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Evaluation of storage stability of poultry by-product meal incorporated fish feed under aerobic storage condition

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Abstract

The research focused on development of fish feed utilising poultry by-product meal (PBM) as a substitute for fish meal (FM) and evaluating its shelf life under aerobic packaging conditions. Initially, a basal diet (C) was formulated to meet the nutritional requirements for optimal growth of Tilapia by using FM as the major protein source. After analysing the nutritional composition of PBM, an isoproteinaceous diet (T) was formulated by replacing FM with PBM. The product was packed in HDPE bags and kept at room temperature and analysed for physico chemical and microbial characteristics for 45 days at weekly intervals. Moisture content of both C and T decreased significantly ($p<0.01$) throughout the storage period and no significant difference ($p>0.05$) was found between C and T. The TBARS value of both C and T followed an increasing trend. This increase in C was not significant ($p<0.01$) between 28th and 35th day, and between 42nd and 45th day but that of T increased significantly ($p<0.01$) throughout the storage period. T consistently displayed the highest TBARS value during storage period. Tyrosine Value (TV) of both C and T also increased during storage and was significant ($p<0.01$) up to 42nd day of storage. C exhibited the highest TV throughout the storage period. Similarly, total viable count (TVC) of both C and T increased on storage. The increase in TVC for C was not significant ($p<0.01$) between 21st and 28th days, whereas that of T was significant ($p<0.01$) throughout the storage period. On 45th day, T exhibited highest count and was significantly different ($p<0.01$) from C. Throughout the 45-day storage period, no observable coliform count or yeast and mould count was noted in either C or T. The present study indicated that fish feed can be effectively prepared by utilising PBM and can be stored under room temperature up to 45 days.

Keywords: Poultry by-product meal, fish meal, fish feed, storage stability

The demand for meat and meat products, notably poultry, is undergoing rapid expansion in recent times. Poultry meat is contributing about 51.44 per cent of the total meat production in India (BAHS, 2023). In 2022, the global production of poultry meat increased by 1.9 per cent to 141 million tonnes and Asia experienced the largest rise of production (FAO, 2023). In fulfilling this consumer demand, a substantial quantity of poultry slaughter by-products is produced daily which accounts to 33 per cent of slaughter weight of chicken (Woodgate and van der Veen, 2014). There

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are enormous amounts of solid wastes such as bedding material, feathers, hatchery wastes, blood, offal, shells and poultry manure/litter apart from wastewater produced by the poultry industry (Muduli *et al*., 2019). These by-products possess great nutritional value and high value addition potential both in agricultural and industrial sector. Jayathilakan *et al*. (2012) stated that for the profitability of the meat industry, efficient utilisation of meat by-products is important. Apart from this, non-utilisation or underutilisation of these waste materials pose environmental pollution and may cause serious health hazards.

According to Subasinghe *et al*. (2009), aquaculture stands as the rapidly expanding sector in food production that provides superior protein to consumers and is a source of income for many people. The major share of aquaculture expense is attributed to aquafeed since the success of aquaculture depends on the provision of nutritionally balanced feed. The cost of production of aquatic animals is on the increase as a result of the sharp rise in aquafeed cost in recent years (El Basuini *et al*., 2017). Fishmeal has historically served as the primary protein source utilised in the formulation of aquaculture feed, making up approximately 68 per cent of the worldwide production of FM (Tacon and Metian, 2015). In addition to being dependent on wild fish stocks to produce, the high demand for FM and limited supply are the key factors driving up the cost of aquafeed (Salin *et al*., 2018).

Poultry by-product meal (PBM), derived from the waste of poultry slaughter, is a readily accessible and most economical protein source for animals, particularly as a feed ingredient in pet and aquaculture nutrition. The protein in PBM has high biological value with a digestibility coefficient of 82 per cent and contains several amino acids that are absent from plant proteins (Wisman *et al*., 1958). Ravindran and Blair (1993) reported that good-quality PBM is thought to include between 58 and 63 per cent crude protein, between 12 and 20 per cent ether extract and between 18 and 23 per cent ash. Studies on apparent digestibility of PBM have shown that a variety of fish species may easily digest this substance (Bureau *et al*., 1999). Therefore, the aim of this study was to develop fish feed utilising PBM as a substitute for FM and evaluating its shelf life under aerobic packaging conditions.

Materials and methods

Ingredients

Poultry by-product meal was procured from the local market in Thrissur, Kerala. The product was ground, sieved, packed in LDPE bags and stored at ambient temperature until further processing. Other feed ingredients such as FM, fish oil, clam meal, soyabean meal, groundnut oil cake (GNOC), wheat bran, wheat powder, sunflower oil and multivitamin supplement (SUPPLEVITE-M) were purchased from local shops in Ernakulam. Food grade chemicals such as vitamin C, mineral mixture, lysine, methionine, dicalcium phosphate (DCP), butylated hydroxytoluene (BHT) and sodium metabisulphite (SMB) were obtained from NICE chemicals Pvt. Ltd., Kochi. Wheat gluten was purchased online. For the subsequent steps, all of these materials were ground, sieved and stored at room temperature in sterile, dry plastic containers.

Analysis of poultry by-product meal (PBM)

Poultry by-product meal was analysed for its nutritional composition such as moisture, crude protein, ether extract, crude fibre, total ash, acid insoluble ash and nitrogen free extract as per AOAC (2016) at the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy.

Product formulation

A basal diet was formulated to meet the nutritional requirements for optimal growth of Tilapia according to the guidelines of FAO (2015) by using FM as the major protein source and used as the study control (C). The isonitrogenous treatment fish feed (T) was formulated by replacing FM in the control formulation with PBM as seen in Table 1.

Table 1. Formulation of control and treatment feeds

Ingredients	C	T
Wheat gluten (%)	3	3
Soyabean meal (%)	20.6	22.75
GNOC (%)	15	15
Wheat bran (%)	21	14
Wheat powder (%)	20	20
Fish meal* (%)	8	0
Clam meal (%)	4	0
PBM* (%)	0	18.6
Fish oil (%)	1.75	2
Sunflower oil (%)	2	0
Vitamin (%)	1.5	1.5
Mineral (%)	1.5	1.5
SMB+BHT (%)	0.2	0.2
Methionine (%)	0.5	0.5
Lysine (%)	0.2	0.2
Vitamin C (%)	0.25	0.25
DCP (%)	0.5	0.5

*FM from C was replaced by PBM in T; Protein content of FM:68 per cent

C – control (Fish feed as per FAO guidelines); T - Fish feed by replacing FM from C with PBM.

Mixing of the ingredients

The processing and preparation were done at the Central Marine Fisheries Research Institute, Kochi. The ingredients, sieved beforehand, were weighed as per the formulation requirements. These weighed dried components were then placed in a plastic container and manually mixed using a stirrer. Liquid ingredients, such as fish oil and sunflower oil, were then added and thoroughly blended. Subsequently, 20 per cent water was added and mixed thoroughly. The mixture underwent homogenisation using a homogeniser (FOSS HM297, Labtec line, Denmark), operating at 1500 and 3000 rpm for one minute each. It was then transferred to a bowl mixer (HOBART, HL 200, USA) for the final mixing stage, where it was blended for a duration of 12 min (6 min at 107 rpm followed by 6 min at 198 rpm). Following this, the mix was sieved and relocated to a plastic container. This same mixing process was applied to both treatment and control feed ingredients.

Extrusion process

The blended ingredients were introduced into the feed hopper of twin screw extruder by fixing the die diameter at 1 mm. The extruder was set to operate at 110 °C, and each experimental diet was extruded separately. The extruded materials exited through the die were cut into pellets by a rotating blade. The pellets were collected in trays and labelled accordingly.

Drying

Immediately after extrusion, the pellets collected in trays were transferred to hot air oven (LABLINE SPW, India) for overnight drying at 50 °C. The dried pellets were sieved through 1 mm diameter sieve to remove any unextruded particles, packed in HDPE bags and stored in ambient temperature conditions.

Product analysis

The feed was then subjected to physicochemical and microbiological quality evaluations during storage for 45 days, with assessments conducted at weekly intervals (on days 0, 7, 14, 21, 28, 35, 42 and 45). Physicochemical parameters assessed included moisture content (AOAC, 2016), thiobarbituric acid reactive substance value (Witte *et al.,* 1970), tyrosine value (Strange *et al.,* 1977) and microbiological qualities such as aerobic plate count, yeast and mould count and total coliform count (APHA, 2015) to ensure the storage stability.

Moisture content of the feed pellets was analysed as per AOAC (2016) on the day of preparation. About 10 gm of powdered sample was taken in petri dish and kept in hot air oven (ROTEK, Mumbai) set at 105°C for 16 - 18 h. The weight of the dried sample was taken after cooling in a desiccator. The difference in the weight was the moisture content of the sample and it was expressed as percentage of the feed.

Thiobarbituric acid reactive substances (TBARS) value of feed samples was determined by the extraction method described by Witte *et al*. (1970). Twenty gram of the sample was added with 50 ml of a chilled extracting solution containing 20 per cent trichloroacetic acid in 2M orthophosphoric acid and was homogenised for about 1.5 to 2 min. Volume of the resultant slurry was then made up to 100 ml with deionised distilled water and filtered through Whatman No.1 filter paper. Five millilitres of the filtrate, trichloroacetic acid (TCA) extract, was mixed with equal volume of 2- thiobarbituric acid solution (0.005 M in distilled water). Blank was constituted with five millilitres of distilled water and 2- thiobarbituric acid solution. The constituted samples along with blank in tightly closed test tubes were placed in a boiling water bath (100°C) for 30 min and cooled for 10 min under cold running water. The developed colour was measured as absorbance value at 530 nm (Systronics-119, UV-visible spectrophotometer, Ahmadabad, India) and expressed as thiobarbituric acid number (expressed as mg malonaldehyde per kg of feed).

Tyrosine value (TV) of the samples was estimated as per the method followed by Strange *et al.* (1977). Two-gram sample was weighed, placed in a beaker and 40 millilitres of five per cent TCA solution was added. After homogenisation for two minutes the sample was filtered and the filtrate collected. To 2.5 ml of TCA extract, equal quantity of distilled water was added in a test tube and shaken with 10ml of 0.5 N NaOH and three millilitres of diluted Folin and Ciocalteu's phenol (FC) reagent (one millilitre of concentrated FC reagent and two millilitres of distilled water). After mixing, the contents were allowed to stand for 15 min at room temperature. The optical density was measured at 660 nm in UV-Vis Spectrophotometer 119 (Systronics, India) using a blank containing 2.5 ml of five per cent TCA. An equal quantity of distilled water was added in a test tube and shaken with ten millilitres of 0.5 N NaOH and three ml of diluted FC reagent for comparison. Tyrosine value was analysed and expressed as mg/100g of feed.

For the estimation of total viable count (TVC), coliform count and yeast and mould count, control and treatment feed samples were prepared by taking five grams of sample aseptically and homogenised with 45 ml of sterile peptone water in a stomacher (Seward Stomacher 400 Circulator) to obtain an initial dilution of 10^{-1}. Serial ten-fold dilutions were made up to 10^{-4} in pre-sterilised tubes containing nine millilitres of peptone water. The microbiological analysis of processed samples was determined by the method described by American Public Health Association (APHA, 2015).

Total viable count of aerobic bacteria of each sample was estimated by using Standard Plate Count

Agar (HiMedia, Mumbai) and the plates were incubated at 37°C for 48 h and the count expressed as $\log_{10}$ CFU/g. Coliform count of each feed sample was estimated by using Violet red bile agar (HiMedia, Mumbai), the plates were incubated at 37°C for 24 h and the count expressed as $\log_{10}$ CFU/g. Yeast and mould count per gram of the sample was analysed using Potato Dextrose Agar (HiMedia, Mumbai). The plates were incubated at 25-27°C for three days and the count expressed as log10 CFU/g.

Statistical analysis

All the experiments were replicated six times. The data obtained from the experiments were assessed statistically by repeated measures ANOVA, one-way ANOVA and independent sample T test using the SPSS software version 26.0 version (Snedecor and Cochran, 1994).

Results and discussion

Proximate composition of poultry by-product meal (% DM basis)

The findings indicated that PBM exhibited a moisture content of 5.40±0.07, a crude protein content of 57.95±0.74, ether Extract of 25.20±0.22, total ash of 6.27±0.20, crude fibre of 2.25±0.07, nitrogen-free extract of 8.33±0.64 and acid-insoluble ash of 2.34±0.05 percent. The protein content of the PBM utilised for this study closely approximated the findings of Ravindran and Blair (1993), yet there was an elevated fat content and reduced ash content. These variations in the nutritional profile may be attributed to differences in processing techniques, nutrient levels and the quantities of constituents like bone, meat, blood, etc. (Nengas *et al*., 1999). Elevated fat levels might result from the use of raw materials with a high fat content, while reduced ash content could be attributed to lower bone content.

Moisture content

On storage, the moisture content of both C and T decreased significantly ($p<0.05$) throughout the storage period (Table 2). The moisture content of C decreased from 5.57±0.14 to 4.90±0.14 and that of T from 5.33±0.14 to 4.68±0.14. Despite variations in moisture content observed over different storage days, no significant disparity ($p>0.05$) in moisture content was found between the control and treatment feed throughout the storage period. The decreases in moisture content of the feed samples in the current study may be due to evaporation of moisture through the permeable HDPE film, this reduction may also be influenced by the escalating room temperature. Razak *et al*. (2018) reported that the temperature and relative humidity of the environment during transportation and storage can have an impact on the moisture content of food products made from local herbal raw materials.

Thiobarbituric Acid Reactive Substances (TBARS) value

The TBARS value of both C and T followed an increasing trend throughout storage period (Table 3). The TBARS value of C increased from 1.29±0.01 to 2.37±0.01 mg of malonaldehyde/kg and was not significant ($p<0.0$) between 28th and 35th day, and between 42nd and 45th day. Treatment feed demonstrated an increase in TBARS value from 1.30±0.01 to 2.60±0.01 mg of malonaldehyde/kg and was significant ($p<0.01$) throughout the storage period. Corresponding outcomes were identified by Karthik *et al*. (2010) in relation to pet food formulations with 10 and 20 percent spent hen meal. Treatment feed consistently displayed the highest TBARS value during storage period. The increased TBA concentration detected in T might be attributed to the inclusion of poultry by-products (PBM). Porter *et al*. (1995) emphasized that the degree of fat unsaturation has a noteworthy impact on the rate of lipid oxidation. According to Aydin *et al*. (2015), monounsaturated and polyunsaturated (n–6) fatty acids exhibited an uptick in diets containing PBM. Both of these explanations indicate that the presence of a high PBM content results in elevated lipid oxidation, thereby contributing to greater TBA values. The lack of a substantial rise of TBARS between the 42nd and 45th days in the current study could be due to the narrow assessment window of just 3 days in contrast to the preceding one-week timeframe.

Tyrosine Value (TV)

Tyrosine value of both C and T increased during storage period (Table 4). The value of C increased from 51.98±0.40 to 72.15±0.39 mg of tyrosine/100 g of feed and was significant ($p<0.01$) up to 42nd day. The TV of T increased from 47.04±0.40 to 69.75±0.39 mg of tyrosine/100 g of feed and was significant ($p<0.01$) up to 42nd day. The TV of T significantly differed ($p<0.01$) from control feed throughout the storage days. Control feed exhibited the highest TV throughout the storage period. Comparable outcomes were recorded by Pame *et al*. (2017) in their study, where the tyrosine content in pet kibbles incorporating slaughterhouse by-products rose from 37.59 to 104.92 mg tyrosine/100 g during a 60-day storage period. The highest TV of control feed may stem from the abundance of FM in control feed and its protein quality. The susceptibility of fish protein to deteriorative changes is quite high (Shahidi, 2004). Additionally, the lack of significant increase between 42nd and 45th day might be linked to shorter assessment interval of only 3 days compared to the previous one-week interval.

Microbiological examination

The TVC of both C and T increased throughout the storage period (Table 5). Initially, the microbial load of the C and T displayed significant difference ($p<0.01$) in TVC and were notably higher in T. The count for C

Table 2: Effect of storage period on moisture content of control and treatment feeds stored at ambient temperature

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 45	F value (p value)
C	5.47±0.14^{A}	5.40±0.15^{B}	5.32±0.14^{C}	5.25±0.15^{D}	5.18±0.14^{E}	5.08±0.14^{F}	4.98±0.14^{G}	4.90±0.14^{H}	27.554 (0.003)**
T	5.32±0.14^{A}	5.23±0.15^{B}	5.12±0.14^{C}	5.00±0.15^{D}	4.93±0.14^{E}	4.85±0.14^{F}	4.77±0.14^{G}	4.68±0.14^{H}	16.234 (0.009)**
t value (p value)	0.884 (0.369)^{ns}	0.380 (0.551)^{ns}	0.532 (0.483)^{ns}	0.824 (0.385)^{ns}	0.859 (0.376)^{ns}	0.777 (0.399)^{ns}	0.690 (0.426)^{ns}	0.688 (0.426)^{ns}	

Table 3: Effect of storage period on TBARS value of control and treatment feeds stored at ambient temperature

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 45	F value (p value)
C	1.29±0.01^{A}	1.52±0.01^{B}	1.76±0.01^{Cb}	1.98±0.04^{Db}	2.13± 0.01^{Eb}	2.15±0.01^{Eb}	2.36±0.01^{Fb}	2.37±0.01^{Fb}	1328.594 (<0.001)**
T	1.30±0.01^{A}	1.53±0.01^{B}	1.80±0.01^{Ca}	2.09±0.04^{Da}	2.18±0.01^{Ea}	2.38±0.01^{Fa}	2.54±0.01^{Ga}	2.60±0.01^{Ha}	2678.832 (<0.001)**
t value (p value)	0.324 (0.582)^{ns}	0.776 (0.399)^{ns}	5.216 (0.045)**	4.380 (0.063)*	23.777 (0.001)**	162.162 (<0.001)**	226.532 (<0.001)**	162.284 (<0.001)**	

** Significant at 0.01 level; * significant at 0.05 level; ns – non- significant at 0.05 level

Means with different uppercase superscripts in rows have significant difference at 0.05 level. Means with different lowercase superscripts in columns have significant difference at 0.05 level.

The values are expressed as their Mean ± Standard error.

Number of observations = 6

C – control (Fish feed as per FAO guidelines)

T - Fish feed by replacing FM from C with PBM

Table 4: Effect of storage period on TV of control and treatment feeds stored at ambient temperature

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 45	F value (p value)
C	51.98±0.29^{Aa}	58.67±0.44^{Ba}	62.20±0.63^{Ca}	64.87±0.21^{43Da}	66.92±0.43^{Ea}	69.02±0.67^{Fa}	71.25±0.37^{Ga}	72.15±0.34^{Ga}	326.343 (<0.001)**
T	47.04±0.29^{Ab}	55.79±0.44^{Bb}	58.23±0.63^{Cb}	59.90±0.21^{Db}	60.93±0.43^{Eb}	64.77±0.67^{Fb}	68.64±0.37^{Gb}	69.75±0.34^{Gb}	338.218 (<0.001)**
t value (p value)	143.470 (<0.001)**	21.266 (0.001)**	19.617 (0.001)**	295.516 (<0.001)**	98.867 (<0.001)**	20.085 (0.001)**	25.273 (0.001)**	24.762 (0.001)**	

Table 5: Effect of storage period on TVC of control and treatment feeds stored at ambient temperature

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 45	F value (p value)
C	1.20±0.01^{Ab}	1.36±0.03^{B}	1.53±0.02^{C}	1.63±0.02^{D}	1.66±0.00^{Db}	1.77±0.01^{Eb}	1.83±0.01^{Fb}	1.87±0.01^{G}	231.25 (<0.001)**
T	1.28±0.01^{Aa}	1.38±0.03^{B}	1.51±0.02^{C}	1.62±0.02^{D}	1.70±0.00^{Ea}	1.79±0.01^{Fa}	1.86±0.01^{Ga}	1.89±0.01^{H}	205.33 (<0.001)**
t value (p value)	13.152 (0.005)**	0.125 (0.731)^{ns}	0.876 (0.371)^{ns}	0.462 (0.512)^{ns}	45.000 (<0.001)**	6.102 (0.033)**	14.307 (0.004)**	2.469 (0.147)^{ns}	

** Significant at 0.01 level; ns – non- significant at 0.05 level

Means with different uppercase superscripts in rows have significant difference at 0.05 level. Means with different lowercase superscripts in columns have significant difference at 0.05 level.

The values are expressed as their Mean ± Standard error.

Number of observations = 6

C – control (Fish feed as per FAO guidelines)

T - Fish feed by replacing FM from C with PBM

increased from 1.20±0.02 to 1.87±0.01 log_{10} CFU/g and was not significant ($p<0.01$) between 21st and 28th day. The increase in TVC of T ranged from 1.28±0.02 to 1.89±0.01 log_{10} CFU/g and was significant ($p<0.01$) throughout the storage period. Karthik *et al*. (2010) observed a comparable pattern in pet food containing 10 and 20 per cent spent hen meal, with the total viable count escalating from 2.5 to 5.9 log cfu/g and 2.5 to 6.0 log cfu/g respectively during a 45-day storage period. The continuous growth in microbial count implies contamination during post-processing or handling, aligning with the conclusions of Fischer *et al*. (2007), who emphasised the importance of maintaining hygienic practices despite the challenges associated with the limited suitability of dry extruded pet food as a substrate for microbial development. On 45th day, T exhibited highest count but was not significantly different ($p>0.05$) from control. Jayathilakan *et al*. (2012) stated that meals containing poultry by-products are highly susceptible to microbiological and oxidative degradation. The insignificant increase noted between 42nd and 45th days, as reflected in TBARS and TV, could be attributed to the reduced assessment interval (only 3 days) as opposed to the followed one-week interval. Throughout the 45-day storage period, no observable coliform count or yeast and mould count was noted in either C or T.

Conclusion

Observations indicated a reduction in moisture content across both feed types, while TBARS value, TV and TVC exhibited an upward trend. No significant variance ($p<0.01$) in moisture content was observed between the PBM-based and FM-based feeds. The feed incorporating PBM exhibited a lower TV in comparison to the FM-based feed, though it had a marginally higher TBARS value and microbial count, likely due to the increased fat content of PBM. Nonetheless, there were no detectable signs of spoilage, such as changes in colour, odour or texture, affirming the viability of utilising PBM in fish feed production. The study concludes that PBM serves as an effective alternative to FM in fish feed formulations, demonstrating that such feeds can be stored for up to a 45-day period without compromise, assuming meticulous storage practices are maintained under aerobic conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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