

Enhancement In Nutritive Value And *invitro* Digestability Of Keratinase Treated Feather Meal

P.Jeevana Lakshmi and V.V.Lakshmi

ABSTRACT: Feather is produced in bulk quantity as a by-product of poultry industry. Feather meal produced by traditional means has disadvantage of low nutritional value as well as low digestibility. Improving the nutritive value and digestibility by adopting novel biological techniques can result in high quality feed supplement from feather waste. Earlier studies in our laboratory resulted in isolation and characterization of four improved *Bacillus* spp. - MBF11, MBF20, MBF21 and MBF45 which produced keratinase enzyme that could degrade feather completely. Keratinase treated feather meal (KTF) was analyzed for the *invitro*-digestibility and compared with feather meal produced by traditional methods like heat treated, acid treated and trypsin digested feather. Total nitrogen, non-protein nitrogen, total free amino acids and essential amino acids were estimated for all the treatments. Non- protein nitrogen increased four folds upto ~3.5-4fold with keratinase hydrolysis where as heat and acid treatments resulted in low free nitrogen of a maximum of ~2. The *invitro* digestibility of untreated feather powder was 24% where as for KTF it increased to ~61-72%. The digestibility of the traditionally treated samples was around 27-32%. There was ~2-3 fold increase in amino acids like proline and glycine, ~2 fold increase in lysine and cystine methionine and histidine content in KTF as compared to controls. Thus, feather meal prepared by keratinase treatment was significantly better than the feather meal produced by traditional methods and this could be an important technique to convert feather waste into value added product.

Index terms: Application potential, *Bacillus*, feed, keratinase treated feather

1 INTRODUCTION:

Feather is generated in bulk quantities as a by-product of poultry industry. It is estimated that 400 million chickens are processed every week. Typically as each bird has upto 125gms of feather, the weekly worldwide production of feather waste is about 3000 tons. Elemental composition analysis of feather show that it is constituted of 45% carbon, 14% Nitrogen, 2.9gkg⁻¹, phosphorus, 1.5gkg⁻¹ potassium and 0.8gkg⁻¹ magnesium. Traditionally feather is processed by heat and acid treatment. These processes result in a low

nutritive value feed supplement thereby undermining the product value [1]. Feathers and the meal derived from it are poorly digested by animals, mainly due to poor and limited degradation of the highly ordered structure of keratins by digestive enzymes. Further the traditional feather meal produced barely covers the cost to its production at best and is not in good demand.

Hence, bulk of feather produced is underutilized and or wasted. Feather meal produced by biological treatment was found to be significantly superior in nutritive value compared to ones produced by traditional means thus increasing their economic value [2],[3],[4]. Application of keratinase to feather processing has several benefits. Bioconversion using keratinase results in production of structurally modified feather keratin that is less resistant to attack by other digestive enzymes. Secondly, significant nutritional enrichment of the feather meal is achieved due to the addition of microbial protein biomass, which serves as complementary additive value. Thirdly, further increased levels of feed- grade lysine and other amino acids can be targeted in biologically treated feather meal by supplementing with additional microorganisms that can produce amino acid by fermentation also. Biological conversion of feather into feather meal is thus now seriously considered as a source for production of high value dietary proteins [5]. Keratinases are considered to have definite advantage as enzyme supplements in feed along with other enzymes mixture as they attack broad variety of proteins.

Keratinase producing *Bacillus* isolates MBF11, MBF20, MBF21 and MBF45 exhibiting high keratinase production due to strain improvement and

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optimization of parameters of fermentation were selected for producing feather meal. These were analyzed *invitro* digestibility and nutritional parameters to evaluate the application potential as feed supplement.

2 MATERIALS AND METHODS:

Application potential of the keratinase from the MBF isolates was analyzed by comparing the digestibility of the keratinase treatment ball milled feather with the traditional methods of feather meal production like treatments like acid (6N hydrochloric acid for 12 hours) and heat adopting method of Papadopoluset *al.* [6]. The activity was also compared with commercial available feather meal (CFM) and commercial trypsin (1.6mg/ml-1) treatment for 12 hours. The nutritional value of the various feather meals was compared by estimating the amount of total nitrogen, free nitrogen and protein nitrogen of the feather meal obtained from the various treatments adopting Micro-Kjeldahl method of nitrogen estimation [7].

2.1 Estimation of nitrogen by Micro-Kjeldahl estimation of Nitrogen:

To treated and control samples in digestion flask, potassium sulphate, mercuric oxide and concentrated H₂SO₄ were added. The samples were digested after addition of glass beads in the digestion flasks. The digests were cooled and the volume made up with ammonia free water. The sample was transferred to distillation apparatus along with sodium hydroxide-sodium thiosulphate solution. The samples were distilled and the ammonia released was collected conical flask containing boric acid and few drops of methyl red - methylene blue indicator. The resulting boric acid was titrated against the 0.02N HCl until the first appearance of violet colour (the end point). The nitrogen content in NgKg-1 of the sample was calculated using the formula [7].

The non- protein nitrogen 100mg of test sample was extracted with 10ml of 10% TCA. The precipitate was washed once with TCA. The pooled supernatant was made upto 25ml and the sample was distilled as given procedure described above. The non-protein nitrogen (NgKg-1) content was calculated using the formula [7]. Protein nitrogen was calculated by deducting the non-protein nitrogen from the total nitrogen and then multiplied with the factor 6.25.

2.2 *vitro* digestibility of the feather meal:

Invitro digestibility of the treated and untreated feather meal was compared by treating powdered treated and untreated feather samples with pepsin

(Sigma) for 2 hours at 37° C followed by pancreatintreatment (Sigma) additional 16 hours [6]. After digestion, samples were centrifuged and protein concentration was determined in supernatants before and after digestion. Protein digestibility (D) was calculated by dividing Content of protein released after digestion with Content of the total protein before digestion.

2.3 Amino acid analysis:

Selected amino acids were estimated in the treated and untreated feather meal and percentage of each amino acid in the total free amino acids was calculated. Cystine was estimated as per the method of Ramakrishna *et al.*, 1979[8] and Methionine[9] and total free amino acids Rosen, 1957[10] respectively. Proline was estimated by adopting the method of Bates *et al.*, 1973[11]. Lysine was estimated with the procedure of Balasubramanian and Sadasivan, 1987 [12] and Tyrosine and Histidine were estimated as per the method adopted in Hanke, 1925[13].

3 RESULTS AND DISCUSSION

The results of total nitrogen, non protein free nitrogen, *invitro* digestibility and amino acid content of KTF, CFM and feather powder treated with traditional methods like acid and heat. Untreated feather powder was maintained as control. There was a significant difference in non-protein nitrogen in the various treatments. The non- protein was zero in the untreated feather as well as in commercially procured feather meal and in heat and acid treated controls. As majority of the non-protein nitrogen is in the form of amino acids, peptides and degraded nitrogen products it justifies their absence in undegraded feather. The conventional methods of feather treatment like heat and acid resulted in destruction of amino acids. Very low non-protein nitrogen indicated that harsh treatment leads to the destruction of these amino acids. The low non -protein nitrogen has been observed to be the main reason for the poor digestibility of conventionally made feather meal (acid and heat treated). The treatment of feather with enzymes like trypsin increased the non-protein nitrogen to ~1.5-2g/Kg. However, the feather meal prepared by treatment with keratinases showed significant increase up to 4g/Kg of non-protein nitrogen (Fig.1). The *invitro* digestibility of untreated feather powder and controls like heat treated, acid treated and commercially procured feather meal was in the range of 24%-32%. In the trypsin treated feather meal the *invitro* digestibility doubled to 50% in comparison to treatment with acid and heat.

With keratinasetreatment the *invitro* digestibility of the feather meal increased to ~65-80% (Fig. 2). *Invitro* digestibility increased by 2.5 folds in the KTF when compared to control where the digestibility was around 24-32%. Comparison among the digestibility of KTF prepared from keratinase produced by the four bacillus species showed that the highest digestibility was exhibited by MBF20 keratinase followed by MBF11, MBF21 and MBF45.

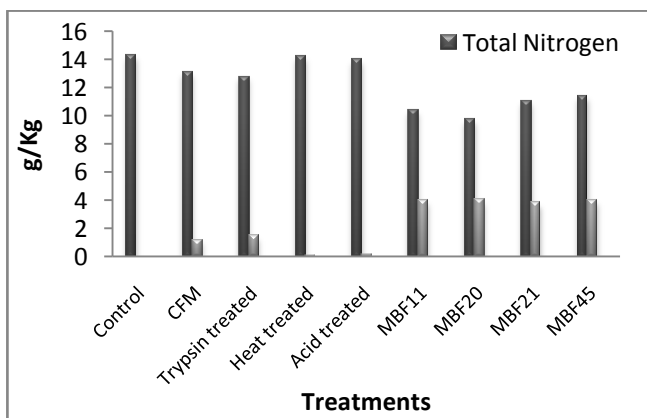


Fig1: Analysis of Nitrogen content for all the treatments

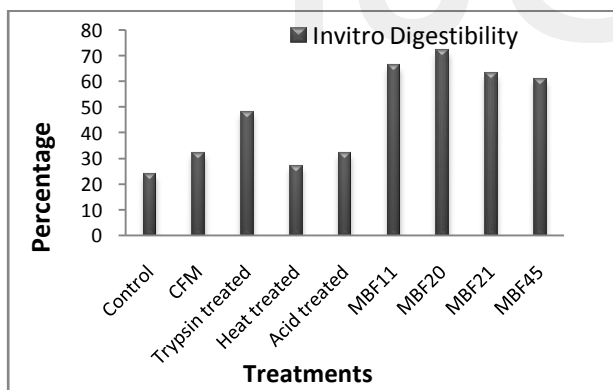


Fig2: Analysis of *Invitro* digestibility (%) content for all the treatments

Further to assess the quality the quality of feathermeals obtained by different treatments some of the essential amino were estimated and the results are shown in Fig.3. There was ~2-3 fold increase in concentration of proline and glycine in KTF as compared to the controls which was highly significant (Fig. 3A), ~2 fold increase in lysine and cystine which are important determinants of the feed quality (Fig. 3A). An increase in concentration of methionine and histidine

content in KTF was also observed though the fold increase was slightly less as compared to other amino acids (Fig. 3C). The overall amino acid concentration was higher in KTF prepared from MBF 20 followed by MBF45, MBF21 and MBF11.

Treatment of feather with protease has been shown to influence the nitrogen availability, *invitro* digestibility and free essential amino acids in earlier studies to limited extents by Papadopoulos *et al.* [6]. Trends of increased digestibility were observed with keratinase treated feather suggesting the potential for improving its acceptability as poultry feed or possible fertilizer [5], [14], [15]. The chicken fed with biologically treated feather showed significantly improved growth rates as compared to those fed with soya bean meal. The resulting product also had an improved overall amino acid availability, digestibility and absorption [5], [16], [17]. Supplementation of poultry diets with enzyme mixtures, containing keratinase and amylases has produced significant improvement in growth performance [18], [19].

The results of the present study showed that there is a significant improvement in the nutritive value of the K feather meal when compared to Control treatment and commercial feather meal procured. The increase in the nutritive value with keratinases from MBF cultures was comparable to or much higher as compared to earlier studies. In terms of overall digestibility and availability of non protein nitrogen as well as presence of essential amino acids and thus could be considered as a better source of dietary protein supplement..

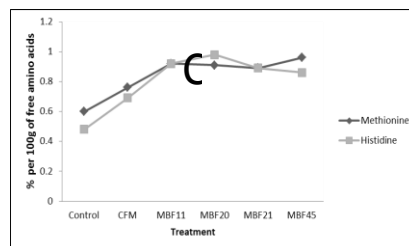
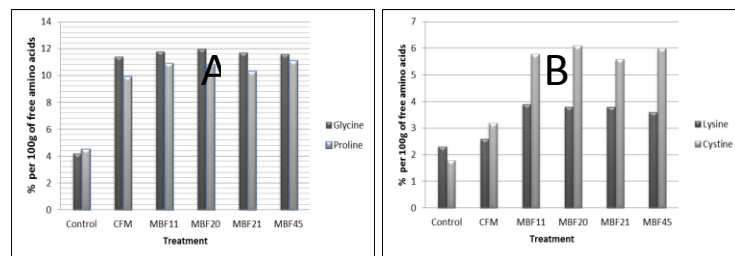


Fig3 :Analysisofselected amino acids A) Glycine and proline B) L ysine and cystineC) Methione and Histidine

4CONCLUSIONS:

Estimation of various parameters of keratinase treated feather meal in comparison to commercial feather meal and trypsin digested and traditional treatments of feather meal reveal that keratinase treated feather meal has very good potential to be used as feed supplement and organic manure. Hence, the present work establishes the application potential biodegradation of feather waste to environmental friendly products of commercial importance.

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6REFERENCES:

- [1] X. Wang and C.M. Parsons, "Effect of processing systems on protein quality of feather meal and hair meals", *Poultry Science*, vol. 76, pp. 491-496, 1997.
- [2] A. A. Onifade, N. A. Al-Sane, A. A. Al-Mussallam and S. Al-Zarban, "Potential for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as live stock feed resources", *Bioresource Technology*, vol. 66, pp. 1-11, 1998.
- [3] X. Lin, G. D. Inglis, L. J. Yanke, and K. J. Cheng, "Selection and characterization of feather-degrading bacteria from canola meal compost", *Journal of Industrial Microbiology and Biotechnology*, Vol. 23, pp. 149-153, 1999.
- [4] A. Riffel, F. Lucase, P. Heeb and A. Brandelli, "Characterization of a new keratinolytic bacterium that completely degrades native feather keratin", *Archives of Microbiology*, Vol. 179, pp. 258-265, 2003.
- [5] A. Grazziotin, F. A. Pimental, E. V. de Jong and A. Brandelli, "Nutritional improvement of feather protein by treatment with microbial keratinase", *Animal Feed Sciences and Technology*, Vol. 126, pp. 135-144, 2006.
- [6] M. C. Papadopoulos, A. R. Elboushy, A. E. Roodbeen and E. H. Ketelors, "Effects of processing time and moisture content on amino acid composition and nitrogen characteristics of feather meal", *Animal Feed Science Technology*, vol. 14, pp. 279-296, 1986.
- [7] AOAC, "Official methods of analysis of the association of official analytical chemists", 14th edition, *The Washington, DC Association*, 1984.
- [8] R. Ramakrishna, P. Siraj and P. Shastri, "Spectrophotometric method for the direct determination of cysteine and cystine in the presence of other naturally occurring amino acids", *Current Science*, Vol. 48, 1979.
- [9] H. H. Elmayergi, and R. E. Smith, "Influence of growth of *Streptomyces fradiae* on pepsin-HCL digestibility and methionine content of feather meal", *Canadian Journal of Microbiology*, vol. 17, pp. 1067-1072, 1971.
- [10] H. Rosen, "A modified ninhydrin colorimetric analysis for amino acids. *Archives of Biochemistry and Biophysics*", vol. 67, pp. 10-15, 1957.
- [11] L. S. Bates, R. P. Waldeen, and I. D. Theare, *Plant and Soil*, Vol. 39, pp. 205, 1973.
- [12] T. Balasubramanian, and S. Sadasivan, *Plant Foods and Human Nutrition*, Vol. 37, pp. 41, 1987.
- [13] M. T. Hanke, "The quantitative estimation of tyrosine and histidine in protein", *The Journal of Biological Chemistry*, vol. 7, pp. 475-488, 1925.
- [14] A. Hadas and L. Kautsky, "Feather meal, a semi-slow-release nitrogen fertilizer for organic farming", *Fertilizer Research*, vol. 38, pp. 165-170, 1994.
- [15] N. H. Odetallah, J. J. Wang, J. D. Garlich and J. C. H. Shih, "Keratinase in starter diets improves growth of broiler chicks", *Poultry Science* chemists, 14th edition. The Association: Washington, DC, vol. 82, pp. 664-670, 2003.
- [16] J. K. AppleBoger, et al., "Effect of feather meal on live animal performance and carcass quality and composition of growing finishing swine", *Journal of Animal Science*, vol. 81, pp. 172-181, 2003.
- [17] M. W. Greenwood, C. A. Fritts, and P. W. Waldroup, "Utilization of enzyme 1502 in corn-soyabean meal diets with and without antibiotics", *Poultry Science*, vol. 81, pp. 25, 2002.
- [18] A. Bertsch, and N. Coello, "A biotechnological process for treatment and recycling poultry feathers as a feed ingredient", *Bioresource Technology*, vol. 96, pp. 1703-1708, 2005.
- [19] H. Burrows, M. Hurby, D. Hung and O. Adeola, "Addition of enzymes to corn-soy diets for ducks: a performance and digestibility study", *Poultry Science*, vol. 81, pp. 29, 2002.