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Juvenile tench (*Tinca tinca* L.) response to practical diets with different replacement levels of fish meal by pea protein concentrate supplemented with methionine

José M. Carral [ORCID] | Teresa García | María Sáez-Royuela [ORCID] | Jesús D. Celada

Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Spain

Correspondence
José M. Carral, Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain.
Email: jmcarl@unileon.es

Abstract

The effects of methionine supplementation in diets with different replacement levels of fish meal (FM) by pea protein concentrate (PPC) on survival, growth performance and body composition of juvenile tench (0.39 g of initial weight) were studied in a 90-day experiment. Six practical diets (50% crude protein) differing in replacement level of FM by PPC were tested: 0% (control diet), 35%,45%, 60%, 75% or 85%, corresponding to 0, 285, 366, 487, 608 and 685.4 g PPC kg^{-1} diet respectively. To provide the same amount as in control diet, methionine was included from the 45% substitution level. Survival rates were high, between 93.7% and 100%, without differences among treatments. Juveniles fed 75% and 85% of replacement diets showed lower ($p < 0.05$) weight and specific growth rate, which cannot be attributed to methionine deficiency. In all treatments, percentages of fish with externally visible deformities were low (under 0.1%). The increase in PPC diet content resulted in a significant reduction of lipid content in whole-body juvenile. Minimum methionine requirements for juvenile tench could be estimated in 10 g kg^{-1} diet and supplementation over this amount would be not necessary. Juvenile tench exhibited a high tolerance to PPC dietary content, up to 487 g kg^{-1}, without affecting growth performance, which make possible to consider this vegetal source of protein as a suitable substitute to FM.

KEYWORDS
juvenile tench rearing, methionine supplementation, pea protein concentrate, practical diets

1 | INTRODUCTION

Aquaculture is currently one of the fastest growing food-production sectors in the world and plays a key role in achieving many of United Nations Sustainable Development Goals in 2030 (FAO, 2020). Although agriculture and livestock farming are less diversified in terms of the number of species and culture systems than aquaculture, it is officially recognized that the development of diversification strategies is essential to improve the culture of aquatic species

(FAO, 2017, 2019). Among freshwater fish, tench (*Tinca tinca* L.) is considered one of the most promising species for the development of inland culture (Kujawa et al., 2011). This cyprinid is highly appreciated in many European countries, mainly for its gastronomic quality but also as an attractive sport fishing species (Garcia et al., 2015; Pula et al., 2018; Wedekind et al., 2003). However, because extensive culture systems are predominant, growth is often limited, leading to usually low and unpredictable production (Kamiński et al., 2017; Pula et al., 2018). Thus, the development of intensive culture

techniques is necessary not only to satisfy its foreseeable increase in demand, but also to comply with the diversification target.

Adequate feeding in early growth phases is a mandatory factor to establish rearing procedures under controlled conditions. The availability of a specific diet is essential to face nutritional studies. For this reason, García et al. (2015) proposed a fish meal-based practical diet for juvenile tench and later González-Rodríguez et al. (2014) estimated the protein requirements between 48 and 52%. In both studies, good survival and growth performance were achieved.

During the past decades, commercial aquafeeds have relied on high concentrations of fishmeal (FM) as protein source. Although its inclusion shows a downward trend, it is still an important component for many carnivorous fish (Herman & Schmidt, 2016; Hua et al., 2019). In fact, the aquaculture sector consumes more than 70% of the FM (Jannathulla et al., 2019). Considering that the forage fish catches, the main contribution to FM production, remain stagnant since 1980, Cottrell et al. (2020) estimated that demand for the expected aquaculture growth is unsustainable. In the search of alternative protein sources, plants are increasingly incorporated to satisfy the growing demands of the aquafeed industry (Malcorps et al., 2019). Between plant protein sources, pea protein concentrate (PPC) is a promising alternative to FM (Gatlin et al., 2007; Hartviksen et al., 2014). This product is obtained using a dry fractionation process, which employs milling and further air classification to separate smaller protein-rich from larger fibre and starch-rich granules (Pelgrom et al., 2013; Schutyser et al., 2015). Between its main advantages of PPC stand out the high protein content, between 51% and 55% (Pelgrom et al., 2013), which allows for formulations with high fish meal replacement and good digestibility (Øverland et al., 2009). However, as other vegetal sources, their nutritional value can be negatively affected by the presence of antinutritional factors (ANFs), low palatability and amino acid imbalance (Daniel, 2018; Torstensen et al., 2008).

Replacement of FM by PPC has been tested by Tibaldi et al. (2005) in European sea bass (*Dicentrarchus labrax*), by Sánchez-Lozano et al. (2011) in marine gilthead sea bream (*Sparus aurata*), by Gao et al. (2013) in black sea bream (*Acanthopagrus schlegelii*), by Cantril (2015) in Nile tilapia (*Oreochromis niloticus*), by Carter and Hauler (2000) and Øverland et al. (2009) in Atlantic salmon (*Salmo salar*) and by Thiessen et al. (2003), Collins et al. (2012) and Zhang et al. (2012) in rainbow trout (*Oncorhynchus mykiss*). Most of these studies reported the feasibility to include between 200 and 340 g of PPC kg^{-1} diet without significant negative effects on growth performance.

In juvenile tench, González-Rodríguez et al. (2016) obtained similar growth in juveniles fed a control diet (without PPC) and those supplied with diets with FM replacements up to 35% (290.4 g PPC kg^{-1} diet). However, when PPC inclusion substituted 45% of FM (373.3 g PPC kg^{-1} diet), a reduced growth performance was reported. Comparing essential amino acid (EAA) content of the diets, a significant decrease in methionine was evidenced in the 45% FM replacement diet, suggesting a possible deficiency of this amino acid as the main cause of growth reduction. On this basis, the aim of this study was to evaluate if supplementation with methionine would allow higher FM replacement with PPC without affecting juvenile tench survival and growth.

TABLE 1 Proximate composition and amino acid profiles of fish meal (FM) and pea protein concentrate (PPC) (g kg^{-1}, wet basis). Analyses were performed in duplicate

Proximate composition	FM	PPC
Moisture	75 ± 1.4	96 ± 3.9
Protein	668 ± 3.1	535 ± 2.5
Lipids	96 ± 3.0	53.5 ± 2.1
Carbohydrates	0	254.8 ± 4.0
Ash	161 ± 4.6	60.7 ± 2.7
Essential amino acids (EAAs)		
Arginine	38.1 ± 1.1	36.5 ± 2.8
Histidine	18.6 ± 2.4	8.4 ± 1.4
Isoleucine	23.3 ± 1.9	21.2 ± 2.0
Leucine	44.3 ± 3.3	32.9 ± 2.6
Lysine	51.2 ± 4.0	31.4 ± 2.4
Methionine	19.2 ± 2.2	7.5 ± 1.0
Phenylalanine	28.0 ± 2.3	27.0 ± 2.0
Threonine	24.1 ± 2.1	22.7 ± 2.3
Tryptophan	7.1 ± 1.1	5.2 ± 0.8
Valine	28.2 ± 2.4	22.2 ± 2.0
Non-essential amino acids (NEAAs)		
Alanine	43.4 ± 3.1	28.6 ± 2.1
Aspartate	67.8 ± 4.2	61.2 ± 4.4
Cysteine	6.0 ± 0.9	1.6 ± 0.3
Glutamate	85.4 ± 5.1	79.0 ± 4.8
Glycine	48.9 ± 3.9	27.5 ± 2.6
Proline	47.1 ± 2.2	24.3 ± 2.1
Serine	30.5 ± 3.0	29.1 ± 2.6
Tyrosine	23.1 ± 1.9	18.0 ± 1.4

Note: Values are mean ± standard deviation.

2 | MATERIALS AND METHODS

2.1 | Fish, facilities and experimental procedure

Larvae were obtained by hatching under artificial reproduction techniques (Rodríguez et al., 2004) and reared in outdoor tanks. From five days after hatching, when first feeding started, larvae were fed decapsulated fresh *Artemia salina* cysts during the two first months, followed by a two-week period of transition from cysts to the control diet described in Table 2 and afterwards only received the control diet until they were four months old. Then, 540 juvenile tench from a homogenous pool were randomly distributed as groups of 30 fish in 18 fibreglass tanks (0.5 × 0.25 × 0.25 m) containing 25 L of water to

obtain replicates corresponding to the different feeding treatments. Prior to tank distribution, a sample of 120 juveniles were anaesthetized with tricaine methanesulphonate (MS-222; Ortoquímica S.L.) to measure initial total length (TL) and weight (W). The number of animals of the sample was calculated assuming a minimum acceptable level of statistical power of 80% (Buhjel, 2008) in order to guarantee equal mean initial length and weight in all tanks. TL was measured with a digital calliper (to the nearest 0.01 mm) and, after removing excess water with tissue paper, W was determined by a precision balance (to the nearest 0.001 g). Values of 30.7 ± 0.43 mm, 0.39 ± 0.02 g (mean ± standard deviation) were obtained. Following a monofactorial design, fish diet was the experimental factor with three replicates per level of treatment.

At the beginning of the experiment, a sample of 70 g was taken from the same pool to determine juvenile whole-body proximate chemical composition and amino acid profile (see section 2.3)

Artesian well water was supplied in an 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. Measures of the incoming water quality, ammonia, nitrites, hardness and total

suspended solids were performed once a week with a spectrophotometer HACH DR2800 (Hach Lange GMBH). Dissolved oxygen in tanks was measured with a multi-metre HACH HQ30d (Hach Lange GMBH). Water temperature was recorded twice a day. The mean values of water quality were pH 7.5, hardness 5.3 German degrees (calcium 32.8 mg L^{-1}), total suspended solids 35.0 mg L^{-1}, dissolved oxygen ranged between 5.8 and 7.3 mg L^{-1}, ammonia <0.10 mg L^{-1} and nitrites <0.013 mg L^{-1}. Water temperature was 25 ± 1°C.

A photoperiod of 16 hours light: 8 hours dark was maintained. Tanks were cleaned of faeces and uneaten feed every two days. The Ethics Committee of the León University (Spain) approved all procedures used in the study. The experiment lasted for 90 days.

2.2 | Diets and feeding

PPC was obtained using dry fractionation process and FM from anchoveta. Before formulating the experimental diets, proximate composition and amino acid profiles of main protein sources were analysed (Table 1), following the methodology described in section

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

	Replacement (%)					
	0	35	45	60	75	85
Ingredients (g kg^{-1})						
Fish meal[a]	645	419	354	258	161.2	95
Pea protein concentrate[b]	–	285	366	487	608	685.4
Corn meal[c]	166	116	88	62	37.3	25.1
Dried *Artemia* cysts[d]	100	100	100	100	100	100
Carboxymethyl cellulose[e]	30	30	30	30	30	30
Cod liver oil[f]	20	20	20	20	20	20
L-ascorbyl-2-monophosphate-Na[g]	5	5	5	5	5	5
Dicalcium phosphate[g]	10	10	10	10	10	10
Choline chloride[g]	3	3	3	3	3	3
Soy lecithin[h]	10	10	10	10	10	10
Sodium chloride[i]	1	1	1	1	1	1
Methionine[g]			3	4	4.5	5.5
Mineral and Vitamin premix[j]	10	10	10	10	10	10

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

[b]Yantai Oriental Protein Tech, Jincheng Road, Zhaoyuan City, Shandong Province, China.

[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]Cargill., 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; cyanocobalamin, 5; retinol, 2400; α-tocopherol, 30000; cholecalciferol, 6.25; naphthoquinone, 5000; butylated hydroxytoluene, 1500; MgSO$_4$-7H$_2$O, 300000; ZnSO$_4$-7H$_2$O, 11000; MnSO$_4$-H$_2$O, 4000; CuSO4-5H2O, 1180; CoSO$_4$, 26; FeSO$_4$-7H$_2$O, 77400; KI, 340; Na$_2$SeO$_3$, 68.

2.3. Considering the data obtained, six diets (nearly isonitrogenous and isoenergetic) with different replacement levels of FM by PPC were formulated: 0% (control), 35%, 45%, 60%, 75% or 85%, corresponding to 0, 285, 366, 487, 608 and 685.4 g PPC kg^{-1} diet respectively. Based on González-Rodríguez et al. (2016) results, diets were supplemented with methionine from 45% substitution level in order to provide the same amount of this EAA as in the control diet. As no differences were found between control diet and 35% FM replacement, this diet was not supplemented with methionine.

The ingredients were ground in a rotary BRABENDER mill (Brabender GmbH & Co. KG), mixed in a STEPHAN UMC5 mixer (Stephan Food Service Equipment) and extruded using a stand-alone extruder BRABENDER KE19/25D (Brabender GmbH & Co. KG) at a temperature range between 100°C and 110°C. Pellets (1 mm diameter) were dried during 24 hours at 30°C and after received a coating of cod liver oil. Formulation of practical diets is presented in Table 2. Fish were fed manually three times a day (at 10:00, 14:00 and 18:00 hours) to apparent satiation.

2.3 | Chemical analysis of diets and fish

Samples (70 g) of FM, PPC and juveniles at the beginning and at the end of the experiment were stored at −30°C. Juveniles were fasted for 14 hours before sampling. Analyses were performed in duplicate by Analiza Calidad Laboratory following Commission Regulation (EC) 152/2009. Moisture was determined by drying at 105°C, crude protein according to the Kjeldahl method, crude lipid by extraction with light petroleum and further distillation, ash by calcination at 550°C and gross energy according to EU regulation 1169/2011. The content of carbohydrates was calculated by subtracting the content of moisture, protein, lipid and ash from the wet weight.

Amino acid profiles were analysed by HPLC using the AccQTag method from Waters. Amino acids were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate reagent (AQC) using the method of Cohen and Michaud (1993) and Cohen and De Antonis (1994) and 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.

2.4 | Data collection and statistical analysis

Juvenile tench behaviour was observed and registered after cleaning, feeding and measuring the water quality parameters.

In order to have information about the growth performance and evolution, a sample of 15 fish per tank (45 per treatment, 50% of total) was anaesthetized to be individually weighed and measured every thirty days. TL and W were measured as described in section 2.1, and then juveniles were gently returned to their respective tanks.

At the end of the experiment, surviving fish were anaesthetized and observed one by one using a magnifying glass to detect externally

visible deformities affecting spinal axis, operculum, mouth and tail fin. Individual weight and total length of all fish were calculated.

After 90 days of experiment, the following indexes were calculated:

- Survival rate (%): final number of juveniles × initial number of juveniles^{-1}
- Specific growth rate, SGR (% day^{-1}) = 100 × [(ln final W − ln initial W) × days^{-1}]
- Daily increment in total length, ITL (mm day^{-1}) = (final TL − initial TL) × days^{-1}
- Fulton's coefficient or condition factor (K) = 100 × [(W × (TL^{-3})$^{-1}$)]
- Feed conversion ratio (FCR) = total amount of feed fed × W gain^{-1} (Fornshell & Hinshaw, 2008)

Using the SPSS version 26.0 software package (IBM, SPSS Statistics), analysis of survival, growth performance and whole-body composition data were done using one-way analysis of variance (ANOVA) and significant differences among means ($p < 0.05$) were determined using the Tukey's multiple range test. Orthogonal polynomial contrasts were performed to determine whether trends were linear, quadratic or cubic and regression coefficients calculated.

Percentages were arcsine-transformed prior to statistical analysis. All treatments were replicated three times, and the experimental unit was a tank.

3 | RESULTS

Proximate composition and amino acid profile of diets are presented in Table 3. A significant linear reduction of lipid and ash content was observed when inclusion of PPC increased, while carbohydrates showed a significant increase. When FM replacement increases, the content of arginine, histidine, leucine, lysine, valine, alanine, aspartate, glutamate and proline decreases ($p < 0.05$).

From observations of juvenile tench behaviour, diets were equally ingested throughout the experiment, independently of PPC amount included. Abnormal behaviour was not observed.

Total length and weight after 30 and 60 days of experiment are presented in Table 4. There were non-significant differences between mean values of TL and W neither after 30 (35.69–38.39 mm; 0.67–0.85 g) nor after 60 days of experiment (46.11–47.33 mm; 1.43–1.69 g). Mean TL increases of 20% and 67% were registered after 30 and 60 days respectively. The mean W was doubled after 30 days while at 60 days was almost four times higher.

The survival rates, growth performance values and indexes and percentage of animals with externally visible deformities over 90 days are in Table 5. There were no differences between survival rates, which were close to 100% (range 100–93.7%).

A reduction of growth was evidenced with the increase in PPC in diets, but there were not significant differences in juvenile TL

TABLE 3 Proximate composition and amino acid profiles of the practical diets with different levels of replacement of fish meal by pea protein concentrate supplemented with methionine (g kg^{-1} diet, wet basis)

	Replacement (%)							
	0	35	45	60	75	85	SEM	ANOVA
Moisture	61.01	61.4	62.5	63.1	61.2	59.8	0.57	0.737
Protein	501.0	500.5	500.2	500.6	501	500.3	1.07	1.000
Lipids	107.5[a]	102.7[ab]	95.5[abc]	95.9[abc]	92.0[bc]	87.1[c]	2.31	0.007
Carbohydrates	200.1[a]	225.3[b]	237.7[bc]	243.0[b]	257.3[cd]	271.2[d]	6.96	0.000
Ash	130.4[a]	110.1[b]	104.1[bc]	97.4[bcd]	88.5[cd]	81.6[d]	4.88	0.001
Energy (MJ)	15.8	16.0	15.9	16.1	16.1	15.2	0.12	0.244
EAAs								
Arg	25.1[a]	23.8[ab]	22.2[abc]	21.0[bcd]	20.3[cd]	19.7[d]	0.67	0.002
His	12.3[a]	10.5[b]	10.0[b]	9.2[bc]	8.6[c]	8.0[c]	0.43	0.000
Ile	27.1	26.8	26.6	26.1	25.8	25.7	0.19	0.172
Leu	36.1[a]	32.8[b]	32.6[b]	32.3[b]	32.1[b]	31.9[b]	0.44	0.000
Lys	41.3[a]	32.0[b]	31.7[bc]	31.0[bcd]	30.2[cd]	30.1[d]	1.18	0.000
Met	12.7[a]	10.3[b]	12.6[a]	12.5[a]	12.5[a]	12.6[a]	0.27	0.050
Phe	31.8	31.1	30.2	31.2	30.6	30.8	0.18	0.115
Thr	19.9	19.7	19.6	19.4	19.1	19.0	0.21	0.384
Trp	4.3	4.2	4.2	4.2	4.1	4.1	0.07	0.986
Val	23.3[a]	23.0[a]	22.6[a]	21.4[b]	18.7[c]	18.1[c]	0.63	0.000
NEAAs								
Ala	28.3[a]	25.4[b]	25.2[b]	24.2[b]	22.6[c]	20.4[d]	0.74	0.000
Asp	59.3[a]	57.3[ab]	57.8[ab]	57.7[ab]	57.5[ab]	56.5[b]	0.27	0.021
Cys	4.3	4.2	4.2	4.1	4.0	4.1	0.80	0.468
Glu	67.8[a]	67.5[a]	67.3[a]	65.4[b]	65.3[b]	64.4[b]	0.41	0.002
Gly	32.4	31.9	31.6	31.4	30.7	30.8	0.21	0.110
Pro	30.5[a]	27.0[b]	26.1[b]	24.5[d]	24.5[d]	22.9[e]	0.74	0.000
Ser	20.6	20.7	20.6	20.3	20.2	20.3	0.12	0.860
Tyr	15.0	14.7	14.6	14.0	14.1	13.8	0.16	0.136

Note: Values are presented as mean and pooled standard error of the mean (SEM). Means in the same row with different superscripts are significantly different ($p < 0.05$).

TABLE 4 Total length and weight in juvenile tench fed practical diets with different levels of substitution of fish meal by pea protein concentrate supplemented with methionine after 30 and 60 days of experiment

									Polynomial contrasts		
Replacement (%)	0	35	45	60	75	85	SEM	ANOVA	Linear	Quadratic	Cubic
30 days											
TL (mm)	36.46	35.69	37.3	38.39	35.94	37.39	0.326	0.133	0.271	0.496	0.825
W (g)	0.81	0.73	0.79	0.85	0.67	0.82	0.022	0.156	0.879	0.685	0.732
60 days											
TL (mm)	46.89	47.29	46.13	47.33	46.40	46.11	0.302	0.788	0.439	0.734	0.830
W (g)	1.69	1.60	1.53	1.56	1.45	1.43	0.040	0.462	0.056	0.858	0.782

Note: Values are presented as mean and pooled standard error of the mean (SEM).

between control, 35%, 45%, 60% and 75% fish meal replacement diets (285, 366, 487 and 608 g PPC kg^{-1} diet respectively). However, W was significantly lower from 75% of replacement.

Compared to control diet, SGR and FCR were significantly lower when animals were fed with replacement levels of 75% and 85%, whereas ITL presented significant differences only with the highest

TABLE 5 Survival, growth performance and percentages of deformed juvenile tench fed practical diets with different levels of substitution of fish meal by pea protein concentrate supplemented with methionine over 90 days

									Polynomial contrasts		
Replacement (%)	0	35	45	60	75	85	SEM	ANOVA	Linear	Quadratic	Cubic
Survival (%)	100	95.8	97.9	97.9	93.7	96.5	0.845	0.411	0.13190	0.712	0.799
TL (mm)	61.23[a]	59.17[ab]	59.08[ab]	57.66[ab]	57.03[ab]	55.96[b]	0.529	0.028	0.001	0.743	0.870
W(g)	3.47[a]	3.17[ab]	3.14[ab]	2.85[ab]	2.70[b]	2.61[b]	0.876	0.010	0.000	0.604	0.751
SGR[1] (% day^{-1})	2.24[a]	2.12[ab]	2.13[ab]	2.03[abc]	1.98[bc]	1.90[c]	0.031	0.003	0.000	0.331	0.774
ITL[2] (mm day^{-1})	0.34[a]	0.32[ab]	0.31[ab]	0.30[ab]	0.29[ab]	0.28[b]	0.006	0.028	0.001	0.743	0.870
K[3]	1.39	1.39	1.39	1.37	1.34	1.30	0.010	0.085	0.170	0.105	0.587
FCR[4]	1.15[a]	1.30[ab]	1.31[ab]	1.46[abc]	1.51[bc]	1.67[c]	0.047	0.003	0.000	0.271	0.717
Deformed fish (%)	0.04	0.10	0.01	0.03	0.01	0.04	0.010	0.123	0.358	0.672	0.186

Note: Initial TL and W: 30.7 ± 0.43 mm, 0.39 ± 0.02 g (mean ± standard deviation).

Values are presented as mean and pooled standard error of the mean (SEM). Means in the same row with different superscripts are significantly different ($p < 0.05$).

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

[2]Daily increment in total length (mm day^{-1}) = (final TL − initial TL) × days^{-1}

[3]Fulton's coefficient or condition factor = 100 × [final W × (final TL^3)$^{-1}$]

[4]Feed conversion ratio = total amount of feed fed × W gain^{-1}

replacement level. There were no differences between treatments for condition factor (range 1.30–1.39).

Polynomial contrasts indicate a significant negative linear trend in TL ($r^2 = 0.60$), W ($r^2 = 0.66$), SGR ($r^2 = 0.71$), ITL ($r^2 = 0.60$) and FCR ($r^2 = 0.69$).

The diets did not affect the percentages of fish with externally visible deformities which were under 0.1%. Body deformities affected the spinal column and the caudal peduncle (break in the axis of the tail).

The proximal composition and the amino acid profile in the whole-body juvenile tench at the beginning and end of the experiment are summarized in Table 6. A significant linear decrease in lipid content in juveniles was evidenced as the amount of PPC included in diets increased. Although diets provided significant different content of nine amino acids in diets (Table 3), there were no differences in AA content on whole-body tench.

4 | DISCUSSION

Studies on the possibilities of pea protein concentrate inclusion aquafeeds show important interspecific differences. In juvenile sharpsnout sea bream (*Diplodus puntazzo*), Nogales-Merida et al. (2016) reported that all dietary PPC amounts tested, from 160 to 487 g kg^{-1} diet, were inappropriate to substitute FM. However, higher amounts (325 and 340 g kg^{-1}) have been included without negative effects on growth performance in the Nile tilapia (Cantril, 2015) and in gilthead sea bream (Sánchez-Lozano et al., 2011). In rainbow trout, Zhang et al. (2012) recommended not to include amounts greater than 300 g kg^{-1}. In other species such as European sea bass (Tibaldi et al., 2005) and Atlantic salmon (Carter & Hauler, 2000; Øverland et al., 2009), lower PPC amounts, between 200 and

258 g kg^{-1}, were tolerated. In all cases, the initial weight was bigger than juvenile tench used in this experiment. Despite this, the inclusion of 487 g kg^{-1} of PPC (60% of FM replacement) did not affect their growth performance, showing a great tolerance of this species to this vegetal protein source.

Interspecific differences in tolerance could be attributed to the main constraints for the vegetal-based protein sources to replace FM in aquafeeds which, following Daniel (2018), are unbalanced essential amino acid composition, low palatability, presence of antinutritional factors (ANFs) and low digestibility. Concerning to EAA, replacement of fish meal by plant proteins in aquaculture is often limited by the low level of methionine (Séité et al., 2018) which plays critical roles in fish growth performance and feed utilization (Gao et al., 2019). Pea pulses have low content of this essential amino acid, even after being processed to obtain concentrate products (Cabuk et al., 2018). In a study to test the possibilities to replace FM by PPC in juvenile tench, González-Rodríguez et al. (2016) obtained similar growth performance with a control diet (without PPC) and 25% and 35% FM replacement. However, when FM substitution level tested increased from 35% (290.4 g PPC) to 45%, (373.3 g of PPC), a significant decline in growth was registered concurring with a decrease in methionine content, from 9.8 to 8.9 g kg^{-1} diet, suggesting a possible deficiency of this EAA. On this basis, in this experiment 35% FM replacement diet was not supplemented with methionine, but 45% or higher replacement diets were supplemented to reach similar methionine content to the control diet (12.7 g kg^{-1}). Growth performance was not significantly different among the control diet and FM replacement levels up to 60%, so it can be deduced that the lower content of methionine provided by the amino acid non-supplemented diet, 10.2 g kg^{-1}, covered the juvenile tench requirements. Combining the results by González-Rodríguez et al. (2016) and the present study, it can be

TABLE 6 Proximate composition and amino acid profiles of the whole body of juvenile tench fed practical diets with different levels of replacement of fish meal by pea protein concentrate (g kg^{-1}, wet basis)

Initial[1]		Replacement (%)								Polynomial contrasts		
		0	35	45	60	75	85	SEM	ANOVA	Linear	Quadratic	Cubic
Moisture	767.4 ± 9.7	733.6	733.8	734.1	748.2	754.8	752.4	3.50	0.209	0.034	0.333	0.338
Protein	133.54 ± 8.7	160.0	164.0	162.0	159.0	160	159.0	2.03	0.990	0.805	0.684	0.717
Lipid	73 ± 4.7	80.5[a]	63.4[b]	56.1[b]	48.9[b]	49.7[b]	47.5[b]	3.61	0.002	0.000	0.081	0.431
Ash	24.5 ± 2.1	24.6	25.7	24.3	23.2	23.8	23.1	0.68	0.893	0.981	0.720	0.284
Essential amino acids												
Arg	9.5 ± 0.4	11.6	11.3	11.3	11.4	11.4	11.1	0.15	0.984	0.594	0.940	0.284
His	2.5 ± 0.2	4.6	4.4	4.2	3.9	4.4	4.3	0.09	0.470	0.326	0.228	0.530
Ile	20.1 ± 2.2	25.2	26.2	23.3	22.6	21.4	21.1	0.72	0.233	0.040	0.505	0.382
Leu	4.9 ± 0.7	5.8	5.9	4.9	5.2	4.9	5.2	0,15	0.178	0.062	0.744	0.207
Lys	10.2 ± 0.8	11.8	11.9	11.6	12.1	11.5	11.7	0.22	0.990	0.867	0.867	0.948
Met	3.9 ± 0.2	5.4	5.1	4.4	4.9	4.9	4.2	0.14	0.052	0.014	0.936	0.173
Phe	11.2 ± 0.9	12.0	11.9	11.7	12.2	11.6	11.8	0.20	0.985	0.738	0.986	0.968
Thr	5.5 ± 0.5	5.8	6.2	6.2	6.2	5.9	6.4	0.15	0.993	0.559	0.769	0.577
Trp	0.4 ± 0.1	0.6	0.6	0.6	0.5	0.6	0.6	0.04	0.981	0.904	0.812	0.621
Val	6.1 ± 0.9	6.2	6.4	6.2	6.2	6.2	6.7	0.21	0.989	0.758	0.802	0.621
Non-essential amino acids												
Ala	7.7 ± 0.7	8.8	9.2	9.2	8.9	9.1	9.1	0.20	0.996	0.812	0.783	0.747
Asp	13.0 ± 1.3	13.9	14.2	13.9	13.9	14.1	14.2	0.34	1.000	0.891	0.996	0.846
Cys	0.2 ± 0.0	0.7	0.6	0.6	0.6	0.6	0.5	0.04	0.870	0.295	0.990	0.589
Glu	17.7 ± 1.9	20.1	21.1	19.4	20.5	19.8	20.1	0.36	0.900	0.846	0.823	0.712
Gly	2.5 ± 0.6	8.7	8.3	8.2	7.8	8.5	8.4	0.16	0.796	0.625	0.338	0.722
Pro	4.9 ± 0.7	5.3	5.2	6.0	6.1	6.2	6.4	0.21	0.553	0.117	0.770	0.607
Ser	6.3 ± 0.4	6.4	6.3	6.2	6.0	6.4	6.7	0.11	0.732	0.690	0.640	0.399
Tyr	2.0 ± 0.3	4.6	4.3	3.8	4.2	4.2	4.0	0.17	0.896	0.465	0.640	0.811

Note: Values are presented as mean and pooled standard error of the mean (SEM). Means in the same row with different superscripts are significantly different (*p* < 0.05).
[1]Initial data presented as mean ± standard deviation were not included in the statistical analysis.

suggested that diets for juvenile tench should provide around 10 g of methionine kg^{-1} diet.

As adverse effects on growth performance when PPC replaced 75% and 85% of FM cannot be attributed to methionine deficiency, other factors should be considered. Palatability of pea protein concentrate is negatively affected by the content of saponins, which are not removed by air classification of pea pulses (Zhang et al., 2012). In the European sea bass, Tibaldi et al. (2005) stated that impaired diet palatability could represent a major limiting factor to achieve an almost complete replacement of FM by PPC. From the observations made when food was supplied all diets were quickly ingested by juvenile tench, leading us to consider that diets were attractive even with high PPC inclusion.

Although the production process of PPC involves a great reduction of ANFs, Schultz et al. (2007) stated that dehulled and air classified process highly increased levels of protease inhibitors, phytic acid and α-galactosides that not only have negative effects in protein utilization, mineral bioavailability and digestive physiology (Francis

et al., 2001), but also can potentially elicit enteritis. In adult Atlantic salmon, Penn et al. (2011) reported an inflammation and reduced relative weight of the distal intestine, similar to those described for soybean meal-induced enteritis, when a diet containing 350 g kg^{-1} of PPC was provided. Although we did not perform observations of intestinal section of juveniles, behavioural symptoms compatible with enteritis were not noticed. Nevertheless, the foreseeable increase in ANFs derived from increasing amounts of PPC could be the cause of the significantly worsening in growth performance. The potential decrease in nutrient absorption derived from the presence of ANFs could also explain the increase in FCRs as dietary PPC does.

Using similar diets, González-Rodríguez et al. (2016) reported lower SGR values, between 1.73 and 1.88% day^{-1}, than in the present study, 1.90–2.24% day^{-1}. As specific growth rate tends to slow down with age (Lugert et al., 2016), differences in age/size must be considered to establish comparisons between the values obtained. Considering this, the higher SGRs in this study could be partially affected by the differences between initial weight of tench juveniles

in this study (0.39) and those of González-Rodríguez et al. (2016), 0.46 g. Daily increment in total length (ITL) is considered by Kamiński et al. (2017) a more reliable indicator of somatic growth than SGR, because it does not depend on fat deposition in fish. In this study, ITL shows a similar trend that SGR showing that both measures give valuable information in nutritional studies.

The inclusion of PPC resulted in significant decrease in lipid and an increase in carbohydrate content in the diets. The lipid content in all diets was in the range recommended, between 84.7 and 118 g kg^{-1} diet, by Sáez-Royuela et al. (2015) for juvenile tench, leading us to consider that its reduction does not affect growth. There are not data about maximum tolerance of carbohydrates in tench feeding but, considering that in natural habitats juvenile fed mainly zooplankton and other small invertebrates (Pyka, 1997), the high carbohydrate content in 75% and 85% of replacement diets could be considered to explain the lower growth recorded.

Jobling (2001), Sargent et al. (2002) and Sáez-Royuela et al. (2015) reported that lipid content of diets is positively correlated with whole-body composition of fish. In agreement with this statement, the decrease in lipid content in diets with PPC (Table 3) led to a significant decrease in juvenile tench fat in comparison with those fed the control diet (Table 6).

In juvenile tench, Kamler et al. (2006), Wolnicki et al. (2006), and Myszkowski et al. (2010) suggested a relationship between high condition factor (1.3–1.4) and incidence of body deformities. In this experiment, even though K values ranged between 1.30 and 1.39, the percentage of animals with externally visible deformities was low (<0.10%). Considering this, high K values do not imply raised incidence of deformities, at least when balanced diets are supplied since, according to Fontagné (2009), adequate feeding especially during early development is important to avoid malformations.

To sum up, minimum methionine requirements for juvenile tench could be estimated in 10 g kg^{-1} diet and supplementation over this amount would be not necessary. Juvenile tench exhibited a high tolerance to PPC dietary content, up to 487 g kg^{-1} without affecting growth performance, which make possible to consider this vegetal source of protein as a suitable substitute to FM.

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CONFLICT OF INTEREST

Not applicable.

ETHICS APPROVAL

All procedures used in the study were approved by the University of León Ethics Committee (Spain).

DATA AVAILABILITY STATEMENT

Data are available by request to corresponding author.

ORCID

José M. Carral https://orcid.org/0000-0003-0928-1702
María Sáez-Royuela https://orcid.org/0000-0002-7503-9810

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