

Article

The Impact of Thermal Treatments up to 140 °C on Amino Acid Digestibility of Fish Meal in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Fish meal (FM) is an essential ingredient in fish feeds, but the thermal treatments used during its production might affect its nutrient availability. Therefore, an in vivo digestibility trial was conducted with three different thermal treated FM, at no heat treatment (control), 70, and 140 °C. A total of 216 rainbow trout (230 ± 6.0 g) were randomly assigned to 12 tanks, and feces samples were collected by stripping. FM was produced by lyophilizing mackerel. The lyophilized FM (94% DM) was then divided into three groups: FM Control, which was not exposed to thermal treatments, and FM 70 °C and FM 140 °C, which were exposed for 1 h to the respective temperatures of its descriptions. A conventional FM-based (FM with 64% CP produced at 110 °C) control diet was used as a basal diet. In the experimental groups, 30% of the basal diet was replaced by one of the tested FMs. No differences in apparent total tract digestibility (ATTD) were observed between FM Control and 70 °C. Protein ATTD of FM 140 °C as well as Arg ATTD were lower compared to FM Control and 70 °C. The same effect was observed for His and Asp ATTD. The current study showed that thermal treatment affects FM macronutrient and amino acid ATTD.

Keywords: fish meal; thermal treatment; apparent total tract digestibility; rainbow trout; arginine; lysine

Key Contribution: A nutritional evaluation of the impact of thermal treatments on fish meal digestibility based on industry processing procedures and European legal requirements.



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1. Introduction

Aquaculture is the fastest-growing agricultural sector [1]. Due to the increased production of fish and shrimp, the demand for fish meal (FM) continues to grow. Fish meal plays a major role in the fish feed industry for its high protein content and amino acid and fatty acid (FA) profile [2–4]. Nonetheless, there are inconsistencies regarding the nutritional value of fish meal. This can be partly due to varying sources of raw material but also to the methods of processing employed [5]. Thermal treatment is essential for FM processing [6]. Most commercial FM is exposed to temperatures between 110 and 120 °C to reduce microbial activity, increase shelf life, and optimize production capacity (t/h).

Moreover, parameters other than temperature, such as pressure, particle size, and time (duration of exposure to thermal treatment), are directly correlated and influence thermal treatments. EC 1774/2002 [7] states that animal byproducts smaller than 50 mm must be

heated to a core temperature of 133 °C for at least 20 min and at a pressure of at least 3 bars. If the animal byproduct is bigger than 50 mm but smaller than 150 mm, the core temperature required is 100 °C for at least 125 min. The variation according to particle size, temperature, and time requirements reinforces the correlation between these parameters. Therefore, to determine the impact of temperature, the other parameters (such as time) must be standardized. Nonetheless, there is a lack of information in the literature regarding standardized values of production parameters other than temperature, making it difficult to compare results and establish processing parameters.

During the production of FM, fish oil is separated and undergoes refining steps before its use as feedstuff. Deodorization is a critical step during fish oil processing that involves high temperatures (180–270 °C) that could cause side reactions [8]. Deodorization primarily removes undesirable volatile substances and converts the oil into the final product. Nevertheless, while deodorization aims to reduce undesirable compounds that affect fish oil sensory characteristics, it may also reduce the integrity of nutritive components, for instance, LC-PUFA (long-chain polyunsaturated fatty acids). Due to the occurrence of numerous methylene-interrupted ethylenic double bonds, LC-PUFA are unstable under thermal treatments and may result in chemical transformations, such as oxidation, polymerization, cyclization, geometrical isomerization, and/or double bond migration [9].

New processing strategies used in Norway produce FM with a thermal treatment of 70 °C. This temperature is the lowest permitted to produce FM according to EU-Regulation EC 1774/2002. The manufacturers of such low-temperature FM claim that gentle thermal treatment avoids losses in amino acid content. The higher availability of amino acids is directly connected to better fish performance [10,11]. Yet, the effect of low-temperature treatment on FM digestibility has not been well investigated. Excessive thermal treatment likely reduces the availability of amino acids, as the epsilon amino group of lysine and the guanidinium group of arginine, for example, are sensitive to elevated temperatures [12]. A lower temperature during production could, therefore, lead to an increased availability of amino acids and, thus, proteins, which, in turn, improves the quality of FM as a feed ingredient for aquaculture.

Due to the novelty of this processing method, there has been little systematic evaluation of the effects of thermal treatment on the apparent total tract digestibility of FM. The digestibility of energy and nutrients can be used to determine the nutritional value of a raw material. The findings from this trial can help to improve the use of FM in commercial feed. As a result, FM can be spared in fish feed while maintaining the same fish performance rate, making the feed use more sustainable and efficient. The availability of amino acids plays a particularly important role here, as a suboptimal amino acid composition (which depends largely on their availability) leads to reduced protein retention and, thus, reduced growth [13].

Therefore, the present study aimed to comprehensively evaluate the nutritive value of fishmeal produced under different thermal treatments. In a subsequent in vivo trial, apparent total tract digestibility of nutrients and energy was determined in rainbow trout (*Oncorhynchus mykiss*). We hypothesized that a low thermal treated fish meal (70 °C) would improve the apparent total tract digestibility of energy and protein. Specifically, we expected that low thermal treatments would benefit the digestibility of amino acids, especially lysine and arginine, due to their heat sensitivity.

This trial is of the utmost importance to understand the effects of thermal treatments on the digestibility of FM nutrients. Also, to improve the commercial use of FM, as protein is one of the most cost-intensive ingredients in fish feed [14], an improvement in its digestibility could lead to a lower level of inclusion in the diet.

2. Material and Methods

2.1. Diet Formulation and Preparation

FM was produced at the Core Facility Food & Bio Processing of the BOKU University Vienna. To produce FM, frozen Atlantic mackerel (*Scomber scombrus*) was obtained at a local

commercial market (METRO, Vienna, Austria) and subsequently freeze dried (Sublimator 30 EKS—R, Zirbus Technology, Harz, Germany, set with condenser temperature of -45°C and radiation temperature of 30°C) over three days. The dried product (94% DM) was divided into three groups. The first group was not exposed to heat after freeze drying, described hereafter as FM Control. The second and third groups were placed in a pre-heated laboratory oven (Heratherm, Thermo Scientific, Waltham, MA, USA) for 1 h at 70°C and 140°C , respectively.

In total, four diets were prepared (Table 1). A conventional FM (64%CP and 20.5 MJ kg^{-1} GE; produced at 110°C) was used to formulate a basal diet (49% CP and 17.5 MJ kg^{-1} GE). Plus, three test diets with three different thermal treated FM (supplemented with TiO_2 as an inert marker) were produced by replacing 30% of the basal diet with the tested FM. The apparent total tract digestibility coefficients (ATTD, %) were determined according to methodologies already established in the literature [15].

Table 1. Formulation (g kg^{-1} feed), proximate composition (g kg^{-1} dry matter), and amino acid composition (g kg^{-1} crude protein) of experimental diets.

	Basal	FM Control	FM 70°C	FM 140°C
<i>Ingredients</i>				
Fish meal	572.4	400.7	400.7	400.7
Corn gluten meal (60% CP)	197.6	138.4	138.4	138.4
Wheat starch	130	91	91	91
Wheat bran	80	56	56	56
Fish meal Control	0	300	0	0
Fish meal 70°C	0	0	300	0
Fish meal 140°C	0	0	0	300
Vit./Min. mix ¹	10	7	7	7
TiO_2	10	7	7	7
<i>Proximate composition</i>				
Dry matter	905.5	947.0	938.0	978.9
Crude protein	490.5	492.9	491.6	508.0
Ether extract ²	60.0	180.0	190.0	190.0
Ash	97.0	97.9	94.3	96.8
Gross energy (MJ kg^{-1})	17.6	22.4	21.	22.4
<i>Essential amino acids</i>				
Arginine	27.7	28.9	29.1	30.3
Histidine	10.2	15.9	12.6	12.9
Isoleucine	18.5	19.6	20.0	20.9
Leucine	39.9	39.3	40.4	42.1
Lysine	24.7	28.8	29.9	30.4
Methionine	12.7	13.0	13.8	14.3
Phenylalanine	20.7	20.8	21.1	22.0
Threonine	17.2	18.6	18.8	19.9
Valine	20.8	22.0	22.5	23.5
<i>Non- and semi-essential AAs</i>				
Alanine	35.3	35.0	35.2	37.1
Aspartic acid	37.7	40.0	50.6	41.3
Cysteine	5.7	2.9	3.9	5.2
Glutamic acid	72.7	74.0	74.0	76.6
Glycine	46.2	42.6	44.3	45.5
Proline	40.8	35.6	36.5	39.7
Serine	22.1	24.5	22.9	23.4

¹ Vitamin and mineral premix nutritional composition: vitamin A (3a672a) 800,000 I.E.; vitamin D3 (3a671) 500,000 I.E.; Betaine (betaine anhydrate 3a920) 84,000 mg; Choline chloride (3a890) 80,000 mg; Vitamin E all-rac-alpha- tocopheryl acetate (3a700) 40,000 mg; Vitamin C (L-ascorbic acid 3a300) 30,000 mg; Niacinamide (3a315) 30,000 mg; Calcium D- Pantothenate (3a841) 10,000 mg; Vitamin B2 (3a825) 4000 mg; Vitamin B6 (3a831) 3000 mg; Vitamin B1(3a821) 3000 mg; Vitamin K3 (3a710) 2000 mg; Folic acid (3a316) 1,600,000 μg ; Biotin (3a880) 160,000 μg ; Vitamin B12 (3a) 10,000 μg ; Iron(II) sulfate monohydrate (3b103) 30,000 mg; Zinc (zinc oxide 3b603) 12,000 mg; Manganese (manganese(II) oxide 3b502) 5000 mg; Iodine (calcium iodate, anhydrous 3b202) 800 mg; Copper (copper(II) sulfate), pentahydrate (3b405) 600 mg; Selenium (sodium selenite 3b801) 40 mg. ² with acid hydrolysis.

All diet ingredients were conditioned and mixed (100 L mixer, 6.0 min conditioning time, 95 °C mixing temperature, 4.6 kg of water addition, and 2.8 kg/h of steam addition). Experimental diets were extruded (OEE 8, Amandus Kahl, Reinbek, Germany) using a 4 mm diameter matrix, ~60 kWh/t, 1:2.5 press ratio, 51 Hz extruder frequency, 83 bar hydraulic pressure, 5.0 kW of performance, throughput of 150.0 kg/h, ~105 °C temperature on extruder head, and ~80 °C after extruder.

2.2. Experimental Setup

The present trial was approved by the ethics committee of the local authority of Schleswig-Holstein (V 244-63725/2022). The trial was conducted at the Fraunhofer IMTE aquaculture facility in Büsum, Germany. Rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial fish farm (Forellenzucht Troststadt, Germany) and transferred to the research facilities in Büsum. Afterwards, fish were stocked randomly into a recirculating aquaculture system (RAS) consisting of 12 rectangular tanks with 150 L water capacity each. Water treatment consisted of a mechanical and biological filter and a disinfection unit (UV filter). The photoperiod was set at a 16 L: 8 D cycle. Trout were adapted to the environmental conditions for 2 weeks and were fed with commercial dry feed (Aller Aqua Gold, 3 mm, Aller Aqua, Golssen, Germany). After acclimatization, the 216 fish were weighed individually, and 18 trout with an initial weight of 230.1 ± 5.8 g (mean \pm standard deviation) were placed into each of the tanks. In total, 12 tanks were assigned to the four experimental groups (basal plus three diets with heat-treated FM) with three replicates (tanks) each. The number of fish per tank is adapted to an optimal stocking density of at least 15 kg/m³ in the individual tanks. This stocking density is necessary for trout to prevent aggression that occurs at lower stocking densities.

Trout were fed manually 1.5% of their body weight once a day at 8:30 am for four weeks. The tanks were cleaned, and water parameters were checked daily (15.6 ± 0.7 °C temperature; 7.1 ± 0.2 pH; 4.2 ± 1.0 ppt salinity, HI 96822 Seawater Refractometer, Hanna Instruments Inc., Woonsocket-RI-USA; 9.1 ± 0.6 mg L⁻¹ O₂, Handy Polaris; OxyGuard International A/S, Birkerød, Denmark; 0.7 ± 0.4 mg L⁻¹ NH₄-N, 1.1 ± 0.4 mg L⁻¹ NO₂-N, Microquant test kit for NH₄ and NO₂; Merck KGaA, Darmstadt, Germany).

Fecal material was collected once a day (at 1:30 pm) by manual stripping of each fish for 28 days. Therefore, fish were transferred to a separate tank and anesthetized using clove oil (1 mL per 40 L of water). Subsequently, gentle pressure on the abdomen was applied to strip feces out of the posterior intestinal area. Daily feces samples from each tank were pooled and stored at -20 °C pending analyses.

Feed and feces samples were analyzed in duplicate for the proximate composition of dry matter (DM), gross energy (GE), ether extract (EE), crude protein (CP), amino acids (AA), and phosphorus, applying standard methods [16]. TiO₂ was measured with a standard method [17], in which 0.5 g of dried samples were digested with 25 mL of H₂SO₄ (98%) and one Kjeldahl tablet for 60 min at 400 °C. After digestion, samples were let overnight to allow crystallization and then filtered in 100 mL PE bottles. A total of 5 mL of the filtrate was mixed with 1 mL of 1M H₂SO₄ and 1 mL of H₂O₂. Samples were placed in disposable cuvettes and measured against distilled water on the spectrophotometer at a wavelength of 405 nm. The concentration of TiO₂ was then calculated with the following formula:

$$\text{TiO}_2 \text{ concentration (\% of DM)} = \frac{\left(\frac{y-b}{a}\right) \times \text{volume of test sample (mL)}}{0.5994 \times \text{weight of sample (g DM)} \times 10,000}$$

In which y is the absorbance, factor 0.5994 represents the Ti concentration in TiO_2 , and the volume of the test sample corresponds to the respective volume of the volumetric flask used after digestion.

In fish meal samples, the content of amino acids was conducted after acid hydrolysis and subsequent pre-column derivatization of the extracts with Waters AccQ-Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidylcarbamate, ACQ). The individual amino acids were separated and determined using reversed-phase HPLC gradient elution and subsequent fluorescence detection at $\lambda_{\text{ex}} = 250 \text{ nm}$ and $\lambda_{\text{em}} = 395 \text{ nm}$. FA profile was additionally analyzed using the one-step methylation method [18]. Briefly, 0.5 g of samples were extracted and converted to methyl esters for 2 h at 70°C with toluene and 5% fresh methanolic HCl using nonadecanoic acid ($\text{C}_{19:0}$, Sigma Aldrich, Munich, Germany) as internal standard. Subsequently, 5 mL of 6% K_2CO_3 was added, followed by another 2 mL of toluene. After centrifugation, 1 mL of the organic supernatant was transferred to a GC- Vial and was analyzed on a gas chromatograph (Agilent Technologies 7890A, Santa Clara, CA, USA) equipped with an Agilent 7693 autosampler and an Agilent G4514A injector turret. For separation of the FA Agilent HP-88 capillary column ($100 \text{ m} \times 0.25 \text{ mm}$ of internal diameter and $0.2 \mu\text{m}$ film thickness) was used. Hydrogen served as a carrier gas at a constant pressure of 11 psi. The flow rates for the FI-Detector were set at 35 mL/min (hydrogen) and 350 mL/min (synthetic air) at a temperature of 260, respectively. Conditions: Inlet temperature was set to 250°C , Split ratio to 50:1, and the injection volume was $1 \mu\text{L}$. The start temperature of the oven was set to 100°C , held for 5 min, and after that, ramped at a rate of 4°C/min to 240°C and held constant for 30 min (total runtime 70 min). Commercial standard fatty acid methyl esters (FAME) mixtures (Supelco 37 Component FAME Mix, Supelco, PA, USA) were used for the identification of individual FA. Data processing was performed using the Agilent Open Lab V3.6 software.

Apparent total tract digestibility (ATTD) of different nutrients in diets was calculated according to methods established in the literature [19] after feces and diet nutrient and marker analysis:

$$\text{ATTD}_{\text{Nutrient,Diet}} (\%) = 100 \times (1 - (\text{Marker}_{\text{Diet}} / \text{Marker}_{\text{Faeces}}) \times (\text{Nutrient concentration}_{\text{Faeces}} / \text{Nutrient concentration}_{\text{Diet}}))$$

The formula for calculation of ATTD of test substance follows the established calculation for fish in the literature [20]:

$$\text{ATTD}_{\text{Test substance}} = (\text{ATTD}_{\text{Test diet}} - \text{ATTD}_{\text{Base diet}}) \times ((30 \times \text{Nutrient concentration}_{\text{Test diet}} + 70 \times \text{Nutrient concentration}_{\text{Base diet}}) / 30 \times \text{Nutrient concentration}_{\text{Test diet}})$$

2.3. Statistics

The water tank was considered as the experimental unit, and the experiment design was completely randomized. Digestibility data were analyzed using the software R Studio (2022.12.0 + 353, R Development Core Team). After the test of normal distribution (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test), a one-way analysis of variance (ANOVA) and a parametric post hoc test (Tukey's HSD test) of ATTD data were carried out. Values for each feeding group are presented as least square means (LS means) and standard error of the mean (SEM). Differences were assumed as significant at $p \leq 0.05$.

3. Results

The evaluation of growth parameters was not the focus of this trial; therefore, the results were not considered. The proximate composition of the FM is presented in Table 2. No significant differences were observed for CP, EE after hydrolysis, GE, or ash content. Similarly, no significant differences were observed for most essential (Arg, Iso, Leu, Met, Phe, Thr, Val) and non-essential (Ala, Asp, Cys, Glu, Gly, Ser) AAs. Nevertheless, FM Control and 70°C had 1.2 g kg^{-1} crude protein more His than FM 140°C . Lys content was

similar between FM Control and FM 70 °C, but it was 1.6 g kg⁻¹ crude protein lower in FM 140 °C than in FM 70 °C. Pro was the only AA to present a linear reduction as thermal treatment increased, reducing from 20.0 g kg⁻¹ crude protein in FM Control to 19.4 g kg⁻¹ crude protein in FM 70 °C and 18.7 g kg⁻¹ crude protein in FM 140 °C.

Table 2. Proximate composition (g kg⁻¹ dry matter) and amino acid composition (g kg⁻¹ crude protein) of fish meal under different thermal treatments.

	FM Control	FM 70 °C	FM 140 °C
Proximate composition			
Dry matter	945.5	932.9	946.2
Crude protein	498.7	516.8	507.9
Ether extract ¹	292.6	294.8	297.4
Ash	110.4	108.4	117.4
Gross energy (MJ kg ⁻¹)	26.4	25.8	26.3
Essential amino acids			
Arginine	26.1	26.9	26.5
Histidine	16.1	16.1	14.9
Isoleucine	17.4	17.5	17.6
Leucine	31.7	32.1	31.9
Lysine	35.7	36.3	34.7
Methionine	11.3	11.9	11.3
Phenylalanine	17.4	16.7	16.5
Threonine	18.6	19.0	19.1
Valine	22.0	22.0	21.2
Non- and semi-essential AAs			
Alanine	26.8	26.8	26.8
Aspartic acid	40.1	40.9	40.7
Cysteine + cystine	4.8	5.0	4.6
Glutamic acid	58.2	58.7	57.7
Glycine	31.1	30.0	30.5
Proline	20.0	19.4	18.7
Serine	18.7	18.7	18.9

¹ with acid hydrolysis.

FA composition (Table 3) varies among FM. No linear effects on increasing thermal treatment were observed. FM Control had ~2.5% more MUFA than FM 70 °C and FM 140 °C. SFA on FM 70 °C was ~3% higher than FM Control (35.29%) and ~7.5% higher than FM 140 °C. Moreover, similar results were found for PUFA between FM Control and FM 70 °C, with less than 1% variation. Nonetheless, PUFA concentration on FM 140 °C was ~7% higher than the others.

The apparent total tract digestibility coefficient (ATTD) of EE after hydrolysis did not differ ($p > 0.05$) among FM. ATTD of DM, CP, and GE did not differ ($p > 0.05$) between FM Control and FM 70 °C. Nonetheless, the ATTD of these parameters was lower ($p < 0.05$) in FM 140 °C in comparison to FM Control and 70 °C (Table 4). ATTD of essential and non-essential AAs (Tables 5 and 6) did not differ ($p > 0.05$) between FM Control and FM 70 °C. On the other hand, Arg ATTD of FM 140 °C was lower ($p < 0.05$) than that of FM Control and FM 70 °C. His ATTD did not differ ($p > 0.05$) between FM 70 °C and FM 140 °C, but the latter had 3.3% lower His ATTD than FM Control. No statistical differences ($p > 0.05$) were observed for Lys nor other essential AA (Table 5).

Table 3. Fatty acid composition and the sum of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of fish meal under different thermal treatments (% of total fatty acids).

Fatty Acids		FM Control	FM 70 °C	FM 140 °C
C 4:0	Butyric acid	0.08	0.04	0.03
C 6:0	Caproic acid	0.31	0.39	0.29
C 8:0	Caprylic acid	n.d.	0.09	n.d.
C 12:0	Lauric acid	n.d.	n.d.	0.02
C 13:0	Tridecanoic acid	n.d.	0.04	n.d.
C 14:0	Myristic acid	8.75	9.39	7.83
C 14:1	Myristicolenic acid	0.15	0.21	0.09
C 15:0	Pentadecanoic acid	0.70	0.74	0.59
C 16:0	Hexadecanoic acid	20.16	21.70	17.96
C 16:1	Palmitoleic acid	4.86	4.40	4.62
C 17:0	Margaric acid	0.49	1.47	0.31
C 17:1	Margoleic acid	0.34	0.45	0.46
C 18:0	Stearic acid	4.20	4.19	3.61
C 18:1n9t	Elaidic acid	0.19	0.17	0.08
C 18:1n9	Oleic acid	19.65	18.05	18.37
C 18:2n6t	Rumic acid	0.40	0.74	0.26
C 18:2n6c	Linoleic acid	0.97	0.61	1.53
C 20:0	Arachidic acid	0.34	0.31	0.30
C 18:3n6	γ -Linolenic acid	0.28	0.54	0.30
C 20:1n9	Gadoleic acid	13.87	13.20	13.22
C 21:0	Heneicosanoic acid	0.04	0.09	n.d.
C 20:2	Eicosanedioic acid	0.16	0.21	0.19
C 22:0	Behenic acid	0.14	0.09	0.10
C 20:3n6	Dihomolinolenic acid	0.14	0.26	0.17
C 20:3n3	Dihomogamma-linolenic acid	19.80	19.19	18.16
C 22:1n9	Erucic acid	n.d.	n.d.	n.d.
C 20:4n6	Arachidonic acid	0.09	0.09	n.d.
C 23:0	Tricosanoic acid	0.04	n.d.	0.38
C 22:2	Docosadienoic acid	0.15	0.19	0.76
C 20:5n3	Eicosapentaenoic acid	0.70	0.45	3.73
C 24:1n9	Nervonic acid	2.06	2.11	1.84
C 22:6n3	Docosahexaenoic acid	0.95	0.53	4.78
Σ SFA		35.29	38.70	31.05
Σ MUFA		41.12	38.58	38.68
Σ PUFA		23.59	22.72	30.27

Table 4. Apparent total tract digestibility (ATTD, %) of fish meal under different thermal treatments.

	Dry Matter ¹	Energy ²	Crude Protein ²	Ether Extract ²
FM Control	74.4 ^a	93.6 ^a	96.5 ^a	90.9
FM 70 °C	75.2 ^a	93.5 ^a	96.1 ^a	92.6
FM 140 °C	69.9 ^b	85.9 ^b	86.6 ^b	87.3
SEM	0.55	1.30	0.82	1.58
	0.0002	<0.001	<0.001	>0.05

^{a,b} LS means within a column not sharing a common superscript differ regarding thermal treatment effect ($p \leq 0.05$) according to Tukey post hoc test. ¹ ATTD of dry matter of diet (%) = $100 \times [1 - (\text{dietary TiO}_2 / \text{fecal TiO}_2)]$. ² ATTD of nutrient (%) = $(\text{ATTD test diet} - \text{ATTD basal diet}) \times ((30 \times \text{nutrient concentration test diet} + 70 \times \text{nutrient concentration basal diet}) / 30 \times \text{nutrient concentration test diet})$. SEM Standard error of the mean.

Table 5. Apparent total tract digestibility (ATTD, %) of essential amino acids of fish meal under different thermal treatments.

	Met	Lys	Arg	His	Val	Iso	Leu	Phe	Thr
FM Control	91.4	91.8	93.5 ^a	92.5 ^a	90.1	91.6	93.2	92.7	87.5
FM 70 °C	91.8	91.3	93.1 ^a	89.9 ^{ab}	90.0	91.6	93.3	92.6	87.0
FM 140 °C	88.5	91.5	91.7 ^b	89.2 ^b	89.2	90.9	92.7	92.0	86.2
SEM	1.01	0.61	0.37	0.34	0.33	0.30	0.25	0.27	0.40
	<i>p</i> -value								
	0.196	0.866	0.044	0.002	0.192	0.273	0.306	0.296	0.188

^{a,b} LS means within a column not sharing a common superscript differ regarding thermal treatment effect ($p \leq 0.05$) according to Tukey post hoc test. SEM Standard error of the mean.

Table 6. Apparent total tract digestibility (ATTD, %) of non-essential amino acids of fish meal under different thermal treatments.

	Ala	Asp	Gly	Glu	Ser	Tyr	Pro
FM Control	91.9	81.0 ^a	86.6	92.6 ^(a)	90.3	92.2	97.7
FM 70 °C	91.7	79.9 ^a	86.1	92.2 ^(a)	89.1	92.1	97.6
FM 140 °C	91.3	78.4 ^b	85.0	91.6 ^(b)	88.5	91.6	97.1
SEM	0.35	0.50	0.55	0.27	0.52	0.28	0.34
	<i>p</i> -value						
	0.525	0.044	0.221	0.090	0.123	0.375	0.438

^{a,b} LS means within a column not sharing a common superscript differ regarding thermal treatment effect ($p \leq 0.05$) according to Tukey post hoc test. ^(a,b) LS means within a column not sharing a common superscript tends to differ regarding thermal treatment effect ($p \leq 0.10$) according to the Tukey post hoc test. SEM Standard error of the mean.

4. Discussion

Although extrusion has high-temperature inputs on the feed and modifies its protein structure, it does not have the same effect expected from thermal treatments during FM production. Extrusion is a high-temperature-short-term process that results in physical and chemical changes, such as ingredient particle size reduction, starch gelatinization, and inactivation of enzymes, but with low exposure time to high temperatures [21–23]. The thermal treatments used in FM production are considered long-term processes that seek the optimization of its production (t/h) and reduction of microbiological activity. The use of extruded feeds in fish nutrition has shown positive effects on the physical properties of the feed and nutrient digestibility, especially for carnivorous species, as they lack enzymes to digest starch [23–26]. On the other hand, thermal treatments result in the binding, denaturation of proteins, and/or destruction of free amino groups and other active groups within the protein molecule, with the severity depending on the exposure time of high temperature [5]. During the production of experimental diets used in the current trial, the feed mixture reaches ~95 °C in the extruder head and ~74 °C after extrusion for a few seconds. Meanwhile, FM was kept under thermal treatments for 1 h after reaching the core temperature set for each treatment. Thus, the use of extruded feed was not considered to be a source of influence that could affect the results of the FM exposed to low thermal temperatures.

We observed a significant ATTD reduction on DM, CP, and GE in FM 140 °C. As most of the studies regarding the effect of thermal treatments on FM focus on AA digestibility, no studies evaluate the effect of FM thermal treatments on DM, gross energy, and EE ATTD. CP content did not vary significantly among FM. Nonetheless, its ATTD was ~8% lower in FM 140 °C than in FM Control and FM 70 °C. The authors reported a similar effect, in which CP was similar among FM, but N total tract true digestibility reduced by 7% comparing a low-temperature FM and an autoclaved FM at 130 °C for 30 min [6]. Although the authors have worked with mink, their results can be extrapolated to salmonid species as they have similarities in protein digestive physiology [27–29]. ATTD of EE, on the other

hand, was not affected by thermal treatments. FA profile also suffered a transformation due to thermal treatments, in which we observed an increase in PUFA percentage at FM 140 °C in comparison to FM Control and FM 70 °C. A study reported that PUFAs are unstable under thermal treatments due to numerous methylene-interrupted ethylenic double bonds, which may result in FA chemical transformations [9]. Contrarily, we observed that FM 140 °C had a lower SFA but higher PUFA profile in comparison to FM Control and FM 70 °C. It is believed that SFAs were destroyed during thermal treatments and that more double bonds could have been formed, although the specific mechanism is still unknown.

The effect of thermal treatments on amino acids ATTD reported in the literature varies depending on the raw material used and the method of heating. Thermal treatments, in the current study, did not affect the ATTD of lysine in fish meal. Lysine is known as a heat-sensitive amino acid, and several studies have already reported its reduction on vegetable feedstuffs exposed to thermal treatments of ≥ 120 °C with different exposure times, varying from 30 min to 100 h [6,30–32]. Nonetheless, a reduction in lysine content in vegetable proteins is expected due to the Maillard reaction, in which amino acids react with reducing sugars, resulting in a lower lysine ATTD [31,32]. This reaction is not expected in fish meal production due to the lack of reducing sugars in fish. Additionally, the exposure time might not have been long enough to cause any damage to the lysine ATTD. A study reported a reduction of 23% on F.D.N.B. (1-Fluor-2,4-dinitrobenzol)-available lysine of fish meal heated in an autoclave at 120 °C for 12 h and 41% when heated for 24 h [5]. The authors reported a plateau in F.D.N.B.-available lysine reduction only after 48 h, confirming the effect of exposure time on nutrient availability. Therefore, thermal treatments for 1 h did probably not affect lysine ATTD, contrasting with other results reported in the literature, in which additional factors (time and pressure) may have influenced lysine ATTD.

The guanidinium group in the side chain of Arg makes it more susceptible to interactions with other amino acids. Arg interacts with acidic amino acids before engaging in cation- π interactions with aromatic residues [33]. It explains why Asp also had a lower ATTD at 140 °C and Glu showed the same trend. Additionally, it could be expected that Arg would bond with FA because of its flexible molecular structure. The guanidine group and α -amino group in the structure can be protonated, while the carboxyl group can be deprotonated. These characteristics result in the self-assembly of long-chain saturated, mono-unsaturated, and polyunsaturated FA with Arg. Nonetheless, in the current study, we observed a decrease in SFA and an increase in PUFA profile, which might be a consequence of AA-FA interaction. Nonetheless, little has been investigated in this regard yet.

His content in fish meal can be decreased during processing, not necessarily due to thermal treatment, but intensified by it. His is mainly found in stick water because of its water solubility. Therefore, it is extracted from raw fish during processing but not destroyed. However, during the current trial, all FM was freeze dried prior to thermal treatment, which would result in the same extraction degree of His in all tested FM. Nonetheless, FM 140 °C presented a reduction of 7.5% of His in comparison to FM Control and 70 °C. This result shows that His was destroyed due to high-temperature thermal treatment. A study reported that His in tuna fish was partially destroyed by boiling at 102 °C for 3 h and destroyed in cans sterilized at 116 °C for 90 min [34]. Our study shows that dry thermal treatment of 140 °C partially reduced His content and made it less digestible by fish, possibly due to interactions with other amino acids and racemization.

5. Conclusions

The study evaluated the apparent total tract digestibility (ATTD) of lyophilized fish meal (FM) exposed to different thermal treatments for 1 h. FM 70 °C had higher ATTD for dry matter, crude protein, and energy in comparison to FM 140 °C, as well as for Arg, His, and Asp. Nonetheless, no differences were observed between FM Control (lyophilized only) and FM 70 °C, which shows that although protein may be denatured at 70 °C, it does not increase its ATTD nor its AA.

However, this study has also raised questions that should be further investigated, such as the effect of thermal treatments on Lys ATTD and the mechanisms behind fatty acid transformation. Overall, the study showed that FM 70 °C, as recommended by the EC regulations as the minimum temperature for FM production, presents a good nutrient ATTD in comparison to high thermal treatments.

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