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

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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 $FCR = D_t/(W_t\text{-}W_0)$, 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 $PPV = 100\ [((W_t \times C_1)\text{-}(W_0 \times C_0))/(C_{diet} \times D_t)]$, 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.

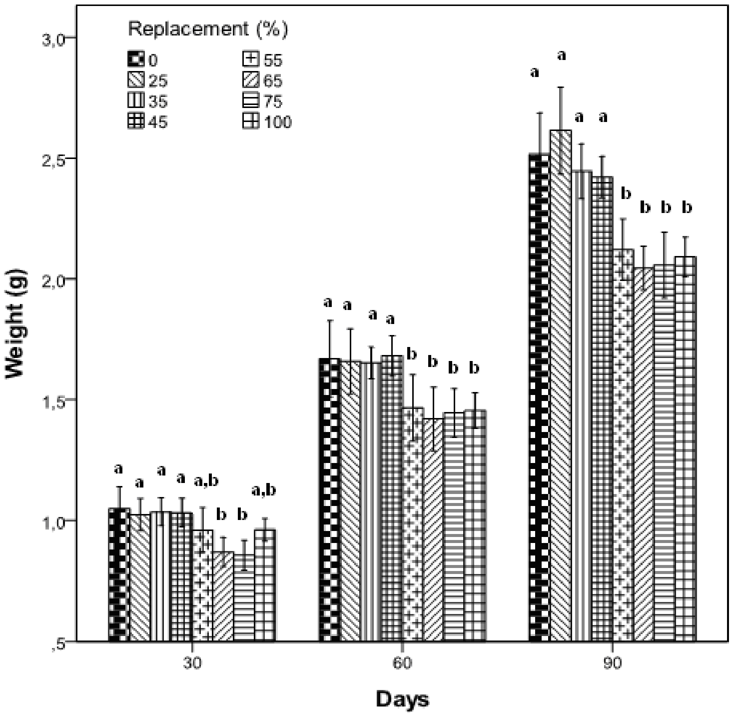

Figure 1. Mean weight of juvenile tench (*T. tinca*) at various periods fed practical diets with different replacement levels of FM protein by SPC protein over 90 days. At day 30 and 60, data derived from 10 fish sampled per replicate (n = 30). At the end of the experiment (day 90), data derived from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

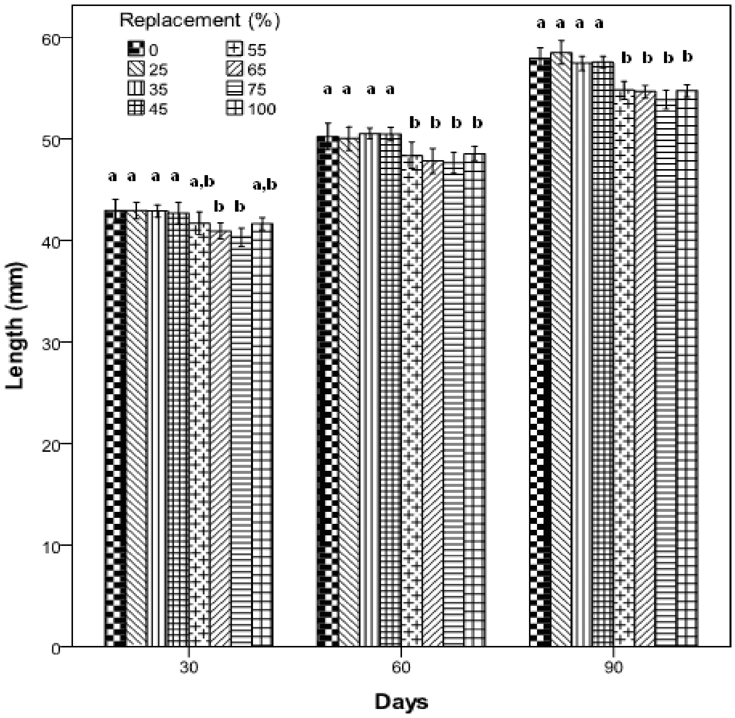

Figure 2. Mean length of juvenile tench (*T. tinca*) at various periods fed practical diets with different replacement levels of FM protein by SPC protein over 90 days. At day 30 and 60, data derived from 10 fish sampled per replicate (n = 30). At the end of the experiment (day 90), data derived from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