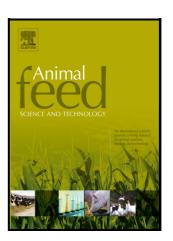
Journal Pre-proof

Effect of Different Extrusion Conditions and Pellet Size on the Physical Properties of Extruded Fish Feeds

Romi Novriadi, T. Gibson Gaylord, Guillaume Salze, D. Allen Davis



PII: S0377-8401(25)00248-2

DOI: https://doi.org/10.1016/j.anifeedsci.2025.116453

Reference: ANIFEE116453

To appear in: Animal Feed Science and Technology

Please cite this article as: Romi Novriadi, T. Gibson Gaylord, Guillaume Salze and D. Allen Davis, Effect of Different Extrusion Conditions and Pellet Size on the Physical Properties of Extruded Fish Feeds, *Animal Feed Science and Technology*, (2025) doi:https://doi.org/10.1016/j.anifeedsci.2025.116453

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Effect of Different Extrusion Conditions and Pellet Size on the Physical

Properties of Extruded Fish Feeds

Romi Novriadi^{1*}, T. Gibson Gaylord², Guillaume Salze³ and

D. Allen Davis⁴

Department of Aquaculture, Fisheries Business Expert Polytechnic (Politeknik Ahli Usaha

Perikanan), Ministry of Marine Affairs and Fisheries, Republic of Indonesia, Jl. Raya Pasar

Minggu, Jati Padang, Jakarta – 12520, Indonesia

U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, 4050 Bridger Canyon

Road, Bozeman, Montana 59715, United States

Knip Inc., 110 Canal St, Lowell, MA 01852, United States

School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL,

United States

* Corresponding author: Romi Novriadi

E-mail: novriadiromi@yahoo.com

Abstract

This work presents useful information on how different temperature profiles during the

extrusion process affect the physical quality properties of the extrudates. In this study, feed

mixtures were extruded using a twin-screw extruder; the barrel temperature profile was set at

110°C for cooking extrusion process, and no additional heat for cold process. Four extruded

diets were designed: D2LT and D4LT for 2 mm and 4 mm diet produced with cold process,

respectively, and D2HT and D4HT for 2 mm and 4 mm diet produced with cooking extrusion.

Pellet durability index (PDI), expansion ratio (ER), sinking velocity (SV), water stability (WS)

and water absorption index (WAI) were affected with the temperature condition. The pellets

Journal Pre-proof

produced with cooking extrusion had a higher PDI, SV, WAI and WS compared to cold process (p < 0.0001). There was no significant difference in terms of ER between the extruded diet processed with different temperature at the same particle size. Reducing particle size from 4 mm to 2 mm significantly affect the PDI, WAI. SV, WAI and WS in cooking extrusion diet. In cold process, the significant differences were found in PDI, ER, and WS, but no significant differences in SV and WAI.

Keywords: Cooking extrusion, Cold process, Physical quality, Size, Extrudates,

1. Introduction

Aquaculture is one of the fastest growing food production sectors in agriculture and plays a significant role in improving national food security, income and nutritional status of people in many regions (Yu et al., 2025; Kannadhason & Muthukumarappan, 2010;). As intensive aquaculture continues to expand, research on diet quality and feeding strategies is being continually refined (Thornburg, 2025; Bu et al., 2024; Hertrampf & Piedad-Pascual, 2012). In the evaluation of diet quality, producers must consider an extensive array of fish with different feeding habits (Hyatt, 1979). Since aquaculture species occupy different strata within the water column, a comprehensive knowledge on physiology and feeding behavior is required to maximize the opportunity for the fish to consume the diets being offered, avoid the loss of nutrients due to disintegration and leaching in the water (Turchini &Hardy, 2024; Parker, 2011) and maximize feed conversion efficiencies (Stark, 2012; Guillaume, 2001). Thus, the feed manufacturing process must accommodate all the specific nutritional requirements to ensure optimal growth performance and health condition of the cultured species.

In commercial aquaculture feed, important physical properties that constitute feed quality include the hardness, water stability, absorption, buoyancy, and resiliency (Cheng et al., 2024; Sørensen, 2012). These properties ensure the feed remains intact during production,

transportation, and until it reaches the feeding devices in fish farms (Sørensen et al., 2009). Durability and water stability are affected by various biochemical changes that occur inside the extruder barrel (Sørensen et al., 2009; Thomas et al., 1999), while the floatability of the diet is affected by the expansion achieved during the extrusion process (Adeparusi & Famurewa, 2011). Extrusion processes improve the water stability, durability, hardness and buoyancy control compared to steam pelleted diets (Sørensen et al., 2009), making extrusion the most effective manufacturing technology for compound fish feed (Bowzer et al., 2016; Brown et al., 2015; Hertrampf & Piedad-Pascual, 2012; Khater et al., 2014).

Extrusion processing integrate multiple operations, including simultaneously mixing, cooking, kneading shearing, shaping and forming (Yadaf et al., 2021; Riaz, 2000; Riaz, 2008). According to Pennels et al. (2025) and Guillaume (2001), Extrusion processing uses a barrel housing one or two screws that compress a mixture of raw materials using a combination of pressure and heat along the length of the barrel over a short period. At the end of the barrel, the mixture is shaped by being forced through one or several openings in a die, with the resulting strands cut by a knife. The product is then cooled and dried (Bowzer et al., 2016; Guillaume, 2001). During extrusion, a combination of moisture, pressure and heat can partially denature the protein and gelatinize the starch in raw materials (Friesen et al., 1992; Jeong et al., 1991; Kim et al., 2006). This process significantly affects starch chemistry, feed digestibility, expansion, and water stability of the pellets (Rosentrater et al., 2009).

The present study evaluates the effect of two different barrel temperatures during twinscrew extrusion processing and the effects of two different pellet sizes on the durability index, expansion ratio, sinking velocity, water stability, and water absorption index of the extruded products.

2. Material and Methods

2.1 Feed formulation and preparation

Four diets with similar compositions were manufactured using commercial methods with a twin-screw extruder (DNDL-44, Buhler Inc., Plymouth, MN, USA) by the U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, MT, USA. The ingredients (excluding the Menhaden fish oil, which was applied to the feeds post-extrusion; see below) were mixed in a paddle mixer (Marion Mixers, Inc., Marion, IA, USA) in a 100-kg batch followed by grinding to a particle size of <200 µm using an air-swept pulverizer (Model 18H; Jacobsen, Minneapolis, MN, USA) (**Table 1**). Two-extrusion processes were evaluated: (1) extrusion- cooking, defined as using temperatures above 110°C to gelatinize the starch and (2) cold process, defined when the starch is not gelatinized, and ingredients are primarily pressed into a form. The cooking-extrusion diets were exposed to an average of 110°C for approximately 14 seconds in five-barrel sections, with the last section maintained at 62°C. Pressure at the die head was approximately 50 bars, and screw speed was maintained at 423 rpm. Portions of the feeds were extruded through a 3 mm die for 4.0 mm pellets (D4HT) and 1.5 mm die for 2 mm pellets (D2HT). The diets were dried in a pulse bed drier (Buhler AG) until moisture readings were below 6%. Pellets were dried at approximately 107°C with an upper limit outflow air temperature of approximately 88°C. The diets were then cooled at ambient air temperatures for final moisture levels of less than 10%. Fish oil was then applied using a Phlauer vacuum infusion coater (A & J Mixing, Ontario, Canada) after the pellets were cooled.

The cold process diet consisted of two distinct sizes of feeds, with all the oil mixed in the mash prior to pelleting, and also manufactured with the Buhler twin-screw extruder. No additional heat was added, and barrel tempering units were set at 15°C, which resulted in an average barrel temperature of 23.2°C mid-way through production. The cold-extruded diet's solids feed rate was half that of the cooking extruded pellets, which resulted in a longer

retention time in the barrels (28 seconds), but only 13 bars of pressure at the end plate. The 2mm (D2LT) and 4mm pellets (D4LT) were manufactured with 2- and 4-mm dies, respectively. All finished diets were bagged and stored in a temperature-controlled room until analysis and shipment.



2.2 Proximate and amino acid composition of diets

The protein, moisture, lipid, fiber and ash content were determined using standard methods described by Association of Official Analytical Chemists (AOAC, 1990). The amino acid composition of the diet was quantified by Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO, USA) and the mean of each treatment was taken.

2.3 Analysis of physical properties

2.3.1 Pellet durability index (PDI)

Approximately 500 g of each diets were manually sieved (U.S.A. standard testing, ASTM E-11 specification, Daigger, Vernon Hills, IL, USA) to remove initial fines, and then tumbled in a pellet durability tester (Model PDT-110, Seedburo Equipment Company, Chicago, IL, USA) for 10 min. Afterwards, the samples were again sieved, and then weighed on an electronic analytical balance (Mettler Toledo ML6001E precision balances, Switzerland). Pellet durability index was calculated as follow:

Pellet Durability Index (%) =
$$\frac{Mat}{Mht} \times 100$$

Where: Mat is the mass of pellet after tumbling (g) and Mbt is the mass before tumbling (g)

2.3.3 Expansion ratio

Expansion ratio (ER) was determined as the ratio of the extrudate diameter to the diameter of die nozzle (Conway & Anderson, 1973). The diameter of the extrudates for each treatment was measured with a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan)

2.3.4 Sinking velocity

Sinking velocity (SV) was measured using the method developed by Das et al. (1993) and was determined by monitoring the time taken for an extrudate to reach the bottom of a 1000 mL measuring cylinder filled with distilled water. Distance travelled for the time taken gave the sinking velocity (ms⁻¹)

2.3.5 Water absorption index (WAI)

Approximately, 2 g of extrudates for each diet was placed in 20 mL of distilled water and stirred with a magnetic stirrer (Southeast Science, Model H4000-HS, Korea) at low speed, which simulates the movement of water of pond, until the extrudates broke or disintegrated over a period of 30 min, and then centrifuged at 3000 x g for 10 min. The mass of remaining gel was weighed, and WAI was calculated as the ratio of gel mass (*Wg*) to the sample mass (*Wds*) (Jones et al., 2000).

Water absorption index (unitless) =
$$\frac{Wg}{Wds}$$

2.3.6 Water stability

Water stability (WS) was measured as the ratio of pellet retained on a wire screen after immersion of 3-5 g of each replicate diet in 100 mL water for 20 min and oven drying at 105^0 C for 24 h to the initial pellets (Lim & Cuzon, 1994).

2.4 Statistical analysis

Mean results per physical properties were expressed as a mean \pm standard deviation (SD) and subjected to two-way analysis of variance (ANOVA) with interaction using diet size and extrusion temperature condition as the independent variables. Prior to analysis, Cramervon Mises test and Anderson-Darling test were performed to analyze the normal distribution of the physical parameters. Student's t test was applied to assess any difference in PDI, expansion ratio, sinking velocity, WAI and water stability were compared between two different diet sizes subjected to the same treatment. Statistical significance was defined at p<0.05 and analysed using the General Linear Model procedure in the SAS system (V9.4, SAS Institute, Cary, NC, USA).

3. Results

3.1 Nutrient composition of experimental diets

The proximate composition of crude protein, moisture, crude fat, crude fiber and ash are presented in **Table 2.** The level of crude fat was higher in D4LT compared to D2LT. However, crude protein, moisture and ash content showed comparable levels among the treatments. In addition, no differences were observed on the amino acid profile of the diet produced by using two different extrusion processes, cooking-extrusion and cold process.

3.2 Physical properties of diets

Two-way ANOVA showed that the expansion ratio, sinking velocity (SV), water absorption index (WAI) and water stability (WS) were significantly influenced by temperature conditions during the extrusion process and the size of the diet (p < 0.0001). However, for the Pellet Durability Index (PDI, %)., barrel temperatures during twin-screw extrusion processing had a more significant effect (p < 0.0001, by two-way ANOVA) compared to the size of the diet (p = 0.1663).

Based on student *t* test results to determine the differences between two different sizes of diet subjected to the same treatment, bigger size (4 mm) significantly increased the expansion ratio, SV and WS compared to lower size (2 mm) of diet either produced by using cooking-extrusion condition or cold process. In addition, smaller size of diet produced by using cooking-extrusion process had higher water absorption index and no significant differences in diet extruded by using cold process. Finally, 2 mm diet extruded in cold process had the higher PDI (%) compared to 4 mm.

Discussion

The choice of feed production methods for the aquaculture industry largely depends on species characteristics, production cost, and their impact on the culture environment (Bektursunova et al., 2023; Ebbing et al., 2022; Espinoza-Ortega et al., 2024; Guillaume, 2001; Wang et al., 2021). In a study with rainbow trout (*Salmo gairdneri* R.), extruded diets had better physical properties than steam pellets and resulted in prolonged gastric emptying and higher feed efficiency compared to the group of fish reared on steam pellets (Hilton et al., 1981). In addition, a study performed by Misra et al. (2002) showed that the extruded pellets induced better feed conversion ratio (FCR) and protein utilization of post-larvae of *Macrobrachium rosenbergii* in comparison to those maintained with steam pellets. While steam pelleting can sometimes replace the function of extruded diet for aquaculture purposes, extrusion remains the preferred method (Guillaume, 2001; Riaz, 2023; Xing et al., 2024).

In the present study, changing the conditioning temperature in the barrel section of double-screw extrusion process and pellet size significantly affected the physical properties of the diets. Results shown in Table 3 indicated that the PDI of the extrudates processed with cooking-extrusion were higher than those produced with the cold process. Increasing pellet size from 2 to 4 mm resulted in an increase in durability of extrudate produced by using cooking-

extrusion process. However, at the cold-process, smaller size yielded significantly higher PDI compared to bigger sizes. The effects of barrel and die temperature of pellet mill have been reviewed (Tumuluru et al., 2010), highlighting the ability of the densified biomass to remain intact during the handling process as die temperature increased. Moreover, the presence of non-starch polysaccharides (NSP) in the diet might also contribute to the pellets not being crushed during the handling process (Kraugerud et al., 2011). The effect of changing size on resulting extruded strength was observed by others with different ingredients. For example, Khater et al. (2014) reported that the mean durability was increased as the pellet size decreased from 3.0 to 1.0 mm at two different protein ratios. Likewise, the PDI of extruded catfish feed formulated by using 47.3% of soybean meal as the protein source was increased when the particle size reduces from 1.2 mm to 0.7 mm (Rolfe et al., 2001). However, considering the interaction effect on this study, changes in durability are more attributed to changes in temperature rather than changes in size.

In aquaculture, manufacturing low-polluting diets and avoiding the risk of leaching nutrients caused by disintegration of pellets has received much attention with significant quality innovations (Guillaume, 2001). In the present study, changes in temperature, die pressure, and screw speed during cooking extrusion produced more stable feed compared to the cold process. In agreement with current results, better WAI was also observed in pellets produced with cooking extrusion with chamber temperature condition raised up to 90% compared to steam pelleting (Larrea et al., 2005). According to Misra et al. (2002) The superior water stability of the extruded diet might be attributed to starch gelatinization under high temperature, combined with high pressure and shear during the manufacturing process. Additionally, the density and moisture content of extruded diet might play a role in determining the absorption index (Misra et al., 2002; Singh & Muthukumarappan, 2016) and can have significant implications in the storage stability of pellets (Chevanan et al., 2007).

Although no clear trend was observed, this study indicated that when the pellet size of cooking extrusion pellet increased from 2 mm to 4 mm, the mean WAI was decreased from 3.60 to 2.52. On the other hand, no significant difference was observed between 2 mm and 4 mm pellet produced with cold process. Since WAI values could vary depending on the diet composition and processing (Thomas et al., 1999), changes in this study were mostly influenced by the temperature.

In this study, an increase in SV (cm s⁻¹) was observed in diets produced by the cooking extrusion process compared to those produced by the cold process. Within the same treatment, bigger size also caused significant increase in SV. Since SV was associated with air entrapped in particles to reduce the specific gravity and capability of diet to absorb the water (Chevanan et al., 2010; Hilton et al., 1981), higher numerical value gives the impression that cooking-extrusion produces a more dense and compact diet compared to cold-process. Indeed, the heavier density of 4 mm diet compared to 2 mm will cause a significant increase in SV for extruded diet either produced with cooking-extrusion or cold process.

The highest water stability was obtained in cooking-extrusion process and bigger size of diet subjected to different extrusion process. The poorer stability found in cold process of 2 mm diet might be related to the compacted conditions and density of the diet (Rout & Bandyopadhyay, 1999). Meanwhile, extrusion process and size of the diet also had a significant interaction to the expansion ratio. Several studies have indicated that the expansion ratio of the extrudates depends on extrusion condition (Chevanan et al., 2007; Miller, 1985; Moore et al., 1990), residence time (Fan et al., 1994; Mitchell et al., 1994) and the die design (Bouzaza et al., 1996). Among the variables, barrel temperature plays a critical role in controlling the expansion properties by lowering the melt viscosity and increasing the longitudinal expansion (Chinnaswamy, 1993; Singh et al., 2014).

4. Conclusion

In present study, changes in extrusion conditions, specifically the barrel temperature, significantly affected the physical properties of extruded diets, including the durability index, expansion ratio, sinking velocity, water absorption index and water stability. A significant interaction was also found between the extrusion conditions and size of the extruded diet on these physical properties when all ingredients exposed to an average of 110°C for approximately 14 seconds in five-barrel sections.

5. Acknowledgement

We wish to thank to: the U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, MT, USA for supplying the extruded diets, Mr. Ruben Kriseldi for the technical assistance, and Fulbright Scholar Program with IIE grantee ID#151510910.

References

- Adeparusi, E. O., & Famurewa, J. A. V. (2011). Water temperature and surface coating effect on floatability, water absorption and thickness swelling of feed. *Journal of Agricultural Science*, *3*(4), 254.
- AOAC (1990). Official methods of Analysis. Association of Official Analytical Chemists, 15th edition Washington DC.
- Bektursunova, M., Sidorova, V., Zhiyenbayeva, S., Mashentseva, N., & Assylbekova, S. (2023). Effect of extrusion process parameters on pellet crumbliness in fish feed production. *Slovak Journal of Food Sciences*, 17.
- Bouzaza, D., Arhaliass, A., & Bouvier, J. (1996). Die design and dough expansion in low moisture extrusion-cooking process. *Journal of Food engineering*, 29(2), 139-152.
- Bowzer, J., Page, M., & Trushenski, J. T. (2016). Extrusion Temperature and Pellet Size Interact to Influence Growth Performance of Hybrid Striped Bass Fed Industrially Compounded Aquafeeds. *North American Journal of Aquaculture*, 78(4), 284-294.
- Brown, M., Fallahi, P., Muthukumarappan, K., Singha, P., & Sindelar, S. (2015). A Comparative Study of the Effects of Non-starch Polysaccharide Gums on Physical

- Properties of Single-screw Extruded Aquafeed. *Journal of Food Processing & Technology*, 6(6), 457.
- Bu, X., Li, Y., Lai, W., Yao, C., Liu, Y., Wang, Z., ... & Ai, Q. (2024). Innovation and development of the aquaculture nutrition research and feed industry in China. *Reviews in Aquaculture*, *16*(2), 759-774.
- Cheng, H., Samuelsen, T. A., Sørensen, M., Xue, M., & Li, J. (2024). Prediction of extruded aquafeed physical quality parameters through a dough viscosity model. *Journal of Food Process Engineering*, 47(4), e14604.
- Chevanan, N., Muthukumarappan, K., Rosentrater, K. A., & Julson, J. L. (2007). Effect of die dimensions on extrusion processing parameters and properties of DDGS-based aquaculture feeds. *Cereal chemistry*, 84(4), 389-398.
- Chevanan, N., Rosentrater, K. A., & Muthukumarappan, K. (2010). Effects of processing conditions on single screw extrusion of feed ingredients containing DDGS. *Food and Bioprocess Technology*, *3*(1), 111.
- Chinnaswamy, R. (1993). Basis of cereal starch expansion. *Carbohydrate Polymers*, 21(2-3), 157-167.
- Conway, H., & Anderson, R. (1973). Protein-fortified extruded food products. *Cereal science today*.
- Das, H. K., Hattula, M. T., Myllymäki, O. M., & Mälkki, Y. (1993). Effects of formulation and processing variables on dry fish feed pellets containing fish waste. *Journal of the Science of Food and Agriculture*, 61(2), 181-187.
- Ebbing, M. A., Yacoubi, N., Naranjo, V., Sitzmann, W., Schedle, K., & Gierus, M. (2022). Towards Large Particle Size in Compound Feed: Using Expander Conditioning Prior to Pelleting Improves Pellet Quality and Growth Performance of Broilers. *Animals*, 12(19), 2707.
- Espinoza-Ortega, M., Molina-Poveda, C., Jover-Cerdá, M., & Civera-Cerecedo, R. (2024). Feeding frequency effect on water quality and growth of Litopenaeus vannamei fed extruded and pelleted diets. *Aquaculture International*, 32(1), 413-429.
- Fan, J., Mitchell, J., & Blanshard, J. (1994). A computer simulation of the dynamics of bubble growth and shrinkage during extrudate expansion. *Journal of Food engineering*, 23(3), 337-356.
- Friesen, K., Nelssen, J., Behnke, K., & Goodband, R. (1992). Ingredient processing: Improving pig feed by extrusion. *Feed management*, *43*(5), 33-38.

- Guillaume, J. (2001). *Nutrition and feeding of fish and crustaceans*. Springer Science & Business Media.
- Hertrampf, J. W., & Piedad-Pascual, F. (2012). *Handbook on ingredients for aquaculture feeds*. Springer Science & Business Media.
- Hilton, J., Cho, C., & Slinger, S. (1981). Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (Salmo gairdneri R.). *Aquaculture*, 25(2-3), 185-194.
- Hyatt, K. D. (1979). Feeding strategy. Fish physiology, 8, 71-119.
- Jeong, K., Takeuchi, T., & Watanabe, T. (1991). Improvement of nutritional quality of carbohydrate ingredients by extrusion process in diets of red sea bream. *Nippon Suisan Gakkaishi*, *57*(8), 1543-1541.
- Jones, D., Chinnaswamy, R., Tan, Y., & Hanna, M. (2000). Physiochemical properties of ready-to-eat breakfast cereals. *Cereal Foods World*, 45(4), 164-168.
- Kannadhason, S., & Muthukumarappan, K. (2010). Effect of starch sources on properties of extrudates containing DDGS. *International Journal of Food Properties*, 13(5), 1012-1034.
- Khater, E.-S. G., Bahnasawy, A. H., & Ali, S. A. (2014). Physical and mechanical properties of fish feed pellets. *Journal of Food Processing & Technology*, 5(10), 1.
- Kim, J., Tanhehco, E., & Ng, P. (2006). Effect of extrusion conditions on resistant starch formation from pastry wheat flour. *Food Chemistry*, 99(4), 718-723.
- Kraugerud, O. F., Jørgensen, H. Y., & Svihus, B. (2011). Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. *Animal Feed Science and Technology*, 163(2), 244-254.
- Larrea, M., Chang, Y., & Martinez-Bustos, F. (2005). Some functional properties of extruded orange pulp and its effect on the quality of cookies. *LWT-Food Science and Technology*, 38(3), 213-220.
- Lim, C., & Cuzon, G. (1994). Water stability of shrimp pellet: a review. *Asian fisheries science*, 7(2-3), 115-126.
- MILLER, R. C. (1985). Low moisture extrusion: Effects of cooking moisture on product characteristics. *Journal of Food Science*, *50*(1), 249-253.
- Misra, C. K., Sahu, N., & Jain, K. (2002). Effect of extrusion processing and steam pelleting diets on pellet durability, water absorption and physical response of Macrobrachium rosenbergii. *ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES*, *15*(9), 1354-1358.

- Mitchell, J., Fan, J., & Blanshard, J. (1994). The shrinkage domain. *Extrusion Communiqué*, *March*, 10-12.
- Moore, D., Sanei, A., Hecke, E., & Bouvier, J. (1990). Effect of ingredients on physical/structural properties of extrudates. *Journal of Food Science*, 55(5), 1383-1387.
- Parker, R. (2011). Aquaculture science. Cengage Learning.
- Pennells, J., Salini, M., Rombenso, A., Simon, C., & Ying, D. (2025). The State-of-the-Art of Aquafeed Extrusion: Mechanisms, Challenges and Opportunities. *Reviews in Aquaculture*, 17(2), e70002.
- Riaz, M. (2000). Introduction to extruders and their principles. *Extruders in food applications*.

 Technomic Publishing Co., Inc., Lancaster, PA.
- Riaz, M. (2008). Extruding full fat soy for maximum quality [J]. Pigs and Poultry, 4, 027.
- Riaz, M. N. (2023). Extrusion of Aquafeeds Formulated with Alternative Protein Sources. In *Alternative Protein Sources in Aquaculture Diets* (pp. 523-546). CRC Press.
- Rolfe, L., Huff, H., & Hsieh, F. (2001). Effects of particle size and processing variables on the properties of an extruded catfish feed. *Journal of Aquatic Food Product Technology*, 10(3), 21-34.
- Rosentrater, K. A., Muthukumarappan, K., & Kannadhason, S. (2009). Effects of ingredients and extrusion parameters on aquafeeds containing DDGS and potato starch. *Journal of Aquaculture Feed Science and Nutrition*, *I*(1).
- Rout, R. K., & Bandyopadhyay, S. (1999). A comparative study of shrimp feed pellets processed through cooking extruder and meat mincer. *Aquacultural engineering*, 19(2), 71-79.
- Singh, R. R., Majumdar, R. K., & Venkateshwarlu, G. (2014). Optimum extrusion-cooking conditions for improving physical properties of fish-cereal based snacks by response surface methodology. *Journal of food science and technology*, *51*(9), 1827-1836.
- Singh, S. K., & Muthukumarappan, K. (2016). Effect of feed moisture, extrusion temperature and screw speed on properties of soy white flakes based aquafeed: a response surface analysis. *Journal of the Science of Food and Agriculture*, 96(6), 2220-2229.
- Sørensen, M. (2012). A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. *Aquaculture Nutrition*, 18(3), 233-248.
- Sørensen, M., Stjepanovic, N., Romarheim, O., Krekling, T., & Storebakken, T. (2009). Soybean meal improves the physical quality of extruded fish feed. *Animal Feed Science and Technology*, *149*(1-2), 149-161.

- Stark, C. R. (2012). Feed processing to maximize feed efficiency. *Feed efficiency in swine*, 131-151.
- Thomas, M., Huijnen, P., Van Vliet, T., Van Zuilichem, D., & Van der Poel, A. (1999). Effects of process-conditions during expander processing and pelleting on starch modification and pellet quality of tapioca. *Physical quality of pelleted feed. A feed model study*, 114.
- Thornburg, J. (2025). Feed the fish: A review of aquaculture feeders and their strategic implementation. *Journal of the World Aquaculture Society*, 56(2), e70016.
- Tumuluru, J. S., Wright, C. T., Kenney, K. L., & Hess, R. J. (2010). A technical review on biomass processing: densification, preprocessing, modeling and optimization. 2010 Pittsburgh, Pennsylvania, June 20-June 23, 2010
- Turchini, G. M., & Hardy, R. W. (2024). Research in Aquaculture Nutrition: What Makes an Experimental Feeding Trial Successful?. *Reviews in Fisheries Science & Aquaculture*, 1-9.
- Wang, H., Ma, S., Yang, J., Qin, Y., Cheng, H., Xue, M., Li, J., & Li, J. (2021). Optimization of the process parameters for extruded commercial sinking fish feed with mixed plant protein sources. *Journal of Food Process Engineering*, 44(1), e13599.
- Xing, S., Liang, X., Wierenga, P. A., Wang, H., Hou, A., Wang, J., Schrama, J. W., & Xue, M. (2024). Effects of protein sources and extrusion processing conditions via pellet physical quality on the feed intake, gastrointestinal emptying and digestion of spotted seabass (Lateolabrax maculatus). *Aquaculture Reports*, *37*, 102243.
- Yadav, D. N., Tushir, S., Guru, P. N., Yadav, D. K., & Vishwakarma, R. K. (2021). Technological advancements in processing of legumes and pulses. In *Advances in Cereals Processing Technologies* (pp. 79-107). CRC Press.
- Yu, X., Faggion, S., Liu, Y., Wang, B., Zeng, Q., Lu, C., ... & Bao, Z. (2025). Role of multiomics in aquaculture genetics and breeding: current status and future perspective. *Science China Life Sciences*, 1-14.

Table 1. Composition (g 100 g⁻¹ as is) of diet manufactured using two-extrusion process.

Ingredient	Inclusion rate (% as-is)
Menhaden fishmeal, Special Select®	12
Corn protein concentrate, Empyreal® 75	10
Soybean meal 48% CP	10
Chicken 42 – ADF	10
Wheat gluten meal	4

Blood meal, spray dried	2.5
Wheat flour, durum	30.03
Menhaden fish oil ^a	10.01
Lecithin (Yelkinol AC dry)	1
Vitamin C (Rovimax® Stay-C® 35)	0.15
Vitamin premix, ARS 702	1
Trace mineral premix, ARS 1440	0.1
Sodium chloride	0.28
Magnesium oxide	0.06
Potassium chloride	0.56
Monocalcium phosphate	2.28
Choline Chloride 50%	1
DL-Methionine	0.56
Lysine HCl	2.66
Threonine	0.63
Taurine	1
Yttrium oxide	0.1
Carophyll® Pink, 10% astaxanthin,	0.08

^a incorporated via post extrusion top-coating

Table 2. Proximate and Amino acid (AA) composition (g kg⁻¹, dry matter) of experimental diets. D2LT: 2-mm pellets processed with low temperature (cold process); D2HT: 2-mm pellets processed with high temperature (cooking-extrusion); D4LT: 4-mm pellets processed with low temperature; D4HT: 4-mm pellets processed high temperature.

AA (g kg ⁻¹ , dry matter)	D2LT	D2HT	D4LT	D4HT
Taurine	1.11	1.12	1.24	1.23
Hydroxyproline	0.26	0.24	0.24	0.23
Aspartic Acid	3.08	3.10	3.02	3.07
Threonine	2.03	2.05	1.96	1.97
Serine	1.46	1.52	1.47	1.45
Glutamic Acid	7.75	7.84	7.86	7.94
Proline	3.00	2.99	3.00	3.01
Lanthionine	0.00	0.00	0.00	0.00
Glycine	1.99	1.97	1.91	1.87
Alanine	2.42	2.43	2.37	2.39
Cysteine	0.53	0.54	0.52	0.53
Valine	2.13	2.15	2.11	2.14
Methionine	1.29	1.29	1.26	1.28
Isoleucine	1.66	1.68	1.66	1.69

Leucine	3.94	3.97	3.93	3.99
Tyrosine	1.52	1.51	1.51	1.53
Phenylalanine	2.18	2.19	2.16	2.20
Hydroxylysine	0.10	0.10	0.09	0.10
Ornithine	0.01	0.01	0.01	0.01
Lysine	4.14	4.19	4.09	4.16
Histidine	1.00	1.01	0.99	1.00
Arginine	2.08	2.10	2.07	2.11
Tryptophan	0.44	0.45	0.38	0.35
Crude Protein	45.11	45.82	44.73	45.64
Moisture	3.37	2.42	3.71	3.56
Crude Fat	14.84	14.31	17.02	12.70
Crude Fiber	0.92	0.93	0.97	1.39
Ash	7.42	7.59	7.38	7.51

Table 3. Physical properties of extruded diets produced by using two different extrusion processes

Extrusion	Diet	Pellet	Expansion	Sinking	Water	Water
	Size	Durability	ratio	velocity	absorption	stability (%)
		Index (%)		(cm s ⁻¹)	index	
Cooked	2	99.41±0.19	1.01±0.02	12.92 ± 9.46	$3.60\pm0.06^*$	86.20 ± 0.15
(D2HT)	mm	~?				
Cooked	4	99.87±0.09*	1.13±0.04*	64.35±4.49*	2.52 ± 0.07	$88.18\pm0.08^*$
(D4HT)	mm					
Cold	2	97.53±0.11*	1.06 ± 0.02	0.40 ± 0.09	2.23 ± 0.15	82.60 ± 0.54
(D2LT)	mm					
Cald	4	07.24+0.07	1.11±0.02*	0.55+0.16	2 20 +0 22	97.70 . 0.10*
Cold	4	97.34±0.07	1.11±0.02	0.55 ± 0.16	2.28 ± 0.23	87.72±0.18*
(D4LT)	mm					
(D-LI)	111111					
Goodness-o	of-Fit fo	r Normal distrib	oution			
Cramer-voi	n	p > 0.250	p > 0.250	p = 0.034	p > 0.250	p > 0.250
	•	P > 0.200	P > 0.200	P 0.021	P > 0.200	P > 0.200
Mises						

Anderson-	p > 0.250	p > 0.250	p = 0.033	p > 0.250	p > 0.250
Darling					
Two-way ANOVA	(Type I SS)				
Extrusion Process	p < 0.0001	p = 0.0181	p < 0.0001	p < 0.0001	p < 0.0001
Diet Size	p = 0.1663	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001
Interaction	p = 0.0058	p < 0.0001	p < 0.0001	p <0.0001	p < 0.0001

^{* =} Significant differences (t test, p<0.05) between two different size produce within the same treatment

Different superscript letters denote significant differences among the treatments

Author's Statement

Types of contribution
Research conceptualization, Methodology,
Validation, formal analysis, investigation,
Draft creation, supervision and Writing the
original draft
Research conceptualization, Methodology,
Validation, formal analysis, investigation,
Draft creation, supervision and Writing the
original draft
Research conceptualization, Methodology,
Validation, formal analysis, investigation,
Draft creation, supervision and Writing the
original draft
Research conceptualization, Methodology,
Validation, formal analysis, investigation,
Draft creation, and visualization

Conflicts of Interest

All authors state no conflict of interest

