

# BENEFICIAL EFFECTS OF FEATHER MEAL BY KERATINASE OF MARINE ACTINOMYCETES- A FEED ADDITIVE FOR FISH

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**Abstract:** The present study was carried out to investigate the fermented feather meal (FFM) as a replacement of dietary fish meal protein in the diet for shark. FFM was produced by keratinase of *S. acrimycini* NGP 1, *S. albogriseolus* NGP 2 and *S. variabilis* NGP 3 which was isolated from the marine sediments of South Indian coastal region. The physical and biochemical characterization were carried out for the sacrificed large and small shark fish, which were fed with commercial fish meal (TRIO) and FFM for 30 days. The characterization studies such as weight, percentage of weight gain, length, daily weight gain, survival rate, protein efficiency ratio, protein and lipid content indicated that, feather meal fed fish were more effective than fish meal which was produced by keratinase of *S. albogriseolus* NGP 2 than others. But in length, large and small fish fed with feather meal were found in the range of 7.5 to 8.0 and 6.4 to 6.7 cm respectively which were not shown significant growth than fish meal fed large (8.3 cm) and small fish (6.7 cm). The present study revealed that the growth and health status of shark fed by FFM and commercially available fish meal. It is the basic, alternative and innovative research platform for commercially available fish feed.

**IndexTerms** - Fermented feather meal, Fish meal, Actinomycetes keratinase, Shark fish.

## I. INTRODUCTION

Actinomycetes play a significant role in the biomass turnover in nature; many genus in the actinomycetes taxa are important polysaccharide degrading organisms. Discovery of enzymes from marine actinomycetes are gaining significance in recent years. Many commercially important extra and intra cellular enzymes such as amylases, protease, cellulase, lipase, keratinase, xylanase and L-asparaginase were produced from marine actinomycetes (El-Sersy et al. 2010; Haritha et al. 2010). Among them keratinase gain more attention in industrial applications especially in the conversion of feather to feather meal and used for aquatic feeds. Feathers are by products of the poultry meat processing which become the raw materials for production of 'feather meal'. Annually large quantities of feather are available. Totally 10, 00, 000 tons of crude feathers are producing which are equipped to 3, 20, 000 tons of feather meal. Mostly those feathers are land filled or brunt which cause environmental problems (Vasileva-Tonkova et al. 2009). In the production of feather meal, most popular method called 'hydrothermal processes' in which feather are worked under the high pressure at high temperature. By this treatment, the most essential fatty acids and nutritional value of the feather are decreased (Wang and Parson 1997). In this respect, microbial degradation of feathers employed to overcome the above mentioned problem. Generally the microorganisms such as bacteria (*Bacillus* and *Streptomyces*) fungi (*Rhyopus* and *Aspergillus*) are active in room temperature and optimum conditions (moisture more than 20%) and these microorganisms can ferment feather meal (Grazziotin et al. 2006). During fermentation the nutritional value of feather meal was decreased; to combat this, focus of the research is changing towards developing of enzymatic methods of feather degradation using keratinase from *Bacillus licheniformis* ER-15

was observed to degrade feather completely to feather meal (Tiwary and Gupta 2010) and the processed feather meal was used as a feed for aquatic animals (Grazziotin et al. 2006).

Currently, the animal feed industry is the main consumer for keratin hydrolysates from agroindustrial byproducts. Recycling of feathers is a subject of great interest for animal nutrition because of its potential as an inexpensive and alternative protein source. Despite the limited nutritional value of keratin, both the digestibility and amino acid balance of feather protein might be improved by microbial fermentation (Grazziotin et al. 2006). Versazyme (VZ) is an approved keratinase based feed additive produced naturally as a fermentation product of *Bacillus licheniformis* PWD-1. The experiment was designed to test the recovery and efficacy of VZ using typical feed industry pelleting parameters (Stark et al. 2009). Hence, the present study aims to conversion of feathers to feather meal by keratinase and used as feed for fish. The parameters such as effect of time and temperature for feather degradation were performed to scale up the production of feather meal in larger amount.

## II. METHODS

Chicken feathers 30.0 g were collected from the local market and washed with detergent followed by distilled water and dried at 80°C for 6 hrs and were used for subsequent experimentation.

### Process parameters

#### Effect of time and temperature

Feather degradation was studied for the period of 1 - 8 hrs at room temperature. 1.0 g of the feathers were taken in test tube containing 5.0 ml of crude keratinase enzyme and incubated. After every one hour the residual feathers were dried and calculate feather degradation (FD), protein release and keratinase activity. Effect of temperature on feather degradation was analyzed at different ranges (30 - 60°C). The time intervals which showed the maximum degradation of feathers were calibrated to this parameter (Munawar et al. 2012).

#### Scanning Electron Microscopy (SEM)

Structural changes in the feather at the optimum time interval and temperature were also analyzed by scanning electron microscopy (SEM). Feathers were washed with 50 mM phosphate buffer and dried at room temperature for scanning studies. It was coated with gold particle and observed using scanning electron microscopy (Hitachi High-Tech SU 3500 model).

#### Feather meal production

The feather meal production was carried out by Tiwary and Gupta (2012). 25.0 g of feathers were soaked in 250 ml water for 2 hrs and boiled for 10 - 20 min in the three separate 1000 ml conical flasks. It was cooled to room temperature and add 250 ml of crude keratinase enzyme along with add 250 ml of phosphate buffer (25 mM; pH 8.0) and mixed properly. It was kept at 45 - 50°C of 150 rpm for the complete degradation of feathers and filtered. The filtrate was served as a feather meal and it was dried at 80°C, then ground to form homogenous powder. The aminoacid in the feather meal was tested by thin layer chromatography (TLC).

#### Experimental procedure

Eight shark fish were bought from fish aquarium, Coimbatore, Tamilnadu, India. Then the fish were reared in four glass aquarium tank (2 fish / tank) in a close water flow system with aeration (Arunlertaree and Moolthongnoi 2008).

#### Feed formulation

Feather meal powder 12.0 g was blended with water for 15 min to form pellets at desirable size of 1.0 to 3.0 mm and oven dried. Sixteen pellets (12 pellets - feather meal; 4 pellets - fish meal) were fed to the fish (4 pellets / tank) for one time a day at 9.30 AM till 30 days. Control pellet used for this study was

commercially available “TRIO”. At the end of experiment, fish were sacrificed and physical parameters such as weight, length, daily weight gain, survival rate and protein efficiency ratio were analyzed (Ahmad et al. 2012).

### Physical parameters

The physical parameters of the fish were carried out by the method of Arunlertaree and Moolthongnoi (2008).

#### Percentage of weight gain

The initial weight of the fish in control and test tank was calculated in the electronic weigh balance (Shimadzu AY220). After 30 days, the final weight was calculated by the following formula,

$$\text{Percentage of weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

#### Length

The lengths of the fish were measured by yard scale on first day and it was measured again after 30 days and noted.

#### Daily weight gain

The daily weight gain of the fish in the both tank (control and test) were analyzed by the following formula,

$$\text{Daily weight gain (g/day)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Culture period (days)}}$$

#### Survival rate (%)

Survival rate of the fish in the tank was calculated by,

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

#### Protein efficiency ratio (PER)

The presence of protein in the diet were influenced the growth and metabolic activities of the fish. The PER calculated by formula,

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Increasing weight in mass fish (g)}}{\text{Protein intake in diet (g)}}$$

#### Sample preparation

Muscle part was carefully dissected (free from skin and scales) and homogenized in mortar and pestle. The prepared sample was subjected for protein and lipid estimation (Jan et al. 2012).

### Biochemical parameters

#### Protein estimation

The protein content was estimated by lowry et al. (1951). The protocol was explained in detail in the page of 56. This is the most widely used method for protein estimation.

## Lipid estimation

The lipid content was estimated by Folch et al. (1957) method. The sample was solvent extracted with 20 - 50 ml of chloroform / methanol (2:1 v/v) and agitated for 20 min in an orbital shaker at room temperature. The homogenate was filtered by filter paper to recover the liquid phase and rinsed twice with methanol / chloroform (1:1 v/v). The lower chloroform phase containing lipid was filtered and evaporate the solvent under vacuum in a rotatory evaporator. The lipid extracted from the sample was covered with foil papers and then stored in a refrigerator at -4°C.

## RESULTS AND DISCUSSION

### Effect of time and temperature

Feather degradation studies were carried out for the period of 1 to 8 hrs at room temperature. After every one hour the degree of feather degradation (DFD), protein release and keratinase activity were calculated and the results were presented in Fig.1. Partially purified keratinase from *S. acrimycini* NGP 1 was exposed for various time intervals. The results revealed that, the maximum degradation of 19.0 % was obtained on 6 hrs of incubation. The keratinase activity and protein were 28.0 U/ml and 0.190 mg/ml respectively. While increasing the time interval feather degradation, enzyme activity and protein release were drastically decreased. Similar to *S. acrimycini* NGP 1 keratinase, the maximum degradation of feathers were brought up to 61.0 % at 6 hrs by keratinase from *S. albogriseolus* NGP 2. The keratinase activity and protein were 66.96 U/ml and 0.581 mg/ml respectively. The feather degradation, enzyme activity and protein release were stable up to 6 hrs. The increased feather degradation and enzyme activity was obtained in the range of 1 to 5 hrs of time interval. The maximum degradation of feathers were brought about by keratinase was 24.5 % at 5 hrs of time interval; enzyme activity was increased up to 35.52 U/ml with protein release of 0.395 mg/ml by the keratinase of *S. variabilis* NGP 3. Tiwary and Gupta (2012) found that, feather degradation was studied as a function of time for the period with enzyme under standard conditions to determine feather degradation, protein release and residual keratinase activity.



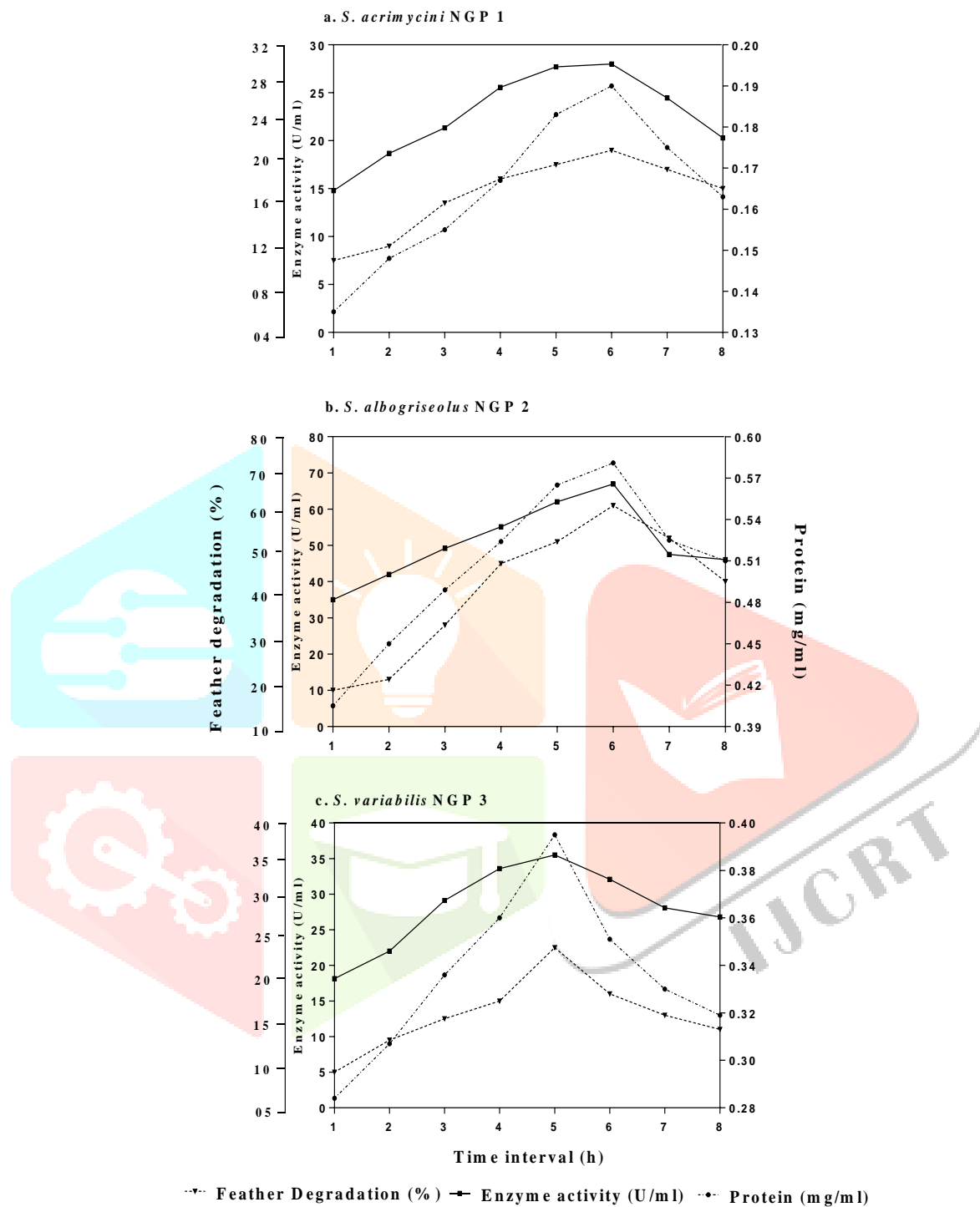


Figure 1: Effect of time interval on feather degradation by keratinase from marine actinomycetes

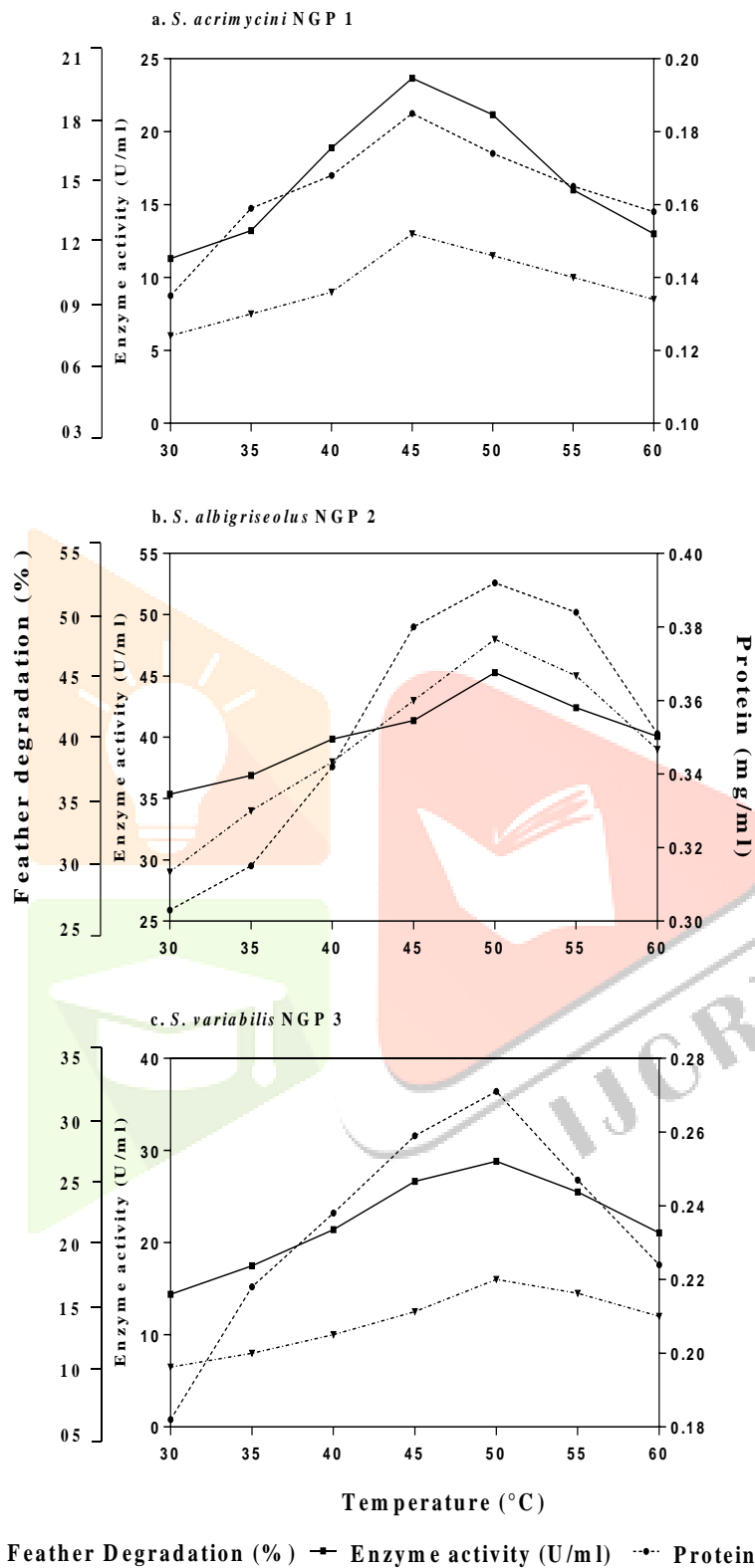


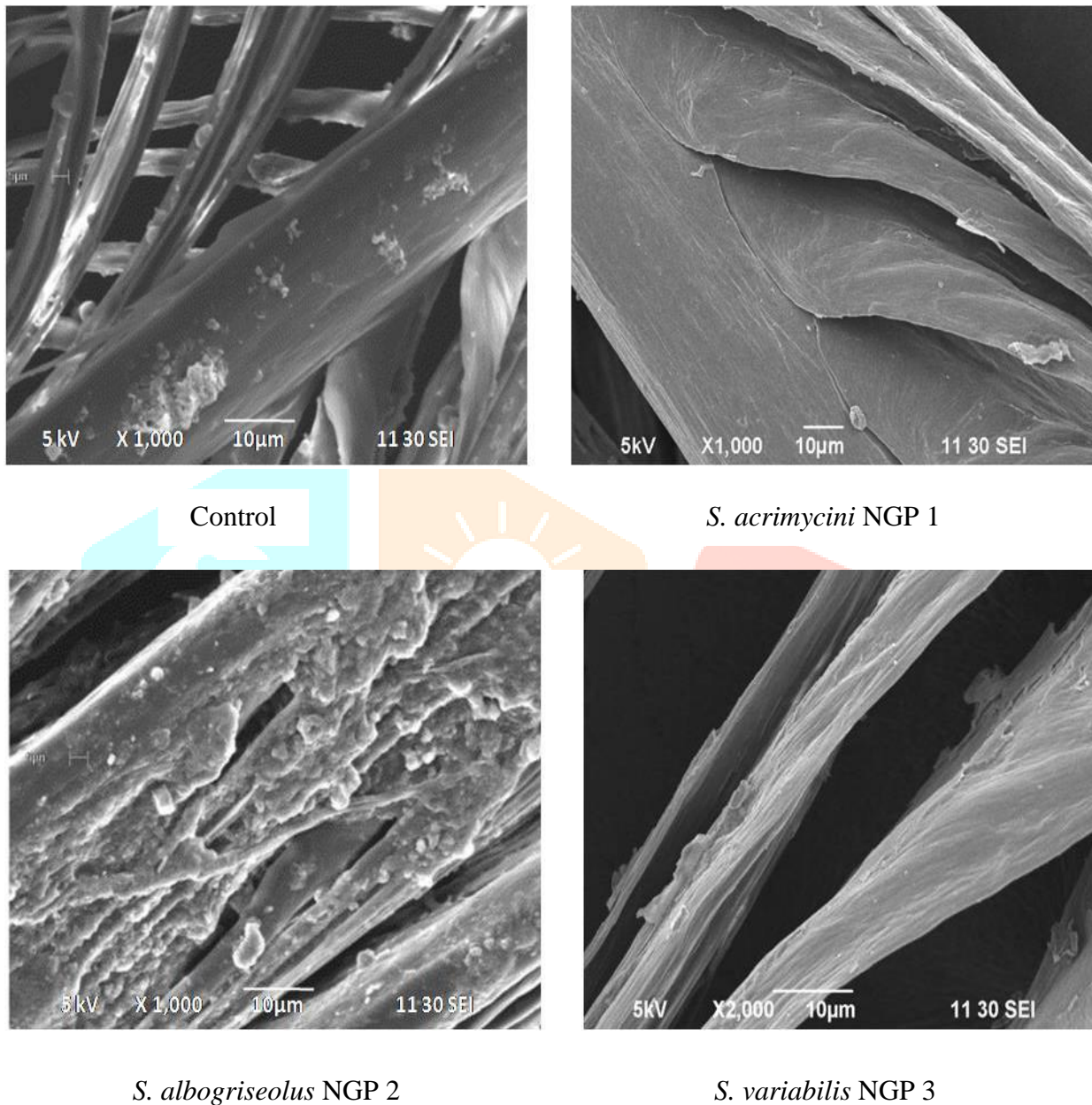
Figure 2: Effect of temperature on feather degradation by keratinase from marine actinomycetes

Effect of temperature on feather degradation was analyzed at different ranges from 30 to 60°C (Fig. 2). Keratinase from *S. acrimycini* NGP 1 showed the increased feather degradation at the temperature of 45°C and the degradation was found to be 12.0 %. The enzyme activity and protein were found to be 23.66 U/ml and 0.185 mg/ml respectively. Above the range of 45°C the feather degradation percentage and enzyme activity were decreased. At the temperature of 50°C the enzyme keratinase from *S. albogriseolus* NGP 2 brought about the feather degradation, enzyme activity and protein up to 48.0 %, 45.28 U/ml and 0.392 mg/ml respectively. While increasing the temperature above 50°C the degradation and enzyme activity were declined. Similar to *S. albogriseolus* NGP 2, the maximum feather degradation, enzyme activity and protein were observed at the temperature of 50°C. The degradation was found to be 16.0 %. Enzyme activity and protein was observed as 28.84 U/ml and 0.271 mg/ml respectively.

Feather degradation by keratinase of *B. subtilis* was studied at 37°C and 50°C and >90% degradation was observed after 24h at 37°C and 8h at 50°C. Further dissolution of shaft was observed only at 50°C. Complete degradation at 50°C may be a result of faster breakdown of disulfide bonds at higher temperature which may have resulted in dissolution of shaft (Onifade et al. 1998). Further, the enzyme has optimal activity at 70°C with >90% activity at 50°C and 67% at 37°C (Tiwary and Gupta 2010). Thus, the faster degradation at 50°C may be result of both enzyme concentration and temperature.

### Scanning Electron Microscopy (SEM)

The structural change of the feather by keratinase was analyzed by scanning electron microscopy at the optimum time interval and temperature of the respective actinomycetes. Keratinase from *S. albogriseolus* NGP 2 degraded the feather barbules completely and rachis was also attacked severely after 6 hrs at 50°C. Followed by, keratinase from *S. variabilis* NGP 3 moderately degraded the feather barbules and rachis after 5 hrs at 50°C. Besides, very slight degradation of feather barbules was observed and there was no attack on rachis of feather by keratinase of *S. acrimycini* NGP 1 after 6 hrs at 45°C (Plate 1). The production of feather meal by *S. acrimycini* NGP 1 contained the amino acids such as lysine and phenyl alanine. The feather meal produced by *S. albogriseolus* NGP 2 had highest content of essential amino acids such as glycine, leucine, histamine and valine. In addition, presence of amino acids in the feather meal produced by *S. variabilis* NGP 3 was lysine, histamine and serine. These findings indicated that the feather meal may be used as feed for livestock and aquatic animals. Structural changes in feather were also analyzed by scanning electron microscopy (Tiwary and Gupta 2012). Enzymatic hydrolysis of feather meal was observed to be rich in essential amino acids in comparison to steam cooked/ acid hydrolyzed feather meal. Amino acid content of present feather meal was comparable to culture supernatant hydrolyzed (CSH) produced by keratinolytic bacterium *Vibrio* kr6 (Grazziotin et al. 2006).



**Plate 1: SEM analysis of feather degradation by keratinase producing marine actinomycetes**

### Feather meal formulation for fish

The homogenized form of feather meal powder produced by marine actinomycetes were blended and formed as pellets at a desired size of 1.0 to 3.0 mm. Shark fish were fed with fish and feather meal pellets for 30 days (Plate 2). After 30 days the sharks were sacrificed to analyze physical and biochemical parameters. Feather meal produced through thermal processing has low nutritional value (Wang and Parsons 1997). However, feather protein has been considered as an excellent source of metabolisable protein. Klemersrud et al. (1998) stated that, the nutritional value of feather hydrolysate was found to be similar to that of soybean meal (Williams et al. 1991), thus could find application as an animal or poultry feed supplement (Grazziotin et al. 2006; 2007).





**Feather meal**



**Fish meal**



**Fish tank**

Plate 2: Feed formulation for fish

## Physical parameters

Parameters	Fish meal		Feather meal					
	Large fish	Small fish	Large fish			Small fish		
			Sa	Sag	Sv	Sa	Sag	Sv
<b>Physical characterization</b>								
Initial weight (g)	2.0	1.4	2.1	2.5	2.0	1.4	1.6	1.4
Final weight (g)	2.5	1.6	2.4	3.3	2.5	1.6	2.0	1.6
Percentage of weight gain (%)	25.0	14.28	14.28	32.0	25.0	14.28	33.3	14.28
Initial length (cm)	7.9	6.5	7.5	7.8	7.8	6.5	6.5	6.4
Final length (cm)	8.3	6.7	7.5	8.0	7.8	6.5	6.7	6.4
Daily weight gain (g/day)	0.017	0.007	0.010	0.027	0.017	0.007	0.017	0.007
Survival rate (%)	100	100	100	100	100	100	100	100
Protein efficiency ratio (PER)	1.736	0.694	0.892	2.381	1.488	0.595	1.489	0.595
<b>Biochemical characterization</b>								
Protein (mg)	0.118	0.074	0.115	0.200	0.117	0.093	0.172	0.098
Lipid (%)	0.075	0.100	0.090	0.115	0.094	0.151	0.170	0.158

Sa: *Streptomyces acrimycini* NGP 1; Sag: *Streptomyces albogriseolus* NGP 2; Sv: *Streptomyces variabilis* NGP 3

Table 1. Physical and biochemical characterization of fish fed with fish and feather meal

## Percentage of weight gain

The physical parameters of the fish were presented in table 1. The results revealed that, the weight gain percentage was maximum for the fish fed with feather meal (test) than the fish meal (control). In control, the weight gain percentage of the shark fish were 25.0 and 14.28 % respectively for large and small fish respectively on 30<sup>th</sup> day. The weight gain percentages of large and small fish were similar as 14.28 % where the feather meal from *S. acrimycini* NGP 1 supplemented in diet. The feather meal from *S. albogriseolus* NGP 2 increased the weight gain percentage of large and small fish than control. The weight gain percentages were 32.0 and 33.3 respectively. Similarly, the weight gain percentage of the large and small shark fish in the test were 25.0 and 14.28 % by the feather meal from *S. variabilis* NGP 3. This result agreed with the study of Bishop et al. (1995) on *Oreochromis niloticus* reported that tilapia fry fed with diet that replace more than 66 % of fish meal by feather meal had high final weight gain. Fowler (1990) also reported that juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) fed with diets that replaced 15 % of fish meal by feather meal, weight gain did not differ from fed with diets that replaced 0 and 5 % of fish meal by feather meal at the end of experiment. Percentage of weight gain in fish fed with diets containing fermented feather meal were showed increased weight gain percentage (Arunlertaree and Moolthongnoi 2008).

## Length

The length of the control and test fish was determined by yard scale. The length of the control fish were increased than the test fish. In control, the initial and final lengths of large and small fish were 7.9, 6.5 and 8.3, 6.7 respectively. There were no significant changes for the final length of large (7.5 and 7.8 cm) and small fish (6.5 and 6.4) supplemented with feather meal from *S. acrimycini* NGP 1 and *S. variabilis* NGP 3; whereas fish fed with feather meal from *S. albogriseolus* NGP 2 showed the initial and final lengths of large and small fish were 7.8, 6.5 and 8.0, 6.7 respectively.

## Daily weight gain

In this parameter, the daily weight gain of the test fish were increased one fold than the control fish. Daily weight gain of the fish indicated that, the availability and digestibility of the feed in their diet. In control, the large and small fish daily weight gain was 0.017 and 0.007 g/day. The daily weight gain of the large fish fed with feather meal synthesized by marine actinomycetes were 0.010, 0.027 and 0.017 g/day respectively. For the small fish it was found to be 0.007, 0.017 and 0.007 respectively from marine actinomycetes. So, feather meal is more effective than fish meal. Average daily weight gain in fish feds with feather meal was significantly higher than fermented feather meal of *Oreochromis Niloticus* (Arunlertaree and Moolthongnoi 2008).

## Survival rate

Survival rate of the fish was 100 % on both diet groups. This result agreed with the study of the diet of *Oreochromis Niloticus* (Arunlertaree and Moolthongnoi 2008)

## Protein efficiency ratio (PER)

Protein efficiency ratio (PER) of control and test fish were analyzed on 30<sup>th</sup> day of culture period and tabulated in table 21. For control fish the PER was found to be 1.736 and 0.694 respectively for the large and small fish. PER of the large fish fed with feather meal by keratinase from actinomycetes were 0.892, 2.381 and 1.488 respectively. Similarly, PER of the small fish were 0.595, 1.489 and 0.595 respectively by keratinase from actinomycetes. The results showed similarly to the study of Fasakin et al. (2005) that presented data on the hybrid tilapia which reported protein efficiency ratio of fish decreased with feather meal partial replacement of fish meal. The study of Tacon et al. (1983) on sub-adult Nile tilapia also reported that the 30 % feather meal replacement of fish meal with addition L-methionine or L-histidine or L-lysine or all 3 amino acid together have better protein efficiency ratio than without addition.

## Biochemical parameters

### Protein estimation

The protein content of shark's muscle was determined over the period of one month and the obtained results were presented in table 1. For control fish, the large and small fish had 0.118 and 0.074 mg/g protein respectively in their muscles. The highest protein content was noted in the test fish were about 0.200 and 0.172 mg/g protein respectively from *S. albogriseolus* NGP 2. Feather meal from the actinomycetes *S. acrimycini* NGP 1 and *S. variabilis* NGP 3 showed the protein in large and small fish muscles were 0.115, 0.117 and 0.093, 0.098 mg/g protein. Fish is rich in protein with amino acid composition very well suited to human dietary requirements comparing favorably with egg, milk and meat in the nutritional value of its protein (Tacon et al. 1983). The protein composition of *Schizothorax niger* was determined over the period of one year and the obtained results are varied from 0.0947 to 0.220 mg/g tissue (Jan et al. 2012).

## Lipid estimation

The control fish were found slightly lesser amount of lipid content than test fish and the lipid content was found to be 0.075 and 0.100 % respectively. In test tank, large fish fed with feather meal were 0.090, 0.115 and 0.094 respectively from marine actinomycetes; for small fish, lipid content was found as 0.151, 0.170 and 0.158 % respectively. Kaga et al. (2009) found that, a total of 175 immature chum salmon caught in the Bering Sea were examined to compare the estimated lipid contents and response values of microwave meter. A regression relationship was obtained between the total lipid content of fish muscles measured by chemical analyses and microwave meter values. Large fish had higher initial lipid contents and therefore period of acclimatization to rapid consumption of feather meal for brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* (Thompson and Bergersen 1991).

## III. CONCLUSION

The study on replacement of fish meal with fermented feather meal was conducted on Shark fish. Based on the results obtained from this study, it could be concluded that feather meal which produced the fermentation process by *S. albogriseolus* NGP 2 could increase efficiency of feather meal utilization in fish diet. Based on the physical and biochemical parameters, feather meal diet is more significant than fish meal

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