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RESEARCH ARTICLE

WATER STABILITY AND PALATABILITY OF PROBIOTIC ENRICHED FEED PELLETS FOR INDIAN MAJOR CARP CIRRHINUS MRIGALA

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ABSTRACT

Experimental trails were conducted to analyze water stability and palatability of probiotic enriched supplementary feeds. The results show that among the six types of diet feed-A,B,C,D and E exhibited moderate stability after 8 hours of immersion, except yeast included feed (feed-F) The maximum stability (71.66±2.08) was recorded in feed-B and the lowest (59.67±6.66) was noted in feed-F. The physical structure of all feeds was cracked at the end of the experiment.

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INTRODUCTION

Pellet water stability is an important parameter in the manufacture of aquaculture diets. High pellet water stability is defined as the retention of pellet physical integrity with minimal disintegration and nutrient leaching while in the water until consumed by the animal. The success of any fish farming depends largely on the provision of suitable and economical fish feed. Fish feed are lost in water system due to early disintegration and leaching, thus making nutrient unavailable to fish. The implications are poor weight gain, unhealthy environment and economic losses to farmers. Therefore fish feed must be bonded well to ensure stability in water and nutrients retention for a considerable period of time (Hilton and Slinger, 1981). Binders are used in fish feed to improve the feed consistency, minimize wastage, reduce disintegration and loss of nutrients thereby increasing feed efficiency (Hastings, 1971; Storebakan, 1985). According to Stiver (1970) there are at least three actions by which binders increase the hardness, help the feed to float and increase water stability of pellets.

The water stability of aquatic feed pellets depends upon the nature and quantity of the binding material used. If the pellet is too hard it is difficult for the animals to ingest and if it is loosely blend, the pellet would disintegrate faster, leading to wastage of the feed and spoilage of the water. Factors that affect the physical quality of feeds include the method of diet preparation and processing, types of ingredients, diet composition and types of binding agents (Obaldo *et al*., 1999; Aarseth, 2004). Hardness and water stability are important factors which determine nutrient retention capacity well as the sinking velocity of pellets (Baeverfjord *et al*., 2006; Chevanan *et al*., 2009; Kraugerud *et al*., 2011). The hardness of pellets generally affects their preference and acceptability by fish, where softer pellets are usually preferred to harder pellets (Aas *et al*., 2011; Skoch *et al*., 1983). High water-stable diets are known to prolong digestion and intestinal absorption of nutrients in fish (Pillay and Kutty, 2005; Venou *et al*., 2009). Fish feed pellets should therefore aim to have physical properties that promote high feed intake and efficient digestion. Water stabilities as well as sinking velocities must be adjusted to the eating habits of the cultured fish species (Lovell, 1989; Baeverfjord *et al*., 2006; Sørensen, 2012).

Application of probiotics in aquaculture is such a technology and research on probiotics as bioremediation and bio control agents is increasing with the demand for eco-friendly aquaculture (Dimitroglou *et al*., 2011) The use of probiotic as feed supplements has attracted considerable attention by feed manufactures as mean of improving livestock performance. In the present study an attempt is made to study the water stability and palatability in probiotic pelleted fish feed.

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MATERIALS AND METHODS

Feed preparation

Ingredient's formulations of the experimental feeds are presented in table-1.All feeds were formulated with ingredients commonly used including fish meal, wheat gram, rice bran, yeast powder, corn flour, fish oil, ground nut cake, vitamins and minerals mix. Agar agar (2%) and tapioca powder (10%)were used as binding agent. Feed formulation was done basically by "Pearson's square method" using determined values of protein content. The ingredients were added factorally (%weight). The agar agar was melted in boiling water .The feeds were prepared by mixing the ingredient with tapioca, melted agar agar and water into dough. The dough was steam cooked for 10 minutes and it was then extruded in the form of pellets using a hand operated pelleting machine, with3mm diameter perforations in the die. All feeds were oven dried at70^{o}C to constant weight.

For probiotic enriched feeds, the required amount of spirullina and yeast were added after steam cooked the ingredients. probiotic bacteria l (*Lactobacillus acidophilus, Bacillus cereus*) suspension (10x10$^{-10CFU.ml}$) were sprayed into the feed slowly mixing part by part in a drum mixer, after which it was air dried in an sterile environment for 12 hours. The pellets were dried in an oven at 30^{0}C for 24hours, packed and stored in a freezer (20^{0}C) until used.

Determination of water stability and palatability of feeds

Water stability of all the experimental feeds were tested in triplicate for different exposure periods in fresh water by dip, string and pouch methods (Ahamad Ali *et al.,* 2006). Stability of the experimental feeds was tested for specified time periods (2, 4, 6 & 8hrs) and the palatability was determined based on the diet acceptance in terms of consumption by *cirrhinusmrigala* fingerlings. The percentage of dry matter leaching was recorded as an index of water stability. Percentage stability of different feeds were tested based on stability grades as: mild (representing pellet stability ranging from 50 to60%), moderate(61 to 80%)good(81to90%).At the end of specific hours pouch along with pellets was carefully taken out from the water. After noting the physical shape of the feeds, loss in the weight of pellets was calculated by the difference in the weight before and after immersion of the pellets.

The percentage of nutrient leaching was estimated using following formula;

$$\% \text{ Nutrient leaching} = \frac{\text{Initial pellet weight - Final pellet weight x 100}}{\text{Initial pellet weight}}$$

Palatability

After an acclimatization period, fish feeding experiments were conducted (in duplicate) for a week to determine the palatability of the experimental feeds. The mrigal fingerlings were stocked (3 numbers/tank). Aeration was provided continuously in all the tanks throughout the experimental period. Feeding was done atlibitum as a single dose daily at 24hrs. Every day morning, left over feed and fecal matters were siphoned out from each tank. The experimental fishes were carefully monitored to record feed acceptance.

Statistical analysis

Statistical analysis conducted using SPSS 15 for Windows and Microsoft Excel.

RESULTS AND DISCUSSION

The water stability of pellets prepared using different ingredients are presented in Table 2. At the initial of 2hours soaked in fresh water all diet recoveries were stable more than 80% .Water stability was decreased by increase the immersion period (8hours), while at the end of 8hours, the stability of all experimental diets was found to be same (moderate) except Feed-F(mild). For the first two hours, the stability of all experimental diets was between 82.00 and89.00%.But it was ranged from 73.67 to 80.33% in the fourth hour. The loss of weight was rapid in the first two hours and then gradual until 8hours.The highest water stability 73.00% was recorded in Feed-B followed by 72.00% in Feed-A at 6 hours immersion. Diets differed significantly in terms of their water stabilities and dry matter retention .Feed-A,B,C,D,E and F recorded 61.66,71.66,69.00,63.00,63.00 and59.67% respectively after 8hours.Among the all diets ,feed-B exerts highest stability 71.66%.The lowest stability 59.67% was noted in Feed-F.

Ryther *et al.* (1988) and Kanazawa (1994) reported that feed pellets for fishes should be hard and should remain stable in water for several hours till they are consumed.

Table 1. Presence of Ingredients in experimental feeds

S.No	Ingredients	Feed-A	Feed-B	Feed-C	Feed-D	Feed-E	Feed-F
1	Fish meal	+	+	+	+	+	+
2	Soybean flour	+	+	+	+	+	+
3	Wheat flour	+	+	+	+	+	+
4	Rice bran	+	+	+	+	+	+
5	Fish oil	+	+	+	+	+	+
6	Groundnut oil cake	+	+	+	+	+	+
7	Vitamins & minerals mix	+	+	+	+	+	+
8	Agar agar	+	+	+	+	+	+
9	Tapioca	+	+	+	+	+	+
10	Spirulina	-	+	-	-	+	+
11	Probiotic bacteria	-	-	+	-	+	-
12	Yeast	-	-	-	+	-	+

(+)-Presence; (-)-Absence
Feed-A-Control; Feed-B, C, D, E, F-Probiotic enriched feeds.

Table 2. Pellet stability and feed stability grade of probioticdiets in fresh water

S.No	Feed type	Mean feed pellet stability				Feed stability grade
		2Hours	4Hours	6Hours	8Hours	
1	Feed-A	87.33 ±3.05	80.33±2.08	72.00±2.00	61.66±2.08	Moderate
2	Feed-B	89.00±1.00	78.00±2.00	73.00±1.00	71.66±2.08	Moderate
3	Feed-C	86.66±4.16	78.33±3.05	71.00±2.64	69.00±1.00	Moderate
4	Feed-D	85.67±2.52	76.00±1.73	70.67±1.53	63.33±2.52	Moderate
5	Feed-E	83.00±2.00	73.67±2.08	69.33±1.15	63.00±2.00	Moderate
6	Feed-F	82.00±2.00	74.67±2.08	64.33±6.65	59.67±6.66	Mild

Table 3. Feed palatability and physical shape of experimental pellets

S.No	Feed Type	Palatability	Physical shape of feed pellets			
			2Hours	4Hours	6Hours	8Hours
1	Feed-A	Accepted	IN	IN	IN	CR
2	Feed-B	Accepted	IN	IN	IN	CR
3	Feed-C	Accepted	IN	IN	IN	CR
4	Feed-D	Accepted	IN	IN	IN	CR
5	Feed-E	Accepted	IN	IN	IN	CR
6	Feed-F	Accepted	IN	IN	CR	CR

IN-Intact; CR- Cracked

The water stability of the pellets mainly depends on the binders used in the feed (Meyers and Zein-Eldin, 1972; Goswamy and Goswamy, 1979; Forman and Lauterio, 1982; Ahamed Ali, 1988). Apart from the nature of binder used, the water stability of feed pellets depends upon the nature of ingredients constituting the feed. Feeds having altered soluble and rough ingredients disintegrate faster (Meyers and Zei-Eldin, 1972) and require higher amount of binder. The use of tapioca as binder has double advantage. While good water stability of the feed pellets could be achieved using tapioca, it enhances the calorific value of the feed simultaneously by supplying good source of carbohydrate (Effiong *et al*., 2009) Tapioca is very cheap compared to any other chemical binder and its use will help in bringing down the final cost of the feed (Keri AlhadiIghwela *et al*., 2013)

The physical shape of pellets was intact upto 6hours in all experiments feeds, but it was cracked at the end of the 8hours. In the present study palatability was recorded with stability, All types of probiotic enriched feeds and control feed were found palatable for *mrigala* fingerlings (Table 3). The physical qualities of feed pellets depend largely on how well particles of the individual ingredients bond together (Behnke, 1996). Based on inherent binding capabilities, MacMahon and Payne (1991) assigned pellet ability indices to different ingredients to indicate the effects of diet with higher water stability. The feed acceptability and intake by fishes also being influenced by pellet structural integrity and hardness. Pellets with very high water stability are generally known to affect preference and accept ability by animals (Kwasi Adu Obirikorang *et al*., 2015). In feed formulation, water stability and nutrient leaching are the main issues. Findings showed that feed- B added with tapioca (10%), agar agar (2%) and probiotic (spirullina) had good water stability followed by probiotic bacterial enriched supplementary feed (feed-C).

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