



An Evaluation of Water Pellet Stability of two Iso-proteineous Floating and Sinking Diets

Thaddeus Zaabwe^{1,3,*}, Wales Singini¹, Jeremiah Kang'ombe², Fanuel Kapute¹ and David Mbamba⁴

¹Department of Fisheries and Aquatic Sciences, Mzuzu University, Mzuzu, Malawi

²Department of Aquaculture and Fisheries Science, Lilongwe University of Agriculture and Natural Resources, Bunda College Campus, Lilongwe, Malawi.

³National Agricultural Research Organisation, Bulindi Zonal Agricultural Research and Development Institute, Hoima, Uganda.

⁴Ministry of Agriculture, Irrigation and Water Development, Department of Fisheries, Lilongwe 3

*Corresponding Author: zthaddeus@yahoo.com

ARTICLE INFO

Article History:

Received: Sept. 3, 2020

Accepted: Oct. 18, 2020

Online: Oct. 28, 2020

Keywords:

Fish feed;
Water Pellet Stability;
Floating diets;
Sinking diets;
Aquaculture.

ABSTRACT

Nutrient leaching from fish feeds when subjected to water in aquaculture systems leads to fish missing out on essential nutrients, thus minimized fish growth. A laboratory experiment was conducted to evaluate the water pellet stability of two iso-proteins (32%) fish feeds. The experiment was arranged in a completely randomized block design (CRBD) with 3 replicates of each time-duration. Floating and sinking pellets were subjected to water stability tests at different time durations of 5, 10, 15, 20, 25, and 30 minutes. Thirty-six borosilicate beakers (250 ml) were used. The beakers were each filled with 150 ml of tap water. Two-gram pellets of each diet were immersed in water-filled beakers, after which were stirred one revolt every minute to mimic disturbance while feeding.

Results showed a significant interaction between diets and time duration $F(5,24) = 7.298$, $p < .001$. Significant differences were observed in the feed, $F(1,24) = 574.877$, $p < .001$ and Duration $F(5, 24) = 58.077$, $p < .001$. A strong positive correlation between feed and water pellet stability ($R = 0.78$), $p < .001$ was observed. The highest stabilities recorded were $90.05 \pm 0.20\%$ and $83.08 \pm 0.20\%$ for sinking and floating feeds, respectively. Improving on the efficiency of pelleting fish feeds together with different trials is paramount. Aqua feeds' water stability is affected by duration, thus, a pre-requisite in fish farming.

INTRODUCTION

The principal purpose of feeding fish is the correct utilization of nutrients and minimal leaching of essential nutrients to the water as feed pellets when subjected to water disintegrate (Kiki *et al.*, 2017). The disintegration leads to nutrient leaching in the water column and fish miss out on the essential nutrients required for growth and other body bio-chemical processes and further pollute the water (Meyer, 2001 and Oke *et al.*,

2017). In order to minimize disintegration and loss of nutrients upon exposure to water, quality aqua feeds should be highly stable in water (Ballarin & Heler, 2010). Feed is expensive to waste, so pellet quality has an economic value (Heidenreich, 2001). Obaldo *et al.* (2002) defined high pellet water stability as the retention of pellet physical integrity with minimal disintegration and nutrient leaching while in water until consumed by the animal.

Pellet water stability is affected by a number of factors which range from feed ingredients, pellet size, type of binder used, starch content, rate of pulverization among others (Heidenreich, 2001; Onada & Ogunola, 2020). A study by Keysuke *et al.* (2015) highlighted heat processing and feed formulation especially fat inclusion level as the major factors that influence pellet stability and quality. Solomon *et al.* (2011) revealed that, high pellet stabilities were achieved for the time duration of 5 minutes which were > 80% for floating and sinking diets. A study by Keri *et al.* (2013) on inclusion of different maltose levels indicated improved water pellet stability.

In the current study, the laboratory experiment aimed at evaluating water pellet stability of two iso-proteineous pelleted fish diets. The information generated will help to guide in the manufacturing of aqua feeds for improved fish growth and economic returns.

MATERIALS AND METHODS

Study area

The study was carried out at the Mzuzu University Fisheries and Aquatic Sciences laboratory. The experimental sinking diets were formulated and processed at the Ministry of Agriculture, Irrigation and Water Development, Department of Fisheries, Mzuzu Fish Farming Center in Mzuzu City, Malawi. The facility has a fish feed processing plant.

Study design

The experiment was set in a completely randomized block design (CRBD). A 2 x 6 (two feeds and six-time durations) factorial design was adopted. A total of 36 borosilicate beakers (Model: B07VB8P6YY, Adamas-Beta) with a capacity 250 ml each (18 for each feed) were used in the experiment. The beakers were filled with 150 ml of tap water (3/4 full). Triplicate 2 grams of whole individual pellets for each experimental feed were weighed using an analytical digital scale (Model: ATY224, Shimadzu, Philippines) fixed to the nearest 0.1 milligrams. These were immersed in water-filled beakers at room temperature (25⁰C) and observed for different time durations (of 5, 10, 15, 20, 25 and 30 minutes). Timing was done using a digital stop watch (Model: 4.4+, IOS 8.4, LED, Android, USA). The water and pellets were mixed one full revolt by a spatula once every minute to stimulate water disturbance.

Experimental diets used

A 32% crude protein and 1 mm pellet sized floating fish feed was purchased and imported from Novatek Animal Feeds, Lusaka, Zambia. Another 32% crude protein sinking fish feed was formulated (**Table 1**) using Pearson's method from locally purchased and available ingredients from Mzuzu market in Malawi.

Table 1: Ingredients used in formulation of the sinking diets.

Ingredient	Inclusion rate (%)
Fish meal	10
Soya bean	50
Wheat flour	13.4
Maize	25.5
Fish oil	0.4
Assorted Vitamins	0.2
Assorted minerals	0.3
Normal Salt	0.2
Total	100

The ingredients for the formulation were chosen according to their cost, availability and suitability for use in aquafeeds, contamination, adulteration, damage and make of the most cost-effective of the raw-materials. Principal animal and plant protein sources consisted of fish meal and soya bean, respectively. Other ingredients included maize, wheat flour, common salt, vitamin, mineral premixes and fish oil.

Sinking diets formulation

Soya bean grains were roasted in a frying pan to remove anti-nutritional factors like trypsin inhibitors. Temperature was monitored using a thermometer at 105⁰C. Silver fish was sun-dried on a synthetic tarpaulin to reduce its moisture content. Maize and wheat bran were prepared by pulverizing their grains into bran using a hammer mill (Model: Y2-160M-4, Dongxiang Hongxiang, China). The soya bean and fish meal were milled into powder. The milled ingredients were mixed manually using a spade to form a homogenized mixture. Water, salt, vitamin premix, minerals and cooking oil were added to the homogenized mixture. The mixture was steam pelleted at 90-120⁰C using an electric powered pelletizer (Model: Y2-160M-4, Dongxiang Hongxiang, China) to form 1mm sinking fish feed pellets. Formulated pellets were spread on a clean synthetic tarpaulin and dried under the shade for three days to rid them of moisture. The pellets

were always turned using a plastic rake with minimal energy to avoid breakage. This was done to further reduce on growth of molds.

Proximate analysis of ingredients used

One gram of each ingredient in triplicate was subjected to proximate analysis (Table 2) for essential nutrients using standard procedures of AOAC (2003).

Table 2: Proximate composition of the essential ingredients for sinking feed formulation
(Values are presented as Mean \pm S.D).

Ingredient	% Dry Matter	% Crude protein	% Crude fat	% Crude fibre	% Ash	% Moisture
Fish meal	88.86 \pm 0.08	63.15 \pm 0.17	20.56 \pm 0.11	0.36 \pm 0.01	12.02 \pm 0.04	11.14 \pm 0.00
Soya bean	92.59 \pm 0.09	41.06 \pm 0.42	22.27 \pm 0.60	3.57 \pm 0.09	5.86 \pm 0.02	7.41 \pm 0.00
Wheat bran	93.52 \pm 0.22	7.99 \pm 0.57	0.97 \pm 0.01	1.17 \pm 0.05	3.22 \pm 0.01	6.48 \pm 0.01
Maize bran	93.57 \pm 0.09	11.35 \pm 0.08	1.11 \pm 0.01	1.06 \pm 0.08	2.54 \pm 0.01	6.43 \pm 0.00

Nutrients included moisture, crude protein, crude lipid, crude fiber, ash and nitrogen free extract. Moisture was determined by drying the sample in an oven to a constant weight. The sample was dried in an oven at 65⁰C for 36 hours, cooled in a desiccator and weighed. Crude protein was determined by Johan Kjeldahl method. Percentage protein was calculated by multiplying apparent nitrogen by 6.25. Lipid extraction was done using Soxhlet apparatus. Crude fibre was determined by transferring the residue into a crucible and placed in a muffle furnace at 400-600⁰C for 4 hours. This was cooled in a desiccator and weighed. Ash was determined by weighing the sample in a pre-heated crucible, which was further put in a muffle furnace at 400-600⁰C for 4 hours until a white-grey ash was obtained. The crucible was placed in a desiccator and weighed. Nitrogen free extract were calculated by subtracting the sum of the percentages of all the nutrients determined from 100.

Data collection

Quantitative data on water pellet stability (%) was obtained by drying the residue in borosilicate flasks for each duration in an incubator (Model: AVI-412, Avishkar International PVT. LTD, Mumbai, India) at 105⁰C for 1 hour to remove moisture. Further drying was done to a constant weight at 65⁰C for 24 hours according to Keri *et al.* (2013). Cooling was done in a desiccator at room temperature (25⁰C). The contents were weighed using an analytical digital scale (Model: ATY224, Shimadzu, Philippines) fixed to the nearest 0.1 milligrams. This was the weight of the pellet left from the original weight after immersion due to disintegration for each duration. The leaching rate was

used to determine pellet stability. Pellet water stability was calculated as a percentage of the retained residue to the original weight of the pellets before immersion. Percentage dry matter loss, the measure of water pellet stability (%) was calculated from the following formula:

$$\text{Leaching rate (x)} = \frac{\mathbf{A} \times (\mathbf{1} - \mathbf{r}) - \mathbf{R}}{\mathbf{A} \times (\mathbf{1} - \mathbf{r})} \times \mathbf{100}$$

Where; **A** = Weight of pellets before immersion,

r = Moisture content of pellets, and

R = Dry weight of the remaining solid.

Data analysis

Data entry and analysis were carried out using IBM SPSS (Version 20) Inc., Chicago, USA. Data were subjected to normality and homogeneity of variance using Shapiro-Wilk's and Levine's tests, respectively. This was done to safeguard against violation of parametric test statistics. A Two-way Analysis of Variance (ANOVA) was used to compare treatment means at 5% level of significance. Statistically significant treatment means were separated using Tukey's-b multiple comparison.

RESULTS

Percentage pellet water stabilities at different time durations for the floating and sinking diets are shown in **Table (3)**. There was a statistically significant interaction between the effects of feed and time duration on pellet water stability $\mathbf{F(5,24) = 7.298}$, $p < .001$. Significant differences were observed in the feed, $\mathbf{F(1,24) = 574.877}$, $p < .001$ and duration $\mathbf{F(5,24) = 58.077}$, $p < .001$. Sinking feed had the highest stability value ($90.05 \pm 0.20\%$) compared to ($77.11 \pm 0.00\%$) for floating feed. Results further indicated a positive correlation between feed and water pellet stability ($R = .78$), $p < .001$. Overall, pellet water stability decreased with increased duration for both diets.

Table 3: Water pellet stability for Floating and Sinking feed pellets at different time durations.

Duration (Minutes)	Water pellet stability (%)	
	Floating	Sinking
5	$83.08 \pm 0.20^{\text{a}}$	$90.05 \pm 0.20^{\text{b}}$
10	$81.76 \pm 0.51^{\text{ab}}$	$88.39 \pm 0.12^{\text{ab}}$
15	$79.93 \pm 0.65^{\text{c}}$	$88.06 \pm 0.35^{\text{c}}$
20	$77.28 \pm 0.51^{\text{d}}$	$86.07 \pm 0.20^{\text{d}}$
25	$77.78 \pm 0.12^{\text{e}}$	$86.24 \pm 0.25^{\text{e}}$
30	$77.11 \pm 0.00^{\text{f}}$	$80.60 \pm 0.54^{\text{g}}$

Values in rows with different superscripts are significantly different at $p < .05$. (Mean \pm S.E)

DISCUSSION

Pellet stability significantly decreased with increase in time duration for both diets. Five minutes duration had high pellet stabilities (83.08 ± 0.20 & 90.05 ± 0.20 for floating and sinking diets, respectively). This could be attributed to the lubricants (fish oil) and binding agent (wheat) used, smaller pellet size, pre-heating and vapor conditioning. This is supported by results obtained by **Solomon *et al.* (2011)**. Results from water stability studies by **Kannadhasan *et al.* (2009)** showed high stability values similar to this study for the different time durations. On the other hand, a study on ingredient substitution on water stability and floatation by the same author reported higher stability values ($86.67 \pm 5.77\%$) for the time duration of 5 minutes as reported in the present study. The use of specific binders like wheat reduces on the abrasion between the die and ingredients hence could be a factor for the high stabilities.

Kannadhasan *et al.* (2007) reported a durability index value of 88.66% when starch sources of distillers dried grain (DDG) were used to improve pellet water stability. The use of cassava starch as a binder for one hour gives a water stability as high as 82.81% (**Effiong *et al.*, 2009**). This was however; lower than the reported values in this study for the time durations of 10, 15, 20, 25 and 30 minutes for the floating feed. In addition, it was only higher for the time duration of 30 minutes for the sinking feed. The use of wheat as a binder in the sinking feed could be the reason for the high stability values. Studies on the use of wheat as binders by **Solomon *et al.* (2011)** generated high stability values ($> 90\%$) for the times durations of 5 minutes. These results are in agreement with this study for sinking feed for the 5 minutes' duration but not with other durations. The biopolymer of starch consists macromolecules of amylose and amylopectin (**Brouillet-Fourmann *et al.*, 2003**).

The high stability values in this study could be accounted for by starch gelatinization due to the intermolecular breakdown of starch in the presence of water and heat, thus allowing hydrogen bonding sites to absorb more water. This improves feed stability, digestibility and expansion. This is in conformity with the results by **Kannadhasan *et al.* (2009)** on effects of ingredients and extrusion parameters on aquafeeds (stability, digestibility and expansion) containing distiller dried grain with soluble and Tapioca starch. The good pulverization of ingredients and steam addition can as well account for the high stabilities. This is re-affirmed by **Heidenreich (2001)** on factors that affect pellet stability, and results ascent that, finer grinding leads to larger surface areas thus, improved liquid absorption and effectiveness of saturated steam hence firm pellets. Saturated steam enhances on conditions for creating bonds between particles thus improving pellet stability.

Results from water pellet stability studies by **Secchi *et al.* (2020)** showed high stability values similar to this study for the different time durations. Ingredient

substitution could be attributed to the different stability in pellets when subjected to water. On the other hand, a study on ingredient substitution on water pellet stability and floatation of farm-made fish feed by **Momoh *et al.* (2016)** reported higher stability values ($86.67 \pm 5.77\%$) for time duration of 5 minutes. This is in agreement with the results of this study. Different carbohydrate sources have different contributions to water pellet stability and the various stability levels could be attributed to this.

Results by **Fashina *et al.* (2019)** on effects of fish feed carbohydrate sources on floatation and water stability of fish feeds pellets between 5 and 30 minutes revealed stability ranges of 90% and 52.4%. Furthermore, inclusion of cassava and brewer yeast on water pellet stability for time durations from 20 to 40 minutes generated stability values $>70\%$ (**Olawale & Oluniyi, 2019**). These were in line with this study.

CONCLUSION

Sinking feed pellets had a better water stability than floating feeds. Improving on the efficiency of pelleting fish feeds coupled with alternative procedures like increasing moisture, fat inclusion, expansion processes and their interaction with stability need to be investigated. Aqua feeds need to be subjected to water stability tests depending on the feeding behavior of the target fish species.

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