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Study on the Stability of Probiotic (*Spirulina fusiformis*) Incorporated Fish Diets

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aquaculture is one of the fastest-growing food production sectors in the world. The use of probiotics might be a good option to reduce the risk of disease and enhance fish productivity. Probiotics thus are opening a new era in the health management strategy for aquatic species including finfish and shellfish. Probiotics are beneficial microorganisms that help in maintaining the well-being of the host animal. The development of non-antibiotic and environment-friendly probiotic feeds is one of the key factors for health management in aquaculture. The benefits of probiotic supplements include improved feed value, enzymatic contribution to digestion, and inhibition of pathogenic microorganisms. Feed stability is of paramount importance in the manufacture of aquaculture diets. The water stability of the feed pellet depends on the individual ingredients that bind together. Hence the study was designed to investigate the stability of probiotic feeds in water. Probiotic Spirulina added feed samples were soaked in a glass beaker containing water and allowed to remain for time intervals ranging from 10 to 60 minutes to study the stability of feed. The results showed that the control feed pellet (40.00 + 1.00) was the least stable among the types of experimental feed (SF2 and SF3) in 60 minutes of immersion. The increased level of stability (71.66 %) was recorded in (2 & 3% inclusion) feed type.

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1. INTRODUCTION

Feed is the principal operating cost in fish production. Fish need proper nutrition to grow and survive. Good nutrition in the fish production system is essential to economically produce a healthy, high-quality product. In aquaculture practices, supplementary feed is necessary. Feed must also be palatable to the particular fish, thus the constituents may be in quite good proportions with special reference to size, taste, and nutritional status [1]. The artificial feed of fish must be resistant to crumbling and water-soluble.

Fish nutrition has advanced dramatically in recent years with the development of new, balanced artificial diets that promote optimal fish growth and health. The development of new probiotic feed formulations supports the aquaculture industry. The development of probiotic feed applies to commercial use in aquaculture also. For alternative feed formulation ingredient selection is necessary and they must meet the fish's nutritional requirements, be less expensive, and be sustainable in the aquatic environment. Thus, the utilization of multifunctional probiotic Spirulina incorporated feed has emerged as a solution with numerous applications in aquaculture.

Potential feed additives (probiotics, prebiotics and synbiotics) are frequently incorporated into the diet that significantly ameliorates the health status (morphological as well as microbial) of the host's intestineThe supplementary feed has become inevitable for the success of fish culture. Supplementary feeding is known to increase the carrying capacity of culture systems and can promote fish production by many folds [2].

Spirulina acts as a growth promoter, and probiotic food booster of the immune system in fishes [3]. Spirulina is a freshwater microalgae belonging to the crossing of cyanobacterium or cyanophytes [4]. Spirulina is rich in nutrients, with high proportional protein content per cell, vitamins, essential amino acids, and fatty acids, as well as bioactive pigments [5]. Spirulina is used as a health supplement for aquaculture. Spirulina is called a “Superfood”.

Water stability is an important physical parameter in aquatic feed. It is defined as the retention of the pellet integrity with minimal disintegration and nutrient leaching, while it is immersed in the water and until it is consumed by fishes [6]. The addition of a specific feed binder improves the water stability of the pellet. Binders are substances that are used within aquaculture feeds to enhance the efficiency of the feed. Palatability, texture, water stability, and adequate digestibility of fish feed promote the growth of fin fishes [7]. Very little information is available on water stability and palatability of probiotic fish feeds. Hence the present study was designed to investigate feed stability of probiotic *Spirulina fusiformis* incorporated supplementary feed.

2. MATERIALS AND METHODS

2.1 Feed Ingredients

The ingredients were selected based on quality and steady availability in local markets. The experimental probiotic feed could be prepared using ingredients such as corn flour, wheat flour, rice bran, groundnut oil cake, tapioca, agar agar, cod liver oil, vitamins, and minerals. Tapioca powder and agar was used as effective binder in experimental feeds. Commercial cod liver oil was also incorporated into the diet. *Spirulina fusiformis* was added as a probiotic source of feed.

2.2 Feed Formulation

The experimental diets were formulated with ingredients as per “Pearson’s square method” using a predetermined value of 50% protein content. Groundnut oil cake was used as a protein source. Corn flour, wheat flour, and rice bran was used as carbohydrate source. Cod liver oil was used as a lipid source. Tapioca powder and agar were utilized as binding agents. Vitamins and minerals were also added. The proportions of each ingredient were calculated using Pearson’s formula (Table 1).

2.3 Probiotic Feed Preparation

The ingredients were dried in a hot air oven at 55^{o}C and then made into fine powder. Then the ingredients were weighed according to the feed formulation. The dough was prepared by adding

Table 1. Ingredient composition of probiotics containing *S. fusiformis* experimental diet

Ingredients	Feed types and quantity of each ingredient (g/100g)					
	Control (0%)	SF1 (1%)	SF2 (2%)	SF3 (3%)	SF4 (4%)	SF5 (5%)
Groundnut oil cake	26.28	26.51	26.11	25.91	25.99	24.91
Rice bran	24.75	21.93	21.93	22.83	22.93	22.83
Corn flour	17.35	17.17	16.17	16.17	15.17	15.17
Wheat flour	17.35	17.17	17.17	17.17	17.17	17.17
Tapioca powder	10.27	10.63	10.63	10.63	10.63	10.63
Cod liver oil	2.00	2.00	2.00	2.00	2.00	2.00
Vitamins & Minerals	1.00	1.00	1.00	1.00	1.00	1.00
Agar	1.00	1.00	1.00	1.00	1.00	1.00
S. fusiformis powder		1.00	2.00	3 .00	4.00	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

a sufficient quantity of distilled water. The dough was autoclaved for 15 minutes and subsequently cooled for the preparation of probiotic experimental feeds. Control and experimental feed was prepared using the ingredients. Diet without probiotics was used as control. A dry powder form of *S. fusiformis* was added at different proportions (1, 2, 3, 4, and 5%) into the cooled steam-cooked basal diet and thoroughly mixed. The dough was placed in the hand pelletizer and pushed out through 2mm die holes. The pellets were dried in a thermostatic oven at 40 ^{o}C until they reached constant weight, and stored in airtight jars at room temperature (28 ^{o}C). The pellets were broken into small sizes before being stored [8].

2.4 Determination of Feed Stability

A feed stability test was conducted using triplicate feed samples. 25g of oven dried feed pellets (Control, SF1,SF2,SF3,SF4 and SF5) were weighed using digital balance and tied in nylon sieve material of 0.1mm mesh. Then the experimental feeds were slowly immersed in 1000 ml of distilled water. Triplicate samples for each feed were soaked in a glass beaker and allowed to remain for time intervals ranging from 10 to 60 minutes. At the end of every test time, the samples for each triplicate were lifted slowly with the aid of the twine and allowed to drain for 3 minutes. The water was filtered through filter paper and soaked pellets were dried in a hot air oven at 105^{o}C for at least 12 hrs. After drying, the filter paper with pellets was again weighed. The weight obtained is the leftover from the original weight after immersion due to disintegration for each test period. The water stability was determined as the percentage of the weight [9].

Percentage feed stability = Weight of retained experimental pellet / Initial weight of experimental pellets * 100.

2.5 Statistical Methods

Data obtained were subjected to the analysis of variance (ANOVA). Differences between means were determined and compared by the Duncan multiple range test (DMRT) and the significances mentioned. The data are represented as mean $\pm$ standard deviation.

3. RESULTS AND DISCUSSION

3.1 Feed Stability (%)

The fish feed pellets' quality was evaluated by stability test. The results of the feed stability test are presented in Table - 2. At the first 10 minutes, the stability of all probiotic *Spirulina* diet SF1, SF2, SF3, SF4, and SF5 ranged between 93.00 ± 1.73 and 98.00 ± 1.00 %, whereas the lowest percentage of feed stability (80.66 ± 1.52 %) was recorded in control feed. This could be achieved by uniform particle size (0.5mm) of the ingredient that was used in feed production. A similar type of observation was also reported about the feed stability of all probiotic feeds at an initial 10 minutes was less than 100 percent [10]. This may be due to the inclusion of local binders and plant ingredients in the experimental diets. The reduction in feed stability may be caused by plant-based nutrient (tapioca flour, wheat flour, rice bran, etc) compounds [11,12].

At the end of 20 minutes, all probiotic *Spirulina* feed types (SF1, SF2, SF3, SF4, and SF5) except SF1 were found to be quite stable with the range of 92.33 ± 0.58 %. The lowest percentage of feed stability

Table 2. Feed stability (%) of probiotic *S. fusiformis* containing feed

Time of immersion (minutes)	Feed types/ Percentage of stability					
	Control	PS1	PS2	PS3	PS4	PS5
10	80.66 ± 1.52	98.00 ± 1.00[b]	93.00 ± 1.73[c]	92.33 ± 3.79[d]	93.33 ± 3.06[d]	95.00 ± 1.00[b]
20	70.66 ± 0.57	92.33 ± 0.58[a]	86.16 ± 1.26[a]	87.66 ± 3.21[c]	86.33 ± 3.06[b]	85.33 ± 1.53[a]
30	63.66 ± 1.15	83.00 ± 1.00[a]	80.33 ± 1.53[b]	82.66 ± 2.52[b]	80.66 ± 3.51[b]	81.00 ± 1.00[a]
40	53.33 ± 2.88	76.66 ± 2.08[b]	75.00 ± 1.00[b]	75.33 ± 3.51[b]	75.33 ± 3.21[b]	74.66 ± 1.53[b]
50	48.66 ± 3.51	70.00 ± 2.65[b]	71.66 ± 0.58[b]	71.66 ± 2.52[b]	71.16 ± 3.33[b]	71.33 ± 0.58[b]
60	40.00 ± 1.00	62.33 ± 4.93[b]	59.33 ± 1.15[c]	60.66 ± 0.58[a]	61.00 ± 3.61[b]	55.33 ± 2.08[b]

Each value is mean ± standard deviation of triplicate observations
Mean values within the same row sharing different superscript are significantly different (P = .05)
Mean value within the same column sharing as same superscript are not significantly different (P = .05)

(70.66 ± 0.57) was recorded in the control feed. At the end of 30 minutes of test time, the percentage of dry matter that remained in the feed was 63.66 ± 1.15, 83.00 ± 1.00, 80.33 ± 1.53, 82.66 ± 2.52, 80.66 ± 3.51, and 81.00 ± 1.00 in control, SF1, SF2, SF3, SF4, and SF5, respectively.

In 40 minutes, there was a significant decrease in the stability of all the pelleted *Spirulina* probiotic feeds. Among the all probiotic *Spirulina* feed, the higher percentage of stability 76.66 ± 2.08 was noted in the SF1 and a lower percentage of stability 74.66 ± 1.53 was observed in the SF5. At the time of 50 minutes of immersion, a similar percentage of stability was observed in probiotic *Spirulina* incorporated diets SF1, SF2, SF3, SF4, and SF5, with corresponding values of 70.00 ± 2.65, 71.66 ± 0.58, 71.66 ± 2.52, 71.16 ± 3.33 and 71.33 ± 0.58, %, respectively. The increased level of stability (71.66 %) was recorded in SF2 and SF3. A similar pattern of results was reported that the residual moisture content, relative humidity, and storage temperature have significant influence on the viability and water stability of probiotics feed [13].

At the end of the experimental hour (60 minutes), the control feed exhibited less stability (40.00 ±1.00) than the experimental feed. The lowest percentage of stability in the control feed occurred may be due to the presence of high moisture content (9.30%). Among all probiotic *Spirulina* feeds, the poor stability was 55.33 ± 2.08 in the SF5. These feed pellets have water-soluble and rough ingredients. So it disintegrated faster at 60 minutes. The length of stability time is directly related to the composition of feed ingredients that will change every time and optimize the temperature [14]. Similar patterns of values were also reported [15,16]. In 60 minutes of immersion, the highest percentage of feed stability was 62.33 ± 4.93 noted in the SF1 . Diets with high dietary fiber content are relatively difficult to bind and this will result in fractures and cause a decrease in the feed stability of the feed. The consistency of nutrient availability and the enhancement of fish feed's palatability are contingent upon the stability of the feed [17].

In all feeds, the disintegration rate increased proportionately with the exposure time to water. The percentage of stability was found to be significantly increased in all probiotic-enriched feeds when compared with the control diet. The results of the water stability test indicated that the control feed pellet (40.00 + 1.00) was the least stable among the types of experimental feed. The control feed's moisture content was found to be higher (9.30%) than that of the probiotic experimental feeds, which may have contributed to the control feed's faster disintegration in water stability during the experiment. These results correlated with the findings of Keri et al. [18] who reported that the stability of formulated feed composition, nature of ingredients, and moisture content.

The feed stability results (62%) in the SF1 were consistent with the research conducted by Falayi et al. [10], which concluded that the best feed ingradients should have the highest possible feed stability in order to reduce feed loss, limit nutrient leaching, and prevent water pollution. The stability of dried probiotic feed is also highly dependent on their final humidity and moisture content [19]. The difference in the proportion also plays vital role in feed stability.

4. CONCLUSION

The length of water stability of fish feed has a noticeable effect on the probiotics *S.fusiformis*. The stability time is beneficial to the feed's chemical, biological, and physical states (SF2 and SF3). Among the all experimental feeds, the superior percentage of stability 76.66 ±2.081 was recorded in SF1 at 60 minutes of immersion. This feed contained high fat and fiber content. In all probiotic feeds, the disintegration rate increased proportionately with the exposure time to water. The percentage of stability was found to be significantly increased in all probiotic-enriched feeds when compared with the control diet. The results of the water stability test indicated that the control feed pellet (40.00 ± 1.00) was the least stable among the type of experimental feed. In conclusion, we recommend to conduct additional research on fish feed stability in freshwater medium with various probiotic microbes to compare.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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