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The impacts of physical properties of extruded feed on the digestion kinetics, gastrointestinal emptying and stomach water fluxes of spotted seabass *(Lateolabrax maculatus)*

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ABSTRACT

This study investigated the effect of extruded feed physical pellet quality (PPQ) on the physiological response of spotted seabass *(Lateolabrax maculatus)*. Two batches of pellets with the same formula but contrasting PPQ were produced by extrusion. The contrast in PPQ was created by applying two levels of water (14% versus 20%) into the feed mixture prior to extrusion. This resulted in a low-water addition treatment (LW) with a short pellet hydration time versus a high-water addition treatment (HW) with a long pellet hydration time. Fish (average weight, 159 g) were fed to satiation twice daily for 48 days. Faeces were collected during the experimental period for digestibility measurements. At the end of the experiment, chyme was collected from the stomach and intestine at 0.5, 2, 4, 6, 8, 10, 12, 16 and 24 h after the last meal during which fish were also fed to satiation. Chyme dry matter, crude protein, and inert marker (Y_2O_3) content were analysed for the calculation of gastrointestinal evacuation, kinetics of digestion and water fluxes. A significantly larger stomach water influx led to a lower stomach DM content at 2 h postprandial in the LW-pellets compared to the HW-pellets. During the last meal, fish fed LW-pellets consumed more feed and had a higher plasma α-melanocyte stimulating hormone concentration at postprandial 2 h. On a fresh basis, LW-pellets generated a faster stomach digesta evacuation, while on a DM basis stomach emptying was similar for both treatments. Fish fed LW-pellets had a lower feed conversion ratio, a higher protein efficiency ratio, hepatosomatic index and postprandial plasma glucose level than fish fed HW-pellets. Nevertheless, PPQ did not statistically influence on the stomach digestion kinetics, faecal digestibility values and feed intake during the experimental period. Our results show that the PPQ can alter stomach water fluxes and digesta evacuation rate in spotted seabass. Additionally, it is suggested that PPQ, particularly hydration time, can affect fish's feed intake regulation and metabolic responses.

1. Introduction

Extrusion has become the primary way to produce aquafeed in recent decades (Khater et al., 2014). Physical pellet quality (PPQ) refers to the texture and structure of pellets, which determine the pellet's integrity during transportation and in the water prior to consumption by fish (Sørensen, 2012). Currently, standards for PPQ of extruded aquafeeds mainly focus on aspects prior to consumption by fish (e.g., transportation and feeding systems), but not on post-prandial aspects. High water stability, durability and hardness are considered favourable PPQ characteristics. A poor PPQ relates to nutrient losses/leaching and pellet breakages, which cause economic losses and water pollution in aquaculture (Aas et al., 2021; Sørensen, 2012).

The relationship between PPQ and fish performance has still yet to be thoroughly explored (Aas et al., 2020). Although some studies showed that pellet water stability and hardness affected fish's digestion and feed

Abbreviations: PPQ, Physical pellet quality; DM, Dry matter; CP, Crude protein; WAI, Water absorption index; WSI, Water solubility index; NSI, Nitrogen solubility index; PT, Pellet type; RWF, Relative water flux; GIT, Gastrointestinal tract; α-MSH, α-melanocyte stimulating hormone; ADC, Apparent digestibility coefficient; SGR, Specific growth rate; FCR, Feed conversion ratio; PER, Protein efficiency ratio; HSI, Hepatosomatic index; VSI, Viscera somatic index.

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intake, results are sometimes conflicting among studies (Aas et al., 2011, 2020, 2021; Adamidou et al., 2009; Baeverfjord et al., 2006; Bogevik et al., 2021; Samuelsen et al., 2021). Baeverfjord et al. (2006) reported that lowering the water stability of extruded pellets, which had the same formula, decreased lipid digestibility without affecting growth in rainbow trout. In contrast, Aas et al. (2021) found in salmon a negative relation between pellet water stability and lipid digestibility, but no effect on feed intake was present. Whereas pellet hardness was negatively correlated with feed intake and growth of salmon and shrimp (Cerecer-Cota et al., 2005; Jacobsen et al., 2018). However, a harder pellet, which was induced by the inclusion of lupin into the diet, increased feed intake of barramundi (Glencross et al., 2011). Samuelsen et al. (2021) reported that pellet hardness is an indefinite factor in the feed intake and growth of Atlantic salmon (*Salmo salar L*).

Natural food and prey items of fish have a water content above 70% (Kristiansen and Rankin, 2001), while extruded pellets typically have a dry matter (DM) content above 90% (i.e., a water content below 10%). In trout, it is suggested that gastric emptying starts when chyme DM is below 35–40% (Kristiansen and Rankin, 2001). A proper water influx into the stomach seems vital for moisturizing pellets leading to disintegration, after which the gastric evacuation can start. This process might be influenced by PPQ characteristics, such as pellet hydration time and water stability. Currently, limited information is available on the impact of PPQ on stomach water influx. In African catfish, it was shown that water fluxes in the gastrointestinal tract (GIT) are affected by dietary composition (Elesho et al., 2022; Harter et al., 2013). Harter et al. (2013) demonstrated that replacing dietary fat with starch changed the chyme characteristics and affected the water balance in various parts of the GIT in African catfish. Elesho et al. (2022) hypothesized that in addition to dietary macronutrient composition, PPQ may affect stomach water fluxes and digestion.

The moment stomach evacuation starts and the gastrointestinal evacuation rates determine when chyme is present in and passes through the intestine (Mazumder et al., 2020). The gastrointestinal evacuation rate is a major determinant of feed intake (Bromley, 1987; Tyler, 1970). A faster gastrointestinal passage together with a higher feed intake were observed when salmon were fed pellets that disintegrated more rapidly (Bogevik et al., 2021). Aas et al. (2017) showed that feeding salmon with soaked feed accelerated gastric evacuation at 2 h postprandial. Oehme et al. (2014) observed that soaking pellets increased feed intake in salmon. Both studies indicate that soaking pellets before feeding accelerated pellet moisturization and gastric evacuation, leading to a higher feed intake. Therefore, we hypothesize that pellet hydration time, water absorption and porosity-related physical characteristics affect stomach water fluxes and gastric evacuation by altering the pellet moisturization process.

The kinetics of digestion is described by the disappearance rate of nutrients as digesta passes through the GIT. Knowledge of digestion kinetics helps to understand differences in final apparent digestibility. In poultry, the kinetics of nutrient digestion in the GIT influenced feed utilization (Chen, 2017). For fish, such information is scarce. Elesho et al. (2022) reported that the type of dietary protein sources (fishmeal versus hydrolysed fishmeal) and energy sources (starch versus fat) affected dry matter and crude protein digestibility through the GIT of African catfish at 4 h postprandial. We assume that pellet hydration time may affect the kinetics of digestion by altering the digesta passage rate through the GIT and thereby the kinetics of nutrient absorption (e.g., postprandial blood glucose levels).

This study assessed the effect of PPQ on (1) the stomach water fluxes, chyme DM content, gastrointestinal emptying and kinetics of digestion; (2) feed intake, feed utilization and growth performance; and (3) postprandial blood glucose levels and plasma α-melanocyte stimulating hormone (α-MSH) concentrations. So far, the impact of PPQ on fish performance and feed digestibility has only been studied in salmon and trout. This study focused on spotted sea bass (*Lateolabrax maculatus*), a carnivorous species and an economically important species in China (Cai et al., 2020).

2. Materials and methods

During the experimental period, all fish were maintained in compliance with the Laboratory Animal Welfare Guidelines of China (Decree No. 2 of Ministry of Science and Technology, issued in 2021).

2.1. Experimental diets

In order to have two batches of pellets with contrasting PPQ characteristics, two levels of water (14% versus 20%) were added to the meal mixture prior to extrusion. This resulted in a moisture content of the meal mixture of 22% versus 28%, respectively, for the LW and HW treatment. Water was gradually added to the meal with a paddle mixer (CH-100, The New Standard Powder Machinery Manufacturing Co., Ltd., Wuxi, China) for 15 min. The mixed materials were thereafter stored at room temperature (25–28 °C) for 1 h to create an evenly distribution of water in the mixture before being transferred to the extruder. These meal mixtures were extruded using a twin-screw extruder (TSE65, Yanggong, Beijing, China) with a screw diameter of 65 mm and a length-to-diameter (L/D) ratio of 20:1. The die plate had two dies of 3 mm. During production, the die and barrel temperature, as well as screw speed, were set at the same values for both batches. The measured extrusion parameters are given in Table 2. After extrusion, the extrudates were cooled and air-dried with fans at room temperature (25 °C) for 48 h. Thereafter, pellets were coated with oil using a vacuum coater (ZJB-40, New Profit Food Machinery Co., Ltd., Weifang, China). Coated pellets were stored at 4 °C until use. The diet formula was identical for both batches of pellets (Table 1). Yttrium oxide (Y_2O_3) was included in the diet to measure stomach water flux and digestibility.

2.2. Analysis of physicochemical properties

2.2.1. Physical properties of intact pellets

Hardness (i.e., strength at rupture) was measured by Texture-Analyzer (TA-XT2i, Model 1000 R, Stable Micro Systems, Blackdown Rural Industries, Surrey, UK), which fitted with a 25 kg load cell and a PC-operated remote control. A cylindrical probe (10 mm) was pressed on the pellet at a speed of 0.8 mm/s to achieve 60% compression with gradually increasing strength until the pellet fractured. Strength–time graphs were recorded and analysed in Texture Expert for Windows (version 2.54, Stable Micro Systems, Blackdown Rural Industries, Surrey, UK). Per replicate measurement, thirty pellets were tested. Hardness was expressed in Newton.

Hydration time was assayed following the method of Wang et al. (2020). A total of 200 pellets were put in 400 mL of distilled water.

Table 1
Ingredient composition of the experimental diet.

Ingredient	Inclusion level (g/kg)
Fish meal[a]	550
Wheat flour	160
Fish oil	30.0
Soybean oil	40.0
Wheat middling	200
Monocalcium phosphate	5.00
Vitamin and mineral premix [b]	10.0
Choline chloride (70%)	4.00
Yttrium oxide	1.00
Total	1000

[a] Fishmeal: anchovy, prime. Tecnológica de Alimentos S.A., Ltd. (Peru).
[b] Vitamin and mineral premix (mg/kg diet): Vitamin A 20; Vitamin B1 10; Vitamin B2 15; Vitamin B6 15; Vitamin B12 (1%) 8; Niacinamide 80; Vitamin C phosphate (35%) 600; Calcium pantothenate 40; Biotin (2%) 2; Folic acid 10; Vitamin E (50%) 300; Vitamin K3 20; Vitamin D3 10; Inositol 150; Wheat middling 220; CuSO4·5H2O 30; FeSO4·H2O 300; ZnSO4·H2O 200; MnSO4·H2O 25; KI (2.9%) 3; Na2SeO3 (10% Se) 5; CoCl2·6H2O (10% Co) 5; NaCl 100; MgSO4·7H2O 1200; Antioxidant 50; Fungicide 200; Zeolite 6382.

Three pellets were taken out every minute. The cross-sections were cut and examined to determine whether the pellets were totally soaked with water and thus without a hardcore. Hydration time was recorded as the time pellets needed to fully penetrate water. This procedure was repeated three times per treatment.

Pellet water solubility on a dry matter basis was measured according to the method of Baeverfjord et al. (2006) with some modifications. Approximately 10 g of pellets were placed into the custom-made steel-mesh basket (0.85 mm mesh size). This basket was weighed and placed into a glass beaker filled with 300 mL distilled water. This beaker with the steel-mesh basket and pellets was placed in a water bath and shaken for 20 min at 25 °C. Afterwards, the baskets with the remaining pellets were oven dried at 135 °C for 5 h and weighed. Measurements were done in triplicate for each treatment. Pellet water solubility (WS) was calculated as follows:

$$WS(\%) = \frac{M1 \times (1-x) - M2}{M1 \times (1-x)} \times 100 \quad (1)$$

where x is the pellet water content before incubation; M_1 and M_2 are the basket weights with pellets, respectively, prior to incubation and after drying.

Expansion rate was assayed according to Wang et al. (2020). Thirty pellets were randomly selected, and their diameter was measured with a digital calliper. Triplicates were done for each treatment. Expansion rate (ER) was calculated by:

$$ER = \frac{D_p}{D_d} \quad (2)$$

where D_p is the pellet diameter (mm); D_d is the die diameter (3.0 mm).

Pellet floatability was measured by randomly taking 150 pellets and placing them in a 500 mL beaker filled with 400 mL distilled water. The number of pellets floating on the water surface was recorded after 30 min at room temperature. Triplicates were performed for each treatment. Floatability was calculated as follows (Khater et al., 2014):

$$\text{Floatability } (\%) = \frac{N_f}{N_t} \times 100 \quad (3)$$

where N_f is the quantity of floating feed after 30 min; N_t is the total number of pellets in the water.

Sinking velocity was determined according to the method described by Kannadhason et al. (2011) with some modifications. A pellet was taken randomly and placed in a glass cylinder (diameter: 75 mm, height: 1.0 m) filled with distilled water. The time taken for each pellet to reach the bottom was recorded. Sinking velocity (SV) was measured in 25 pellets and determined using the equation:

$$SV\ (m/s) = \frac{H}{T} \quad (4)$$

where H is the height of the water column (m); T is the time taken by the pellet to reach the bottom of the cylindrical container (s).

2.2.2. Chemical properties of grounded pellets

The extruded pellets were grounded with a lab grinder and sieved through a 70-mesh sieve (215 μm). The sieved fraction was used to measure the water solubility index (WSI), water absorption index (WAI), nitrogen solubility index (NSI) and starch gelatinization degree.

WSI and WAI were determined according to Anderson (1982). About 2.5 g of grounded pellets were suspended in 30 mL distilled water in a centrifuge tube and vibrated for 20 min. The suspension was centrifuged at 3000 *g* for 10 min. Thereafter, the supernatant was dried at 135 °C for 2.5 h and then the dissolved solids was weighted. Weighing was also performed on the sediment in the centrifuge tube. WAI was calculated according to the following equation (Singh and Muthukumarappan, 2016):

$$WAI(\%) = \frac{W_g}{W_{ds}} \times 100 \quad (5)$$

and WSI was calculated as:

$$WSI(\%) = \frac{W_{ss}}{W_{ds}} \times 100 \quad (6)$$

where W_{ds} is the amount of grounded material used for the measurement; W_g is the weight of the material remaining after centrifugation and decanting in the tube; and W_{ss} is the dried weight of the dissolved solids in the supernatant.

The nitrogen solubility index (NSI) was measured according to the AACC approved method 46–23 with modifications (AACC, 1995). About 1 g of grounded pellets were suspended in 30 mL of distilled water at room temperature. They were mechanically shaken at 2500 rpm for 30 min and then centrifuged at 3000 *g* for 10 min. The supernatant was analysed for nitrogen by the standard Kjeldahl method without a dry step. The nitrogen solubility index was calculated by:

$$NSI(\%) = \frac{N1}{N2} \times 100 \quad (7)$$

where N1 is the amount of nitrogen in the supernatant; and N2 is the amount of nitrogen in the analysed sample.

Starch gelatinization degree was assayed by enzymatic hydrolysis using amyloglucosidase (Sigma, 10115-5G-F, from *Aspergillus niger*) according to Xiong et al. (1990) with modifications.

2.3. Fish and housing conditions

The experiment was conducted at National Aquatic Breeding Centre (Beijing, China) where the experimental fish were produced. Two weeks prior to the experimental start, the spotted seabass (*Lateolabrax maculatus*) were moved to the recirculating aquaculture system, which was used in the experiment. During this acclimation period, fish were housed in one tank and fed a commercial extruded diet. Six 1000-L circular tanks were used during the experiment. Each tank was equipped with six air stones for aeration. The six tanks were connected to the same recirculation system. Tanks were randomly assigned to one of the PPQ treatments (3 replicates per diet). At the start of the experiment, 50 fish with a mean weight of 158.7 ± 0.12 g were stocked per tank. Fish were randomly distributed to the tanks. Water quality was measured every second day. Water temperature was on average 22 ± 0.9 °C and ranged between 20 and 24 °C. pH was on average 8.2 ± 0.07 and ranged from 8.2 to 8.5. Dissolved oxygen was on average 8.6 ± 0.5 mg/L and ranged between 8 and 9 mg/L. Ammonia nitrogen was below 0.1 mg/L. Fish were hand-fed to apparent satiation twice daily at 7:00 and 13:00 for 48 days. The amount of feed given per tank was recorded at each feeding moment. Additionally, 1 h after the start of feeding, uneaten pellets in the tanks were gently removed, dried to constant weight at 70 °C and reweighted. Feed intake of fish in each tank was calculated as the difference between the amount fed and the amount of uneaten diet recovered.

2.4. Sampling

Fish were fasted for 48 h before the experiment ended to ensure the gastrointestinal tract was completely empty. After this fasting on the last experimental day, fish were fed to apparent satiation the morning meal for 0.5 h. The sampling moments were 0.5, 2, 4, 6, 8, 10, 12, 16 and 24 h after the start of the last meal. Four fish per tank were randomly selected and anesthetized with tri-chlorobutanol (300 mg/L) at each sampling moment. Within 1 min after catching, blood was sampled from the dorsal vein. Afterwards, the fish was individually weighed and dissected to collect chyme from the stomach and intestine. Chyme samples from each fish were weighted and stored at −20 °C for further analysis. After

the last sampling moment (24 h), the remaining fish in each tank were batch-weighed and counted.

Faeces were collected daily during the last five weeks. The faeces were collected by syphoning approximately three hours after each feeding. After decanting excessive water, the faeces were frozen and stored at −20 °C. Throughout the experiment, daily samples were pooled per tank.

2.5. Chemical analysis

Chemical analysis for dry matter, crude protein, fat, ash and energy were carried out for samples of feed, stomach chyme and faeces according to AOAC (2006). Dry matter was measured by drying samples to a constant weight at 105 °C. Crude protein was determined following the Kjeldahl method (Kjeltec™ 2300 Unit, Foss, Denmark). Crude lipid was analysed after acid hydrolysis by Soxhlet extraction (Soxtex System 1043, Foss, Hillerød, Denmark). Ash was assayed through combustion at 550 °C for 6 h (CWF 1100 muffle furnace, Carbolite, UK). Gross energy was determined by bomb calorimetry (IKA C2000 Calorimeter, IKA, Germany). Yttrium (the inert digestibility marker, Yt) in the faeces and chyme was analysed using inductively coupled plasma atomic emission spectrometry at Guobiao Testing & Certification Co., Ltd. (Beijing, China). Plasma glucose was measured by enzymatic colorimetric method with the assay kit (Nanjing Jiancheng Bioengineering Institute, China, No. F006). Plasma α-MSH concentration was determined by radioimmunoassay (RIA) kit at Beijing Sino-UK institution of Biological Technology (China).

2.6. Calculations

The calculation of performance parameters are presented in Table 3. The amount of chyme in the stomach was regressed against time postprandial per diet using the exponential model with some modification (Elliott, 1972):

$$W_t = Ae^{-kt} \tag{8}$$

where W_t is the relative amount of chyme in the stomach at t hours postprandial; A is the amount of chyme in the stomach at zero hours postprandial, which is an estimation of the feed intake of the fish during the last meal; k is the stomach evacuation rate constant.

Since the amount of chyme in the stomach might differ between fish having different body weights, these relationships were estimated based on the relative chyme content parameters (i.e., chyme content per g body weight). The stomach evacuation relationships were estimated for both chyme expressed on a fresh and dry matter basis.

The apparent digestibility coefficient (ADC, %) of nutrients in the faeces was calculated by:

Table 2
The measured extrusion processing parameters of two batches of pellets.

Processing parameters	LW [a]	HW [a]
Preconditioning water content (wt /%)	22.0	28.0
Die temperature (°C)	141	141
Extrusion zone 1 (°C) [b]	98	94
Extrusion zone 2 (°C) [b]	118	120
Screw speed (HZ)	23.1	23.1
Die diameter (mm)	3.00	3.00
Electric current (Am)	22.0	19.0
Throughput (kg/h) [c]	55.8	49.1

[a] LW-pellets and HW-pellets are produced with respectively a low and high amount of water added to the meal mixture prior to extrusion. The added amount of water was 14% and 20%, respectively.

[b] Aimed temperature in barrel zone 1 (entrance side) and zone 2 (die side) was 100 and 120 °C.

[c] The throughput was measured on the wet basis.

Table 3
Fish performance calculation.

Parameters	Formula
Survival (%)	$Survival = 100 \times N_f/N_i$
Total weight gain (TWG, g)	$TWG = W_f - W_i$
Daily weight gain (DWG, g/d)	$DWG = [(W_f/N_f) - (W_i/N_i)]/t$
Specific growth rate (SGR, %/d)	$SGR = 100 \times (Ln(W_f) - Ln(W_i))/t$
Feeding rate (FR, %)	$FR = 100 \times FI/[(W_f + W_i + W_d)/2]/t$
Feed conversion ratio (FCR)	$FCR = FI/(W_f + W_d - W_i)$
Protein efficiency ratio (PER)	$PER = (W_f + W_d - W_i)/(FI \times DPC)$
Condition factor (CF, g cm^{-3})	$CF = 100 \times BW/BL^3$
Hepatosomatic index (HSI %)	$HSI = 100 \times LW/BW$
Viscera somatic index (VSI, %)	$VSI = 100 \times VW/BW$

W_f, final fish weight (g); W_i, initial fish weight (g); W_d, dead fish weight; t, length of experimental period (d); N_f, final fish number per tank; N_i, initial fish number per tank; BW, body weight of fish (g); BL, body length of fish (cm); FI, Feed intake per tank (g); DPC, dietary protein content (%); LW, liver weight (g); VW, viscera weight (g); N, fish number per tank.

$$ADC = 100 \times \left[1 - \frac{Dietary\ Y_t\ concentration \times Faecal\ nutrient\ concentrition}{Faecal\ Y_t\ concentration \times Dietary\ nutrient\ concentration}\right] \tag{9}$$

The ADC of protein and dry matter in the chyme was calculated as:

$$ADC = 100 \times \left[1 - \frac{Dietary\ Y_t\ concentration \times Chyme\ nutrient\ concentrition}{Chyme\ Y_t\ concentration \times Dietary\ nutrient\ concentration}\right] \tag{10}$$

The stomach relative water flux was calculated according to the method described by Harter et al. (2013) with some modifications. The relative water influx (RWF, mL/g of ingested DM) was calculated from the relative water content in the chyme minus the relative water content in the feed at each sampling moment divided by the relative ingested dry matter, using the following equation:

$$RWF = (RW - RW_i)/(DM_i/M) \tag{11}$$

where the relative water content (RW) was calculated from the chyme water content divided by its marker content at each sampling moment. RW_i was the relative water content in the feed. DM_i was ingested DM on the sampling day; M was the marker content of the ingested feed.

2.7. Statistical analysis

Statistical analyses were carried out using SPSS Statistics 25.0. All parameters were evaluated for normal distribution and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. These checks for normality and homogeneity of variance were done on the residuals of the respective statistical models used. If normality was not met, data were transformed to meet normal distribution. Relative water fluxes and HSI were cosine transformed. Relative intestinal chyme content and plasma glucose level data were square root transformed. Parameters measured at each time point were analysed for the effect of diet by *t*-test. Data measured at different time moments were analysed by 2-way ANOVA for the effect of diet, time and their interaction. Transformations failed to meet the requirement of normal distribution of water content in the stomach and intestine. These data were analysed at each sampling moment postprandial for the effect of diet by the Mann-Whitney *U* test. All the data were reported as mean value ± standard error (SE). $P < 0.05$ was considered statistically different. The graphics were drawn using GraphPad Prism 8.

3. Results

3.1. Physicochemical properties of experimental diets

The contrast between the two process conditions did not change the macronutrient composition of the diet (Table 4). Process conditions affected the hardness, water solubility, hydration time and floatability of intact pellets ($P < 0.05$; Table 4). LW-pellets were less hard than HW-pellets. The hydration time of HW-pellets was more than double that of LW-pellets. LW-pellets had higher water solubility than HW pellets (10.5 versus 5.7%). Similarly, the water solubility index of grounded pellets was higher at the LW treatment than at the HW treatment ($P < 0.05$; Table 4), but the relative differences were much smaller than the difference in pellet water solubility. The water absorption index and nitrogen solubility index of ground pellets were similar for both treatments ($P > 0.05$; Table 4).

3.2. Gastrointestinal chyme water content

At the first sampling moment (0.5 h postprandial), stomach chyme moisture content was already higher at the LW treatment than at the HW treatment and stayed equally different between treatments at 2 h postprandial ($P < 0.05$; Fig. 1 a). At the next sampling moment (4 h), stomach chyme moisture content was slightly higher at the HW than at the LW treatment. From 6 h postprandial onward, stomach chyme moisture contents were equal between treatments and declined slightly with time, from 72.9 to 68.1% between 6 h and 16 h postprandial (Fig. 1 a). At 24 h postprandial, no chyme was present in the stomach.

At 0.5 h postprandial, the intestines were still empty. The highest intestinal chyme moisture content was observed 2 h postprandial at both diets (Fig. 1 b) and was numerically higher at the LW- than at the HW treatment. This difference was also present at 4 and 6 h postprandial but was significant at these moments. Between 8 and 16 h postprandial, there was no difference between treatments in the intestinal chyme moisture content. At 24 h postprandial, LW-pellets had again a higher intestinal moisture content than the HW-pellets ($P < 0.05$). At this sampling moment, the amount of chyme collected in the intestine was small, which might explain the larger variability between samples (Fig. 1 b).

3.3. Stomach water flux

The two types of pellets affected the relative water flux into the stomach, but this effect of pellet type was dependent on the time postprandial, which is indicated by the interaction effect ($P < 0.01$). At 2 h postprandial, the influx of water in the stomach was larger in fish fed LW-pellets (2.58 mL/g DM) than those fed HW-pellets (1.86 mL/g DM). While at 4 h postprandial, the water influx in the stomach was higher at the HW treatment (2.77 mL/g DM) than at the LW treatment (2.28 mL/g DM) (Fig. 2).

Table 4
Physicochemical properties of whole and grounded pellet as affected by process condition.

	LW*	HW*
Whole pellets:		
Water solubility (%)	10.5 ± 0.26 [a]	5.7 ± 0.06 [b]
Hydration time (min)	19.92 ± 0.30 [a]	52.94 ± 0.11[b]
Hardness (N)	79.32 ± 1.51[a]	112.05 ± 4.31[b]
Expansion rate	1.43 ± 0.01	1.43 ± 0.01
Floatability (%)	100 ± 0.0 [a]	37 ± 1.0 [b]
Sinking velocity (m/s)	/	0.05 ± 0.01
Grounded pellets:		
Water absorption index (%)	302 ± 4.06	317 ± 3.70
Water solubility index (%)	15.5 ± 0.20 [a]	14.2 ± 0.08 [b]
Nitrogen solubility index (%)	18.5 ± 0.27	18.2 ± 0.04
Starch gelatinization degree (%)	87.0 ± 2.38	89.4 ± 2.22
Dry matter (g/kg)	943	945
Crude protein (g/kg DM)	462	462
Crude lipid (g/kg DM)	110	112
Crude ash (g/kg DM)	122	122
Gross energy (MJ/kg DM)	22.1	22.2

[ab] Means (± SE) within rows having a differed superscript differ ($P < 0.05$).
* LW, low water addition; HW, high water addition.

3.4. Gastrointestinal evacuation

At 0.5 h postprandial, no chyme was present in the intestine in both treatments, which indicates that gastric evacuation started somewhere between 0.5 and 2 h postprandial. At 24 h postprandial, the stomachs of fish at both treatments were empty. Thus between 16 and 24 h postprandial, the consumed feed during the last meal was completely transferred to the intestine. In the statistical analysis of the amount of chyme over time, the zero values at 24 h postprandial for the stomach and 0.5 h postprandial for the intestine were excluded.

On a fresh basis, the relative amount of chyme in the stomach (Fresh-SCW) was affected by the interaction effect of time postprandial and pellet type ($P < 0.05$; Fig. 3 a). For both pellet types, Fresh-SCW declined with time. Between 0.5 and 6 h postprandial, Fresh-SCW was higher in fish fed LW-pellets than those fed HW-pellets; thereafter, the opposite happened (Fig. 3 a). Per pellet type, the following exponential relationships between Fresh-SCW (%BW) and time (h) were estimated:

$$\mathrm{LW-pellets: Fresh-SCW} = 2.256\, e^{-0.102\, t},\ R^2 = 0.593.$$

$$\mathrm{HW-pellets: Fresh-SCW} = 1.690\, e^{-0.068\, t},\ R^2 = 0.335.$$

The estimated intercepts (amount of fresh chyme at $t = 0$) were different between both pellet types ($P < 0.05$), being higher for the LW-pellets. The stomach evacuation rate was higher in fish fed LW-pellets than in fish fed HW-pellets (0.102 and 0.068 h^{-1}; $P < 0.05$). In contrast to Fresh-SCW, the relative amount of chyme on a DM basis in the stomach (DM-SCW) was only affected by time postprandial ($P < 0.001$; Fig. 3 b). DM-SCW averaged over sampling moments and for each time point postprandial did not differ between LW- and HW-pellets ($P > 0.05$). Per pellet type, the following exponential relationships between DM-SCW (% BW) and time (h) were estimated:

$$\mathrm{LW-pellets: DM-SCW} = 0.774\, e^{-0.13t},\ R^2 = 0.662.$$

$$\mathrm{HW-pellets: DM-SCW} = 0.654\, e^{-0.11t},\ R^2 = 0.520.$$

On a DM basis, the stomach evacuation rate did not differ between both pellet types ($P > 0.05$). However, the intercept estimates that the DM feed intake during the last meal differed between both pellet types ($P < 0.05$). The amount of DM-SCW at t = 0 was 18% higher in fish fed the LW-pellets than in fish fed the HW-pellets.

On a fresh basis, the relative amount of chyme in the intestine (Fresh-ICW) was influenced by pellet type ($P<0.01$) and time postprandial ($P<0.001$) but not by their interaction effect (Fig. 3 c). Averaged over all sampling moments, fish fed LW-pellets had a higher Fresh-ICW than fish fed HW-pellets (0.436 versus 0.364% BW). Between 0.5 and 8 h postprandial, Fresh-ICW increased and peaked at 8 h postprandial in both treatments. This suggested that the amount of chyme that entered the intestine was larger than the sum of digestion and faecal discharge before 8 h postprandial. The relative amount of chyme in the intestine on dry matter (DM-ICW) was only affected by time postprandial ($P < 0.001$; Fig. 3 d). At every postprandial time point, the DM-ICW was similar for both pellet types. The pattern in DM-ICW with time postprandial paralleled the pattern of Fresh-ICW.

3.5. Kinetics of digestion in stomach

The apparent digestibility coefficient (ADC) of DM and crude protein in the stomach were only affected by the time postprandial ($P < 0.001$;

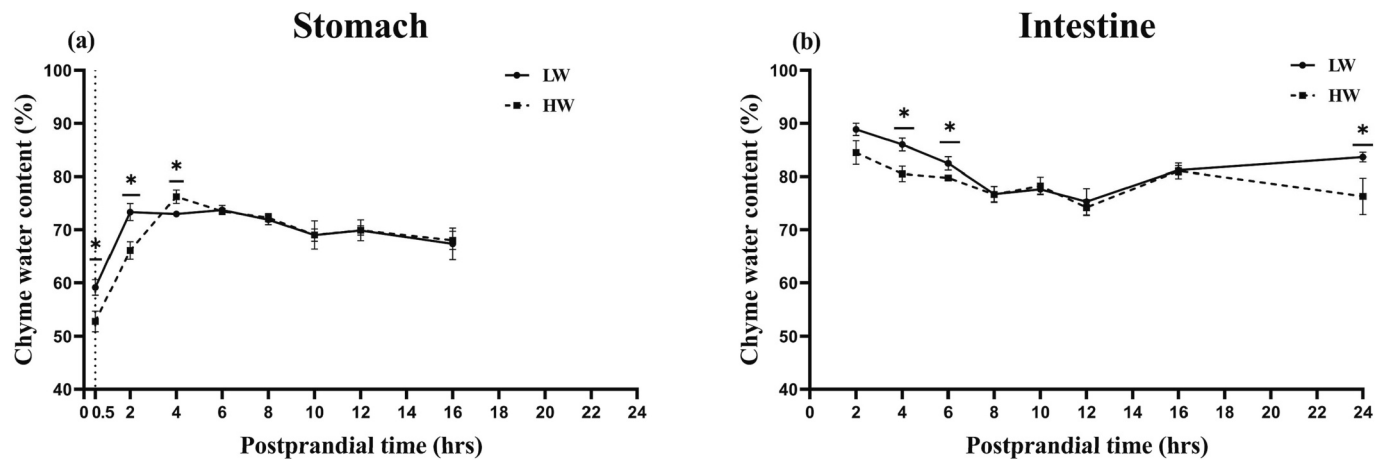

Fig. 1. Chyme water content in the stomach (a) and the intestine (b) as affected by pellet type. The different pellet types were created by a contrast in process conditions during extrusion: LW, low water addition; HW, high water addition. * Indicates a significant difference between treatments at the respective sampling moment ($P < 0.05$); Presented values are means ± standard error ($n = 12$).

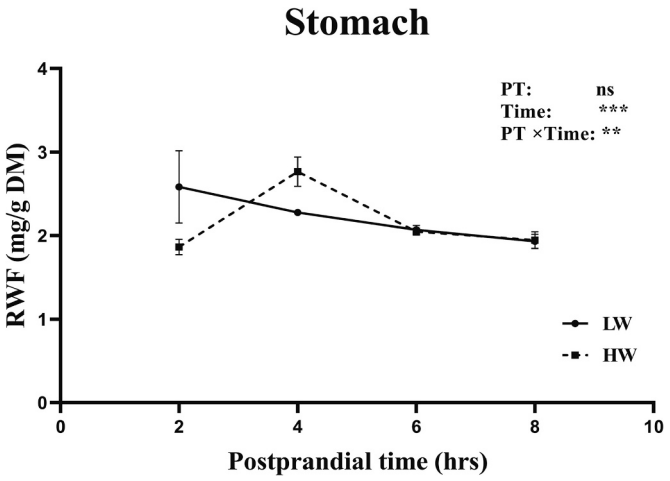

Fig. 2. Relative water flux (RWF) in the stomach as affected by time postprandial and pellet type. The different pellet types were created by a contrast in process conditions during extrusion: LW, low water addition; HW, high water addition. PT, pellet type; **, $P < 0.01$; ***; $P < 0.001$; ns, not significant; Presented values are means ± standard error (n = 3).

Fig. 4). At each sampling moment, the ADC of DM and protein were similar for both pellet types. The ADC of DM increased from 5.8 to 23.3% in LW-pellets, and 5.6 to 24.8% in HW-pellets. The ADC of CP increased from 9.1 to 32.6% in LH-pellets, and 7.7 to 33.5% in HW-pellets. Over the all the sampling moments per pellet type, the ADC of CP was higher than that of DM; 22.0% versus 16.0% in LW-pellets and 22.6% versus 16.8% in HW-pellets. This observation suggested that protein moved faster out of the stomach than dry matter.

3.6. Apparent digestibility coefficient of nutrients

The result of final digestibility showed that pellet type did not affect the ADC of dietary crude protein, energy, lipid and dry matter between treatments ($P > 0.05$, Fig. 5).

3.7. Growth performance, feed intake and morphology

Survival was high (> 98%) and unaffected by pellet type ($P > 0.1$; Table 5). The feed intake was also equal at both treatments ($P > 0.1$). Daily weight gain was numerically higher by 13% for fish fed LW-pellets than fish fed HW-pellet, but this difference was insignificant ($P > 0.1$). Fish fed LW-pellets had a lower feed conversion ratio (FCR) and a higher protein efficiency ratio (PER) than fish fed HW-pellets ($P < 0.01$). The condition factor and viscera somatic index were not influenced by pellet type, but the hepatosomatic index differed between pellet types ($P < 0.01$; Table 5). Fish fed LW-pellets had a 39% higher hepatosomatic index than fish fed HW-pellets.

3.8. Plasma glucose and α-MSH levels

Plasma glucose levels were influenced by pellet type ($P < 0.05$) and time postprandial ($P < 0.05$), but not by their interaction effect (Fig. 6 a). Fish fed LW-pellets had higher postprandial plasma glucose levels during all the sampling moments (13.3 versus 11.9 mmol/L). In fish fed LW-pellets, plasma glucose concentrations peaked at 0.5 h postprandial, while in fish fed HW-pellets the peak occurred later (at 2 h postprandial). With time after reaching this peak, plasma glucose levels decreased at both treatments ($P < 0.05$). However, even after 24 h postprandial fish were still in a hyperglycaemia state at both treatments as blood glucose levels were above 8.5 mmol/L.

Postprandial plasma α-melanocyte-stimulating hormone (α-MSH) was affected by the pellet type, but this difference was dependent on the time postprandial, indicated by the observed interaction effect ($P < 0.01$; Fig. 6 b). At 2 h postprandial, α-MSH concentrations were higher in fish fed LW-pellets than in those fed HW-pellets ($P < 0.05$). Thereafter a sharp decline of the α-MSH level was observed in fish fed LW-pellets from 19.7 to 13.2 pg/mL between 2 h and 8 h postprandial, while no noticeable drop was observed in fish fed HW-pellets during this time.

4. Discussion

In the present study, we produced two types of pellets with the same formula (a high fish meal diet). These two pellet types had contrasting physical pellet quality, created by changing the amount of water added to the meal mixture prior to extrusion. The higher water addition before extrusion led to higher hardness, longer hydration time and lower water solubility of feed pellets (Table 4). This observation suggested that for the high fish meal formula, sufficient moisture facilitated the intermolecular force and dense microstructure of the pellets (Kaliyan and Morey, 2009). Inconsistent with our study, Ma et al. (2022) reported that a higher moisture content generated a looser structure, such as lower pellet hardness and bulk density when using *Clostridium autoethanogenum* protein, soybean protein concentrate (SPC) and cottonseed protein concentrate (CPC) as the protein sources in the formula. In commercial production of sinking fish feeds, which apply formulas with

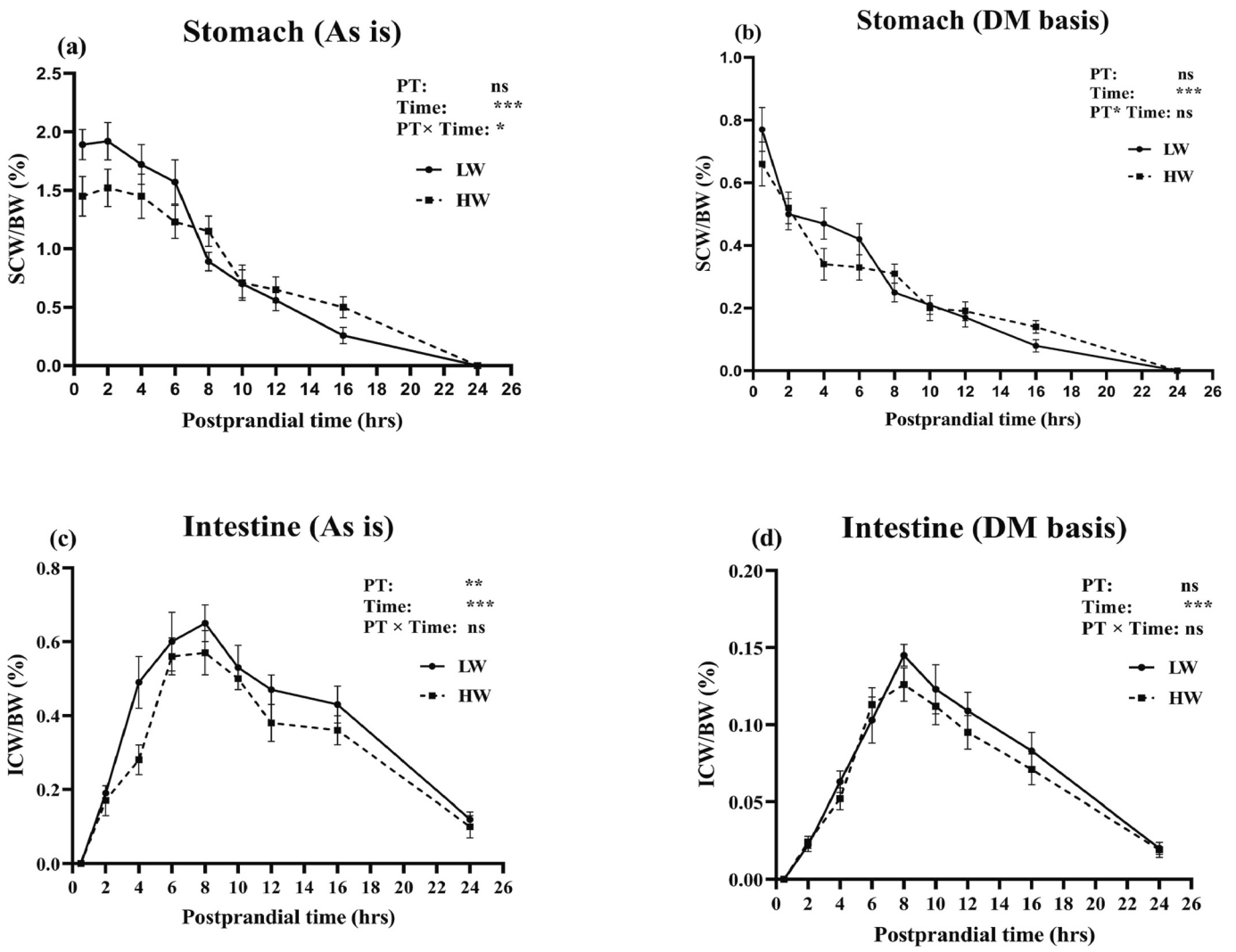

Fig. 3. Postprandial stomach relative chyme content on fresh basis (a) and dry matter basis (b) and postprandial intestinal relative chyme content on fresh basis (c) and dry matter basis (d) as affected by time postprandial and pellet type. The different pellet types were created by a contrast in process conditions during extrusion: LW, low water addition; HW, high water addition. SCW, stomach chyme weight; ICW, intestinal chyme weight; BW, body weight; PT, pellet type; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant; Presented values are means ± standard error (n = 12).

a low fish meal content and large amounts of soybean meal (42%) and other plant protein sources (e.g., SPC and CPC), the water content was negatively correlated with pellet hardness and water solubility, while being positively correlated with pellet hydration time (Wang et al., 2020). Contradicting results between studies may relate to the physiochemical properties of ingredients included in the formula. For example, plant and bacterial protein sources have a higher water absorption index and water-holding capacity than fish meal. Therefore, the influence of water addition on the pellet quality might be dependent on the feed ingredient composition and the physicochemical properties of the included ingredients. Different formulas may explain the observed variability in responses between studies, but also other factors like extruder configuration may have induced differences in responses regarding pellet quality between studies (Sørensen et al., 2010; Sørensen, 2012).

The low-water addition treatment resulted in higher electric current and throughput (on a wet basis) than the high-water addition treatment (Table 2). During feed production, the conditioned mash was fed to the extruder by a screw conveyor. The constant screw speed in both treatments led to the constant volume flow rate of the mixture from the conditioner into the extruder. Higher water addition resulted in a larger volume and lower density of the conditioned mash due to the water-absorbent swelling, thus reducing the mass flow rate and throughput. The friction between the feed mash, screw and barrel of the extruder was lower in the high-water addition treatment than in the low-water addition treatment due to lubrication and plasticization by water (Hayashi et al., 1992; Blanche and Sun, 2004). This resulted in reduced torque and a lower electric current in the treatment with more water addition to the meal mixture in the present study.

In this study, the differences in pellet characteristics led to differences in stomach chyme DM content inside the fish. The stomach chyme DM was lowest at 2 h and 4 h postprandial in fish fed LW- and HW-pellets, respectively. This observation regarding chyme DM suggested that pellet hydration time inside the fish relates to the pellet hydration time measured outside the fish. DM content in the stomach is also affected by factors like diet composition, feed pre-treatment (e.g., soaking) and processing technology (Aas et al., 2017; Elesho et al., 2022; Hilton et al., 1981). The current study shows that differences in physical pellet quality induced by process conditions can also influence the DM content in the stomach.

The observed difference in stomach chyme DM between the two pellet types was related to the water fluxes. The measured water fluxes into the stomach theoretically originate from moisture in the feed, water absorption into the pellets prior to being ingested, water drinking by fish

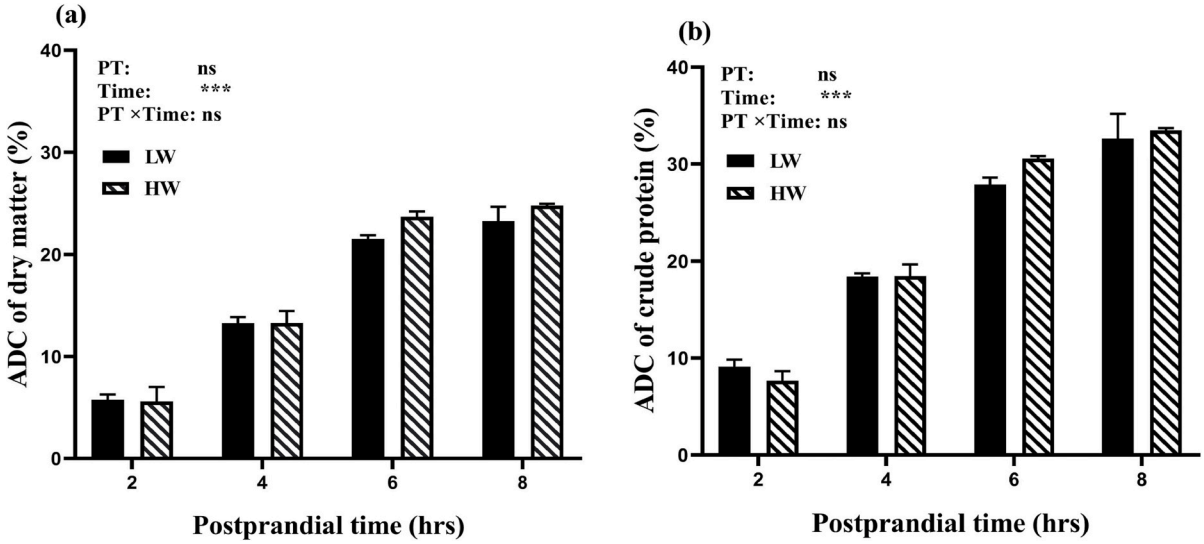

Fig. 4. The apparent digestibility coefficient (ADC) of dry matter (a) and protein (b) in the stomach as affected by time postprandial and pellet type. The different pellet types were created by a contrast in process conditions during extrusion: LW, low water addition; HW, high water addition. PT, pellet type; ***, $P < 0.001$; ns, not significant; Presented values are means ± standard error ($n = 3$).

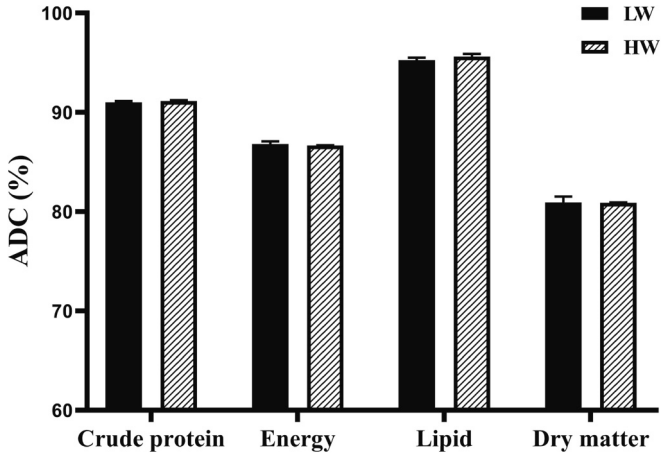

Fig. 5. The final apparent digestibility coefficient (ADC) of dietary protein, energy, lipid and dry matter as affected by pellet type. The different pellet types were created by a contrast in process conditions during extrusion: LW, low water addition; HW, high water addition. Presented values are means ± standard error (n = 3).

Table 5
Effects of pellet type on growth performance, feed utilization, condition score and organ indexes of spotted seabass (means ± SE, $n = 3$).

	LW*	HW*	*P-value*
Survival (%)	98.7 ± 1.33	98.0 ± 1.15	0.725
Final body weight (g)	225.9 ± 6.35	218.0 ± 2.84	0.324
Total weight gain (g)	3209 ± 405	2758 ± 76	0.343
Daily weight gain (g/d)	1.40 ± 0.13	1.24 ± 0.06	0.330
Specific growth rate (%/d)	0.73 ± 0.06	0.67 ± 0.03	0.331
Feed intake (g)	4493 ± 369	4455 ± 116	0.926
Feeding rate (%/d)	0.97 ± 0.06	0.99 ± 0.02	0.810
Feed conversion ratio	1.35 ± 0.02	1.53 ± 0.03	0.008
Protein efficiency ratio	1.61 ± 0.03	1.42 ± 0.03	0.008
Condition factor (g cm^{-3})	1.30 ± 0.02	1.26 ± 0.02	0.325
Hepatosomatic index (%)	2.31 ± 0.21	1.66 ± 0.08	0.003
Viscera somatic index (%)	10.35 ± 0.49	9.78 ± 0.28	0.318

* LW, low water addition; HW, high water addition.

and endogenous liquid secretion by the stomach wall (Kristiansen and Rankin, 2001). In the present study, the fish consumed the pellets within several seconds. Therefore, it can be assumed that pellet water absorption before being swallowed did not or only marginally contribute to the differences in water fluxes. Thus, most likely the differences in water fluxes in the stomach were either due to differences in drinking or fluid secretion in the stomach. Limited studies have investigated the relationship between water fluxes and diet composition. Harter et al. (2013) and also Elesho et al. (2022) found that African catfish fed a starch-rich diet had larger stomach water influxes than those fed a fat-rich diet. The former study also showed that the difference in water influx into the stomach between diets increased with time postprandial. The measured stomach water flux is the net amount of water entering and leaving the stomach at a certain time. In addition to the stomach water influx, the water evacuation in the intestine also affects the stomach water flux. The present results showed a higher stomach water influx in fish fed LW-pellets than those fed HW-pellets at 2 h postprandial; this agrees with the differences in pellet hydration time. However, at 4 h postprandial, a higher stomach water flux was found in the HW-pellets group. This finding was unexpected and might suggest a faster water evacuation rate in the LW-pellets group. It is also surprising that stomach water influx decreased after the peak value (maximum value, Fig. 2) at both treatments. Two possible explanations are: firstly, water moved faster than yttrium from the stomach to the intestine; secondly, the amount of water evacuated from the stomach was larger than the water influx into the stomach. Overall, the present study shows that physical pellet quality can alter postprandial stomach water fluxes.

In freshwater trout, it is suggested that gastric evacuation starts at around 1 h postprandial only when the chyme DM content is <35–40% (Kristiansen and Rankin, 2001). Likewise, in salmon and European seabass, which were kept in marine water and fed extruded pellets, gastric evacuation started before 2 h and 4 h postprandial, respectively (Aas et al., 2017; Bonvini et al., 2018). In the present study, the gastric evacuation of both treatments started between 0.5 and 2 h postprandial. The gastric evacuation rate has been shown to depend on water temperature, fish species, fish size, feed composition, feeding regime and feed particle size (Azaza and Dhraief, 2020; Azaza et al., 2010; Hossain et al., 2000; Koed, 2001). The water temperature in this study was between 20 and 24 °C, which is within the preferred range of spotted seabass (12.7 to 26.3 °C). Since fish in both treatments were kept in the same system, water temperature (and all other water quality

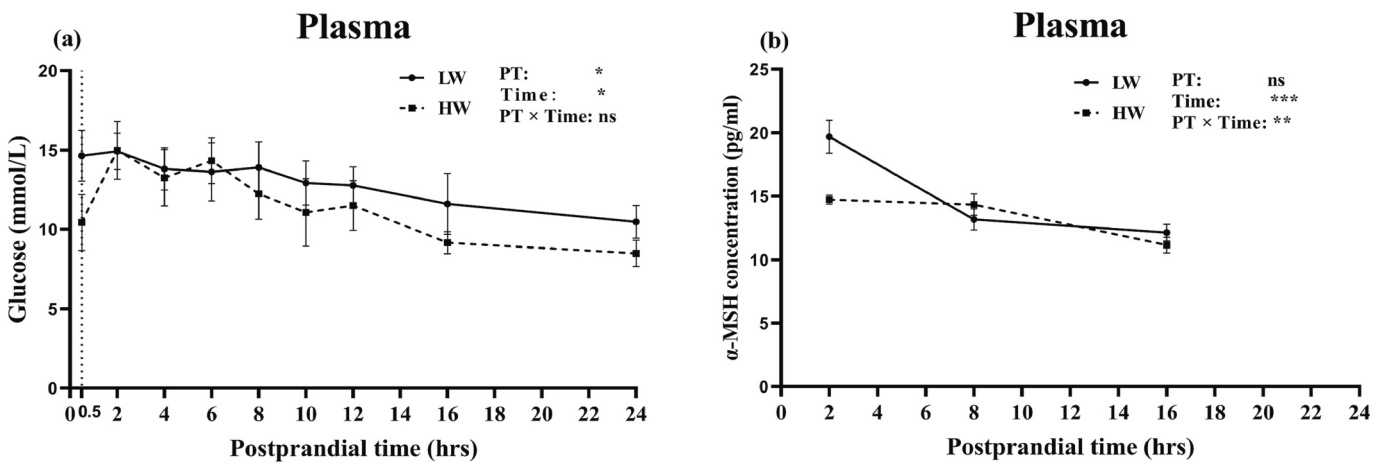

Fig. 6. Glucose (a) and α-MSH levels (b) in plasma as affected by time postprandial and pellet type. The different pellet types were created by a contrast in process conditions during extrusion: LW, low water addition; HW, high water addition. PT, pellet type; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. Presented values are means ± standard error ($n = 7$).

parameters) were equal and thus could not have caused potential differences between treatments (e.g., gut transit time). The current study demonstrated that treatments effects on chyme evacuation rate depend on the units in which chyme is expressed (DM versus fresh basis). On a fresh basis, differences in pellet quality affected the stomach emptying rate, whereas on a DM basis, evacuation rates were similar between treatments (Fig. 3). Meanwhile, there is a lag phase at the early stage of evacuation on a fresh but not on a dry matter basis; similar observations were also found in rockfish fed shrimp and crab (Hopkins and Larson, 1990). So far, some reported studies evaluated fish gastrointestinal evacuation on the DM chyme basis (Venou et al., 2003; Aas et al., 2017; Bonvini et al., 2018; Azaza et al., 2010; Gao et al., 2022), while others analysed it on the fresh chyme basis (Mazumder et al., 2020; Lee et al., 2000; Andersen and Beyer, 2005; Jia et al., 2021; Kim et al., 2018). In such cases, different evaluation methods can therefore lead to inconsistent results between studies. In our opinion, gastric evacuation on a fresh basis reflects the physiological condition as it occurs throughout the GIT and thus is the most appropriate method to study the effect of PPQ on digestive physiology. A faster gastric evacuation rate of fresh digesta in fish fed LW-pellets implies that the LW-pellets generated a faster water evacuation throughout the GIT than the HW-pellets. In general, the present study highlights that physical pellet quality can affect fresh digesta and water evacuation throughout the GIT but does not alter the DM evacuation rate.

Macro-nutrient absorption is not expected to take place in the stomach (Uys and Hecht, 1987). We noticed the positive and with time postprandial increasing disappearance of DM and CP from the stomach between 2 and 8 h after feeding, indicated by the measured ADCs in the stomach (Fig. 4). This observation implies that DM and CP were evacuated from the stomach faster than the inert marker yttrium oxide. Similarly, Aas et al. (2017) found that yttrium and nutrients were transported at different rates through the GIT. Next to this difference in evacuation rates between nutrients and marker, various types of markers also moved at different rates through the GIT (e.g., yttrium oxide versus ytterbium oxide) (Hatlen et al., 2015; Bucking and Wood, 2006; Warner et al., 2014; Brand and Morgan, 1975). Averaged over both treatments, a higher ADC of CP than DM was observed (Fig. 4), which suggests a faster stomach evacuation of CP than that of DM. This might relate to the hydrolysis of protein into smaller peptides or amino acids by pepsin in the stomach, leading to a higher water-soluble fraction of crude protein. As discussed above, water was evacuated faster than DM and consequently, the water-soluble protein fraction evacuated with water at a fast rate. This hypothesis is supported by the increasing difference in stomach ADC between CP and DM with time postprandial (Fig. 4). A higher stomach ADC for CP than DM has also been found in salmon and African catfish (Aas et al., 2017; Elesho et al., 2022). In the current study, pellet type did not affect stomach chyme DM and CP digestibility during 2–8 h postprandial. This observation is in line with Aas et al. (2017) that soaking pellets did not affect the postprandial kinetics of digestion in salmon. Only a few studies have addressed the topic of digestion kinetics in different gut segments of fish. Elesho et al. (2022) found that dietary protein sources (conventional FM versus hydrolyzed FM) and energy sources (starch versus fat) affected stomach DM and CP digestibility in African catfish at 4 h postprandial. This suggests that compared to physical pellet quality, dietary ingredients and macro-nutrients have a greater influence on the kinetics of digestion. Overall, the kinetics of nutrient digestion were unaffected by the pellet type in the current study, though nutrients ADC of the stomach were slightly higher at postprandial 6 and 8 h in fish fed HW-pellets than in those fed LW-pellets, but this did not affect the final faecal digestibility values.

Amirkolaie et al. (2006) found a negative correlation between chyme DM content in the stomach and the final ADC of macro-nutrients (protein and fat) in freshwater Nile tilapia. This result is not in accordance with the current study, where the difference in the stomach and intestinal chyme DM content between 0.5 and 6 h postprandial (Fig. 1) did not result in differences in the final ADC of macro-nutrients (CP, fat and energy; Fig. 5) digestibility. Possible explanations for the differences between studies could be the difference in fish species (tilapia versus spotted seabass), feeding strategy (continue belt feeding versus a single meal), feeding level (restrictive versus satiation), feed processing method (steam pelleting versus extrusion) and dietary nutrient/ingredient content, e.g., differences in starch sources and levels used in the former study. In line with the results of the present study, Oehme et al. (2014) and Aas et al. (2020) claimed that pellet quality characteristics did not affect the final ADC of DM, CP, energy and fat in salmon.

In fish, α-MSH is an appetite-suppressive peptide that is derived from proopiomelanocortin. It acts via binding to melanocortin-4 receptors (MC4R) in the hypothalamus (Leder and Silverstein, 2006; Schwartz et al., 2000; Volkoff, 2016). The higher plasma α-MSH levels at 2 h postprandial observed in fish fed LW-pellets than those fed HW-pellets (Fig. 6) could be related to the higher amount of fresh digesta in the stomach at this moment (Fig. 3 a). At this time, the amount of chyme DM in the stomach was similar between the two treatments. This might suggest that the amount of stomach fresh chyme rather than the amount of DM influenced the anorexigenic hormone level of α-MSH in spotted seabass. In other words, pellet quality characteristics regarding water absorption and hydration time may have an impact on feed intake response of fish. However, fish feeding behaviour is regulated jointly by

the central nervous system and peripheral organs, and it involves many types of peptide secretion, neural and endocrine pathways and signal transmissions (Liang et al., 2019; Volkoff, 2016; Volkoff and Peter, 2006). The impact of pellet quality characteristics on feed intake regulation in fish requires further investigation. Despite the difference in α-MSH, feed intake averaged over the experimental period was unaffected by pellet type in the current study. The absence of an effect of pellet quality characteristics on feed intake has been found in studies in salmon and trout (Aas et al., 2021; Baeverfjord et al., 2006; Oehme et al., 2014).

In the current study, both final body weight and growth rate were not significantly different between the two pellet types. This seems in line with long-term growth trials in salmon (>3 months), where contrasting physical properties of pellets did not affect growth (Aas et al., 2020; Bogevik et al., 2021; Oehme et al., 2014). However, in our present study, the numerical growth rate was 13% higher for fish fed LW-pellets than for fish fed HW-pellets. The absence of significant differences might be due to the relatively short duration of the current experiment (7 wk) with the consequence that fish weight did not double their weight during the experimental period. Even though growth and nutrients digestibility were not statistically affected, FCR and PER were significantly influenced by pellet type (Table 5). This indicates that differences in pellet quality led to variations in feed utilization. The improvement in feed utilization might be related to a faster fresh chyme evacuation throughout the GIT by shortening pellet hydration time. In other words, rapid fluid evacuation and water turnover facilitate the post-absorption of nutrients, and the digestion and absorption of water-soluble vitamins and minerals. In humans, the rate at which ingested fluid is evacuated from the stomach to the intestine will affect the rate of absorption (Leiper et al., 2000). In fish, this assumption needs further studying. Another plausible reason could be that the contrast in pellet processing led to the modification of macro-nutrients, such as dietary starch gelatinization and dextrinization, which alter the bioavailability of carbohydrates. The degree of starch gelatinization in LW- and HW-pellets were 87.0% and 89.4%, respectively. However, the starch gelatinization degree was determined by the amyloglucosidase hydrolysis method in this study, which also includes the dextrinized starch. Therefore, the difference between gelatinized and dextrinized starch cannot be distinguished by this method. Starch dextrinization is another stage beyond gelatinization when the feed is produced under higher temperatures and low moisture conditions. Dextrinization breaks down and rearranges the starch molecules, resulting in increased solubility and lower viscosity (Shrestha and Halley, 2014). Studies have shown that the water absorption index (WAI) and water solubility index (WSI) positively relate to starch gelatinization and dextrinization, respectively (Vijayagopal, 2004; Singh and Muthukumarappan, 2016; Bortone, 2004; Van den Einde et al., 2003). In the present study, WAI was similar between treatments, while WSI was higher in the LW-pellets, suggesting that more starch dextrinization occurred in the LW-pellets. In addition, the observed differences in plasma glucose levels (Fig. 6) and liver weights (Table 5) were further evidence that LW-pellets contain more bioavailable polysaccharides, further supporting the assumption of starch dextrinization. Impacts of the feed processing method on liver weights were also reported in trout (Hilton et al., 1981). Trout fed with pellets produced by extrusion had a higher liver weight than those produced by steam pelleting (2.2% versus 1.6%). In general, extrusion processing conditions not only affected the physical pellet quality, but also altered the bioavailability of nutrients. This implies that in future nutrition research, process conditions in combination with physical pellet characteristics should be considered and reported for a better interpretation of the obtained results.

5. Conclusion

The current study shows that physical pellet quality can affect stomach water fluxes, water evacuation throughout the gastrointestinal tract and feed utilization in spotted seabass. In addition, physical pellet quality potentially affects fish feed intake regulation and metabolic responses. Feed intake and digestion kinetics were not affected by the current contrast in physical pellet quality created in this study.

CRediT authorship contribution statement

Shujuan Xing: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Xiaofang Liang:** Conceptualization, Methodology. **Hao Wang:** Methodology, Software. **Xiaoze Xie:** Resources, Methodology. **Peter A. Wierenga:** Conceptualization, Supervision, Writing – review & editing. **Johan W. Schrama:** Conceptualization, Supervision, Writing – review & editing. **Min Xue:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Data availability

Data will be made available on request.

Acknowledgments

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