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REPLACING FISH MEAL WITH UNTREATED AND ENZYMATICALLY TREATED TORULA YEAST (*CYBERLINDNERA JADINII*) AFFECTS PELLETING DIE FLOW RESISTANCE AND PHYSICAL PROPERTIES OF THE FEED PELLETS

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Abstract: Yeast is gaining importance as a novel feed ingredient. Although fishmeal generally provides better feed conversion in farmed aquatic animals, research suggests that the torula yeast *Cyberlindnera jadinii* (CJ) is a promising alternative to fishmeal. This study examines the effects of replacing fishmeal with torula yeast (CJ), both untreated and treated with protease and endo-exo 1.3-beta-glucanase, on pellet production. The first experiment evaluated changes in flow resistance and pellet quality when fishmeal was replaced with yeast. The second experiment focused on how enzyme-treated yeast influenced these factors. Pellets containing 20% CJ, whether treated or not, showed increased flow resistance and higher pellet strength. Pellets formulated with 10% and 20% CJ, as well as pellets composed solely of yeast material used as a control, exhibited water-repellent properties, potentially enhancing feed intake, reducing waste, and supporting sustainable production. However, pellets with 10% and 20% enzyme-treated CJ showed fat-repellent behavior, which may hinder post-production processes. Enzymatic treatment reduced underwater swelling in these pellets, and minimal hydrolysis is recommended to limit disintegration. Enzyme treatment reduced surface roughness, with pellets containing 20% treated CJ exhibiting the smoothest texture.

Key words: *fishmeal alternative, protease, glucanase, single die pelleting, pellet strength, underwater swelling*

INTRODUCTION

The expansion of aquaculture requires more sustainable protein sources (Aslaksen et al., 2007). Fishmeal remains a key component in aquatic feeds, and the industry continues to seek alternatives (Tacon & Metian, 2015; Malcorps et al., 2019). Research institutions are exploring optimal replacements (Froehlich, Jacobsen, Essington, Clavelle & Halpem, 2018; Pelletier, Klinger, Sims, Yoshioka & Kittinger, 2018). Over the past decade, various yeast species have gained attention as nutritionally valuable feed ingredients (Langeland et al., 2014; Vidakovic et al., 2015, 2020; Huyben et al., 2017; Agboola, Øverland, Skrede & Hansen, 2020).

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Yeasts offer high protein content and amino acid profiles suitable for farmed aquatic animals like tilapia, salmon, and shrimp (Olvera-Novoa, Martinez-Palacios & Olivera-Castillo, 2002; Øverland, Karlsson, Mydland, Romarheim & Skrede, 2013; Gamboa-Delgado, Fernández-Díaz, Nieto-López & Cruz-Suarez, 2015). They can replace fishmeal or soybean meal up to 24% without affecting growth or health (Guo, Qiu, Salze & Davis, 2019; Jin et al., 2018).

Replacing conventional feed ingredients can affect physical pellet quality and water stability, potentially leading to nutrient loss and pollution (Tantikitti, 2014; Ferreira, Bonetti & Seiffert, 2011). Pellet durability, hardness, and swelling are key indicators of feed efficiency. These properties are influenced by the behavior of novel ingredients during pelleting, including flow resistance, hydrophilicity, and surface characterristics (Stokes, Boehm & Baier, 2013). Flow resistance during compaction (p_{max}) affects energy use and product consistency.

Temperature changes in the pelleting die, combined with water content, can alter nutrients and microstructure (Hoseney, William, Lai & Guetzlaff, 1992). Shearing during pelleting improves density and cohesion by redistributing particles and reducing air pockets (Hermansson, 2000). Enzymatic treatment can enhance nutritional and physical quality (Inborr & Bedford, 1994). Yeast cell walls, rich in mannoproteins and β-glucans, absorb water and form viscous gels (Feldmann, 2012; Klis, Mol, Hellingwerf, & Brul, 2002). Enzymes like protease and β-glucanase can reduce viscosity by breaking down these components (Li et al., 2025), improving pellet compaction. However, short conditioning times and low water content may limit these effects.

There is limited data on how enzymes affect flow resistance and pellet quality during pelleting, especially regarding exo-endo-1,3-β-glucanase and protease. Changes in flowability, measured by pressure at incipient flow (p_{max}), influence energy consumption and pellet quality (Miladinovic, Storebakken, Lekang & Salas-Bringas et al., 2021). Water activity affects texture, shelf life, and compaction behavior (Slade & Levine, 1991; Lowe & Kershaw, 1995). Higher water activity can reduce flow resistance, making compression easier. These factors impact pelleting efficiency, pellet quality, and energy use.

This study investigates how replacing fishmeal in shrimp feed formulation with *Cyberlindnera jadinii* (CJ), with or without enzymes, affects feed mash flow and pellet quality. It hypothesizes that yeast and enzymes (protease and exo-endo-1,3-β-glucanase) can lower p_{max}, improving pellet durability and underwater stability. Two experiments were conducted: one to optimize yeast dosage, and another to assess enzyme effects on physical feed properties.

MATERIALS AND METHODS

Experiment 1

Raw materials

All raw ingredients presented in Table 1, except CJ and fishmeal, were obtained from the Center for Feed Technology, Norwegian University of Life Sciences, Ås, Norway. Inactivated CJ yeast was obtained from Lallemand, Salutaguse, Estonia in powder form with a dry matter of 970 g/kg, ash 78 g/kg, crude protein 470 g/kg, crude fat 16 g/kg, and gross energy 19.9 MJ/kg. Fishmeal was obtained from Norsildmel AS, Egersund, Norway with dry matter 917 g/kg, ash 145 g/kg, crude protein 684 g/kg, crude fat 73 g/kg, and gross energy of 19.4 MJ/kg. All materials used for the experimental diets, except CJ and vitamin/mineral premix, were milled through a 1 mm screen size installed on an Alpine mill (model 160 UPZ, 1988, No. 13580.1). Yeast and vitamin/mineral premix were not milled due to their particle size below 1mm.

Preparation of experimental mixes

Mixing of the raw materials was done using a mixer Diosna P1/6 (DIOSNA Dierks & Söhne GmbH, Osnabrück, Germany), with mixing tools based on three agitating paddles and a tulip-form knife. The speed of the paddles during mixing was 250 rpm and the knife 500 rpm. During the mixing of the formulated diets, 10% of distilled water was added into the mixing mash with a spraying nozzle, model 970, Düsen-Schlick GmbH (Untersiemau/Coburg, Germany). Water was added to integrate enough moisture for further processes. All diets were mixed thoroughly for 800 seconds. From each mixed diet, three samples were randomly taken and thereafter mixed to obtain a representative homogeneous sample. Representative samples were analyzed for moisture content in triplicate by the EU, No. 152/2009 method. The average moisture of the samples for all the trials before

Table 1.
Formulation of the experimental aquatic feed model for benthic animals, shrimps, presented as the percentage of the inclusion of each raw material

	***Cyberlindnera jadinii* (CJ) inclusion level (%)**				
Raw materials[*]	**0 CJ**	**2.5 CJ**	**5 CJ**	**10 CJ**	**20 CJ**
Wheat flour	30	30	30	30	30
Fishmeal	22.5	20	17.5	12.5	2.5
Soybean meal	10	10	10	10	10
Poultry meal	8.5	8.5	8.5	8.5	8.5
Rice flour	6	6	6	6	6
Soy protein concentrate	6	6	6	6	6
Squid meal	5	5	5	5	5
Cyberlindnera jadinii	-	2.5	5	10	20
Monocalcium phosphate	1.6	1.6	1.6	1.6	1.6
Magnesium oxide	0.3	0.3	0.3	0.3	0.3
Manganese dioxide	0.01	0.01	0.01	0.01	0.01
Monosodium phosphate	0.56	0.56	0.56	0.56	0.56
Vit/Min premix[**]	0.5	0.5	0.5	0.5	0.5
Water content (%)	9	9	9	9	9
TOTAL (%)	100	100	100	100	100

[*]*Batch size 300 gram;* [**]*DSM - OVN*™

the trials was 13% (+/- 1 % w/w). After moisture analyses were performed the mixed mash was vacuum-packed to avoid moisture loss and stored at room temperature of 18°C. Each package was opened right before the pelleting trial.

Steam conditioning and pelleting

To ensure uniformity in size and weight, 0.13 g of feed mash was weighed for each pellet and placed into thirty (30) Eppendorf tubes per trial. Subsequently, all sealed Eppendorf tubes containing the specific feed were immersed in boiling water at atmospheric pressure.

The feed mash in the Eppendorf tube was conditioned for 3 minutes in boiling water to allow the water in the feed mash to become steam. Subsequently, the Eppendorf tubes were placed in the fridge at 4 °C to cool down for 20 minutes before pelleting so that water could condense swiftly within the entire feed sample in the Eppendorf tube after conditioning. The single-die pelleting method described by Salas-Bringas et al. (2010) was used for the compaction of the mixed feed into cylinder-shaped pellets.

Steam-conditioned material from each Eppendorf tube was used to produce a single pellet by pouring it into a blank die channel preheated to 81°C. The 81°C temperature of the pelleting die is recommended to eliminate potential salmonella contamination following Norwegian law (VKM, 2006). Poured feed mash was heated for 3 minutes before compaction to ensure an equal temperature of the mash particles. Pelleting was done with a compression rod having a diameter of 5.45 mm. The same pelleting temperaturewas used during material compaction and the extraction of the pellet from the die. During pelleting, an initial pre-load pressure of 240 kPa was used. The setting for a maximal force load for each pellet was 285 N and applied compressibility was 12 MPa. The compacting force load was chosen according to the densities of commercial animal feed pellets produced on ring-die pellet press. This is explained in detail by Salas-Bringas, Misljenovic, Wicklund, Lekang and Schüller (2011) and Salas-Bringas et al. (2015).

The compaction was done at a speed of 10 mm/min with a compression rod inserted in a 5.5-mm die channel with a closed end. Successively after reaching the maximal force load, the closed part of the die was removed. Subsequently, the pellet was discharged from the pelleting die at a pace of 2 mm/min. The selected pace was set to avoid getting beyond the compacting forces and thus avoiding additional compaction. The total time of the material retaining in the pelleting channel was 9 minutes. Compacted pellets were 5.5 mm in diameter and had about 0.1 grams of weight. The loss of weight was due to high pressures and temperatures causing moisture evaporation. Pellets were stored at 4 ºC, each in the Eppendorf tube prior to further analysis.

Experiment 2

Preparation of experimental mixes and sampling

The remaining mixed feed from Experiment 1, which was vacuum-packed and stored at room temperature, was used. Each diet was then individually poured into the mixer Diosna P1/6 (DIOSNA Dierks & Söhne GmbH, Osnabrück, Germany). Before mixing, the enzymatic cocktail was prepared with buffer solution and enzymes, non-commercial protease (AB Vista, Marlborough, UK) derived from *Fusarium equiseti*, and endo-exo 1,3-β-glucanase produced by Megazyme, Ireland and derived from *Trichoderma sp.* A pre-prepared 100 mmol buffer solution (pH 5.5) was stored overnight at 4 ℃ and used as a carrier for the enzymes. About 0.9 ml of protease and 0.75 ml of endo-exo 1,3-β-glucanase were added to 15 ml of buffer solution.

The pH of the mixture was 5.8, close to the producer's recommendation of pH 5.5 for both enzymatic products. The enzymatic cocktail of 16.65 ml was sprayed on the feed mash batch of 150 g for each feed mixture. The control diet was sprayed only with 16.65 ml of distilled water without enzymes. Spraying was done with a spraying nozzle (Model 970, Düsen-Schlick GmbH, Germany) built into the mixer Diosna P1/6 (Osnabrück, Germany). During the spraying of the enzymatic solution, the mixer was operated with the speed of the paddles set at 250 rpm. The speed of the knife was 500 rpm. All diets were mixed thoroughly for 800 seconds.

Three samples were randomly taken from each mixed diet and subsequently combined to create a representative homogeneous sample. The representative samples were used to analyze moisture content in triplicate for each enzymatically enriched feed mash (EU method No. 152/2009).

The average moisture content for all feed diets was 15% (0.3% w/w). The remaining treated feed mash was removed from the mixer, vacuum-packed, and stored at 4 °C to preserve moisture content until further steam conditioning and pelleting. Steam conditioning and pelleting were done in the same way as in Experiment 1.

Analytical techniques and measurements

Particle size distribution

The laser diffraction method was used to verify the particle size distribution of the feed-mixed mash. The device used for measuring particle size distribution was Mastersizer S instrument (Malvern Instruments Ltd, Worcestershire, UK). Calculating the volumetric particle size distribution of the light energy on the detector was done by deflecting the light based on the theory for spherical particles (Hetland, Svihus & Olaisen, 2002).

Measurement of compaction, pellet discharge, and pressure at initial flow (p_{max})

Maximum compaction pressure (Pa) at maximum force (N) during densification, compacttion, and pellet ejection from the die were observed and recorded. The recording was done with NEXIGEN Plus software connected to a Lloyd texture analyzer (LR 5K Plus, Lloyd Instruments, U.K.). Measuring maximum compaction pressure at maximum force might point to possible alterations in electrical energy consumption connected to alterations in the resistance of the feed material to move through the die (p_{max}). The p_{max} was identified as the pressure needed to initiate pellet ejection from the pelleting die when the close end from the die channel was removed. Measurements were done as a flow resistance indicator to measure variations between trials, caused by friction on the contact area between the die and compacted pellet. The initial movement of the pellets through the die was recorded as a force needed for the pellet to start moving through the open 5.5 mm die hole. Measurements were done after compaction and when the blank part of the die was removed.

The pellet discharge speed was set to 2 mm/min to guarantee that the pressure would be under the previous compaction pressures. The analytical results for maximal pressure before the pellet started moving through the die, were obtained using a Lloyd LR 5K Plus texture analyzer and assessed with Eq. 1.

$$p_{max} = F / \pi r^2 \qquad \text{Eq. 1}$$

where:

F – Load force (Nm)
r - radius of a pellet (mm)

Tensile strength

Results of the tensile strength analyses were done by applying maximum force (F) on the compacted feed pellet under diametral compression. Three randomly chosen pellets for each treatment were used. Tensile strength was measured for each pellet by recording the first peak force (F) while compressing the pellet across the diameter at a speed of 1 mm/min¯. The stress (σ) was estimated by using Eq. 2, the Brazilian test. The Brazilian test is a geotechnical laboratory method used to indirectly measure the tensile strength of materials, by applying diametrical compression to a disc-shaped specimen until it splits. A probe with a surface of 60 mm in diameter was used to measure σ (Eq. 2) while being connected to a Lloyd LR 5K Plus texture analyzer (Lloyd Instruments, U.K.)

$$\sigma t = 2F/\pi Dl \qquad \text{Eq. 2}$$

where:

σt - maximum tensile strength (MPa)
F - load at fracture of the first peak force (N)
D – shrimp feed pellet diameter (mm)
l - shrimp feed pellet length (mm)

Measuring contact surface angle (θ)

The contact surface angle measures how a liquid droplet interacts with a solid surface, indicating wettability. Droplets are used because their shape reveals the balance between adhesive and cohesive forces. The droplets of oil and water were dispensed from a dosing needle and placed at the diametral plain surface of a feed pellet, defined as the upper plain being at the side of a compression rod during pel-leting. The analyses of liquid droplets from a dosing needle, defined as contact surface angle, outline the surface energy between the adhering liquid and the pellet surface. Surface wetting of the shrimp pellets, the effect of added yeast, and the enzyme treatment on θ for both oil and water were evaluated with a video-based device OCA 15EC (Data Physics Instruments GmbH, Germany) (Fig. 1).

The θ analyses were completed on three randomly chosen pellets to compare how liquid sorption of the pellets with rape seed oil or distilled water could be affected by the experimental factors. The droplet volume for distilled water was 1 μl and for rapeseed oil 5 μl. Due to the larger surface tension of rapeseed oil, it was more difficult to detach the oil droplet from the needle, thus a larger droplet volume was needed. The recorded droplet absorption is measured in seconds and presented in the results as the initial surface angle (T0) and final droplet age. Rapeseed oil θ and distilled water θ were recorded within 3 and 1.2 seconds, respectively.

The time frame was decided for both oil and water droplets by full penetration of the liquids at the upper diametral plain surface of a shrimp pellet. The θ measurements were done at a temperature of 18 °C. The software SCA20 (Dataphysics instruments GmbH, Germany) was used to record the change of θ at different time intervals. When the initial θ was < 90°, the surface was considered as hydrophilic, however, if θ > 90° a hydrophobic surface was considered, described by Förch, Schönherr and Jenkins(2009) and Mišljenović, Mosbye, Schüller, Lekang and Salas-Bringas (2015).

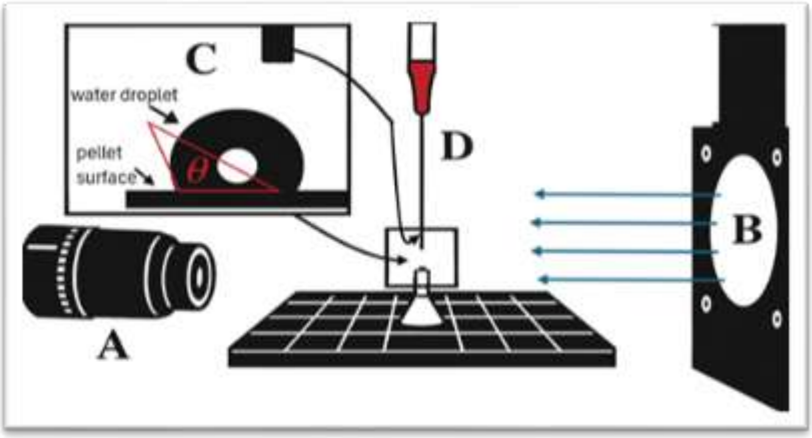

Figure 1. Setup for surface angle measurement. Letters indicating: A - video camera; B – light source; C – an image of a droplet on top of a pellet surface; D – dosing syringe

Figure 2. Full setup instrument for the UPS measurements (1 - Krüss Tensiometer; 2 - Water glass container with the pellet; 3 - Allen zoom compact video microscope lenses; 4 - Microviper portable computer)

Underwater pellet swelling rate analyses (UPS)

The UPS measurements explain if increasing yeast content in the feed instead of fishmeal and enzymatic treatments could influence changes in the physical properties of the pellets. Also, the UPS indicates pellet cohesiveness (particle detachment), linked to the swelling rate of the pellet. A special prearrangement to monitor the swelling of shrimp feed pellets underwater was used as described by Miladinovic et al. (2021).

The constructed system consisted of a video microscope (Microviper) and a microscope lens (Allen ¼”) mounted on the optical tensiometer (OCA 15EC, Data Physics Instruments GmbH, Germany) as shown in Fig. 2 was used for the UPS measurements.

Optical monitoring of pellet swelling was done in accordance with Ferreira and Rasband (2012) with the image processing software Fiji. Distilled water at a temperature of 20 °C was added to the experimental glass container. For each treatment, four randomly chosen pellets were used for measuring UPS. The reference of a starting point for UPS measurement was a cross-sectional view of the feed pellet with a diameter of 5.5 mm. An image of a cross-sectional view of the feed pellet was taken every 60 seconds for an observation time of 40 minutes. The length of the observation time was chosen as the time required for shrimp to consume the pellet in the open-pond shrimp farming system (Lovell, 1998). The observation area defining the cross-section of a swollen shrimp feed pellet underwater during observation time implies the UPS results.

Surface roughness analyses

Surface roughness analyses were done to understand if the replacement of the fish meal with yeast or the addition of enzymes may influence any changes to particle packing and thus surface characteristics of the pellets.

Results are represented by the irregularities existing on the surface of the feed pellets in the diametral and longitudinal directions. The diametral surface roughness has been created by interactions between feed particles during compaction.

However, longitudinal surface roughness is created as the interaction between particles and the die wall. Surface roughness is further presented as a numerical scale of the surface condition influenced by novel materials, enzymes, and diverse packing-ability of the particle in the pellets. The analyses were done with a surface roughness tester (Surftest SJ-210, Mitutoyo, Japan).

Data analyses

The software used for statistical analyses was Minitab v.17 and for plotting the figures was utilized Microsoft Excel. ANOVA analyses were used to examine the possible effects of the increased percentage of yeast and enzyme addition. Tukey–Kramer method, using a 95 % confidence interval, was used to show significant differences between treatments. Pearson correlation test with a 95 % confidence interval was used to analyze correlations between variables.

RESULTS AND DISCUSSION

Experiment 1 – replacement of the fish meal with CJ

Particle size of the mash prior to compaction

All diets with yeast inclusion had similar particle size distribution, where large particles were between 400 µm and 500 µm. Medium size particles were found to be between 80 and 120 µm with a significant decrease when adding more yeast to the feed. The same was observed with the smallest particles under 20 µm (Fig. 3).

Flow resistance in the die during discharge of the shrimp feed pellet (p_{max})

The p_{max} results presented in Table 2 do not show any distinctive change when yeast is added and compared to control feed without added yeast. However, pelleting only yeast as a negative control, increased p_{max} over 17 folds compared to all other diets ($p<0.05$). Adding from 2.5% to 20% yeast did not change p_{max}.

Tensile strength of pellets

The hardness of pellets measured as tensile stress increased significantly ($p<0.05$) by including 20% yeast in the feed when compared to the control diet and the diets with 2.5% and 5% yeast. Pelleting only yeast without other ingredients showed increased hardness of the pellets by over 9 folds (Table 2). Tensile strength showed to be moderately correlated to p_{max} (Fig. 4), where almost half of the tensile strength as a dependent variable may be explained by flow resistance during discharge of the shrimp feed pellet from the die ($R^2 = 0.49$).

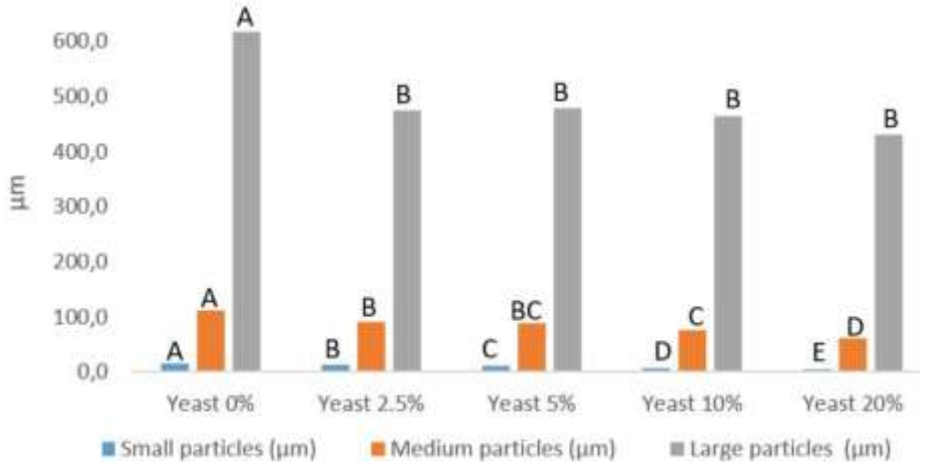

Figure 3. Particle size distribution of the shrimp feed diets. Data representing means based on two replications. Different sequential letters (A, B, C, D, E) from the ANOVA-Tukey statistical method indicate differences at a 5% level

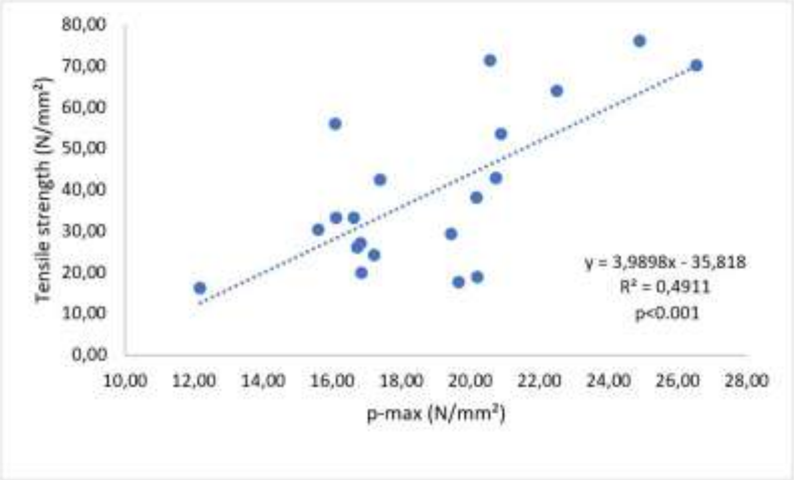

Figure 4. Correlation between p_{max} and tensile strength in experiment 1 ($p<0.001$)

By adding 2.5% and up to 20% yeast in the feed, the θ of the oil at zero time was not different from that of the control feed. A difference at zero time for oil θ was, however, observed between 100% yeast and 0% yeast ($p<0.05$).

Pellets made of 100% yeast were more lipophobic as compared to pellets with no added yeast. At 47 seconds the lipophilicity was more pronounced ($p<0.05$) for feed pellets without yeast as compared to feed with 20% yeast and 100% yeast. Similar results for oil θ were observed when pellets with no added yeast were compared with 10%, 20%, and 100% yeast at 94 seconds.

Pellets with 10%, 20%, and 100% yeast at time zero showed to have pronounced aquaphobic behavior when compared to control pellets without added yeast. Similar effects were observed at 47 seconds. However, at 94 seconds the water θ showed to be significantly lower ($p<0.01$) in the pellets with from 2.5% and up to 100% yeast (Table 2).

Underwater pellet swelling (UPS)

The UPS did not differ when control pellets (0% yeast) were compared to other pellets at the first minute after submersion under water. Differences were observed between pellets with 2.5% and 100% yeast at minute 1. Pellets containing 100% yeast tended to swell poorly at the first minute. After 20 minutes the pellets with 20% yeast had swollen 1.8 folds and the pellets with 100% yeast about 1.3 folds. Pellets with 100% yeast showed significantly ($p<0.05$) slower swelling when compared to pellets with 20% added yeast (Table 2). UPS results showed having low correlation to tensile strength ($R^2 = 0.3$) in Experiment 1.

Surface roughness in the diametral and longitudinal direction

Irregularities at the surface of the pellets defined as surface roughness, are presented in Fig. 5. Diametral surface roughness was different when yeast was added to the feed as compared to the control feed with no yeast. However, no significant change in surface roughness was observed for the diet with 5% yeast. Longitudinal roughness difference was observed only for the pellets with 20% yeast in comparison with the control pellets, and pellets with 2.5% or 100% yeast.

A correlation between p_{max} and longi-tudinal surface roughness was observed. More than half of the longitudinal surface roughness, as a dependent variable, may be explained by flow resistance in the pelleting die during shrimp feed pellet discharge ($R^2 = 0.5$).

The correlation between the diametral surface roughness and p_{max} was not observed ($R^2 = 0.07$). However, the correlation between longitudinal surface roughness and p_{max} was observed ($R^2 = 0.5$) (Fig. 6).

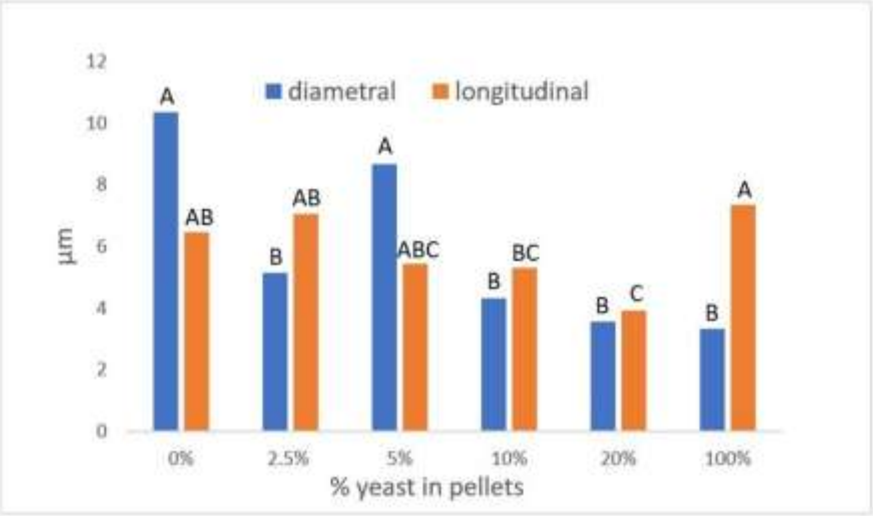

Figure 5. Surface roughness of the pellets with no added enzymes for both diametral and longitudinal analyses (Experiment 1). Data representing means based on 25 repetitions. Different sequential letters (A, B, C) from ANOVA-Tukey statistical method signify difference at a 5% level separately for diametral and for longitudinal measurements, where p-values were lower than 0.001

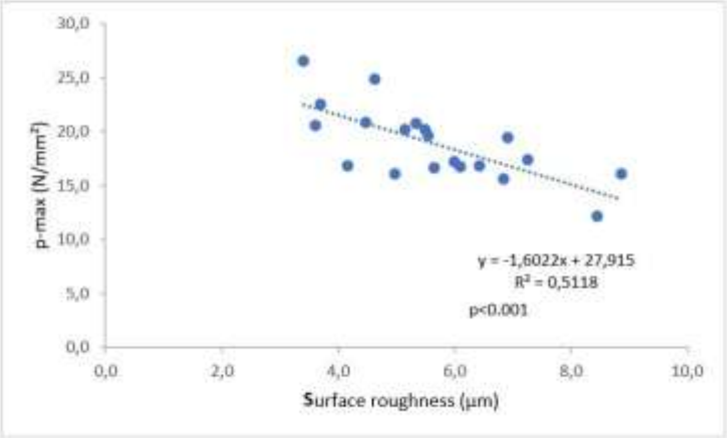

Figure 6. Correlation between longitudinal surface roughness and p_{max} based on % of added yeast from 0 to 100 %

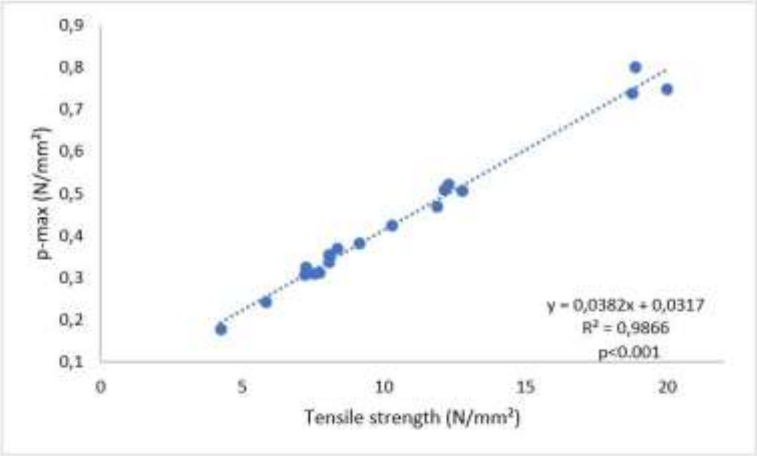

Figure 7. Correlation between p_{max} and tensile strength in experiment 2 ($p<0.001$) based on % of added yeast whether treated or not treated with enzymes

Experiment 2 – Enzymatic treatment of the feed and its effect on physical pellet properties

Flow resistance in the die during discharge of the feed pellet (p_{max})

By adding enzymes in the feed with the inclusion of up to 5% yeast, no change in p_{max} was detected. However, when enzymes were included in the feed with 10% and 20% yeast a significant increase in p_{max} was identified ($p<0.001$) when compared to the control feed with added enzymes (Table 3).

Tensile strength of pellets

The tensile strength of the pellets was significantly increased in the feed containing 20% yeast and added enzymes ($p<0.001$). This increase was not observed in other yeast-containing feeds with added enzymes when compared to the control feed (Table 3). Additionally, it was found that p_{max} had a very strong influence on the tensile strength of the pellets (Fig. 7).

Surface contact angle (θ) measurements for oil and water (CA)

At zero time, pellets with 10% and 20% yeast and added enzymes showed a lipophobic behavior ($p<0.01$) when compared to other treatments (Table 3, Fig. 8). After 5 seconds the lipophobic behavior was also observed on the surface of pellets containing 5% yeast and added enzymes. At 10 seconds, this was also seen for enzymatically treated pellets containing 2.5% yeast and 0% yeast. A similar response was observed for the rest of the analytical time, i.e. after 15 and 20 seconds (Table 3).

Table 2.
Characteristics of pellets supplemented with CJ (Experiment 1)

Added yeast	**p_{max} (MPa)**	**aW**	**Tensile strength (N/mm^2)**	**CA - oil at 0 sec.**	**CA - oil at 47 sec.**	**CA - oil at 94 sec.**	**CA - water at 0 sec.**	**CA - water at 47 sec.**	**CA - water at 94 sec.**	**UPS (mm^2) at 1 min.**	**UPS (mm^2) at 20 min.**
0 %	18.2^{b}	0.35^{a}	18.1^{c}	64.0^{a}	62.9^{a}	61.6^{a}	63.1^{a}	61.3^{a}	59.5^{a}	30.3^{ab}	40.2^{ab}
2.5%	16.6^{b}	0.46^{a}	34.1^{c}	63.3^{ab}	60.7^{ab}	58.8^{ab}	59.7^{ab}	53.2^{ab}	47.9^{b}	36.1^{a}	40.8^{ab}
5 %	17.4^{b}	0.35^{a}	30.7^{c}	57.5^{ab}	54.2^{ab}	57.4^{ab}	59.1^{ab}	49.3^{ab}	43.7^{b}	28.4^{ab}	49.1^{ab}
10 %	19.7^{b}	0.40^{a}	44.2^{bc}	61.2^{ab}	59.3^{a}	57.6^{bc}	51.0^{b}	44.0^{bc}	40.1^{bc}	33.0^{ab}	47.2^{ab}
20 %	23.9^{b}	0.40^{a}	70.3^{b}	50.7^{ab}	49.6^{bc}	48.7^{bc}	49.9^{bc}	44.9^{bc}	42.5^{bc}	28.8^{ab}	51.0^{a}
100 %	128.7^{a}	0.35^{a}	173.8^{a}	49.4^{b}	48.1^{c}	47.00^{c}	38.3^{c}	33.2^{c}	31.0^{c}	25.1^{b}	32.8^{b}

Data representing means from 6 measurements. Different alphabetical letters in superscripts from ANOVA-Tukey statistical method indicate a significant difference at 5% level.
Presented p-values for p_{max} ($p<0.001$); a_W ($p>0.05$); Tensile strength ($p<0.001$); CA-oil ($p<0.05$); CA-water ($p<0.01$) and UPS ($p<0.05$)

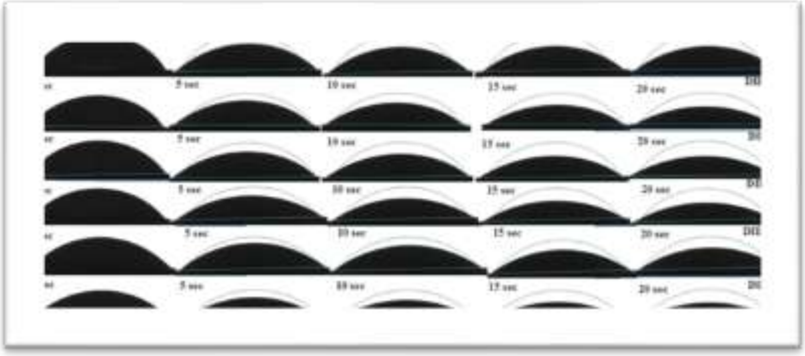

Figure 8. Change of contact angle (CA) from oil drop on the pellet surface at different time intervals. The curved line symbolizes the initial oil drop profile.

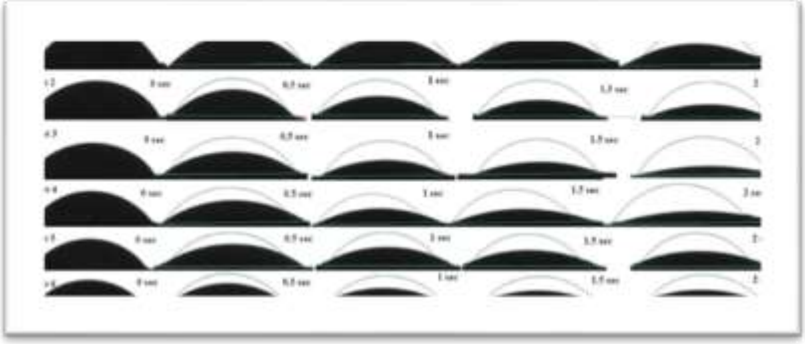

Figure 9. Change of contact angle CA from water drop on the pellet surface at different time intervals. The curved line symbolizes the initial water droplet profile.

CA of the water drop placed at the pellet surface, measured every 0.5 seconds for a total measuring time of 2 seconds, showed similar results for all feed pellets at time zero. However, after 0.5 seconds, the enzymatically treated pellet with 5% yeast had a significantly lower contact angle when compared to the control feed without enzymes and pellets treated with enzymes containing 20% yeast (Table 2, Fig. 9). Similar observations were seen after 1, 1.5 and 2 seconds of CA measurements.

Underwater pellet swelling rate (UPS)

The UPS analyses showed that by adding enzymes to the control feed without yeast there was a significant increase in pellet swelling one minute after submersion in water. After 40 minutes, the control feed with and without added enzymes was equally swollen. Conversely, the inclusion of 10% and 20% of yeast significantly decreased the UPS when compared to the enzymatically treated control feed (p=0.003) (Table 3). A similar trend was observed after 20 and 40 minutes after submersion in stagnant water. The UPS linearly decreased by increasing yeast addition in the shrimp feed, indicating that the addition of 20% yeast may result in a UPS decrease of about 31%. The UPS of the feed pellets showed to be independent of the tensile strength ($R^2 = 0.24$).

Water activity (a_w)

Differences in a_w values were not observed in experiment 2 (Table 3). Thus, the a_w analyses showed that the substitution of fishmeal with yeast and added enzymes in shrimp feed did not influence a_w.

Surface roughness at the diametral and longitudinal direction of pellets treated with enzymes

In this work, irregularities at the surface of pellets compressed with the same forces are defined in this work as surface roughness. Fig. 10 shows that longitudinal surface roughness presented as the interaction between particles and the pelleting die wall is lowered in feed containing 5% and up to 20% yeast and added enzymes. The longitudinal surface roughness results indicate a linear decrease of surface roughness along the longitudinal pellet wall by replacing fishmeal with yeast when the feed mash was treated with enzymes.

Table 3.
Characteristics of pellets supplemented with enzyme-treated CJ (Experiment 2)

Experiment 2

Added yeast	**p_{max} (MPa/mm^2)**	**Tensile strength (N/mm^2)**	**CA - oil at 0 sec.**	**CA - oil at 5 sec.**	**CA - oil at 10 sec.**	**CA - oil at 15 sec.**	**CA - oil at 20 sec.**	**CA - water at 0 sec.**	**CA - water at 0.5 sec.**	**CA - water at 1 sec.**	**CA - water at 1.5 sec.**	**CA - water at 2 sec.**	**UPS (mm^2) at 1 min.**	**UPS (mm^2) at 20 min.**	**UPS (mm^2) at 40 min.**
0% no enz	0.4^{bc}	9.3^{bc}	53.4^{a}	42.2^{a}	41.3^{a}	38.5^{a}	38.0^{a}	53.1^{a}	46.5^{a}	40.4^{a}	36.6^{a}	33.2^{a}	48.3^{b}	80.5^{abc}	106.5^{ab}
0% + enz.	0.3^{c}	6.3^{c}	52.1^{ab}	41.5^{a}	37.7^{b}	35.2^{b}	31.7^{b}	58.1^{a}	45.4^{ab}	37.9^{a}	31.8^{ab}	25.7^{ab}	72.6^{a}	109.6^{a}	116.4^{a}
2.5% + enz.	0.3^{bc}	8.3^{bc}	51.0^{b}	41.4^{a}	36.7^{c}	33.9^{b}	31.6^{bc}	59.0^{a}	39.7^{ab}	32.2^{ab}	26.7^{ab}	22.3^{ab}	62.5^{ab}	100.3^{ab}	105.2^{ab}
5% + enz.	0.3^{bc}	7.9^{c}	52.1^{ab}	35.5^{b}	33.5^{c}	30.7^{c}	28.7^{d}	56.2^{a}	29.3^{b}	19.2^{b}	10.2^{b}	4.6^{b}	53.5^{ab}	74.0^{abc}	97.0^{abc}
10% + enz.	0.5^{b}	12.4^{b}	45.9^{c}	36.1^{b}	32.7^{c}	31.1^{c}	29.7^{d}	57.1^{a}	35.3^{ab}	30.6^{ab}	27.1^{ab}	24.5^{ab}	37.3^{b}	73.9^{bc}	92.1^{bc}
20% + enz.	0.8^{a}	19.2^{a}	46.4^{c}	38.1^{b}	35.1^{bc}	32.0^{c}	29.9^{cd}	58.9^{a}	49.7^{a}	49.7^{a}	42.1^{a}	40.1^{a}	36.7^{b}	66.4^{c}	80.3^{c}

Data representing mean values from 6 measurements. Different alphabetical letters in superscripts from the ANOVA-Tukey statistical method indicate a significant difference at 5% level. p-values: p-max $p<0.001$;
Tensile strength $p<0.001$; CA-oil $p<0.001$; CA-water 0' $p=0.6$; CA-water 0.5'' $p=0.01$; CA-water 1'' & 1.5'' $p=0.007$; CA-water 2'' $p=0.003$; UPS 1' and 40' $p=0.003$; UPS 20' $p=0.004$

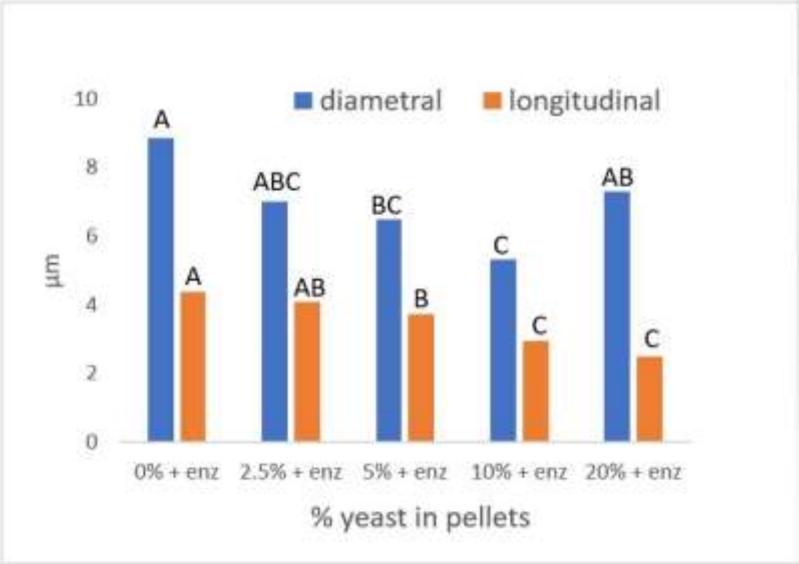

Figure 10. Surface roughness data representing means based on 25 repetitions each for diametral and longitudinal analyses (Experiment 2). Different sequential letters from the ANOVA-Tukey statistical method signify a difference at 5% level separately for diametral and for longitudinal measurements, where p-values were lower than 0.001.

However, for diametral surface roughness, created by interactions between feed particles during compaction, the same can be concluded only for 5% and 10% added yeast. No significant difference in diametral surface roughness between enzyme-treated feed containing 2.5% or 20% yeast. The surface roughness of pellets added enzymes was not influenced p_{max}, neither longitudinally (R^2 = 0.08) nor diametrically (R^2 = 0.01).

Flow resistance during pellet discharge in experiment 1 was unaffected by yeast addition from 2.5% to 20% (Table 2). However, when yeast was pelleted alone, p_{max} increased significantly. This may result from altered particle size distribution affecting densification (particle-particle) and compaction (particle-die). Micro-scale yeast material may diffuse via solid bridges, where cohesion depends on contact area and bridge diameter, contributing to high-density pellet formation. A larger compressed area requires more force to pass through the die. A similar increase in p_{max} was seen in experiment 2 with enzymatically treated feed containing 20% yeast (Table 3), showing a clear correlation between p_{max} and tensile strength (Fig. 7). In experiment 1, up to 20% yeast significantly increased pellet tensile strength (Table 2). In experiment 2, enzymatic hydrolysis with limited water and 20% yeast enhanced pellet hardness (Table 3), likely due to compact packing and strong micro-scale interactions during pelleting, consistent with Miladinovic, Sørensen and Svihus (2013). Improved durability may result from interlocking bonds between fine particles.

Surface contact angle (CA) analysis revealed how compacted shrimp feed absorbs liquids. In Experiment 1, CA for oil and water varied with observation time up to 94 seconds. Oil CA dropped significantly with 10% yeast (Table 2), and water CA decreased after 47 seconds. Even 2.5% yeast affected water CA at 94 seconds. In experiment 2, yeast and enzymatic hydrolysis reduced oil CA from 10 seconds onward, while water CA results were unclear. Only 5% yeast showed a reduction between 0.5–2 seconds (Table 3). These effects may be explained by powder density distribution and packing in the die. Korachkin and Gethin (2004) noted that particle packing can create two regions with up to 10% density variation during die-fill, influencing CA values.

Pellet-liquid interactions depend on friction, adhesion, adsorption, and wettability, aligning with Yuan and Lee (2013). Structural changes from yeast and enzymes altered pellet surface properties, explained by particle diffusion and liquid absorption in inter-particle cavities (Roman-Gutirrez, Sabathier, Guilbert, Galet & Cuq, 2003). Pellet disintegration underwater is a key indicator of shrimp feed usability (Flemming, 1995; Obaldo, Divakaran, Tacon, 2002; Bansemer et al., 2015). Swelling rate measurements via image analysis of undisturbed shrimp feed in stagnant water, as developed by Salas-Bringas, Catargiu. Miladinović, Schüller and Mišljenović (2015) and applied by Miladinovic et al. (2021), proved effective. These measurements help determine the optimal time a pellet remains intact before consumption or disintegration.

Underwater pellet swelling (UPS) is driven by fluid adsorption and desorption, which depend on the physical and chemical properties of compacted solids and raw materials (Saalah, Shapawi, Othman & Bono, 2010). Chemical binding can enhance or inhibit surface adsorption, with molecules continuously attaching and detaching. A 37.3% UPS difference in the first 60 seconds of control treatments (no yeast, no enzymes) across both experiments was linked to a 2% moisture variation. External variables like vapor pressure, temperature, and surface structure influence adsorption/de-sorption rates (Oura, Katayama, Zotov, Lifshits & Saranin, 2003). Free water in the pellet matrix facilitates rapid water movement through porous structures. Molecular adsorption, especially in hygroscopic materials like non-starch polysaccharides, drives swelling (Li et al., 2023). These polysaccharides also affect starch behavior, improving texture and functionality via altered hydrolysis.

Bound water drives molecular adsorption, while capillary adsorption involves free water drawn into pellet walls by attraction forces. As moisture increases, molecular attraction decreases, causing volume expansion nearly equal to the added water. Water equilibrium is reached when molecules migrate from surface to core, after 20 minutes in Experiment 1 (Table 2), and 40 minutes with enzymatic treatment in Experiment 2. Yeast replacement did not affect UPS in Experiment 1, but in Experiment 2, pellets without yeast swelled more in the first 60 seconds (Table 3). Enzymatic treatment with 10–20% yeast reduced UPS by 48.6% in the first minute, with similar effects at 20 and 40 minutes. While yeast alone did not affect swelling, enzyme addition slowed the deterioration of compacted solids. This likely stems from enzyme-facilitated formation of yeast microparticles with fewer cavities, enhancing water transport via capillary adsorption and altering absorption capacity. Consequently, tensile strength tripled with 20% yeast (Table 3).

Surface roughness in the longitudinal direction differed only in pellets with 20% yeast in experiment 1, likely due to better packing of fine particles, aligning with increased tensile strength (Table 1). Enzyme addition reduced roughness in feeds with 5%, 10%, and 20% yeast (Fig. 10). Roughness was influenced by particle size and correlated with p_{max} during pelleting (Fig. 6), consistent with Sarkar, Ang and Liew (2014).

CONCLUSIONS

Including enzymatically untreated yeast at 2.5% to 20% in aquatic feed does not significantly affect maximum pelleting pressure, suggesting it is unlikely to increase energy use compared to fishmeal-based pellets. However, 20% yeast may produce harder pellets with slower swellling, which benefits farms with slow-feeding species. For smoother pellet surfaces, 20% yeast is recommended. Enzymatic treatment increases pelleting pressure at 10% and 20% yeast, potentially raising energy consumption. It also enhances pellet hardness and reduces oil absorption due to the denser structure. Replacing fishmeal with treated yeast lowers surface roughness, which may limit oil coating. Evaluating the technical quality of novel ingredients is essential when adjusting feed formulations. To avoid high pelleting pressure, enzymatic hydrolysis should be avoided at 10% and 20% yeast inclusion. However, to boost pellet strength, enzymatic treatment with 20% yeast is advised. Additionally, to reduce swelling between 20 and 40 minutes underwater, feeds with 10% and 20% treated yeast should be considered.

AUTHOR CONTRIBUTIONS

Conceptualization, D.D.M.; Methodology, D.D.M. and C.S.B.; Investigation, formal analysis, validation, writing-original draft preparation, D.D.M., E.M.J., P.S.; Writing-review and editing, C.S.B. and O.I.L.; Supervision, O.I.L.

DATA AVAILABILITY STATEMENT

Data is contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ZAMENA RIBLJEG BRAŠNA NETRETIRANIM I ENZIMATSKI TRETIRANIM TORULA KVASCEM *(CYBERLINDNERA JADINII)* UTIČE NA OTPOR PROTOKA KROZ MATRICU PRI PELETIRANJU I FIZIČKE OSOBINE PELETA ZA ISHRANU

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Sažetak: Kvasac sve više dobija na značaju kao novi sastojak u ishrani životinja. Iako riblje brašno u osnovi obezbeđuje bolju konverziju hrane kod uzgajanih vodenih organizama, istraživanja ukazuju da torula kvasac *Cyberlindnera jadinii* (CJ) ima dobar potencijal kao zamena ribljem brašnu. Ova studija ispituje efekte zamene ribljeg brašna torula kvascem (CJ), kako netretiranim, tako i tretiranim proteazom i endo-eko 1.3-beta-glukanazom, na proizvodnju peleta. Prvi eksperiment je procenjivao promene u otporu protoka i kvalitetu peleta pri zameni ribljeg brašna kvascem. Drugi eksperiment je ispitivao efekte enzimski tretiranog kvasca na ove parametre. Pelete koje su sadržale 20% kvasca (CJ), tretiranog ili netretiranog, pokazale su povećan otpor protoka i veću čvrstoću peleta. Peleti formulisani sa 10% i 20% CJ, kao i kontrolni peleti sastavljeni isključivo od kvasca (CJ), ispoljili su vodoodbojne osobine, što može doprineti boljem unosu hrane, smanjenju otpada i održivoj proizvodnji. Međutim, peleti sa 10% i 20% enzimski tretiranog kvasca (CJ) pokazali su repelentna svojstva prema mastima, što može otežati postprodukcione procese. Enzimski tretman je smanjio bubrenje peleta pod vodom, a minimalna hidroliza se preporučuje radi ograničavanja dezintegracije. Enzimski tretman je takođe smanjio hrapavost površine, pri čemu su peleti sa 20% tretiranog kvasca (CJ) pokazali najglatkiju teksturu.

Ključne reči: *zamena za riblje brašno, protease, glukanaze, peletiranje sa jednom matricom, čvrstoća pelete, bubrenje po vodom*

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