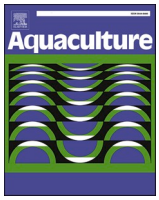
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Impact of extrusion temperature and moisture incorporation on nutrient digestibility in barramundi (*Lates calcarifer*) diet

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ABSTRACT

This study aimed to evaluate the influence of extrusion temperature and moisture incorporation procedure on nutrient digestibility of diets fed to barramundi (*Lates calcarifer*). A single premix was processed under six contrasted extrusion processing conditions, with extruder barrel temperatures set at 100 °C and 110 °C, and liquid water either incorporated in the preconditioner, in the barrel, or in both. In a subsequent 33-day feeding trial, grow-out fish (758.3 ± 24.5 g) were fed the 6 diets and apparent digestibility coefficients (ADC) were assessed for 42 fatty acids, 17 amino acids, protein, lipids and energy. Digestibility of protein (92.3–93.7%), lipid (84.1–89.4%), and energy (69.4–75.2%) also remained unaffected by extrusion settings ($P > 0.05$). However, lower extrusion temperature and moisture incorporation in both preconditioner and barrel resulted in higher digestibility of poly-unsaturated fatty acids (PUFA), long-chain poly-unsaturated fatty acids (LC-PUFA), omega-3, omega-6 and 22:6 docosahexaenoic n-3 (DHA). Moreover, lower extrusion temperature significantly improved ADCs of essential amino acids, except lysine, histidine, and threonine ($P < 0.05$). The results showed no significant impact ($P > 0.05$) of the tested extrusion parameters on fish growth (TGC: 2.11 ± 0.17), feed intake, or feed conversion performance (FCR: 1.67 ± 0.13). The study unveils diverse responses to extrusion temperature and moisture levels regarding amino acid and fatty acid digestibility. These findings highlight the advantages of preconditioning during the extrusion process to enhance fatty acid digestibility.

1. Introduction

Extrusion has been the prevailing method for pelleting fish feeds (Hardy and Brezas, 2022) for over twenty years, see (Glencross et al., 2023) for history. The benefits of extruded feeds are well-documented, including enhanced durability, reduced fines, improved water stability, and the potential to deactivate anti-nutritional factors, enhance nutrient digestibility, kill most bacterial species, and increase the available energy for fish (Hardy and Barrows, 2003; Glencross et al., 2011; Welker et al., 2018). The extrusion process involves converting the feed premix into a molten mixture by employing a combination of high temperature, high pressure, and shear forces. This procedure influences feed composition and nutrient digestibility, with variations being noted based on the specific ingredients employed (Cheng and Hardy, 2003). Therefore, improving the quality of extruded feed can be achieved by increasing the precision control over the processing conditions (Barrows et al., 2007). Conversely, a high temperature within the premix might induce alterations in the physiochemical properties of certain nutrients, reducing the digestibility and availability of proteins and decreasing their nutritional value (Sørensen et al., 2002; Barrows et al., 2007) or cause a decrease in the availability of amino acids transformed through the Maillard reaction (Salazar-Villanea et al., 2018; Van Rooijen et al., 2015). Therefore, controlling the processing conditions is critical to improve the quality of extruded feed while safeguarding valuable nutrients.

During the extrusion process, moisture is incorporated into the feed premix through both direct water addition and steam condensation in both the preconditioner and extruder barrel (Frame, 1994). While some studies have investigated the influence of extrusion temperature or extrusion feed rate on nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) (Barrows et al., 2007; Sørensen et al., 2002, 2005), limited information is available regarding the effects of moisture incorporation on the digestible nutrient contents of the feed. Specifically, the distinct impact of preconditioner settings on feed quality remains insufficiently understood (Chaabani et al., 2022). Moisture incorporation in the preconditioner plays a vital role in ensuring a uniform and continuous mixture of the feed ingredients, effectively hydrating, and heating them (Riaz and Rokey, 2012). This process is

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especially crucial in wet extrusion (Obaldo et al., 2000), contributing to the enhancement of starch gelatinization (Karkle et al., 2012), optimizing heat transfer, minimizing extruder barrel wear, and reducing energy consumption during feed production (Sørensen, 2012).

This study aimed to contrast the effects of three distinct moisture incorporation procedures and two extrusion temperature profiles within a 3 × 2 matrix (factorial) design during the manufacture of the feed pellets. In addition, and as a means of evaluating the nutritional impacts of the processing parameters, the diets were then fed to juvenile barramundi (*Lates calcarifer*) for a short-term assessment. The primary objective was to test the impact of processing parameters on nutrient digestibility. The study aspires to offer insights into refining feed processing conditions, ultimately safeguarding nutritional contents of aquafeeds.

2. Materials and methods

2.1. Ethical statement

All fish handling procedures were conducted in accordance with relevant guidelines on the care and use of animals for scientific purposes developed by the Singapore Institutional Animal Care and Use Committee (approval number: TLL(F)-22–006).

2.2. Diet processing

An experimental diet was formulated with Wittaya software (Wittaya Aqua International Inc, 2022) to fulfil nutritional requirements for total proteins, digestible energy, and lipids (Table 1) for barramundi of the desired size, as advised for finisher fish in RAS by IAFFD (2022). The diets were prepared at the R&D feed mill of Marine Aquaculture Centre (Singapore). First, ingredients were hammer milled (Mikro AP-1SH, Hosokawa Micron, USA) and sieved to a particle size of 500 μm using a vibratory sifter. Subsequently, in 50-kg batches, the ingredients were thoroughly dry-mixed utilizing a powder mixer (KSE-PM100, Kong Shiang Engineering, Singapore). The diet premix was then delivered to a 25-l twin-shaft preconditioner (25 L V1, Clextral, Firminy, France) by a twin-screw feeder (DDSR20, Brabender, Germany) at 150 rpm. From there, the premix went through a twin-screw extruder (Evolum 25, Clextral, Firminy, France), equipped with intermeshing, co-rotating screws measuring 25 mm in diameter and 600 mm in length. Extrusion screw rotation was fixed at 807 rpm. The screw configuration comprised a series of 24 feed screws (FS) and counter-threaded feed screws (CS), arranged in the following sequence from the barrel entrance: 4FS1, 10FS2, 2CS, 4FS2, and 4FS3, leading to the die (6.0 mm). During the extrusion process, fish oil, heated to a temperature range of 50–50.5 °C, was injected at the entrance of the barrel. The extruder barrel, consisting of six sections, ensured precise temperature control in each section using a series of heater collars. Throughout the extrusion process, die and barrel pressures were continuously monitored and recorded three times. The bulk density (BD) of both the pre-processed premix and the pellets coming out from the extruder was estimated by weighing loosely filled 1-l beakers. Pellet sinking speed (PellSS, in cm s^{-1}) was estimated on individual pellet (n = 10) in a graduated 0.5 l cylinder. Pellet diameter (in mm) was averaged from the measurement of 10 pellets, and the expansion rate (Exp) was then expressed relative to the die aperture (i.e., 6.0 mm). Additionally, the dry matter (DM) content was determined using a moisture analyser (Table 2). Subsequently, the resulting pellets were subjected to drying at 60 °C for 180–270 min, employing a boiling dryer (GFG-60, Mecflou, Singapore). In this study, moisture was incorporated solely as liquid water, and steam was not utilized. The total moisture incorporation rate was consistently maintained at 31.4% across all diets. Three distinct moisture incorporation procedures were tested, involving the incorporation of either 25%, 65%, or 90% of the total moisture content in the preconditioner, as detailed in Table 2. Additionally, two temperature profiles were implemented in the barrel, with the maximum temperature for each profile set at either 100 °C or 110 °C (Table 2). Following extrusion and pellet drying, three samples from each diet were subjected to proximate composition analysis, as detailed in the *Chemical analysis* section (Table 3).

Table 1
Experimental diet composition (g kg^{-1}).

Ingredients	
Fish meal[1]	250
Rice flour	211
Soybean meal	177
Soy protein concentrate[2]	148
Microalgae meal[3]	96
Wheat gluten	50
Sardine oil[4]	39
L-lysine	20
Vitamin premix[5]	5
Mineral premix[5]	2.5
Yttrium oxide[6]	1

[1] FF-Classic, Skagen, Denmark, 70% crude protein.
[2] X-Soy 200, C.J. Selecta, Brazil, 60% crude protein.
[3] AlgaPrime DHA (Corbion, Brazil): 9% crude protein, 65.2% lipid, 29.6% 22:6 docosahexaenoic n-3 (DHA).
[4] Sardine oil contained no antioxidants.
[5] ANA Fish Vitamin Premix-199 and ANA Fish Mineral Premix-199 (Zagro, Singapore).
[6] Regent Chemicals Pte Ltd., Singapore.

2.3. Fish and system

One batch of 630 barramundi (*Lates calcarifer*) was grown in the Marine Aquaculture Centre of Singapore Food Agency, transferred to the research facility, and acclimatized for 30 days. Following a light

Table 2
Settings and means (n = 3 unless otherwise indicated) of measured values during the extrusion of experimental diets produced at 100 °C, 110 °C maximum extrusion temperature, when 25%, 65% or 90% of the total moisture was incorporated into the preconditioner[1].

	100 °C			110 °C		
	25%	65%	90%	25%	65%	90%
Barrel temperature (°C)						
Chamber[2]	70 (68.0)	70 (68.0)	70 (67.7)	90 (86.3)	90 (87.0)	90 (88.8)
Chamber[3]	70 (68.7)	70 (69.0)	70 (69.3)	90 (88.0)	90 (89.0)	90 (89.8)
Chamber[4]	80 (78.7)	80 (79.5)	80 (78.0)	100 (96.7)	100 (97.3)	100 (96.8)
Chamber[5]	80 (73.3)	80 (75.5)	80 (72.0)	100 (85.3)	100 (85.3)	100 (86.8)
Chamber[6]	100 (96.7)	100 (96.0)	100 (97.3)	120 (114.0)	120 (114.0)	120 (114.0)
Extruder flow						
Pre-BD[5]	529.2	524.8	531.9	521.0	520.5	520.0
Pre-DM[6]	91.8	91.9	91.7	91.7	92.0	91.8
Post-BD[7]	644.8	626.6	637.2	673.0	626.0	654.4
Post-DM[8]	73.9	77.2	74.1	74.4	76.8	73.8

[1] Means (n = 3) of measured values of the extruder are indicated between brackets.
[2] FR: flow rate of the premix in the extruder in l h^{-1}.
[3] TMR: total moisture incorporation rate in % of premix.
[4] BP: barrel pressure in bar.
[5] Pre-BD: Bulk density (g L^{-1}) of the premix before extrusion.
[6] Pre-DM: Dry matter (g kg^{-1}) of the premix before extrusion.
[7] Post-BD: Bulk density (g L^{-1}) of the pellets, out of the extruder, before drying.
[8] Post-DM: Dry matter (g kg^{-1}) of the pellets, out of the extruder, before drying.

Table 3
Dietary content (g kg^{-1} DM, unless otherwise indicated) of experimental diets (n = 1) produced at 100 °C, 110 °C maximum extrusion temperature, when 25%, 65% or 90% of the total moisture was incorporated into the preconditioner.

	100 °C			110 °C		
	25%	65%	90%	25%	65%	90%
Dry matter[1]	884.0	897.0	881.0	878.0	884.0	886.0
Lipid	157.2	157.2	165.7	149.2	148.2	154.6
Ash	77.3	77.7	76.7	77.9	77.4	76.7
Protein	472.9	478.3	473.3	474.9	477.4	474.0
Fibre	21.2	14.8	19.8	19.1	20.2	19.4
NFE[2]	271.5	272.0	264.5	278.8	276.8	275.2
Gross energy[3]	22.4	22.5	22.6	22.3	22.3	22.4
Yttrium	0.7	0.7	0.7	0.7	0.7	0.7
Essential amino acids						
Arginine	29.0	29.1	29.5	30.5	29.4	27.5
Histidine	11.0	11.1	11.0	11.5	10.8	10.3
Isoleucine	20.6	20.7	20.5	21.4	20.4	19.6
Leucine	35.9	36.1	35.6	37.0	35.3	34.0
Lysine	43.3	45.2	43.4	45.4	47.2	42.6
Methionine	10.7	10.5	10.6	11.0	10.1	10.0
Phenylalanine	21.8	21.7	21.8	22.7	21.2	20.5
Threonine	18.7	18.7	18.6	19.1	18.2	17.5
Valine	23.1	23.2	23.2	24.0	22.6	21.9
Non-essential amino acids						
Alanine	24.1	24.4	24.0	24.7	23.8	22.8
Aspartic acid	43.3	44.1	43.6	44.9	43.6	41.0
Cystine	6.5	6.0	6.2	6.8	6.3	6.4
Glutamic acid	81.1	82.1	81.6	83.9	80.4	77.2
Glycine	21.5	22.3	21.3	22.2	23.0	20.8
Proline	24.5	25.0	24.2	25.5	23.9	23.6
Serine	21.0	21.3	20.5	21.4	20.6	19.8
Tyrosine	15.7	16.2	16.5	17.0	16.4	15.5
Fatty acidsi[4]						
TFA[5]	135.9	134.0	139.9	113.5	119.5	127.9
SFA[6]	42.4	42.1	43.7	38.5	39.8	41.7
MFA[7]	17.9	17.4	18.8	16.2	16.8	17.8
PUFA[8]	64.4	64.0	66.4	49.5	53.4	58.2
LC-PUFA[9]	54.6	54.2	56.6	41.7	45.1	49.4
n-3 LC-PUFA[9]	44.2	43.8	45.8	33.4	36.1	39.7
Omega 3	46.4	45.9	48.0	35.1	37.9	41.7
EPA[10]	7.3	6.5	7.3	5.5	5.6	6.4
DHA[11]	34.9	35.5	36.5	26.5	28.9	31.6
Omega 6	18.0	18.1	18.4	14.4	15.4	16.5
Omega 7	4.8	4.3	4.9	4.2	4.2	4.7
Omega 9	10.7	10.9	11.3	9.7	10.2	10.7

[1] Dry Matter in g kg^{-1}.
[2] NFE: Nitrogen-free extracts.
[3] Gross Energy in MJ kg^{-1} DM.
[4] Refer to Table A1 for the list of measured fatty acids.
[5] TFA: total fatty acids.
[6] SFA: saturated fatty acids.
[7] MFA: mono-unsaturated fatty acids.
[8] PUFA: poly-unsaturated fatty acids.
[9] LC-PUFA: long-chain poly-unsaturated fatty acids.
[10] EPA; 20:5 eicosapentaenoic acid n-3.
[11] DHA: 22:6 docosahexaenoic acid n-3.

sedation with AQUI-S™ (10 ppm), the fish were individually weighed (±0.1 g) and randomly distributed into 18 tanks (35 fish per tank) which were fed in triplicate with one of the six experimental diets. On day 33, the fish were weighed the same way. In this study, circular tanks with a water volume of 1000 l were used, and were supplied with recirculated seawater (29.4 °C ± 0.5). Before transfer to the tanks at a flow rate of 1500 l h^{-1} (150% tank renewal per hour), the recirculated seawater underwent several treatment processes. These included passing through a drum filter, moving bed biofilter, ultra-violet treatment (75 mJ cm^{-2}), ozonation (1.2–2.0 l min^{-1}). Pure oxygen was accumulated with an Oxyport pressure swing adsorption system (Oxywise, Slovakia) and injected to water through a 482-l Speece cone. Salinity, pH, nitrate, nitrite, total ammonia, and alkalinity in the biofilter were measured daily. Dissolved oxygen (in mg l^{-1}) and temperature were measured in each tank every 10 min with standard electrochemical probes (OxyGuard, Denmark). A natural 12:12 h photoperiod was maintained.

2.4. Feeding and fecal samples collection

For 33 days, operators manually distributed the diets to satiation twice a day (9:30–11:30 am and 2:30–4:30 pm), 5 days a week. Feeding was discontinued upon the detection of satiation, which was inferred from a decrease in feed intake and confirmed when the first pellets reached the bottom of the tank. After 30 min, any uneaten pellets were siphoned and quantified. The quantity was subtracted from the distributed diets based on individual pellet weights (Helland et al., 1996), and the resulting value was used to calculate the daily feed intake (DI) for each tank. As per the recommendation of Blyth et al. (2015), barramundi were allowed to adjust to the diets for 7 days before the first fecal collection. In each tank, faeces were accumulating from the conical-shaped bottom tanks and upwelled into a swirl separator that was flushed and cleaned daily. The fecal samples were centrifuged at 3000 relative centrifugal force (rcf) for 15 min, with the supernatant being discarded and the pellets frozen at −20 °C, followed by oven-drying (60 °C, 24 h), and analysis of chemical content.

2.5. Chemical analysis

Chemical analyses of diet and fecal samples were performed by an accredited analytical service laboratory (Eurofins, Singapore) following AOAC methods (Latimer Jr., 2023). Dry Matter (DM) was calculated by gravimetric analysis (AOAC 925.09), following oven drying at 100 °C for 5 h and desiccator cooling. Crude lipid (CL) was estimated by acid hydrolysis (AOAC 922.06). Ash content (Ash) was determined gravimetrically following the loss of mass after combustion of a sample at 550 °C (AOAC 923.03). Total nitrogen (N) was estimated after pyrolysis and combustion (AOAC 968.06) and crude protein level (CP) was based on N × 6.25. Crude fibre (Fibre) was estimated after digestion of the sample with sulfuric acid and sodium hydroxide (AOAC 962.09). Nitrogen-free extract (NFE) was calculated using DM-CP-CL-Fibre-Ash formula and gross energy (GE) using the formula 23.6*CP + 39*CL + 17.6*(Fibre+NFE), as detailed in (National Research Council, 1993). The fatty acid (FA) composition of the samples was assessed by Eurofins in Singapore. Lipid fractions were extracted from the samples using a double extraction method. Subsequently, the FA were methylated to FA methyl-ester (FAME) and quantitively measured by capillary gas chromatography (AOAC 996.06) using Eurofins FAME standard solutions. The complete list of measured FA used to compute omega-3, omega-6, omega-7, omega-9, saturated fatty acids (SFA), mono-unsaturated (MFA), poly-unsaturated (PUFA), long-chain poly-unsaturated (LC-PUFA) and total fatty acids (TFA) sums is provided in Table A1. The amino acid composition of the samples was determined by an acid hydrolysis prior to separation using high-performance liquid chromatography as detailed in (Schuster, 1988). After digestion in hydrofluoric acid at 80 °C, Yttrium was measured (Latech, Singapore) using inductively coupled plasma mass spectrometry (ICP-MS).

2.6. Data collection

The means of the initial and final fish body weights (FBW, in g) was utilized to calculate both the body weight gain (BWG, in g) and the thermal-unit growth coefficient (TGC) for each tank. TGC was used as a standardized measure of growth (Dumas et al., 2007), assumed to be unaffected by body weight, time interval and water temperature differences (Cho, 1992; Iwama and Tautz, 1981), here calculated in base 20 to improve its stability in high temperature (Le Boucher et al., 2023):

$$TGC_a = 1000 * \frac{FBW_a^{(1/3)} - IBW_a^{(1/3)}}{\sum_{i=0}^{33}(T_i - 20)} \quad (1)$$

where FBW_a and IBW_a are the final and initial average fish body weight (g) in tank a and T_i (°C) is the water temperature on day i. Feed conversion ratio (FCR) in tank a was calculated as follows:

$$FCR_a = \left(\sum_{i=1}^{33} TI_{ai} \Big/ qty\ of\ fish_{ai} \right) \Big/ (FBW_a - IBW_a) \quad (2)$$

where TI_{ai} is the tank feed intake (g day^{-1}) in tank a, on day i. The ADCs (%) of DM, CP, CL, GE, nutrients or nutrient groups of the experimental diets were calculated as follows:

$$ADC_{parameter} = \left[1 - \left(\frac{Y_{Diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}} \right) \right] \times 100 \quad (3)$$

where Y_{diet} and Y_{faeces} represent the Yttrium content of the diet and faeces, respectively, and $Parameter_{diet}$ and $Parameter_{faeces}$ are the content of the nutritional parameter of interest (DM, CP, CL, GE, nutrient or nutrient group) of the diet and faeces, respectively. For each tank, the coefficient of variation (CV) was measured as the ratio between the mean and the standard deviation of FBW, and the survival rate (Surv) as the ratio between the final and the initial quantity of fish.

2.7. Statistics

For fish performance, ADC, and digestible contents, a two-way analysis of variance (ANOVA) with a linear model Y = T + M + T*M was employed to investigate the effects of extrusion temperature (T) and moisture incorporation procedure (M). In cases where a significant M*T interaction was observed, separate tests were conducted to assess the individual effects. To compare the diets, pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) multiple range test. An arcsine-square-root transformation was applied to the survival rate, ADC, and coefficient of variation of final body weight (CV). All statistical analyses were performed using the R software (R Core Team, 2021), and effects were considered statistically significant at a significance level of $p < 0.05$. For each ADC, relative distances from the mean across various processing conditions were calculated and employed to construct a heatmap using the *pheatmap* package (Kolde, 2019). Euclidian distances were utilized to build a dendrogram, aiding in the identification of potential clusters.

3. Results

3.1. Pellet physical characteristics

The mean expansion rate of the pellets was 5.4% ± 2.1%, the sinking speed was 14.2 ± 1.2 cms^{-1} and the bulk density was 748.9 ± 20.3 g L^{-1} (Table 4).

Table 4
Mean (±SD) physical pellet characteristics of the experimental diets.

	100 °C			110 °C		
	25%	65%	90%	25%	65%	90%
Exp[1]	3.7 ± 1.3	9.0 ± 1.6	7.0 ± 2.5	4.0 ± 1.8	5.0 ± 2.9	3.8 ± 1.4
PelL[2]	10.5 ± 0.6	9.5 ± 0.4	9.5 ± 0.5	9.4 ± 0.2	9.8 ± 1.0	9.8 ± 0.4
PelSS[3]	14.2 ± 0.7	12.9 ± 1.8	12.9 ± 1.2	15.2 ± 1.3	15.1 ± 1.2	15.7 ± 1.7
BD[4]	748.5 ± 6.6	738.5 ± 7.4	715.2 ± 10.4	772.0 ± 1.8	764.7 ± 8.4	754.7 ± 10.0

[1] Exp: Expansion rate of the pellets (%, n = 10).
[2] PelL: Pellet length (mm, $n = 10$).
[3] PelSS: Sinking speed (cm s^{-1}, n = 10).
[4] BD: Pellet bulk density ((g L^{-1}, $n = 3$).

3.2. Apparent digestibility coefficients

The ADCs for all diets (Table 5) ranged from 92.3% to 93.7% for protein, from 84.1% to 89.4% for lipid, from 69.4% to 75.2% for gross energy, and from 59.5% to 67.0% for dry matter. The mean ADCs for all essential amino acids was 94.9% ± 1.6%, and 94.2% ± 1.2% for non-essential amino acids. The mean ADCs for fatty acids were as follows: 87.9% ± 2.0% for TFA, 81.2% ± 2.5% for SFA, 92.4% ± 0.5% for MFA, and 90.5% ± 2.4% for PUFA. The ADC of protein, lipid and energy were not impacted by extrusion temperature and moisture incorporation procedure ($P > 0.05$), as detailed in Table 5. However, the ADC of PUFA, LC-PUFAn-3 LC-PUFA, omega-3, omega-6 fatty acids and 22:6 docosahexaenoic n-3 (DHA) were significantly higher at 100 °C than 110 °C ($P < 0.05$) and when moisture was incorporated in both the preconditioner and the barrel. The ADC of all essential amino acids except lysine, histidine and threonine were significantly lower at 110 °C than 100 °C ($P < 0.05$).

3.3. Fish performance

In 33 days, the barramundi grew from initial average body weight of 758.3 ± 24.5 g to 868.1 ± 28.3 g, with an average FCR of 1.67 ± 0.13. Extrusion temperature and moisture incorporation procedure seemed to have no effect ($P > 0.05$) on growth, feed intake or feed conversion performance in the tested extrusion range (Table 6). Throughout all dietary treatments, survival rates remained consistently at 100%.

4. Discussion

4.1. Fish performance

In the current study, a cohort of barramundi reached a mean weight of 868.1 ± 28.3 g within 33 days, displaying an average TGC of 2.11 ± 0.17 and a FCR of 1.67 ± 0.13. These performances are in line with findings from studies carried out on barramundi of comparable sizes (Glencross et al., 2003), suggesting that growth and feed conversion during the fecal sample collection period were not suboptimal. Moreover, there was no discernible variations in pellet physical characteristics observed among the tested processing conditions (Table 4). This observation is in partial agreement with prior findings on rainbow trout (*Oncorhynchus mykiss*), where high extruder barrel temperatures (127 °C vs. 93 °C) were noted to have little influence on feed intake or weight gain over 84 days (Barrows et al., 2007). However, the same authors observed an increase in feed conversion ratio (FCR) with higher barrel temperatures. It is important to note that the duration of the present study is limited to 33 days, and as such, might not fully capture the long-term performance effects of the observed differences in digestible contents between diets. Performance differences in growth and feed conversion might become more pronounced when measured over an extended period.

4.2. Impact of extrusion temperature on apparent digestibility

In this study, a diet extrusion at 100 °C, when compared to 110 °C did significantly improve ADCs of most of the essential amino acids and groups of fatty acids. However, there was no discernible impact on the level of lipid, protein and energy ADCs ($P > 0.05$). In previous research, the results on the impact of extrusion temperature on digestibility have been contrasted, probably because they are known to depend on the specific ingredients used (Cheng and Hardy, 2003). In rainbow trout (*Oncorhynchus mykiss*), no ADC differences were measured between 93 °C and 127 °C extrusion (Barrows et al., 2007), as well as between 100 °C and 150 °C for protein, amino acids, and energy ADC (Sørensen et al., 2002, 2005). However, contrasting findings were observed in Atlantic salmon (*Salmo salar*), where increasing diet extrusion temperatures between 110 °C and 150 °C had a significant positive impact on

Table 5
Mean (n = 3) apparent digestibility coefficients (%) of dry matter, crude protein, lipid, energy, amino acids and fatty acid groups in barramundi fed diets extruded at 100 °C, 110 °C, when 25%, 65% or 90% of the total moisture was incorporated into the preconditioner.[1]

	100 °C			110 °C				Effects[2]		
	25%	65%	90%	25%	65%	90%	SEM[3]	M	T	M*T
Dry matter	63.2[a]	67.0[a]	63.3[a]	60.2[a]	59.5[a]	67.0[a]	5.71	ns	ns	ns
Crude protein	93.0[a]	93.7[a]	92.9[a]	92.3[a]	92.4[a]	93.5[a]	0.94	ns	ns	ns
Lipid	86.1[a]	89.4[a]	86.4[a]	84.1[a]	87.0[a]	85.8[a]	3.27	ns	ns	ns
Gross energy	72.2[a]	75.2[a]	72.4[a]	69.6[a]	69.4[a]	74.9[a]	4.41	ns	ns	ns
Essential amino acids										
Lysine	98.4[a]	98.8[a]	98.3[a]	98.2[a]	98.4[a]	98.4[a]	0.51	ns	ns	ns
Arginine	96.4[a]	97.1[a]	96.7[a]	95.9[a]	96.1[a]	96.6[a]	0.64	ns	*	ns
Histidine	95.8[a]	96.2[a]	95.6[a]	95.2[a]	95.2[a]	95.7[a]	0.80	ns	ns	ns
Isoleucine	93.6[a]	94.5[a]	93.6[a]	93.2[a]	93.0[a]	93.7[a]	0.87	ns	*	ns
Leucine	94.8[b]	95.8[a]	95.0[ab]	94.4[b]	94.3[b]	94.6[b]	0.55	ns	**	*
Methionine	94.3[a]	95.2[a]	94.4[a]	93.8[a]	93.3[a]	94.2[a]	1.06	ns	*	ns
Phenylalanine	94.4[ab]	95.3[a]	94.6[ab]	93.8[b]	93.7[b]	94.1[ab]	0.70	ns	**	ns
Threonine	93.5[a]	94.5[a]	93.5[a]	93.0[a]	93.1[a]	93.7[a]	0.93	ns	ns	ns
Valine	93.5[a]	94.4[a]	93.6[a]	93.1[a]	92.8[a]	93.5[a]	0.96	ns	*	ns
Non-essential amino acids										
Alanine	93.5[a]	94.6[a]	93.5[a]	93.0[a]	93.1[a]	93.6[a]	0.92	ns	*	ns
Aspartic Acid	94.9[a]	95.8[a]	94.9[a]	94.5[a]	94.6[a]	94.9[a]	0.86	ns	ns	ns
Cystine	92.4[a]	92.8[a]	92.1[a]	91.8[a]	91.6[a]	93.1[a]	1.56	ns	ns	ns
Glutamic Acid	96.2[a]	96.8[a]	96.2[a]	95.7[a]	95.7[a]	96.1[a]	0.66	ns	*	ns
Glycine	93.0[a]	94.3[a]	93.1[a]	92.6[a]	93.1[a]	93.2[a]	1.07	ns	ns	ns
Proline	95.0[a]	95.7[a]	95.0[a]	94.7[a]	94.5[a]	95.1[a]	0.64	ns	ns	ns
Serine	94.8[ab]	95.6[a]	94.6[ab]	94.0[b]	94.3[ab]	94.6[ab]	0.80	ns	ns	ns
Tyrosine	94.2[a]	95.1[a]	94.5[a]	94.1[a]	93.6[a]	94.0[a]	1.00	ns	*	ns
Fatty acids[4]										
TFA[5]	89.0[ab]	90.6[a]	88.2[ab]	84.4[b]	87.6[ab]	87.3[ab]	3.20	ns	*	ns
SFA[6]	82.6[a]	84.6[a]	81.5[a]	77.0[a]	80.8[a]	80.6[a]	5.92	ns	ns	ns
MFA[7]	93.1[a]	92.4[a]	92.2[a]	91.7[a]	92.3[a]	92.9[a]	2.02	ns	ns	ns
PUFA[8]	91.5[ab]	93.8[a]	91.0[abc]	86.5[c]	90.5[abc]	89.6[bc]	2.44	*	***	ns
LC-PUFA[9]	91.1[ab]	93.9[a]	90.8[ab]	85.6[c]	90.3[abc]	89.0[bc]	2.69	**	***	ns
n-3 LC-PUFA[9]	92.0[ab]	94.5[a]	91.5[ab]	86.8[c]	91.3[abc]	89.9[bc]	2.35	**	***	ns
Omega-3	92.2[ab]	94.6[a]	91.8[ab]	87.2[c]	91.4[abc]	90.2[bc]	2.42	*	***	ns
EPA[10]	98.8[a]	98.8[a]	98.3[a]	98.4[a]	98.5[a]	98.9[a]	0.74	ns	ns	ns
DHA[11]	91.6[ab]	94.7[a]	91.2[ab]	85.7[b]	91.1[ab]	88.9[b]	3.18	**	**	ns
Omega-6	89.6[a]	91.8[a]	89.1[ab]	84.8[b]	88.5[ab]	87.9[ab]	2.50	*	**	ns
Omega-7	96.4[a]	95.9[a]	95.6[a]	95.9[a]	95.9[a]	95.8[a]	1.66	ns	ns	ns
Omega-9	93.1[a]	92.2[a]	92.0[a]	91.5[a]	92.1[a]	92.7[a]	2.01	ns	ns	ns

[1] Arcsine-square-root transformation was applied to all data; significant ($P < 0.05$) differences among means are indicated by different superscript letters.
[2] Tested effects: moisture incorporation procedure (M), extrusion temperature (T), effects interaction (M*T); when M*T interaction was significant, the effects were separately tested; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[3] Pooled standard error.
[4] Refer to Table A1 for the list of measured fatty acids.
[5] TFA: total fatty acids.
[6] SFA: saturated fatty acids.
[7] MFA: mono-unsaturated fatty acids.
[8] PUFA: poly-unsaturated fatty acids.
[9] LC-PUFA: long-chain poly-unsaturated fatty acids.
[10] EPA; 20:5 eicosapentaenoic acid n-3.
[11] DHA: 22:6 docosahexaenoic acid n-3.

the ADCs of lipid, protein, and amino acids (Morken et al., 2012). No other research has been conducted on fish, and only similar studies in terrestrial species, such as mink and chicken, are known. In mink (*Mustela vison*), raising the diet extrusion temperature between 100 °C and 125 °C resulted in decreased protein digestibility (Ljøkjel et al., 2004). Similarly, in broiler chicks, elevating the extrusion temperature between 110 °C and 125 °C led to reduced digestibility of dry matter, crude protein, starch, and energy in diets (Hejdysz et al., 2022). The variability in these results underscores the necessity of considering multi-parameter extrusion factors. Temperature alone cannot fully account for the observed variations and other factors such as pressure and moisture incorporation procedures must also be explored.

4.3. Impact of moisture incorporation procedure on digestibility

The incorporation of moisture in both the preconditioner and the barrel emerged as the most beneficial for nutrient digestibility and can notably enhance the digestibility of fatty acids ($P < 0.05$). Utilizing cluster analysis (Fig. 1) offers insight into the interaction between moisture and temperature, revealing processing sensitivity patterns of fatty acids and amino acids. Two distinct clusters emerge, revealing a shared sensitivity to moisture incorporation procedures at 100 °C extrusion but slightly divergent responses at 110 °C. Much of the prior studies on this topic have focused on examining the influence of the overall moisture content during extrusion. This factor is known to modify the degree of transformation of the feed matrix, leading to differences in starch digestibility and feed microstructure (Karkle et al., 2012). Changes in the extrusion moisture content have also been demonstrated to impact nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) (Sørensen et al., 2002), performance in Pacific white shrimp (*Penaeus vannamei*) (Obaldo et al., 1999, 2000) or in vitro protein digestibility (Wang et al., 2008). However, the impact of the moisture incorporation procedure has remained relatively unexplored. The incorporation of moisture in the preconditioner is, however, expected to extend the hydration time of the premix (Karkle et al., 2012) and facilitate the necessary mixing and retention time for both physical and

Table 6
Mean (n = 3) performance of barramundi fed with diets extruded at 100 °C, 110 °C, when 25%, 65% or 90% of the total moisture was incorporated into the preconditioner.[1]

	100 °C			110 °C				effects[2]		
	25%	65%	90%	25%	65%	90%	SEM[3]	M	T	M*T
FBW[4]	871.4[a]	855.2[a]	880.8[a]	850.2[a]	879.7[a]	873.5[a]	49.9	ns	ns	Ns
BWG[5]	105.9[a]	102.3[a]	118.1[a]	102.4[a]	117.3[a]	110.1[a]	12.5	ns	ns	Ns
TGC[6]	2.0[a]	2.0[a]	2.3[a]	2[a]	2.2[a]	2.1[a]	0.2	ns	ns	Ns
FCR[7]	1.8[a]	1.7[a]	1.7[a]	1.8[a]	1.6[a]	1.7[a]	0.1	ns	ns	Ns
Biom[8]	3.5[a]	3.4[a]	4.0[a]	3.4[a]	3.9[a]	3.6[a]	0.4	ns	ns	Ns
TFI[9]	184.3[a]	173[a]	196.2[a]	180.5[a]	192.8[a]	185[a]	14.7	ns	ns	Ns
Surv[10]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	100.0[a]	ns	ns	Ns
CV[11]	22.8[a]	20.0[a]	20.2[a]	22.5[a]	21.5[a]	20.3[a]	2.3	ns	ns	Ns

[1] Values with differing superscripts are significantly different ($P < 0.05$).
[2] Effects of extrusion temperature (T) and moisture incorporation procedure (M) are indicated (ns: non-significant).
[3] Pooled standard error mean.
[4] FBW: final body weight (g).
[5] BWG: body weight gain (g fish^{-1}). Initial fish body weight was 758.3 ± 24.5 g.
[6] TGC: thermal-unit growth coefficient in base 20.
[7] FCR: feed conversion ratio (g feed (as fed) / g wet weight gain).
[8] Biom: biomass gain per tank (kg tank^{-1}).
[9] TFI: total feed intake (g fish^{-1}).
[10] Survival rate (%).
[11] CV: coefficient of variation (%) for final body weight.

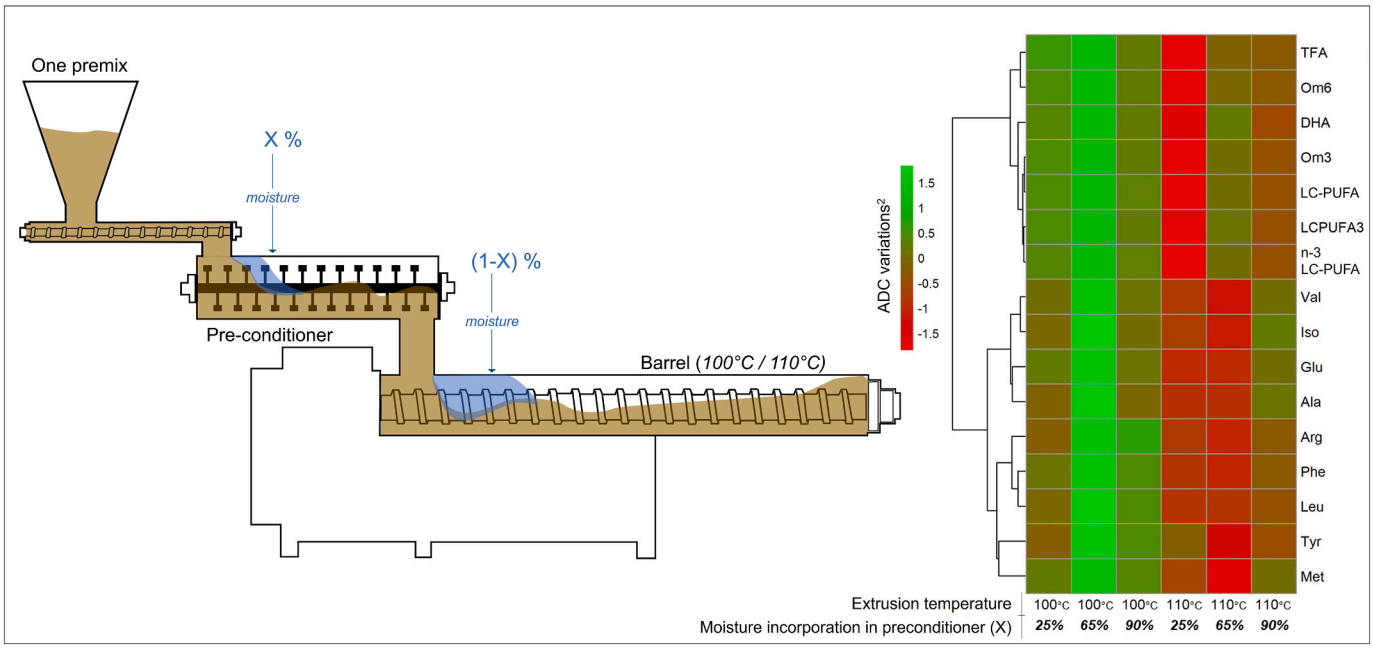


Fig. 1. Impact of extrusion temperature and moisture incorporation procedure on nutrient apparent digestibility coefficients (in %) in barramundi.[1]
[1] Total moisture incorporation is fixed for all diets: 31.4% of the premix.
[2] Dimensionless representations of the variations in normalized nutrient ADC for each respective nutrient or group of nutrients. Euclidian relative distances were utilized to build a dendrogram clusters.

chemical transformations to occur (Obaldo et al., 2000). The targeted inclusion of moisture into the preconditioner has been associated with several changes of the feed, including an increased expansion ratio (Zhang and Hoseney, 1998), lower bulk densities (Mathew et al., 1999), and a more comprehensive starch gelatinization process. Yet, there was insufficient evidence to indicate any alteration in nutrient digestibility (Karkle et al., 2012). Looking ahead, exploring the effects of elevated heat through steam injection in the preconditioner, or enhancing beater interactions using high-intensity preconditioners (HIP) or high-shear conditioners (HSC), could present promising directions for refining the extrusion process and elevating the nutritional qualities of the feed.

4.4. Susceptibility of n-3 LC-PUFA to extrusion process

In the current study, the digestibility of n-3 LC-PUFA, LC-PUFA, omega-3, omega-6 and DHA were notably sensitive to the extrusion settings. The sensitivity of n-3 LC-PUFA to extrusion settings is especially a concern as they are essential dietary nutrients for the majority of aquatic species (Glencross and Rutherford, 2011; Turchini et al., 2009) but their availability is limited due to the constrained supply from traditional sources like fish oil. (Tacon and Metian, 2008; Turchini et al., 2009). The recent utilization of microalgae, such as the one utilized in this study (Table 1), holds great promise as a sustainable alternative source of n-3 LC-PUFA (Alhazzaa et al., 2019; Chauton et al., 2015).

However, it is imperative to ensure the protection of n-3 LC-PUFA during the extrusion process. A first hypothesis to explain the decline in digestibility of n-3 LC-PUFA would come from the limited oxidative and thermal stability of PUFA (Hădărugă et al., 2016). During heat treatment, PUFA undergo a series of chemical transformations, including oxidation, polymerization, cyclization, geometrical isomerization, and double bond migration (Fournier et al., 2006). DHA, in particular, exhibit heightened vulnerability to degradation, even at relatively modest temperatures, such as 50 °C (Hădărugă et al., 2016). A second hypothesis arises from the fact that extrusion has a propensity to promote the generation of amylose-lipid complexes (Cheng and Hardy, 2003; Singh et al., 2007). During its gelatinization, amylose can co-crystallize with fatty acids, capturing them within the core of a helical structure (De Pilli et al., 2008, 2011). This arrangement renders the fatty acids less accessible to digestive enzymes (Arora et al., 2020). Consequently, the development of amylose-lipid complexes during the extrusion process could hinder the effective digestion and absorption of fatty acids. Notably, the creation of these complexes is influenced by extrusion moisture and temperature (De Pilli et al., 2011), the nature of the fatty acid (Bhatnagar and Hanna, 1994; Camire et al., 1990), and may decline with greater premix viscosity and prolonged residence time in the extruder (Singh et al., 2007).

5. Conclusion

Extrusion has been the predominant method for pelleting fish feeds for more than twenty years due to the fact that it offers significant advantages. This study highlights the significance of precision control over processing conditions in preventing overprocessing and minimizing the wastage of precious nutrients. By adopting precision in processing practices, we will improve the potential for sustainable and efficient resource utilization, benefiting both the aquaculture sector and the environment.

CRediT authorship contribution statement

R. Le Boucher: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **W. Chung:** Investigation, Formal analysis, Data curation. **J.K.L. Ng:** Investigation, Data curation. **L.S.E. Tan:** Resources, Project administration, Data curation. **C. Wu:** Investigation, Formal analysis, Data curation. **C.S. Lee:** Investigation, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Richard Le Boucher reports financial support was provided by Singapore Food Story - Theme 2 - IAF-PP Future Foods. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Appendix

Table A1
Fatty acids used in the sum calculation of total fatty acid (TFA) and details of saturated (SFA), mono-saturated (MFA), poly-unsaturated (PUFA), long-chain poly-unsaturated (LC-PUFA), long-chain poly-unsaturated omega-3 (n-3 LC-PUFA) omega-3, omega-6, omega-7, omega-9.

Formula	Fatty acid	SFA	MFA	PUFA	LC-PUFA	n-3 LC-PUFA	n-3	n-6	n-7	n-9
4:0	Butyric	x								
6:0	Caproic	x								
8:0	Caprylic	x								
10:0	Capric	x								
12:0	Lauric	x								
14:0	Myristic	x								
14:1	Myristoleic		x							
15:0	Pentadecanoic	x								
15:1	Pentadecenoic		x							
16:0	Palmitic	x								
16:1	Palmitoleic		x						x	
17:0	Heptadecanoic	x								
17:1	Heptadecenoic		x						x	
18:0	Stearic	x								
18:1 Trans	Oleic trans									
18:1 cis	Oleic cis		x							
18:2 cis	Linoleic cis			x				x		
18:2 trans	Linoleic trans									
18:3 gamma	Gamma linolenic			x				x		
18:3 alpha	Alpha linolenic			x			x			
18:3 trans	Linolenic trans									
18:4	Octadecatetraenoic			x			x			
20:0	Arachidic	x								
20:1	Eicosenoic		x							x
20:2	Eicosadienoic			x	x			x		
20:3	Eicosatrienoic			x	x	x	x			

(*continued on next page*)

Table A1 (*continued*)

Formula	Fatty acid	SFA	MFA	PUFA	LC-PUFA	n-3 LC-PUFA	n-3	n-6	n-7	n-9
20:3	Dihomo-gamma linolenic			x	x			x		
20:4	Arachidonic			x	x	x	x			
20:4	Arachidonic			x	x			x		
20:5	Eicosapentaenoic			x	x	x	x			
21:5	Heneicosapentaenoic			x	x	x	x			
22:0	Behenic	x								
22:1	Erucic		x							x
22:2	Docosadienoic			x	x			x		
22:3	Docosatrienoic			x	x	x	x			
22:4	Docosatetraenoic			x	x			x		
22:5	docosapentaenoic			x	x	x	x			
22:5	Docosapentaenoic			x	x			x		
22:6	Docosahexaenoic			x	x	x	x			
24:0	Lignoceric	x								
24:1	Nervonic		x							x

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