

Microbial Screening and Nutrient Levels of Cottage Compounded Fish Feed Under Different Storage Conditions

Umma Samuel Bem^{*}, Oshimagye Michael Ibagye, Sadiq Hauwa Ohunene, Dauda, Abdullahi Kida

Department of Fisheries and Aquaculture, Federal University Wukari, Taraba, Nigeria

umma@fuwukari.edu.ng

Abstract: Fish feed is known to occupy a huge space as the single most expensive operating cost in aquaculture. Improved availability must be tilted towards door to door cottage production, proper handling and storage in order to fill the gap in the observed high price of feeds. Therefore, this study aimed at investigating some storage conditions for locally produced fish feed. A 40% CP pelleted diet was prepared and placed in uniformly transparent plastic bags in triplicates as Nylon-sealed and Refrigerated at 4°C (NR), Nylon-sealed and under room temperature at 25°C (NRT) and Nylon-Sealed and in the dark at 25°C (ND) for ninety (90) days. Data for nutrient levels and microbial loads at every 30 days were analyzed by subjecting to one way Analysis of Variance. Proximate composition decreased in crude protein from 24.31± 0.02% to 10.03±0.02%. Lipid levels slightly declined from 10.55±0.05% to 6.62±0.07%, Carbohydrate increased from 51.87±0.13% to 63.4±0.36%. Gram positive bacteria identified were staphylococcus, streptococcus and bacillus species within the ranges of 5.31±0.02 to 4.95±0.20 in MCA, 5.24±0.04 to 3.95±0.21 in NA, and 3.70±0.12 to 5.33±0.18 in MSA media respectively. The persisted fungus identified was *Aspergillus niger*, with loads increasing during the storage period in NRT and ND (4.0±0.01 and 4.2±0.01) respectively. This study provided information on the possible microorganisms that can persist and affect the quality of feeds and nutrients levels under different storage conditions. It also concluded with suggestions that proper handling and preparation are prerequisite for qualitative and longer shelf life of feed.

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Keywords: Cottage feed production, storage, nutrient levels, microbial screening

1.0: Introduction

The major solution to the shortage in animal protein in most parts of the world is by no doubt an increase in fish production (Nnaji *et al.*, 2009). Therefore, an urgent step is needed to continually explore aquaculture as a suitable option in sustaining and balancing the demand for this rich and one of the most sought after animal source of protein. Supplementary diets in the form of artificial feed must therefore be provided to complement natural food supply for aquaculture to be sustained and furnish fish with the required nutrients for optimal growth (Karapan, 2002; Umma *et al.*, 2018). Fish feeds has been reported by many researchers as the single most expensive component in aquaculture occupying over 40% of the operating cost (Nwanna, 2002; Partos, 2010; Akinwole and Faturoti, 2014). There is no gainsaying that high-priced and scarce availability of quality feed are major threat to aquaculture in Nigeria. Hence, without doubt, when the challenges of feed affordability, ready acceptability and bioavailability for cultured fish are met, the next important aspect is the proper handling and storage of feeds.

Prepared feeds for fish culture are perishable and in global terms, animal feeds and forages are routinely subjected to wide range of contaminants and toxins arising from anthropogenic and natural sources, including environmental pollution and activities of insects and microbes (FAO, 2004). The activity of insects, microorganisms and animals as well as improper handling plus physical and chemical changes due to variations in temperature and humidity pose serious problem of deterioration in stored fish feeds (Effiong and Fakunle, 2014). According to Zmyslowska (2000), storage conditions especially temperature and humidity are important factors affecting microbial quality of fish feeds. Losses occurring in feedstuff during storage fall under four major categories such as weight loss, quality loss, health risk and economic loss; these losses arise from the foraging activities of microorganism, insects and animals; improper handling; physical and chemical changes, all interwoven (ACF, 2014). High temperature and humidity also predispose fish feeds to microbial spoilage, this can cause feed to decompose and also lead to the mortality of fed fish (Solomon, *et*

al., 2016). The effect of microbial activity in stored feeds reduces nutritional value owing to the loss of dietary lipids, amino acids and vitamins by enzymes digestion (Jones, 1987 and Lim *et al.*, 2008).

The nutrient composition of feed influence feed utilization and ultimately the growth of fish (Adaga, 2014). Feed storage at high temperature results in an increase in both oxidative and hydrolytic rancidity with loss in feed quality (Ramezandeh, *et al.*, 1999). However, storage losses are primarily due to material eaten or destroyed by insects, animal pests and fungi (Aulakh *et al.*, 2013).

Destruction of feedstuffs through recontamination by adventitious microorganism during storage is a major factor promoting poor output by feed processor; these microorganisms thrive at moisture content (15% to 20%) in equilibrium with a relative humidity of 70 to 90% and are singled out as the principal spoilers of feedstuffs in storage (Chow 1980; Effiong and Alatisé, 2009). Poor storage conditions of feeds will enhance microbial activity (Effiong and Fakunle, 2014). The occurrence of these microbial strains in fish feeds have been reported to depend on the storage condition of the feed, particularly temperature (Nwabueze and Nwabueze, 2011). When this occurs, secondary spoilage by bacteria and fungi takes place. Hence this study investigates the levels of nutrients decline and the microbial loads in pelleted feed under different storage conditions for the period of 90 days.

2.0: Materials And Methods

2.1: Feed preparation

Processed feed ingredients: Fish meal 72% CP, Soya bean meal 48% CP, Maize 10% CP, Vitamin and Mineral premixes (Table 1) were purchased from a reputable Agriculture store in Ibadan, weighed and mixed thoroughly to homogenize the ingredients into a dough to produce 40% CP pelleted diet. The pelleted diet was sun dried and packaged into plastic containers.

2.2: Experimental set-up

Feeds placed in uniform transparent plastic bags in triplicate were subjected to the following conditions for ninety (90) days: Nylon-sealed and Refrigerated at 4°C (NR), Nylon-sealed and kept under room temperature at 25°C (NRT), and Nylon-Sealed and

kept in the dark at 25°C (ND). Initial proximate and microbial analyses of the pelleted feed were performed before subjecting the diet into the different conditions. The nutrient composition and microbial loads of the various conditions were analyzed every 30 days under storage for the period of 90 days.

2.3: Proximate analysis of feed samples

Proximate composition of ingredients in the feed was determined according to the methods of Lakin (1978); Midkiff, (1984); AOAC, (1995) and Petterson *et al.*, (1999). Carbohydrate (CHO) was determined by calculating from the remaining sample.

2.4: Microbiological Analysis of feed samples

Peptone water 0.1% was used for serial dilution of homogenate feed samples and subjected to microbial test through asepsis. 1g of each sample was grinded in 9ml sterile peptone water in the mortar and serially diluted (10^{-3}). Each diluent (1ml) was poured in two petri dishes; one received plate count agar for total bacteria count using the pure plate count method according to the standard methods for the examination of water and waste water (APHA, 1985). The second petri dish received MacConkey agar for total coliform count and was incubated for 24 hours at 37°C as described by Hitchins *et al.*, (1995). Total viable count and Enterobacteriaceae was determined and expressed in Log_{10} CFU/ml.

2.5: Biochemical Analysis of feed samples

Oxidase Test, Sugar (glucose) broth with Durham tubes, Methyl Red/Voges-Proskauer (MR/VP), Kliger's Iron Agar (KIA), Nitrate Broth, Motility Agar, MacConkey agar, Simmon's Citrate Agar, Urease test, Sulfur Indole Motility Media (SIM) procedure was carried out according to the methods described by Buchanan and Gibbons, (1974), Cowan, (1974) and Cruickshank *et al.*, (1975).

2.6: Statistical Analysis

Data obtained were subjected to one-way Analysis of Variance (ANOVA) and means separated by Duncan's Multiple Range Test (MRT).

3.0 Results

The results from stored feeds indicates that feeds stored under low temperature (NR) were least in mold count, slightly followed by feed stored under room temperature (NRT) and then feed stored at room temperature in the dark condition (ND).

Table 2: Biochemical characteristics of isolated bacteria load

| Study periods | Plates (10 ⁻³) | Colonies counted | Gram staining reaction (All +ve) | Indo | Cat. | Coag | Oxid | Gluc. | Lac. | Suc. | Cit. | Cultural Characteristics and Tentative. Organisms | |
|----------------------|----------------------------|---------------------|----------------------------------|------|------|------|------|-------|------|------|------|---|---|
| Initial | MCA | 204 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| | NA | 172 | +ve | + | + | + | + | + | + | + | + | Rod shape Bacillus chain | |
| | MSA | 5 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| | NR condition | | | | | | | | | | | | |
| | MCA | 24 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| | NA | 6 | +ve | + | - | + | + | + | + | + | + | Cocci chain (streptococcus) | |
| After 30 Days | MSA | 17 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| | NRT condition | | | | | | | | | | | | |
| | MCA | 136 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| | NA | 0 | - | - | - | - | - | - | - | - | - | - | |
| | MSA | 415 | +ve | + | - | + | + | + | + | + | + | Cocci chains (streptococcus) | |
| | ND condition | | | | | | | | | | | | |
| | MCA | 136 | +ve | + | - | + | + | + | + | + | + | Cocci chains (streptococcus) | |
| | NA | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| | MSA | 24 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| | NR condition | | | | | | | | | | | | |
| | MCA | 36 | +ve | + | - | + | + | + | + | + | + | Cocci chains (streptococcus) | |
| | NA | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| | MSA | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| | NSRT condition | | | | | | | | | | | | |
| | MCA | 0 | -ve | - | - | - | - | - | - | - | - | - | - |
| | NA | 0 | -ve | - | - | - | - | - | - | - | - | - | - |
| | MSA | 0 | -ve | - | - | - | - | - | - | - | - | - | - |
| | After 60 Days | ND condition | | | | | | | | | | | |
| MCA | | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| NA | | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| MSA | | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| NR condition | | | | | | | | | | | | | |
| MCA | | 30 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| NA | | 9 | +ve | + | - | + | + | + | + | + | + | Cocci chains (streptococcus) | |
| MSA | | 9 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |
| NRT condition | | | | | | | | | | | | | |
| MCA | | 101 | +ve | + | + | + | + | + | + | + | + | Rod shape Bacillus | |
| NA | | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| MSA | | 85 | +ve | + | + | + | + | + | + | + | + | Rod shape Bacillus | |
| After 90 Days | ND condition | | | | | | | | | | | | |
| | MCA | 90 | +ve | + | - | + | + | + | + | + | + | Cocci chains (streptococcus) | |
| | NA | 0 | -ve | - | - | - | - | - | - | - | - | - | |
| | MSA | 215 | +ve | + | + | + | + | + | + | + | + | Cocci clusters (staphylococcus) | |

Legend: MCA; Macconkey Agar; NA: Nutrient Agar, MSA; Mannitol Salt Agar

Table 3: Cultural Characteristics of Isolated Fungi

| Parameters | Initial reading | Number of isolates (10 ³ cfu/g) | | | Cultural Characteristics (Potato Dextrose Agar) | Tentative Organisms |
|------------|-----------------|--|-----------------------|-----------------------|--|--------------------------|
| | | 30 days | 60 days | 90 days | | |
| NR | 2.2±0.01 | 2.1±0.02 ^a | 2.3±0.03 ^b | 2.3±0.02 ^b | Whitish to pale yellow, black, with conidial production. | <i>Aspergillus niger</i> |
| NRT | 2.2±0.01 | 2.2±0.03 ^a | 3.8±0.03 ^b | 4.0±0.01 ^c | Whitish to pale yellow, rapidly changing black, with conidial production | <i>Aspergillus niger</i> |
| ND | 2.2±0.01 | 2.2±0.02 ^a | 3.6±0.02 ^b | 4.2±0.01 ^c | Whitish to pale yellow, rapidly changing black, with conidial production | <i>Aspergillus niger</i> |

Means with the same superscript along rows for each duration are not significantly different

Table 4: Proximate composition of compounded feed

| Parameter (%) | Initial value | After 30 days | | | After 60 days | | | After 90 days | | |
|---------------|---------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | | NR | NRT | ND | NR | NRT | ND | NR | NRT | ND |
| C. protein | 24.31±0.02 | 20.92±0.04 ^d | 12.27±0.13 ^b | 10.87±0.02 ^a | 15.56±0.02 ^b | 16.09±0.02 ^c | 10.85±0.03 ^a | 11.11±0.02 ^b | 13.84±0.03 ^c | 10.03±0.02 ^a |
| Lipid | 10.55±0.05 | 9.08±5.75 ^a | 12.52±0.03 ^a | 12.00±0.01 ^a | 10.01±0.01 ^b | 10.92±0.12 ^c | 8.06±0.16 ^a | 10.67±0.15 ^c | 9.42±0.02 ^b | 6.62±0.07 ^a |
| Fibre | 4.45±0.12 | 2.53±0.02 ^a | 2.75±0.06 ^b | 2.98±0.03 ^c | 3.37±0.07 ^c | 2.53±0.15 ^a | 3.35±0.02 ^b | 3.60±0.03 ^c | 3.12±0.12 ^b | 3.01±0.01 ^a |
| Ash | 3.05±0.20 | 4.23±0.20 ^b | 3.95±0.07 ^{ab} | 3.53±0.44 ^a | 3.68±0.01 ^b | 3.87±0.12 ^c | 3.66±0.03 ^a | 3.54±0.04 ^a | 3.73±0.03 ^c | 3.59±0.06 ^b |
| Moisture | 8.77±0.11 | 12.12±0.12 ^b | 12.15±0.07 ^b | 12.32±0.03 ^a | 13.34±0.03 ^c | 12.95±0.03 ^b | 11.53±0.05 ^a | 14.04±0.04 ^c | 12.08±0.03 ^a | 12.10±0.03 ^b |
| CHO | 51.87±0.13 | 47.86±0.10 ^a | 56.52±0.05 ^c | 62.39±0.50 ^d | 54.06±0.04 ^b | 53.64±0.04 ^a | 62.58±0.03 ^c | 57.00±0.20 ^a | 57.86±0.08 ^b | 63.49±0.36 ^c |

Means with the same superscript along rows for each duration are not significantly different

The crude protein (CP) among the diets showed significant difference $P<0.05$, and the quality of the CP in the diets under different storage conditions degraded with time.

After 30 days of storage, feed in Nylon-sealed and Refrigerated (NR) had the highest CP value (20.92±0.04%). There was significant difference

($P<0.05$) in the CHO values as it increased sequentially after 30 days in storage with NSR having the least CHO value (47.86±0.10%) and ND recording the highest CHO (62.39±0.50%). There was no significant difference ($P<0.05$) in the lipid, ash and moisture content across the storage conditions (Table 4).

After 60 days, the CP in the various stored feeds were significantly different $P < 0.05$ with highest values in NR ($15.56 \pm 0.02\%$) and the least values in ND ($10.85 \pm 0.03\%$). There was significant differences ($P < 0.05$) across all the storage condition for lipid, fibre, ash, moisture content and carbohydrate with feeds in ND having the least values except in CHO where it had the highest value ($62.58 \pm 0.03\%$). At the end of the study period, feed in ND had the least values for CP, lipid and fibre which were significantly different from feeds in other storage conditions. Feed in NRT had the highest CP value ($13.84 \pm 0.03\%$) and the least moisture content ($12.08 \pm 0.03\%$) while feed in NR had the highest moisture content ($14.04 \pm 0.04\%$) and the least value for ash ($3.54 \pm 0.04\%$). Carbohydrate was highest in feed of ND ($63.49 \pm 0.36\%$).

The high moisture content in NR may be as a result of condensation of water in the refrigerator. Feed in ND had the highest CHO during the study period and this may be due to enzymatic breakdown of starch in the ingredients to sugar in the presence of no light.

The microbial profile showed significant difference at $P < 0.05$ with the feed in NR (4.38 ± 0.11) against other storage conditions NSRT (5.13 ± 0.03) and ND (5.20 ± 0.10) which had no significant difference among themselves under MSA media. Feeds in NSR reflected the value 3.78 ± 0.26 under NA media and no values recorded in NSRT and ND. However, in MSA media all the storage conditions recorded the presence of microbial load, while the storage condition NR was significantly different from the values of the feeds in NSRT and ND which were not significantly different $P > 0.05$ after 30 days in storage, Table 5. After a period of 60 days under storage, there was hardly any microbial load recorded in the feeds in the various media with the exception of NSR (4.56 ± 0.21) in MSA media. While at 90th day the presence of microbial loads were noticed with NSR and NSRT significantly different, while microbial loads unnoticed in ND at MCA media, in NA media microbial loads were only noticed in NR, however, microbial loads were seen to be significantly different among NSR and NRT, while ND unnoticed, Table 5.

Table 5: Microbial profile of compounded feed in storage (10^3 cfu/g)

| Parameters | Initial Value | After 30 Days | | | After 60 Days | | | After 90 Days | | |
|------------|-----------------|-------------------|----------------------|-------------------|-------------------|-----|----|-------------------|-------------------|----|
| | | NR | NRT | ND | NR | NRT | ND | NR | NRT | ND |
| MCA | 5.31 ± 0.02 | 4.38 ± 0.11^a | 5.13 ± 0.03^b | 5.20 ± 0.10^b | - | - | - | 4.48 ± 0.15^a | 4.95 ± 0.20^b | - |
| NA | 5.24 ± 0.04 | 3.78 ± 0.26^a | - | - | - | - | - | 3.95 ± 0.21^a | - | - |
| MSA | 3.70 ± 0.12 | 4.23 ± 0.09^a | 4.38 ± 0.05^{ab} | 5.30 ± 0.07^b | 4.56 ± 0.21^a | - | - | 3.95 ± 0.21^a | 5.33 ± 0.18^b | - |

Mean on the same column within the same periods with different superscripts are significantly different

4.0: Discussion

Destruction of pelleted feeds through poor handling, exposure to unhealthy environmental conditions and recontamination by adventitious microorganisms during storage is a major factor that if tackled is crucial to the success of fish farming operations (Cruz, 1996), the above research reported a generally no change in colour and appearance of the stored feed with a moderately bad odour, a little bit of soft texture and broken pellets with some little variations among the different stored feeds with the following attributes; a decrease in the crude protein (CP) values in the pelleted feed over time in all the storage conditions, this is as reported by Odunigba (2015), where there were a decrease in the composition of the crude protein, carbohydrate, ash and crude fibre content in stored fish feed and an increase in crude fat and moisture content of the feed. However, the decline in nutrient composition of the feed was reported least in feeds stored in the refrigerator (NR), this can be attributed to the low temperatures playing a role in slowing down the activities of microorganisms as reported by Chow (1980), where high temperatures is seen as one of the

most important factors promoting rancidity in processed feeds, leading to microbial contamination, secondary diseases and mortality.

Lipid in diet is very important as it serves as source of energy, medium for fat soluble vitamins, and partake in some structural and functional role in the fish body (Tocher, 2003), the lipid levels in the results decreased with time under the different storage conditions. Lipids often react under unfavourable conditions, becoming unhealthy compounds known as free radicals that are rather harmful to the fish body (Kubow, 1992). Therefore, where lipids content of feed decreased under storage, it suggests that there are some unhealthy or toxic compounds that are deposited in the feed through chemical reactions and some form of rancidity. Decreased level of lipids was noticed more in feed kept sealed and under room temperature in the dark (ND) leading to the conclusion that, this storage condition is not ideal for fish feed storage. Feeds that are in contact with mold and insects like grain beetles, lesser grain borer, clover weevil, are most likely to be contaminated or faced with recontamination under storage (Kemin-Europa, 2009).

The pelleted feeds investigated under different storage conditions were seen to be infested at various times by different microbial loads, since pelleting may only reduce but not eliminate contamination completely as reported by Jones, (2011). Therefore, there was a trend in the survival of microbial loads in the stored feed, at 60 days under storage; bacteria load where not seen in the stored feed, with the exception of NR and at 90 days bacteria loads resurfaced. This could be as a result of their life cycle where bacteria life span may have ended and their spores re-hatched under favourable conditions leading to recontamination of feeds (Lewandowska, 1999). The nutrient levels during storage period where reported to be generally declining with increase in microbial loads as the storage period persisted. Generally the microbial loads among the different stored feeds were at the ranges of 1.2 to 3.6 (10^3 cfu/g). This was moderately within the same range with the results of Nwabueze and Nwabueze (2011), where it was reported that Durante and Dizengolf feeds after storage had 3.33 and 1.33 (10^3 cfu/g) levels of *S. aureus* respectively. The levels of microbes based on CFS (2014) microbiological guideline for food, colony forming units (cfu/g) ranges of $10^2 \leq 10^4$ is considered to be at the borderline and not safe for consumption especially when at the extreme values, therefore the results of the research reports various values within the borderline and care must be taken in the decision to feed fish with feeds having microbial loads within this ranges.

Observed temperature of stored feed where at 4 to 5°C for the feed under refrigerated condition and between 26-30°C for feeds at room temperature, however according to Zmyslowska and Lewandowska (1999), standard storage conditions for feed is considered at 5 to 20°C, in addition to well and adequate ventilation (FAO, 1987). This may have been the reason for the variations in the results showing unfavorable storage conditions for NRT and ND.

Conclusion

This study clearly indicates that feed stored at 4°C happened to be in almost same state as the initial state before storage unlike the other storage conditions. Therefore, it is suggested that proper handling and preparation are prerequisite for qualitative and longer shelf life of fish feed.

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Corresponding Authors:

Umma, Samuel Bem: Federal University Wukari, Taraba State, Nigeria

Email address: umma@fuwukari.edu.ng

Phone number: +234: 08076334396

Co- Authors:

1. Sadiq Hauwa Ohunene, Federal University Wukari, Taraba State, Nigeria

2. Oshimagye Ibagye Michael, Federal University Wukari, Taraba State, Nigeria

3. Dauda Abdullahi Kida, Federal University Wukari, Taraba State, Nigeria

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References

1. Action Contre La Faim (ACF), Technical Paper on Post-Harvest Losses and Strategies to Reduce them. 2014
https://www.actioncontrelafaim.org/wpcontent/uploads/.../technical_paper_phl_.pdf.
2. Adaga K. Nutrient profile of some commercial feeds under different storage conditions and their effect on growth performance of *Clarias gariepinus*. Unpublished MSc. thesis Department of Fisheries and Aquaculture, University of Agriculture Makurdi Nigeria. 2014, pp125.
3. Akinwale, A. O. and Faturoti, E. O., Cost Characteristics of the African Catfish Culture in Recirculating Production Facilities in Ibadan, Oyo State, Nigeria. *International Journal of Applied Agricultural and Apicultural Research* 2014. 10 (1 & 2):188-197.
4. AOAC Animal feed. Chapter 4. In official methods of analysis; 16th edition. AOACC International, Arlinton, VI, USA. 1995. 30pp.
5. Aulakh J., Regmi A., Fulton J.R., Alexander C. Estimating post-harvest food losses: Developing a consistent global estimation framework; *Proceedings of the Agricultural & Applied Economics Association's 2013 AAEA & CAES Joint Annual Meeting; Washington, DC, USA. 4-6 August 2013*.
6. Chow, K.W. Storage problems of feedstuffs 1980. (215-224). In: *Fish Feed Technology. ADCP/REP/80/11. UNDP/FAO, Rome*.
7. Cruz, P.S. Feed quality problems and management strategies. In: C.B. Santiago, R.M.

- Colso, O.M. Millamena and I.G. Borloutan (Eds.). Feeds for small aquaculture. *Proceedings of the national seminar- workshop on fish nutrition and feeds*. 1996. pp. 64-73. Tigbauan, Ilorilo, Philippines, SEAFDEC Aquaculture Department.
8. Effiong, B.N and Fakunle J.O. Effect of storage and anti-nutritional components in stored pelleted fish feed. *International Journal of Science, Technology and Society*. 2014. 2 (6): 186-189. Doi: 10.11648/j.ijsts.20140206.14.
 9. Effiong, B.N. And Alatise, S. P. Effect of Mold Infested Feeds on the Growth And Survival of *Heterobranchus longifilis* Fingerlings, *Report and Opinion*. 2009. 1 (3): 9 -14.
 10. FAO How should I store my feeds? (Chapter 7). In: Feeds and feeding of fish and shrimp. 1987. *FAO corporate document repository. Fisheries and Aquaculture Department, Rome, Italy*.
 11. FAO, Contaminants and Toxins in Animal Feeds, *Rome (Italy) Physical Description*. 2004. 92-5-105046-5.
 12. Jones F.T.): A review of practical salmonella control measures in animal feed, *The Journal of Applied Poultry Research*, 2011. 20 (1): 102-113.
 13. Karapan A. Studies to optimize poly unsaturated fatty acid composition of tilapia for human consumption in S.E. Asia. *Aquaculture News*, 2002.. 28, 6-7.
 14. Kemin-Europa N.V. Grain storage and spoilage prevention and control, 2009. *Animal feed*.
 15. Kubow S., Routes of formation and toxic consequences of lipids oxidation products in food. *Tree Radicals Biology and Medicine*. 1992. 12 (1): 63-81.
 16. Lakin, A.L. Determination of nitrogen and estimation of protein in foods. In R.D. King, ed. *Developments in food analysis techniques*. 1978.. pp. 43-74. *Applied science publishers, London*.
 17. Midkiff, V. A century of analytical excellence. The history of feed analysis, as chronicle in the development of AOAC official methods, 1884-184. *Journal of the association of official analytical chemists*, 1984. 67, 851-860.
 18. Nwabueze, A.A and R.O. Nwabueze. Microbial flora of fish feeds sold in Asaba, Southern Nigeria. *American journal of Experimental Agriculture*. 2011. 1 (2): 27-32.
 19. Odunigba, S. O., Aflotoxins associated with storage fungi in fish feed, *Ife journal of science*, 2015. 17 (2): 519-523.
 20. Olukunle, O. Home Stead Pond Management, *John Archers Limited Ibadan*. 2004. Pp 40.
 21. Partos, L. FAO, Fish feed costs to remain high. 2010. www.seafoodsource.com/news/aquaculture/fao-fish-feed-costs-to-remain-high accessed 03/03/2017
 22. Petterson, D.S., Harris, D.J; Rayner, C.J., Blakeney A. B. and Choct, M. Methods for the analysis of premium livestock grains. *Australian journal of aquacultural research*. 1999. 50 (5): 775-787.
 23. Solomon S.G., Tihamiyu L.O., Okomoda V.T., Adaga K. Effects of storage conditions on quality characteristics of commercial aquafeeds and growth of African catfish *Clarias gariepinus*. *Journal of Fisheries*, 2016. 74, (1): 30-37. DOI: 10.1515/cjf-2016-0006.
 24. Tocher, D.R Metabolism and functions of lipids and fatty acids in teleost fish, *Reviews in Fisheries Science*. 2003. 11 (2): 107-184.
 25. Umma S.B, Adeyemi-Doro,O., Oshimagye M.I., Igbani F, Uruku, N. M, Dauda, A.K. Assesmmnt of the Optimal Inclusion Level of Dietary Zinc Requirement For Catfish *Clarias Gariepinus* Production. *International Journal of Agricultural Sciences and Natural Resources* 2018. 5 (1): 6-11.
 26. Zmyslowska D, L, Survival of bacterial strains in fish feeds stored at different temperatures. *Polish journal of environmental studies*, 2000. 8 (6): 447-451.
 27. Zmyslowska, I., Lewandowska, D. Survival of bacterial strains in fish stored at different temperatures. *J. Env. Stud.*, 1999. 8 (6): 447- 44.

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