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# Physical feed quality and starch content causes a biological response in Atlantic salmon (*Salmo salar* L)

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ABSTRACT

The aim of this study was to investigate the effect of starch content and pellet physical quality on the biological response in Atlantic salmon. Salmon feeds with a large variation in physical quality were produced by the adjustment of dietary starch inclusion (92 g $kg^{-1}$ or 64 g $kg^{-1}$), preconditioner oil (0.0 or 11.0 kg $h^{-1}$), extruder moisture addition (13.5 or 27 kg $h^{-1}$) and drying temperature (38 °C or 70 °C). The variables impacted physical quality for the 12 feed codes and with hardness S (standing) and Durability measured in the range of 39–212 N and 39–94 %, respectively. Gently dried feed (38 °C) was harder and more durable than conventionally dried (70 °C). This could be explained by the phase transition theory and drying in the mobile rubbery phase above the glass transition temperature ($T_g$) for the gently dried feed codes. All feed codes were evaluated in Atlantic salmon performance and digestibility trials. There was a significant difference between the codes for weight gain (%; $P < 0.001$), specific and thermal growth rate ($P < 0.01$) and protein efficiency ratio ($P < 0.01$). Growth was negatively associated with increased starch content, hardness and durability parameters and reduced solubility, fat content and gross energy. The most important physical properties affecting growth performance were Hardness S, Durability and Doris dust, parameters that may represent feed hydration- and dissolution rates. This study documents that the impact of pellet physical quality should be considered when evaluating the results from biological fish trials.

## 1. Introduction

Extruded feed used in modern aquaculture production must be of a consistent and high physical quality to avoid crushing during transport, bulk storage and pneumatic feeding (Aas et al., 2011; Aarseth et al., 2006). A pellet quality that is optimal for handling may however not optimise the biological response in the fish. Physical product quality can be described by its hardness, durability, and water stability (Sørensen, 2012). Jacobsen et al. (2018) found that hardness of extruded pellet was negatively correlated to feed intake and growth of Atlantic salmon (*Salmo salar*). In another study however, the feed intake in salmon was higher in pellets with low and high hardness compared to intermediate hardness, indicating that combined factors and interactions with process parameters play a role (Aas et al., 2020). Bogevik et al. (2021) concluded that pellet who disintegrated slowly in water prolonged the gastric evacuation rate, and reduced feed intake in Atlantic salmon. This is in line with other published studies that have concluded that dry feed passed the gut slower than either soaked or less water stable feeds, and that soaked feed gave a significantly higher feed intake (Aas et al., 2011b, 2017, 2021; Oehme et al., 2014). It was also reported that less durable pellet with low water stability causes oil separation and accumulation of oil in the stomach of rainbow trout (*Oncorhynchus mykiss;* Baeverfjord et al., 2006). Hilton et al. (1981) concluded that trout fed extruded feed had prolonged gastric emptying, lower weight gain but higher feed efficiency compared to a steam pelleted feed. The effect was attributed to a higher physical quality, water stability and adsorption capacity of the extruded feed. The carbohydrate bioavailability also increased. This may be a result of increased starch gelatinization in the extrusion process and the fact that gelatinized starch is more easily digestible than native starch (Kanmani et al., 2018; Romano and Kumar,

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2019)

Starch in salmon feed is primarily used to facilitate binding (Sørensen et al., 2010; Kanmani et al., 2018; Romano and Kumar, 2019). Salmonids have a limited capacity to digest starch (Krogdahl et al., 2004), and energy originating from starch brings less digestible and metabolic energy than from lipid sources (National Research Council (NRC, 2011). Due to these metabolic limitations in the utilization of starch the feed manufacturing industry reduces the inclusion level of starch sources to a minimum without compromising physical feed quality. A minimum inclusion of carbohydrate in diets for carnivorous fish species is required to optimise the use of protein and lipids for the promotion of growth (Hemre et al., 1995, 2002). The carbohydrate level has been reported to be on average 11 % (including NFE and crude fiber; Aas et al., 2019) in salmon feeds, with whole wheat as the main starch source (8.9 %). Pea (0.8 %), tapioca and other carbohydrate sources has also been used (Aas et al., 2019; Ytrestøyl et al., 2015).

The extrusion of fish feed is a thermomechanical process involving starch gelatinization, protein plasticization and texturization of the dry feed mix and is the preferred technology for the manufacturing of highly durable and expanded feeds. Physical pellet quality and expansion are mainly controlled by water and steam addition, and viscous dissipation of mechanical energy (heat) (Samuelsen and Oterhals, 2016). The physicochemical and rheological properties of the feed ingredients also impact physical feed characteristics. (Sørensen et al., 2009; Glencross et al., 2010; Kraugerud et al., 2011; Samuelsen et al., 2014; Samuelsen and Oterhals, 2016). Lipids can be added in the feed mix or preconditioner step prior to extrusion. Due to its lubrication effect, a total lipid content should not exceed 120 g $kg^{-1}$ (Rokey, 1994). Above this content poor physical pellet quality can be a consequence due to reduced viscous heat dissipation (friction) in the extruder barrel. For high-energy salmon feed most of the lipid therefore must be coated onto the dried feed by use of vacuum coating technology (Strauch, 2005). The wet extrudate from the extruder is dried to prevent mould and bacteria growth and to fix the final porous structure prior to vacuum coating. The most used dryers in fish feed processing are continuous conveyor dryers where the air flows transversely through the product bed in separate zones with the lowest air temperature in the outlet zone (Rokey, 1994). Product depth, air flow, temperature, humidity, and residence time can be adjusted and will impact the final product characteristics. If the drying rate at the pellet surface is too fast, tensile stress may occur due to shrinkage of the inner core resulting in reduced physical pellet quality (Haubjerg, 2016).

The objectives of this study were to (1) produce salmon feeds with a large variation in physical feed quality by adjustment of starch content, preconditioner oil- and extruder moisture addition and drying temperature, (2) investigate the effect of dietary starch content and pellet physical quality on the biological response in Atlantic salmon and (3) discuss the most important pellet physical quality parameters affecting the biological response.

## 2. Materials and methods

### 2.1. Experimental diets formulation and processing

Two experimental diets were formulated according to current commercial specifications expected to meet the nutritional requirement for Atlantic salmon (*Salmo salar* L; Table 1). The diets had either high (HS) or low pea starch (LS) content with target content of 92 and 64 g $kg^{-1}$ starch respectively, 360–385 g $kg^{-1}$ crude protein, 320 g $kg^{-1}$ crude fat and 24.5–24.6 MJ $kg^{-1}$ gross energy (GE). The inclusion of fishmeal, soy protein concentrate, sunflower presscake meal and wheat gluten were increased relative to the reduction in pea starch from the HS to the LS diets (Table 1). The 5% pea starch (as is) was replaced with 5% sum of protein raw materials, and the protein raw materials were added at the same ratio to each other. This was performed in order to have a constant fat content in the feed mix and feed and similar GE. The protein was therefore slightly lower in in the high starch diet (not isonitrogenous diets). Micro-nutrients were formulated according to fish requirement at given size. Increased inclusion of especially soy protein concentrate can reduce the nutritional value of the feed and impact physical quality. (Barrow et al., 2007; Morken et al., 2012; Samuelsen et al., 2018). The latter is however depending on the used moisture content and achieved temperature in the extrusion process (Samuelsen et al., 2018). The increase in SPC is 15 g $kg^{-1}$ and considered to have minor impact on the responses in this study. Yttrium oxide ($Y_2O_3$) was added as an inert marker for digestibility determination. The two diets were conditioned in an atmospheric double differential preconditioner (DDC; Wenger Manufacturing Inc., Sabetha, KS) prior to extrusion on a TX-52 co-rotating, fully intermeshing twin-screw extruder (Wenger). Six experimental codes were produced by varying oil addition at preconditioner outlet or steam/water (moisture) level in the extruder (Table 2). A standard shaft speed in the preconditioner (220 rpm) and extruder (400 rpm) were used in all the experiments. The extruder screw (Samuelsen

**Table 1**
Formulation of the two experimental diets with high (HS) and low (LS) starch content (g $kg^{-1}$ diet, as is).

| Diet code | HS | LS |
|---|---|---|
| Fishmeal[a] | 268 | 292 |
| Soy protein concentrate[b] | 165 | 180 |
| Pea starch[c] | 150 | 100 |
| Sunflower presscake meal[d] | 108 | 118 |
| Wheat gluten[e] | 18 | 20 |
| Fish oil[f] | 283 | 280 |
| Monocalcium phosphate[g] | 5.2 | 3.7 |
| Vitamin and mineral premix[d] | 3.2 | 3.2 |
| Amino acid mix (Lys, Met, Thr[g]) | 3.7 | 5.7 |
| Yttrium[h] | 0.5 | 0.5 |
| Carophyll Pink 10 % CWS[i] | 0.2 | 0.2 |
| Water change | −4.4 | −2.5 |

[a] NorsECO-LT, provided by Norsildmel a/l (Bergen, Norway).
[b] Purchased from EWOS (Florø, Norway).
[c] Provided by Agrimarin Nutrition (Stavanger, Norway).
[d] Purchased from BioMar (Karmøy, Norway).
[e] Provided by Syral Belgium N.V. (Aalst, Belgium).
[f] NorSalmOil, provided by Norsildmel a/l (Bergen, Norway). Lipid content was standardized to 100 g $kg^{-1}$ in the feed mixes. The fish oil was mixed homogeneously into the feed mixes at least 24 h before processing to secure even partitioning and adsorption into the feed matrix.
[g] Provided by AS Norsk Mineralnæring (Hønefoss, Norway).
[h] Alfa Aesar GmbH & Co KG (Karlsruhe, Germany).
[i] G.O. Johnsen AS (Oslo, Norway).

**Table 2**
Processing parameters during preconditioning and extrusion of the two experimental diets[a].

| Sample code | 1HS | 2HS | 3HS | 4LS | 5LS | 6LS |
|---|---|---|---|---|---|---|
| Dry recipe rate (kg $h^{-1}$) | 150 | 150 | 150 | 150 | 150 | 150 |
| Conditioning | | | | | | |
| Steam (kg $h^{-1}$) | 11.6 | 12.0 | 12.0 | 11.7 | 11.6 | 11.6 |
| Water (kg $h^{-1}$) | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 |
| Fish oil (kg $h^{-1}$) | 0.0 | 0.0 | 11.0 | 0.0 | 0.0 | 11.0 |
| Moisture (%)[b] | 19.8 | 20.0 | 18.8 | 19.7 | 19.6 | 18.4 |
| Temperature (°C) | 89 | 84 | 87 | 90 | 88 | 89 |
| Extrusion | | | | | | |
| Steam (kg $h^{-1}$) | 6.0 | 3.0 | 3.0 | 3.0 | 6.0 | 3.0 |
| Water (kg $h^{-1}$) | 21.0 | 10.5 | 10.5 | 10.5 | 21.0 | 10.5 |
| Temp. behind die (°C)[b] | 142 | 144 | 133 | 146 | 143 | 136 |
| SME (Wh $kg^{-1}$)[b] | 18.8 | 27.8 | 19.8 | 25.7 | 20.0 | 20.2 |
| Moisture in extrudates (%)[c] | 27.7 | 20.7 | 20.8 | 21.7 | 27.3 | 20.9 |

SME, Specific Mechanical Energy.
[a] Diets, high (HS) and starch low (LS) as in Table 1.
[b] Calculated according to Riaz (2000) by use of Wenger Extruder Analysis Software (Wenger Manufacturing Inc., Sabetha, KS, USA).
[c] Analysed on a HG53 Halogen Moisture Analyzer (Mettler Toledo, Oslo, Norway) during processing.

and Oterhals, 2016) was adapted for the production of high energy salmon feed and the outlet restricted by two circular 7.0 mm dies. The wet extrudates were cut at the die surface by a 3-blade knife assembly to an equal length. The extrudate were dried to a water content of approximately 80 g $kg^{-1}$, either (1) Conventionally in a hot air dual layer carousel pilot dryer (Model 200.2, Paul Klöckner GMBH, Nistertal, Germany) at constant air temperature of 70 °C (drying time <1.5 h; DH) and (2) Gently in a tray dryer (FUNK 1, Hammerun, Denmark) at constant air temperature of 38 °C (drying time >16 h; DG). The dried pellets were coated with fish oil (45 °C) in a Dinnissen vacuum coater (VC 60 ltr, Sevenum, Netherlands). To avoid floating feed or fat leakage the coated fish oil content had to be varied. The pellet samples were cooled to room temperature and stored in sealed paper bags with plastic lining at 18 °C for further analysis and growth experiment. The achieved proximate chemical composition and GE for the 12 experimental feed codes are found in Table 3.

### 2.2. Chemical analyses

Dry matter (DM) was measured gravimetrically after drying at 103 ± 1 °C (ISO, 6496). Crude protein (N x 6.25) was analysed by the Kjeldahl method (ISO, 5983-2). Lipid content was determined gravimetrically after light petroleum extraction (EU, 98/64/EC) and according to Folch et al. (1957) where lipids were extracted with chloroform/methanol.

Total ash content was determined by combustion of organic matter at 550 °C and gravimetric measurement of the residue remaining (ISO, 5984). Total starch and degree of starch gelatinisation were measured utilising a modification of the glucoamylase methodology described by Chiang and Johnson (1977). Glucose concentration was determined using immobilized glucose oxidase to form hydrogen peroxide which was quantified electrochemically in an YSI 2700 SELECT Biochemistry Analyser (YSI Inc., Ohio, USA). Total starch was determined after total gelatinization with sodium hydroxide (Samuelsen and Oterhals (2016). All chemical measurements were based on averages of duplicate analyses. Yttrium was measured by inductivity coupled plasma (ICP) mass-spectroscopy, as previously described by Refstie et al. (1997). Gross Energy (GE) was determined in a Parr adiabatic bomb calorimeter (ISO, 9831).

### 2.3. Analysis of extruded pellet properties

Diameter and length were measured on coated feed with an electronic calliper and based on averages of 30 pellets. Hardness (peak breaking force) was measured on coated feed by use of a texture analyzer (TA-XT2, Stable Micro Systems Ltd, Surrey, UK) and the peak force before breakage expressed in Newton (N). The speed of the load arm was set to 1 mm/s and the penetration depth 50 % of the diameter or length for laying (Hardness L) and standing (Hardness S) pellet, respectively. The load arm was equipped with a cylindrical flat-ended probe (50 mm diameter). Pellets were treated individually, and reported values based on the average of 20 analyses. The Kahl hardness were measured by use of a motorized Kahl Pellet Hardness Tester (Amandus Kahl GmbH & Co., Reinbek, Germany) on coated laying pellets (Kahl L) and reported as the average of 10 pellets. Durability was measured on uncoated feed using a Holmen pellet tester (Holmen Feed Technology, Berkshire, UK), where a sieved (5.6 mm screen) 100 g pellet sample was conveyed around in a closed circuit by a high velocity air stream (21 m/s for 60 s). Durability was expressed as the weight-percentage of pellets retained on a 5.6 mm screen and based on the average of triplicate measurements. Durability was also measured in a Doris tester (AKVAsmart, Bryne, Norway) where a sieved (5.6 mm screen) 300 g pellet sample of coated pellet was transported in a screw conveyor to a rotating fan. Impact with the fan and the walls downstream the fan generates cracks and dust, which were measured using different screen sizes (5.6, 3.55, 2.35 mm) The following Doris parameters were determined: unbroken (5.6 mm), fracture (3.55−2.35 mm), and dust (<2.35 mm). Doris durability parameters were based on the average of triplicate measurements. Water stability (solubility) was determined utilizing a modified methodology described by Baeverfjord et al. (2006). Duplicate samples of each diet (25 g each) were placed in glass beakers filled with 200 ml distilled water. The beakers were incubated in a thermostat-controlled water bath (25 °C) and shaken (120/min) for 240 min, and the remaining amount of DM, fat and ash was determined after drying (18 h/105 °C).

### 2.4. Fish experiments and calculations

Atlantic salmon (*Salmo salar*) smolts (720 fish) were obtained from the nursery Sævareid in Fusa, Norway. The experimental fish were acclimatized post transportation during 4 weeks whilst they were fed a commercial diet (450−480 g $kg^{-1}$ crude protein; 280−300 g $kg^{-1}$ crude fat; 24.2 MJ $kg^{-1}$ GE). Following the acclimatization period, the fish were starved for 2 days and then counted and weighed in groups and distributed into 24 experimental tanks (2 × 2 × 0.9 $m^3$) in Nofima indoor tank facilities in Austevoll (60o05'N, 05o16'E), Norway, excluding the smallest and biggest individuals, ending with 30 fish per tank. As the aim for the research was to screen pellet with a large variation in physical pellet quality, 2 replicate tanks per diet was used to enable testing of all the 12 pellet codes. The initial fish mean body weight was 810−856 g and the duration of the experiment 6.5 weeks (47 days). The fish populations were fed using automatic feeders twice a day (05:00−08:00 and 14:00−03:00) in intervals of 10 s on and 145 s off feeding during the feeding periods, 7 days a week.

Unconsumed feed was collected daily using a continuous feed collection system (Hølland Teknologi AS, Sandnes, Norway), dried and weighed in order to record the actual feed consumption and regulate the daily feeding rations. Based on the amount of feed fed and uneaten feed collected, the amount of feed fed each day was adjusted to about 10 % in excess in order to cover fish appetite.

Constant illumination was used in the fish tanks. Seawater pumped from a depth of 50 m below sea surface level was supplied to the tanks at a rate of 80 l $min^{-1}$ (flow-through system). The water oxygen levels in the tanks were measured daily and oxygen concentration during the experiment was mostly more than 7.0 mg $l^{-1}$, but dropped to 6.5 mg $l^{-1}$ (70 % saturation) at the end of trial due to high water temperatures. The inlet water was heated up before introduced to the experimental tanks.

**Table 3**
Proximate chemical composition and gross energy of the experimental feeds.

| | 1HS-DH | 1HS-DG | 2HS-DH | 2HS-DG | 3HS-DH | 3HS-DG | 4LS-DH | 4LS-DG | 5LS-DH | 5LS-DG | 6LS-DH | 6LS-DG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DM (g $kg^{-1}$) | 947 | 938 | 944 | 943 | 947 | 944 | 950 | 937 | 945 | 939 | 945 | 938 |
| In DM | | | | | | | | | | | | |
| Crude protein | 422 | 432 | 404 | 397 | 398 | 393 | 427 | 432 | 432 | 458 | 421 | 425 |
| Lipid | 295 | 280 | 333 | 336 | 345 | 346 | 337 | 326 | 321 | 285 | 350 | 349 |
| Total ash | 69 | 69 | 66 | 65 | 64 | 65 | 67 | 68 | 69 | 72 | 67 | 67 |
| Starch | 100 | 99 | 91 | 92 | 92 | 92 | 65 | 67 | 71 | 70 | 65 | 65 |
| Gelatinized starch | 98 | 90 | 89 | 90 | 87 | 76 | 65 | 61 | 70 | 67 | 64 | 58 |
| Gross energy (kJ $g^{-1}$) | 24.0 | 23.5 | 24.5 | 24.4 | 24.7 | 24.7 | 24.8 | 24.1 | 24.3 | 23.4 | 24.8 | 24.5 |

Diets either dried "conventionally (hard)" at 70 °C (DH; drying time <1.5 h) or "gently" at 38 °C (DG; drying time >16 h).

The water temperature in the tanks was recorded daily and ranged from 8.7–11.8 °C throughout the experimental period (mean value 10.56 °C ± 0.74). Water salinity was stable at 32 g $l^{-1}$.

At the end of the experiment, feces samples were collected from regularly fed fish for determination of the apparent digestibility coefficient of dietary protein, lipid and energy. Twenty fish from each tank were stripped and all feces samples were pooled into one sample per tank. Ethoxyquin (400 mg $kg^{-1}$ dry matter; Mundheim et al., 2004) was added as antioxidant to the feces, which were then mixed and immediately frozen and stored at −20 °C. Frozen feces were lyophilized (final plate temperature 24 °C) prior to chemical analyses. Following stripping, all fish in each tank were weighed individually. In order to reduce handling stress, prior to handling and stripping, fish were sedated with a 15 ml anaesthetic solution (50 % isoeugenol, 50 % ethanol) in 1.2 $m^3$ oxygenated water in the tanks and by a dose of benzocaine in smaller tanks (3 ml benzocaine in 20 $l^{-1}$ seawater).

All fish were sampled, and fork length and body weight measurements were taken individually for the determination of the condition factor (CF) of the fish, calculated as CF = fish weight (g) x fish fork $length^{-3}$ x 1000. Other on-growing performance data were calculated per tank: Feed intake (FI) was the mean feed consumption per fish per day as a % of the daily fish body weight (bw). The daily fish bw was calculated using daily SGR values equal to the overall SGR of each tank. Specific growth rate was SGR (%) = ($lnw_2$-$lnw_1$) x 100/ feeding days, where $w_1$ and $w_2$ are initial and final fish weights, respectively, and *ln* the natural logarithm. Feed conversion ratio (FCR) was feed consumed/biomass increase. Specific feed ratio (SFR) was FCR x SGR. Feed efficiency (FE) was biomass increase/feed consumed. Thermal growth coefficient was TGC = ($w_2^{1/3}$- $w_1^{1/3}$) x 1000/ ∑(t x feeding days), where ∑(t x feeding days) is the sum of water temperatures (°C) for every feeding day in the experiment. Protein efficiency ratio (PER) was fish weight gain/protein consumption. Daily growth rate (DGR) = ($w_2$-$w_{1)}$/days, that is weight gain (g/fish) divided by feeding days. Apparent digestibility coefficient protein was ADC = (1-((protein feces/yttrium feces)/(protein feed/yttrium feed)))*100. Apparent digestibility coefficient fat was ADC = (1-((fat feces/yttrium feces)/(fat feed/yttrium feed)))*100. Apparent digestibility coefficient gross energy was ADC = (1-((GE feces/yttrium feces)/(GE feed/yttrium feed)))*100.

### 2.5. Statistical analyses

The results were analysed by one-way analysis of variance (ANOVA). The significant difference between means ($P < 0.05$) were determined using Tukey's multiple range test. Correlation between proximate chemical composition, significant physical pellet quality and on-growing performance parameters were carried out using the Pearson correlation coefficient procedure. Statistical analyses were performed using STATISTICA v13.5 (StatSoft, Inc. Tulsa, USA). To assess the data structure of the responses, Principal component analysis (PCA) was performed. The PCA (Martens and Martens, 2001) were carried out using Unscrambler 10.5 (Camo, Oslo, Norway) on mean centred and standardized variables.

## 3. Results

### 3.1. Feed processing

The aim for the production was different physical feed quality but with similar expansion, pellet length and fat adsorption capacity. Pellet with a combination of high oil and high moister level was therefore not produced due to a high deviation from these criteria. To avoid floating feed or fat leakage, oil adjustment in the vacuum coating process had to be performed resulting in deviation in proximate lipid content between the pellet codes (Table 3) and with GE content positively correlated to the lipid content ($r^2 = 0.87$).

### 3.2. Pellet physical quality responses

The different treatments gave a large span in physical feed quality responses. All responses were significantly different for the experimental codes except for solubility DM, fat and ash (Table 4). In the PCA loading plot visualization (Fig. 1), where principal component (PC) 1 accounts for 46 % and PC2 22 % of the variance, the PC1 primarily explain the variation in physical pellet quality parameters and solubility data, were a harder and more durable pellet affected solubility data negatively. All pellet hardness and durability data were highly explained and clustered to the left in the plot i.e. positive associated. Doris fracture and dust and solubility data were also highly explained and clustered to the right in the plot together with the drying temperature. To the right preconditioner oil addition is also found, however poorly explained by PC1 and PC2. This shows that drier conditions had the most prominent effect on the pellet responses. Hardness S and L, Kahl L, Durability and Doris unbroken were significantly affected by the drying conditions ($r^2 = 0.87$ to $r^2 = 0.45$) were pellets dried gently (DG) gave harder and more durable pellet compared to conventionally dried. Pellets dried conventionally (DH) produced significantly higher Doris fracture and dust compared to gently dried ($r^2 = 0.63$ and 0.44, respectively). Oil addition in the preconditioner affected pellet quality negatively (Fig. 1) and for the conventionally dried pellet, oil addition gave the feed codes with the lowest values of Hardness S and Durability (3HS-DH and 6LS-DH). At the same dryer condition, the high starch content gave the strongest pellet (1 and 2HS-DH, Tables 3 and 4). Moisture level is poorly correlated to physical pellet data (Fig. 1). Diameter is mainly explained by PC2, where the pea starch inclusion affected this parameter positively ($r^2 = 0.46$). Diameter is also negatively associated with the moisture level in the extruder. By extruder knife speed adjustment pellet length were tried to be kept constant however some variations were observed (Table 4).

All pellet samples were properly cooked with high degree of starch gelatinization (94 % ± 5.1 of total starch content). Even low variation (uncertainty for the analytical method is ±4.5 % at a starch gelatinization attainment of 96 %; Samuelsen and Oterhals, 2016) there is a tendency of improved gelatinization for the conventionally dried pellet (Table 3).

### 3.3. Fish performance

All experimental diets were well accepted by the fish. Feed intake (FI %bw/day) and specific feeding ratio (SFR) was good for all the diets and with zero mortality for all groups (Table 5). FI (FI%bw/day) was not significantly different between the codes but were positively correlated to the growth parameters (Table 7). There was a significant difference between the codes for weight gain (g/fish and %), specific and thermal growth rate (SGR and TGC), protein efficiency ratio (PER) and daily growth rate (DGR; Table 5). The above parameters were all positively correlated and also correlated to other measured fish performance parameters (Fig. 2A, Table 6).

The PCA loading plot visualize the main data structure for proximate chemical composition, physical pellet quality and on-growing performance parameters, with principal component (PC) 1 explaining 52 % and PC2 18 % of the variance (Fig. 2A). The on-growing performance parameters were mainly, and highly explained by PC1 and clustered to the left in the plot. Hardness and durability parameters were also highly explained and grouped in the upper right quadrant together with FCR, i.e., negatively associated with growth parameters. FCR were negatively correlated to GE ($r^2 = 0.50$), however there was no significant difference in FCR between the codes. Improved on-growing performance parameters were also associated with higher lipid content and GE combined with low starch content. Starch level is highly explained and grouped together with gelatinized starch and diameter in the lower right quadrant (Fig. 2A). In the PCA, score plot (Fig. 2B), along PC1, the low starch, conventionally dried codes 6LS-DH and 4 LS-DH showed best performance and the high starch, gently dried codes 1HS-DG and 2 HS-DG

**Table 4**
Pellet responses.

| | 1HS-DH | 1HS-DG | 2HS-DH | 2HS-DG | 3HS-DH | 3HS-DG | 4LS-DH | 4LS-DG | 5LS-DH | 5LS-DG | 6LS-DH | 6LS-DG | SEM* | *P*-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diameter (mm) | 8.7$^{e}$ | 8.5$^{de}$ | 9.7$^{f}$ | 9.5$^{f}$ | 8.5$^{d}$ | 8.4$^{cd}$ | 8.3$^{bcd}$ | 8.4$^{cd}$ | 8.1$^{b}$ | 7.8$^{a}$ | 8.2$^{bc}$ | 8.2$^{bc}$ | 0.03 | <0.001 |
| Length (mm) | 11.1$^{ab}$ | 11.3$^{ab}$ | 11.7$^{b}$ | 11.7$^{b}$ | 11.4$^{ab}$ | 11.7$^{b}$ | 10.8$^{a}$ | 11.0$^{ab}$ | 11.3$^{ab}$ | 11.2$^{ab}$ | 11.5$^{b}$ | 11.3$^{ab}$ | 0.04 | <0.001 |
| Hardness S (N) | 91.4$^{cd}$ | 146.7$^{f}$ | 111.9$^{e}$ | 212.3$^{h}$ | 46.0$^{a}$ | 107.7$^{de}$ | 71.8$^{b}$ | 145.6$^{f}$ | 69.6$^{b}$ | 167.6$^{g}$ | 39.2$^{a}$ | 84.0$^{bc}$ | 3.37 | <0.001 |
| Hardness L (N) | 47.3$^{b}$ | 96.6$^{e}$ | 47.2$^{b}$ | 77.4$^{d}$ | 35.0$^{a}$ | 76.9$^{cd}$ | 43.3$^{a\ b}$ | 85.2$^{d}$ | 33.4$^{a}$ | 101.3$^{e}$ | 35.5$^{a}$ | 66.5$^{c}$ | 1.41 | <0.001 |
| Kahl L (N) | 55.8$^{a}$ | 115.1$^{d}$ | 59.8$^{a}$ | 109.4$^{cd}$ | 44.2$^{a}$ | 92.6$^{b\ c}$ | 58.9$^{a}$ | 109.4$^{cd}$ | 44.4$^{a}$ | 123.7$^{d}$ | 43.3$^{a}$ | 84.3$^{b}$ | 2.88 | <0.001 |
| Durability (%) | 79.8$^{h}$ | 94.2$^{j}$ | 74.8$^{e}$ | 79.1$^{gh}$ | 53.9$^{b}$ | 80.6$^{h}$ | 68.6$^{d}$ | 77.3$^{fg}$ | 63.6$^{c}$ | 89.8$^{i}$ | 39.0$^{a}$ | 75.9$^{ef}$ | 2.46 | <0.001 |
| Doris unbroken (%) | 79.1$^{a}$ | 96.3$^{de}$ | 94.7$^{cd}$ | 98.8$^{e}$ | 79.2$^{a}$ | 97.8$^{e}$ | 92.7$^{c}$ | 98.5$^{e}$ | 78.5$^{a}$ | 99.0$^{e}$ | 83.1$^{b}$ | 96.8$^{de}$ | 1.39 | <0.001 |
| Doris fracture (%) | 17.2$^{f}$ | 2.0$^{ab}$ | 3.1$^{bc}$ | 0.4$^{a}$ | 14.4$^{e}$ | 0.2$^{a}$ | 4.5$^{c}$ | 0.2$^{a}$ | 17.8$^{f}$ | 0.3$^{a}$ | 11.0$^{d}$ | 0.9$^{a}$ | 1.14 | <0.001 |
| Doris dust (%) | 2.2$^{c}$ | 0.4$^{a}$ | 1.6$^{b}$ | 0.3$^{a}$ | 5.9$^{e}$ | 1.5$^{b}$ | 2.1$^{bc}$ | 0.8$^{a}$ | 2.3$^{c}$ | 0.3$^{a}$ | 5.2$^{d}$ | 1.8$^{bc}$ | 0.30 | <0.001 |
| Solubility DM (%) | 8.2 | 7.4 | 6.3 | 7.5 | 8.7 | 8.0 | 9.3 | 8.5 | 10.5 | 7.4 | 12.0 | 7.1 | 0.40 | ns*** |
| Solubility fat (%) | 3.0 | 3.7 | 5.6 | 5.5 | 4.7 | 2.6 | 6.5 | 6.2 | 5.7 | 0.0 | 7.0 | 3.0 | 0.55 | ns |
| Solubility ash (%) | 25.1 | 23.4 | 19.9 | 19.7 | 22.2 | 20.9 | 23.0 | 20.1 | 23.4 | 22.7 | 25.6 | 22.1 | 0.47 | ns |

* Pooled standard error of mean.
** One-way ANOVA. Values not sharing common superscript letters are significantly different ($P < 0.05$) determined by ANOVA followed by Tukey post hoc test.
*** Not significant.

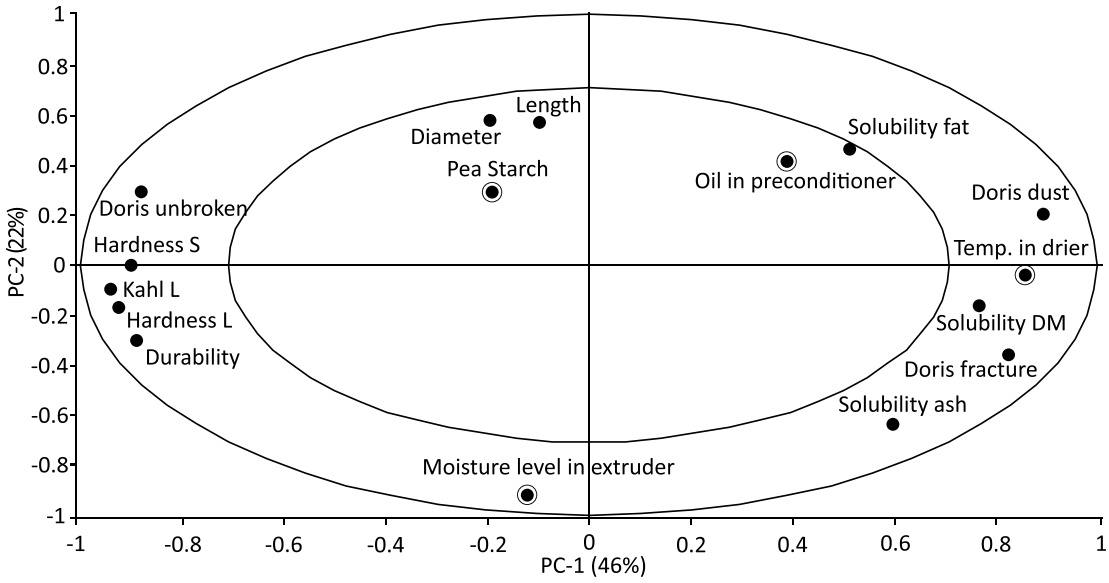


**Fig. 1.** Principal component analysis (PCA) correlation loading plot based on the input variables, and physical pellet quality parameters. The two ellipses represent 50 and 100 % of explained variance.

showed the poorest performance.

Correlation between proximate chemical composition, physical pellet quality, FI and significant growth parameters are shown in Table 7. FI were negatively affected by an increase in Durability and positively correlated to Doris Dust and Solubility DM. Solubility DM was however not significant different between the codes (Table 4). The growth parameters, weight gain (g/fish and %), SGR, TGC, PER and DGR were positively correlated to lipid and GE. The same parameters were negatively correlated to Hardness S and Durability. PER was negatively correlated to total ash. Increased starch content affected FI and growth parameters negatively except of PER. The FI, Weight gain (%), SGR, TGC and PER were all positively correlated to Doris Dust and Solubility DM.

## 4. Discussion

### *4.1. Effect of process conditions and starch content on physical pellet quality*

A large span in the physical pellet property responses were observed with i.e., Hardness S and Durability in the range of 39–212 N and 39–94 %, respectively. Flow rate, preconditioner conditions, screw configuration and screw speed were constant in this study and with oil addition at preconditioner outlet and extruder moisture addition as the only process variables. These changes affected Specific mechanical energy (SME) in the range of 19–28 W h kg$^{-1}$ and with highest values for the codes 2HS and 4LS with both low moisture and low oil addition (Table 2). SME is expressing mechanical energy input which is dissipated into heat (Samuelsen et al., 2018) and with the highest temperature behind the extruder die found for these two codes (144 and 146 °C, respectively; Table 2). Water act as a plasticizer (Samuelsen and Oterhals, 2016), and at low levels higher SME and temperature is needed for proper starch gelatinization and protein plasticization. The high SME and temperature measured for the 2HS and 4LS codes have most likely resulted in properly cooked pellet with physical quality in the same range as the 1HS and 5LS codes extruded at a higher moisture level (Table 4; Fig. 1). Oil addition in the preconditioner prior to extrusion gave low durability and hardness. This can be attributed to the lubrication effect of the lipids resulting in reduced feed melt viscosity and consequently reduced SME and viscous heat dissipation (Table 2; Samuelsen et al., 2018). Improper cooking will increase the level of particulate matter in the extrudates resulting in poorer pellet hardness and durability of the final product (Arèas, 1992; Mitchell and Areas, 1992).

**Table 5**
On-growing performance of experimental fish.

| | 1HS-DH | 1HS-DG | 2HS-DH | 2HS-DG | 3HS-DH | 3HS-DG | 4LS-DH | 4LS-DG | 5LS-DH | 5LS-DG | 6LS-DH | 6LS-DG | SEM* | *P*-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial no. of fish*** | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | | |
| Non-grower fish | 1 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | | |
| Initial fish weight (g) | 832 | 832 | 810 | 856 | 824 | 844 | 832 | 855 | 821 | 848 | 828 | 839 | 5.0 | ns**** |
| Final fish weight (g) | 1520 | 1528 | 1526 | 1560 | 1625 | 1594 | 1683 | 1653 | 1590 | 1576 | 1667 | 1655 | 72.6 | ns |
| Weight gain fish (g) | 688$^{a}$ | 696$^{ab}$ | 716$^{ab}$ | 704$^{ab}$ | 801$^{ab}$ | 750$^{ab}$ | 851$^{b}$ | 798$^{ab}$ | 769$^{ab}$ | 728$^{ab}$ | 839$^{ab}$ | 816$^{ab}$ | 12.9 | <0.05 |
| Weight gain fish (%) | 83$^{a}$ | 84$^{ab}$ | 88$^{abc}$ | 82$^{a}$ | 97$^{bc}$ | 89$^{abc}$ | 102$^{c}$ | 93$^{abc}$ | 94$^{abc}$ | 86$^{ab}$ | 101$^{c}$ | 97$^{bc}$ | 1.53 | <0.001 |
| FI (g/fish) | 642 | 669 | 647 | 675 | 744 | 684 | 768 | 749 | 695 | 696 | 767 | 738 | 11.2 | ns |
| FI (% bw/day) | 1.16 | 1.21 | 1.18 | 1.19 | 1.29 | 1.19 | 1.30 | 1.27 | 1.23 | 1.22 | 1.31 | 1.26 | 0.01 | ns |
| SFR | 1.20 | 1.24 | 1.22 | 1.22 | 1.34 | 1.23 | 1.35 | 1.32 | 1.27 | 1.26 | 1.36 | 1.31 | 0.01 | ns |
| FE | 1.05 | 1.00 | 1.09 | 1.04 | 1.08 | 1.05 | 1.11 | 1.07 | 1.11 | 1.05 | 1.10 | 1.09 | 0.01 | ns |
| FCR | 0.93 | 0.96 | 0.90 | 0.96 | 0.93 | 0.91 | 0.90 | 0.94 | 0.90 | 0.96 | 0.91 | 0.90 | 0.01 | ns |
| SGR | 1.28$^{ab}$ | 1.29$^{ab}$ | 1.35$^{abc}$ | 1.27$^{a}$ | 1.44$^{bc}$ | 1.35$^{abc}$ | 1.50$^{c}$ | 1.40$^{abc}$ | 1.41$^{abc}$ | 1.32$^{ab}$ | 1.49$^{c}$ | 1.45$^{bc}$ | 0.02 | <0.001 |
| TGC | 4.20$^{a}$ | 4.24$^{a}$ | 4.40$^{abc}$ | 4.21$^{a}$ | 4.78$^{abc}$ | 4.48$^{ab\ c}$ | 5.00 | 4.68$^{abc}$ | 4.63$^{abc}$ | 4.36$^{ab}$ | 4.95 | 4.81$^{abc}$ | 0.06 | <0.01 |
| PER | 17.2$^{ab}$ | 17.2$^{ab}$ | 18.8$^{ab}$ | 18.8$^{ab}$ | 21.2$^{b}$ | 20.2$^{ab}$ | 21.0$^{ab}$ | 19.7$^{ab}$ | 18.9$^{ab}$ | 16.9$^{a}$ | 21.1$^{ab}$ | 20.5$^{ab}$ | 0.35 | <0.01 |
| DGR | 14.6$^{a}$ | 14.8$^{ab}$ | 15.2$^{ab}$ | 15.0$^{ab}$ | 17.0$^{ab}$ | 16.0$^{ab}$ | 18.1$^{b}$ | 17.0$^{ab}$ | 16.4$^{ab}$ | 15.5$^{ab}$ | 17.9$^{ab}$ | 17.4$^{ab}$ | 0.27 | <0.05 |
| Fork length (cm) | 46.8 | 47.0 | 46.7 | 47.1 | 47.2 | 46.9 | 47.8 | 47.6 | 47.0 | 47.3 | 47.9 | 47.5 | 0.13 | ns |
| CF | 1.47 | 1.45 | 1.47 | 1.49 | 1.54 | 1.50 | 1.53 | 1.53 | 1.52 | 1.48 | 1.51 | 1.52 | 0.01 | ns |
| ADC protein | 85.2 | 83.9 | 84.6 | 80.6 | 86.3 | 85.2 | 86.1 | 84.3 | 85.0 | 85.6 | 86.1 | 85.6 | 0.42 | ns |
| ADC fat | 91.8 | 91.4 | 90.4 | 88.1 | 94.7 | 92.7 | 93.1 | 92.8 | 93.3 | 93.3 | 94.1 | 94.6 | 0.47 | ns |
| ADC GE | 78.9 | 76.5 | 78.8 | 75.7 | 81.4 | 79.5 | 81.7 | 79.2 | 80.0 | 79.0 | 81.1 | 80.9 | 0.46 | ns |

* Pooled standard error of mean.
** One−way ANOVA. Values not sharing common superscript letters are significantly different (P<0.05) determined by ANOVA followed by Tukey post hoc test.
*** Zero mortality for all groups.
**** Not significant.

The starch content had a positive impact on hardness and durability parameters and also affected pellet diameter and expansion positively. Starch is composed of linked glucose molecules in the form of linear amylose and highly branched amylopectin. Gelatinization of starch is a phenomenon associated with the disruption of the granular starch structure, hydration, swelling and solubilization of starch molecules (Appelqvist and Debet, 1997; Liu, 2005; Romano and Kumar, 2019). Gelatinized starch forms intermolecular hydrogen bonds that creates network structure, strength, elasticity, and expansion to the finished product (Colonna et al., 1989).

### 4.2. Effect of drying conditions on physical pellet quality

The drying conditions had the most prominent effect on the pellet responses. In this study 70 °C were used for conventional drying of the pellet. This is gentler than commercial drying as this most often occur in the range of 94–150 °C (Rokey, 1994). The conventionally dried feeds had significant lower hardness and was less durable than the gently dried feeds. Such differences are also expected for commercial dried feeds. The conventionally dried feeds had slightly higher gelatinized starch level (Table 3). A prolonged higher extrudate temperature at the start of the conventional drying process may be the main explanation for the observed increased starch gelatinization relative to the gently dried codes.

The observed reduction in physical feed quality for the conventionally dried codes can be explained by using the phase transition theory (Haubjerg, 2016). The glass transition ($T_g$) of an amorphous solid is a temperature range where the solid transits from a brittle glassy to a soft rubbery state and where $T_g$ is defined as the onset or midpoint of this transition range. The transition is a function of temperature, time, molecular weight, composition, water activity and moisture content (Abiad et al., 2009). At conventional drying a moisture gradient will be created in the extrudate with a faster drying rate at the pellet surface. If the pellet surface reach $T_g$ with the center still in the rubbery phase, increased tensile stress will occur due to shrinkage of the inner core resulting in reduced physical pellet quality. This may have been prevented for the gently dried pellet due to the long drying time, low temperature and drying in the mobile rubbery phase above the glass transition temperature. A higher degree of extrudate shrinkage when drying in the rubbery phase is expected (Haubjerg, 2016). This was observed for 1, 2, 3HS and 5LS codes in this study (Table 4).

Sinter bridge development during long time gentle drying created by molecular diffusion between the feed mass amorphous particles may also have strengthened the binding network resulting in improved physical quality (Hartmann and Palzer, 2011).

Starch retrogradation can occur during slow drying and lead to textural changes, such as increased hardness and reduced starch digestibility (Svihus et al., 2005; Wang et al., 2015) Starch retrogradation is affected by time, temperature, and water content. The process is slow and depends on water content above 20 % (Wang et al., 2015). Most of the pellet codes had extrudate moisture content ~ 20 % with only 1HS-DH and 5HS-DH at 27 % (Table 2). The two pellet codes do not differ from the other codes in terms of gelatinized starch content reduction, excluding this as an explanation for improved physical pellet quality for the gently dried feed codes.

### 4.3. Fish performance

There was a significant difference between the codes for weight gain (g/fish and %), specific and thermal growth rate (SGR and TGC), daily growth rate (DGR) and protein efficiency ratio (PER; Table 5). Feed intake (FI) was not statistically different, but increase in growth (weight gain, SGR and TGC) may be explained by increased FI and are associated with how the fish utilized the level of protein (PER) and fat content ($ADC_{fat}$) in the feeds (Table 6). Some of the increase in growth may also be explained by the difference in crude protein between the feeds or with increase in digestibility of nutrients ($ADC_{GE}$; Table 6).

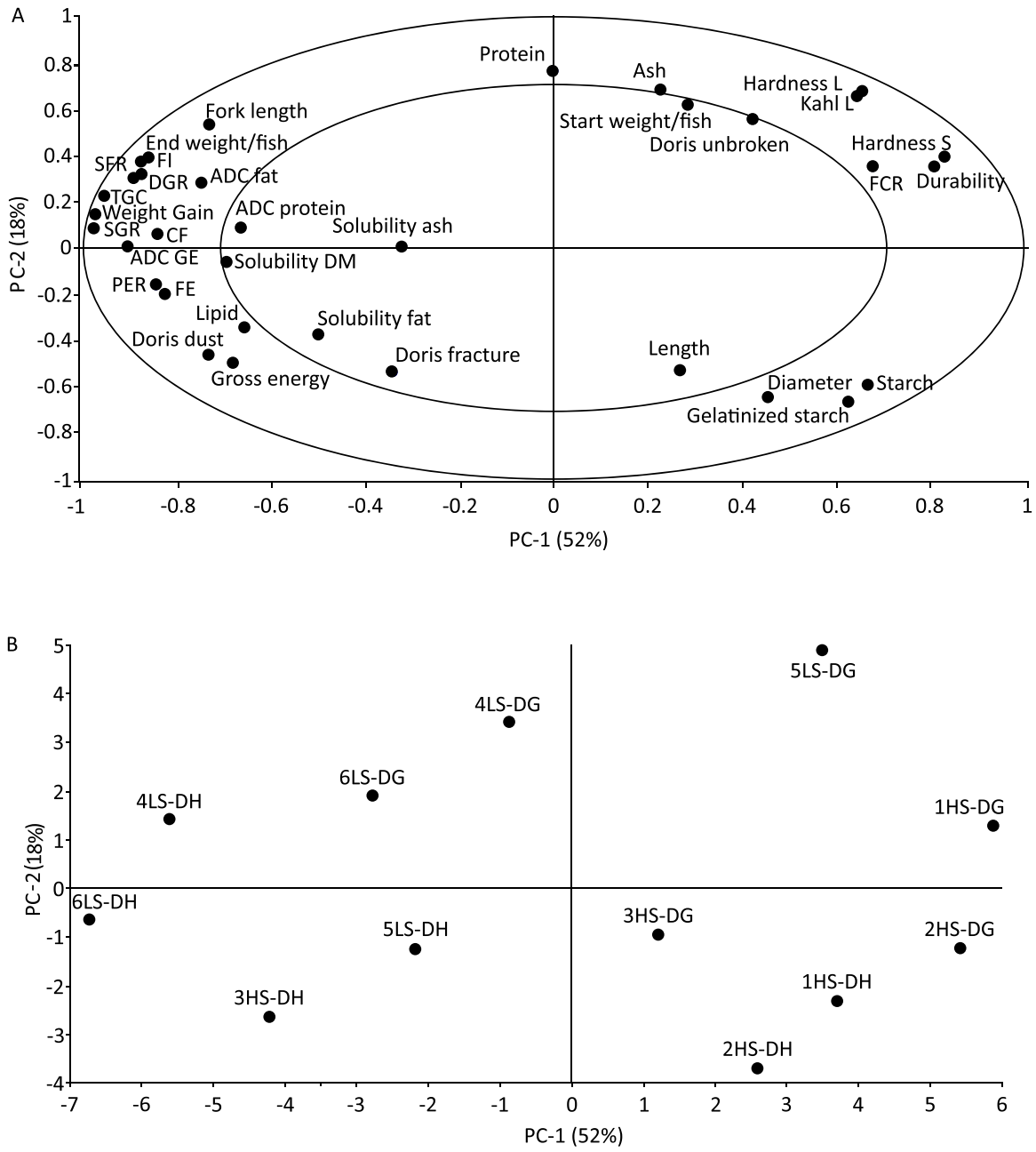


**Fig. 2.** Principal component analysis (PCA). A) Correlation loading plot based on proximate chemical composition, physical pellet quality and on-growing performance parameters. The two ellipses represent 50 and 100 % of explained variance. B) Score plot showing similarities in responses based on the applied experimental diets.

### *4.4. Effect of process on fish performance*

Severe heating above 150 °C in the extrusion process may give degradation of protein primary structure, and formation of covalent bonds that can negatively affect the digestibility of amino acids as arginine, cysteine, lysine, serine, and threonine (Hager, 1984; Sørensen et al., 2002). A temperature in the range of 127−150 °C during extrusion was however found to be positive for growth, FCR and digestibility (Sørensen et al., 2002, 2005; Barrows et al., 2007; Morken et al., 2012). This implies that extrusion is not damaging to the nutritional value of the feed as long as the temperature does not exceed 150 °C (Hager, 1984; Sørensen, 2012). Extrusion is also reported to be an effective method to reduce the activity of heat labile anti-nutritional factors as trypsin inhibitors found in i.e., soya resulting in improved nutritional value (Barrows et al., 2007; Tran et al., 2008). In this study the temperature range was between 133−146 °C (Table 2) and should be optimal for good performance. This can be seen by the high protein digestibility for all codes (Table 5) and confirm that the extrusion process has not impacted the biological response of the feeds in this study.

### *4.5. Effect of starch, fat content and GE on fish performance*

All feeds showed high starch gelatinization which are documented to be positive for the digestion of the starch and fish performance (Kanmani et al., 2018; Romano and Kumar, 2019). Growth and digestibility parameters were positively affected by a reduction in starch content (Table 7). This is in line with other studies (Krogdahl et al., 2004). Lipid level and GE had positive impact on fish performance (Table 7) also reported earlier (Hemre et al., 2002; Hillestad et al., 1998; Glencross et al., 2014, 2017). In most species there is a negative correlation

**Table 6**
Correlation (*r*) between on-growing performance parameters.

| | Weight gain fish (g) | Weight gain fish (%) | FI (g/fish) | FI (% bw/day) | SFR | FE | FCR | SGR | TGC | PER | DGR | Fork length (cm) | CF | ADC protein | ADC fat | ADC GE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weight gain fish (g) | 1.00 | | | | | | | | | | | | | | | |
| Weight gain fish (%) | 0.98[b] | 1.00 | | | | | | | | | | | | | | |
| FI (g/fish) | 0.95[b] | 0.89[b] | 1.00 | | | | | | | | | | | | | |
| FI (% bw/day) | 0.93[b] | 0.90[b] | 0.98[b] | 1.00 | | | | | | | | | | | | |
| SFR | 0.94[b] | 0.92[b] | 0.98[b] | 1.00[b] | 1.00 | | | | | | | | | | | |
| FE | 0.74[b] | 0.80[b] | 0.54 | 0.54 | 0.56 | 1.00 | | | | | | | | | | |
| FCR | −0.58 | −0.68[a] | −0.30 | −0.29 | −0.33 | −0.84[b] | 1.00 | | | | | | | | | |
| SGR | 0.98[b] | 1.00[b] | 0.89[b] | 0.90[b] | 0.92[b] | 0.80[b] | −0.68[a] | 1.00 | | | | | | | | |
| TGC | 0.99[b] | 1.00[b] | 0.92[b] | 0.92[b] | 0.93[b] | 0.78[b] | −0.64[a] | 1.00[b] | 1.00 | | | | | | | |
| PER | 0.86[b] | 0.85[b] | 0.77[b] | 0.74[b] | 0.76[b] | 0.66[a] | −0.61[a] | 0.85[b] | 0.86[b] | 1.00 | | | | | | |
| DGR | 1.00[b] | 0.98[b] | 0.95[b] | 0.93[b] | 0.94[b] | 0.74[b] | −0.58[a] | 0.98[b] | 0.99[b] | 0.86[b] | 1.00 | | | | | |
| Fork length (cm) | 0.85[b] | 0.77[b] | 0.93[b] | 0.89[b] | 0.89[b] | 0.46 | −0.16 | 0.76[b] | 0.80[b] | 0.57 | 0.85[b] | 1.00 | | | | |
| CF | 0.85[b] | 0.81[b] | 0.80[b] | 0.75[b] | 0.76[b] | 0.75[b] | −0.53 | 0.81[b] | 0.83[b] | 0.82[b] | 0.85[b] | 0.58[a] | 1.00 | | | |
| ADC protein | 0.57 | 0.65[a] | 0.46 | 0.53 | 0.54 | 0.49 | −0.56 | 0.65[a] | 0.62[a] | 0.37 | 0.57 | 0.36 | 0.41 | 1.00 | | |
| ADC fat | 0.71[a] | 0.73[b] | 0.67[a] | 0.70[a] | 0.71[a] | 0.46 | −0.44 | 0.73[b] | 0.73[b] | 0.49 | 0.71[a] | 0.52 | 0.61[a] | 0.88[b] | 1.00 | |
| ADC GE | 0.83[b] | 0.88[b] | 0.70[a] | 0.72[b] | 0.74[b] | 0.79[b] | −0.74[b] | 0.88[b] | 0.87[b] | 0.70[a] | 0.83[b] | 0.55 | 0.75[b] | 0.88[b] | 0.86[b] | 1.00 |

[a] Denotes significant correlation coefficients ($P < 0.05$).
[b] Denotes significant correlation coefficients ($P < 0.01$).



**Table 7**
Correlation (*r*) between proximate chemical composition, physical pellet quality, FI and significant growth parameters. Only chemical and physical pellet quality data with significant effect on FI and growth parameters are shown.

| | Lipid (g $kg^{-1}$ DM) | Total ash (g $kg^{-1}$ DM) | Starch (g $kg^{-1}$ DM) | Gelatinized starch (g $kg^{-1}$ DM) | Gross energy (kJ $g^{-1}$) | Hardness S (N) | Durability (%) | Doris Dust (%) | Solubility DM | FI (% bw/day) | Weight gain (%) | SGR | TGC | PER | DGR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lipid (g $kg^{-1}$ DM) | 1.00 | | | | | | | | | | | | | | |
| Total ash (g $kg^{-1}$ DM) | −0.80[b] | 1.00 | | | | | | | | | | | | | |
| Starch (g $kg^{-1}$ DM) | −0.30 | −0.32 | 1.00 | | | | | | | | | | | | |
| Gelatinized starch (g $kg^{-1}$ DM) | −0.34 | −0.27 | 0.95[b] | 1.00 | | | | | | | | | | | |
| Gross energy (kJ $g^{-1}$) | 0.93[b] | −0.80[b] | −0.20 | −0.18 | 1.00 | | | | | | | | | | |
| Hardness S (N) | −0.43 | 0.21 | 0.25 | 0.22 | −0.55 | 1.00 | | | | | | | | | |
| Durability (%) | −0.66[a] | 0.42 | 0.36 | 0.25 | −0.73[a] | 0.75[b] | 1.00 | | | | | | | | |
| Doris Dust (%) | 0.51 | −0.41 | −0.09 | −0.03 | 0.59[b] | −0.84[b] | −0.89[b] | 1.00 | | | | | | | |
| Solubility DM | 0.28 | 0.00 | −0.46 | −0.37 | 0.41 | −0.60[a] | −0.79[b] | 0.62[a] | 1.00 | | | | | | |
| FI (% bw/day) | 0.47 | −0.06 | −0.70[a] | −0.67[a] | 0.41 | −0.54 | −0.66[a] | 0.59[a] | 0.60[a] | 1.00 | | | | | |
| Weight gain (%) | 0.65[a] | −0.20 | −0.70[a] | −0.68[a] | 0.65[a] | −0.75[b] | −0.75[b] | 0.64[a] | 0.60[a] | 0.90[b] | 1.00 | | | | |
| SGR | 0.65[a] | −0.20 | −0.71[a] | −0.68[a] | 0.64[a] | −0.75[b] | −0.74[b] | 0.64[a] | 0.60[a] | 0.90[b] | 1.00[b] | 1.00 | | | |
| TGC | 0.65[a] | −0.19 | −0.73[b] | −0.72[b] | 0.63[a] | −0.71[b] | −0.72[b] | 0.61[a] | 0.60[a] | 0.92[b] | 1.00[b] | 1.00[b] | 1.00 | | |
| PER | 0.91[b] | −0.65[a] | −0.41 | −0.45 | 0.88[b] | −0.60[a] | −0.73[b] | 0.65[a] | 0.44 | 0.74[b] | 0.85[b] | 0.85[b] | 0.86[b] | 1.00 | |
| DGR | 0.65[a] | −0.18 | −0.77[b] | −0.76[b] | 0.61[a] | −0.63[a] | −0.68[a] | 0.55 | 0.58[a] | 0.93[b] | 0.98[b] | 0.98[b] | 0.99[b] | 0.86[b] | 1.00 |

[a] Denotes significant correlation coefficients ($P < 0.05$).
[b] Denotes significant correlation coefficients ($P < 0.01$).

between GE (kJ/g$^{-1}$) and fat in the diet and FCR (Hillestad et al., 1998; Rodehutscord and Pfeffer, 1999) also found in this study ($r^2 = 0.50$ and 0.40, respectively, Fig. 2A). The FCR was also negatively correlated to TGC ($r^2 = 0.41$) and other growth parameters (Fig. 2A). The FCR was however not statistically different between the codes (Table 5).

### 4.6. Effect of physical pellet quality on fish performance

In this study pellet codes with a large span in physical quality parameters were produced to study the effects of physical quality on the fish performance. Hardness S and Durability showed significant negative effects on the growth parameters and PER, while Doris dust gave positive effect. Doris dust is most likely not the causation effect but contains information/is correlated to hardness, durability and solubility parameters (Table 7; Fig. 1). Solubility DM was not significantly different between the codes but was negatively correlated to Hardness S and Durability and improved the performance (Table 7). This is in line with other studies that has shown that physical feed quality have affected fish performance. In Jacobsen et al. (2018) increased extruded pellet hardness impacted feed intake and growth of Atlantic salmon negatively. Bogevik et al. (2021) concluded that pellet who disintegrated slowly in water prolonged the gastric evacuation rate and reduced feed intake in Atlantic salmon compared to faster water disintegration.

### 4.7. Best methods for the evaluation of pellet physical quality

The pellet codes were subjected to several physical pellet quality parameters and Hardness S, Durability and Doris dust were identified as the most important parameters affecting fish performance. Hardness was reported both on lying (L) and standing (S) pellets (texture analyser) and with the use of a Kahl tester. They were positively correlated, but Hardness S showed more normal distributed values and spanned a larger range. The same pattern was observed for Durability compared to Doris unbroken. This may indicate that Hardness S and Durability is best suited for separating the pellet codes and also best suited for the statistical analyses. However, it is not possible to conclude that the true causal variables affecting fish performance have been identified and other studies have discussed hydration- and dissolution rates as important parameters (Aas et al., 2011; Hilton et al., 1981; Jacobsen et al., 2018; Bogevik et al., 2021). Even not significantly different, Solubility DM was positive correlated to Doris Dust and negatively correlated to Hardness S and Durability (Fig. 1) and had a positive effect on fish growth (Table 7, Fig. 2A). Future research should therefore focus on the development of a proper method measuring extruded feed hydration- and dissolution rates that can be used to predict pellet disintegration in the fish stomach and fish performance. In this study, screening of several parameters impacting physical pellet quality was performed, with the different starch content used also impacting the growth and digestibility parameters. A continuation of this study should be conducted with the use of one diet in an optimization design where physical quality is varied by process parameters only (i.e., oil and moisture level and dryer type and conditions).

## 5. Conclusion

The variation in diet starch, preconditioner oil and extruder moisture levels combined with conventional and gentle extrudate drying had significant impact on physical feed quality parameters. The drying conditions had the most prominent effect on the pellet responses. This could be related to phase transition and drying in the mobile rubbery phase above $T_g$ for the gently dried codes. Growth and digestibility were negatively associated with increased starch content, hardness and durability parameters and reduced solubility, fat content and GE. The most important physical parameters affecting growth performance were Hardness S, Durability and Doris dust, parameters that may represent feed hydration- and dissolution rates. Such methods must be developed for better prediction of fish stomach pellet disintegration and fish performance. This study concludes that the impact of physical pellet quality on fish performance should be given more focus. High digestibility is important for increased nutrient retention and for minimizing losses and waste to the environment. Standardized and suitable methods to evaluate pellet physical quality are therefore vital for providing optimal feeds to the fish farms.

## Author statement

Tor Andreas Samuelsen; Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review and editing, Visualization, Supervision, Project administration.

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Hanne Jorun Sixten; Conceptualization, Methodology, Formal analysis, Investigation, Writing - review and editing, Project administration.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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