Effects of dietary tannin on growth, feed utilization and digestibility, and carcass composition in juvenile European seabass (Dicentrarchus labrax L.)

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ABSTRACT

Plant-based products in fish diets are valuable protein alternatives to fishmeal for the aquafeed industry. Many plant feed ingredients contain polyphenolic compounds, including tannins, which can have beneficial or adverse effects. The tolerable threshold of ingested tannins is unknown for marine carnivorous fishes. We studied the effects of tannic acid (TA) supplementation to the diet of juvenile European seabass (*Dicentrarchus labrax*) by measuring growth, feed utilization and digestibility, and carcass composition. We randomly allocated groups of fish (initial mean body weight of 10.2 ± 0.7 g; $n = 40$ fish per tank) to 12 replicate cylindrical-conical tanks (three per treatment). The fish were assigned to one of four dietary treatments for five weeks: control diet (C) with tannin-free protein sources (mostly fishmeal as the base diet, containing 55.7% dry matter (DM) crude protein, gross energy $22.3 \, \text{kJ} \, \text{g}^{-1}$ DM) and three experimental diets supplemented with 10, 20, or $30 \, \text{g} \, \text{TA} \, \text{kg}^{-1}$ (called TA1, TA2, and TA3, respectively). Tannin ingestion resulted in significantly decreased cumulative feed intake, growth, feed and protein efficiencies, apparent digestibility coefficients, hepatosomatic index, and carcass lipids. The protein digestibility in fish fed the $10 \, \text{g} \, \text{kg}^{-1}$ tannin-containing diet was significantly lower than that in fish fed the control diet. This threshold should be taken into account when using novel terrestrial and aquatic plant ingredients for temperate marine fishes.

1. Introduction

The use of plant protein sources in fish feeds has expanded considerably in recent years to meet the demand for feeds and sustain the development of worldwide aquaculture production (Tacon and Metian, 2015). Many trials have tested the inclusion of novel unconventional or underutilized plant species after adequate processing. The sustainable development of aquaculture systems in some regions depends on their regional integration and the local availability of agricultural by-products (Dongmeza et al., 2009; Chebbaki et al., 2010; Altan and Korkut, 2011). Thus, feed formulations containing a broad range of promising ingredients are being developed for farmed tropical herbivorous/omnivorous and temperate carnivorous fishes. These ingredients include plant oils, legume seeds, fruits and their respective by-products (Emre et al., 2008; Azaza et al., 2009b), plant leaves (Dongmeza et al., 2009), aquatic plants (Mandal and Ghosh, 2010a,b), seaweeds (Güroy et al., 2013; Peixoto et al., 2016) and algal biomass (Tulli et al., 2012; Tibaldi et al., 2015).

Plant-based products used to partially or completely substitute fishmeal, have been associated with small amounts of various endogenous secondary compounds and imbalanced nutrient profiles, resulting in poor nutritional value, whereas amino acid supplements lead to better utilization of plant-based feed. These secondary compounds include heat-labile (protease inhibitor, lectin) and heat-stable (phytic acid, phenolic compounds, tannin, and fiber) antinutritional factors (Drew et al., 2007). The properties of such antinutrients are likely to impair digestive function and the metabolic utilization of nutrients, and therefore fish production. Only a few compounds have been studied in teleost species to better understand the antinutritional effects on fish physiology (Francis et al., 2001; Krogdahl et al., 2010; Couto et al., 2015).

Several plant feed ingredients contain appreciable amounts of polyphenolic substances, broadly referred to as tannins. Polyphe-

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nolic tannins show both positive and negative effects; they possess antinutritive properties, but are also beneficial for health due to their role as antioxidants and their ability to stimulate the immune system and various effectors (Chung et al., 1998; Makkar et al., 2007; Quideau et al., 2011). Tannic compound has also been reported to have antimicrobial activity against fish pathogens (Schrader, 2008).

Polyphenolic tannins represent most of the secondary metabolites found in terrestrial plants (Makkar et al., 2007) and several aquatic macrophytes (Arnold and Targett, 2002). Three classes of tannins have been established (Hagerman, 2002; Quideau et al., 2011): 1) hydrolysable tannins (HTs), gallo- and ellagi-tannins derived from gallic and ellagic acids, esterified to a core polyol and galloyl group, that can be esterified or oxidized to more complex HT; 2) condensed tannins (CTs), proanthocyanidin polymeric flavanoids; and 3) phlorotannins (PTs), monomers of phloroglucinol found in brown algae. Tannin levels in feed depend on the plant sources and varieties (Crépon et al., 2010), plant maturity, and the season, as well as the technological processes used for feed manufacturing (Drew et al., 2007). The proportion of HT in feed frequently exceeds that of CT (Gaber, 2006; Dongmeza et al., 2009), and can vary widely. The tannin content of ingredients used for animal feed has been determined to be between 5 and 16 g kg^{-1} dry matter (DM). It is highest in oilseed meals (Heuzé et al., 2015a,b; Sauvant et al., 2015), followed by various legumes tested for temperate fish production, which contain between 4.9 and 11.4 g tannin kg^{-1} DM (Adamidou et al., 2009a; Collins et al., 2013). Estimated tannin content in natural foods and plant ingredients for tropical carp species ranges from 5.4 (coconut oil cake) to 34.3 (phytoplankton) g kg^{-1} dry weight (DW, Mandal and Ghosh, 2010a). The total phenolic content of algal biomass was shown to be between 6.0 and 20.0 g kg^{-1} DM (Tibbetts et al., 2015). The number of varieties and the proportion of plant protein sources in fish diets are steadily increasing. It is thus important to study the effects of tannins in organisms that are currently or likely to ingest tannin-containing food, or polyphenolic tannin supplements.

Ingredients containing tannin give feed an unpleasant taste and reduce consumption due to decreased palatability (Becker and Makkar, 1999). In addition, tannins affect animals through several mechanisms, including the formation of strong complexes with feed components, such as proteins and minerals (Chung et al., 1998), the loss of endogenous proteins (Mansoori and Acamovic, 2007), and inactivation of digestive enzymes, thus interfering with digestion (Chung et al., 1998; Mandal and Ghosh, 2010b).

There is limited literature on the effect of polyphenolic tannins on intensive fish production. Many previous studies on the effect of dietary tannin on tropical fish species (Becker and Makkar, 1999; Prusty et al., 2007; Aiura and de Carvalho 2007) and animals (Mansoori and Acamovic, 2007) used tannic acid (TA) as a model compound for HT. The effects of tannin have been little studied in carnivorous fish, except for recent studies related to the influence of antinutrients from various diets on the physiology of rainbow trout (*Oncorhynchus mykiss*, Collins et al., 2013) and polyphenol-enriched feed on that of seabass (Magrone et al., 2016).

European seabass (*Dicentrarchus labrax*) is one of the most important marine finfish species reared in Europe, especially in the Mediterranean basin, with a production similar to that of gilthead seabream (*Sparus aurata*), comprising 145–150,000 tons in 2014 supplied by Turkey, Greece, and Spain among European countries (FEAP, 2015). European seabass is thus one of the most highly studied temperate marine fish species (Sanchez-Vazquez and Muñoz-Cueto, 2014). Its nutritional requirements and feeding management have been recently established, highlighted by the near replacement of fishmeal by a variety of plant-based ingredients (Kousoulaki et al., 2015).

However, the tolerable threshold of ingested tannin has not yet been determined. The objective of this five-week short-term *in vivo* study was to evaluate the effects of various amounts of TA, added to the base diet, on growth, feed intake and digestibility, and body composition in juvenile European seabass.

2. Materials and methods

This study complied with ethical guidelines (ASAB/ABS, 2006) and regulations concerning animal experimentation, and was carried out with permission from the French national board for animal experimentation.

2.1. Fish and rearing conditions

European seabass were supplied by a fish farm (Aquastream, Ploemeur, France). The trial was conducted in the experimental indoor facilities at Ifremer Centre Bretagne (Plouzané, France). The fish were initially fed Neo Start ½ commercial pellets for pre-growing marine fish (Le Gouessant, Lamballe, France) containing crude protein: 52% DM, crude fat: 17% DM, and a negligible amount of tannin estimated to be below 0.2 g kg^{-1} DM. After a four-week acclimation period, the trial was set up in a complete randomized design with 480 fish. The fish were divided into batches of ten and randomly distributed into replicate tanks (three tanks per treatment, four treatments) at a density of 40 fish per tank. The 12 cylindrical-conical fiberglass tanks (75 L capacity) were exposed to an 8 h light: 16 h dark cycle. They were supplied with a continuous flow of filtered seawater (5.4 L min^{-1}). An individual stone diffuser per tank ensured dissolved oxygen levels above 90% saturation. The salinity averaged 35.3 g L^{-1} and the temperature was held constant at $20.8 \pm 0.2\,^{\circ}$C. The fish were fasted for one day, anaesthetized with 2-phenoxyethanol (at a concentration of 0.125 mL L^{-1} seawater, within a few minutes of exposure), and bulk weighed at the beginning, after two weeks of acclimation in the tanks, and at the end of the trial. For sampling, the fish were humanely killed by a blow to the head.

2.2. Experimental diets

Following acclimation, the fish had an average body weight of 10.2 ± 0.7 g and were fed the experimental diets (Table 1). Four iso-nitrogenous (crude protein: 55.7% DM) and iso-energetic (gross energy: 22.3 kJ g^{-1} DM) diets were prepared. They included fishmeal as the principal protein source, fish protein hydrolysate, small amounts of wheat gluten meal, fish oil, and wheat starch as the base or control diet (C). The base diet was supplemented with 10, 20, or 30 g kg^{-1} tannic acid (Chinese gallnut grass, Sigma-Aldrich T0200, Saint-Quentin Fallavier, France), to constitute the experimental diets, called TA1, TA2, and TA3, respectively, and cellulose was used to replace the tannin in the control diet. Vitamins and minerals were added according to the nutrient requirements of European seabass. For the digestibility study, 10 g kg^{-1} of starch was replaced by the same amount of the inert marker chromic oxide (Cr_2O_3) in each diet. All ingredients were ground for homogeneity (<800 μm), mixed with water, and pelletized. The pellets were dried in a forced-air dryer, at 80 $^{\circ}$C for 10 min, to reduce the moisture content to under 10% DW. The pellets were packed and stored at $-20\,^{\circ}$C until use as feeds or samples for chemical analysis. The fish were hand-fed a daily ration of 3% body weight, which was then visually adjusted to apparent satiety, three times (09:00, 13:00, and 16:00) on working days, and twice on weekends, for five weeks. The feed ingested was recorded.

Table 1
Composition of experimental diets: control (C) diet and diets containing various levels of tannic acid.

	Experimental diets			
Ingredients (g kg^{-1} dry weight)	C	TA1	TA2	TA3
Fish meal[a]	600	600	600	600
CPSP 90[b]	50	50	50	50
Wheat gluten meal[c]	50	50	50	50
Marine fish oil[a]	83	83	83	83
Pregelatinized starch[d,j]	147	147	147	147
Binder[e]	10	10	10	10
Vitamin premix[f]	20	20	20	20
Mineral premix[g]	10	10	10	10
Cellulose[h]	30	20	10	0
Tannic acid[i]	0	10	20	30
Proximate composition (% dry weight; % DM)				
Dry matter (DM)	96.43 ± 0.03	95.09 ± 0.06	94.40 ± 0.01	95.03 ± 0.06
Crude protein	55.21 ± 0.78	55.17 ± 0.38	56.01 ± 0.75	56.46 ± 1.00
Crude fat	14.01 ± 0.27	13.69 ± 0.23	13.28 ± 0.24	13.02 ± 0.03
Starch	15.52 ± 0.10	14.71 ± 0.08	13.36 ± 0.12	13.94 ± 0.10
Ash	11.69 ± 0.15	11.52 ± 0.06	11.75 ± 0.08	11.61 ± 0.12
Gross energy (kJ g^{-1} DM)	22.27 ± 0.15	22.25 ± 0.06	22.44 ± 0.04	22.34 ± 0.02

[a] Norvik LT 70, blue whiting meal (CP 70% DM; CF 12% DM) and cod liver oil, La Lorientaise, Lorient, France.
[b] Soluble fish protein hydrolysate (CP 82% DM; CF 10% DM; ash 6% DM) Sopropêche, Boulogne, France.
[c] Vitalor, Chamtor, Bazancourt, France.
[d] Pregeflo, Roquette, Lestrem, France.
[e] Na Alginate Agrimer, Plouguerneau, France.
[f] Vitamin premix composition (g kg^{-1}): retinyl acetate, 1; thiamin-HCl, 0.1; riboflavin, 0.5; niacin, 1; d-calcium panthothenate, 2; pyridoxine-HCl, 0.3; mesoinositol, 30; d-biotin, 1; folic acid, 0.1; cyanocobalamin, 1; ascorbic acid (ascorbyl polyphosphate), 14.2; cholecalciferol, 0.5; dl-a-tocopheryl acetate, 10; menadione, 2; choline, 167; UPAE, Inra, Jouy-en-Josas, France. (All ingredients were diluted with α-cellulose).
[g] Mineral premix composition (g kg^{-1}): calcium phosphate (bicalcic), 500; calcium carbonate, 215; sodium chloride (salt) 40; potassium chloride, 90; magnesium oxide, 124; ferrous sulfate, 20; zinc sulfate, 4; manganese sulfate, 3; copper sulfate, 3; cobalt sulfate, 0.02; potassium iodide, 0.04; sodium selenite, 0.03; sodium fluoride, 1.00; UPAE, Inra, Jouy-en-Josas, France.
[h] Arbocel, J. Rettenmaier & Söhne (JRS), Saint-Germain en Laye, France.
[i] Natural extract T0200, Sigma-Aldrich, Saint Quentin Fallavier, France.
[j] In the diets used for the digestibility study, 10 g kg^{-1} of starch was replaced by Cr_2O_3 as an inert marker (Merck Millipore, Guyancourt, France).

Table 2
Growth performance and feed utilization in juvenile European seabass fed experimental diets for 5 weeks.

	C	TA1	TA2	TA3		R^2	P
Initial body weight (g)	10.2 ± 0.7	10.5 ± 0.7	10.4 ± 0.8	10.1 ± 0.9			
Final body weight (g)	19.8^a ± 0.7	18.9^a ± 0.9	16.7^b ± 0.6	14.8^c ± 0.5	$y = 20.15 - 0.22\,X$	0.97	<0.014
SGR (% d^{-1})	1.8^a ± 0.2	1.6^{ab} ± 0.2	1.3^{bc} ± 0.1	1.0^c ± 0.0	$y = 1.83 - 0.04\,X$	0.99	<0.005
FI (g kg^{-1} d^{-1})	15.5^a ± 0.4	15.0^{ab} ± 1.3	14.7^{ab} ± 1.3	13.7^b ± 1.6	$y = 15.59 - 0.07\,X$	0.94	<0.030
FE (g g^{-1})	1.1^a ± 0.1	1.0^{ab} ± 0.2	0.9^{ab} ± 0.0	0.7^b ± 0.1	$y = 1.12 - 0.02\,X$	0.96	<0.018
PER (%)	2.0^a ± 0.2	1.8^{ab} ± 0.4	1.6^{ab} ± 0.1	1.3^b ± 0.2	$y = 2.02 - 0.03\,X$	0.99	<0.006

Data are expressed as the mean ± S.D. ($n = 3$). Values in the same row with different superscript letters are significantly different ($P < 0.05$, and $P < 0.10$, FI). The relationship between the tannin concentration in the diets (X, explanatory variable) and the response (Y, dependent variable) is expressed by linear polynomial regression equations, coefficient of determination (R^2) and probability (P). Specific growth rate (SGR); feed intake (FI); feed efficiency (FE); and protein efficiency ratio (PER).

2.3. Sample collection, chemical analysis and digestibility study

For initial carcass composition, 20 fish from the initial stock were randomly sampled. At the end of the study, six fish per tank were randomly collected and individually measured. Fish organs were removed, weighed for somatic index calculations, and carcasses stored for later composition analysis. Before analysis, feed and fish samples were homogeneously ground up, freeze-dried, and the components determined (duplicated sub-samples from diets, $n = 4$, or from fish mass, $n = 3 \times 4$) by standard laboratory methods: DM at 105 °C for 24 h by oven drying, ash content at 550 °C for 12 h by combustion in a muffle furnace (Thermolyne 30400 Furnace, Thermofisher Bioblock, Illkirch, France), crude protein content (N × 6.25) by the Dumas method, using automatic flash combustion, followed by gas chromatographic separation and detection by thermal conductivity (Nitrogen Analyser 2000, Fison Instruments, Arcueil, France), lipid content by extraction with dichloromethane using the Soxhlet method (Avanti 2050 extraction system, Bezons, France), and gross energy content using an adiabatic calorimeter (IKA-Calorimeter System-C4000 Adiabatic, Fontenay-sous-Bois, France).

The digestibility trial, to determine nutrient utilization, was started after the first week of adaptation to the diet and conducted for two weeks under the same conditions as for the fish groups ($n = 40$) by substituting the experimental diets by the diets including the digestibility marker. Sodium alginate as a dietary binder ensured cohesion of the fecal matter. Feces were harvested daily using an automatic system with continuous water filtration (Guillaume and Choubert, 2001). The sieves were placed in the system collector after the last meal of the day, the feces collected for 15 h d^{-1} prior to the next morning meal and pooled per tank in test tubes to obtain a sufficient mass (30 g) for analysis, and stored at −20 °C. Samples were lyophilized at 40 °C and homogenized for analysis. Chromium content in the diet and feces ($n = 3 \times 4$) was determined in duplicate using an atomic absorption spectrophotometer (Perkin Elmer, Villebon-sur-Yvette, France) with a mixture of perchloric acid for digestion and sulfuric acid for concentration. The dichromate concentration was measured at a wavelength of 440 nm using a standard Cr_2O_3 solution.

2.4. Calculations

The apparent digestibility coefficients (ADCs) were calculated, based on the ratio of the marker in the diets and feces, according to the formula (Guillaume and Choubert, 2001):

$$ADC_{DM} = 100 \times [1 - (Cr_{diet}/Cr_{feces})]$$

$ADC_{nutrient\ or\ energy} = 100 \times [1 - (Cr_{diet}/Cr_{feces}) \times (nutrient$ or $energy_{feces}/nutrient\ or\ energy_{diet})]$; $Cr_{diet/feces}$: chromium content in diet or feces, respectively; $nutrient_{diet/feces}$: specific nutritional variable, DM, protein or energy content in diet or feces.

Growth and feed utilization parameters were calculated based on the following criteria: live weight gain (%) = $100 \times [(Wf - Wi)/Wi]$, where Wi, Wf are initial and final average fish body weight (g); specific growth rate (SGR, % body weight day^{-1}) = $100 \times [(\ln Wf - \ln Wi)/t]$, with t, duration in days of the feeding period; feed intake (FI, g kg^{-1} day^{-1}) = feed consumed/biomass/t, biomass: average fish body weight (Wi + Wf)/2; feed efficiency (FE, g g^{-1}) = weight gain/feed consumed; protein efficiency ratio (PER, g g^{-1}) = weight gain/crude protein consumed on a DM basis; percent protein or energy retained (PR or ER, %) = $100 \times$ (protein or energy gain/crude protein or energy consumed on a DM basis). Fulton's condition factor (K) was calculated as: K = Wf/Lfexp3 × 100, Wf, final weight of fish (g), Lf, total length (cm). Somatic indices were calculated as: organ-somatic index (% g g^{-1}) = $100 \times$ (weight of organ/total weight of fish); hepato- (HSI), spleno- (SSI), nephro- (NSI), viscera- (VSI) and intestinal- (ISI) somatic index referring to the relative proportion of liver, spleen, kidney, viscera, and emptied intestine, respectively, (viscera, calculated as the difference between the carcass weight and total weight of fish).

2.5. Statistical analysis

The experimental data are expressed as the mean ± standard deviation (SD). Data transformation was applied if necessary for the statistical analysis. The results of the tannin effects were analyzed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls *post-hoc* multiple-range test, whenever there were statistically significant differences between the means (at $P < 0.05$, or $P < 0.10$). Simple linear regression was applied to determine the relationship between the fish parameters (dependent variable) and included dietary TA levels (independent variable). Statistical analysis was performed using Statistica, version 6 software (StatSoft France, Maisons-Alfort, France).

3. Results

No diet-related mortality occurred during the experiment in any group. However, two fish escaped and this loss was taken into account for the calculations. All diets were readily eaten, but fish fed the TA2 and TA3 diets manifested early signs of reduced feed consumption that persisted until the end of the experiment. The cumulative feed intake was affected by the exposure to dietary TA: fish showed a significant loss of appetite from the 17th day in the TA3 group and the 21st day in the TA2 group, respectively (Fig. 1), whereas there was no difference between the TA1 and control groups. Feed intake was significantly different at $P < 0.10$ between groups.

By the end of the experiment, the growth of the TA2 and TA3 groups was significantly less than that of the control group; their body mass was 15.6 and 25.6% lower than that of the fish fed the C diet, respectively, which doubled their weight during the study (Table 2). The corresponding significant decreases of SGR were 27.8 and 44.4%, respectively. The TA3 diet significantly affected FE and
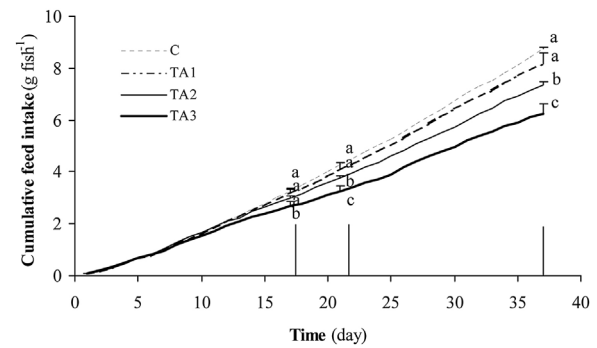
Fig. 1. Cumulative feed intake (g fish^{-1}) in juvenile European seabass fed experimental diets containing various levels of tannic acid. Differences among groups were recorded (vertical lines) from the 17th day (between treatment TA3 and the others) and the 21st day (between treatment TA2, TA3, and the others) until the end of the trial (final point). Data are expressed as the mean values ± SEM ($n = 3$ groups per treatment); the different letters above the vertical lines indicate significant differences between the means ($P < 0.05$).

Table 3
Apparent digestibility coefficient (ADC) of dry matter (DM), crude protein (CP), and energy (E) of experimental diets.

ADC (%)	C	TA1	TA2	TA3
ADC DM	80.2[a] ± 0.4	78.2[ab] ± 0.6	77.7[ab] ± 0.3	75.2[b] ± 3.5
ADC CP	95.8[a] ± 0.2	94.7[b] ± 0.3	93.4[c] ± 0.2	91.6[d] ± 0.2
ADC E	92.8[a] ± 0.3	92.2[a] ± 0.2	91.7[ab] ± 0.7	90.2[b] ± 1.2

Data are expressed as the mean ± S.D. ($n = 3$). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

PER, which were 36.4 and 20% lower than those of the control group, respectively.

The diets were highly digestible, even when supplemented with TA, but the digestibility of DM was significantly different between TA3 and the other treatments, as were the protein ADCs, which were between 91.6 and 95.8%, depending on the quantity of TA (Table 3). The protein ADC (Y) and TA concentration in the diets (X) were related by a simple linear regression by the model, $Y = 95.38 - 0.153 X$ (determination coefficient $R^2 = 0.89$ at $P < 0.036$). The subsequent energy ADC was significantly affected ($P < 0.05$) only by the TA3 diet relative to the C and other two diets.

The K index of fish reared on the TA3 diet (1.17) was non-significantly lower than that of fish from the C (1.29) and TA1 (1.31) groups, showing that they tended to not be in as good condition as the fish from the other groups. Somatic indices and the proximate composition of the fish fed the C and TA-containing diets are presented in Table 4. The hepatosomatic index (HSI) was 38.5% lower in fish of the TA3 group than in control fish, and the difference was significant. There were no significant differences between the other somatic indices of the different groups.

Only TA supplementation at the highest concentration significantly affected carcass composition in terms of lipid and energy content, which decreased by 13.6 and 9.3%, respectively, whereas protein content was not altered by TA (Table 4). However, PR values were significantly lower in fish from the TA2 and TA3 groups than those in the C group (24 and 39.5%, respectively). Energy retention was also significantly lower in fish fed the TA2 and TA3 diets than those fed the C diet (32.4 and 58.7%, respectively).

4. Discussion

The successful use of plant meals as feeds in European seabass diets has been demonstrated by good growth performance, but the effects of TA inclusion have not been studied yet. The tolerable threshold of this polyphenolic compound merited investigation,

Table 4

Somatic indices (%) and proximate carcass composition (% wet weight) of juvenile European seabass fed experimental diets for 5 weeks, and nutrient/energy retention efficiency (% of intake).

		C	TA1	TA2	TA3
Somatic indices					
K		1.29 ± 0.25	1.31 ± 0.18	1.22 ± 0.16	1.17 ± 0.10
HSI		$2.83^a \pm 0.43$	$2.67^{ab} \pm 1.02$	$2.45^{ab} \pm 0.56$	$1.74^b \pm 0.33$
SSI		0.04 ± 0.01	0.06 ± 0.02	0.06 ± 0.03	0.05 ± 0.05
NSI		0.28 ± 0.13	0.25 ± 0.12	0.34 ± 0.13	0.27 ± 0.19
VSI		19.10 ± 7.12	19.13 ± 5.37	18.40 ± 6.95	18.20 ± 2.86
ISI		2.00 ± 3.60	2.51 ± 0.27	2.30 ± 0.90	2.50 ± 1.03
Proximate carcass composition	Initial				
Moisture	67.8	67.5 ± 0.1	67.7 ± 0.6	68.3 ± 0.7	68.9 ± 0.6
Protein	17.3	18.0 ± 0.6	17.8 ± 1.0	17.5 ± 0.3	17.4 ± 0.6
Fat	11.0	$11.0^a \pm 0.2$	$10.8^a \pm 0.6$	$10.1^{ab} \pm 0.4$	$9.5^b \pm 0.6$
Ash	3.6	4.0 ± 0.1	3.8 ± 0.3	4.2 ± 0.2	4.2 ± 0.2
Energy (kJ g^{-1} w. w.)	8.5	$8.6^a \pm 0.2$	$8.6^a \pm 0.3$	$8.3^a \pm 0.3$	$7.8^b \pm 0.2$
Retention efficiency					
PR		$36.1^a \pm 4.0$	$34.3^a \pm 3.7$	$27.4^b \pm 1.0$	$21.8^b \pm 3.4$
ER		$13.8^a \pm 1.5$	$12.9^a \pm 2.6$	$9.3^b \pm 0.9$	$5.7^c \pm 0.8$

especially given the increasing supply of miscellaneous plant-based protein sources by the feed industry. The upper TA concentration tested in the present study was higher than the low to moderate levels normally found in most formulations. This was to increase the accuracy of the results obtained *in vivo* and to enable smaller antinutritional effects to be more clearly detected (Griffith, 1989). All fish grew during the experimental period and no diet-related mortality occurred. The results demonstrate that TA addition to the diet lowered nutritional quality, leading to a significant reduction in growth.

Tannins are widely present in most plant species used as protein substitutes for fishmeal, including soybean, canola-rapeseed, wheat, sunflower, field pea, faba bean, chickpea, sorghum, hazelnut seed, microalgal biomass, and seaweed meals (Oliva-Teles et al., 1998; Valente et al., 2006; Emre et al., 2008; Adamidou et al., 2009a,b; Chebbaki et al., 2010; Altan and Korkut, 2011; Messina et al., 2013; Collins et al., 2013). Several studies have been published, but many without analytical measurements, and only few report the tannin content of the diets.

A high level of incorporation of soybean protein (580 g kg^{-1} DM, Oliva-Teles et al., 1998), and of differently processed soybean meals (480 and 530 g kg^{-1}, Tibaldi et al., 2006) was shown to affect the growth of seabass. Inclusion of chickpeas and faba beans in the diets (340 and 350 g kg^{-1}, with a tannin content in raw ingredients of between 5.9 and 11.4 g kg^{-1} DM) lowered the feed conversion ratio and growth rate (Adamidou et al., 2009a). However, the growth of seabass was not adversely affected by low inclusion of legumes in the diet (160 g kg^{-1}, Adamidou et al., 2009a). Diets for trout containing soybean, canola, and field pea meals or their protein concentrates (300 g kg^{-1}, with tannin levels between 4.9 and 10.6 g kg^{-1} DM), did not affect growth rates (Collins et al., 2013). Currently underutilized fat-extracted hazelnut oil meal contains up to 7.5 g tannin kg^{-1} tannins among the total phenolics in the raw material (Xu and Hanna, 2011). Seabass fed a diet containing this meal (from 75 to 300 g kg^{-1}, which includes from 0.6 to 2.3 g estimated tannin kg^{-1}), exhibited growth comparable to that of fish receiving the control fishmeal diet (Emre et al., 2008).

The incorporation of green algae (50 and 100 g kg^{-1}, Valente et al., 2006) or seaweed (25 and 75 g kg^{-1}, Peixoto et al., 2016) in the seabass diets, and the inclusion of seaweed (up to 100 g kg^{-1}, Güroy et al., 2013), in the trout diets also did not adversely affect the growth. However, incorporation of *Ulva* sp. meal (100–300 g kg^{-1} that brought tannin levels to 1.6–2.2 g kg^{-1} DM) to replace soybean meal in the diet of Nile tilapia (*Oreochromis niloticus*), impaired growth (Azaza et al., 2008), whereas a diet containing 2.9 g tannin kg^{-1} coming from the upper volume of 300 g waste date meal

did not (Azaza et al., 2009b). Inclusion at 15 g kg^{-1} and 25 g kg^{-1} of either hydrolysable or condensed tannins into diets impaired tilapia growth, with a greater negative effect for HT than CT (Buyukcapar et al., 2011).

Furthermore, diets including soybean meal, sunflower, and coconut oil cakes, which contain 6.3 g tannin kg^{-1} (Xavier et al., 2012), or supplementation with tannic acid up to 20 g kg^{-1} diet (Prusty et al., 2007), did not negatively affect the growth of rohu (*Labeo rohita*, Hamilton) fingerlings.

Impaired fish growth appears to be due to reduced palatability, resulting in lower food intake. In the present study, the high dietary tannin levels that reduced voluntary consumption by seabass are in agreement to the 20 g tannin kg^{-1} that lowered feed intake of the common carp (*Cyprinus carpio* L.) for a similar feeding period relative to fish fed CT for a longer period of time (Becker and Makkar, 1999). In contrast, the addition of tannin to trout diets had a small positive effect on feed intake (Collins et al., 2013). Palatability is an important criterion for feeding in ecosystems and in animal nutrition, affecting whether the food is ingested or rejected. For example, extracts of polyphenolic compounds (>20 g kg^{-1} DW) from temperate brown algae are effective feeding deterrents for some tropical herbivorous fishes (Van Alstyne and Paul, 1990). In animals, supplementation of feeds with 30 g kg^{-1} of tannin-rich extract reduced pig feed intake (Candek-Potokar et al., 2015). Substantial quantities of polyphenolic tannins present in ingredients, combined with other antinutrients, contribute to the astringency and bitter taste of the meals that contain them (Mwachireya et al., 1999). Aside from the unpleasant taste, tannins lowered the appetite of rainbow trout fed canola meal (Mwachireya et al., 1999), tilapia fed *V. faba* meal (Gaber, 2006), and seabass fed a high tannin-containing diet (present study).

The availability of nutrients to fish is essentially associated with their digestibility. In the present study, the protein source in the base diet was mostly blue whiting (crude protein: 70% of DM), which is highly digestible and showed the highest ADC for protein (95.8%), in the same range of ADC values found in previous studies for seabass diets (Adamidou et al., 2009b). In contrast, there was a correlation between the gradient of dietary tannin and ADC for protein, that showed an inverse dose-response relationship and ADC for DM and energy were also affected. Altan and Korkut (2011) reported a high ADC for protein (90.1%) in seabass juveniles fed 100 g kg^{-1} plant protein sources in diets using various combinations of low cost feeds. However, CTs in the sorghum control diet, not noticed by the authors, probably resulted in lower nutrient digestibility than for the other meals. Indeed, low *in vitro* digestibility of *V. faba* containing CT, has been reported for trout (Grabner

and Hofer, 1985). In contrast, incorporation of 150 and 300 g kg^{-1} faba bean as a protein source in extruded diets for on-growing seabass significantly improved the ADC for protein, fat, and energy relative to the control diet (Adamidou et al., 2009b), whereas similar or lower ADC values were obtained when the diets included chickpeas and field peas. Inclusion of 360 g kg^{-1} faba bean meal (tannin 21.4 g kg^{-1}; CT 20.4 g kg^{-1}) impaired the growth of Nile tilapia (Azaza et al., 2009a). Tannins from a *Fabaceae* plant extract at levels equal to or greater than 6.3 g kg^{-1} also significantly affected DM and protein digestibility in tilapia (Pinto et al., 2000). Thus, differences in nutrient digestibility appear to be species specific, and depend on the tolerance of the fish to tannin-containing plant protein sources and tannin supplements. Tannin-protein complexes are formed with exogenous dietary proteins coming from feed (Becker and Makkar, 1999), in particular those containing proline, but other amino acids and phospholipids can also interact with tannin. In addition, reduced digestibility has been partially ascribed to endogenous nitrogen or protein and amino acid losses previously observed in small animals (rat or chickens) fed diets containing tannin-rich food (Skopec et al., 2004; Mansoori and Acamovic, 2007), as well as in pigs fed acorn-rich hydrolysable tannin (Cappai et al., 2013). In this study, the amount of TA that bound protein molecules may have reduced the digestibility by lowering gut protein bioavailability. Furthermore, inhibition of digestive enzymes by tannin may have also lowered digestibility and impaired growth (Mandal and Ghosh, 2010b).

Dietary TA did not affected protein levels in the carcass whereas lipid deposition, energy content, and HSI were proportionally lower with increasing TA inclusion levels. Here, the high HSI values were probably related to the proportion of fishmeal used in the base diet. Low palatability of the diets (Lanari and D'Agaro, 2005), reduced dietary starch (Tibaldi et al., 2006; Adamidou et al., 2009b), or incorporation of dried microalgae into the diet (Tulli et al., 2012) have diminished feed intake and induced subsequent depletion of lipid stores and liver glycogen, and have reduced HSI and ADC values, probably due to the modulating effects of dietary elements on seabass lipid metabolism. These observations may partially explain our results on the effects of TA on HSI, whereas the depletion of lipids after tannin exposure may be due to inhibition of lipid synthesis and/or lipid mobilization, resulting from the formation of complexes between tannin and enzymes involved in these processes (Aiura and de Carvalho, 2007). Tannin supplementation also reduced protein and energy retention in this study. Different patterns of carcass fat storage have been observed in other fish species. Aiura and de Carvalho (2007) showed that Nile tilapia fed diets containing from 0.8 to 6 g TA kg^{-1} had more body lipid deposition than when fed low tannin-containing sorghum and than those fed high tannin-containing sorghum. However, graded levels of TA in the diet of rohu did not affect the lipid stores, but significantly increased the HSI (Prusty et al., 2007).

We used commercially available TA in this study to investigate the effects of tannin. However, there are many natural molecules classified as tannins, of which CTs would probably be less toxic to fish than TA (Becker and Makkar, 1999; Buyukcapar et al., 2011). Currently, diets formulated with a mixture of various plant protein sources must compensate for nutrient deficiencies and avoid undesirable compound effects to produce the best performance. Indeed, tannins are also known to interact with other antinutrients, removing the inhibitory action of tannins or the deleterious effects of others (Francis et al., 2001). Genetic improvements have been attempted to select tannin-free or low-tannin plant varieties (Crépon et al., 2010). Various treatments (Xavier et al., 2012; Bhat et al., 2013) and biotechnological applications such as fermentation using fish gut tannase enzyme, have also been tested with the aim

to eliminate or reduce the amount of tannin in feeds to ensure their nutritional value (Mandal, 2012; Ghosh and Mandal, 2015).

5. Conclusion

Tannin supplementation above 10 g kg^{-1} in diets decreased protein digestibility, and above 20 g kg^{-1} growth performance in European seabass juveniles. These results further support the use of a variety of low tannin-containing plant protein sources as nutritionally digestible ingredients for carnivorous fishes, and highlight the need to take precautions when including tannin-rich feeds in diets. Furthermore, among the potentially beneficial phytochemicals for fish farming, polyphenol extracts show promise due to their biological properties. However, the effect of their hydrolyzed derivatives on the health of fish remains to be determined.

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