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PAPER

Tenebrio molitor meal in rainbow trout (*Oncorhynchus mykiss*) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets

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Abstract

This study evaluated the effects of diets containing Tenebrio molitor (TM) larvae meal on growth performances, somatic indexes, nutrient digestibility, dorsal muscle proximate and fatty acid (FA) compositions of rainbow trout. Three hundred sixty fish were randomly divided into three groups with four replicates each. The groups were fed diets differing in TM inclusion: 0% (TM0), 25% (TM25) and 50% (TM50) as fed weight basis. Weight gain was not affected by treatment. Feeding rate was significantly higher in TM0 than TM50. Feed conversion ratio was significantly higher in TM0 than TM25 and TM50, while an opposite trend was observed for protein efficiency ratio and specific growth rate. The survival rate was significantly lower in TM0 than TM25 and TM50. The apparent digestibility of protein was significantly lower in the TM50 group than the other groups, while the apparent digestibility of dry matter, organic matter and lipids was unaffected by treatment. If compared to control, the protein and lipid contents of fillets were respectively increased and decreased following TM inclusion in the diet. The Σn3/Σn6 FA ratio of fish dorsal muscle was linearly (TM0>TM25>TM50) reduced by TM inclusion in the diet. Results suggested that TM could be used during the growing phase in trout farming; however, additional studies on specific feeding strategies and diet formulations are needed to limit its negative effects on the lipid fraction of fillets.

Introduction

In aquaculture, the impressive growth of the use of fishmeal (FM) and fish oil (FO) in feeds for fish farming has jeopardized the sustainability of this production. About 61.3 and 9.9% of wild fish stocks worldwide are fully and under-fished, respectively (FAO, 2014). FM and FO productions seem therefore to have reached a maximum extent, cannot being able to fulfil the increasing world demand anymore (Shepherd and Jackson, 2013). The costs of FM and FO are influenced to a great extent by the available supplies of wild fish stocks, and, as a consequence, fish derivative products are currently the most expensive components of fish feed for aquaculture.

In the last twenty years, the price of FM has increased more than 400% and the trend is still increasing. Consequently, nowadays the main target of research studies in this field is to decrease the dietary level of fish derivative products, with potential associated benefits related to both the curtailment of feed costs and the face of global environmental issues.

The substitution of FM and FO by plant products is a common practice in fish aquaculture feeds (De Francesco *et al*., 2004; Palmegiano *et al*., 2005). Unfortunately, the use of plant products can lead to adverse effects, essentially attributable to their content of anti-nutritional factors and/or unpalatable compounds, inappropriate fatty acid (FA) profile, and shortage of essential amino acids (Gatlin *et al.,* 2007; Gai *et al*., 2012).

The sustainability of insect production is also well documented; when the right farming temperature range is used, the feed conversion rate and the greenhouse gas production of insects are lower than those of other animals, since the former do not use energy to maintain their body temperature in a strict range. Consequently, the interest of researchers in the use of insect meal as aquaculture feed has been growing rapidly.

Recent reviews highlighted that meals derived from some insect larvae may provide adequate nutritional value for fish (Henry *et al.,* 2015) as well as terrestrial animals (Makkar *et al*., 2014). Regarding fish, some trials have been carried out with various fish species to evaluate the use of diets containing different insect meals, often used as FM substitutes (Fasakin *et al.,* 2003; St-Hilaire *et al.,* 2007b; Sealey *et al.,* 2011; Alegbeleye *et al.,* 2012; Kroeckel *et al.,* 2012; Oso and Iwalaye, 2014; Roncarati *et al*., 2015). The results of these trials confirm the possibility of insect meals inclusion in aquaculture feeds, even though there could be some limits related to both insect and fish species (Henry *et al*., 2015).

Tenebrio molitor (TM) belongs to the family of Tenebrionidae. It is currently mainly sold in the larvae stage for pet feeding and for fishing, but also as edible insects for humans (van Huis, 2013). TM is considered one of the most promising insect species suitable for mass production because it is easy to be bred and fed. Some studies have been performed using TM to feed poultry (Ramos-Elorduy *et al.*, 2002; Bovera *et al*., 2015) and fish. In African catfish (*Clarias gariepinus*) Ng *et al.* (2001) demonstrated that an inclusion up to 35% of TM meal (up to 80% replacement of the dietary fish meal component in the experimental diets) still maintained growth performances similar to those achieved using fishmeal diets. Nevertheless when fish have different nutritional requirements (*i.e*., for juveniles), the results may change, as noted by Roncarati *et al.* (2015) who observed a reduction of weight growth and an increase of mortality in com-

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Key words: Insect meal; Mealworm; Rainbow trout; Growth performance; Dorsal muscle composition.

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mon catfish (*Ameiurus melas*) fingerlings fed TM larvae meal containing diets. In European sea bass (*Dicentrarchus labrax*) juveniles, data showed that an inclusion of 25% TM larvae meal did not affect growth performances while a higher inclusion level (50%) compromised the weight gain (Henry *et al.,* 2015). Similar results were obtained in gilthead sea bream (*Sparus aurata*) juveniles (Henry *et al.,* 2015) as, when the inclusion switched from 25 to 50%, weight gain, specific growth rate, feed conversion efficiency and protein efficiency ratio were compromised. To the author knowledge, no trials have been performed using TM in rainbow trout (*Oncorhyncus mykiss*) diets. The aim of this research was therefore to evaluate the effects of dietary inclusion of TM larvae meal on performance, nutrient digestibility and dorsal muscle proximate and FA composition of rainbow trout.

Materials and methods

Experimental diets

A commercial full-fat TM larvae meal purchased from Gaobeidian Shannong Biology CO. LTD (Shannong, China) was been used in this trial. Two diets, with different levels of TM inclusion (25% - TM25; 50% - TM50, as fed basis), were compared to a control diet (TM0). The three experimental diets were formulated to be nearly isoenergetic and isonitrogenous. Hence, with the increase of TM inclusion and the decrease of FM in the diets, some other dietary ingredients were also modified; in particular as cod liver oil has a high fat content, it was dramatically decreased in TM diets (80, 39 and 0 g kg^{-1} in TM0, TM25 and TM50, respectively). The grounded ingredients and the FO were thoroughly mixed; water was then added to the mixture to attain an appropriate consistency for pelleting, which was performed using a 3.5 mm die meat grinder. After pelleting, the diets were dried at 50°C for 48 h and stored in a fresh, dry and dark room until utilisation. The ingredients of the experimental diets are reported in Table 1.

Fish and rearing conditions

A 90-day trial was carried out using and three hundred sixty mixed-sex rainbow trout. Fish were individually weighed (115.6±14.0 g) and randomly divided into twelve fiberglass tanks (W 0.5m; L 0.5m; H 0.4m). Artesian well water (constant temperature of 13±1°C) was supplied in open system (flow-throughout) with each tank having a water inflow of 8 L min^{-1}. Dissolved oxygen (DO) was measured every fortnight and ranged between 7.6 and 8.7 mg L^{-1}. Fish were fed to visual satiety twice a day per 6 days per week. To check growing rate, the biomass tanks were weighed in bulk every fortnight. Mortality was recorded daily.

Growth performance and somatic indexes

At the end of the trial, all fish were individually weighed and individual weight gain (WG) was calculated:

WG (g)=[FBW (final body weight, g) – IBW (initial body weight, g)].

The following performance indexes were calculated for each treatment:

FR (feeding rate, %/day)=[total feed supplied (g DM) * 100%/number of feeding days)]/$e^{(lnIBW+lnFBW)*0.5}$];

FCR (feed conversion ratio)=[total feed supplied (g DM)/ WG (g)];

PER (protein efficiency ratio)=[WG (g)/ total protein fed (g DM)];

SGR (specific growth rate, %/day)=[(lnFBW-lnIBW)/number of feeding days] * 100%.

Two fish per tank (eight fish per treatment) were killed by over anaesthesia (MS-222) and

Table 1. ***Tenebrio molitor*** **proximate composition, and diets ingredients and proximate composition.**

	TM	TM0	TM25	TM50
Ingredients, g kg^{-1}				
Herring fish meal°	-	750	490	250
TM	-	0	250	500
Cod liver oil	-	80	39	0
Corn gluten meal	-	0	0	5
Barley (grinded flakes)	-	0	46	35
Wheat meal	-	63	58	58
Wheat bran	-	57	57	57
Gelatinized starch (D500)	-	30	40	75
Mineral mixture#	-	10	10	10
Vitamin mixture§	-	10	10	10
Proximate composition^				
DM, g kg^{-1}	939	915	911	907
CP, g kg^{-1} DM	519	452	446	448
EE, g kg^{-1} DM	236	150	149	147
Ash, g kg^{-1} DM	47	119	94	76
Gross energy, MJ kg^{-1} DM	24.40	20.98	21.38	21.84

TM, *Tenebrio molitor;* TM0, 0% TM inclusion as fed weight basis; TM25, 25% TM inclusion as fed weight basis; TM50, 50% TM inclusion as fed weight basis; DM, dry matter; CP, crude protein; EE, ether extract. °Herring fish meal purchased by FF Skagen A/S (Skagen, Denmark). #Mineral mixture (g or mg kg^{-1} diet): dicalcium phosphate, 500 g; calcium carbonate, 215 g; sodium salt 40, g; potassium chloride, 90 g; magnesium chloride, 124 g; magnesium carbonate, 124 g; iron sulphate, 20 g; zinc sulphate, 4 g; copper sulphate, 3 g; potassium iodide, 4 mg; cobalt sulphate, 20 mg; manganese sulphate, 3 g; sodium fluoride, 1 g (purchased from Granda Zootecnica, Cuneo, Italy). §Vitamin mixture (U or mg kg^{-1} diet): DL-α tocopherol acetate, 60 U; sodium menadione bisulphate, 5 mg; retinyl acetate, 15,000 U; DL-cholecalciferol, 3000 U; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg (purchased from Granda Zootecnica, Cuneo, Italy). ^Values are reported as mean of duplicate analyses.

dissected. Liver and gut were weighed to determine hepatosomatic (HSI) and viscerosomatic (VSI) indexes as follows:

HSI (%)=[liver weight (g)/fish weight (g)] * 100

VSI (%)=[gut weight (g)/fish weight (g)] * 100.

Digestibility trial

An *in vivo* digestibility trial was performed in order to determine the apparent digestibility of diets nutrients. Forty-eight trout (mean weight 110±12 g) were divided in twelve 40-L cylindroconical tanks; DO (7.6÷8.7 mg L^{-1}) and temperature (13±1°C) were checked weekly. After 14 days of acclimatization with the experimental diets, fish were fed by hand to visual satiety twice a day. The apparent digestibility coefficients were measured using the indirect acid-insoluble ash (AIA) method; 1% celite® (Fluka, St. Gallen, Switzerland) was added to the diets as an inert marker. The faeces were collected daily from each tank for three consecutive weeks, using a continuous automatic device, as described by Palmegiano *et al*. (2006). The faeces were frozen (-20°C) until analyzed. The apparent digestibility coefficients of dry matter (ADC_{DM}), organic matter (ADC_{OM}), crude protein (ADC_{CP}), and ether extract (ADC_{EE}) were calculated following Palmegiano *et al*. (2006).

Chemical analyses of feeds

Feeds were ground using a cutting mill (MLI 204; Bühler AG, Uzwil, Switzerland) and analyzed for dry matter (DM; AOAC# 934.01), crude protein (CP; AOAC# 984.13) and ash (AOAC# 942.05) contents according to AOAC (2000); ether extract (EE; AOAC# 2003.05) was analyzed according to AOAC (2003). The gross energy content was determined using an adiabatic calorimetric bomb (IKA C7000; IKA, Staufen, Germany). The proximate composition and energy level of the TM and of the experimental diets are shown in Table 1.

The FA composition of TM and of the experimental diets was assessed using the method described by Schmid *et al.* (2009). Fatty acid methyl esters (FAME) were separated, identified and quantified on the basis of the chromatographic conditions reported by Renna *et al.* (2014). Tridecanoic acid (C13:0) was used as internal standard. The results were expressed in absolute values as g 100 g^{-1} DM (Table 2).

All analyses were performed in duplicate.

Chemical analyses of fish dorsal muscle

At the end of the trial, the dorsal muscle of eight fish per treatment was collected, freeze-dried (Edwards MF 1000, Milan, Italy) and ground (cutting mill MLI 204; Bühler AG). Dry matter, crude protein, ether extract and ash contents of the fillets were determined according to the same procedures used for feed analyses (AOAC, 2000, 2003).

The freeze-dried and ground samples of fish muscles were also used to assess their FA composition, as reported by Schmid *et al.* (2009). FAME were separated and quantified using the same analytical instruments and temperature programme previously described for the analysis of feed. The results were expressed as percentage of total detected FA. All analyses were performed in duplicate.

Table 2. Fatty acid profile (g 100 g^{-1} dry matter) of *Tenebrio molitor* larvae meal and experimental diets.

	TM	TM0	TM25	TM50
C14:0	0.51	0.58	0.45	0.34
C14:1 *c*9	0.03	0.06	0.05	0.04
C16:0	3.43	2.03	2.26	2.49
C16:1 *c*9	0.40	0.63	0.44	0.25
C18:0	0.64	0.54	0.53	0.50
Σ18:1	7.58	2.63	3.55	4.39
C18:2 n6	6.97	0.72	2.70	4.71
C18:3 n3	0.27	0.21	0.21	0.20
C20:0	0.31	0.05	0.04	0.04
ΣC20:1	-	0.52	0.15	0.04
C20:2 n6	-	0.17	0.10	0.04
C22:1 n9	-	0.14	0.08	0.01
C20:5 n3	-	1.02	0.62	0.18
C22:5 n3	-	0.17	0.10	0.03
C22:6 n3	-	1.23	0.81	0.31
Other FA°	-	0.05	0.27	0.21
ΣSFA	4.94	3.36	3.43	3.50
ΣMUFA	8.01	3.75	4.32	4.80
ΣPUFA	7.24	3.64	4.61	5.49
Σn3	0.27	2.64	1.74	0.72
Σn6	6.97	1.00	2.87	4.77
Σn3/Σn6	0.04	2.64	0.61	0.15
TFA	20.19	10.75	12.36	13.78

TM, T*enebrio molitor*; TM0, 0% TM inclusion as fed weight basis; TM25, 25% TM inclusion as fed weight basis; TM50, 50% TM inclusion as fed weight basis; ΣC18:1, C18:1 *t* + C18:1 *c*9 + C18:1 *c*11; ΣC20:1, C20:1 *c*9 + C20:1 *c*11; FA, fatty acid; SFA, saturated fatty acids (=C12:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C22:0); MUFA, monounsaturated fatty acids (=C14:1 *c*9 + C16:1 *c*9 + C17:1 *c*9 + Σ C18:1 + Σ C20:1 + C22:1 n9); PUFA, polyunsaturated fatty acids (=C18:2 n6 + C18:3 n3 + C20:2 n6 + C20:3 n6 + C20:4 n6 + C20:5 n3 + C22:5 n3 + C22:6 n3); TFA, total fatty acids. All values are reported as mean of duplicate analyses. °Other FA (all less than 0.05 g 100 g^{-1} DM): C12:0, C15:0, C17:1 *c*9, C20:0, C22:0, C20:3 n6, C20:4 n6.

The atherogenicity (AI) and thrombogenicity (TI) indexes of fish muscles were calculated as reported in Palmegiano *et al*. (2006):

$$AI=(C12:0 + 4 * C14:0 + C:16)/[\Sigma MUFA + \Sigma n6 + \Sigma n3]$$

$$TI=(C14:0 + C16:0 + C18:0)/[0.5 * \Sigma MUFA + 0.5 * \Sigma n6 +3 * \Sigma n3 + (\Sigma n3/\Sigma n6)],$$

where MUFA stands for monounsaturated fatty acids.

Statistical analysis

Data were analyzed by one-way ANOVA using IBM SPSS Statistics 20.0. Differences were evaluated by Turkey's post-hoc test. Significance was declared at P≤0.05.

Results and discussion

The experimental diets were well accepted by the fish. The productive traits of the fish are summarized in Table 3. No significant differences were observed for individual FBW and growth WG. The trout of TM0 group showed significantly higher feed intake than those of TM50 group (FR: 1.40 *vs* 1.31%; P=0.05); intermediate values were observed for TM25. Consequently, the TM0 group showed less favorable performance traits. In fact, FCR was significantly higher (1.20 *vs* 1.00 and 1.00 for TM0, TM25 and TM50, respectively), while PER (1.69 *vs* 2.06 and 2.04) and SGR (1.13 *vs* 1.30 and 1.27% day^{-1}) were significantly lower (P=0.01) in TM0 than TM25 and TM50. The survival rate observed in TM0 was significantly lower than that of TM25 and TM50 (P=0.02), even if no specific pathological symptoms of disease were experienced. Moreover the TM0 group showed a significantly higher value of HSI than TM25 and TM50 (P≤0.01), while no significant difference was observed for *VSI* among groups.

Such results indicate that the inclusion of 25 or 50% of TM in rainbow trout diets does not affect growth WG, but significantly ameliorates performance parameters. The effects on FCR, PER and SGR can be explained by the observed decreasing trend of voluntary intake for the TM diets, set at about -3.6% (TM25) and -6.4% (TM50) compared with the control diet.

Literature shows different responses to the use of insects in fish diets. Indeed, Ng *et al.* (2001) observed a decrease in growth performances when insect meal exceeded the level of 40% of FM substitution in African catfish fingerlings. Similar results were also observed by Roncarati *et al.* (2015) in fingerlings of common catfish. Moreover, some studies reported that the use of insects decreased growth performances and diet digestibility (St-Hilaire *et al.,* 2007b; Kroeckel *et al.,* 2012), while no differences were observed in other trials (Fasakin *et al.,* 2003).

Our results confirm that the productive traits are not negatively affected by dietary inclusion of TM and that the latter seems to be able to reduce the fish voluntary intake, possibly due to the high quantity of fat and the type of fatty acids composing the fat. As reported in Table 3, the HSI decreased with the increasing dietary level of TM. This trend is similar to those observed in other studies, where FM was totally or partially substituted by insect meal or vegetable proteins (Sealey *et al.,* 2011).

Among the considered apparent digestibility coefficients, only that of ADC_{CP} was significantly lower for TM50 than TM25 and TM0

Table 3. Growth performances, survival rate and somatic indexes of rainbow trout fed the experimental diets.

	TM0	TM25	TM50	SEM	P
IBW, g	116.5	115.2	115.9	0.40	0.44
FBW, g	313.8	316.6	308.2	2.51	0.42
WG, g	197.3	201.5	192.4	2.62	0.40
FR, % day^{-1}	1.40^{a}	1.35^{ab}	1.31^{b}	0.02	0.05
FCR	1.20^{a}	1.00^{b}	1.00^{b}	0.04	0.01
PER	1.69^{b}	2.06^{a}	2.04^{a}	0.06	0.01
SGR, % day^{-1}	1.13^{b}	1.30^{a}	1.27^{a}	0.03	0.01
Survival rate, %	86.7^{b}	96.7^{a}	97.5^{a}	1.94	0.02
HSI, %	2.18^{a}	1.79^{b}	1.61^{b}	0.07	0.00
*VS*I, %	8.04	7.70	7.71	0.13	0.50

TM, *Tenebrio molitor;* TM0, 0% TM inclusion as fed weight basis; TM25, 25% TM inclusion as fed weight basis; TM50, 50% TM inclusion as fed weight basis; SEM, standard error of mean; IBW, initial body weight; FBW, final body weight; WG, weight gain; FR, feeding rate; FCR, feed conversion ratio; PER, protein efficiency ratio; SGR, specific growth rate; HSI, hepatosomatic index; VSI, viscerosomatic index. Sample size equal to: i) 120 treatment^{-1} for IBW, FBW, WG, survival rate; ii) 4 treatment^{-1} for FR, FCR, PER, SGR; iii) 8 treatment^{-1} for HSI and VSI. ^{a,b}Different superscripts in the same row indicate significant differences (P≤0.05).

Table 4. Proximate composition of the dorsal muscle of rainbow trout fed the experimental diets.

	TM0	TM25	TM50	SEM	P
DM, %	23.9^{a}	22.4^{b}	22.7^{b}	0.24	0.01
CP, % DM	81.2^{b}	84.7^{a}	85.0^{a}	0.67	0.02
EE, % DM	13.9^{a}	9.5^{b}	9.1^{b}	0.67	0.01
Ash, % DM	4.7	5.0	5.0	0.05	0.57

TM, *Tenebrio molitor*; TM0, 0% TM inclusion as fed weight basis; TM25, 25% TM inclusion as fed weight basis; TM50, 50% TM inclusion as fed weight basis; DM, dry matter; CP, crude protein; EE, ether extract; SEM, standard error of mean. Sample size equal to 8 treatment^{-1}. ^{a,b}Different superscripts in the same row indicate significant differences (P≤0.05).

groups (90.1 *vs* 91.5 and 92.2%, respectively; P=0.05). No significant differences were observed for ADC_{DM}, ADC_{OM} and ADC_{EE} among groups. Similarly to the results of St-Hilaire *et al.* (2007b), our results showed an absolute high digestibility rate for lipids and protein. This occurred without significant differences for lipids apparent digestibility, but with a significant decrease in the protein apparent digestibility following increasing TM inclusion in fish diets. Nevertheless, even if protein digestibility decreased, the productive performances were not compromised and PER was improved. The reduction of ADC_{CP} could be an effect of increasing quantity of chitin that reduces the apparent digestibility of dietary protein, mainly affecting Kjeldahl method to estimate protein content. Indeed, it has been shown that about 5-6% of nitrogen in TM is bound to chitin, and this leads to a protein overestimation of 1.1-1.3% on a fresh weight basis (Yi *et al.,* 2013). The improvement of PER could instead be due to the increasing quantity of cysteine in the TM diets. It has to be mentioned that some studies showed how chitin could also interfere with lipid digestibility (Olsen *et al.,* 2006; Kroeckel *et al.,* 2012), but in the present study no differences were found for ADC_{EE}.

The proximate composition of the trout dorsal muscle showed significant differences in DM, CP and EE contents, while the ash content remained unaffected by treatment (Table 4). The dietary inclusion of TM significantly decreased the DM and EE contents (P=0.01), contemporarily increasing the CP content (P=0.02). Similarly, a study conducted using black soldier fly (*Hermetia illucens*) showed a decrease in DM and EE in fish fillets with the inclusion of insect meal (Sealey *et al.,* 2011).

As reported in Table 2, the sum of octadecenoic acids (Σ C18:1=Σ Z18:1 *t* + C18:1 *c*9 + C18:1 *c*11), thanks to the high amount of oleic acid (C18:1 *c*9), as well as linoleic acid (C18:2 n6) were the most represented FA in TM (7.58 and 6.97 g 100 g^{-1} DM, respectively). The Σ C18:1 and C18:2 n6 increased in the experimental diets with the increase of TM inclusion (for C18:1 *c*9: 2.17, 3.24 and 4.24 g 100 g^{-1} DM, for TM0, TM25 and TM50 respectively). Therefore, the concentration of total MUFA and total PUFA increased, while the Σ n3/Σ n6 FA ratio decreased in the diets following the increase of TM inclusion. Very long chain n3 PUFA, such as eicosapentaenoic (EPA, C20:5 n3), docosapentaenoic (DPA, C22:5 n3) and docosahexaenoic (DHA, C22:6 n3) acids, were not detected in TM.

The FA composition of trout dorsal muscle was significantly altered by diet, showing significant differences for all individual FA and groups of FA except for C16:0 and total MUFA percentages (Table 5). The greatest differences were recorded for Σ C18:1 (Σ C18:1 *t* + C18:1 *c*9 + C18:1 *c*11) and C18:2 n6 that increased with the dietary inclusion of TM. Conversely, EPA, DPA and DHA significantly decreased as a consequence of both increase of TM and reduction of FM and FO in the diets. The atherogenicity and thrombogenicity indexes showed significant differences among treatments with opposite trends (a decrease for AI and an increase for TI) at the increase of TM inclusion in fish diets.

It is well known that the FA profile of diets significantly affects the FA profile of fish tissues. In particular, in the current trial linoleic acid was increased in the dorsal muscles of trout fed diets containing TM, with the highest value recorded in the TM50 group, where cod liver oil was absent. In the same way, a reduc-

Table 5. Fatty acid composition (% of total fatty acids) of the dorsal muscle of rainbow trout fed the experimental diets.

	TM0	TM25	TM50	SEM	P
C14:0	4.33 ^{a}	3.30^{b}	2.53^{c}	0.18	0.00
C16:0	18.89	19.38	19.69	0.18	0.18
C16:1	5.00^{a}	3.47^{b}	2.67^{c}	0.24	0.00
C18:0	4.99^{a}	4.40^{b}	4.18^{b}	0.09	0.00
ΣC18:1	27.05^{c}	28.31^{b}	30.45^{a}	0.42	0.00
C18:2 n6	8.87^{a}	17.38^{b}	22.85^{a}	1.41	0.00
ΣC20:1	2.22^{a}	1.43^{b}	0.85^{c}	0.14	0.00
C18:3 n3	1.96^{a}	1.44^{b}	0.96^{c}	0.10	0.00
C20:5 n3	4.73^{a}	2.82^{b}	1.27^{c}	0.35	0.00
C22:5 n3	1.79^{a}	0.98^{b}	0.47^{c}	0.14	0.00
C22:6 n3	16.14^{a}	12.63^{b}	8.26^{c}	0.84	0.00
Other FA°	4.03^{b}	4.46^{b}	5.81^{a}	0.15	0.00
ΣSFA	29.27^{a}	27.92^{b}	27.02^{b}	0.27	0.00
ΣMUFA	35.38	34.23	34.74	0.32	0.37
ΣPUFA	35.37^{b}	37.85^{a}	38.24^{a}	0.40	0.00
Σn3	24.62^{a}	17.87^{b}	11.04^{c}	1.38	0.00
Σn6	10.75^{c}	19.98^{b}	27.20^{a}	1.64	0.00
Σn3/Σn6	2.31^{a}	0.90^{b}	0.41^{c}	0.20	0.00
AI	0.51^{a}	0.45^{b}	0.40^{c}	0.01	0.00
TI	0.36^{c}	0.42^{b}	0.54^{a}	0.02	0.00

TM, T*enebrio molitor*; TM0, 0% TM inclusion as fed weight basis; TM25, 25% TM inclusion as fed weight basis; TM50, 50% TM inclusion as fed weight basis; SEM, standard error of mean; ΣC18:1, C18:1 *t* + C18:1 c9 + C18:1 *c*11; ΣC20:1, C20:1 *c*9 + C20:1 c11; FA, fatty acid; SFA, saturated fatty acids (=C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0); MUFA, monounsaturated fatty acids (=C14:1 *c*9 + C16:1 *c*9 + C17:1 *c*9 + ΣC18:1 + ΣC20:1 + C22:1 n9); PUFA, polyunsaturated fatty acids (=C18:2 n6 + C18:3 n3 + C18:3 n6 + C20:2 n6 + C20:3 n6 + C20:3 n3 + C20:4 n6 +C20:5 n3 + C22:6 n3 + C22: 6n); AI, atherogenicity index; TI, thrombogenicity index. °Other FA (less than 1% of TFA): C12:0 + C14:1 *c*9 + C15:0 + C17:0 + C17:1 c9 + C20:0 + C18:3 n6 + C20:2 n6 + C20:3 n6 + C22:1 n9 + C20:3 n3 + C20:4 n6. Sample size equal to 8 treatment^{-1}. $^{a-c}$Different superscripts in the same row indicate significant differences (P≤0.05).

tion of EPA, DPA and DHA was recorded in fish fed TM. Even though the three experimental diets contained the same concentration of α-linolenic acid (ALA), a reduced amount of ALA was observed in the dorsal muscle of trout belonging to both the TM25 and TM50 groups (-26.5% and -51.0% if compared to control diet, respectively).

The data reported in the present trial differ from what described by Sealey *et al.* (2011) who found increasing levels of ALA in dorsal trout muscles even if fish were fed diets containing similar ALA content. St-Hilaire *et al*. (2007b) found that fatty acids were affected by diets with proportionally less ALA, EPA, and DHA in fish fed the hermetia illucens diets. These results are in agreement with those reported in the present study, as a decreasing of EPA and DHA in diets led to a consistent reduction of the same FA in fillets.

In fish fed increasing level of FM substitution, fillets showed consistent increase and decrease of Σ n6 and Σ n3 FA, respectively. This led to a significant reduction of the Σ n3/Σ n6 FA ratio, which ranged from 2.31 (TM0) to 0.41 (TM50) ($P \leq 0.01$), thus negatively affecting the nutritional value of the fillets. Palmegiano *et al*. (2006) reported a similar trend for the Σ n3/Σ n6 FA ratio in muscle of rainbow trout fed diets containing increasing levels of a plant concentrate protein source. As far as the health indices are concerned, AI improved with increasing levels of TM inclusion, due to increasing percentage of Σ n6 PUFA, while TI worsened, due to the lower Σ n3 PUFA percentage. Nevertheless, the values are lower than those registered for other food products derived from terrestrial animals (Palmegiano *et al*., 2006).

Evidence from the present study indicates that FM can be partially substituted in trout diets using different inclusion levels of TM, without modifying growth performance. The inclusion of TM in the trout diets significantly decreased the relative fat quantity but also led to modifications of the dorsal muscle lipid profile. To balance the negative effects of diets poor in Σ n3 PUFA, and overcome the worsening of fillets FA profile, a finishing feeding strategy (*i.e*., period of starvation, followed by a short Σ n3 rich diet period at the end of the rearing period), might be applied, as already reported in previous trials (Zoccarato *et al*., 1994; Gause and Trushenski, 2013). Another solution would be to increase the Σ n3 FA concentration of insect meals by means of a modification of the insect rearing substrate, as already shown (St-Hilaire *et al*., 2007a).

Conclusions

T. molitor meal is an innovative raw material and seems to be promising to be used as alternative feedstuffs to FM in trout diets. Further research on specific feeding strategies and diet formulations is still needed to limit the negative effects of TM on the nutritional value of the lipid fraction of trout fillets.

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