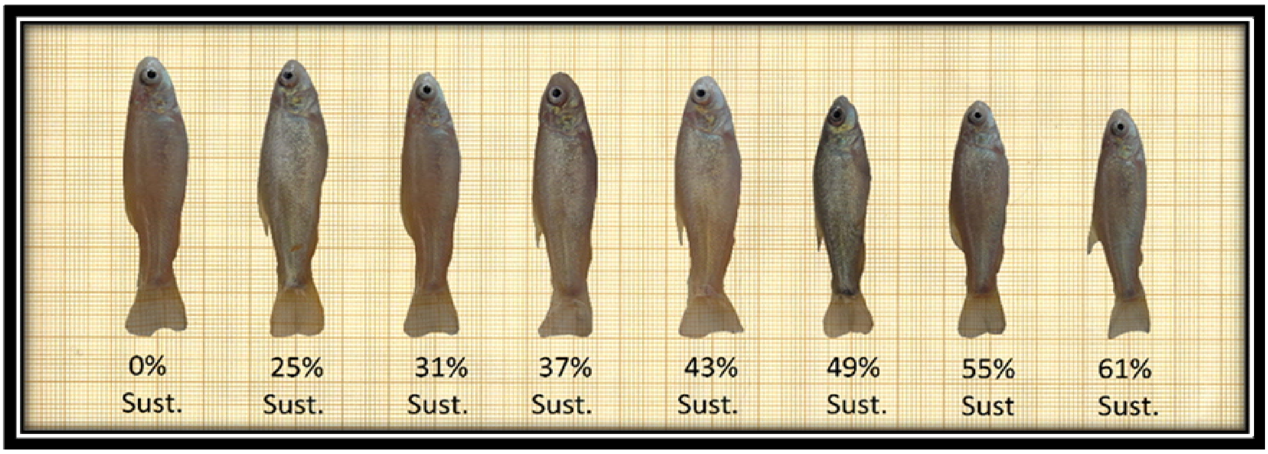

Foto 15. Juveniles alimentados con dietas con diferente nivel de sustitución de proteína de pescado por proteína de harina de subproductos de pollo al final del experimento III.

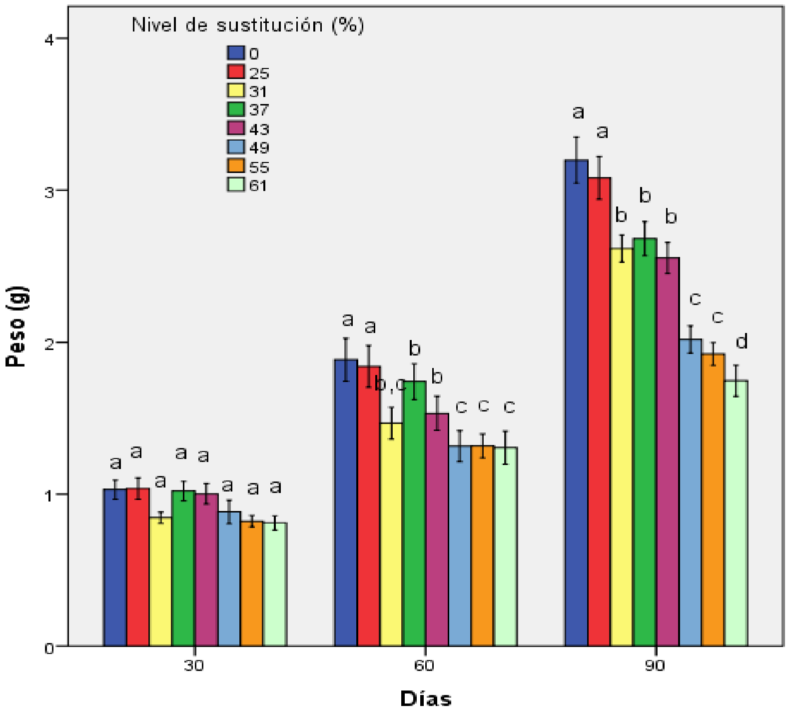

Gráfico 4. (Experimento III) Peso de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de subproductos de pollo durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas ($P < 0,05$).

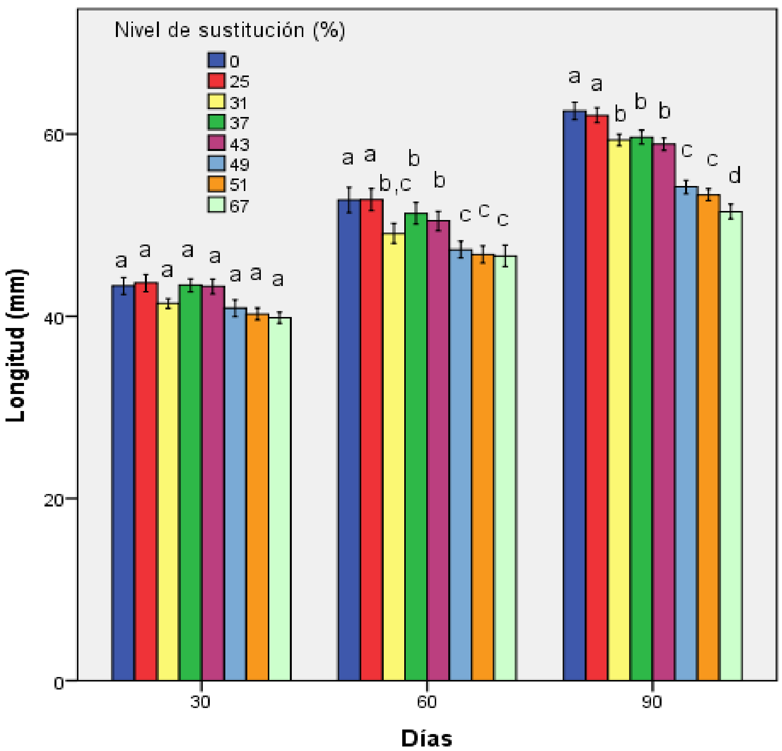

Gráfico 5. (Experimento III) Longitud de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de subproductos de pollo durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas ($P < 0,05$).

6.3.3. Discusión

Según Rodehutscord et al. (1995), considerando que la composición corporal de los peces está relacionada con la composición de la dieta, la retención proteica y de aminoácidos esenciales son los indicadores más sensibles de un suministro inadecuado de aminoácidos. En el experimento III, la retención de proteína (estimada por medio del valor productivo de la proteína) evidenció una disminución significativa al incluir cantidades por encima de 184,8 g de PBM/kg dieta (25% de sustitución), lo que sugiere un suministro insuficiente de algún o algunos aminoácidos esenciales. En este sentido, la sustitución de harina de pescado por harina de subproductos de pollo en la dieta condujo a una reducción significativa de lisina y de metionina (Tabla 12). El crecimiento de los juveniles no fue afectado por el 25% de sustitución, pero con niveles más altos (31% o superiores) se detectó una disminución del crecimiento. Teniendo en cuenta que el contenido de lisina y metionina en el cuerpo de los juveniles no mostró diferencias significativas entre los alimentados con la dieta control y los que recibieron la del 25% de sustitución, podría inferirse que ambas dietas cubren los requerimientos de juveniles de tenca de ambos aminoácidos esenciales. Sin embargo, cuando el nivel de sustitución aumentó hasta el 31% (229,2 g PBM/kg dieta) o niveles superiores, se evidenció una disminución significativa del contenido de metionina tanto en las dietas como en el cuerpo de los juveniles. Esto permite plantear la hipótesis de un aporte de este aminoácido inferior a las necesidades de los juveniles de tenca. De hecho, el contenido de metionina en la dieta del 31% de sustitución (9,7 g/kg) estaba por debajo de los requerimientos determinados (NRC 2011) para la perca amarilla (*P. flavescens)* y carpa mrigal (*C. mrigala)*.

6.4. ESTUDIO IV: EVALUACIÓN DE UN CONCENTRADO PROTEICO DE GUISANTE COMO SUSTITUTO PARCIAL DE HARINA DE PESCADO

Evaluation of pea protein concentrate as partial replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.)

Á. González-Rodríguez, J.D. Celada, J.M. Carral, M. Sáez-Royuela, J.B. Fuertes

Aquaculture Research (En revisión)

6.4.1. Planteamiento experimental

En los últimos años, la formulación de dietas para especies piscícolas ha tendido a incorporar materias primas de origen vegetal con objeto de reemplazar la proteína de la harina de pescado. Entre ellas se encuentran los guisantes, con elevada producción mundial y, por ello, con potencial para reemplazar proporciones significativas de proteína de pescado (Gatlin et al. 2007). Los avances recientes en la tecnología de procesamiento de los guisantes han permitido la obtención de varios productos, como el concentrado proteico de guisante (PPC), cuyo elevado porcentaje de proteína bruta (52%) permitiría un alto nivel de sustitución de la harina de pescado en la formulación de alimentos piscícolas. Este ingrediente se ha probado únicamente en peces adultos como salmón del Atlántico, *Salmo salar* (Øverland et al. 2009, Penn et al. 2011), lubina, *Dicentrarchus labrax* (Tibaldi et al. 2005), y trucha arco iris, *O. mykiss* (Zhang et al. 2012), mostrando variaciones en el nivel de inclusión óptimo entre un 20% y un 36%. El objetivo de este estudio fue evaluar los efectos de una sustitución parcial de harina de pescado por un concentrado proteico de guisante en dietas para juveniles de tenca sobre supervivencia, valores de crecimiento y composición corporal.

Experimento IV. Tomando como referencia la dieta base propuesta en el estudio I, se probaron varios niveles de sustitución de proteína de pescado por proteína de guisante. Los niveles de sustitución se elegieron teniendo en cuenta las recomendaciones para diferentes especies piscicolas (Reigh 2008). Por ello, se formularon y fabricaron cuatro dietas prácticas con diferentes niveles de sustitución: 0% (control), 25%, 35% y 45%, que correspondían a 0, 207,5, 290,4 y 373,3 g PPC/kg de dieta. La composición proximal y el perfil de aminoácidos de las harinas se muestran en la tabla 15. La formulación y composición proximal de las dietas se muestran en la tabla 16 y su perfil de aminoácidos en la tabla 17.

Tabla 15. (Experimento IV) Composición proximal y perfil de aminoácidos de la harina de pescado (FM) y del concentrado proteico de guisante (PPC) (g/kg).

	FM	PPC
Composición		
Humedad	79,7	78
Proteína bruta	678	522
Lípidos	90,3	18
Carbohidratos	0	330
Cenizas	152	52
Aminoácidos esenciales		
Arginina	81,2	38,8
Histidina	12,6	9,5
Isoleucina	25,2	14,1
Leucina	44,2	38,3
Lisina	53,2	33,4
Metionina	16,1	7,6
Fenilalanina	24,5	23,0
Treonina	35,8	22,6
Triptófano	5,6	5,2
Valina	30,1	19,7
Aminoácidos no esenciales		
Alanina	37,1	26,5
Aspartato	51,1	62,6
Cisteína	6,1	5,2
Glutamato	81,5	105,6
Glicina	54,9	16,9
Prolina	44,0	18,8
Serina	36,9	25,7
Tirosina	14,2	16,2

Tabla 16. (Experimento IV) Formulación y composición proximal de las dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de guisante (g/kg).

	Nivel de sustitución (%)			
	0	25	35	45
Ingredientes				
Harina de pescado [a]	640	480,5	417,6	354,7
Concentrado proteico de guisante [b]	0	207,5	290,4	373,3
Harina de maíz [c]	126	78	58	38
Quistes de *Artemia* decapsulados [d]	120	120	120	120
Carboximetilcelulosa [e]	30	30	30	30
Aceite de hígado de bacalao [f]	20	20	20	20
L-ascorbil-2-monofosfato-Na [g]	5	5	5	5
Fosfato dicálcico [g]	10	10	10	10
Cloruro de colina [g]	3	3	3	3
Lecitina de soja [h]	5	5	5	5
Cloruro de sodio [i]	1	1	1	1
Mezcla de minerales [j]	20	20	20	20
Mezcla de vitaminas [k]	20	20	20	20
Composición				
Humedad	72,3	70,9	70,4	69,8
Proteína bruta	500,4	500,1	500,5	501,0
Lípidos	108,0	94,8	89,3	84,3
Carbohidratos	157,4	186,4	197,5	208,1
Cenizas	161,9	147,8	142,3	136,8
Energía bruta (MJ/kg)	18,1	18,0	18,0	18,0

[a] BioMar Iberia/ProAqua Nutrición S.A., Dueñas (Palencia), España.
[b] Yantai Oriental Protein Tech Co., Ltd., Zhaoyuan City, China.
[c] Adpan Europa S.L., Siero (Asturias), Spain.
[d] INVE Aquaculture Nutrition, High HUFA 430µ, Hoogyeld 91, Dendermonde, Belgium.
[e] HELM IBERICA S.A., Alcobendas(Madrid), España.
[f] ACOFARMA, Terrassa (Barcelona), España.
[g] NUTRAL S.A, Colmenar Viejo (Madrid), España.
[h] BIOVER NV/SA, Brujas, Bélgica.
[i] Unión Salinera de España S.A., Madrid, Spain.
[j] (mg/kg premezcla): $MgSO_4$-$7H_2O$, 150000; $ZnSO_4$-$7H_2O$, 5500; $MnSO_4$-H_2O, 2000; $CuSO_4$-$5H_2O$, 590; $CoSO_4$, 13; $FeSO_4$-$7H_2O$, 37350; KI, 170; Na_2SeO_3, 34.
[k] (mg/kg premezcla): inositol, 25000; tiamina, 250; riboflavina, 400; niacina, 2500; piridoxina, 750; ácido pantoténico, 2500; biotina, 75; ácido fólico, 1750; cianocobalamina, 2,5; retinol, 500; α-tocoferol, 15000; colecalciferol, 3,125; naftoquinonas, 2500; etoxiquina, 35000.

Tabla 17. (Experimento IV) Perfil de aminoácidos de las dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de guisante (g/kg dieta).

	Nivel de sustitución (%)			
	0	25	35	45
Aminoácidos esenciales				
Arginina	58,4[a]	55,5[a]	52,6[b]	50,7[b]
Histidina	8,9	8,8	8,8	8,8
Isoleucina	18,6	17,6	16,7	15,8
Leucina	31,7	31,2	30,7	30,4
Lisina	38,5	37,0	36,4	35,9
Metionina	11,6[a]	10,6[a,b]	9,9[b]	8,9[b]
Fenilalanina	14,7	14,1	14,0	13,8
Treonina	22,4	22,1	22,0	21,9
Triptófano	4,3	4,2	4,2	4,2
Valina	21,2	20,9	20,6	20,3
Aminoácidos no esenciales				
Alanina	27,2	26,7	26,6	26,5
Aspartato	38,2[a]	42,8[b]	45,1[c,d]	46,7[d]
Cisteína	4,2	4,1	4,1	4,0
Glutamato	60,3[a]	68,2[b]	72,3[b,c]	75,6[c]
Glicina	36,0[a]	31,0[b]	29,2[b]	27,4[c]
Prolina	29,9[a]	27,0[a]	26,1[a,b]	25,0[b]
Serina	26,9	26,3	26,2	25,9
Tirosina	10,2	11,0	11,5	11,9

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

Se utilizaron 648 juveniles de 5 meses de edad (35,53 ± 0,28 mm LT y 0,461 ± 0,011 g P (n = 120)), que fueron distribuidos aleatoriamente en 12 tanques de fibra de vidrio con una capacidad de 25 litros de agua. En cada tanque se alojaron 25 g de tencas para obtener las réplicas correspondientes a los diferentes tratamientos (densidad = 1 g/l). El número de animales por cada tanque fue 54. Cada dieta fue probada por triplicado (tres tanques por tratamiento) durante 90 días. Las dietas fueron suministradas manualmente 4 veces al día.

Cada 30 días, se realizaron medidas de longitud y peso sobre 20 juveniles de cada réplica (aproximadamente un 37% del total). En el muestreo final (90 días), se realizaron las medidas mencionadas sobre todos los animales, se procedió al recuento de supervivientes y a la observación individualizada de todos los juveniles con una lupa con objeto de detectar deformidades externamente visibles.

Al comienzo y al final del estudio, se tomó una muestra de juveniles que habían recibido el mismo tratamiento, para ser analizados posteriormente.

6.4.2. Resultados

Los juveniles ingirieron todas las dietas prácticas desde el inicio del experimento. Los valores finales de supervivencia, crecimiento y portentaje de peces deformes se recogen en la tabla 18. El porcentaje de supervivencia varió entre 96% y 99%, sin diferencias significativas entre tratamientos.

En cuanto al crecimiento, no hubo diferencias significativas entre los juveniles alimentados con las dietas con 25% y 35% de sustitución de proteína de pescado por proteína de guisante (207,5 y 290,4 g

PPC/kg de dieta, respectivamente) y los alimentados con la dieta control (promedio de los tres tratamientos: 57,57 mm LT, 2,48 g P y 1,87 %/día TCE). Los gráficos 4 y 5 muestran los cambios en peso y longitud, respectivamente, a lo largo de los 90 días de prueba. Los juveniles alimentados con la dieta del 45% de sustitución presentaron un menor ritmo de crecimiento que el resto, siendo las diferencias significativas a partir del día 30.

El factor de condición (K) osciló entre 1,28 y 1,29. El índice de conversión del alimento (IC) varió entre 1,56 y 1,63, y el valor productivo de la proteína (VPP) entre 22,93% y 23,24%, sin diferencias significativas entre tratamientos. El porcentaje de peces deformes varió entre 0% y 1,5%. Las deformidades afectaron a la columna vertebral y al pedúnculo caudal.

El perfil de aminoácidos de las dietas prácticas se presenta en la tabla 17. Respecto a los aminoácidos esenciales, no hubo diferencias significativas en el contenido de histidina, isoleucina, leucina, lisina, fenilalanina, treonina, triptófano y valina. Las dietas con los mayores niveles de sustitución de proteína de pescado por proteína de guisante (35% y 45%) tuvieron contenidos de arginina y de metionina significativamente menores que los de la dieta control. Con la dieta del 35% de sustitución, a pesar de que los contenidos de los mencionados aminoácidos se redujeron en un 9,9% (arginina) y un 14,7% (metionina), no se produjo una reducción del crecimiento. Sin embargo, un nivel de sustitución del 45% de la proteína de pescado determinó una reducción del contenido de arginina y metionina del 13,2% y 23,3%, respectivamente, que coincidió con una reducción significativa del crecimiento de los juveniles.

La composición proximal y el perfil de aminoácidos del cuerpo de los juveniles de tenca al inicio y al final del experimento se muestran en la tabla 19. No hubo diferencias significativas en el contenido de humedad, proteína bruta y cenizas entre tratamientos. El contenido lipídico de los peces alimentados con la dieta del 45% de sustitución fue significativamente mayor. En cuanto al perfil de aminoácidos esenciales en el cuerpo de los juveniles, se evidenció una reducción a medida que se incrementó el nivel de sustitución de la proteína de pescado por proteína de guisante. Así, los peces alimentados con la dieta del 35% de sustitución (290,4 g PPC/kg de dieta) tuvieron un contenido de leucina, treonina y triptófano similar al de los alimentados con la dieta control, mientras que el resto de aminoácidos fueron significativamente más bajos; sin embargo, el crecimiento no fue afectado. Comparando el contenido de aminoácidos esenciales de los peces alimentados con la dieta control y el de los alimentados con la dieta con 45% de sustitución, las mayores reducciones correspondieron a la metionina (19,2%), isoleucina (10,0%), arginina (9,1%) e histidina (9,1%), y los valores de crecimiento se redujeron significativamente.

Tabla 18. (Experimento IV) Valores finales de supervivencia, crecimiento y porcentaje de peces deformes de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de guisante durante 90 días.

	Nivel de sustitución (%)			
	0	25	35	45
Supervivencia (%)	98,5 ± 0,16	98,0 ± 0,12	97,9 ± 0,13	96,4 ± 0,08
Longitud total (mm)	57,96 ± 0,296[a]	57,42 ± 0,262[a]	57,33 ± 0,345[a]	55,29 ± 0,362[b]
Peso (g)	2,52 ± 0,039[a]	2,46 ± 0,032[a]	2,47 ± 0,046[a]	2,19 ± 0,047[b]
TCE (%/day)	1,88 ± 0,017[a]	1,86 ± 0,014[a]	1,87 ± 0,021[a]	1,73 ± 0,022[b]
K	1,28 ± 0,004	1,29 ± 0,005	1,29 ± 0,005	1,28 ± 0,008
IC	1,57 ± 0,034	1,56 ± 0,024	1,60 ± 0,039	1,63 ± 0,044
VPP (%)	23,24 ± 0,426	22,93 ± 0,367	23,14 ± 0,522	22,94 ± 0,680
Peces deformes (%)	0,0 ± 0,0	0,8 ± 0,08	0,7 ± 0,08	1,5 ± 0,18

Los valores son media ± error estándar.
Valores en la misma fila con diferente superíndice presentaron diferencias significativas (*P* < 0,05).

Tabla 19. (Experimento IV) Composición proximal y perfil de aminoácidos del cuerpo de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de guisante durante 90 días (g/kg).

	Inicial	Nivel de sustitución (%)			
		0	25	35	45
Composición					
Humedad	*772,2*	730,0	733,8	734,1	730,0
Proteína bruta	*149,3*	168,4	167,5	166,8	165,7
Lípidos	*39,4*	62,0[a]	57,6[a]	60,2[a]	68,2[b]
Cenizas	*33,9*	32,3	34,5	32,4	33,0
Aminoácidos esenciales					
Arginina	*24,8*	18,6[a]	18,1[a]	17,4[b]	16,9[b]
Histidina	*4,1*	5,5[a]	5,1[b]	5,0[b]	5,0[b]
Isoleucina	*4,7*	7,0[a]	6,7[a]	6,5[b]	6,3[c]
Leucina	*8,3*	13,5	13,3	13,3	13,2
Lisina	*11,8*	13,9[a]	13,7[a,b]	13,6[b]	13,4[c]
Metionina	*1,4*	2,6[a]	2,5[a]	2,2[b]	2,1[b]
Fenilalanina	*3,3*	7,4[a]	7,1[b]	7,1[b]	7,0[b]
Treonina	*5,2*	6,8	6,6	6,5	6,5
Triptófano	*0,4*	0,6	0,6	0,6	0,6
Valina	*6,5*	7,6[a]	7,3[b]	7,1[b]	7,0[b]
Aminoácidos no esenciales					
Alanina	*8,3*	10,1	10,0	10,0	9,9
Aspartato	*27,7*	24,9[a]	25,9[b]	27,3[c]	28,0[c]
Cisteína	*0,6*	1,2	1,2	1,2	1,3
Glutamato	*19,1*	22,3	22,3	22,4	22,5
Glicina	*3,9*	5,5[a]	5,1[b]	4,9[b]	4,8[b]
Prolina	*4,3*	6,9[a]	6,5[b]	6,5[b]	6,4[b]
Serina	*4,8*	8,4[a]	8,2[a,b]	8,1[b]	8,0[b]
Tirosina	*2,6*	3,5	3,6	3,6	3,6

Valores en la misma fila con diferente superíndice presentaron diferencias significativas (*P* < 0,05).

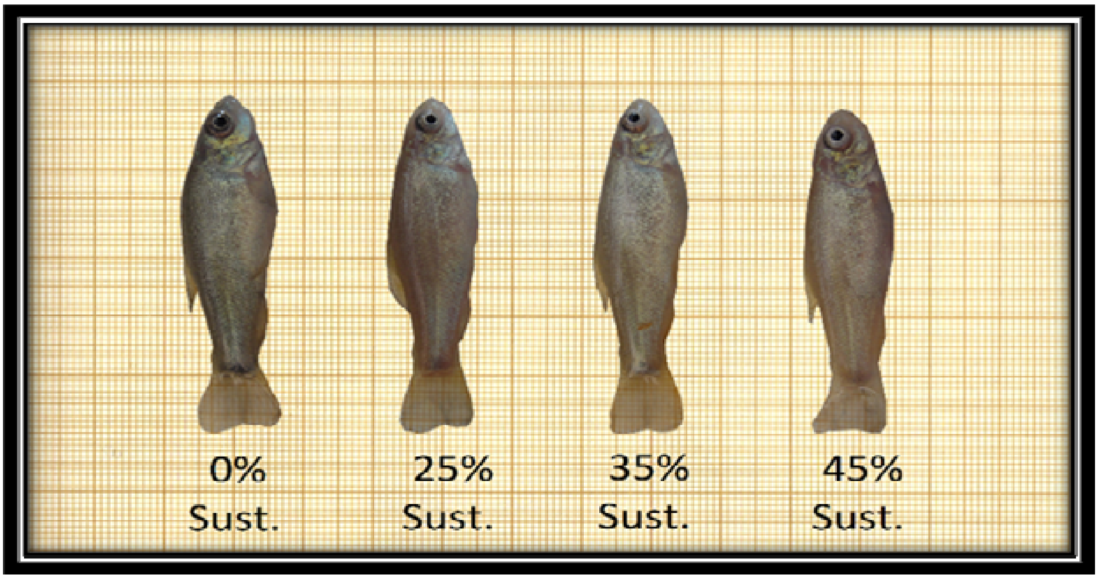

Foto 16. Juveniles alimentados con dietas con diferente nivel de sustitución de proteína de pescado por proteína de concentrado proteico de guisante al final del experimento IV.

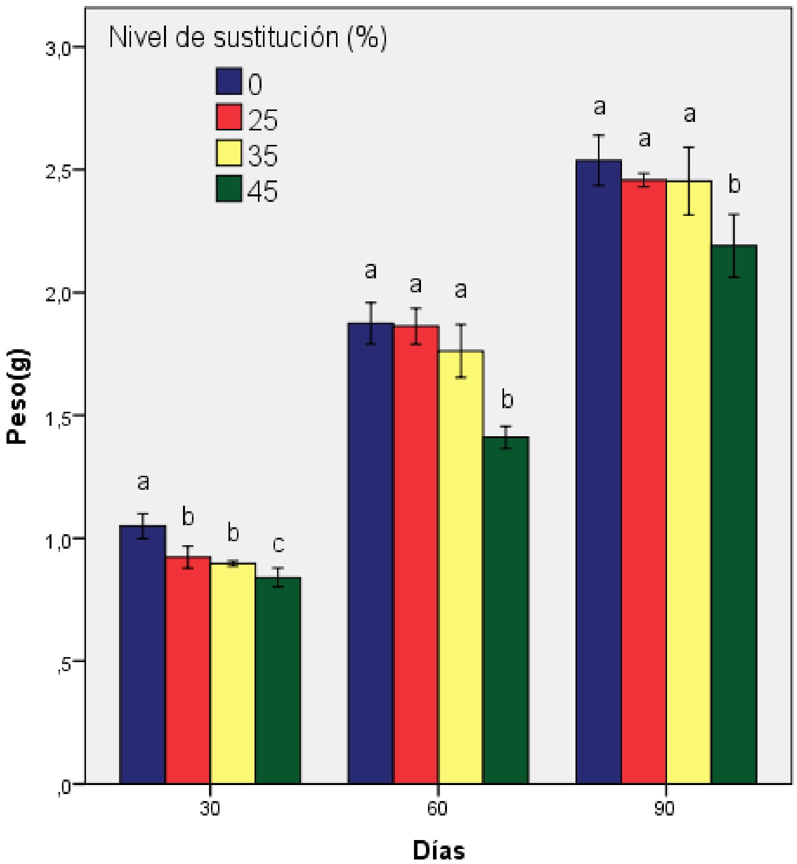

Gráfico 6. (Experimento IV) Peso de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de guisante durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas ($P < 0,05$).

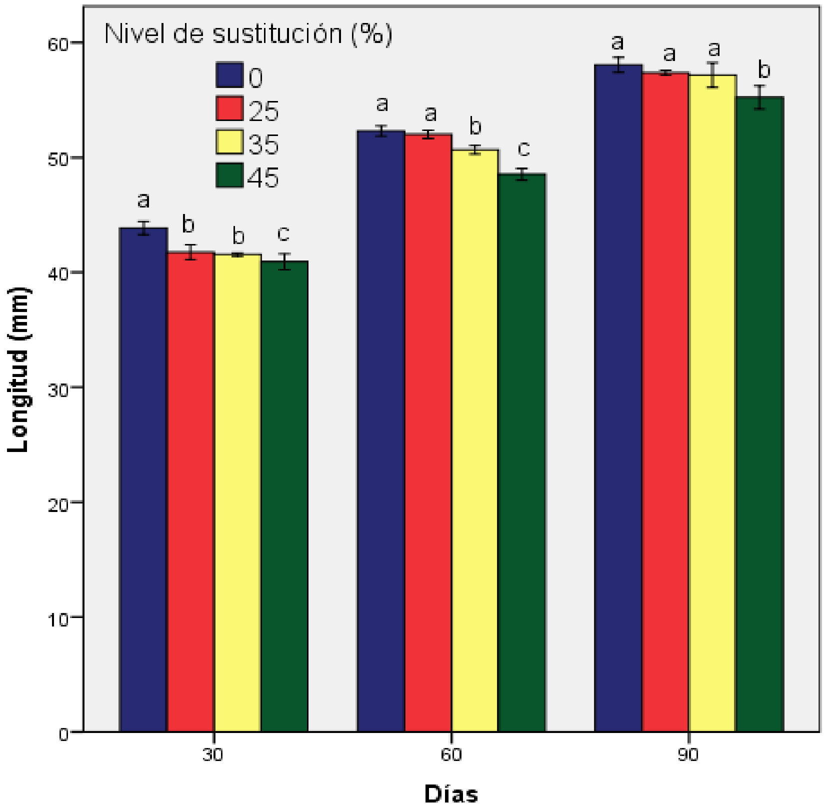

Gráfico 7. (Experimento IV) Longitud de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de guisante durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas ($P < 0,05$).

6.4.3. Discusión

La dieta control (0% de sustitución) utilizada en este estudio, formulada a partir de los resultados obtenidos en el experimento I, permitió nuevamente una aceptable tasa de crecimiento (1,88), bajo índice de conversión (1,57), elevada supervivencia (98,5%) y ausencia de peces deformes, apoyando nuevamente su utilización como base para futuros estudios nutricionales con juveniles de tenca.

La incorporación de materias de origen vegetal para sustituir harina de pescado origina una disminución del contenido de aminoácidos en la dieta (Reigh 2008). En el presente estudio, los contenidos de todos los aminoácidos esenciales disminuyeron cuando el concentrado proteico de guisante fue incluido en la dieta (Tabla 17), lo cual se reflejó en una disminución del contenido de todos los aminoácidos esenciales en el cuerpo de los juveniles de tenca (Tabla 19). Con el 35% de sustitución, los niveles de arginina y metionina fueron significativamente más bajos que los de la dieta control. Sin embargo, no hubo reducción del crecimiento de los juveniles, por lo que se podría asumir que tanto la dieta control (0% sustitución) como la dieta con 25% de sustitución contenían un exceso de estos aminoácidos. Cuando el nivel de sustitución aumentó hasta el 45% (373,3 g PPC/kg de dieta), las cantidades de arginina y metionina (Tabla 17) disminuyeron en comparación con la dieta con 35% de sustitución y se evidenció una disminución significativa del crecimiento de los juveniles. Mientras que el contenido de arginina en la dieta con 45% de sustitución (50,7 g/kg dieta) estaba por encima de los requerimientos estimados para juveniles de otras especies de peces de agua dulce, como carpa común (*C. carpio*), trucha arco iris (*O. mykiss*), y carpa mrigal (*C. mrigala*) (NRC 2011), el contenido de metionina (8,9 g/kg dieta) estaba por debajo del mínimo recomendado para perca amarilla (*P. flavescens*) y carpa mrigal (*C. mrigala*) (NRC 2011). Por ello, podría asumirse que un bajo contenido de metionina, probablemente insuficiente para los requerimientos de juveniles de tenca, podría estar relacionado con la reducción del crecimiento de los peces alimentados con el mayor nivel de sustitución (45%).

Además de la posible deficiencia de aminoácidos esenciales, la reducción del crecimiento de los juveniles que recibieron la dieta con más alto contenido de PPC (45% de sustitución) podría también tener relación con la merma de la digestibilidad y/o la presencia de factores antinutricionales. La inclusión de PPC en dietas para diferentes especies carnívoras de peces no alteró los valores de digestibilidad, que fueron similares a los de las dietas que contenían sólo harina de pescado (Øverland et al 2009, Zhang et al 2012). Por tanto, parece improbable que la digestibilidad del concentrado proteico de guisante afectara negativamente al crecimiento de los juveniles de tenca. En cuanto a los factores antinutricionales, es ampliamente sabido que el concentrado proteico de guisante, como otras fuentes proteicas de origen vegetal, contiene varios factores antinutricionales. A pesar de que el descascarillado de los guisantes utilizado para la obtención del PPC y la extrusión de las dietas disminuyen la concentración de factores antinutricionales (Thiessen et al. 2003), los remanentes podrían afectar negativamente al crecimiento de los juveniles de tenca. Estudios recientes han demostrado que un 35% de concentrado proteico de guisante en dietas para salmón del Atlántico, *S. salar* (Penn et al. 2011), o un 40% para trucha arco iris, *O. mykiss* (Zhang et al. 2012), afectan a la fisiología digestiva y, por tanto, a la salud de los peces. De esta manera, la disminución del crecimiento de los juveniles de tenca alimentados con la dieta del 45% de sustitución podría ser atribuible tanto a una deficiencia de aminoácidos como a la posible presencia de factores antinutricionales.

El aumento del contenido de lípidos registrado en el cuerpo de los juveniles alimentados con la dieta del 45% de sustitución (Tabla 19) podría estar relacionado con el aumento del contenido de carbohidratos en las dietas con mayor contenido de PPC (Tabla 16). En coincidencia con nuestros resultados, se ha observado una relación entre el incremento del contenido de carbohidratos en la dieta y el de lípidos en el cuerpo de los juveniles de otras especies de peces, como lubina europea, *D. labrax* (Moreira et al. 2008), y cobia, *Rachycentron canadum* (Ren et al. 2011). Teniendo en cuenta que la cantidad de síntesis de novo de lípidos a partir de hidratos de carbono es bastante limitada (Hemre y Kahrs 1997), parece más bien que las dietas con alto contenido de carbohidratos estimulan las enzimas implicadas en la lipogénesis, como Likimani y Wilson (1982) y Fynn-Aikins et al (1992) han obsevado en otras especies de peces.

6.5. ESTUDIO V: EVALUACIÓN DE UN CONCENTRADO PROTEICO DE SOJA COMO SUSTITUTO DE HARINA DE PESCADO

Evaluation of soy protein concentrate as replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.).

Á. González-Rodríguez, J.D. Celada, J.M. Carral, M. Sáez-Royuela, V. García, J.B. Fuertes

Turkish Journal of Fisheries and Aquatic Sciences

6.5.1. Planteamiento experimental

Dentro de las fuentes proteicas de origen vegetal, la harina de soja constituye una de las alternativas a la harina de pescado. Sin embargo, su uso en alimentos para especies acuáticas tiene algunas limitaciones, como la presencia de factores antinutricionales, un bajo nivel de metionina y efectos perjudiciales sobre la fisiología intestinal de algunas especies carnívoras (Gatlin et al. 2007). El procesamiento de la harina de soja mediante fraccionamiento para producir concentrado proteico de soja (SPC) puede mejorar su valor nutritivo (Vielma et al. 2000, Collins et al. 2012) y eliminar la mayor parte de los factores antinutricionales. Además, el concentrado de soja presenta un contenido proteico y una digestibilidad aparente de proteína y aminoácidos similar a la harina de pescado (Hardy 2008). La sustitución de harina de pescado (FM) por concentrado proteico de soja ha sido probada en juveniles de varias especies de peces, como trucha arco iris, *O. mykiss* (Kaushik et al. 1995), rodaballo, *Scophthalmus maximus* (Day y González 2000), lenguado Japonés, *P. olivaceus* (Deng et al. 2006), y palometa, *Seriola lalandi* (Bowyer et al. 2013), mostrando variaciones en el nivel de inclusión óptimo según las diferentes especies (entre 185 y 220 g SPC/kg de dieta). El objetivo de este estudio fue evaluar los efectos de la sustitución total o parcial de harina de pescado por un concentrado proteico de soja en dietas para juveniles de tenca sobre supervivencia, crecimiento y composición corporal.

Experimento V. Tomando como referencia la dieta base propuesta en el estudio I, se formularon ocho dietas prácticas con diferentes niveles de sustitución de la proteína de pescado por proteína de soja: 0% (control), 25%, 35%, 45%, 55%, 65%, 75% y 100%, que correspondían a 0, 159, 222, 285, 348, 412, 475 y 634 g SPC/kg dieta, respectivamente. El concentrado proteico de soja (SPC, 70% proteína bruta) fue obtenido mediante la extracción en fase alcohol-agua de copos de soja. La composición proximal y el perfil de aminoácidos de las harinas se muestran en la tabla 20. La formulación y composición proximal de las dietas se muestran en la tabla 21 y su perfil de aminoácidos en la tabla 22.

Tabla 20. (Experimento V) Composición proximal y perfil de aminoácidos de la harina de pescado (FM) y del concentrado proteico de soja (SPC) (g/kg).

	FM	SPC
Composición		
Humedad	79,7	79,9
Proteína bruta	678,0	700,0
Lípidos	90,3	8,1
Carbohidratos	0,0	147,2
Cenizas	152,0	64,8
Aminoácidos esenciales		
Arginina	96,2	55,3
Histidina	15,7	18,9
Isoleucina	27,6	35,8
Leucina	45,9	61,5
Lisina	48,6	43,5
Metionina	18,2	9,1
Fenilalanina	19,8	39,3
Treonina	39,5	28,0
Triptófano	5,5	10,2
Valina	32,0	33,5
Aminoácidos no esenciales		
Alanina	39,4	32,1
Aspartato	62,9	99,7
Cisteína	6,2	10,4
Glutamato	85,8	96,4
Glicina	26,2	30,6
Prolina	26,7	35,2
Serina	37,2	35,9
Tirosina	16,2	21,8

Tabla 21. (Experimento V) Formulación y composición proximal de las dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de concentrado proteico de soja (g/kg).

	Nivel de sustitución (%)							
	0	25	35	45	55	65	75	100
Ingredientes								
Harina de pescado [a]	656	492	426	361	295,5	230	164	0
Concentrado proteico de guisante [b]	0	159	222	285	348	412	475	634
Harina de maíz [c]	150	155	153	152,5	155	154	154,5	157
Quistes de *Artemia* decapsulados [d]	100	100	100	100	100	100	100	100
Carboximetilcelulosa [e]	30	30	30	30	30	30	30	30
Aceite de hígado de bacalao [f]	30	30	30	30	30	30	30	30
L-ascorbil-2-monofosfato-Na [g]	5	5	5	5	5	5	5	5
Fosfato dicálcico [g]	10	10	10	10	10	10	10	10
Cloruro de colina [g]	3	3	3	3	3	3	3	3
Lecitina de soja [h]	5	5	10	12,5	12,5	15	17,5	20
Cloruro de sodio [i]	1	1	1	1	1	1	1	1
Mezcla vitamínico-mineral [j]	10	10	10	10	10	10	10	10
Composición								
Humedad	74,6	74,7	74,2	74,0	74,1	73,8	73,6	73,5
Proteína bruta	500,5	500,9	500,3	500,4	500,2	500,6	500,0	500,4
Lípidos	117,5	103,8	103,1	100,0	94,5	91,4	88,3	76,7
Carbohidratos	173,3	200,6	208,3	217,3	228,4	237,1	246,8	272,3
Cenizas	134,1	120,0	114,1	108,3	102,8	97,1	91,3	77,1
Energía bruta (MJ/kg)	74,6	74,7	74,2	74,0	74,1	73,8	73,6	73,5

[a] BioMar Iberia/ProAqua Nutrición S.A., Dueñas (Palencia), España.
[b] C.D.A. S.L., Burguillos (Sevilla), España.
[c] Adpan Europa S.L., Siero (Asturias), Spain.
[d] INVE Aquaculture Nutrition, High HUFA 430µ, Hoogyeld 91, Dendermonde, Belgium.
[e] HELM IBERICA S.A., Alcobendas(Madrid), España.
[f] ACOFARMA, Terrassa (Barcelona), España.
[g] NUTRAL S.A, Colmenar Viejo (Madrid), España.
[h] BIOVER NV/SA, Brujas (Bélgica).
[i] Unión Salinera de España S.A., Madrid, Spain.
[j] (mg/kg premezcla): inositol, 50000; tiamina, 500; riboflavina, 800; niacina, 5000; piridoxina, 1500; ácido pantoténico, 5000; biotina, 150; ácido fólico, 3500; cianocobalamina, 5; retinol, 2400; α-tocoferol, 30000; colecalciferol, 6,25; naftoquinonas, 5000; etoxiquina, 70000; $MgSO_4$-$7H_2O$, 300000; $ZnSO_4$-$7H_2O$, 11000; $MnSO_4$-H_2O, 4000; $CuSO_4$-$5H_2O$, 1180; $CoSO_4$, 26; $FeSO_4$-$7H_2O$, 77400; KI, 340; Na_2SeO_3, 68.

Tabla 22. (Experimento V) Perfil de aminoácidos de las dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de soja (g/kg dieta).

	Nivel de sustitución (%)							
	0	25	35	45	55	65	75	100
Aminoácidos esenciales								
Arginina	71,4^{a}	64,4^{b}	61,2^{c}	58,9^{d}	55,4^{e}	53,1^{f}	50,3^{g}	43,5^{h}
Histidina	11,3^{a}	11,7^{a,b}	11,8^{a,b,c}	11,9^{a,b,c}	12,1^{a,b,c}	12,3^{a,b,c}	12,4^{b,c}	12,8^{c}
Isoleucina	20,5^{a}	21,6^{a,b}	22,1^{b,c}	22,5^{b,c,d}	22,9^{c,d,e}	23,4^{d,e}	23,7^{e}	25,0^{f}
Leucina	33,6^{a}	35,8^{b}	36,7^{b,c}	37,6^{c,d}	38,4^{d,e}	39,4^{e,f}	40,2^{f}	42,4^{g}
Lisina	36,4^{a}	35,4^{b}	34,9^{b,c}	34,5^{b,c,d}	34,0^{c,d}	33,6^{c,d}	33,2^{d,e}	32,1^{e}
Metionina	13,3^{a}	11,7^{b}	11,1^{c}	10,5^{d}	9,8^{e}	9,2^{f}	8,6^{g}	7,1^{h}
Fenilalanina	14,3^{a}	17,3^{b}	18,4^{c}	19,6^{d}	20,8^{e}	22,0^{f}	23,2^{g}	26,2^{h}
Treonina	29,5^{a}	27,3^{b}	26,4^{b,c}	25,5^{c,d}	24,6^{d,e}	23,7^{e,f}	22,8^{f}	20,6^{g}
Triptófano	4,2^{a}	4,9^{a,b}	5,2^{b,c}	5,5^{b,c,d}	5,7^{c,d,e}	6,0^{d,e}	6,3^{e,f}	7,0^{f}
Valina	23,3	23,4	23,4	23,4	23,4	23,5	23,5	23,5
Aminoácidos no esenciales								
Alanina	29,3^{a}	27,9^{b}	27,3^{b,c}	26,8^{b,c,d}	26,2^{c,d,e}	25,7^{d,e}	25,1^{e}	23,8^{f}
Aspartato	46,9^{a}	52,5^{b}	54,6^{b}	56,8^{c}	59,0^{d}	61,2^{e}	63,4^{e}	68,9^{f}
Cisteína	4,2^{a}	4,9^{a,b}	5,1^{a,b,c}	5,4^{b,c,d}	5,6^{b,c,d}	5,9^{c,d,e}	6,1^{d,e}	6,7^{e}
Glutamato	64,7^{a}	66,0^{a,b}	66,3^{b,c}	66,8^{b,c,d}	67,3^{b,c,d}	67,8^{c,d}	68,3^{d,e}	69,5^{e}
Glicina	19,1^{a}	19,6^{a,b}	19,8^{a,b}	20,0^{a,b}	20,2^{b,c}	20,4^{b,c}	20,6^{b,c}	21,1^{c}
Prolina	20,1^{a}	21,3^{b}	21,8^{b,c}	22,2^{b,c,d}	22,7^{c,d,e}	23,2^{d,e}	23,7^{e}	24,9^{f}
Serina	28,6^{a}	28,0^{a,b}	27,7^{a,b}	27,5^{a,b,c}	27,2^{a,b,c}	26,9^{b,c}	26,6^{b,c}	26,1^{c}
Tirosina	12,2^{a}	12,9^{a,b}	13,2^{a,b,c}	13,4^{b,c,d}	13,7^{b,c,d}	14,0^{c,d,e}	14,3^{d,e}	15,0^{e}

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

Se utilizaron 720 juveniles de 5 meses de edad (31,95 ± 0,14 mm LT, 0,396 ± 0,005 g P (n = 120)), que fueron distribuidos aleatoriamente en 24 tanques de fibra de vidrio con una capacidad de 25 litros de agua. En cada tanque se alojaron 30 juveniles de tenca para obtener las réplicas correspondientes a los diferentes tratamientos. Cada una de las dietas fue probada por triplicado (tres tanques por tratamiento) durante 90 días. Las dietas fueron suministradas manualente 4 veces al día.

Cada 30 días, se realizaron medidas de longitud y peso sobre 10 juveniles de cada réplica (aproximadamente un 33% del total). En el muestreo final (90 días), se realizaron las medidas mencionadas sobre todos los animales y se procedió a la observación individualizada de todos los juveniles con una lupa con objeto de detectar deformidades externamente visibles.

Al comienzo y al final del estudio, se tomó una muestra de juveniles que habían recibido el mismo tratamiento, para ser analizados posteriormente.

6.5.2. Resultados

Los juveniles ingirieron todas las dietas prácticas desde el inicio del experimento. Los valores de supervivencia, crecimiento y porcentaje de peces deformes al final del experimento se muestran en la tabla 23. La supervivencia fue 100% con todas las dietas.

En términos de crecimiento, no existieron diferencias significativas entre los juveniles alimentados con las dietas del 25%, 35% o 45% de sustitución de proteína de pescado por proteína de soja (159, 222 o 285 g SPC/kg en dieta, respectivamente) y los alimentados con la dieta control (0% sustitución) (media de los cuatro tratamientos: 57,86 mm LT, 2,50 g P y 2,02 %/día TCE). Con mayores niveles de sustitución (desde 55% a 100%), todos los valores de crecimiento fueron significativamente menores. Los gráficos 6 y 7 muestran los cambios en peso y longitud, respectivamente, a lo largo de los 90 días de prueba. Los juveniles alimentados con las dietas del 0%, 25%, 35% y 45% de sustitución presentaron un mayor ritmo de crecimiento, siendo las diferencias significativas a partir del día 30.

El factor de condición (K) osciló entre 1,24 y 1,28, sin diferencias significativas entre tratamientos. El índice de conversión del alimento (IC) varió entre 1,35 y 1,57, siendo el de los peces alimentados con las dietas entre 0% y 45% de sustitución de proteína de pescado significativamente menor (promedio: 1,37) que el del resto. El valor productivo de la proteína varió entre 21,96% y 27,65%. Las dietas desde 0% a 45% de sustitución permitieron valores productivos de la proteína significativamente mayores (media: 26,46) que las dietas con niveles de sustitución más altos (55% a 100%). El porcentaje de peces deformes varió entre 1,1% y 4,4%. Las deformidades afectaron a la columna vertebral y al pedúnculo caudal.

Los perfiles de aminoácidos de las dietas prácticas se presentan en la tabla 24. Teniendo en cuenta los aminoácidos esenciales, los contenidos de arginina, lisina, metionina y treonina disminuyeron al aumentar el nivel de sustitución de la proteína de pescado por proteína de soja. Comparando la dieta del 35% con la dieta del 45%, hubo reducciones significativas de los contenidos de arginina (3,8%) y de metionina (5,4%), que no fueron acompañadas por una disminución del crecimiento. Sin embargo, cuando el nivel aumentó del 45% al 55%, las reducciones de los contenidos de arginina y metionina (5,9% y 6,7%, respectivamente) coincidieron con una merma significativa del crecimiento.

La composición proximal y el perfil de aminoácidos del cuerpo de los juveniles de tenca al inicio y al final del experimento se muestran en la tabla 24. No hubo diferencias significativas en la composición proximal. En cuanto al perfil de aminoácidos, los contenidos de arginina, lisina y metionina disminuyeron en el cuerpo con el incremento del nivel de sustitución de la proteína de pescado por proteína de soja. Los peces alimentado con la dieta del 45% de sustitución (285 g SPC/kg dieta) tuvieron un contenido de arginina significativamente menor que los alimentados con la dieta control; sin embargo, el crecimiento no fue afectado. Los peces alimentados con la dieta del 55% (348 g SPC/kg dieta) tuvieron un contenido de metionina significativamente menor que los alimentados con la dieta del 45% de sustitución, coincidiendo con una reducción significativa del crecimiento.

Tabla 23. (Experimento V) Valores finales de supervivencia, crecimiento y porcentaje de peces deformes de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de soja durante 90 días.

	Nivel de sustitución (%)							
	0	25	35	45	55	65	75	100
Supervivencia (%)	100	100	100	100	100	100	100	100
Longitud total (mm)	57,95 ± 0,50[a]	58,49 ± 0,58[a]	57,44 ± 0,36[a]	57,55 ± 0,30[a]	54,80 ± 0,44[b]	54,65 ± 0,31[b]	53,88 ± 0,46[b]	54,74 ± 0,31[b]
Peso (g)	2,52 ± 0,09[a]	2,61 ± 0,09[a]	2,45 ± 0,06[a]	2,42 ± 0,04[a]	2,12 ± 0,08[b]	2,04 ± 0,05[b]	2,06 ± 0,07[b]	2,09 ± 0,04[b]
TCE (%/ d)	2,01 ± 0,03[a]	2,05 ± 0,03[a]	2,00 ± 0,02[a]	2,00 ± 0,02[a]	1,83 ± 0,03[b]	1,80 ± 0,02[b]	1,78 ± 0,03[b]	1,83 ± 0,02[b]
K	1,26 ± 0,01	1,27 ± 0,01	1,27 ± 0,01	1,26 ± 0,01	1,26 ± 0,01	1,24 ± 0,01	1,28 ± 0,01	1,26 ± 0,01
IC	1,39 ± 0,05[a]	1,35 ± 0,05[a]	1,37 ± 0,03[a]	1,36 ± 0,03[a]	1,56 ± 0,05[b]	1,57 ± 0,04[b]	1,57 ± 0,05[b]	1,56 ± 0,04[b]
VPP (%)	26,86 ± 1,02[a]	27,65 ± 1,05[a]	25,85 ± 0,68[a]	25,49 ± 0,55[a]	22,81 ± 0,74[b]	22,15 ± 0,60[b]	23,27 ± 0,84[b]	21,96 ± 0,52[b]
Peces deformes (%)	2,2 ± 0,2[a]	2,2 ± 0,2[a]	1,1 ± 0,2[b]	3,3 ± 0,3[c]	2,2 ± 0,2[a]	2,2 ± 0,2[a]	4,4 ± 0,4[d]	4,4 ± 0,3[d]

Los valores son media ± error estándar.
Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

Tabla 24. (Experimento V) Composición proximal y perfil de aminoácidos del cuerpo de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de soja durante 90 días (g/kg).

	Inicial	Nivel de sustitución (%)							
		0	25	35	45	55	65	75	100
Composición									
Humedad	*770,8*	741,8	742,3	741,9	742,7	743,1	743,0	743,8	744,2
Proteína bruta	*138,9*	162,2	162,1	162,1	161,7	160,6	159,7	159,2	158,7
Lípidos	*48,3*	62,8	62,4	59,8	59,8	60,9	60,3	57,4	58,9
Carbohidratos	2,1	4,0	3,3	6,0	5,9	5,1	5,4	7,9	6,4
Cenizas	*39,9*	29,2	29,9	30,2	29,9	30,3	31,6	31,7	31,8
Aminoácidos esenciales									
Arginina	*23,6*	$21,9^{a}$	$21,8^{a,b}$	$21,6^{b,c}$	$21,6^{b,c}$	$21,4^{c,d}$	$21,3^{d}$	$21,2^{d}$	$21,2^{d}$
Histidina	*5,1*	7,0	6,9	6,9	6,8	6,8	6,8	6,8	6,7
Isoleucina	*6,3*	8,9	8,9	8,8	8,8	8,7	8,7	8,7	8,6
Leucina	*9,2*	13,3	13,2	13,2	13,1	13,1	13,1	13,0	13,0
Lisina	*10,2*	$17,0^{a}$	$16,9^{a,b}$	$16,9^{a,b}$	$16,9^{a,b}$	$16,8^{a,b}$	$16,8^{a,b}$	$16,8^{a,b}$	$16,7^{b}$
Metionina	*1,5*	$3,0^{a}$	$2,9^{a}$	$2,9^{a}$	$2,8^{a}$	$2,6^{b}$	$2,6^{b}$	$2,5^{b}$	$2,4^{b}$
Fenilalanina	*6,1*	9,4	9,3	9,3	9,3	9,3	9,3	9,3	9,2
Treonina	*4,2*	7,3	7,2	7,2	7,2	7,2	7,2	7,1	7,0
Triptófano	*0,1*	0,7	0,7	0,6	0,6	0,6	0,6	0,6	0,6
Valina	*5,1*	8,2	8,2	8,1	8,1	8,1	8,0	8,1	8,0
Aminoácidos no esenciales									
Alanina	*9,1*	$11,2^{a}$	$11,1^{a,b}$	$11,1^{a,b}$	$11,1^{a,b,c}$	$10,8^{b,c}$	$10,8^{b,c}$	$10,8^{b,c}$	$10,7^{c}$
Aspartato	*25,5*	10,6	10,5	10,5	10,4	10,4	10,3	10,4	10,3
Cisteína	*0,5*	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,6
Glutamato	*10,6*	10,6	10,5	10,4	10,4	10,3	10,3	10,2	10,2
Glicina	*4,2*	8,2	8,3	8,4	8,4	8,5	8,5	8,5	8,5
Prolina	*4,9*	6,7	6,8	6,8	6,9	6,9	6,9	6,9	6,9
Serina	*4,3*	$8,5^{a}$	$8,4^{a,b}$	$8,4^{a,b}$	$8,4^{a,b}$	$8,2^{b,c,d}$	$8,1^{c,d}$	$8,1^{c,d}$	$8,1^{c,d}$
Tirosina	*3,6*	5,1	5,1	5,0	5,0	5,0	5,0	4,9	4,9

Valores en la misma fila con diferente superíndice presentaron diferencias significativas ($P < 0,05$).

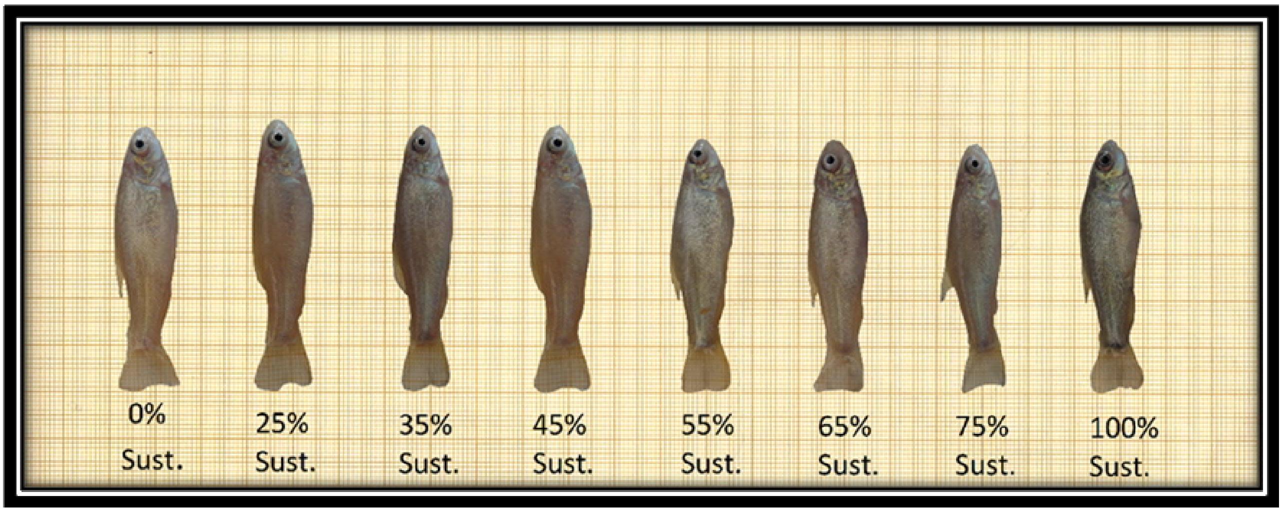

Foto 17. Juveniles alimentados con dietas con diferente nivel de sustitución de la proteína de pescado por proteína de concentrado proteico de soja al final del experimento V.

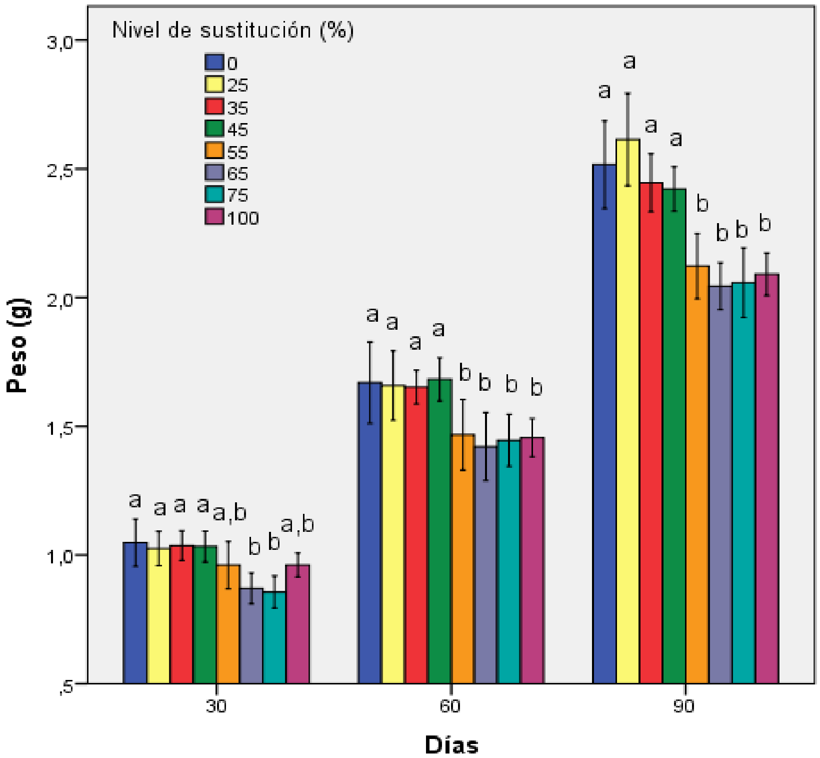

Gráfico 8. (Experimento V) Peso de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de soja durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas ($P < 0,05$).

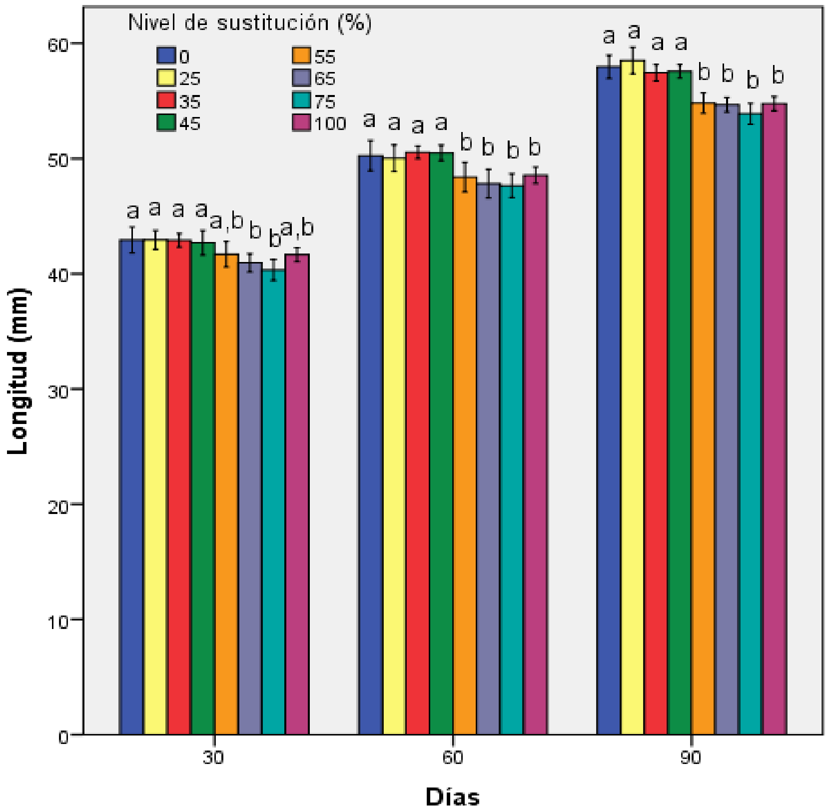

Gráfico 9. (Experimento V) Longitud de juveniles alimentados con dietas prácticas con diferentes niveles de sustitución de proteína de pescado por proteína de soja durante 90 días. Las barras de error representan el error estándar de la media. Letras diferentes en el mismo día denotan diferencias significativas (*P* < 0,05).

6.5.3. Discusión

La dieta control (0% de sustitución) utilizada en este estudio, formulada a partir de los resultados obtenidos en el experimento I, permitió una buena tasa de crecimiento (2,01), bajo índice de conversión (1,39), alta supervivencia (100%) y bajo porcentaje de peces deformes (2%), mostrando una vez mas su utilidad para el desarrollo de futuros estudios nutricionales con juveniles de tenca.

En el presente experimento, fue posible incluir hasta 285 g SPC/kg dieta para sustituir el 45% de la proteína de la harina de pescado. Con mayores niveles de sustitución (desde 55% a 100%) el crecimiento se redujo significativamente. Los contenidos dietéticos de arginina, lisina, metionina y treonina se redujeron significativamente cuando se incluyó el concentrado proteico de soja en la dieta (Tabla 22). Con el 45% de sustitución (285 g SPC/ kg dieta), los contenidos de arginina y metionina fueron significativamente inferiores (21,9% y 21,1%, respectivamente) que los contenidos en la dieta control; sin embargo, no hubo una disminución significativa del crecimiento. Por lo tanto, cabe suponer que al menos las dietas con 0%, 25% y 35% de sustitución tenían un exceso de estos aminoácidos. Cuando el nivel de sustitución de proteína de pescado por proteína de soja aumentó hasta el 55% (348 g SPC/kg dieta), los contenidos de arginina y metionina fueron significativamente más bajos que los de la dieta control, y se evidenció una disminución significativa del crecimiento de los juveniles. Considerando que el contenido de arginina en la dieta con 55% de sustitución (55,4 g/kg) fue superior a los requerimientos determinados para juveniles de otras especies de agua dulce, como carpa común (*C. carpio)* trucha arco iris (*O. mykiss),* y carpa mrigal (*C. mrigala* (NRC, 2011), y que el contenido de metionina (9,8 g/kg) estaba por debajo del mínimo recomendado para perca amarilla (*P. flavescens)* y carpa mrigal (*C. mrigala)* (NRC 2011), se podría inferir que el contenido de metionina estuvo por debajo de los requerimientos para juveniles de tenca (*T. tinca*).

Según Rodehutscord et al. (1995), la composición corporal esta relacionada con la composición de la dieta, y la retención de proteína y aminoácidos esenciales son los indicadores más sensibles de un suministro inadecuado de aminoácidos. En este sentido, se evidenció una disminución significativa del valor productivo de la proteína cuando se incluyeron cantidades superiores a 285 g SPC/kg dieta. Esta disminución coincidió con un menor contenido de metionina en el cuerpo de los juveniles. La deficiencia de este aminoácido esencial en las dietas con los niveles más altos de sustitución de proteína de pescado podría explicar tanto la disminución del VPP como el aumento del índice de conversión del alimento, probablemente debido a que la proteína de la dieta era utilizada para procesos catabólicos en lugar de procesos anabólicos.

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11. ANEXO

Publicaciones

I. Á. González-Rodríguez, J. D. Celada, J. M. Carral, M. Sáez-Royuela, J. B. Fuertes (2014). Evaluation of a practical diet for juvenile tench (*Tinca tinca* L.) and substitution possibilities of fish meal by feather meal. **Animal Feed Science and Technology 187**, 61– 67.

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V. Á. González-Rodríguez, J. D. Celada, J. M. Carral, M. Sáez-Royuela, V. García, J. B. Fuertes (2104). Evaluation of soy protein concentrate as replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.). **Turkish Journal of Fisheries and Aquatic Sciences** 14, 807-815.

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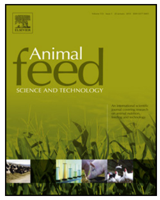

Evaluation of a practical diet for juvenile tench (*Tinca tinca* L.) and substitution possibilities of fish meal by feather meal

Á. González-Rodríguez*, J.D. Celada, J.M. Carral, M. Sáez-Royuela, J.B. Fuertes

Dpto. de Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain

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ABSTRACT

A basal diet for juvenile tench (*Tinca tinca*) was formulated and used to test possibilities of replacement of fish meal (FM) by feather meal (FeM). A 120-day experiment was conducted with 5 month-old juveniles (27.45 mm total length, 0.308 g body weight). Three diets (500 g crude protein/kg) differing in the level of replacement of FM protein by FeM protein were prepared: 0 (basal diet), 0.25 (148 g FeM/kg) or 0.35 (210 g FeM/kg). Survival ranged from 0.96 to 0.97 (P=0.061). The basal diet enabled higher growth (59.3 mm total length, 2.6 g body weight, 1.74%/d specific growth rate; P<0.001) and lower feed conversion ratio (1.36; P=0.032) than the FeM diets. There were not significant differences in body weight and specific growth rate between 0.25 or 0.35 replacement of FM protein by FeM protein. The basal diet enabled satisfactory performance and low proportion of deformed fish (0.03), showing to be feasible for future studies on juvenile tench. The FeM inclusion at the levels tested reduced growth and increased the proportion of deformed fish.

1. Introduction

The tench (*Tinca tinca* L. 1758), a freshwater fish belonging to the family Cyprinidae, has a great potential for aquaculture (Wang et al., 2006; Celada et al., 2009; García et al., 2010). At present, juveniles are usually reared under extensive or semi-extensive systems using earthen ponds where fish management is difficult and yield depends on uncontrollable factors (Celada et al., 2007). The major obstacle for the increase of tench production is a deficit of young fish for stocking outdoor ponds or open waters (Wolnicki et al., 2006; Celada et al., 2009; García et al., 2010). Consequently, special attention is needed to find effective techniques for rearing juvenile tench under controlled conditions, focusing mainly on feed as an essential factor.

Tench are carnivorous (Kennedy and Fitzmaurice, 1970) and juveniles fed zooplankton and other small invertebrates (Pyka, 1997). In culture, the use of manufactured feed is limited by the lack of knowledge on nutritional requirements, forcing the use of dry diets formulated for other species. Fish meal (FM) is the main protein ingredient used for aquafeeds (Tacon and Metian, 2008). Feather meal (FeM) is an economical protein ingredient that has found increasing use in aquafeeds (Poppi et al., 2011). It has been tested with juveniles of several species as chinook salmon, *Oncorhynchus tshawytscha* (Fowler, 1990), Japanese flounder, *Paralichthys olivaceus* (Kikuchi et al., 1994), Indian major carp, *Labeo rohita* (Hasan et al., 1997) or common carp, *Cyprinus carpio* (Jahan et al., 2001), showing that adequate inclusion levels of FeM in diet are different

Abbreviations: BW, body weight; CP, crude protein; FCR, feed conversion ratio; FeM, feather meal; FI, feed intake; FM, fish meal; K, Fulton's coefficient; SGR, specific growth rate; TL, total length.

* Corresponding author. Tel.: +34 987291187; fax: +34 987291187.
E-mail address: agonr@unileon.es (Á. González-Rodríguez).

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depending on the studied species. The aim of this study was the formulation and manufacturing of a basal practical diet to test substitution possibilities of FM protein by FeM protein for juvenile tench.

2. Materials and methods

2.1. Fish, facilities and experimental procedures

A 120-day experiment was carried out with juvenile tench. Larvae were obtained by hatching under artificial reproduction techniques (Rodríguez et al., 2004) and were reared for five months until the juvenile stage in which the experiment started. From five days after hatching, when first feeding started, larvae were maintained in outdoor fiberglass tanks (2500 L) and fed decapsulated *Artemia* cysts for two weeks (Celada et al., 2013). Then, fish were fed a combination of a dry carp starter diet and decapsulated *Artemia* cysts. After 150 days, 932 juvenile tench with a mean initial body weight (BW) of 0.308 ± 0.011 g and a total length (TL) of 27.45 ± 0.47 mm (n = 120) were transferred to indoor facilities and stocked in nine fiberglass tanks (0.5 m × 0.25 m × 0.25 m) containing 25 L of water. Juvenile tench were randomly distributed to obtain replicates. Fish were anesthetized with tricaine methanesulfonate (MS-222; Ortoquímica S.L., Barcelona, Spain), bulk weighing for each tank was carried out (to the nearest 0.001 g) and number of fish was counted. The stocking density was 1 g BW/L and the mean number of animals in each tank was 103 ± 1.6 (mean ± standard error). All experimental groups were in triplicate (three tanks per treatment).

Artesian well water was supplied in open system (flow-throughout) and each tank had a water inlet (inflow 0.30 L/min) and outlet (provided with a 250 μm mesh filter) and light aeration. The variables of the incoming water quality (measured once a week) were: pH = 7.8, hardness 5.3°dH (German degrees, calcium 33.1 mg/L), total dissolved solids 112.2 mg/L and total suspended solids 37.3 mg/L. Dissolved oxygen content in tanks was measured with a meter HACH HQ30d (Hach Lange GMBH; Vigo, Spain) throughout the trial and values ranged between 5.7 and 7.2 mg/L. Ammonia and nitrites were measured with a spectrophotometer HACH DR2800 (Hach Lange GMBH; Vigo, Spain) from water samples taken inside the tanks (values were always ammonia <0.15 mg/L and nitrites <0.02 mg/L). Water temperature (measured twice a day) was 24 ± 1 °C and a 16 h light:8 h dark photoperiod was maintained throughout the experiment. Mortality was immediately removed from the tanks. Tanks were cleaned of feces and uneaten feed every two days.

2.2. Diets and feeding

A basal diet was formulated according to current knowledge on carnivorous fish nutrition and juvenile tench feeding. Dietary protein was provided by FM (662 g/kg diet). Decapsulated *Artemia* cysts (100 g/kg diet) were included in this diet (García et al., 2010). Crude fat was adjusted at relatively low level, as suggested by Wolnicki et al. (2006).

Triplicate groups of juvenile tench (*ca.* 103 per replicate) were fed one of three diets in which fish meal (FM) protein was substituted by hydrolyzed feather meal (FeM) protein. FM was from anchoveta. Hydrolyzed FeM was obtained by cooking under pressure broiler poultry feathers, and then grinding and drying. Proximate composition and amino acid profiles of the FM and FeM used are presented in Table 1. The maximum level of FM substituted by FeM was chosen considering the recommendations of Yu (2008). Thus, three diets (nearly isoproteic and isoenergetic) were formulated to test different levels of replacement of FM protein by FeM protein: 0 (basal diet), 0.25 (148 g FeM/kg) or 0.35 (210 g FeM/kg). Ingredients were ground in a rotary mill BRABENDER (Brabender GmbH & Co. KG, Duisburg, Germany), mixed in a mixer STEPHAN UMC5 (Stephan Food Service Equipment, Hameln, Germany) and extruded using a stand-alone extruder BRABENDER KE19/25D (Brabender GmbH & Co. KG, Duisburg, Germany) at a temperature range between 75 °C and 90 °C. Pellets (1 mm diameter) were obtained and dried during 24 h at 30 °C. Then, pellets received a coating of cod liver oil (20 g/kg diet). Formulation, proximate composition and amino acid profiles of the diets are summarized in Table 2.

Fish were fed manually four times a day (at 10:00, 14:00, 18:00 and 22:00 h), in equal portion. A ration level of 3% live weight per day was adjusted based on the biomass calculated from the samples (see subsection 2.4). This ration was slightly in excess, thus fish were fed to satiation.

2.3. Chemical analysis of the diets

Proximate composition of FM and FeM (Table 1) and practical diets (Table 2) were analyzed according to the International Standards Organization: moisture to ISO R-1442 (ISO, 1979), protein to ISO R-937 (ISO, 1978), lipid to ISO R-1443 (ISO, 1973) and ash to ISO R-936 (ISO, 1998). Gross energy was determined using a calorimeter. Samples were stored at −30 °C until analysis.

Amino acid profiles of FM and FeM (Table 1) and practical diets (Table 2) were analyzed by HPLC using AccQTag method from Waters (Milford, MA, USA). Amino acids were derivatized with 6-aminoquinolyl-N-hydrosysuccinimidyl carbamate reagent (AQC) by the method of Cohen and Michaud (1993) and Cohen and De Antonis (1994), and were detected by Dual λ Absorbance Detector Waters 2487 from Waters (Milford, MA, USA) at 254 nm. Quantification was carried out with Empower Pro 2.0 software from Waters (Milford, MA, USA). All analyses were performed in duplicate.

Table 1
Proximate composition and amino acid profiles of fish meal and feather meal (g/kg, wet basis).

	Fish meal	Feather meal
Proximate composition		
Moisture	79.9	53.3
Crude protein	680.0	761.0
Crude fat	90.1	158.7
Ash	150.0	3.4
Indispensable amino acids		
Arginine	113.8	62.5
Histidine	12.7	42.1
Isoleucine	25.6	26.5
Leucine	44.2	26.8
Lysine	44.0	11.2
Methionine	19.7	7.2
Phenylalanine	19.3	24.9
Threonine	36.3	29.5
Tryptophan	0.1	0.1
Valine	30.1	55.8
Dispensable amino acids		
Alanine	38.2	34.1
Aspartate	51.3	89.8
Cysteine	1.1	12.1
Glutamate	79.9	93.3
Glycine	24.0	20.3
Proline	24.7	64.5
Serine	36.9	86.4
Tyrosine	14.0	8.7

2.4. Data collection and statistical analysis

Every 30 days, a sample of 20 fish per tank (20% of the initial number) was taken and the fish were anesthetized. The excess water was removed with tissue paper and fish were weighed and measured individually. Total length (TL) was measured with a digital caliper (to the nearest 0.01 mm) and individual body weight (BW) was determined by precision balance (to the nearest 0.001 g). After the measurements, juveniles were returned to their respective tanks. At the end of the experiment (day 120), surviving fish were anesthetized, counted, and observed one by one using a magnifying glass in order to detect externally visible deformities. Survival rates were calculated, and individual weight and length of a sample of 40 fish per tank (40% of the initial number) were determined. Specific growth rate (SGR) was expressed as $SGR = 100\,[(\ln W_t - \ln W_0)/t]$ where W_t is the mean final weight, W_0 is the mean initial weight, and t is the duration of the experiment (days). Fulton's coefficient (K), also named condition coefficient, was used to determine the fish condition with $K = 100(W_t/TL^3)$. Feed intake (FI) was expressed as $FI = Dt/\text{fish}$ where D_t is the total amount of feed fed (g). Feed conversion ratio (FCR) was calculated as $FCR = D_t/(W_t - W_0)$ where $W_t - W_0$ is the weight gain (g) over 120 days (Fornshell and Hinshaw, 2009).

All treatments were replicated three times and the experimental unit was a tank with *ca.* 103 fish. Results were examined by analysis of variance (ANOVA) using the computer program SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Tukey test was applied to compare means at P<0.05 level of significance.

3. Results

Juvenile tench readily accepted all practical diets. Data on survival rate, growth and proportion of fish with externally visible deformities after 120 days of experiment are presented in Table 3. Survival rate ranged from 0.96 to 0.97 and was not affected by treatment (P=0.061). The highest growth (59.3 mm TL, 2.6 g BW and 1.74%/d SGR) was observed for fish fed the basal diet with significant differences (P<0.001) from the diets with 0.25 or 0.35 replacement of FM protein (148 or 210 g FeM/kg, respectively). Table 4 shows the changes in weight and length of the fish fed the practical diets from 0 to 90 days. Tench fed the basal diet grew faster than those fed the diets with 0.25 or 0.35 replacement of FM protein (148 or 210 g FeM/kg, respectively), being significant different (P<0.001) from day 60 onwards.

The FCR was not affected (P>0.05) by dietary treatment from 0 to 90 days (Table 4). For the entire experimental period (0 to 120 days) FCR was lower (P=0.032) for fish fed the basal diet than for fish fed the FeM diet (1.36 vs 1.47; Table 3). The feed intake (FI) was higher (P<0.001) for fish fed the basal diet (3.07 g/fish) than for fish fed the diets with 0.25 or 0.35 replacement of FM protein (average: 2.68 g/fish). Condition coefficient ranged from 1.22 for the basal diet to 1.29 for the 0.35 replacement diet (210 g FeM/kg). The proportion of fish with externally visible deformities was higher (P<0.001) for the diets containing FeM (average: 0.15) than for the basal diet (0.03). Body deformities affected to the caudal peduncle (break in the tail axis).

Table 2
Ingredient composition and determined nutrient content of the experimental diets (g/kg diet, wet basis).

	Replacement[a]		
	0	0.25	0.35
Ingredient			
Fish meal (680 g CP/kg)	662	490	422.6
Feather meal (760 g CP/kg)	–	148	210
Maize	121	147	153.4
Cod liver oil	20	20	20
Dried *Artemia* cysts	100	100	100
Dicalcium phosphate	10	10	10
Choline chloride	2	2	2
Soy lecithin	5	3	2
Mineral premix[b]	20	20	20
Vitamin premix[c]	20	20	20
L-Ascorbyl-2-monophosphate-Na	10	10	10
Carboxymethyl cellulose	30	30	30
Determined analysis			
Moisture	74.0	70.0	68.0
Crude protein	502.2	501.7	502.3
Crude fat	108.6	113.1	114.2
Ash	170.4	150.2	143.4
Gross energy (MJ/kg)	19.0	19.1	19.4
Indispensable amino acids			
Arginine	88.3	79.9	72.1
Histidine	11.6	14.8	16.1
Isoleucine	19.0	18.6	18.3
Leucine	35.0	34.1	33.6
Lysine	39.8	34.7	31.7
Methionine	13.2	10.9	10.1
Phenylalanine	15.0	15.4	15.2
Threonine	22.0	21.4	20.9
Tryptophan	0.2	0.2	0.2
Valine	25.2	27.9	28.2
Dispensable amino acids			
Alanine	31.5	30.4	29.6
Aspartate	40.5	45.0	45.8
Cysteine	1.1	2.7	3.5
Glutamate	63.3	63.7	62.4
Glycine	16.2	14.9	14.2
Proline	18.3	23.5	25.3
Serine	24.3	30.7	32.1
Tyrosine	10.1	9.0	8.4

[a] Proportion of dietary crude protein from fish meal substituted by hydrolyzed feather meal protein.
[b] Provided (mg/kg diet): $MgSO_4{-}7H_2O$, 500; $ZnSO_4{-}7H_2O$, 25; $MnSO_4{-}H_2O$, 13; $CuSO_4{-}5H_2O$, 3; $CoSO_4$, 0.1; $FeSO_4{-}7H_2O$, 150; KI, 2.5; Na_2SeO_3, 0.3.
[c] Provided (mg/kg diet): myoinositol, 500; thiamin, 5; riboflavin, 8; niacin, 50; pyridoxine, 15; pantothenic acid, 50; biotin, 1.5; folic acid, 35; cyanocobalamin, 0.05; retinol, 10; α-tocopherol, 300; cholecalciferol, 0.0625; naphthoquinone, 50; ethoxyquin, 700.

Table 3
Survival, growth values and percentages of deformed juvenile tench (*Tinca tinca*) fed practical diets with different levels of replacement of fish meal protein by feather meal protein over 120 days.

	Replacement[a]			SEM[b]	P value
	0	0.25	0.35		
Survival	0.97	0.97	0.96	0.129	0.061
Total length (mm)	59.3^{A}	56.3^{B}	54.4^{C}	0.252	<0.001
Body weight (g)	2.6^{A}	2.2^{B}	2.1^{B}	0.033	<0.001
SGR[c]	1.74^{A}	1.62^{B}	1.58^{B}	0.012	<0.001
FI[d]	3.07^{A}	2.76^{B}	2.56^{C}	0.013	<0.001
FCR[e]	1.36^{A}	1.48^{B}	1.46^{B}	0.024	0.032
Condition factor[f]	1.22^{A}	1.24^{A}	1.29^{B}	0.009	0.002
Deformed fish	0.03^{A}	0.16^{B}	0.14^{B}	0.330	<0.001

[A–C] Values in the same row having different superscripts are significantly different ($P<0.05$).
[a] Proportion of dietary crude protein from fish meal substituted by hydrolyzed feather meal protein.
[b] Pooled standard error of the mean: survival = 3 tanks; growth values = 120 juveniles; deformed fish = *ca.* 300 juveniles.
[c] Specific growth rate (%/day) = 100 × [(ln final mean body weight − ln initial mean body weight)/days].
[d] Feed intake = total feed fed (g)/fish.
[e] Feed conversion ratio = feed fed/(final weight − initial weight).
[f] $100 \times (\text{body weight}/\text{body length}^3)$.

Table 4
Total length, body weight and feed conversion ratio (FCR) of juvenile tench (*Tinca tinca*) fed different levels of replacement of fish meal protein by feather meal protein over 90 days.

		Replacement[a]			SEM[b]	P value
Day		0	0.25	0.35		
30	Total length (mm)	34.97	35.20	34.46	0.162	0.162
	Body weight (g)	0.48	0.51	0.47	0.008	0.101
	FCR	0.88	0.78	0.97	0.053	0.370
60	Total length (mm)	44.73[A]	43.11[B]	40.78[B]	0.300	<0.001
	Body weight (g)	1.05[A]	0.94[B]	0.82[B]	0.018	<0.001
	FCR	0.90	0.97	1.05	0.031	0.145
90	Total length (mm)	53.89[A]	50.96[B]	49.59[C]	0.291	<0.001
	Body weight (g)	1.90[A]	1.62[B]	1.50[B]	0.030	<0.001
	FCR	0.95	1.06	1.10	0.031	0.105

[A–C]Values in the same row having different superscripts are significantly different (P<0.05).
[a] Proportion of dietary crude protein from fish meal substituted by hydrolyzed feather meal protein.
[b] Pooled standard error of the mean: growth values = 60 juveniles.

The diets showed no significant differences in the content of isoleucine, leucine, phenylalanine, threonine, tryptophan and valine (Table 2). The inclusion of FeM resulted in higher histidine content (P<0.006), but arginine (P<0.001), lysine (P<0.001) and methionine (P<0.005) contents were lower than those of the basal diet. With 0.25 replacement of FM protein (148 g FeM/kg), the contents of the mentioned amino acids decreased 9.5% for arginine, 12.9% for lysine and 17.8% for methionine. Growth of juvenile tench fed the FeM diets was reduced (P<0.001).

4. Discussion

Most of the studies on the intensification of juvenile tench rearing have started with fish aged 3–7 months. Growth rates (SGR, % per day) from 0.70 to 1.98 have been reported, being higher when dry diets for other species were supplemented with natural feed (García et al., 2010). In the present study, 5 month-old juveniles were fed the basal practical diet as the sole feed and the SGR observed was of 1.74. Thus, it is possible to use successfully this extruded diet without any natural supplements.

There is scarce information on FCR of juvenile tench, and all previous data have been calculated considering the total amount of diet supplied to the fish. In the intensive rearing of 3 month-old juveniles (around 0.45 g at the beginning of the experiment) fed a commercial trout feed for some 450 days, Rennert et al. (2003) reported FCRs ranging from 1.75 to 3.56. Mareš et al. (2007) tested three dry diets with 7 month-old juveniles (0.8–1.2 g) during 63 days, and FCRs ranged from 1.84 to 4.15. In the present 120-day trial with 5 month-old juveniles (0.3 g), FCRs ranged from 1.36 to 1.48. These values are more favorable than those previously reported and are in the range of the levels of feed conversion in the intensive culture of well studied species, with FCR values typically ranging from 0.8 to 1.5 (Hardy and Barrows, 2002).

Regarding substitution possibilities of FM by FeM, it must be taken into account that juvenile fishes have a higher requirement for most nutrients and greater sensibility to nutrient deficiency compared with older fish. For this reason, most research trials have used juveniles as experimental animal (Yu, 2008). The fish species studied have different tolerance for dietary FeM. For instance, Fowler (1990) recommended 150 g FeM/kg in diet for chinook salmon (*O. tshawytscha*) without affecting growth. Lower levels of FeM in diet, as 120 g/kg for Japanese flounder, *P. olivaceus* (Kikuchi et al., 1994), or 104 g/kg for Indian major carp, *L. rohita* (Hasan et al., 1997), have been successfully included. However, body weight of common carp (*C. carpio*) was reduced when 50 g FeM/kg in diet was incorporated (Jahan et al., 2001). In the present study with juvenile tench, the lowest inclusion of FeM in diet (148 g/kg) led to a decrease in growth rate. This replacement (0.25 of FM protein) derived in a significantly lower content of arginine, lysine and methionine (Table 2) compared with the control diet. Dietary deficiency of indispensable amino acids results in reduced growth such has been reported in some freshwater fish: for arginine in common carp, *C. carpio* (NRC, 2011), chinook salmon, *O. tshawytscha* (NRC, 2011) or Indian major carp, *Cirrhinus mrigala* (Ahmed and Khan, 2004a), and for lysine in chinook salmon, *O. tshawytscha* (NRC, 1993) or Indian major carp, *C. mrigala* (Ahmed and Khan, 2004b). In the present trial, when 148 g FeM/kg were included in diet, the contents of arginine and lysine were 79.9 and 34.7 g/kg diet, respectively. In spite of these amounts were higher than the requirements of the above mentioned species, growth of juvenile tench decreased. Considering the indispensable amino acids, only methionine (10.9 g/kg diet) was below the requirements of chinook salmon, *O. tshawytscha* (NRC, 1993), common carp, *C. carpio* (NRC, 1993) or rainbow trout, *Oncorhynchus mykiss* (Bae et al., 2011). Besides the deficiency in some indispensable amino acids, another limitation for the use of FeM in diets for aquatic animals may be its difficult digest, which has been attributed to the disulfide bonds (NRC, 2011). Despite the FeM hydrolyzation process led to a significant improvement in the digestibility of protein (Bureau et al., 1999, 2000), FM apparent digestibility recorded in different fish species is higher (NRC, 2011). Thus, the decrease in growth of juvenile tench fed the diets including FeM could be attributable to both a deficiency of some indispensable amino acids and a lower digestibility of FeM compared with FM.

The potential benefit of FM substitution by FeM depends not only on the biological response of fish, but also the relative cost of both sources of protein (Yu, 2008). As current prices of FeM are less than half of FM prices, a small reduction in growth could be assumed by savings in the most expensive ingredient of the diet. From this consideration, the main concern may be not a decrease in SGR of juvenile tench (from 1.74 to 1.62) but the increase of the proportions of deformed fish from 0.03 to 0.15 when FeM was included in the diet. Body deformities are an undesirable but inherent problem in intensive aquaculture, which entail severe losses to the production sector and have the more detrimental effects on the consumers' image of aquaculture (Zambonino-Infante et al., 2009). Inadequate feeding, especially during early development, has been reported to induce malformations in fish (Cahu et al., 2003; Fontagné, 2009). High proportions of deformed fish have been reported (0.37–0.92) for other juvenile cyprinids (Kaminski et al., 2005; Myszkowski et al., 2002) and for juvenile tench (0.23–0.96) (Rennert et al., 2003; Wolnicki et al., 2006; Myszkowski et al., 2010) when were fed commercial diets formulated for other species. In juvenile *T. tinca*, a relationship among the use of commercial diets for other species and fish with elevated condition coefficient (1.3–1.4) and body deformities has been suggested (Kamler et al., 2006; Wolnicki et al., 2006; Myszkowski et al., 2010), and fast growing juveniles were more endangered with body deformities (Rennert et al., 2003; Kamler et al., 2006; Myszkowski et al., 2010), probably due to imbalanced feed composition for tench. In the present experiment, the basal diet enabled good growth rate (1.74) and low proportion of deformed fish (0.03), indicative that this diet was adequate for proper growth of juvenile tench.

5. Conclusions

The basal diet enabled high survival, satisfactory growth and low proportion of deformed fish. This diet might be a starting point for nutritional studies on juvenile tench. The incorporation of FeM in diet at the levels tested reduced growth and increased proportion of deformed fish.

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Effects of varying protein level in practical diets on survival, growth, feed utilization and body composition of juvenile tench (*Tinca tinca* L.)

Á. González-Rodríguez · J. D. Celada · J. M. Carral · M. Sáez-Royuela · J. B. Fuertes

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Abstract The aim of this study was to evaluate the effects of practical diets with different protein content on survival, growth, feed utilization and body composition of juvenile tench (*Tinca tinca*). A 90-day experiment was conducted with 6-month-old juveniles (34.35 mm total length, 0.411 g weight). Six practical diets differing in the protein level were tested: 40, 44, 48, 52, 56 or 60 %. Survival rates ranged from 96.7 to 100 %. The 52 % protein enabled the highest growth (55.49 mm total length, 2.11 g weight, 1.80 % day^{-1} specific growth rate) and the lowest feed conversion ratio (1.61) without significant differences ($P > 0.05$) from the 48 %. Protein productive value ranged from 15.64 to 22.01. The percentages of fish with visible deformities ranged from 1.1 to 4.4 %. The relationship among amino acid profiles of the diets, growth of juveniles, body composition and amino acid requirements of other fish species is discussed. Second-order polynomial regression analysis showed that the optimum dietary protein requirement for maximum growth of juvenile tench may be 52.7 %.

Keywords Juvenile · Practical diet · Protein requirement · Tench · *Tinca tinca*

Abbreviations

EAA	Essential amino acids
FCR	Feed conversion ratio
FM	Fish meal
K	Fulton's coefficient
NRC	National Research Council
PPV	Protein productive value
SGR	Specific growth rate
TL	Total length
W	Weight

Á. González-Rodríguez (✉) · J. D. Celada · J. M. Carral · M. Sáez-Royuela · J. B. Fuertes
Dpto. de Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain
e-mail: agonr@unileon.es

Introduction

Tench, *Tinca tinca* (Linnaeus 1758), a freshwater fish belonging to the family Cyprinidae, has a great potential for aquaculture (Wang et al. 2006; Celada et al. 2009; García et al. 2013); it is demanded in the market and appreciated by consumers as a tasty fish with healthy meat. Originally, occurring in the waters of Europe and Siberia, today tench occurs in the inland waters of all the continents (Freyhof and Kottelat 2008). In natural habitats, tench are carnivorous (Kennedy and Fitzmaurice 1970), and gut content analysis shows that juveniles fed zooplankton and other small invertebrates (Pyka 1997). Traditionally, juvenile tench are cultured in extensive or semi-extensive systems using earthen ponds with usually low yields because both growth and survival depend on uncontrolled environmental factors. The deficit of young fish for stocking in outdoor ponds or open waters is considered as the major obstacle to increasing tench production (Celada et al. 2009; García et al. 2010), being necessary to find effective techniques for rearing juvenile tench under controlled conditions, focusing mainly on feed as an essential factor (Celada et al. 2009).

In the search of efficient, low cost and environmental friendly commercial feeds, protein is considered the most important component in fish diets. Protein amounts over fish requirements are metabolized and used for energy, and nitrogenous waste material excreted increases due to an excess of protein provided in diets (Hardy and Gatlin 2002). Thus, for economical and environmental reasons, determining the minimum dietary protein level which allows optimum growth is a major challenge in nutrition research. Protein requirements of several juvenile carnivorous freshwater fish species have been studied, and optimum dietary protein ranges from 36 to 54.9 % (Webster et al. 1997; Gunasekera et al. 2000; Portz et al. 2001; Martínez-Palacios et al. 2007; Schulz et al. 2007; NRC 2011). In juvenile tench, when dry diets formulated for other species have been supplied, crude protein content ranged from 35 to 64 % (García et al. 2013). Considering that optimum level of protein has not been established in this species, the aim of the present study was to evaluate the effects of practical diets with different protein content on survival, growth, feed utilization and body composition of juvenile tench.

Materials and methods

Fish, facilities and experimental procedures

A 90-day experiment was carried out with juvenile tench. Larvae were obtained by hatching under artificial reproduction techniques (Rodríguez et al. 2004) and were reared for 6 months until the juvenile stage in which the experiment started. From 5 days after hatching, when first feeding started, larvae were maintained in outdoor fiberglass tanks (2,500 L) and fed decapsulated *Artemia* cysts for 2 weeks (Celada et al. 2013). Then, fish were fed a combination of a dry carp starter diet and decapsulated *Artemia* cysts. After 6 months, 630 juvenile tench (34.35 ± 0.38 mm total length, 0.411 ± 0.015 g weight, mean ± SE, $n = 120$) were transferred to indoor facilities. Fish were anesthetized with MS-222 (Ortoquímica S.L., Barcelona, Spain), bulk weighed and randomly distributed as groups of 30 fish in 21 fiberglass tanks (0.5 × 0.25 × 0.25 m) containing 25 L of water to obtain replicates corresponding to the different feeding treatments. All experimental groups were in triplicate. The juveniles were acclimated to experimental conditions for 10 days before the trial.

Artesian well water was supplied in open system (flow-throughout) and each tank had a water inlet (inflow 0.30 L min^{-1}) and outlet (provided with a 250 μm mesh filter) and light aeration. The variables of the incoming water quality (measured once a week) were: pH 7.7, hardness 5.3 °dH (German degrees, calcium 32.5 mg L^{-1}), total dissolved solids 112.5 mg L^{-1} and total suspended solids 36.9 mg L^{-1}. Dissolved oxygen content in tanks was measured with a meter HACH HQ30d (Hach Lange GMBH, Vigo, Spain) throughout the trial and values ranged between 5.7 and 8.2 mg L^{-1}. Ammonia and nitrites were measured with a spectrophotometer HACH DR2800 (Hach Lange GMBH, Vigo, Spain) from water samples taken inside the tanks (values were always ammonia <0.08 mg L^{-1} and nitrites <0.015 mg L^{-1}). Water temperature (measured twice a day) was 24 ± 1 °C and a 16 h light: 8 h dark photoperiod was maintained throughout the experiment. Dead animals were immediately removed from the tanks. Every other day, the bottom of the tanks was cleaned by siphoning. All procedures used in the study were approved by the León University Ethics Committee (Spain).

Diets and feeding

A basal practical diet was prepared taking as reference the formulation of García et al. (2013). Vitamins and minerals were included taking as reference the recommendations for other freshwater fish species better known than tench. Triplicate groups of juvenile tench were fed one of six practical diets prepared to test different protein levels. Fish meal (FM, 68 % crude protein) was used as principal source of protein. The different protein levels were obtained by reducing the amount of corn meal and increasing the amount of FM. Thus, six experimental diets (nearly isoenergetic) were prepared to test 40, 44, 48, 52, 56 or 60 % dietary protein. Ingredients were ground in a rotary mill BRABENDER (Brabender GmbH & Co. KG, Duisburg, Germany), mixed in a mixer STEPHAN UMC5 (Stephan Food Service Equipment, Hameln, Germany) and extruded using a stand-alone extruder BRABENDER KE19/25D (Brabender GmbH & Co. KG, Duisburg, Germany) at a temperature range between 100 and 110 °C (speed of supply 60 rpm and speed of the spindle 40 rpm). Pellets (1 mm diameter) were obtained and then dried during 24 h at 30 °C. Then, pellets received a coating of cod liver oil. During the trial, it was observed that the extruded diets quickly sank to the bottom and remained apparently intact in the water at least 8 h. Formulation and proximate composition of practical diets are summarized in Table 1 and amino acid profiles are in Table 2. As reference, another triplicate group was fed a commercial carp starter (ALLER AQUA, produced by Aller Aqua A/S Allervej, Christiansfeld, Denmark. Proximate composition: moisture 71.4 g kg^{-1}, crude protein 638 g kg^{-1}, crude fat 121.2 g kg^{-1}, carbohydrates 44.9 g kg^{-1}, ashes 124.5 g kg^{-1}, gross energy 20.9 MJ kg^{-1}, pellet diameter 0.9–1.6 mm).

Fish were fed by means of automatic feeders (Automated Microdiet Dispenser AMD™, Perth, Australia) four times a day (at 10:00, 14:00, 18:00 and 22:00 h) to apparent satiation.

Chemical analysis of diets and fish

Proximate composition of practical diets (Table 1) and the whole body of juvenile tench (Table 4) were analyzed according to the Norms of the International Standards Organization (1973–1998): moisture to ISO R-1442, protein to ISO R-937, lipid to ISO R-1443, ash to ISO R-936 and gross energy to ISO 9831. Carbohydrates content was calculated by

Table 1 Formulation and proximate composition of the practical diets with different protein content (g kg^{-1} diet, wet basis)

	Protein content (%)					
	40	44	48	52	56	60
Ingredients						
Fish meal[a]	506	566	626	686	746	806
Corn meal[b]	295	235	180	130	75	25
Cod liver oil[c]	40	40	35	25	20	10
Dried *Artemia* cysts[d]	100	100	100	100	100	100
Carboxymethyl cellulose[e]	30	30	30	30	30	30
L-Ascorbyl-2-monophosphate-Na[f]	5	5	5	5	5	5
Dicalcium phosphate[f]	10	10	10	10	10	10
Choline chloride[f]	3	3	3	3	3	3
Sodium chloride[g]	1	1	1	1	1	1
Mineral–vitamin premix[h,i]	10	10	10	10	10	10
Proximate composition						
Moisture	79.1	75.9	74.1	75.0	74.3	73.0
Crude protein	402.8	440.8	481.6	520.7	559.6	601.9
Crude lipid	115.0	118.0	116.1	109.5	106.6	101.0
Carbohydrates	289.8	243.0	198.0	156.8	112.5	70.3
Ash	113.3	122.3	130.2	138.0	147.0	153.8
Gross energy (MJ kg^{-1})	19.2	19.3	19.5	19.4	19.5	19.5

[a] Skretting España, S.A., Ctra. de la Estación s/n 09620 Cojóbar, Burgos, España

[b] Adpan Europa, S.L., ES-33186 El Berrón, Siero, Asturias, Spain

[c] Acofarma distribution, S.A., ES-08223 Terrassa, Barcelona, Spain

[d] INVE Aquaculture Nutrition, High HUFA 430µ, Hoogyeld 91, Dendermonde, Belgium

[e] Helm Iberica, S.A., ES-28108 Alcobendas, Madrid, Spain

[f] Nutral, S.A., ES-28720 Colmenar Viejo, Madrid, Spain

[g] Unión Salinera de España, S.A., Serrano, 21– 2,28001 Madrid, Spain

[h] Mineral premix (mg kg^{-1} premix): $MgSO_4 \cdot 7H_2O$, 300,000; $ZnSO_4 \cdot 7H_2O$, 11,000; $MnSO_4 \cdot H_2O$, 4,000; $CuSO_4 \cdot 5H_2O$, 1,180; $CoSO_4$, 26; $FeSO_4 \cdot 7H_2O$, 77,400; KI, 340; Na_2SeO_3, 68

[i] Vitamin premix (mg kg^{-1} premix): inositol, 50,000; thiamin, 500; riboflavin, 800; niacin, 5,000; pyridoxine, 1,500; pantothenic acid, 5,000; biotin, 150; folic acid, 3,500; cianocobalamin, 5; retinol, 2,400; α-tocopherol, 30,000; cholecalciferol, 6.25; naphthoquinone, 5,000; ethoxyquin, 70,000

subtracting the content of moisture, protein, lipid and ash from wet weight. Samples were stored at −30 °C until analysis.

Amino acid profiles of the practical diets (Table 2) and the whole body of juvenile tench (Table 4) were analyzed by HPLC using AccQTag method from Waters (Milford, MA, USA). Amino acids were derivatized with 6-aminoquinolyl-*N*-hydrosysuccinimidyl carbamate reagent (AQC) by the method of Cohen and Michaud (1993) and Cohen and De Antonis (1994) and were detected by Dual λ Absorbance Detector Waters 2487 from Waters (Milford, MA, USA) at 254 nm. Quantification was carried out with Empower Pro 2.0 software from Waters (Milford, MA, USA). All analyses were performed in duplicate.

Table 2 Amino acid profiles of the practical diets with different protein content (g kg^{-1} diet, wet basis)

	Protein content (%)					
	40	44	48	52	56	60
Essential amino acid						
Arginine	55.70[a]	64.48[b]	71.00[c]	79.20[d]	86.78[e]	94.57[f]
Histidine	6.38[a]	7.55[b]	8.82[c]	9.56[d]	10.74[e]	12.23[f]
Isoleucine	19.09[a]	21.08[b]	23.69[c]	25.07[d]	26.67[e]	27.77[f]
Leucine	34.33[a]	37.12[a]	41.28[b]	44.71[c]	47.71[c]	51.51[d]
Lysine	37.04[a]	40.53[b]	43.46[c]	46.76[d]	49.85[e]	51.54[f]
Methionine	9.21[a]	9.89[b]	10.65[c]	11.32[d]	12.25[e]	13.02[f]
Phenylalanine	15.76[a]	18.55[b]	20.20[c]	21.70[d]	23.21[e]	25.32[f]
Threonine	20.13[a]	22.02[b]	24.06[c]	26.21[d]	27.67[e]	28.07[f]
Tryptophan	2.28[a]	2.51[a,b]	2.70[b,c]	2.82[b,c]	2.88[c]	3.05[c]
Valine	22.71[a]	23.20[a]	25.22[b]	26.78[b]	27.73[c]	28.32[c]
Non-essential amino acid						
Alanine	32.15[a]	34.30[b]	36.58[c]	38.81[d]	40.29[e]	41.53[f]
Aspartate	36.15[a]	39.67[b]	43.36[c]	47.19[d]	51.82[e]	55.60[f]
Cysteine	1.30[a]	1.52[a,b]	1.62[b]	1.73[b,c]	1.94[c,d]	2.05[d]
Glutamate	41.05[a]	44.53[b]	47.83[c]	50.93[d]	53.89[e]	57.71[f]
Glycine	13.79[a]	14.88[b]	15.90[c]	17.23[d]	19.39[e]	20.92[f]
Proline	17.03[a]	18.74[b]	20.25[c]	22.11[d]	23.27[e]	24.54[f]
Serine	24.09[a]	26.38[b]	29.27[c]	31.57[d]	33.84[e]	36.06[f]
Tyrosine	11.05[a]	12.66[b]	13.93[c]	14.59[d]	15.23[e]	16.35[f]

Asparagine and glutamine contents were not determined

In the same row, values with different superscript are significantly different ($P < 0.05$)

Data collection and statistical analysis

Every 30 days, a sample of 15 juveniles from each replicate (45 per treatment, 50 % of total) was collected to register total length (TL) and weight (*W*). After being anesthetized, excess water was removed with tissue paper and fish were weighed and measured individually. Total length (TL) was measured with a digital caliper (to the nearest 0.01 mm) and individual wet weight (*W*) was determined by precision balance (to the nearest 0.001 g). After measurements, fish were returned to their respective tanks. At the end of the experiment (day 90), all surviving tench were individually weighed, measured and observed one by one using magnifying glass in order to detect externally visible deformities. Specific growth rate (SGR) was expressed as $\text{SGR} = 100 \times (\ln W_t - \ln W_0) \times t^{-1}$, where W_t is the mean final weight, W_0 is the mean initial weight and t is the duration of the experiment (days). Fulton's coefficient (K) was used to determine the fish condition with $K = 100 \times W_t \times (\text{TL}^3)^{-1}$. According to Fornshell and Hinshaw (2009), feed conversion ratio (FCR) was calculated as $\text{FCR} = D_t \times (W_t - W_0)^{-1}$, where D_t is the total amount of feed fed (g) and $W_t - W_0$ is the weight gain (g) over 90 days. Protein productive value (PPV) was expressed as $\text{PPV} = 100\ [((W_t \times C_1) - (W_0 \times C_0))/(C_{diet} \times D_t)]$, where C_1 and C_0 are the final and initial protein concentration in the whole body, respectively, and C_{diet} is the concentration in the diet.

Results were examined by analysis of variance (ANOVA) using the computer program SPSS 16.0 (SPSS, Chicago, IL, USA). Duncan test was applied to compare means at $P < 0.05$ level of significance. Percentages were arcsine-transformed prior to statistical analysis.

Results

Juvenile tench readily accepted all practical diets, as well as the commercial carp starter. Final values (90 days) of survival, growth, feed utilization and percentages of fish with externally visible deformities are presented in Table 3. Survival ranged from 96.7 to 100 %.

Regarding growth, the 52 % protein enabled the highest values (55.49 mm TL, 2.11 g W, 1.80 % day^{-1} SGR) without significant differences ($P > 0.05$) from the 48 %. Fish fed lower (40 or 44 %) or higher (56 or 60 %) protein contents had lower growth ($P < 0.05$). Figures 1 and 2 show the changes in weight and length, respectively, throughout the 90 days of trial. Considering the different samplings, significant differences ($P < 0.05$) were only found at the end of the experiment (day 90). Second-polynomial regression analysis based on SGR (Fig. 3) showed that the optimum dietary protein level for maximum growth may be 52.7 %.

Condition factor (*K*) ranged from 1.20 to 1.26 with practical diets (Table 3). Fish fed carp starter had the highest condition coefficient (1.34). FCR ranged from 1.61 to 1.95. The 52 % protein enabled the lowest feed conversion ratio (1.61) without significant differences ($P > 0.05$) from the 48 %. PPV ranged from 15.64 to 22.01. PPV of fish fed dietary protein levels ranging from 40 to 52 % was significantly higher ($P < 0.05$; average: 21.28) than those fish fed diets with higher protein contents (56 or 60 %).

After 90 days, the percentages of fish with externally visible deformities were low for all practical diets, ranging from 1.1 to 4.4 % (Table 3) while in the group fed carp starter, it was significantly higher ($P < 0.05$; 47.8 %). Body deformities affected to the spinal column and caudal peduncle (break in the tail axis).

Amino acid profiles of practical diets are presented in Table 2. Comparing 52 with 48 % dietary protein, there were reductions in the essential amino acids (EAA) contents that ranged from 4.25 % (tryptophan) to 10.35 % (arginine), but they were not accomplished with reduction in growth. Comparing 48 with 44 % dietary protein, there were reductions in the EAA contents that ranged from 6.74 % (lysine) to 14.40 % (histidine) and growth was significantly reduced ($P < 0.05$). With levels above 52 % dietary protein, the EAA contents in diet increased but growth was not improved.

Proximate composition and amino acid profiles of the whole body of juvenile tench at the start and end of the experiment are presented in Table 4. Protein content of fish fed practical diets ranging from 52 to 60 % dietary protein was higher ($P < 0.05$) than those fish fed diets with lower protein contents (40, 44 or 48 %). No differences ($P > 0.05$) in moisture, lipid and ash were found among fish fed practical diets. Regarding amino acids profiles of the whole body, the content of all amino acids increased up to 52 % dietary protein and remained without significant differences ($P > 0.05$) at higher levels.

Discussion

Most of the studies on the intensification of juvenile tench rearing have started with fish aged 3–7 months and growth rates (SGR, % day^{-1}) from 0.70 to 1.98 have been reported,

Table 3 Survival, growth performance and percentages of deformed juvenile tench fed practical diets with different protein content and carp starter (CS) over 90 days

	Protein content (%)						CS
	40	44	48	52	56	60	
Survival (%)	96.7 ± 0.3	98.9 ± 0.2	96.7 ± 0.3	100 ± 0.0	97.8 ± 0.2	100 ± 0.0	100 ± 0.0
Total length (mm)	51.64 ± 0.37[a]	51.94 ± 0.36[a]	54.74 ± 0.36[b,c]	55.49 ± 0.22[c]	53.83 ± 0.37[b,d]	53.28 ± 0.26[d]	52.24 ± 0.21[a]
Weight (g)	1.70 ± 0.05[a]	1.73 ± 0.05[a]	2.03 ± 0.05[b]	2.11 ± 0.04[b]	1.99 ± 0.05[b,c]	1.88 ± 0.03[c]	1.92 ± 0.03[b,c]
SGR (% day^{-1})[1]	1.54 ± 0.03[a]	1.56 ± 0.03[a]	1.74 ± 0.03[b,c]	1.80 ± 0.02[c]	1.72 ± 0.03[b]	1.67 ± 0.02[b]	1.70 ± 0.02[b]
K[2]	1.20 ± 0.01[a]	1.21 ± 0.01[a,b]	1.21 ± 0.01[a,b]	1.23 ± 0.01[a,b]	1.26 ± 0.01[c]	1.24 ± 0.01[b,c]	1.34 ± 0.01[d]
FCR[3]	1.95 ± 0.07[a]	1.91 ± 0.06[a]	1.71 ± 0.05[b,c]	1.61 ± 0.04[b]	1.79 ± 0.06[a,c]	1.83 ± 0.04[a,c]	1.71 ± 0.03[b,c]
PPV[4]	22.01 ± 0.78[a]	21.07 ± 0.71[a]	21.47 ± 0.70[a]	20.57 ± 0.44[a]	17.94 ± 0.54[b]	15.64 ± 0.30[c]	15.06 ± 0.26[c]
Deformed fish (%)	4.4 ± 0.1[a]	3.3 ± 0.2[b]	2.2 ± 0.2[c,d]	1.1 ± 0.2[c]	2.2 ± 0.2[c,d]	1.1 ± 0.2[c]	47.8 ± 1.2[e]

Values are mean ± SE. In the same row, values with different superscript are significantly different ($P < 0.05$). Growth data derived from all surviving fish (ca. 30 per replicate, $n = 90$)

[1] Specific growth rate (% day^{-1}) = 100 × [(ln final body weight − ln initial body weight) × $days)^{-1}$]

[2] Condition factor = 100 × (body weight × body $length^{3-1}$)

[3] Feed conversion ratio = total amount of feed fed × weight $gain^{-1}$

[4] Protein productive value = (fish protein gain × crude protein $intake^{-1}$) × 100

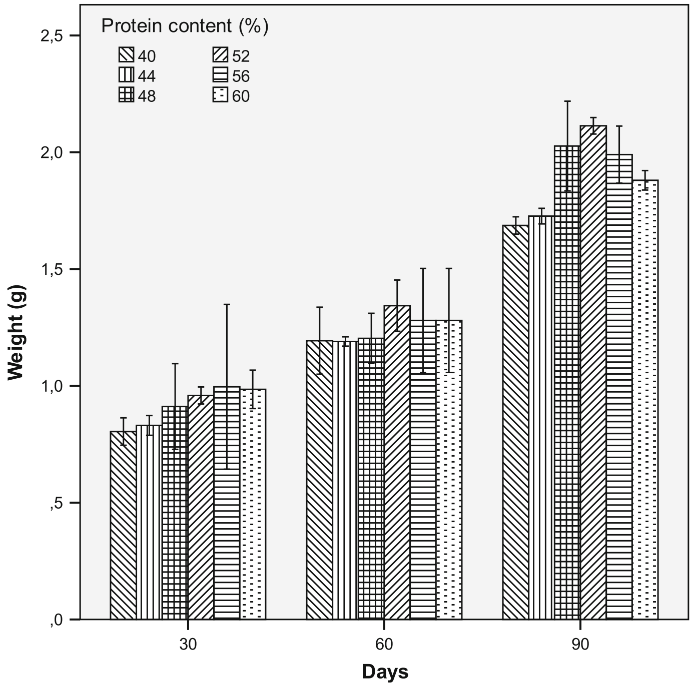

Fig. 1 Weight (mean ± SEM) of juvenile tench (*T. tinca*) at various periods fed practical diets with different protein contents over 90 days. At day 30 and 60, data derived from 15 fish sampled per replicate ($n = 45$). At the end of the experiment (day 90), data derived from all surviving fish (ca. 30 per replicate, $n = 90$)

being higher when dry diets for other species were supplemented with natural feed (García et al. 2013). In the present study, 6-month-old juveniles were fed practical diets as the sole feed and SGRs (1.54–1.80) are in the range of the highest reported. Thus, it is possible to use successfully these extruded diets without any natural supplements.

There is a scarcity of information on FCR of juvenile tench nutrition. All previous data have been calculated using the total amount of diet supplied to the fish. In the intensive rearing of 3-month-old juveniles (around 0.45 g at the beginning of the experiment) fed a commercial trout feed for some 450 days, Rennert et al. (2003) reported FCRs ranging from 1.75 to 3.56. Mareš et al. (2007) tested three dry diets with 7-month-old juveniles (0.8–1.2 g) during 63 days, and FCRs ranged from 1.84 to 4.15. In the present trial with 6-month-old juveniles (0.4 g), FCRs were 1.6–1.9, which are values more favorable than those previously reported and close to the feed conversion rates in the intensive culture of well-studied species, with values typically ranging from 0.8 to 1.5 (Hardy and Barrows 2002).

Body deformities are an undesirable but inherent problem in intensive aquaculture, which entail severe losses to the production sector and have the more detrimental effects on the consumers' image of aquaculture (Zambonino-Infante et al. 2009). Inadequate feeding, especially during early development, has been reported to induce malformations in fish (Cahu et al. 2003; Fontagné 2009). Different authors have reported high percentages (22.7–96.4 %) of deformed juvenile tench under intensive conditions (Rennert et al. 2003;

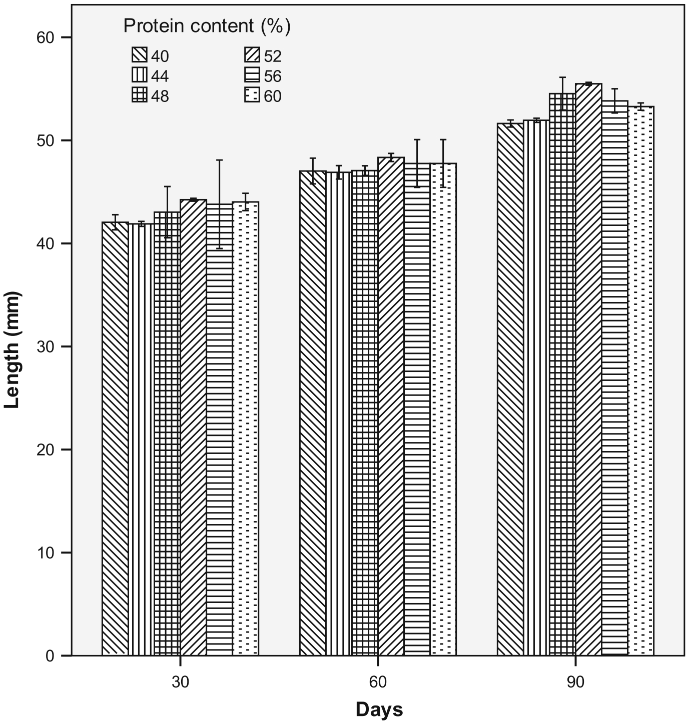

Fig. 2 Length (mean ± SEM) of juvenile tench (*T. tinca*) at various periods fed practical diets with different protein contents over 90 days. At day 30 and 60, data derived from 15 fish sampled per replicate ($n = 45$). At the end of the experiment (day 90), data derived from all surviving fish (ca. 30 per replicate, $n = 90$)

Wolnicki et al. 2006; Myszkowski et al. 2010), probably due to imbalanced feed composition for this species (García et al. 2013). In the present experiment, juvenile tench that received carp starter had a too high condition coefficient (1.34), which means a growth in length proportionally lesser than growth in weight, and 47.8 % of them showed deformities. In contrast, the practical diets with 48–60 % protein enabled similar or higher growth rate and very low percentage of deformed fish was recorded (average: 1.7 %). This enormous difference shows that the practical diets were better balanced for juvenile tench than the carp starter.

As tench are carnivorous in natural habitats, it would be expected that this feeding will be reflected in their protein requirement. Dietary protein had a clear effect on growth of juvenile tench, being 52 % the optimum level. This value is in the range of the requirements reported for other carnivorous juvenile freshwater fish species, such as Murray cod, *Maccullochella peelii peelii* (50 %, Gunasekera et al. 2000), pike perch, *Sander lucioperca* (54.9 %, Schulz et al. 2007), or rainbow trout, *Onkorhynchus mykiss* (48 %, NRC 2011), and even it was higher than the dietary protein requirements reported for some other carnivorous juvenile freshwater fish species, such as hybrid bluegill, *Lepomis cyanellus* × *L. macrochirus* (36 %, Webster et al. 1997), largemouth bass, *Micropterus salmoides* (43.6 %, Portz et al. 2001), or Mexican silverside, *Menidia estor* (40.9 %, Martínez-Palacios et al. 2007).

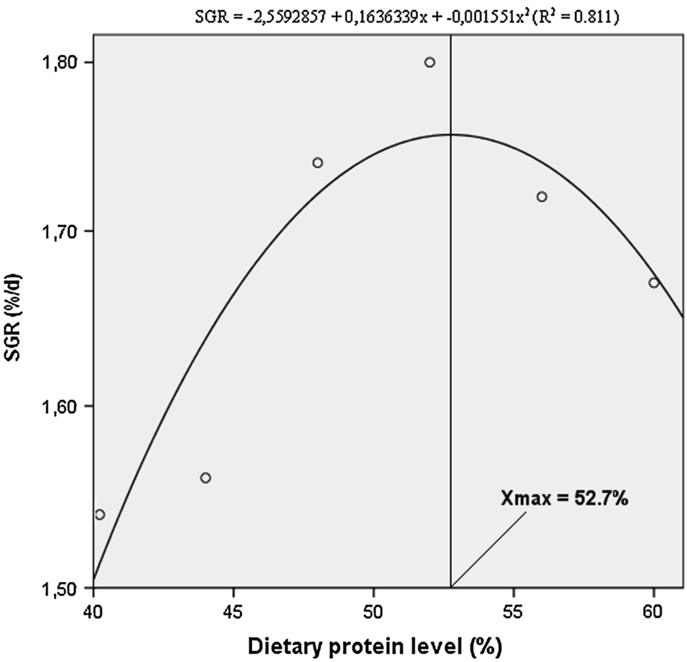

Fig. 3 Second-order polynomial relationship between dietary protein level and SGR of juvenile tench

There were no significant differences in growth between 52 and 48 % dietary protein. When protein decreased up to 44 %, significant growth reduction was observed, probably because this protein content did not cover the requirements to enable an optimum growth. No improved growth was observed beyond 52 % dietary protein and even a slightly decrease was found (Table 3). The reduction in growth with protein levels above the optimum has also been observed in other fish species such as tilapia, *Sarotherodon mossambicus* (Jauncey 1982), largemouth bass, *M. salmoides* (Portz et al. 2001) or masheer, *Tor putitora* (Islam and Tanaka 2004). It has been related to a reduction in the dietary energy available for growth due to the energy required to metabolize the excess amino acids absorbed (Jauncey 1982).

Both protein and amino acid contents of the body of juvenile tench (Table 4) increased with increasing dietary protein levels up to 52 %, but remained unchanged thereafter. In the same way, PPV did not improve above 52 % protein (Table 3). Similar trends have been also observed in other freshwater fish species such as tilapia, *S. mossambicus* (Jauncey 1982), brown trout, *Salmo trutta* (Arzel et al. 1995), silver perch, *Bidyanus bidyanus* (Yang et al. 2002), masheer, *Tor putitora* (Islam and Tanaka 2004) or Asian red-tailed *Hemibagrus wyckioides* (Deng et al. 2011). It is known that the body protein can increase until the requirement level is met and higher protein levels are metabolized for energy rather than used to build tissue protein (Hardy and Gatlin 2002).

With 48 % protein, all EAA contents were higher than the requirements determined for juvenile freshwater species summarized in NRC (2011). However, when dietary protein was reduced to 44 %, the content of histidine (7.55 g kg^{-1} diet) was below the requirements of common carp, *Cyprinus carpio* (Nose 1979), or mrigal carp, *Cirrhinus mrigala*

Table 4 Proximate composition and amino acid profiles of the whole body of juvenile tench fed practical diets with different protein contents (g kg^{-1}, wet basis)

	Initial	Protein content (%)					
		40	44	48	52	56	60
Proximate composition							
Moisture	772.9	762.9	757.5	753.8	754.3	753.8	752.8
Protein	138.9	150.8^{a}	154.9^{b}	158.1^{c}	159.8^{d}	159.9^{d}	159.7^{d}
Lipid	48.3	57.6	57.4	58.5	55.6	56.3	57.0
Ash	39.9	28.7	30.2	29.6	30.3	30.0	30.5
Essential amino acid							
Arginine	26.58	20.09^{a}	20.76^{b}	20.91^{b}	21.26^{c}	21.21^{c}	21.28^{c}
Histidine	5.16	6.99^{a}	7.15^{b}	7.27^{b}	7.43^{c}	7.42^{c}	7.49^{c}
Isoleucine	6.27	8.29^{a}	8.48^{a}	8.63^{b}	8.75^{b}	8.80^{b}	8.77^{b}
Leucine	10.18	12.91^{a}	13.22^{b}	13.37^{b}	13.55^{c}	13.59^{c}	13.57^{c}
Lysine	12.21	16.98^{a}	17.32^{a}	17.56^{a}	17.87^{b}	17.83^{b}	17.82^{b}
Methionine	1.47	2.55^{a}	2.71^{b}	2.82^{c}	2.89^{c}	2.93^{c}	2.91^{c}
Phenylalanine	7.02	8.75^{a}	8.98^{a}	9.07^{a}	9.29^{b}	9.30^{b}	9.27^{b}
Threonine	4.61	6.86^{a}	6.99^{a}	7.15^{b}	7.25^{b}	7.30^{b}	7.24^{b}
Tryptophan	0.06	0.61	0.62	0.62	0.63	0.65	0.67
Valine	5.71	8.19^{a}	8.30^{b}	8.48^{c}	8.60^{c}	8.59^{c}	8.58^{c}
Non-essential amino acid							
Alanine	9.08	10.97^{a}	11.25^{b}	11.43^{c}	11.56^{c}	11.52^{c}	11.50^{c}
Aspartate	29.85	10.08^{a}	10.39^{b}	10.46^{b}	10.67^{c}	10.69^{c}	10.63^{c}
Cysteine	0.49	0.56	0.56	0.57	0.59	0.62	0.59
Glutamate	11.26	10.39^{a}	10.97^{b}	11.06^{c}	11.21^{d}	11.19^{d}	11.11^{d}
Glycine	4.81	7.21^{a}	7.46^{b}	7.50^{b}	7.66^{c}	7.64^{c}	7.62^{c}
Proline	5.07	5.79^{a}	5.89^{a}	6.12^{b}	6.24^{b}	6.26^{b}	6.30^{b}
Serine	4.33	8.41^{a}	8.73^{b}	8.83^{c}	8.90^{c}	8.94^{c}	8.90^{c}
Tyrosine	3.91	4.18^{a}	4.44^{b}	4.53^{c}	4.59^{c}	4.60^{c}	4.57^{c}

Asparagine and glutamine contents were not determined

In the same row, values with different superscript are significantly different ($P < 0.05$)

Initial data are not included in the statistical analysis

(Ahmed and Khan 2005), and the methionine content (9.89 g kg^{-1} diet) was below the requirements of yellow perch, *Perca flavescens* (Twibell et al. 2000), or mrigal carp, *C. mrigala* (Ahmed et al. 2003). From these considerations, it could be hypothesized that histidine and methionine contents were probably also below the requirements of juvenile tench.

Besides the amino acid deficiency, another possible reason for the lower growth performance of juvenile tench fed 40 or 44 % protein could be related to carbohydrates content. As protein level decreased, carbohydrates increased steadily due to higher levels of corn meal in the diets (Table 1). Reduced growth was also reported in some juvenile freshwater species such as Mexican silverside, *M. estor* (Martínez-Palacios et al. 2007) or bagrid catfish, *Horabagrus brachysoma* (Giri et al. 2011) fed high carbohydrates contents. This decrease in growth has been explained by a negative correlation between high carbohydrates content and protein digestibility (Hepher 1985), due to a decrease in protease activity with high dietary carbohydrates (Mohanta et al. 2009).

To summarize, the present results indicate that juvenile *T. tinca* require 52 % dietary protein for optimum growth and feed utilization. Methionine and histidine may be the most limiting amino acids.

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Evaluation of poultry by-product meal as partial replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.)

Álvaro González-Rodríguez, Jesús D Celada, José M Carral, María Sáez-Royuela, Vanesa García & Juan B. Fuertes

Dpto. de Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, León 24071, Spain

Correspondence: Á González-Rodríguez, Dpto. de Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, León 24071, Spain. E-mail: agonr@unileon.es

Abstract

The aim of this study was to evaluate different replacement levels of fish meal (FM) by poultry by-product meal (PBM) on survival, growth performance and body composition of juvenile tench (*Tinca tinca*). A 90-day experiment was conducted with 5 month-old juveniles (31.95 mm total length, 0.396 g weight). Eight practical diets (50% crude protein) differing in the level of replacement of FM protein by PMB protein were tested: 0% (control), 25%, 31%, 37%, 43%, 49%, 55% and 61% corresponding to 0, 184.8, 229.2, 273.5, 317.8, 362.1, 406.5 or 450.8 g PBM kg^{-1} diet respectively. Significant differences were not found ($P > 0.05$) between 25% replacement of FM protein by PBM protein (184.8 g kg^{-1} PBM in diet) and control diet. At higher replacement levels, fish had significantly lower growth, higher feed conversion ratio and lower protein productive value ($P < 0.05$). Fish with externally visible deformities ranged from 1.1% to 3.3%. The relation among amino acid profiles of the diets, body composition, growth performance of juveniles and amino acid requirements of other fish species is discussed. Up to 184.8 g PBM kg^{-1} diet can be included in diets for juvenile tench without impairing growth performance.

Keywords: juvenile tench, nutrition, poultry by-product meal, *Tinca tinca*, feeding

Introduction

Tench, *Tinca tinca* (Linnaeus 1758), a freshwater fish belonging to the family Cyprinidae, has great potential for aquaculture (Wang, Min, Guan, Gong, Ren, Huang, Zheng, Zhang, Liu & Han 2006; Celada, Aguilera, García, Carral, Sáez-Royuela, González & González 2009; García, Celada, González, Carral, Sáez-Royuela & González 2013). Originally occurring in the waters of Europe and Siberia, today tench occurs in the inland waters of all the continents (Freyhof & Kottelat 2008). In natural habitats, tench are carnivorous (Kennedy & Fitzmaurice 1970), and gut content analysis shows that juveniles fed on zooplankton and other small invertebrates (Pyka 1997). In culture, the use of manufactured feed development is limited by the lack of knowledge on nutritional requirements, forcing the use of dry diets formulated for other species. From the practical point of view, research should be conducted to solve this pressing problem by studying specifically compounded diets for juvenile tench.

When developing a diet, to choose the right ingredients, the current availability and price of different feedstuffs should be taken into account. Finfish and crustacean aquaculture is highly dependent upon marine capture fisheries to supply fishmeal (FM), the most important and expensive protein ingredient used in aquafeeds (Tacon & Metian 2008). This fact has led to a double concern, on the one hand the non-sustainability of the fisheries pressure on wild stocks to cover the increasing demand of FM (Naylor, Hardy, Bureau, Chiu, Elliot, Farrell, Forster, Gatlin, Goldburg, Hua & Nichols 2009). On the other hand, the rising prices because of the growing demand make this ingredient less feasible to use at current inclusion levels (Tacon & Metian 2008; FAO 2009). Thus, the aquafeed industry is forced to search for alternative protein sources to reduce its dependence of FM (Naylor *et al.* 2009; Hardy 2010). Alternative proteins, including plant and animal protein, have been studied by many fish nutritionists and the feed industries (Tacon & Jackson 1985). Protein feedstuffs of animal origin are generally of better quality than most plant protein sources (Li, Manning & Robinson 2002). Considering the different possibilities, one of the most promising animal protein sources for the replacement of FM is poultry by-product meal (PBM) (Tacon & Jackson 1985) mainly due to their similarity in terms of protein level and amino acid profile (National Research Council 2011) and also to the reasonable price and steady supply (Hu, Wang, Wang, Zhao, Xiong, Qian, Zhao & Luo 2008). The substitution of FM with PBM has been tested with several juvenile freshwater fish species such as Nile tilapia, *Oreochromis niloticus* L. (El-Sayed 1998), African catfish, *Clarias gariepinus* Burchell (Abdel-Warith, Russell & Davies 2001), mirror carp, *Cyprinus carpio* L. (Emre, Sevgili & Diler 2003), gibel carp, *Carassius auratus* L. (Yang, Xie, Cui, Lei, Zhu, Yang & Yu 2004), and grass carp, *Ctenopharyngodon idella* Valenciennes (Tabinda & Butt 2012), showing that adequate inclusion levels of PBM in diet are different depending on the studied species. The aim of this study was to evaluate the effects of partial replacement of fish meal (FM) with poultry by-product meal (PBM) in practical diets for juvenile tench on survival, growth performance and body composition.

Materials and methods

Fish, facilities and experimental procedures

A 90-day experiment was carried out with juvenile tench. Larvae were obtained by hatching under artificial reproduction techniques (Rodríguez, Celada, Sáez-Royuela, Carral, Aguilera & Melendre 2004) and were reared for 5 months up to the juvenile stage used for the experiment. From 5 days after hatching, when first feeding started, larvae were maintained in outdoor fibreglass tanks (2500 L) and fed decapsulated *Artemia* cysts for 2 weeks (Celada, García, Carral, Sáez-Royuela, González & González 2013). Then, fish were fed a combination of a carp starter diet and decapsulated *Artemia* cysts. After 5 months, 720 juvenile tench (31.95 ± 0.14 mm total length, 0.396 ± 0.005 g weight, mean ± SE, $n = 120$) were transferred to indoor facilities. Fish were anaesthetized with tricaine methanesulfonate (MS-222; Ortoquímica S.L., Barcelona, Spain; 0.15 mg L^{-1}) and randomly distributed as groups of 30 fish in 24 fibreglass tanks (0.5 × 0.25 × 0.25 m) each containing 25 L of water, to provide replicates corresponding to the different feeding treatments. All experimental groups were in triplicate (three tanks per treatment). The juveniles were acclimated to experimental conditions for 10 days before the trial.

The water used in this study comes from an artesian well (200 m deep) and is pumped to a surface tank and then conducted to experimental facilities. The water was supplied in an open system (flow-through, without recirculation) and each tank had a water inlet (inflow 0.30 L min^{-1}) and outlet (provided with a 250 μm mesh filter) and light aeration. The variables of the incoming water quality (measured once a week) were: pH = 7.6, hardness 5.3°dH (German degrees, calcium 32.8 mg L^{-1}), total dissolved solids 115.2 mg L^{-1} and total suspended solids 34.6 mg L^{-1}. Dissolved oxygen content in tanks was measured with a HACH HQ30d meter (Hach Lange GMBH, Vigo, Spain) throughout the trial and values ranged between 5.7 and 7.2 mg L^{-1}. Ammonia and nitrites were measured with a HACH DR2800 spectrophotometer (Hach Lange GMBH) from water samples taken inside the tanks (ammonia values were always <0.10 mg L^{-1} and nitrites <0.013 mg L^{-1}). Water temperature (measured twice daily) was 24 ± 1°C. A 16 h light: 8 h dark photoperiod was maintained throughout the experiment. Tanks were cleaned of faeces and uneaten feed every 2 days. All procedures used in the study were approved by the León University Ethics Committee (Spain).

Diets and feeding

Based on the practical diet proposed by González-Rodríguez, Celada, Carral, Sáez-Royuela and Fuertes (2013), different practical diets (experimental diets formulated with ingredients commonly used in the manufacturing of commercial aquafeeds, 50% crude protein) were prepared to test substitution possibilities of FM protein by PBM protein. Proximate composition and amino acid profiles of FM and PBM are presented in Table 1. To obtain initial information on the response of juvenile tench to maximum levels of substitution, the values tested were chosen considering the recommenda-

Table 1 Proximate composition and amino acid profiles of fish meal (FM) and poultry by-product meal (PBM) (g kg^{-1}, wet basis)

	FM	PBM
Proximate composition		
Moisture	79.7	50.3
Crude protein	678.1	601.2
Crude fat	90.3	190.8
Carbohydrates	0.0	34.3
Ash	151.9	123.4
Essential amino acid		
Arginine	88.8	82.4
Histidine	13.9	11.0
Isoleucine	35.0	29.2
Leucine	45.3	44.8
Lysine	60.6	38.9
Methionine	19.6	13.0
Phenylalanine	28.1	22.1
Threonine	37.8	28.5
Tryptophan	5.7	4.6
Valine	27.6	32.7
Non-essential amino acid		
Alanine	43.2	37.8
Aspartate	62.1	53.0
Glutamate	85.4	55.2
Glycine	27.2	17.6
Proline	25.8	47.6
Serine	38.3	42.3
Tyrosine	21.0	15.4
Cysteine	4.3	6.5

tions of PBM substitutions in other studies performed with different fish species (Yu 2008). Thus, eight diets (nearly isonitrogenous and isoenergetic) were formulated to test different replacement levels of FM protein by PBM protein: 0% (control), 25%, 31%, 37%, 43%, 49%, 55% and 61% corresponding to 0, 184.8, 229.2, 273.5, 317.8, 362.1, 406.5 or 450.8 g PBM kg^{-1} diet respectively. Ingredients were ground in a BRABENDER rotary mill (Brabender GmbH & Co. KG, Duisburg, Germany), mixed in a STEPHAN UMC5 mixer (Stephan Food Service Equipment, Hameln, Germany) and extruded using a stand-alone BRABENDER KE19/25D extruder (Brabender GmbH & Co. KG, Duisburg, Germany) at a temperature range between 100 and 110°C. Pellets (1 mm diameter) were obtained and dried during 24 h at 30°C. Pellets then received a coating of cod liver oil (25 g kg^{-1} diet). Formulation and proximate composition of practical diets are summarized in Table 2 and amino acid profiles are in Table 3.

During the 90-day trial, fish were fed manually four times a day (at 10:00, 14:00, 18:00 and 22:00 hours, in equal portions) to satiation. This ration was about 3% live weight per day.

Chemical analysis of diets and fish

Proximate composition of FM and PBM (Table 1), practical diets (Table 2) and whole-body composition of juvenile tench (Table 5) were analysed according to the Norms of the International Standards Organization (1973, 1978, 1979, 1998a, b): moisture to ISO R-1442, protein to ISO R-937, lipid to ISO R-1443, ash to ISO R-936 and gross energy to ISO 9831. Samples were stored at −30°C until analysis. The content of carbohydrates was calculated by subtracting the content of moisture, protein, lipid and ash from the wet weight. Fish were left to fast for 14 h before harvest.

Amino acid profiles of FM and PBM (Table 1), practical diets (Table 3) and whole-body of juvenile tench (Table 5) were analysed by HPLC using the AccQTag method from Waters (Milford, MA, USA). Amino acids were derivatized with 6-aminoquinolyl-N-hydrosysuccinimidyl carbamate reagent (AQC) by the method of Cohen and Michaud (1993) and Cohen and De Antonis (1994), and were detected by Dual λ Absorbance Detector Waters 2487 from Waters (Milford, MA, USA) at 254 nm. Quantification was carried out with Empower Pro 2.0 software from Waters (Milford, MA, USA). All analyses were performed in duplicate.

Data collection and statistical analysis

Every thirty days, a sample of 10 juveniles from each replicate (30 per treatment, 33% of total) was taken and the fish were anesthetized. The excess water was removed with tissue paper and fish were weighed and measured individually. Total length (TL) was measured with a digital calipers (to the nearest 0.01 mm) and individual wet weight (W) was determined using a precision balance (to the nearest 0.001 g). After measurement, juveniles were gently returned to their respective tanks. At the end of the experiment (day 90), fish were anaesthetized and observed one by one using a magnifying glass to detect externally visible deformities. Individual weight and length of all fish (90 per treatment) were determined. Specific growth rate (SGR) was expressed as SGR = 100 $[(\ln W_t - \ln W_0)/t]$ where W_t is the mean final weight, W_0 is the mean initial weight, and t is the

Table 2 Formulation and proximate composition of the practical diets with different levels of replacement of FM protein by PBM protein (g kg^{-1} diet, wet basis)

	Replacement (%)							
	0	25	31	37	43	49	55	61
Ingredients								
Fish meal*	655	491.5	452.0	412.7	373.4	334.1	294.8	255.5
Poultry by-product meal*	0	184.8	229.2	273.5	317.8	362.1	406.5	450.8
Corn meal†	131	114.7	114.8	114.8	114.8	114.8	112.2	108.2
Dried *Artemia* cysts‡	100	100	100	100	100	100	100	100
Carboxymethyl cellulose§	30	30	30	30	30	30	30	30
Cod liver oil¶	25	25	25	25	25	25	25	25
L-ascorbyl-2-monophosphate-Na6	5	5	5	5	5	5	5	5
Dicalcium phosphate**	10	10	10	10	10	10	10	10
Choline chloride**	3	3	3	3	3	3	3	3
Soy lecithin††	30	25	20	15	10	5	2.5	1.5
Sodium chloride‡‡	1	1	1	1	1	1	1	1
Vitamin-Mineral premix§§	10	10	10	10	10	10	10	10
Proximate composition								
Moisture	84.3	85.5	84.8	86.1	85.2	83.9	84.7	83.9
Crude protein	499.4	500.1	500.0	499.9	499.8	499.9	500.0	499.8
Crude lipid	134.9	149.2	149.6	147.4	148.4	149.1	152.2	155.1
Carbohydrates	122.3	122.0	125.1	128.2	131.0	133.0	134.4	134.7
Ash	159.1	143.2	140.5	138.4	135.6	134.1	128.7	126.5
Gross energy (MJ/kg)	20.3	20.5	20.6	20.6	20.7	20.6	20.7	20.8

*Skretting España, S.A., Ctra. de la Estación s/n 09620 Cojóbar, Burgos, España.
†Adpan Europa, S.L., ES-33186 El Berrón, Siero, Asturias, Spain.
‡INVE Aquaculture Nutrition, High HUFA 430μ, Hoogyeld 91, Dendermonde, Belgium.
§Helm Iberica, S.A., ES-28108 Alcobendas, Madrid, Spain.
¶Acofarma distribution, S.A., ES-08223 Terrassa, Barcelona, Spain.
**Nutral, S.A., ES-28720 Colmenar Viejo, Madrid, Spain.
††Biover N.V., Monnikenwerve 109, B-8000 Brugge, Belgium.
‡‡Unión Salinera de España, S.A., Serrano, 21 – 2,28001 Madrid Spain.
§§Provides mg Kg−1 premix: $MgSO_4$-$7H_2O$, 300 000; $ZnSO_4$-$7H_2O$, 11 000; $MnSO_4$-H_2O, 4000; $CuSO_4$-$5H_2O$, 1180; $CoSO_4$, 26; $FeSO_4$-$7H_2O$, 77 400; KI, 340; Na_2SeO_3, 68; inositol, 50 000; thiamin, 500; riboflavin, 800; niacin, 5000; pyridoxine, 1500; pantothenic acid, 5000; biotin, 150; folic acid, 3500; cianocobalamin, 5; retinol, 2400; α-tocopherol, 30 000; cholecalciferol, 6.25; naphthoquinone, 5000; ethoxyquin, 70 000.

duration of the experiment (days). Fulton's coefficient (K) was used to determine the fish condition, where $K = 100\ (W_t/TL^3)$. Following Fornshell and Hinshaw (2009), feed conversion ratio (FCR) was calculated as $FCR = D_t/(W_t - W_0)$, where D_t is the total amount of feed fed (g) and $W_t - W_0$ is the weight gain (g) over 90 days. Protein productive value (PPV) was expressed as $PPV = 100\ [((W_t \times C_1) - (W_0 \times C_0))/(C_{diet} \times D_t)]$, where C_1 and C_0 are the final and initial protein concentration in the whole-body, respectively, and C_{diet} is the concentration in the diets.

All treatments were replicated three times and the experimental unit was a tank with 30 fish. Results were examined by analysis of variance (ANOVA) using the computer program SPSS 16.0 (SPSS, Chicago, IL, USA). The Duncan test was applied to compare means at $P < 0.05$ level of significance. Percentages were arcsine-transformed prior to statistical analysis.

Results

Juvenile tench readily accepted all practical diets. Final values (90 days) of survival, growth performance and percentages of fish with externally visible deformities are presented in Table 4. No mortality was noted during the experiment in all groups.

Regarding growth, significant differences were not found ($P > 0.05$) between 25% replacement of FM protein by PBM protein (184.8 g kg^{-1} PBM in diet) and the control diet (average of the two feeding treatments: 62.28 mm TL, 3.14 g W and

Table 3 Amino acid profiles of the practical diets with different levels of replacement of FM protein by PBM protein (g kg^{-1} diet, wet basis)

	Replacement (%)							
	0	**25**	**31**	**37**	**43**	**49**	**55**	**61**
Essential amino acid								
Arginine	66.6	67.3	67.4	67.7	67.7	67.9	68.1	68.3
Histidine	9.9	9.7	9.6	9.6	9.5	9.4	9.4	9.3
Isoleucine	25.2	24.9	24.8	24.7	24.6	24.5	24.5	24.4
Leucine	33.2	34.1	34.2	34.5	34.6	34.8	35.0	35.2
Lysine	44.2[a]	41.5[b]	40.8[b,c]	40.2[b,c,d]	39.5[b,c,d]	39.4[b,c,d]	38.7[c,d]	37.6[d]
Methionine	11.1[a]	10.4[b]	9.7[c]	9.3[c]	9.2[c]	8.4[d]	8.3[d,e]	7.8[e]
Phenylalanine	19.7	19.2	19.0	18.9	18.8	18.7	18.6	18.4
Threonine	27.4	26.7	26.5	26.3	26.0	25.8	25.6	25.4
Tryptophan	4.3	4.2	4.2	4.2	4.2	4.1	4.1	4.1
Valine	20.4	21.9	22.3	22.7	23.0	23.4	23.8	24.1
Non-essential amino acid								
Alanine	31.7	31.7	31.6	31.6	31.6	31.6	31.5	31.5
Aspartate	46.4	46.1	45.9	45.9	45.7	45.7	45.6	45.5
Glutamate	64.3[a]	60.6[b]	59.6[b,c]	58.8[b,c,d]	57.8[c,d,e]	56.9[c,d,e]	56.0[d,e]	55.1[e]
Glycine	19.5	18.4	18.0	17.8	17.4	17.2	16.9	16.6
Proline	19.5[a]	24.0[b,c]	25.0[b,c]	26.1[b,c,d]	27.2[b,c,d]	28.4[c,d]	29.6[c,d]	30.6[d]
Serine	28.4[a]	30.0[a,b]	30.3[b,c]	30.7[b,c]	31.0[b,c]	31.4[b,c]	31.8[b,c]	32.2[c]
Tyrosine	15.0	14.4	14.2	14.1	13.9	13.8	13.7	13.5
Cysteine	3.0[a]	3.5[a,b]	3.6[a,b]	3.7[a,b]	3.8[b]	4.0[b]	4.1[b]	4.2[b]

In the same row, values with different superscript are significantly different ($P < 0.05$).
Asparagine and glutamine content were not determined.

2.27% per day SGR). At higher replacement levels (from 31% to 61%), fish had significantly lower growth ($P < 0.05$). Figures 1 and 2 show the changes in weight and length, respectively, throughout the 90 days of trial. Juveniles fed the control diet (0% replacement) or 25% replacement of FM protein grew faster than those fed diets with higher replacement levels (from 31% to 61%). These differences were significant ($P < 0.05$) from day 60 onwards.

Condition factor (K) ranged from 1.24 to 1.29 without significant differences ($P > 0.05$) among treatments. Feed conversion ratio (FCR) ranged from 1.25 to 1.99 (Table 4). FCRs of fish fed 0% (control) or 25% replacement of FM protein were significantly lower (average: 1.25; $P < 0.05$) than those fish fed diets with higher replacement levels (from 31% to 61%). Protein productive value (PPV) ranged from 17.56 to 28.98 and fish fed the control diet or 25% replacement diet showed a PPV significantly higher (average: 28.84; $P < 0.05$) than those fish fed diets with higher replacements levels (from 31% to 61%). The percentages of fish with externally visible deformities (Table 4) were low for all practical diets, ranging from 1.1% to 3.3%. Body deformities affected the spinal column and caudal peduncle (break in the tail axis).

The amino acid profiles of the practical diets are presented in Table 3. Considering the EAA (essential amino acids), there were no significant differences ($P > 0.05$) in the content of arginine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan and valine. Compared with the control diet, the 25% replacement of FM protein led to a significant reduction ($P < 0.05$) in the lysine and methionine contents that were 6.1% and 6.3% respectively. When the replacement of FM protein increased to 31%, the methionine content decreased 12.6% and growth performance was significantly reduced ($P < 0.05$).

The proximate composition and the amino acid profiles of the whole-body of juvenile tench at the start and at the end of the trial are presented in Table 5. No significant differences ($P > 0.05$) in moisture and ash were found among dietary treatments. Fish fed the control diet and 25% replacement diet had significantly lower ($P < 0.05$) lipid content and higher ($P < 0.05$) protein content than those fed diets with higher replacements levels (from 31% to 61%). Regarding the amino acid profile, there were only significant differences

Table 4 Survival, growth performance and percentages of deformed juvenile tench fed practical diets with different levels of substitution of FM protein by PBM protein over 90 days

	Replacement (%)							
	0	25	31	37	43	49	55	61
Survival (%)	100	100	100	100	100	100	100	100
Total length (mm)	62.52 ± 0.47^{a}	62.04 ± 0.41^{a}	59.33 ± 0.33^{b}	59.64 ± 0.39^{b,c}	58.86 ± 0.34^{c}	54.17 ± 0.36^{d}	53.32 ± 0.32^{d}	51.49 ± 0.40^{e}
Weight (g)	3.20 ± 0.08^{a}	3.08 ± 0.07^{a}	2.62 ± 0.04^{b}	2.68 ± 0.06^{b}	2.55 ± 0.05^{b}	2.02 ± 0.05^{c}	1.92 ± 0.04^{c}	1.75 ± 0.05^{d}
*SGR (%/d)	2.29 ± 0.03^{a}	2.25 ± 0.03^{a}	2.08 ± 0.02^{b}	2.10 ± 0.02^{b}	2.05 ± 0.02^{b}	1.79 ± 0.02^{c}	1.74 ± 0.02^{c}	1.61 ± 0.03^{d}
†K	1.29 ± 0.01	1.27 ± 0.01	1.24 ± 0.01	1.25 ± 0.01	1.24 ± 0.01	1.25 ± 0.01	1.26 ± 0.01	1.25 ± 0.01
‡FCR	1.25 ± 0.03^{a}	1.25 ± 0.03^{a}	1.41 ± 0.03^{b}	1.46 ± 0.04^{b}	1.40 ± 0.03^{b}	1.63 ± 0.04^{c}	1.68 ± 0.04^{c}	1.99 ± 0.07^{d}
§PPV (%)	28.98 ± 0.77^{a}	28.70 ± 0.74^{a}	23.90 ± 0.48^{b}	23.53 ± 0.57^{b}	24.47 ± 0.57^{b}	20.68 ± 0.57^{c}	19.64 ± 0.47^{c}	17.56 ± 0.66^{d}
Deformed fish (%)	1.1 ± 0.2^{a}	2.2 ± 0.2^{a,b}	1.1 ± 0.2^{a}	3.3 ± 0.3^{b}	1.1 ± 0.1^{a}	2.2 ± 0.2^{a,b}	3.3 ± 0.3^{b}	2.2 ± 0.2^{a,b}

Values are mean ± SE. In the same row, values with different superscript are significantly different ($P < 0.05$). Growth data derived from 30 fish sampled per replicate ($n = 90$).
*Specific growth rate (% per day) = 100 × [(ln final mean body weight − ln initial mean body weight) × days)$^{-1}$].
†Condition factor = 100 × (body weight × body length$^{3-1}$).
‡Feed conversion ratio = total amount of feed fed × weight gain^{-1}.
§Protein productive value = (fish protein gain × crude protein intake^{-1}) × 100.

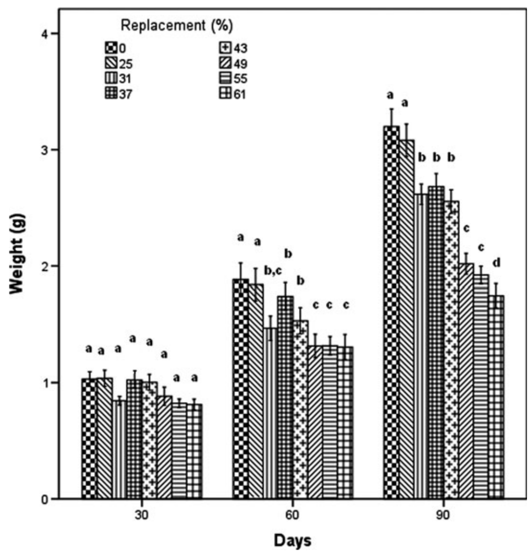

Figure 1 Mean weight of juvenile tench (*T. tinca*) at various periods fed practical diets with different replacement levels of FM protein by PBM protein over 90 days. At day 30 and 60, data derived from 10 fish sampled per replicate ($n = 30$). At the end of the experiment (day 90), data derived from all fish (30 per replicate, $n = 90$). Error bars represent the standard error of the mean. Means in columns without a common superscript differ at $P < 0.05$.

($P < 0.05$) in the lysine and methionine contents. Compared with the control diet, the juveniles fed 31% or higher replacement levels of FM protein had lower ($P < 0.05$) lysine and methionine contents and slower growth ($P < 0.05$).

Discussion

Most of the studies on the intensification of juvenile tench rearing started with fish aged 3–7 months. Growth rates (SGR, % per day) from 0.70 to 1.98 have been reported, being higher when dry diets for other species were supplemented with natural feed (García *et al.* 2013). In the present study, 5 month-old juveniles were fed practical diets as the sole feed and SGRs (1.61–2.29% per day) are in the range of the highest reported.

There is scarce information on FCR of juvenile tench, and all previous data have been calculated considering the total amount of diet supplied to the fish. In the intensive rearing of 3 month-old juveniles (around 0.45 g at the beginning of the experiment) fed a commercial trout feed for some

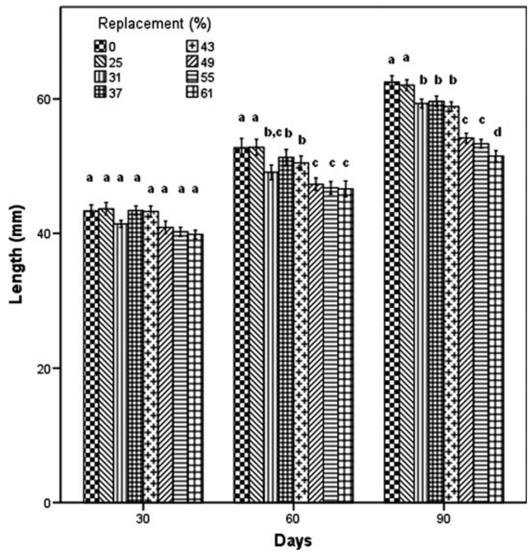

Figure 2 Mean length of juvenile tench (*T. tinca*) at various periods fed practical diets with different replacement levels of FM protein by PBM protein over 90 days. At day 30 and 60, data derived from 10 fish sampled per replicate ($n = 30$). At the end of the experiment (day 90), data derived from all fish (30 per replicate, $n = 90$). Error bars represent the standard error of the mean. Means in columns without a common superscript differ at $P < 0.05$.

450 days, Rennert, Kohlmann and Hack (2003) reported FCRs ranging from 1.75 to 3.56. Mareš, Jirásek, Baránek, Fiala and Copp (2007) tested three dry diets with 7 month-old juveniles (0.8–1.2 g) during 63 days, and FCRs ranged from 1.84 to 4.15. The FCRs obtained in the present study ranged from 1.25 to 1.99 and were similar to those reported by González-Rodríguez *et al.* (2013), starting also with 5 month-old juveniles and using the same basal diet. In both cases, values are more favourable than those previously reported and are close to the feed conversion rates in the intensive culture of well-studied species, with values typically ranging from 0.8 to 1.5 (Hardy & Barrows 2002).

Regarding substitution possibilities of FM by PBM, it must be taken into account that juvenile fish have a higher requirement for most nutrients and greater sensibility to nutrient deficiency compared with older fish. For this reason, most research trials have used juveniles as experimental animals (Yu 2008). The fish species studied have different tolerance for dietary PBM. For instance, PBM can totally replace FM in diets for Nile tilapia, *O. niloticus* (El-Sayed 1998), or for grass carp, *C. idella* (Tabinda & Butt 2012), including 470 and 300 g of PBM kg^{-1} diet, respectively, without negative effects on growth. Lower levels of replacement of FM protein with PBM protein in diets, such as 50% (359 g of PBM kg^{-1} diet) for gibel carp, *C. auratus* (Yang *et al.* 2004), or 40% (170 g of PBM kg^{-1} diet) for African catfish, *C. gariepinus* (Abdel-Warith *et al.* 2001), have been successfully included. By contrast, a reduction in growth was observed in mirror carp, *C. carpio* (Emre *et al.* 2003) even at the minimum replacement level tested (120 g of PBM kg^{-1} diet). In the present study with juvenile tench, 25% FM protein was replaced by PBM protein (184.8 g of PBM kg^{-1} diet) without harmful effects on growth performance.

As fish composition is related to diet composition, protein and essential amino acid retention have been considered the most sensitive indicators of an inadequate supply of amino acids (Rodehutscord, Mandel, Pack, Jacobs & Pfeffer 1995). In the present experiment, the retention of protein, estimated by means of PPV, evidenced a significant decrease when amounts above 184.8 g of PBM kg^{-1} (25% replacement) were included, suggesting an insufficient supply of essential aminoacids. The replacement of FM by PBM in our diets led to a reduction in two essential aminoacids, lysine and methionine, in comparison with the control diet (Table 3). Juvenile growth performance was unaffected at 25% replacement, but with higher levels (31% or more) depressed growth was detected. Content of methionine and lysine in the body of juvenile tench did not show significant differences between control and 25% replacement diets. Thus, it could be assumed that both diets have enough amounts of the mentioned essential aminoacids. However, when substitution of FM increased up to 31% (229.2 g PBM kg^{-1} diet) or higher levels, a significant decrease in methionine content in diets and juvenile body was evidenced. From this, it could be hypothesized that methionine content was probably below the requirement of juvenile tench. In fact, the methionine content of 31% replacement diet (9.7 g kg^{-1}) was below the minimum recommended for yellow perch (*Perca flavescens* Mitchell) or mrigal carp (*Cirrhinus mrigala* Hamilton) (National Research Council 2011).

In this experiment, the proximate composition of the practical diets (Table 2) showed an increase

Table 5 Proximate composition and amino acid profiles of the whole-body of juvenile tench fed practical diets with different levels of replacement of FM protein by PBM protein (g kg^{-1}, wet basis)

		Replacement (%)							
	Initial	0	25	31	37	43	49	55	61
Proximate composition									
Moisture	794.1	743.3	744.4	741.9	738.7	739.4	743.7	741.1	741.0
Protein	147.2	166.6^{a}	165.2^{a}	159.7^{b}	160.2^{b}	160.2^{b}	156.1^{b,c}	154.2^{c}	154.3^{c}
Lipid	32.2	58.1^{a}	59.0^{a}	66.7^{b}	68.1^{b}	68.2^{b}	68.1^{b}	72.4^{c}	71.6^{c}
Ash	26.5	32.0	31.4	31.7	33.0	32.2	32.1	32.3	33.1
Essential amino acid									
Arginine	24.3	21.5	21.6	21.5	21.6	21.7	21.7	21.7	21.8
Histidine	4.8	6.1	6.1	6.0	6.0	6.0	5.8	5.7	5.6
Isoleucine	6.9	8.2	8.2	8.1	8.1	8.0	8.0	7.9	7.8
Leucine	9.8	13.2	13.3	13.3	13.3	13.4	13.4	13.5	13.6
Lysine	11.6	15.1^{a}	14.8^{a,b}	14.4^{b,c}	14.3^{c,d}	14.2^{c,d}	14.2^{c,d}	14.2^{c,d}	14.0^{d}
Methionine	1.4	2.6^{a}	2.5^{a}	2.3^{b}	2.3^{b}	2.3^{b}	2.3^{b}	2.2^{c}	2.2^{c}
Phenylalanine	6.6	8.6	8.4	8.4	8.4	8.3	8.3	8.2	8.2
Threonine	4.5	7.3	7.2	6.9	6.8	6.7	6.6	6.6	6.5
Tryptophan	0.2	0.7	0.7	0.6	0.6	0.5	0.5	0.5	0.5
Valine	5.6	8.0	8.1	8.1	8.2	8.3	8.3	8.4	8.5
Non-essential amino acid									
Alanine	8.9	11.8	11.8	11.9	12.0	12.0	12.0	12.1	12.3
Aspartate	27.3	16.1	16.0	16.0	15.8	15.8	15.8	15.7	15.6
Cysteine	0.5	0.6^{a}	0.6^{a}	0.6^{a}	0.7^{a,b}	0.7^{a,b}	0.7^{a,b}	0.7^{a,b}	0.8^{b}
Glutamate	12.8	11.1^{a}	11.0^{a,b}	10.8^{a,b,c}	10.7^{a,b,c}	10.7^{a,b,c}	10.6^{b,c}	10.5^{b,c}	10.4^{c}
Glycine	4.4	7.4	7.3	7.1	7.0	7.0	6.9	6.9	6.9
Proline	5.0	6.5^{a}	6.4^{a,b}	6.2^{a,b,c}	6.1^{b,c}	6.1^{b,c}	6.0^{c}	5.9^{c}	5.9^{c}
Serine	4.2	8.3	8.5	8.5	8.6	8.6	8.6	8.7	8.7
Tyrosine	3.6	3.1	3.0	3.0	3.0	2.9	2.9	2.7	2.6

In the same row, values with different superscript are significantly different ($P < 0.05$).
Initial data are not included in statistical analysis.
Asparagine and glutamine content were not determined.

in lipid content with increasing dietary PBM. This could be related to a higher body lipid content in juvenile tench fed the highest PBM levels (Table 5). This increase in body lipid content has also been observed in other fish species as fall Chinook salmon *Oncorhynchus tschawytscha* Walbaum (Fowler 1991) noting that it could be due to excess lipid in the diets.

The practical diets tested enabled not only acceptable growth and high survival but also no deformed fish. Body deformities are an undesirable but inherent problem in intensive aquaculture, which entail severe losses to the production sector and have detrimental impacts on the consumers' image of aquaculture (Zambonino-Infante, Koumoundouros & Tandler 2009). Inadequate feeding, especially during early development, has been reported to induce malformations in fish (Cahu, Zambonino-Infante & Takeuchi 2003; Fontagné 2009). High percentages of deformed fish (37–92%) have been reported for other juvenile cyprinids (Myszkowski, Kamisnki, Quirós, Stanny & Wolnicki 2002; Kaminski, Korwin-Kossakowski, Kusznierz, Myskowski, Stanny & Wolnicki 2005) and for juvenile tench (23–96%) (Rennert *et al.* 2003; Wolnicki, Myszkowski, Korwin-Kossakowski, Kaminski & Stanny 2006; Myszkowski, Kamler & Kwiatkowski 2010) when were fed commercial diets formulated for other species, probably due to imbalanced feed composition. The deformity rates recorded in the present study (1.1–3.3%) were much lower, suggesting that the practical diets used were better balanced for juvenile tench than the aquafeeds for other species used so far.

To summarize, up to 184.8 g PBM kg^{-1} diet can be included in extruded diets (50% crude protein) for juvenile tench to replace 25% of FM protein without impairing growth performance.

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Evaluation of pea protein concentrate as partial replacement of fish meal in practical diets for juvenile tench (*Tinca tinca* L.)

Álvaro González-Rodríguez, Jesús D Celada, José M Carral, María Sáez-Royuela & Juan B Fuertes

Dpto. de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Spain

Correspondence: Á González-Rodríguez, Dpto. de Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, León 24071, Spain. Email: agonr@unileon.es

Abstract

With the aim to evaluate different replacement levels of fish meal (FM) by pea protein concentrate (PPC) on survival, growth performance and body composition of juvenile tench (*Tinca tinca*), a 90-day experiment was conducted with 6-month-old juveniles. Four practical diets (50% crude protein) differing in the level of replacement of FM protein by PPC protein were tested: 0% (control), 25%, 35% or 45%, corresponding to 0, 207.5, 290.4 or 373.3 g PPC kg^{-1} diet respectively. Survival rates ranged from 96.4% to 98.5%. The 25% and 35% replacement diets resulted in similar growth values ($P > 0.05$) to those obtained with the control diet (average of the three feeding treatments: 57.57 mm total length, 2.48 g weight and 1.87% day^{-1} specific growth rate). The 45% replacement diet had the lowest growth ($P < 0.05$). Fish with externally visible deformities ranged from 0% to 1.5%. The relation among amino acid profiles of the diets, body composition, growth performance of juveniles and amino acid requirements of other fish species is discussed. An amount of 290.4 g PPC kg^{-1} diet (35% replacement of FM protein) can be included in juvenile tench diets without impairing growth performance.

Keywords: juvenile, nutrition, pea protein concentrate, *Tinca tinca*, tench

Introduction

Tench (*Tinca tinca* L. 1758), a freshwater fish belonging to the family Cyprinidae, has a great potential for aquaculture (Wang, Min, Guan, Gong, Ren, Huang, Zheng, Zhang, Liu & Han 2006; Celada, Aguilera, García, Carral, Sáez-Royuela, González & González 2009; García, Celada, González, Carral, Sáez-Royuela & González 2013), it is in demand in the market and appreciated by consumers as a tasty fish with healthy meat. Originally occurring in the waters of Europe and Siberia, today tench occurs in the inland waters of all the continents (Freyhof & Kottelat 2008). In natural habitats, tench are carnivorous (Kennedy & Fitzmaurice 1970), and gut content analyses show that juveniles feed on zooplankton and other small invertebrates (Pyka 1997). Traditionally, juvenile tench are cultured in extensive or semi-extensive systems using earthen ponds with usually low yields because of both growth and survival depend on uncontrolled environmental factors. A deficit of young fish for stocking outdoor ponds or open waters is considered as a major obstacle to increasing tench production (Celada *et al.* 2009; García, Celada, Carral, Sáez-Royuela, González & González 2010), and it is necessary to find effective techniques for rearing juvenile tench under controlled conditions, focusing mainly on feed as an essential factor (Celada *et al.* 2009).

When developing a diet, to choose the right ingredients, the current availability and price of different feedstuffs must be considered. Finfish and crustacean aquaculture is highly dependent upon marine capture fisheries to supply fishmeal (FM), the most important and expensive protein ingredient used in aquafeeds (Tacon & Metian 2008). This has led to a double concern, on the one hand the non-sustainability of the fisheries pressure on wild stocks to cover the increasing demand of FM (Naylor, Hardy, Bureau, Chiu, Elliot, Farrell, Forster, Gatlin, Goldburg, Hua & Nichols 2009), and on the other hand, the rising prices derived from the growing demand make this ingredient less feasible to use at current inclusion levels (Tacon & Metian 2008; FAO 2009). Thus, the aquafeed industry is forced to search for alternative protein sources to reduce its dependence on FM (Naylor *et al.* 2009; Hardy 2010). Peas are common plants produced in significant quantities throughout the world, and have the potential to replace significant proportions of FM protein in aquafeeds (Gatlin, Barrows, Brown, Dabrowski, Gaylord, Hardy, Herman, Hu, Krogdahl, Nelson, Overturf, Rust, Sealey, Skonberg, Souza, Stone, Wilson & Wurtele 2007). Recent advances in pea processing technology have provided several products, such as pea protein concentrate (PPC, 52% crude protein), which could allow for a high level of FM protein replacement in fish feed formulation. To our knowledge, PPC has been only tested in adult fish, such as Atlantic salmon, *Salmo salar* L. (Øverland, Sorensen, Storebakken, Penn, Krogdahl & Skrede 2009; Penn, Bendiksen, Campbell & Krogdahl 2011), European sea bass, *Dicentrarchus labrax* L. (Tibaldi, Tulli, Messina, Franchin & Badini 2005) or rainbow trout, *Oncorhynchus mykiss* Walbaum (Zhang, Øverland, Sørensen, Penn, Mydland, Shearer & Storebakken 2012). The aim of this study was to evaluate the effects of partial replacement of fish meal (FM) with pea protein concentrate (PPC) in practical diets for juvenile tench (*T. tinca*) on survival, growth performance and body composition.

Materials and methods

Fish, facilities and experimental procedure

A 90-day experiment was carried out with juvenile tench. Larvae were obtained by stripping and hatching as described Rodríguez, Celada, Sáez-Royuela, Carral, Aguilera and Melendre (2004) and were reared for 6 months until the juvenile stage in which the experiment started. From 5 days after hatching, when first feeding started, larvae were maintained in outdoor fibreglass tanks (2500 L) and fed decapsulated *Artemia* cysts for 2 weeks (Celada, García, Carral, Sáez-Royuela, González & González 2013). Then, fish were fed a combination of a dry carp starter diet and decapsulated *Artemia* cysts. After 180 days, 648 juvenile tench with a mean initial body weight (W) of 0.461 ± 0.011 g and a total length (TL) of 35.53 ± 0.28 mm ($n = 120$) were transferred to indoor facilities. Fish were anaesthetized with MS-222 (Ortoquímica S.L., Barcelona, Spain) and randomly distributed in groups of 54 fish in 12 fibreglass tanks ($0.5 \times 0.25 \times 0.25$ m) containing 25 L of water to obtain replicates corresponding to the different feeding treatments. Each replicate was bulk weighed and the stocking density was around 1 g L^{-1}. All experimental groups were in triplicate (three tanks per treatment). The juveniles were acclimated to experimental conditions for 10 days before the trial.

Artesian well water was supplied in open system (flow-throughout) with each tank having a water inlet (inflow 0.30 L min^{-1}) and outlet (provided with a 250 μm mesh filter) and light aeration. The variables of the incoming water quality (measured once a week) were: pH = 7.6, hardness 5.2°dH (German degrees, calcium 32.3 mg L^{-1}), total dissolved solids 115.7 mg L^{-1} and total suspended solids 35.2 mg L^{-1}. Dissolved oxygen content in tanks was measured with a Hach meter HQ30d (Hach Lange GMBH, Vigo, Spain) every day throughout the trial and values ranged between 5.9 and 7.8 mg L^{-1}. Ammonia and nitrites were measured with a spectrophotometer Hach DR2800 (Hach Lange GMBH, Vigo, Spain) from water samples taken inside the tanks (values were always ammonia <0.10 mg L^{-1} and nitrites <0.015 mg L^{-1}). Water temperature (measured twice a day) was 24 ± 1°C and a 16 h light: 8 h dark photoperiod was maintained throughout the experiment. Dead animals were immediately removed from the tanks. Every other day, the bottom of the tanks was cleaned by syphoning. All procedures used in the study were approved by the León University Ethics Committee (Spain).

Diets and feeding

Based on the practical diet proposed by González-Rodríguez, Celada, Carral, Sáez-Royuela and Fuertes (2013), different practical diets (50% crude protein) were prepared to test substitution possibilities of FM protein by PPC protein. FM was from anchoveta. The PPC was obtained by physical isolation technology (around 52% crude protein). Proximate composition and amino acid profiles of FM and PPC are presented in Table 1. The values tested were chosen considering the recommendations of pea product substitutions in other studies

Table 1 Proximate composition and amino acid profiles of fish meal (FM) and pea protein concentrate (PPC) (g kg^{-1}, wet basis)

	FM	PPC
Proximate composition		
Moisture	79.7	78
Crude protein	678	522
Crude fat	90.3	18
Carbohydrates	0	330
Ash	152	52
Essential amino acid		
Arginine	81.2	38.8
Histidine	12.6	9.5
Isoleucine	25.2	14.1
Leucine	44.2	38.3
Lysine	53.2	33.4
Methionine	16.1	7.6
Phenylalanine	24.5	23.0
Threonine	35.8	22.6
Tryptophan	5.6	5.2
Valine	30.1	19.7
Non-essential amino acid		
Alanine	37.1	26.5
Aspartate	51.1	62.6
Cysteine	6.1	5.2
Glutamate	81.5	105.6
Glycine	54.9	16.9
Proline	44.0	18.8
Serine	36.9	25.7
Tyrosine	14.2	16.2

performed with different fish species (Reigh 2008). Thus, four diets (nearly isonitrogenous and isoenergetic) with different replacement levels of FM protein by PPC protein were tested: 0% (control), 25%, 35% or 45%, corresponding to 0, 207.5, 290.4 or 373.3 g PPC kg^{-1} diet respectively. Ingredients were ground in a rotary mill Brabender (Brabender GmbH & Co. KG, Duisburg, Germany), mixed in a mixer Stephan UMC5 (Stephan Food Service Equipment, Hameln, Germany) and extruded using a stand-alone extruder Brabender KE19/25D (Brabender GmbH & Co. KG) at a temperature range between 100°C and 110°C (speed of supply 40 rpm and speed of the spindle 25 rpm) during 5 min. Pellets (1 mm diameter) were obtained and then dried for 24 h at 30°C. Later, pellets received a coating of cod liver oil. Formulation and proximate composition of practical diets are summarized in Table 2 and amino acid profiles are in Table 3.

Fish were fed manually four times a day (at 10:00, 14:00, 18:00 and 22:00 hours) to apparent satiation.

Chemical analysis of diets and fish

All analyses were performed in duplicate. Proximate composition of FM and PPC (Table 1), practical diets (Table 2) and whole body of juvenile tench (Table 5) were analysed according to the Norms of the International Standards Organization (1973–1998): moisture to ISO R-1442, protein to ISO R-937, lipid to ISO R-1443, ash to ISO R-936 and gross energy to ISO 9831. The content of carbohydrates was calculated by subtracting the content of moisture, protein, lipid and ash from the wet weight. Samples were stored at −30°C until analysis. The fish were fasted for 14 h before harvest.

Amino acid profiles of FM and PPC (Table 1), practical diets (Table 3) and whole body of juvenile tench (Table 5) were analysed by HPLC using AccQTag method from Waters (Milford, MA, USA). Amino acids were derivatized with 6-aminoquinolyl-N-hydrosysuccinimidyl carbamate reagent (AQC) by the method of Cohen and Michaud (1993) and Cohen and De Antonis (1994), and were detected by Dual λ Absorbance Detector Waters 2487 from Waters at 254 nm. Quantification was carried out with Empower Pro 2.0 software from Waters.

Data collection and statistical analysis

Every 30 days, a sample of 20 juveniles from each replicate (60 per treatment, around 37% of total) was collected to register total length (TL) and weight (W). After being anaesthetized, excess water was removed with tissue paper and fish were weighed and measured individually. Total length (TL) was measured with a digital calipre (to the nearest 0.01 mm) and individual wet weight (W) was determined by a precision balance (to the nearest 0.001 g). After measurement, juveniles were gently returned to their respective tanks. At the end of the experiment (day 90), surviving fish were anaesthetized, counted and observed one by one using a magnifying glass to detect externally visible deformities. Survival rates were calculated and individual weight and length of all fish (around 160 per treatment) were determined. Specific growth rate (SGR) was expressed as $SGR = 100\ [(\ln W_t - \ln W_0)/t]$ where W_t is the mean final weight, W_0 is the mean initial weight and t is the duration of the experiment (days). Fulton's coefficient (K) was used to determine the fish

Table 2 Formulation and proximate composition of the practical diets with different levels of replacement of fish meal protein by pea protein concentrate protein (g kg^{-1} diet, wet basis)

	Replacement (%)			
	0	25	35	45
Ingredients (g kg^{-1})				
Fish meal*	640	480.5	417.6	354.7
Pea protein concentrate†	–	207.5	290.4	373.3
Corn meal‡	126	78	58	38
Dried *Artemia* cysts§	120	120	120	120
Carboxymethyl cellulose¶	30	30	30	30
Cod liver oil**	20	20	20	20
L-ascorbyl-2-monophosphate-Na††	5	5	5	5
Dicalcium phosphate††	10	10	10	10
Choline chloride††	3	3	3	3
Soy lecithin‡‡	5	5	5	5
Sodium chloride§§	1	1	1	1
Mineral premix¶¶	20	20	20	20
Vitamin premix***	20	20	20	20
Proximate composition (g kg^{-1})				
Moisture	72.3	70.9	70.4	69.8
Crude protein	500.4	500.1	500.5	501.0
Crude lipid	108.0	94.8	89.3	84.3
Carbohydrates	157.4	186.4	197.5	208.1
Ash	161.9	147.8	142.3	136.8
Gross energy (MJ kg^{-1})	18.1	18.0	18.0	18.0

*Biomar Iberia/Proaqua Nutrición, S.A., ES-34210 Dueñas, Palencia, Spain.
†Yantai Oriental Protein Tech, Jincheng Road, Zhaoyuan City, Shandong Province, China.
‡Adpan Europa, S.L., ES-33186 El Berrón, Siero, Asturias, Spain.
§INVE Aquaculture Nutrition, High HUFA 430 μ, Hoogyeld 91, Dendermonde, Belgium.
¶Helm Iberica, S.A., ES-28108 Alcobendas, Madrid, Spain.
**Acofarma distribution, S.A., ES-08223 Terrassa, Barcelona, Spain.
††Nutral, S.A., ES-28720 Colmenar Viejo, Madrid, Spain.
‡‡Biover N.V., Monnikenwerve 109, B-8000 Brugge, Belgium.
§§Unión Salinera de España, S.A. Serrano, 21 – 2, 28001 Madrid Spain.
¶¶Provides mg kg^{-1} premix: $MgSO_4$-$7H_2O$, 150000; $ZnSO_4$-$7H_2O$, 5500; $MnSO_4$-H_2O, 2000; $CuSO_4$-$5H_2O$, 590; $CoSO_4$, 13; $FeSO_4$-$7H_2O$, 37350; KI, 170; Na_2SeO_3, 34.
***Provides mg kg^{-1} premix: inositol, 25000; thiamin, 250; riboflavin, 400; niacin, 2500; pyridoxine, 750; pantothenic acid, 2500; biotin, 75; folic acid, 1750; cianocobalamin, 2.5; retinol, 500; α-tocopherol, 15000; cholecalciferol, 3.125; naphthoquinone, 2500; ethoxyquin, 35000.

condition with $K = 100\ (W_t/TL^3)$. According to Fornshell and Hinshaw (2009), feed conversion ratio (FCR) was calculated as $FCR = D_t/(W_t - W_O)$, where D_t is the total amount of feed fed (g) and $W_t - W_0$ is the weight gain (g) over 90 days. Protein productive value (PPV) were expressed as $100\ [((W_t \times C_1) - (W_O \times C_O))/(C_{diet} \times D_t)]$, where C_1 and C_0 are the final and initial protein concentration in the whole body, respectively, and C_{diet} is the concentration in the diets.

Results were examined by analysis of variance (ANOVA) using the computer program SPSS 16.0 (SPSS, Chicago, IL, USA). Duncan test was applied to compare means at $P < 0.05$ level of significance. Percentages were arcsine-transformed prior to statistical analysis.

Results

Juvenile tench readily accepted all practical diets. Final values (90 days) of survival, growth, feed conversion ratio and percentages of fish with externally visible deformities are presented in Table 4. Survival ranged from 96.4% to 98.5%. Regarding growth, significant differences were not found ($P > 0.05$) among 25% or 35% replacement of FM protein by PPC protein (207.5 or 290.4 g PPC kg^{-1} diet, respectively) and the control diet (average of the three feeding treatments:

Table 3 Amino acid profiles of the practical diets with different levels of replacement of fish meal protein by pea protein concentrate protein (g kg^{-1} diet, wet basis)

	Replacement (%)			
	0	25	35	45
Essential amino acid				
Arginine	58.4^{a}	55.5^{a}	52.6^{b}	50.7^{b}
Histidine	8.9	8.8	8.8	8.8
Isoleucine	18.6	17.6	16.7	15.8
Leucine	31.7	31.2	30.7	30.4
Lysine	38.5	37.0	36.4	35.9
Methionine	11.6^{a}	10.6^{a,b}	9.9^{b}	8.9^{b}
Phenylalanine	14.7	14.1	14.0	13.8
Threonine	22.4	22.1	22.0	21.9
Tryptophan	4.3	4.2	4.2	4.2
Valine	21.2	20.9	20.6	20.3
Non-essential amino acid				
Alanine	27.2	26.7	26.6	26.5
Aspartate	38.2^{a}	42.8^{b}	45.1^{c,d}	46.7^{d}
Cysteine	4.2	4.1	4.1	4.0
Glutamate	60.3^{a}	68.2^{b}	72.3^{b,c}	75.6^{c}
Glycine	36.0^{a}	31.0^{b}	29.2^{b}	27.4^{c}
Proline	29.9^{a}	27.0^{a}	26.1^{a,b}	25.0^{b}
Serine	26.9	26.3	26.2	25.9
Tyrosine	10.2	11.0	11.5	11.9

In the same row, values with different superscript are significantly different ($P < 0.05$).

57.57 mm TL, 2.48 g W and 1.87% day^{-1} SGR). Fish fed the 45% replacement diet had significantly lower growth ($P < 0.05$). Figures 1 and 2 show the changes in weight and length, respectively, throughout the 90 days of trial. Juveniles fed the control diet (0% replacement), 25% and 35% replacement of FM protein grew faster than those fed the 45% replacement diet. These differences were significant ($P < 0.05$) from day 30 onwards.

Condition factor (K) ranged from 1.28 to 1.29. Feed conversion ratio (FCR) ranged from 1.56 to 1.63 and protein productive value (PPV) ranged from 22.93% to 23.24%, without significant differences ($P > 0.05$) among feeding treatments. The percentages of fish with externally visible deformities (Table 4) were low for all practical diets, ranging from 0% to 1.5%. Body deformities affected the spinal column and caudal peduncle (breaks in the tail axis).

The amino acid profiles of the practical diets are presented in Table 3. Considering the EAA (essential amino acids), there were no significant differences ($P > 0.05$) in the content of histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan and valine. The highest replacement levels of FM protein by PPC protein (35% and 45%) resulted in arginine and methionine contents significantly lower ($P < 0.05$) than those of the control diet. With 35% replacement of FM protein, reductions in the mentioned amino acids were 9.9% (arginine) and 14.7% (methionine), but this fact was not accompanied by a significant reduction in growth. When 45% of FM protein

Table 4 Survival, growth performance and percentages of deformed juvenile tench fed practical diets with different levels of substitution of fish meal protein by pea protein concentrate protein over 90 days

	Replacement (%)			
	0	25	35	45
Survival (%)	98.5 ± 0.16	98.0 ± 0.12	97.9 ± 0.13	96.4 ± 0.08
Total length (mm)	57.96 ± 0.296^{a}	57.42 ± 0.262^{a}	57.33 ± 0.345^{a}	55.29 ± 0.362^{b}
Weight (g)	2.52 ± 0.039^{a}	2.46 ± 0.032^{a}	2.47 ± 0.046^{a}	2.19 ± 0.047^{b}
SGR (% day^{-1})*	1.88 ± 0.017^{a}	1.86 ± 0.014^{a}	1.87 ± 0.021^{a}	1.73 ± 0.022^{b}
K†	1.28 ± 0.004	1.29 ± 0.005	1.29 ± 0.005	1.28 ± 0.008
FCR‡	1.57 ± 0.034	1.56 ± 0.024	1.60 ± 0.039	1.63 ± 0.044
PPV (%)§	23.24 ± 0.426	22.93 ± 0.367	23.14 ± 0.522	22.94 ± 0.680
Deformed fish (%)	0.0 ± 0.0	0.8 ± 0.08	0.7 ± 0.08	1.5 ± 0.18

Values are mean ± standard error. In the same row, values with different superscript are significantly different ($P < 0.05$). Growth data derived from ca. 54 fish sampled per replicate ($n = 160$).

*Specific growth rate(%day^{-1}) $= 100 \times \frac{(\text{ln final body weight} - \text{ln initial body weight})}{\text{days}}$.

†Condition factor $= 100 \times \frac{\text{body weight}}{\text{body length}^3}$.

‡Feed conversion ratio $= \frac{\text{dry feed fed}}{\text{wet weight gain}}$.

§Protein productive value $= 100 \times \frac{(\text{final weight} \times \text{final body protein}) - (\text{initial weight} \times \text{initial body protein})}{(\text{total protein intake})}$.

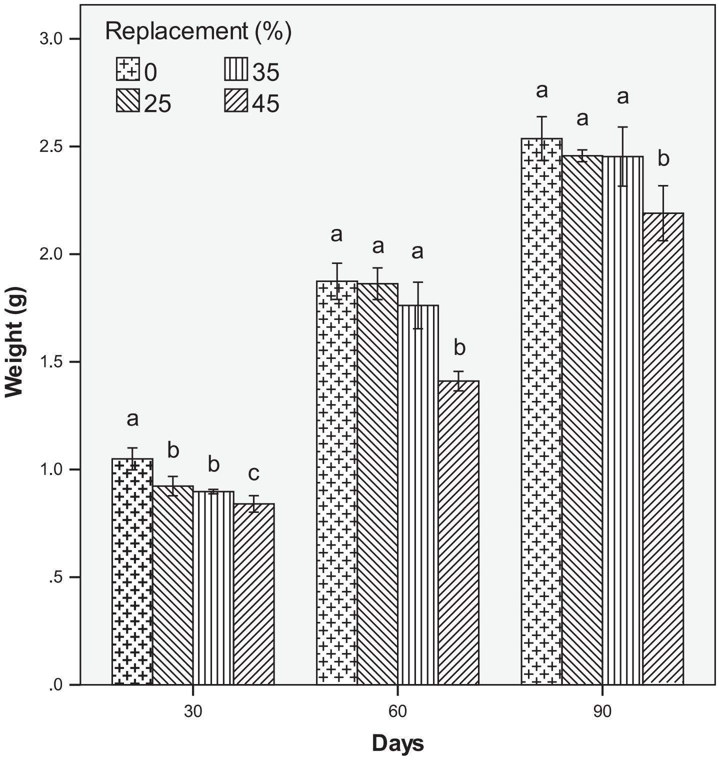

Figure 1 Mean weight of juvenile tench (*Tinca tinca*) at various periods fed practical diets with different replacement levels of FM protein by PPC protein over 90 days. At day 30 and 60, data derived from 20 fish sampled per replicate ($n = 60$). At the end of the experiment (day 90), data derived from all surviving fish (ca. 54 per replicate, $n = 160$). Error bars represent the standard error of the mean. Means in an organ without a common superscript differ at $P < 0.05$.

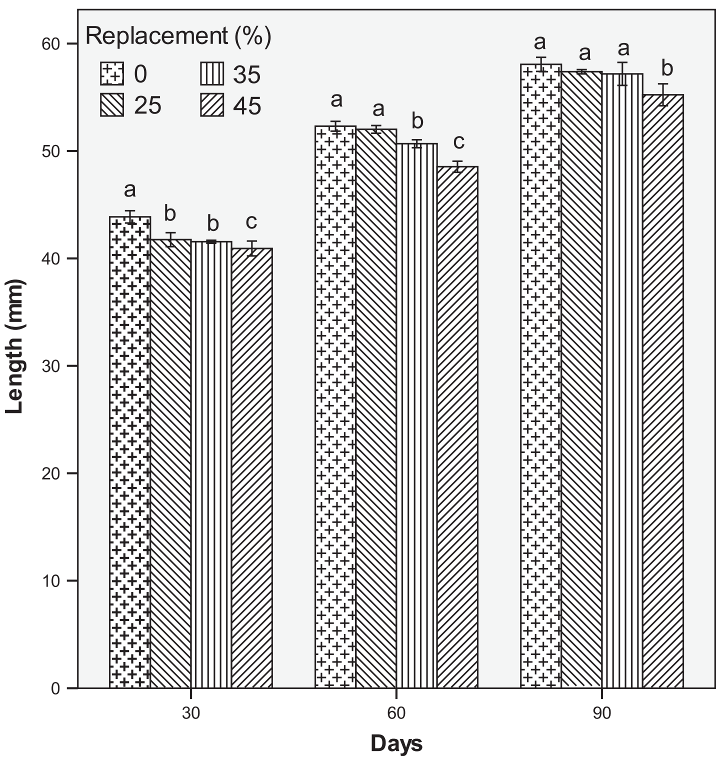

Figure 2 Mean length of juvenile tench (*Tinca tinca*) at various periods fed practical diets with different replacement levels of FM protein by PPC protein over 90 days. At day 30 and 60, data derived from 20 fish sampled per replicate ($n = 60$). At the end of the experiment (day 90), data derived from all surviving fish (ca. 54 per replicate, $n = 160$). Error bars represent the standard error of the mean. Means in an organ without a common superscript differ at $P < 0.05$.

was replaced, the content of arginine and methionine decreased by 13.2% and 23.3%, respectively, and growth of juvenile tench was significantly reduced ($P < 0.05$).

The proximate composition and amino acid profiles of the whole body of juvenile tench at the start and at the end of the trial are presented in Table 5. No significant differences ($P > 0.05$) in moisture, crude protein and ash were found among dietary treatments. The lipid content of the fish fed the 45% replacement diet was significantly higher ($P < 0.05$) than the rest. Regarding the amino acid profiles, EAA contents decreased in the body with the increase in the replacement level of FM protein by PPC protein in diet. Fish fed the 35% replacement diet (290.4 g PPC kg^{-1} diet) had contents of leucine, threonine and tryptophan similar to the fish fed the control diet, while the rest of EAA were significantly lower ($P < 0.05$), but grow performance showed no significant differences. Comparing EAA contents of the fish fed the control diet with the fish fed the 45% replacement diet, the highest reductions corresponded to methionine (19.2%), isoleucine (10.0%), arginine (9.1%) and histidine (9.1%) and growth values were significantly reduced ($P < 0.05$).

Table 5 Proximate composition and amino acid profiles of the whole body of juvenile tench fed practical diets with different levels of replacement of fish meal protein by pea protein concentrate protein (g kg^{-1}, wet basis)

		Replacement (%)			
	Initial	0	25	35	45
Proximate composition					
Moisture	772.2	730.0	733.8	734.1	730.0
Protein	149.3	168.4	167.5	166.8	165.7
Lipid	39.4	62.0^{a}	57.6^{a}	60.2^{a}	68.2^{b}
Ash	33.9	32.3	34.5	32.4	33.0
Essential amino acid					
Arginine	24.8	18.6^{a}	18.1^{a}	17.4^{b}	16.9^{b}
Histidine	4.1	5.5^{a}	5.1^{b}	5.0^{b}	5.0^{b}
Isoleucine	4.7	7.0^{a}	6.7^{a}	6.5^{b}	6.3^{c}
Leucine	8.3	13.5	13.3	13.3	13.2
Lysine	11.8	13.9^{a}	$13.7^{a,b}$	13.6^{b}	13.4^{c}
Methionine	1.4	2.6^{a}	2.5^{a}	2.2^{b}	2.1^{b}
Phenylalanine	3.3	7.4^{a}	7.1^{b}	7.1^{b}	7.0^{b}
Threonine	5.2	6.8	6.6	6.5	6.5
Tryptophan	0.4	0.6	0.6	0.6	0.6
Valine	6.5	7.6^{a}	7.3^{b}	7.1^{b}	7.0^{b}
Non-essential amino acid					
Alanine	8.3	10.1	10.0	10.0	9.9
Aspartate	27.7	24.9^{a}	25.9^{b}	27.3^{c}	28.0^{c}
Cysteine	0.6	1.2	1.2	1.2	1.3
Glutamate	19.1	22.3	22.3	22.4	22.5
Glycine	3.9	5.5^{a}	5.1^{b}	4.9^{b}	4.8^{b}
Proline	4.3	6.9^{a}	6.5^{b}	6.5^{b}	6.4^{b}
Serine	4.8	8.4^{a}	$8.2^{a,b}$	8.1^{b}	8.0^{b}
Tyrosine	2.6	3.5	3.6	3.6	3.6

In the same row, values with different superscript are significantly different ($P < 0.05$). Initial data are not included in the statistical analysis.

Discussion

Most of the studies for the intensification of juvenile tench rearing have started with fish aged 3–7 months and growth rates (SGR, % day^{-1}) from 0.70 to 1.98 have been reported, being higher when dry diets for other species were supplemented with natural feed (García *et al.* 2013). In this study, 6-month-old juveniles were fed practical diets as the sole feed and SGRs (1.73–1.88% day^{-1}) are in the range of the highest reported. Thus, the possibility to avoid natural feed as supplement to guarantee acceptable results on juvenile tench under controlled conditions represents an important advantage as the extruded diets tested in this study can be handled as a commercial aquafeed.

There is scarce information on FCR of juvenile tench, and all previous data have been calculated considering the total amount of diet supplied to the fish. In the intensive rearing of 3-month-old juveniles (around 0.45 g at the beginning of the experiment) fed a commercial trout feed for some 450 days, Rennert, Kohlmann and Hack (2003) reported FCRs ranging from 1.75 to 3.56. Mareš, Jirásek, Baránek, Fiala and Copp (2007) tested three dry diets with 7-month-old juveniles (0.8–1.2 g) over 63 days, and FCRs ranged from 1.84 to 4.15. In the present 90-day trial with 6-month-old juveniles (0.5 g), FCRs ranged from 1.56 to 1.63. These values are more favourable than those previously reported and are close to the feed conversion rates in the intensive culture of well-studied species, with values typically ranging from 0.8 to 1.5 (Hardy & Barrows 2002).

Pea can be processed throughout physical isolation technology to obtain pea protein concentrate (PPC, around 52% crude protein). This raw material has been tested in FM replacement experiments performed with adults of several fish species (Tibaldi *et al.* 2005; Øverland *et al.* 2009; Penn *et al.* 2011; Zhang *et al.* 2012) with successful inclusion levels between 20% and 36%. To our knowledge, only Schulz, Wickert, Kijora, Ogunji and Rennert (2007) have tested on juvenile fish an isolate pea protein (IPP, 80% crude protein) in a 8-week trial with tilapia (*Oreochromis niloticus* L.) noting that it is possible to include 120 g of IPP protein kg^{-1} diet (30% replacement of FM protein by IPP protein) without negative effects on growth. In this study with juvenile tench (*T. tinca*), 35% FM protein was replaced by PPC protein (151 g of PPC protein kg^{-1} diet) without harmful effects on growth performance.

The inclusion of high levels of plant protein in fish feeding is associated with reduced growth performance (Schulz *et al.* 2007), probably because alternative plant proteins possess poorer amino acids profiles than the ingredients they replace (Reigh 2008). In this study, the content of all EAA in the diets including PPC were lower than those of the control diet (Table 3), which was reflected in a decrease in the EAA contents of the body of juvenile tench (Table 5). Regarding the 35% replacement diet (290.4 g PPC kg^{-1} diet), arginine and methionine contents were significantly lower than those of the control diet. However, this was not accompanied by a significant decrease in growth performance. Therefore, it can be assumed that both the control diet and the 25% replacement diet had an excess of these amino acids. When the substitution level of FM protein increased up to 45% (373.3 g PPC kg^{-1} diet), the amounts of arginine and methionine (Table 3) again decreased compared with the 35% replacement diet, and significant growth reduction in juvenile tench was evidenced. Although the arginine content of the 45% replacement diet (50.7 g kg^{-1} diet) was above the requirements estimated for juveniles of other freshwater fish species such as common carp (*Cyprinus carpio* L.), rainbow trout (*O. mykiss*) or mrigal carp (*Cirrhinus mrigala* Hamilton) (National Research Council 2011), the methionine content (8.9 g kg^{-1} diet) was below the minimum recommended for yellow perch (*Perca flavescens* Mitchill) or mrigal carp (*C. mrigala*) (National Research Council 2011). From these considerations, it may be hypothesized that a low methionine content, probably below the requirement of juvenile *T. tinca*, could be related to the reduction in growth observed with the highest replacement level (45%).

Besides the amino acid deficiency, other factors have been related to poor growth of fish fed high levels of plant protein sources, such as digestibility and presence of anti-nutritional factors (ANF). Taken into account that there are studies reporting that feeding PPC to different carnivorous species gave similar digestibility values compared with diets containing only FM (Øverland *et al.* 2009; Zhang *et al.* 2012), it is improbable that the digestibility of PPC did negatively affect juvenile tench growth. Regarding ANF, it is outstanding that pea meal, like other sources of protein from plants, has several ANF. Although processes such as pea dehulling to obtain PPC and extrusion decrease concentrations of ANF (Thiessen, Campbell & Adelizi 2003), the remaining ANF could also adversely affect growth with the highest inclusion level of PPC in diet. Recent studies have showed that a 35% PPC in diets for Atlantic salmon, *S. salar* (Penn *et al.* 2011) or a 40% PPC in diets for rainbow trout, *O. mykiss* (Zhang *et al.* 2012) can affect digestive physiological parameters and fish health indicating that PPC may be contraindicated at high dietary inclusion levels. Thus, the decrease in growth of juvenile tench fed the 45% replacement diet could be mainly attributable to both an amino acid deficiency and the possible presence of ANF.

The proximate composition of the practical diets (Table 2) showed an increase in carbohydrate content with increasing dietary PPC. This could be related to a higher body lipid content in juvenile tench fed the highest PPC level (37% in diet, Table 5). This relationship between dietary carbohydrate and body lipid content has also been observed in other fish species, such as juvenile European sea bass, *D. labrax* (Moreira, Peres, Couto, Enes & Oliva-Teles 2008) or juvenile cobia, *Rachycentron canadum* L. (Ren, Ai, Mai, Ma & Wang 2011) noting that it may be due to an increase in deposition of fat in the body with the increase in available carbohydrates. Considering that the amount of *de novo* synthesis of lipids from carbohydrate is quite limited (Hemre & Kahrs 1997), it rather seems that high-carbohydrate diets stimulated enzymes involved in lipogenesis, as Likimani and Wilson (1982) and Fynn-Aikins, Hung, Liu and Li (1992) have reported in other fish species.

The practical diets tested enabled not only acceptable growth and high survival but also very low percentages of deformed fish. Body deformities are an undesirable but inherent problem in intensive aquaculture, which entail severe losses to the production sector and have the most detrimental effects on the consumers' image of aquaculture (Zambonino-Infante, Koumoundouros & Tandler

2009). Inadequate feeding, especially during early development, has been reported to induce malformations in fish (Cahu, Zambonino-Infante & Takeuchi 2003; Fontagné 2009). Different authors have reported high percentages (27–96.4%) of deformed juvenile tench under intensive conditions, probably due to imbalanced feed composition for this species (García *et al.* 2013). The deformity rates recorded in this study (0–1.5%) were much lower. This may support that the practical diets used in this trial were better balanced for juvenile tench than the aquafeeds for other species used so far.

This study provides the first information on substitution possibilities of FM by PPC in the tench (*T. tinca*). Despite the drawbacks that limit the use of plants as alternative protein source for fish feeds, PPC has shown to be a promising protein ingredient for this species. Up to 290.4 g PPC kg^{-1} diet can be included in extruded diets (50% crude protein) for juvenile tench to replace 35% of FM protein without impairing growth performance.

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PROOF

Evaluation of Soy Protein Concentrate as Replacement of Fish Meal in Practical Diets for Juvenile Tench (*Tinca tinca* L.)

Álvaro González-Rodríguez[1,*], Jesús Domingo Celada[1], José Manuel Carral[1], María Sáez-Royuela[1], Vanesa García[1], Juan Bautista Fuertes[1]

[1] Universidad de León, Facultad de Veterinaria, Dpto. de Producción Animal, Campus de Vegazana s/n, 24071, León, Spain.

* Corresponding Author: Tel.: +34.987 291187; Fax: +34.987 291187;
E-mail: agonr@unileon.es

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Abstract

The aim of this study was to evaluate different replacement levels of fish meal (FM) by soy protein concentrate (SPC) on survival, growth performance and body composition of juvenile tench (*Tinca tinca*). A 90-day experiment was conducted with 5 month-old juveniles (31.95 mm total length, 0.396 g weight). Eight practical diets (50% crude protein) differing in the level of replacement of FM protein by SPC protein were tested: 0% (control diet), 25%, 35%, 45%, 55%, 65%, 75% or 100% corresponding to 0, 159, 222, 285, 348, 412, 475 or 634 g SPC kg^{-1} diet, respectively. Survival rate was 100% for all diets. Significant differences were not found (P>0.05) in growth values among 25%, 35% or 45% replacement diets and the control diet. At higher replacement levels (from 55% to 100%), fish had significantly lower growth (P<0.05). Fish fed from 0% to 45% replacement diets had significantly lower (P<0.05) feed conversion ratio and higher (P<0.05) protein productive value than those fed diets with higher replacement levels. The percentages of fish with externally visible deformities ranged from 1.1% to 4.4%. The relation among amino acid profiles of the diets, body composition, growth performance of juveniles and amino acid requirements of other fish species are discussed. Up to 285 g SPC kg^{-1} diet can be included in diets for juvenile tench without impairing growth performance.

Keywords: Soy protein concentrate, juvenile rearing, practical diet, tench.

Introduction

Tench, *Tinca tinca* (Linnaeus, 1758), a freshwater fish belonging to the family Cyprinidae, has a great potential for aquaculture (Wang *et al*., 2006; Celada *et al*., 2009; García *et al*., 2013). Originally occurring in the waters of Europe and Siberia, today tench occurs in the inland waters of all continents (Freyhof and Kottelat, 2008). In natural habitats, tench are carnivorous (Kennedy and Fitzmaurice, 1970), and gut content analysis show that juveniles fed zooplankton and other small invertebrates (Pyka, 1997). In culture, the use of manufactured feed development is limited by the lack of knowledge on nutritional requirements, forcing the use of dry diets formulated for other species. From the practical point of view, research should be conducted to solve this pressing problem by studying specifically compounded diets for juvenile tench.

Plant protein sources are increasingly used to satisfy the growing demands of the aqua-feed industry (Hardy, 2010). Considering the different possibilities, soybeans are a logical protein source for use in aquaculture diets (Brown *et al*., 2008). Soybean meal (SBM) has been one of the most studied alternatives to fish meal (FM), but has several limitations, including antinutritional factors, low level of methionine and adverse effects on the intestinal integrity of some carnivorous species (Gatlin *et al*., 2007). The nutritive value of SBM can be improved using fractionation to produce soy protein concentrate (SPC) (Vielma *et al*., 2000; Collins *et al*., 2012). The highly refined SPC has a similar protein content and apparent dietary protein and amino acid digestibility to FM (Hardy, 2008). Furthermore, as SPC is a highly refined ingredient, most of the antinutritional factors present in SBM have been removed during processing (Bowyer *et al*., 2013). The substitution of FM with SPC has been tested in several juvenile fish species, such as rainbow trout, *Oncorhynchus mykiss* (Kaushik *et al*., 1995), turbot, S*cophthalmus maximus* (Day and González, 2000), Japanese flounder, *Paralichthys olivaceus* (Deng *et al*., 2006) or kingfish, *Seriola lalandi* (Bowyer *et al*., 2013), showing that adequate inclusion levels of SPC in diet are different depending on the studied species**.** The aim of this study was to evaluate the effects of partial or total replacement of fish meal with soy protein concentrate in practical

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diets for juvenile tench (*T. tinca*) on survival, growth performance and body composition.

Materials and Methods

Fish, Facilities and Experimental Procedure

A 90-day experiment was carried out with juvenile tench. Larvae were obtained by hatching under artificial reproduction techniques (Rodríguez *et al*., 2004) and were reared for five months until the juvenile stage in which the experiment started. From five days after hatching, when first feeding started, larvae were maintained in outdoor fiberglass tanks (2500 L) and fed decapsulated *Artemia* cysts for two weeks (Celada *et al*., 2013). Then, fish were fed a combination of a carp starter and decapsulated *Artemia* cysts. After five months, 720 juvenile tench (31.95±0.14 mm total length, 0.396±0.005 g weight, mean ± standard error, n = 120) were transferred to indoor facilities. Fish were anesthetized with tricaine methanesulfonate (MS-222; Ortoquímica S.L., Barcelona, Spain) and randomly distributed as groups of 30 fish in 24 fiberglass tanks (0.5 x 0.25 x 0.25 m) containing 25 L of water to obtain replicates corresponding to the different feeding treatments. All experimental groups were in triplicate. The juveniles were acclimated to experimental conditions for 10 days before the trial.

Artesian well water was supplied in open system (flow-throughout) and each tank had a water inlet (inflow 0.30 L min^{-1}) and outlet (provided with a 250 μm mesh filter) and light aeration. The variables of the incoming water quality (measured once a week) were: pH = 7.6, hardness 5.3 ºdH (German degrees, calcium 32.8 mg L^{-1}), total dissolved solids 115.2 mg L^{-1} and total suspended solids 34.6 mg L^{-1}. Dissolved oxygen content in tanks was measured with a meter HACH HQ30d (Hach Lange GMBH, Vigo, Spain) throughout the trial and values ranged between 5.7 and 7.2 mg L^{-1}. Ammonia and nitrites were measured with a spectrophotometer HACH DR2800 (Hach Lange GMBH, Vigo, Spain) from water samples taken inside the tanks (values were always ammonia <0.10 mg L^{-1} and nitrites <0.013 mg L^{-1}). Water temperature (measured twice a day) was 24±1°C and a 16 h light: 8 h dark photoperiod was maintained throughout the experiment. Tanks were cleaned of faeces and uneaten feed every two days. All procedures used in the study were approved by the León University Ethics Committee (Spain).

Diets and Feeding

Based on the practical diet proposed by González-Rodríguez *et al*. (2013), different diets (50% crude protein) were formulated and prepared to test substitution possibilities of FM protein by SPC protein. The SPC (70% crude protein) was obtained through aqueous ethanol extraction of soy flakes. FM was from anchoveta. Proximate composition and amino acid profiles of FM and SPC are presented in Table 1. Eight diets (nearly isonitrogenous and isoenergetic) were formulated to test different replacement levels of FM protein by SPC protein: 0% (control), 25%, 35%, 45%, 55%, 65%, 75% or 100%, corresponding to 0, 159, 222, 285, 348, 412, 475 or 634 g SPC kg^{-1} diet, respectively. Ingredients were

Table 1. Proximate composition and amino acid profiles of fish meal (FM) and soy protein concentrate (SPC) (g kg^{-1}, wet basis)

	FM	SPC
Proximate composition		
Moisture	79.7	79.9
Crude protein	678.0	700.0
Crude fat	90.3	8.1
Carbohydrates	0.0	147.2
Ash	152.0	64.8
EAA		
Arginine	96.2	55.3
Histidine	15.7	18.9
Isoleucine	27.6	35.8
Leucine	45.9	61.5
Lysine	48.6	43.5
Methionine	18.2	9.1
Phenylalanine	19.8	39.3
Threonine	39.5	28.0
Tryptophan	5.5	10.2
Valine	32.0	33.5
NEAA		
Alanine	39.4	32.1
Aspartate	62.9	99.7
Cysteine	6.2	10.4
Glutamate	85.8	96.4
Glycine	26.2	30.6
Proline	26.7	35.2
Serine	37.2	35.9
Tyrosine	16.2	21.8

ground in a rotary mill BRABENDER (Brabender GmbH & Co. KG, Duisburg, Germany), mixed in a mixer STEPHAN UMC5 (Stephan Food Service Equipment, Hameln, Germany) and extruded using a stand-alone extruder BRABENDER KE19/25D (Brabender GmbH & Co. KG, Duisburg, Germany) at a temperature range between 100°C and 110°C. Pellets (1 mm diameter) were obtained and dried during 24 hours at 30°C. Then, pellets received a coating of cod liver oil (30 g kg^{-1} diet). Formulation and proximate composition of practical diets are summarized in Table 2 and amino acid profiles are in Table 3. Fish were fed manually four times a day (at $10:^{00}$, $14:^{00}$, $18:^{00}$ and $22:^{00}$ hours) to apparent satiation.

Chemical Analysis of Diets and Fish

Proximate composition of FM and SPC (Table 1), practical diets (Table 2) and whole-body of juvenile tench (Table 4) were analyzed according to the Norms of the International Standards Organization (1973-1998): moisture to ISO R-1442, protein to ISO R-937, lipid to ISO R-1443, ash to ISO R-936 and gross energy to ISO 9831. The content of carbohydrates was calculated by subtracting the content of moisture, protein, lipid and ash from the wet weight. Samples were stored at -30 °C until analysis. Fish were fasted for 14 h before harvest.

Amino acid profiles of FM and SPC (Table 1), practical diets (Table 3) and whole-body of juvenile tench (Table 4) were analyzed by HPLC using AccQTag method from Waters (Milford, MA, USA). Amino acids were derivatized with 6-aminoquinolyl-N-hydrosysuccinimidyl carbamate reagent (AQC) by the method of Cohen and Michaud (1993) and Cohen and De Antonis (1994), and were detected by Dual λ Absorbance Detector Waters 2487 from Waters (Milford, MA, USA) at 254 nm. Quantification was carried out with Empower Pro 2.0 software from Waters (Milford, MA, USA). All analyses were performed in duplicate.

Data Collection and Statistical Analysis

Every thirty days, a sample of 10 fish per tank (33% of total) was taken and fish were anesthetized. The excess water was removed with tissue paper and fish were weighed and measured individually. Total length (TL) was measured with a digital caliper (to the nearest 0.01 mm) and individual weight (W) was determined by a precision balance (to the nearest 0.001 g). After measurement, juveniles were gently returned to their respective tanks. At the end of the experiment (day 90), fish were anesthetized and observed one by one using a magnifying glass in order to detect externally visible deformities. Individual weight and length of all fish (90 per

Table 2. Formulation and proximate composition of the practical diets with different levels of replacement of FM protein by SPC protein (g kg^{-1} diet, wet basis)

	Replacement (%)							
	0	25	35	45	55	65	75	100
Ingredients								
Fish meal [a]	656	492	426	361	295.5	230	164	-
Soy protein concentrate [b]	-	159	222	285	348	412	475	634
Corn meal [c]	150	155	153	152.5	155	154	154.5	157
Dried *Artemia* cysts [d]	100	100	100	100	100	100	100	100
Carboxymethyl cellulose [e]	30	30	30	30	30	30	30	30
Cod liver oil [f]	30	30	30	30	30	30	30	30
L-ascorbyl-2-monophosphate-Na [g]	5	5	5	5	5	5	5	5
Dicalcium phosphate [g]	10	10	10	10	10	10	10	10
Choline chloride [g]	3	3	3	3	3	3	3	3
Soy lecithin [h]	5	5	10	12.5	12.5	15	17.5	20
Sodium chloride [i]	1	1	1	1	1	1	1	1
Vitamin-Mineral premix [j]	10	10	10	10	10	10	10	10
Proximate composition								
Moisture	74.6	74.7	74.2	74.0	74.1	73.8	73.6	73.5
Crude protein	500.5	500.9	500.3	500.4	500.2	500.6	500.0	500.4
Crude lipid	117.5	103.8	103.1	100.0	94.5	91.4	88.3	76.7
Carbohydrates	173.3	200.6	208.3	217.3	228.4	237.1	246.8	272.3
Ash	134.1	120.0	114.1	108.3	102.8	97.1	91.3	77.1
Gross energy (MJ kg^{-1})	74.6	74.7	74.2	74.0	74.1	73.8	73.6	73.5

[a] Skretting España S.A., Ctra. de la Estación s/n 09620 Cojóbar. Burgos. España.
[b] C.D.A. S.L., Pol. Ind. El Estanquillo. Burguillos. Sevilla. España.
[c] Adpan Europa S.L., ES-33186 El Berrón. Siero. Asturias. Spain.
[d] INVE Aquaculture Nutrition. Hoogyeld 91. Dendermonde. Belgium.
[e] Helm Iberica S.A., ES-28108 Alcobendas. Madrid. Spain.
[f] Acofarma distribution S.A., ES-08223 Terrassa. Barcelona. Spain.
[g] Nutral S.A., ES-28720 Colmenar Viejo. Madrid. Spain.
[h] Biover N.V., Monnikenwerve 109. B-8000 Brugge. Belgium.
[i] Unión Salinera de España S.A., ES-28001 Madrid. Spain.
[j] Provides mg kg^{-1} premix: inositol, 50000; thiamin, 500; riboflavin, 800; niacin, 5000; pyridoxine, 1500; pantothenic acid, 5000; biotin, 150; folic acid, 3500; cianocobalamin, 5; retinol, 2400; α-tocopherol, 30000; cholecalciferol, 6.25; naphthoquinone, 5000; ethoxyquin, 70000; $MgSO_4$-$7H_2O$, 300000; $ZnSO_4$-$7H_2O$, 11000; $MnSO_4$-H_2O, 4000; $CuSO_4$-$5H_2O$, 1180; $CoSO_4$, 26; $FeSO_4$-$7H_2O$, 77400; KI, 340; Na_2SeO_3, 68.

Table 3. Amino acid profiles of the practical diets with different levels of replacement of FM protein by SPC protein (g kg^{-1} diet, wet basis)

	Replacement (%)							
	0	25	35	45	55	65	75	100
EAA								
Arginine	71.4^{a}	64.4^{b}	61.2^{c}	58.9^{d}	55.4^{e}	53.1^{f}	50.3^{g}	43.5^{h}
Histidine	11.3^{a}	$11.7^{a,b}$	$11.8^{a,b,c}$	$11.9^{a,b,c}$	$12.1^{a,b,c}$	$12.3^{a,b,c}$	$12.4^{b,c}$	12.8^{c}
Isoleucine	20.5^{a}	$21.6^{a,b}$	$22.1^{b,c}$	$22.5^{b,c,d}$	$22.9^{c,d,e}$	$23.4^{d,e}$	23.7^{e}	25.0^{f}
Leucine	33.6^{a}	35.8^{b}	$36.7^{b,c}$	$37.6^{c,d}$	$38.4^{d,e}$	$39.4^{e,f}$	40.2^{f}	42.4^{g}
Lysine	36.4^{a}	35.4^{b}	$34.9^{b,c}$	$34.5^{b,c,d}$	$34.0^{c,d}$	$33.6^{c,d}$	$33.2^{d,e}$	32.1^{e}
Methionine	13.3^{a}	11.7^{b}	11.1^{c}	10.5^{d}	9.8^{e}	9.2^{f}	8.6^{g}	7.1^{h}
Phenylalanine	14.3^{a}	17.3^{b}	18.4^{c}	19.6^{d}	20.8^{e}	22.0^{f}	23.2^{g}	26.2^{h}
Threonine	29.5^{a}	27.3^{b}	$26.4^{b,c}$	$25.5^{c,d}$	$24.6^{d,e}$	$23.7^{e,f}$	22.8^{f}	20.6^{g}
Tryptophan	4.2^{a}	$4.9^{a,b}$	$5.2^{b,c}$	$5.5^{b,c,d}$	$5.7^{c,d,e}$	$6.0^{d,e}$	$6.3^{e,f}$	7.0^{f}
Valine	23.3	23.4	23.4	23.4	23.4	23.5	23.5	23.5
NEAA								
Alanine	29.3^{a}	27.9^{b}	$27.3^{b,c}$	$26.8^{b,c,d}$	$26.2^{c,d,e}$	$25.7^{d,e}$	25.1^{e}	23.8^{f}
Aspartate	46.9^{a}	52.5^{b}	54.6^{b}	56.8^{c}	59.0^{d}	61.2^{e}	63.4^{e}	68.9^{f}
Cysteine	4.2^{a}	$4.9^{a,b}$	$5.1^{a,b,c}$	$5.4^{b,c,d}$	$5.6^{b,c,d}$	$5.9^{c,d,e}$	$6.1^{d,e}$	6.7^{e}
Glutamate	64.7^{a}	$66.0^{a,b}$	$66.3^{b,c}$	$66.8^{b,c,d}$	$67.3^{b,c,d}$	$67.8^{c,d}$	$68.3^{d,e}$	69.5^{e}
Glycine	19.1^{a}	$19.6^{a,b}$	$19.8^{a,b}$	$20.0^{a,b}$	$20.2^{b,c}$	$20.4^{b,c}$	$20.6^{b,c}$	21.1^{c}
Proline	20.1^{a}	21.3^{b}	$21.8^{b,c}$	$22.2^{b,c,d}$	$22.7^{c,d,e}$	$23.2^{d,e}$	23.7^{e}	24.9^{f}
Serine	28.6^{a}	$28.0^{a,b}$	$27.7^{a,b}$	$27.5^{a,b,c}$	$27.2^{a,b,c}$	$26.9^{b,c}$	$26.6^{b,c}$	26.1^{c}
Tyrosine	12.2^{a}	$12.9^{a,b}$	$13.2^{a,b,c}$	$13.4^{b,c,d}$	$13.7^{b,c,d}$	$14.0^{c,d,e}$	$14.3^{d,e}$	15.0^{e}

Values in the same row having different superscripts are significantly different ($P<0.05$).

Table 4. Proximate composition and amino acid profiles of the whole-body of juvenile tench fed practical diets with different levels of replacement of FM protein by SPC protein (g kg^{-1}, wet basis)

	Initial[a]	Replacement (%)							
		0	25	35	45	55	65	75	100
Proximate composition									
Moisture	*770.8*	741.8	742.3	741.9	742.7	743.1	743.0	743.8	744.2
Protein	*138.9*	162.2	162.1	162.1	161.7	160.6	159.7	159.2	158.7
Lipid	*48.3*	62.8	62.4	59.8	59.8	60.9	60.3	57.4	58.9
Carbohidrates	2.1	4.0	3.3	6.0	5.9	5.1	5.4	7.9	6.4
Ash	*39.9*	29.2	29.9	30.2	29.9	30.3	31.6	31.7	31.8
EAA									
Arginine	*23.6*	21.9^{a}	$21.8^{a,b}$	$21.6^{b,c}$	$21.6^{b,c}$	$21.4^{c,d}$	21.3^{d}	21.2^{d}	21.2^{d}
Histidine	*5.1*	7.0	6.9	6.9	6.8	6.8	6.8	6.8	6.7
Isoleucine	*6.3*	8.9	8.9	8.8	8.8	8.7	8.7	8.7	8.6
Leucine	*9.2*	13.3	13.2	13.2	13.1	13.1	13.1	13.0	13.0
Lysine	*10.2*	17.0^{a}	$16.9^{a,b}$	$16.9^{a,b}$	$16.9^{a,b}$	$16.8^{a,b}$	$16.8^{a,b}$	$16.8^{a,b}$	16.7^{b}
Methionine	*1.5*	3.0^{a}	2.9^{a}	2.9^{a}	2.8^{a}	2.6^{b}	2.6^{b}	2.5^{b}	2.4^{b}
Phenylalanine	*6.1*	9.4	9.3	9.3	9.3	9.3	9.3	9.3	9.2
Threonine	*4.2*	7.3	7.2	7.2	7.2	7.2	7.2	7.1	7.0
Tryptophan	*0.1*	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6
Valine	*5.1*	8.2	8.2	8.1	8.1	8.1	8.0	8.1	8.0
NEAA									
Alanine	*9.1*	11.2^{a}	$11.1^{a,b}$	$11.1^{a,b}$	$11.1^{a,b,c}$	$10.8^{b,c}$	$10.8^{b,c}$	$10.8^{b,c}$	10.7^{c}
Aspartate	*25.5*	10.6	10.5	10.5	10.4	10.4	10.3	10.4	10.3
Cysteine	*0.5*	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Glutamate	*10.6*	10.6	10.5	10.4	10.4	10.3	10.3	10.2	10.2
Glycine	*4.2*	8.2	8.3	8.4	8.4	8.5	8.5	8.5	8.5
Proline	*4.9*	6.7	6.8	6.8	6.9	6.9	6.9	6.9	6.9
Serine	*4.3*	8.5^{a}	$8.4^{a,b}$	$8.4^{a,b}$	$8.4^{a,b}$	$8.2^{b,c,d}$	$8.1^{c,d}$	$8.1^{c,d}$	$8.1^{c,d}$
Tyrosine	*3.6*	5.1	5.1	5.0	5.0	5.0	5.0	4.9	4.9

Values in the same row having different superscripts are significantly different ($P<0.05$).
[a] Initial data are not included in the statistical analysis.

treatment) were determined. Specific growth rate (SGR) was expressed as $SGR = 100\ [(\ln W_t - \ln W_0)/t]$ where W_t is the mean final weight, W_0 is the mean initial weight, and t is the duration of the experiment (days). Fulton's coefficient (K) was used to determine the fish condition with $K = 100\ (W_t/TL^3)$. According to Fornshell and Hinshaw (2009), feed conversion ratio (FCR) was calculated as $FCR = D_t/(W_t-W_0)$, where D_t is the total amount of feed fed (g) and W_t-W_0 is the weight gain (g) over 90 days. Protein productive value (PPV) were expressed as $PPV = 100\ [((W_t \times C_1)-(W_0 \times C_0))/(C_{diet} \times D_t)]$, where C_1 and C_0 are the final and initial protein concentration in the whole-body, respectively, and C_{diet} is the concentration in the diets. Results were examined by analysis of variance (ANOVA) using the computer program SPSS 16.0 (SPSS, Chicago, IL, USA). Duncan test was applied to compare means at P<0.05 level of significance. Percentages were arcsine-transformed prior to statistical analysis.

Results

Final values (90 days) of survival, growth performance and percentages of fish with externally visible deformities are presented in Table 5. Survival rate was 100% for all diets.

Regarding growth, significant differences were not found (P>0.05) among 25%, 35% or 45% replacement of FM protein by SPC protein (159, 222 or 285 g kg^{-1} SPC in diet, respectively) and the control diet (average of the four feeding treatments: 57.86 mm TL, 2.50 g W and 2.02 % d^{-1} SGR). On the other hand, at higher replacement levels (from 55% to 100%), fish had significantly lower growth (Figures 1 and 2). Juveniles fed the control diet (0%

Table 5. Survival, growth performance and percentages of deformed juvenile tench fed practical diets with different levels of substitution of FM protein by SPC protein over 90 days

	Replacement (%)							
	0	25	35	45	55	65	75	100
Survival (%)	100	100	100	100	100	100	100	100
Total length (mm)	57.95±0.50[a]	58.49±0.58[a]	57.44±0.36[a]	57.55±0.30[a]	54.80±0.44[b]	54.65±0.31[b]	53.88±0.46[b]	54.74±0.31[b]
Weight (g)	2.52±0.09[a]	2.61±0.09[a]	2.45±0.06[a]	2.42±0.04[a]	2.12±0.08[b]	2.04±0.05[b]	2.06±0.07[b]	2.09±0.04[b]
SGR (% d^{-1}) [a]	2.01±0.03[a]	2.05±0.03[a]	2.00±0.02[a]	2.00±0.02[a]	1.83±0.03[b]	1.80±0.02[b]	1.78±0.03[b]	1.83±0.02[b]
K [b]	1.26±0.01	1.27±0.01	1.27±0.01	1.26±0.01	1.26±0.01	1.24±0.01	1.28±0.01	1.26±0.01
FCR [c]	1.39±0.05[a]	1.35±0.05[a]	1.37±0.03[a]	1.36±0.03[a]	1.56±0.05[b]	1.57±0.04[b]	1.57±0.05[b]	1.56±0.04[b]
PPV (%) [d]	26.86±1.02[a]	27.65±1.05[a]	25.85±0.68[a]	25.49±0.55[a]	22.81±0.74[b]	22.15±0.60[b]	23.27±0.84[b]	21.96±0.52[b]
Deformed fish (%)	2.2±0.2[a]	2.2±0.2[a]	1.1±0.2[b]	3.3±0.3[c]	2.2±0.2[a]	2.2±0.2[a]	4.4±0.4[d]	4.4±0.3[d]

Values are mean±standard error. Growth data derived from all fish (30 per replicate, n = 90). Values in the same row having different superscripts are significantly different (*P*<0.05).
[a] Specific growth rate (% d^{-1}) = 100 x [(ln final mean body weight - ln initial mean body weight) x $days^{-1}$)].
[b] Condition factor = 100 × (body weight x body $length^{-1\ 3}$).
[c] Feed conversion ratio = total amount of feed fed x weight $gain^{-1}$.
[d] Protein productive value = (fish protein gain x crude protein $intake^{-1}$) x 100.

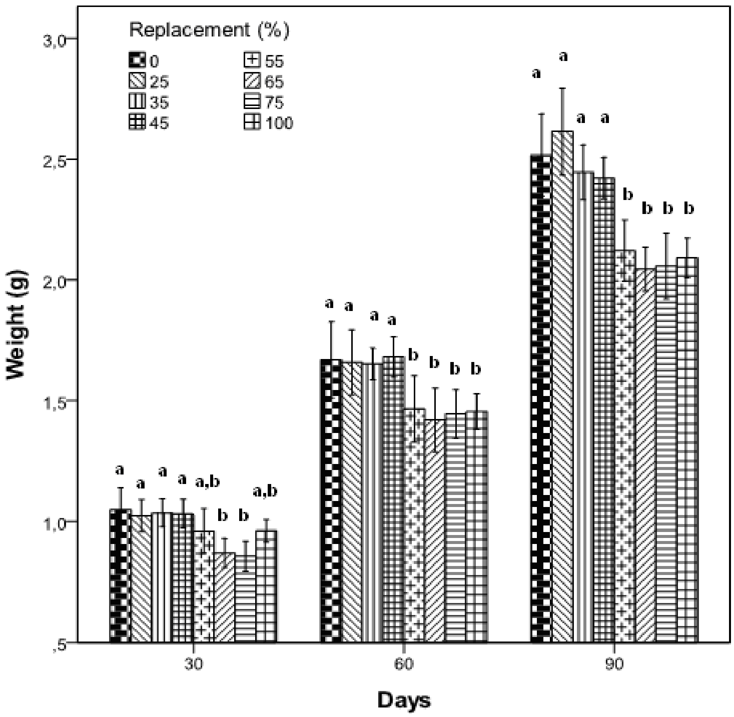

Figure 1. Mean weight of juvenile tench (*T. tinca*) at various periods fed practical diets with different replacement levels of FM protein by SPC protein over 90 days. At day 30 and 60, data derived from 10 fish sampled per replicate (n = 30). At the end of the experiment (day 90), data derived from all fish (30 per replicate, n = 90). Error bars represent the standard error of the mean.

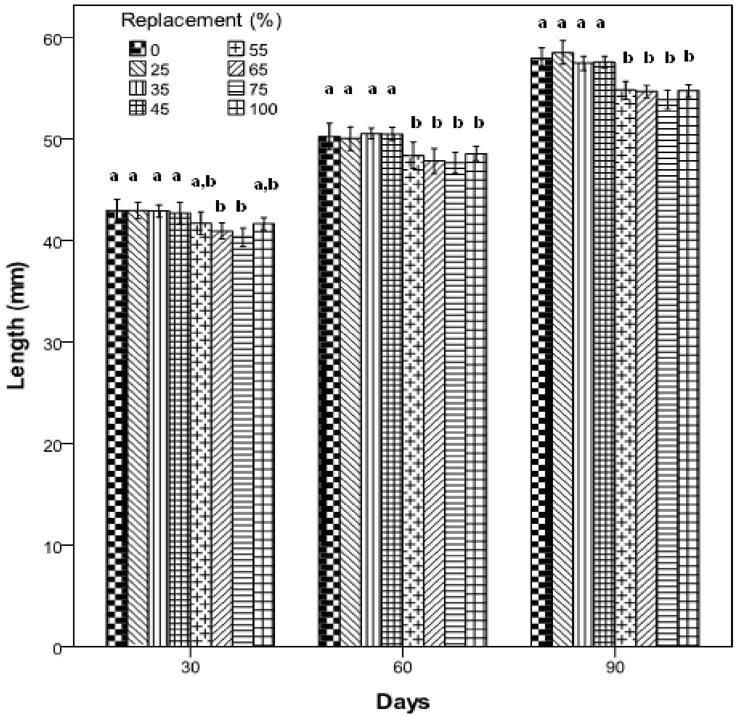

Figure 2. Mean length of juvenile tench (*T. tinca*) at various periods fed practical diets with different replacement levels of FM protein by SPC protein over 90 days. At day 30 and 60, data derived from 10 fish sampled per replicate (n = 30). At the end of the experiment (day 90), data derived from all fish (30 per replicate, n = 90). Error bars represent the standard error of the mean.

replacement), 25%, 35% or 45% replacement of FM protein grew faster than those fed with higher replacement levels (from 55% to 100%). These differences were significant (P<0.05) from day 60 onwards.

Condition factor (K) ranged from 1.24 to 1.28 without significant differences (P>0.05) among treatments. Feed conversion ratio (FCR) ranged from 1.35 to 1.57 (Table 5). FCRs of fish fed from 0% to 45% replacement of FM protein by SPC protein were significantly lower (average: 1.37; P<0.05) than those fish fed diets with higher replacement levels (from 55% to 100%). Protein productive value (PPV) ranged from 21.96 to 27.65 and fish fed from 0% to 45% replacement diets showed a PPV significantly higher (average: 26.46; P<0.05) than those fish fed diets with higher replacements levels (from 55% to 100%). The percentages of fish with externally visible deformities (Table 5) were low for all practical diets, ranging from 1.1% to 4.4%. Body deformities affected to the spinal column and caudal peduncle (break in the tail axis).

The amino acid profiles of the practical diets are presented in Table 3. Considering the essential amino acids (EAA), the arginine, lysine, methionine and threonine contents decreased with the increase of the replacement level of FM protein by SPC protein in diet. Comparing 35% with 45% replacement of FM protein, there were significant reductions (P<0.05) in the arginine and methionine contents that were 3.8% and 5.4%, respectively, but they were not accomplished with a reduction in growth. When the replacement of FM protein by SPC protein in diets increased from 45% to 55%, there were significant reductions (P<0.05) in arginine and methionine contents (5.9% and 6.7%, respectively) and growth was significantly reduced (P<0.05).

The proximate composition and the amino acid profiles of the whole-body of juvenile tench at the start and at the end of the trial are presented in Table 4. Differences among feeding treatments were not significant for each macronutrient (P>0.05). Regarding the EAA, arginine, lysine and methionine contents decreased in the body with the increase of the replacement level of FM protein by SPC protein in diet. Fish fed the 45% replacement diet (285 g SPC kg^{-1} diet) had a content of arginine significantly lower (P<0.05) than the fish fed the control diet. However, growth performance showed no significant differences (P>0.05). Fish fed the 55% replacement diet (348 g SPC kg^{-1} diet) had a content of methionine significantly lower (P<0.05) than the fish fed the 45% replacement diet, and growth values were significantly reduced (P<0.05).

Discussion

Most of the studies on the intensification of juvenile tench rearing have started with fish aged 3 to 7 months. Growth rates (SGR, % day^{-1}) from 0.70 to 1.98 have been reported, being higher when dry diets for other species were supplemented with natural feed (García *et al.*, 2010). In the present study, 5 month-old juveniles were fed practical diets as the sole feed and SGRs (1.78-2.05% d^{-1}) are in the range of the highest reported.

There is scarce information on FCR of juvenile tench, and all previous data have been calculated considering the total amount of diet supplied to the fish. In the intensive rearing of 3 month-old juveniles (around 0.45 g at the beginning of the experiment) fed a commercial trout feed for some 450 days, Rennert *et al*. (2003) reported FCRs ranging from 1.75 to 3.56. Mareš *et al*. (2007) tested three dry diets with 7 month-old juveniles (0.8-1.2 g) during 63 days, and FCRs ranged from 1.84 to 4.15. The FCRs obtained in the present study ranged from 1.35 to 1.57 and were similar to the reported by González-Rodríguez *et al*. (2013) starting also with 5 month-old juveniles and using the same basal diet. In both cases, values are more favorable than those previously reported and are close to the feed conversion rates in the intensive culture of well studied species, with values typically ranging from 0.8 to 1.5 (Hardy and Barrows, 2002).

Soybean meal can be processed to obtain soy protein concentrate (SPC, around 70% crude protein). This ingredient has been tested in FM replacement experiments performed with juveniles of several fish species which have different tolerance for dietary SPC. For instance, Kaushik *et al*. (1995) tested SPC with rainbow trout, (*O. mykiss*) noting that it is possible to include 220 g of SPC kg^{-1} diet without negative effect on growth. Lower levels of SPC in diet, as 200 g kg^{-1} for kingfish, *S. lalandi* (Bowyer *et al*., 2013), or 185 g kg^{-1} for turbot, S. *maximus* (Day and González, 2000), have been successfully included. By contrast, a growth reduction was observed in Japanese flounder, *P. olivaceus* (Deng *et al*., 2006), even at the minimum replacement level tested (159 g of SPC kg^{-1}). In the present study with juvenile tench (*T. tinca*), 285 g of SPC kg^{-1} (45% replacement of FM protein) were included in diet without harmful effects on growth performance.

The inclusion of high levels of plant protein in fish feeding is associated with reduced growth performance (Schulz *et al*., 2007), probably due to alternative plant proteins possess poorer amino acids profiles than the ingredients they replace (Reigh, 2008). In the present study, the content of arginine, lysine, methionine and threonine in the diets including SPC were lower than those of the control diet (Table 3). Regarding the 45% replacement diet (285 g SPC kg^{-1} diet), arginine and methionine contents were significantly lower (21.9% and 21.1%, respectively) than those of the control diet. However, this was not accomplished with a significant decrease in growth. Therefore, it could be assumed that the control diet and the 25%, 35% or 45% replacement diets had an excess of these amino acids. When the substitution level of FM protein increased up to 55% (348 g SPC kg^{-1} diet), the amounts of arginine and methionine (Table 3) newly decreased compared with the 45% replacement diet, and significant growth reduction of juvenile tench was evidenced. Whereas the arginine content of the 55% replacement diet (55.4 g kg^{-1}) was above the requirements estimated for juveniles of other freshwater fish species such as common carp (*Cyprinus carpio*), rainbow trout (*O. mykiss*) or mrigal carp (*Cirrhinus mrigala*) (NRC, 2011**),** the methionine content (9.8 g kg^{-1}**)** was below the minimum recommended for yellow perch (*Perca flavescens*) or mrigal carp (*C. mrigala*) (NRC, 2011)**.** From these considerations, it could be suggested that the methionine content was probably below the requirement of juvenile *T. tinca*. Considering that body composition is related to diet composition and that protein and essential amino acid retention are the most sensitive indicators of an inadequate supply of amino acids (Rodehutscord *et al*. 1995), a significant decrease of deposition of protein in the juvenile tench, estimated by means of the PPV, was evidenced when amounts above 285 g of SPC kg^{-1} diet were included. This PPV decrease coincided with a significantly lower content of methionine in the whole-body of tench. The deficiency of this essential amino acid in diets with the highest replacement levels of FM protein could explain the decrease of PPV and the increase of FCR, probably due to the use of dietary protein for catabolic rather than anabolic processes.

The practical diets tested enabled not only acceptable growth and high survival but also very low percentages of deformed fish. Body deformities are an undesirable but inherent problem in intensive aquaculture, which entail severe losses to the production sector and have the more detrimental effects on the consumers' image of aquaculture (Zambonino-Infante *et al*., 2009). Inadequate feeding, especially during early development, has been reported to induce malformations in fish (Cahu *et al*., 2003; Fontagné, 2009). Different authors have reported high percentages (27-96%) of deformed juvenile tench under intensive conditions (Wolnicki *et al*., 2006; Rennert *et al*., 2003; Myszkowski *et al*., 2010), probably due to imbalanced feed composition for this species (García *et al*., 2013). The deformity rates recorded in the present study (1.1-4.4%) were much lower, suggesting that the practical diets used were better balanced for juvenile tench than the aquafeeds for other species used so far.

The present study provides the first information on substitution possibilities of FM by SPC in tench (*T. tinca*). Despite the drawbacks that limit the use of plants as alternative protein source for fish feeds, SPC has shown to be a promising protein ingredient for this species. Up to 285 g SPC kg^{-1} diet can be included in extruded diets (50% crude protein) for juvenile tench to replace 45% of FM protein without impairing growth performance.

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