

Development of Fish Hydrolysate (Bind-Add⁺) incorporated extruded pellets and its performance in Tilapia (*Oreochromis niloticus*) feeding trial

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Abstract—Fish hydrolysate (FH) based Bind-Add⁺ is a emulsion type feed binder cum additive which contains un-denatured proteins and calcium is capable of reasonable level of gelatinization and contribute to the binding properties which is seen from water absorptive capacity, buoyancy, bulk density and water stability characteristics during extrusion pellet production..

Bind-Add⁺ has good amount of bio-available primary nutrients, secondary nutrients, micro nutrients, anti-oxidants and pro-biotic in a soluble liquid form and most suitable as additive or feed supplement.

Tilapia (*Oreochromis niloticus*) feeding trial indicate that growth rate and feed utilization efficiency in Bind-Add⁺ incorporated diets increases significantly in comparison to controlled diet.

Keywords—Fish hydrolysate, Binder, Additive, *Oreochromis niloticus*, Extrusion Engineering and mechanical properties of pellet, Floating extruded pellet.

I. INTRODUCTION

The extrusion technology is gaining popularity day by day for production of floating feed for fish. It improves nutrient digestibility, palatability, pellet durability, water stability and pellet storage life (Barrows and Hardy, 2001). It also increases the digestibility of dry matter and digestibility of energy as measured in vivo using rainbow trout (Cheng and Hardy, 2003) and high physical and nutritional quality of the fish feed is maintained by this technique (Hilton et al. 1981). Extrusion technology provides a number of major benefits over the traditional pellet milling. It provides a control over “cooking” and product density; which finally results in floating or sinking characteristics (¹Autin, 1997, ²Ammar et Al., 2008 and ³Paoluci et al., 2008). Since the material through the extruder is subjected to high temperature and pressure for a very limited period of time, it is argued that extrusion causes less damage to nutrients like amino acids

resulting in a finished product of higher biological value (Sorensen et al., 2016). Cost effective floating feed could be produced by inclusion of locally available ingredients (Das, 2016).

Binders are used in aquaculture feeds to improve the quality and to reduce wastage by producing water stable diet. Binders reduce void spaces resulting in a more compact and desirable pellet. It also acts as adhesive by sticking particles together. Binders stabilize feed pellets and ensure minimum nutrient leaching and disintegration (Sinha et al., 2011). Fish protein hydrolysates (FPH) are products of hydrolysis reaction on peptide bonds in proteins and result in shorter peptides or amino acids which are easy for animal to absorb. Fish hydrolysate belongs to binders of protein origin and have carbohydrate source. Fish processing wastes are grounded into liquid phase where cleavage of natural bonds may be enacted through various biological processes like natural fermentation, enzyme action and bacterial inoculation. Cold and natural fermentation or fish water slurries with molasses and starter culture produce acids and alcohol which lower the pH of product up to 4.5. Molasses based fish hydrolysate have good binding properties in formulated feed and contribute in obtaining feed pellets with desired strengths (Kristinsson and Rasco, 2008 and Sahu, et al., 2014). The fish hydrolysate fed fish showed increased leucocrit and lower haematocrit level. Innate cellular responses were increased after feeding fish by-product and fish hydrolysate (Murray et al., 2003, Refstie et al., 2004 and Dauksas et al., 2004) reported partial dietary replacement of fish meal by a novel feeding stimulant in Atlantic salmon was highly digestible and well utilized for growth. Dauksas et al. (2004) reported that enzymatic hydrolysis process for recovery of protein from underutilized fish biomass often creates bitter taste or the product. The bitterness restricts the practical use of the hydrolysate. Fish processing wastes are potentially

valuable sources of nutrients (Swanson, 2004). The disposals of fish processing wastes have become restrictive. This is adding to the problem and costs associated with discarding these materials and consequently recycling techniques are becoming more and more viable.

Fish hydrolysate from its low end used as animal, fish feed or as a fish based organic fertilizer for agriculture, horticulture and aquaculture (Sahu et. al., 2014, Sahu et. al., 2016, Stone and Hardy, 1986).

The rest of the paper is organized as follows. Materials and Methods are explained in section II. Results and Discussion are presented in section III followed by conclusion in section IV.

II. MATERIALS AND METHODS

Fish feed was formulated using fish feed ingredients i.e. maize, soya bean meal, groundnut oil cake (GOC) and de-oiled rice bran(DORB) with minerals and vitamin mixture. Tilapia grow out feeds were produced in the Feed Mill of ICAR-CIFA, Bhubaneswar, India by using fish hydrolysate at a level of 2% with extrusion temperatures of 130^oc and moisture of 20 percentage maintaining constant pressure (10 kg/cm²). Few other processing parameters as maintained during production of the experimental feed were screw speed 90 rpm, barrel screw speed 430 rmp, culter speed 1100 rpm and die hole diameter 2 mm. The feed ingredients were grinded, mixed and fed to the pilot twin screw extruders for production of floating pellets replacing 2% water with FH (CIFA-BIND ADD⁺) (Fig. 1.). Similar extruded floating feed was also produced in the feed mill without inclusion of fish hydrolysate. The feed so produced with and without fish hydrolysate (FH) (Sahu et al., 2014; Sahu et al., 2015) were then analyzed for physical and chemical characteristics to ascertain the quality (Fig. 2.). A feeding experiment of three months duration was also conducted in Nile Tilapia to know the overall performance of the feed. Fish Hydrolysate (Sahu et al., 2015; Salu et al., 2016) was conducted in the laboratory of ICAR-CIFA, Kausalyaganga, Bhubaneswar. Floating extruded pellets (Ciflylate forte) were prepared in the feed mill of ICAR-CIFA. (Fig. 3.)

2.1 Physical Evaluation of Fish Feed –

Physical, mechanical and engineering properties of the extruded feed pellets were conducted as follows (Fig 5. and Fig 6.)

2.1.1 Water stability:

Feed samples of 5g each in duplicate were placed in wire net container immersed the 2L beaker containing water. The beaker was kept in a magnetic stirrer to simulate mild water flowing condition for period or 0.5, 1,2,4,6,8,10

and 12. After each time interval, the feed samples from containers were collected by draining water and dried at 600C till complete drying.

Water Stability was calculated from the following formula.

$$\text{Water Stability \%} = \frac{\text{Dry weight of pellet after immersion}}{\text{Dry weight of pellets before immersion}} \times 100 \dots\dots(1)$$

2.1.2 Water absorption rate:

Feed samples of 5g each in duplicates were placed in wire net container and immersed in 2L beaker. Containing water at room temperature for period of 1, 3, 5 and 10 minutes. After each specified time period the feed samples were removed and allowed to drain for one minute followed by weighing. The water absorption rate was calculated by water absorption.

$$\text{Water absorption (\%)} = \frac{\text{Dry weight of pellet after immersion}}{\text{Dry weight of pellets before immersion}} \times 100 \dots\dots (2)$$

2.1.3. Expansion ratio:

Expansion ratio (%) was calculated as described by Oliveria et al., 1992. The average pellet a diameter was determined by Vernier Caliper. The expansion ratio was calculated as increase in the pellet cross sectional area compared to the die cross sectional area as follows.

$$\text{Expansion ratio (\%)} = \{(\text{D}^{\text{Pellet}})^2 / (\text{D}^{\text{die}})^2 - 1\} \times 100$$

Where

^DPellet = Pellet diameter

^Ddie = diameter of die

2.1.4 Bulk density:

The cylinder has inner diameter of 7 cm and was made or plastic. Three replication or each feed were prepared by pouring the material through a funnel. The excess feed was gently trimmed by passing a serape, one time on the edge of the cylinder. The pellets in cylinder were weighed. Bulk density of pellet were calculated as follows

$$\text{Bulk Density (g/cm}^3\text{)} = \frac{M}{A \times L} \dots\dots\dots (3)$$

Where

M = Mass (g) of pellet

L = Length (cm) or the pellet

A = Cross sectional area (cm)² of the pellet.

2.2 Chemical Evaluation of Fish Feed and Fish

2.2.1 Hydrolysate:

The chemical evaluation of fish feed and Fish Hydrolysate i.e. Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fibre (CF), Nitrogen Free extract (NFE) and Total ash (TA) were done as per AOAC (1990). Crude Protein(CP)of the feed was determined using micro-kjeldhalapparatus where as crude fiber(CF)and Ether Extract(EE) in fish feed were

determined by using Fiber tech (Model: M- 1017, Tecator) and Soxtec system (Model: ST2, 1045, Tecator) respectively (AOAC, 1980; APHA, 1992).

2.2.2 Feeding Experiment

A feeding experiment of three months duration was carried out in 20 liter glass pool (30cm × 45cm) at Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar. The water from pond was filled in eight nos of tanks to maintain the water column. Five number of fingerlings of tilapia (*Oeochromis niloticus*) were stocked in each tank and one dietary treatment (with and without FH incorporated floating feed) was allocated randomly to four jars. Aerators were used in each tank to provide sufficient oxygen and half of water was changed daily. Water quality parameters were also checked and found to be optimum. The bio-mass in each tank was recorded weekly interval and feed intake was recorded daily. Finally the FCR and cost of three floating feeds was calculated based on the ingredients cost purchased from the local market. (Fig. 4)

III. RESULTS AND DISCUSSION

3.1 Physico-Chemical and nutrition properties of FH:

Fish Hydrolysate was analyzed for chemical composition and is presented in Table 1. The bio available protein was found to be 9.2 % and it is a good source of many trace minerals like Iron, Manganese, Copper and Zinc. Fish hydrolysate contains the full spectrum of nutrients. Liquid fish contain significant quality or protein nitrogen, as well as a healthy balance of all 18 nutrients known to be significant for plant, animal and fish growth.

Table.1: Chemical composition of Fish Hydrolysate (FH, on DM basis)

Sl. No.	Parameters	Composition
1	Bioavailable Protein (%)	9.47 ± 0.23
2	Bioavailable Phosphorus (%)	0.52 ± 0.11
3	Bioavailable Potassium (%)	0.40 ± 0.15
4	Iron (ppm)	240.5 ± 32.2
5	Manganese (ppm)	6.2 ± 0.3
6	Copper (ppm)	3.5 ± 0.5
7	Zinc (ppm)	1.8 ± 0.3
8	Organic Carbon (%)	2.2 ± 0.2
9	Available nitrogen (mg/100ml)	392 ± 0.21
10	C/N ratio	1.5
11	Carbohydrate (molasses)	45 brix

Table.2: Elements of bio-available nutrients of Bind-Add⁺ in water soluble liquid form

1.	Amino acids	9.	Beta Carotene
2.	Fatty acids	10.	Vitamin A

3.	Peptides	11.	Chitosan
4.	Hormones	12.	Chito lipo ligo saccharides
5.	Taurine	13.	Chitin deacetylases
6.	Antioxidant	14.	Lactic acid bacteria
7.	Trace minerals	15.	Photo synthetic bacteria
8.	Astaxanthine	16.	Yeast

Extruded floating feed prepared with Maize, Soyabean meal, Ground nut oil cake (GOC), de-oiled rice bran (DORB), minerals and vitamin mixture with incorporation of liquid fish Hydrolysate (FH). The formulation of the fish feed is given in Table 3.

3.2 Feed Formulation:

Table.3: The Formulation of Fish feed during the experiment.

Ingredients	Extruded Pellets
Maize	23.0%
Soya bean meal	40.0%
Groundnut oil cake (GOC)	25.0%
De-oiled rice bran (DORB)	10.0%
Liquid fish Hydrolysate	2.0%
Minerals and vitamin	2.0%

Control: - without fish hydrolysate

Treatment: with 2% liquid fish hydrolysate (Bind-Add⁺)

Extruded pellets produced after incorporation of liquid fish Hydrolysate (FH) by replacing from the total amount of water required during extrusion cooking and were analyzed for chemical composition (Table 4.) (AOAC, 1980; APHA, 1992)

Table.4: Chemical composition of experimental feed (% on DM basis)

Sl. No.	Parameters	Extruded Pellets
1	Crude proteins	35.0
2	Ether Extract	8.0
3	Crude fiber	7.0

3.3 Physical and mechanical evaluation of fish feed

3.3.1 Water Stability:

The water stability of the feed pellets after incorporation of fish hydrolysate is presented in Table 5. The water stability of experimental feed was higher than the control feed. Water stability in FH incorporated feed was highest within half an hour of water contact and stability was not reduced significantly within 2 hours of water contact. This indicated that, there was fairly good water stability of the FH 2% incorporated pellets with normal processing conditions.

Table.5: Water Stability (%) of control and fish hydrolysate incorporated extruded pellet.

Sl. No.	Time Interval (h)	Water Stability (%)	
		Control	5% FH
1	0.5	90.15	98.05
2	1.0	88.12	96.50
3	2.0	86.12	95.01
4	4.0	79.12	87.15
5	6.0	74.12	83.13
6	8.0	70.12	80.15
7	10.0	69.05	75.01
8	12.0	68.01	71.50

3.3.2 Water Absorption Capacity:

The water absorption capacity as measured after production of extruded pellets is given in Table 6. Water absorption was better in FH incorporated feed compared to control feed.

Table.6: Water Absorption (%) of control and fish hydrolysate incorporated extruded pellet.

Sl. No.	Time Interval (h)	Water absorption pellets (%)	
		Control	2% FH
1	1	30.83	32.15
2	3	42.18	44.12
3	5	53.15	55.12
4	10	64.87	66.18

3.3.3 Bulk Density:

The bulk density of control and experimental feed is presented in Table 6. The bulk density of 2%FH incorporates extruded pellets (3 mm size) was reported to be 550 gm/L and was having good buoyancy. There was expansion of the pellet as pellets leave the die from high pressure inside extruder to outside atmospheric pressure. The control samples produced partial floating pellets and bulk density above 650g/L produced sinking pellets (Sorensen et al., 2009). Addition of 2% FH has produced floating pellet of bulk density 500g/L.

Table.7: Bulk density (gm/L) of 55 FH incorporated and control pellets

Sl. No.	Trait	Control pellets	2% FH incorporated pellets
1	Bulk density (gm/L)	550.5	500.0

3.3.4 Growth performance of tilapia (*Oreochromis niloticus*) in the feeding trial

A 150 days (5 months) feeding trial was conducted in square glass jars of 20 liters capacity to evaluate the effect of fish hydrolysate incorporation on growth performance in tilapia (*Oreochromis niloticus*) fingerling. Five fingerlings in each jar were fed with iso-nitrogenous

extruded pellets with or without 5% FH incorporation. Fingerlings of initial average weight of 23.2 gram were fed daily with fish hydrolysate incorporated diet at 3% of the fish biomass. Result indicated that growth rate and feed utilization efficiency of Nile tilapia increased significantly with administration of 2% FH incorporated extruded pelleted feed compared to non- incorporated feed. The average body weight of Tilapia (*Oreochromis niloticus*) during experimental period is given in Table 8.

Table.8: The average body weight of Tilapia (*Oreochromis niloticus*) during experimental period

Items	Expt. Period	Control	Treatment(FH incorporated)
0 day	Weeks	23.5 ± 1.24	23.2 ± 1.25
15d	2	37.9 ± 3.2	38.2 ± 2.1
30	4	55.0 ± 5.1	58.0 ± 3.5
45	6	69.2 ± 5.17	73.5 ± 5.1
60	8	85.0 ± 6.53	90.0 ± 5.3
75	10	100.5 ± 6.93	105.5 ± 7.5
90	12	117.8 ± 7.04	123.8 ± 6.9
105	14	135.0 ± 7.15	142.5 ± 8.2
120	16	150.5 ± 8.15	165.6 ± 7.7
135	18	185.0 ± 11.2	198.1 ± 8.4
150	20	215.0 ± 12.8	232.2 ± 11.4

Table.9: Performance of tilapia (*Oreochromis niloticus*) during feeding final with control extruded pellets and F H 2% in incorporated pellets n = 5 in glass pool in triplicate.*

Parameter	Control	Treatment(FH incorporated)
Average initial body weight (g)	23.5	23.2
Average final body weight (g/ fish)	215.0 ^a	232.2 ^b
Weight gain (g/fish)	191.5 ^a	209.0 ^b
Total weight gain (kg)	2.873 ^a	3.135 ^b
Total production (kg)	3.225 ^a	3.483 ^b
Total feed consumed (kg)	4.735	4.791
Feed Conversion Ratio (FCR)	1.47 ^a	1.38 ^b

*Different superscript in a row differ significantly (P<0.05)

Improved growth performance in FH incorporated feed was because of high nutrition in treatment fish feed compared to control fish feed. Fish hydrolysate contains more than 60 trace minerals which have positive effects on animal cells, plant cell, chlorophyll and plankton health. Again, Fish hydrolysate can be rapidly assimilated when applied as feed supplement, fertilizer, animal fish and plant feeding as foliar spray. Water quality in the glass jar experiment revealed that water quality was

maintained better in FH incorporated feed. It also produced good quality plankton. (Sahu et al., 2014; Sahu et al., 2016)

3.3.5 Bind-add+ role as binder, additive as feed supplement in feed technology

Fish is rich in taurine especially in fish processing byproducts. Taurine is a sulphoric acid found in high concentration in animal tissue. Taurine is involved in bile salt formation, membrane stability, immune modulation, anti-oxidation, mitochondrial function and calcium signaling. Dietary taurine supplementation increased buoyancy, fertilization and hatching rate in fish. The role of taurine in fish embryonic and larval development has been reported. Taurine deficiency in fish leads to green liver syndrome (Saize and Davis, 2015). In addition to that, nutrients in FH do not leach due to oils and collagen. All of these nutrients are in protein chelated form and emulsion resistant to leaching. FH feed containing trace minerals and taurine with higher binding capacity might be responsible for higher growth performance in Tilapia. (Rhodes and Davis, 2011)

Till now, fish meal has been a major ingredient and primary source of proteins in most fish diets. However, increase cost of fish meal in the market, are forcing the farmers and feed mill entrepreneurs to replace the fish meal with other sources. Commercial fish feed manufacturer are trying to substitute fish meal by alternate protein sources from plants and animals like soy bean, feather meal, blood meal, bone meal or sea products (Sorensen et al., 2009). However, such alternate ingredients are often devoid or contain very low concentration of taurine compared to fish meal (El-Sayad, 2013). It has been reported that taurine play an important role in production and reproduction behavior of the species (Gaylord et al., 2014).

3.3.6 FH (BIND-ADD⁺) extruded pellet quality

Bind-Add⁺ belongs to binder of protein origin and carbohydrate source. Fish hydrolysate contains well balanced protein, fatty acids, amino acids, macro and micro minerals. FH is a natural product, biodegradable and renewable and this may be advantageous from environmental and economic point of view. Fish hydrolysate has reduced void space in extrusion process resulting is compact and desirable pellet. It has worked as adhesive agent in sticking the particles together. They exert a chemical coating on the ingredients resulting in more durable and water resistant pellet and ease in feed manufacture. Molasses based fish hydrolysate have good binding properties and Bind-Add⁺ extruded pellets nutritive quality makes it a good feed additive. It also helps in obtaining feed pellets with desired strengths (Sorensen et al., 2010). (Fig. 7 and Fig. 8)

Extrusion processed and fish hydrolysate incorporated fish feed has been shown to meet criterion like resistant to mechanical strength during transportation, texture and size that can facilitate high feed utilization (Hardy and Barows, 2000, Arvanitogannis et al., 2008), efficient digestion by fish (Sinha et al., 2011), water stability as well as bulk density in order to control sinking velocity and buoyancy. Tilapia feeding experiment has showed that extruded pellets are durable and remain in one piece until eaten by fish and small fractures of feed are not ingested and result in poor feed conversion efficiency. It has also been observed that fish does not physically disrupt the feed in the oral cavity but gulp the prey whole. Samuelsen and Oterhols (2015) used water soluble fish protein for fish feed processing industry. Serving multiple purposes as nutrient, plasticizer and binder, water soluble fish protein has a significant effect on the fish feed extrusion process. Extruded pellets with and without 2% FH were prepared and were evaluated for water stability and water absorption test. The extruded pellets were water stable up to 5 hrs. The diameter of the pellet is negatively co-related to bulk density. FH incorporated floating pellets expanded at least 50% more than the die opening which was only 40% in case of the control pellets. The degree of expansion has been positively affected by the level of fat and protein in FH in feed formulation. High temperature and short time extrusion cooking produced positive effect on feed durability. Water soluble fish protein, starch and fat has significant influence on fish feed extrusion process, gelatinization and physical pellet quality (Samuelsen and Oterhols, 2015). The length of the pellet is negatively allocated with the diameter of the pellet. They have a plasticizing effect and increased floatability and cooking efficiency.

IV. CONCLUSION

Fish meal is the major ingredient and primary source of protein in most fish and poultry diets. Fish meal can be replaced by *Bind-Add⁺*, binder cum additive by commercial feed manufacturer. *Bind-Add⁺* contain well balanced protein, fatty acid, amino acids, macro, micro and trace minerals and is advantageous from environmental and economic point of view.

Bind-Add⁺ incorporation at 2% level improves gelatinization and binding properties which is reflected in water absorption capacity, buoyancy, bulk density and water stability characteristics in extruded feed pellets.

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Fig.1: CIFA BIND-ADD+ Binder cum additive in feed extrusion



Fig.2: 2% replacement of water with CIFA BIND-ADD+ during extrusion cooking



Fig. 3: Floating extruded pellets with Bimd-Add+



Fig. 4: Oreochromis niloticus during the feeding trial in glass pool



Fig. 5: 6 mm extruded floating pellets



Fig. 6: 3 mm extruded floating pellets



Fig. 7: Fish hydrolysate emulsion incorporated feed (CIFLYSATE FORTE)