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Application of Fermentation Technology to Use Slaughterhouse Blood as Potential Protein Supplement in Fish Feed

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Abstract

Slaughterhouse Blood (SHB) is a rich source of protein, but it requires a suitable processing before its use as an ingredient in animal feed formulations. In this study, SHB was fermented using whey or pure culture of *Lactobacillus acidophilus* as the fermentation inoculums. Mixtures of SHB, fermentation inoculums and molasses were incubated at 35°C and microbiological and biochemical changes were determined every day until the end of fermentation. The results revealed that the fermentation of SHB by both the inoculums required 4 days to complete. The harmful microorganisms were removed or reduced to a negligible level after fermentation. Both the fermented products were also aerobically stable. Protein, lipid and essential amino acid contents of SHB were almost completely recovered after fermentation. The *L. acidophilus* fermented SHB (LFSHB) exhibited a better microbiological quality than the whey fermented SHB (WFSHB), while WFSHB exhibited a higher amount of free amino acid than the LFSHB. The present study concludes that fermentation is an appropriate technique to convert SHB into a nutritionally sound and microbiologically safe ingredient for the use in fish feed formulation. Whey can be used as an easily available and cost effective inoculum to carry out the fermentation.

Keywords: Organic Wastes, Fermentation, Lactic Acid Bacteria, Feed, Crude Protein, Amino Acids

1. Introduction

Disposal and management of organic wastes generated from slaughterhouses are tough jobs because of the huge volume in which these wastes are generated everyday in solid and liquid forms (Bergleiter, 2011). In India, there are 2,702 registered slaughterhouses which generate about 21 lakh tones of wastes per annum (Jayathilakan *et al*., 2012). The solid slaughterhouse wastes consist mainly of inedible parts of animal while the liquids comprise blood, urine, water, dissolved solids and gut contents. Fearon *et al*. (2014) observed that about 0.7 ton blood was produced from slaughtering of 125 livestock comprising 55 cattle, 50 sheep and 20 goats. In India, cattle, sheep and goat slaughtering is the major sources of slaughterhouse blood (SHB). Most of the SHB generated from these slaughterhouses is discarded as wastes and disposed into the environment in an unorganized manner. Otherwise, these wastes can be used as protein supplement in the formulation of feed for fish. However, nutrient compositions, recovery of the nutrients and microbiological status of SHB generated in India are poorly documented. The idea of recycling these wastes through agriculture and animal husbandry is gaining importance because of multiple benefits like reduction of environmental pollution arising out of disposal of the wastes, prevention of loss of huge nutrients contained in these wastes and curtailing in production cost in agriculture and animal husbandry. Since the past decade, many studies have actively been engaged in developing suitable techniques to recycle animal wastes in the formulation of feed for fish because of an urge to find suitable cost effective alternative of fishmeal, the most dependable but a costly source of protein in aquaculture feed (De Silva and Anderson, 2009; Zhu *et al*., 2011).

Slaughterhouse blood (SHB) is a rich source of protein. However, SHB contains a high amount of moisture and huge load of unwanted microbes, the two most important factors responsible for the breakdown of protein and the generation of unpleasant odors (Jayathilakan *et al*., 2012). Therefore, processing of SHB is essential before its use as an ingredient in aquaculture feed formulation. In spite of supplying a high quality protein in the feed, inclusion of unprocessed SHB exhibited lower digestibility in some fish (Zhu *et al*., 2011), while processed SHB or Blood Meal (BM) was always considered as a quality protein source for the use in aquaculture feed industry (Agbedi *et al*., 2009). Therefore, a suitable technique to produce a cost effective and a nutritionally improved SHB to be used as an ingredient in aquaculture feed is in demand. Spray dried or oven dried blood meals are frequently used in the formulation of feed for fish (Ribeiro *et al*., 2011;

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Aladetohun and Sogbesan, 2013). However, heat treatment often results in the loss of nutrients of the ingredients.

The success of converting organic wastes into feedstuff depends on the treatment used to process the wastes. Fermentation has been used as a reliable biotechnological tool in processing nutrient rich organic wastes to reduce the inherent problems contained in animal wastes and to make them nutritionally sound feedstuff (Kader *et al.,* 2012). Lactic Acid Bacteria (LAB) serve as the main players in the fermentation and preservation of such wastes (Pyar *et al.,* 2011). Improvisation of a fermentation technique to produce a cost effective, microbiologically safe and nutritionally sound fermented product suitable for use as feed ingredient in the formulation of feed has been given importance in recent years (Samaddar and Kaviraj, 2014). The objective of the present study is to develop an eco-friendly and efficient fermentation technique acceptable to aquaculturists to process SHB and to use it as a low cost protein supplement in the formulation of feed for fish without compromising nutrient quality of the feed.

2. Materials and Methods

2.1. Collection of Sample

Fresh Slaughterhouse Blood (SHB) was collected from a few selected slaughterhouses immediately after slaughtering sheep and goats. The blood was allowed to clot and only the clotted portion of the blood was collected and brought to the laboratory in an ice bucket for fermentation. Two inoculums were used for the fermentation of the SHB: One is a pure culture of *Lactobacillus acidophilus*,a representative microorganism of Lactic Acid Bacteria (LAB) and the other is whey, a byproduct of milk industry and a cheap source of LAB. Samaddar and Kaviraj (2014) discussed in detail the potential of whey and pure culture of *L. acidophilus* as fermentation inoculums and their procurement. Cane molasses was used, as discussed previously (Samaddar and Kaviraj, 2014), as a source of fermentable carbohydrate for microorganisms involved in the fermentation and was collected from the local market.

2.2. Fermentation Process

Fermentation of SHB was carried out in 1 L Erlenmeyer flasks, each containing 400 g of fermentation mixture. The mixture contained 80% of SHB, 15% of cane molasses and 5% of fermentation inoculum containing either whey or actively growing culture [109 colony forming units (CFU)/ml] of *Lactobacillus acidophilus* (NCIM 2903) procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. The pre-fermented mixture was subjected to biochemical and microbiological analyses before the incubation was started. All analyses were made following standard analytical methods described below. The fermentation mixture (mean moisture level = 72 %) was then incubated at 35°C. Seven replicates were maintained for each of the fermentation groups. Every day one replicate from each group was opened and pH, Water Soluble Carbohydrates (WSC) and quantity of Lactic Acid (LA) and Acetic Acid (AA) produced in the fermentation mixture were determined. Determinations were continued till the mixture attained a pH value in between 4.0 and 4.2, after which the fermentation was discontinued. The entire experiment was repeated thrice. The fermented products were subjected to microbiological and biochemical analyses. Detailed analytical methods are described below.

2.3. Microbiological Analyses

Determinations of Standard Plate Count (SPC), population of LAB, yeast and hazardous microorganisms such as total and faecal coliform, *Staphylococcus* and *Clostridium* were made in the pre-fermented mixture and in the fermented products, Whey-Fermented Slaughterhouse Blood (WFSHB) and *L. acidophilus*-Fermented Slaughterhouse Blood (LFSHB). Ten g of each sample was blended with 90 ml of saline water (NaCl, 0.85 % w/v), diluted serially (10-folds), plated with 1 ml of the suspension in appropriate media using pour plating technique (Cappuccino and Sherman, 2007) and incubated at the appropriate temperature. The colonies that appeared on the selected plates after incubation were enumerated as CFU/g or ml of sample. Samples were incubated in Plate Count Agar (Standard Methods Aagar) (HiMedia, Mumbai, India) at 30° C for 2–3 days for enumeration of SPC, in Potato Dextrose Agar (PDA) (HiMedia, Mumbai, India) acidified by LA solution to pH 3.5 at 30°C for 72 h for enumeration of yeast, in *Lactobacillus* MRS agar (HiMedia, Mumbai, India) at 30°C for 48 h for enumeration of LAB, in MacConkey broth (HiMedia, Mumbai, India) supplemented with agar, at 37°C for 24 h for enumeration of total coliform bacteria and again in the same media at 44°C for 24 h for determination of faecal coliform bacteria. For enumeration of *Staphylococcus*, the samples were incubated in Mannitol salt agar (HiMedia, Mumbai, india) at 37°C for 24 h. Yellow colonies were enumerated and checked by Gram stain and catalase reactions. For enumeration of *Clostridium* colonies, the samples were heated at 80°C for 10 min, immediately followed by cooling on ice water. The cooled samples were serially diluted, plated and incubated at 44°C for 24 h in Reinforced clostridial agar (HiMedia, Mumbai, India).

Aerobic stability against the growth of moulds and yeast on the pre-fermented and fermented products was checked by a short-term experiment. Ten grams of dry sample were taken in a plate of 90 mm diameter, spread in a very thin layer and kept in open air or covered by a lid in the laboratory. Two control sets, one containing Potato Dextrose Agar (PDA) and another containing PDA acidified by LA to pH 3.5 (acidified PDA) in similar plates, were also maintained. The temperature and humidity were recorded during the experiments. Samples of the prefermented and fermented products and the control media were taken out from the respective plates after 1 h and 5 days of incubation, diluted serially (1:10) in saline water (NaCl, 0.85 % w/v), plated following spread plating technique and incubated for 3 days at 30°C separately in two media: One containing PDA and another containing acidified PDA to grow a mixture of mould and

yeast and only yeast respectively. CFU were enumerated in the plates after 3 days.

2.4. Biochemical Analyses

The samples were diluted with water (1:10) for determination of pH in a direct reading digital pH meter. Water-Soluble Carbohydrate (WSC) was determined by modified spectrophotometric method described by Murphy *et al.* (2007). For the determination of Lactic Acid (LA) and Acetic Acid (AA), samples were diluted with water (1:10), homogenized and filtered through Whatman-2 filter paper. The filtrate was centrifuged at 14,400 g for 15 min and the supernatant was further filtered through 0.45 µm membrane filter. LA in the filtrate was determined by the method of McHugh *et al*. (2006) in an HPLC system (Waters) using a 4.6 9 250 mm, 5 µm, column (ZORBAX Rx-C18; Agilent Technologies), a Waters 2996 photodiode array UV detector programmed at 210 nm and a mixture of acetonitrile:buffer (3:97) as the mobile phase with a flow rate of 1.2 ml/min. The buffer used was 25 mM KH_2PO_4 (adjusted to pH 2.5 by H_3PO_4). AA in the filtrate was determined in the same HPLC system, but 50 mM KH_2PO_4 (adjusted to pH 2.5 by H_3PO_4) buffer alone was used as the mobile phase with a flow rate of 1 ml/min (Dremetsika et al., 2005). Retention time of the standard LA and AA was found at 2.2 and 2.9 min., respectively. The quantity of both LA and AA in the samples was determined using Empower software associated with the HPLC system. Proximate compositions were determined by AOAC methods (Helrich, 1990). Samples were oven dried at 55–60° C for 18–20 h before analysis. Moisture was determined by oven drying at 105° C for 24 h; crude protein (nitrogen × 6.25) was determined by Kjeldahl method after acid hydrolysis; lipid was extracted by petroleum ether (boiling point 40–60° C) for 7–8 h in a Soxhlet apparatus followed by determination of lipid gravimetrically; crude fiber was determined as loss on ignition of dried lipid-free residues after digestion with 1.25 % H_2SO_4 and 1.25 % NaOH and ash was determined by combustion at 550° C in a muffle furnace till a constant weight was achieved. Nitrogen-free extract (NFE) was calculated by taking the sum of values for crude protein, crude fiber, crude lipid, moisture and ash and subtracting this from 100. Essential and non-essential amino acids were determined in the pre-fermented mixture and in fermented products by the method of Kwanyuen and Burton (2010) in a HPLC system (Waters, USA) equipped with UV detector programmed at 254 nm. The analysis was performed by using similar column and software as discussed above. Analytical grade amino acid standard (AAS18, Fluka) was purchased from Sigma-Aldrich (St. Louis, USA). During acid hydrolysis tryptophan was partially destroyed. Therefore, the level of tryptophan was not determined in the samples. Free amino acid was determined by the method of Moore and Stein (1948) using ninhydrin reagent dissolved in methyl cellosolve (2-methoxyethanol).

2.5. Statistical Analyses

Single factor analysis of variance and then the least significant difference test (Gomez and Gomez, 1984) were employed to test the significant difference in pH, WSC, LA and AA content of the two fermentation mixtures between initial and terminal days of fermentation as well as between the two final fermented products (WFSHB and LFSHB). Significant differences in other biochemical compositions between dried WFSHB and LFSHB as well as between pre-fermentation mixture and the final product of each category (WFSHB and LFSHB) were also determined.

3. Results

3.1. Microbiological Status

Standard Plate Count (SPC) and population of Lactic Acid Bacteria (LAB), yeast and hazardous microorganisms (total and faecal coliform bacteria, *Staphylococcus* and *Clostridium*) determined in pre-fermented mixture, WFSHB and LFSHB have been presented in Table 1. A high degree of contamination of hazardous microorganisms was found in the pre-fermented SHB mixture. Interesting microbiological changes occurred during fermentation. As a result, SPC, LAB and yeast population increased and *Staphylococcus*, *Clostridium* and coliform population decreased after fermentation in both WFSHB and LFSHB. *Clostridium* and feacal coliforms were totally removed from both the fermented products, while other coliforms were also totally removed from LFSHB and reduced to a negligible level in WFSHB. *Staphylococcus* was also reduced to a negligible level in both the fermented products. LAB became the dominating microflora due to fermentation in both the fermented products. The aerobic stability test showed that both moulds and yeast grew profusely in the control and moderately in pre-fermented SHB, but failed to grow on the fermented products (Table 2).

Table 1. Microbiological profile[a] of the pre-fermented and fermented slaughterhouse blood (SHB).

	Pre-fermented SHB (log CFU per g)	LFSHB (log CFU per g)	WFSHB (log CFU per g)
Standard plate count	7.13 ± 0.07	7.71 ± 0.05	7.73 ± 0.06
Total coliform	5.32 ± 0.03	ND[b]	1.2 ± 0.07
Faecal coliform	3.87 ± 0.03	ND	ND
Staphylococcus	4.61 ± 0.02	0.84 ± 0.01	1.5 ± 0.02
Clostridium	3.78 ± 0.02	ND	ND
Lactic acid bacteria	6.91 ± 0.02	7.57 ± 0.02	7.65 ± 0.06
Yeast	1.06 ± 0.05	6.04 ± 0.04	6.88 ± 0.03

[a]Data are mean of three replications and presented as number of colony forming units (CFU) per unit w/vol of the sample with ±SD; [b]Not detected

Table 2. Mould and yeast population (log CFU/g)[a] in the fresh and fermented waste.

Treatments	After 1 hour exposure to air		After 5 days of incubation	
	Mixture of moulds and yeast	Yeast	Mixture of moulds and yeast	Yeast
Prefermented SHB	0.60±0.03	ND[b]	2.71±0.1	2.42±0.06
LFSHB[c]	ND	ND	ND	ND
WFSHB[d]	ND	ND	ND	ND

[a]Data are mean of three replications and presented as number of colony forming units (CFU) per unit w/vol of the sample with ±SD; [b] ND=Not detected; [c] *L. acidophilus* fermented slaughter house blood; [d] Whey fermented slaughter house blood.

3.2. Biochemical Status

Changes observed in the level of pH, Water Soluble Carbohydrate (WSC), Lactic Acid (LA) and Acetic Acid (AA) contents during fermentation have been presented in Figure 1. pH of the fermentation mixture reduced to the range indicative of completion of fermentation (4.0-4.2) within 4 days of incubation. Reduction of pH was accompanied by an increase in LA and AA concentration and a reduction of WSC in both the treatments. There was no change in biochemical parameters between Whey Fermented SHB (WFSHB) and *L. acidophilus* Fermented SHB (LFSHB), except for a higher AA level in WFSHB at the end of incubation as compared to LFSHB.

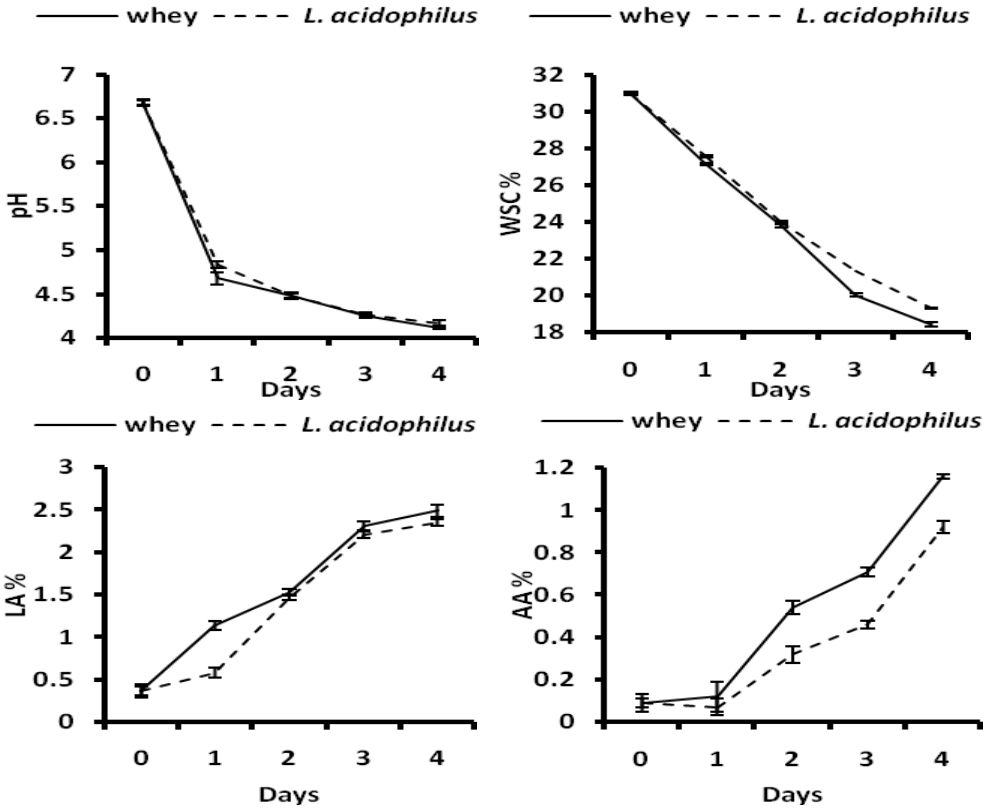

Figure 1. Biochemical changes during fermentation of slaughter house blood mixture.

Table 3 shows a proximate composition of the pre-fermented mixture containing SHB and changes in these parameters after fermentation with *L. acidophilus* and whey. The pre-fermented mixture showed an excellent content of crude protein, leucine and lysine. The level of arginine, phenylalanine, threonine and valine was moderate and the percentage of other essential amino acids was lower. Among non-essential amino acids, amount of alanine, aspartic acid and glutamic acid were higher in comparison to others.

Crude protein level decreased slightly in both LFSHB and WFSHB as compared to pre-fermented mixture.

Crude lipid level remained similar, but crude fiber level significantly reduced in both LFSHB and WFSHB after fermentation. Higher amount of free amino acid was found in WFSHB as compared to LFSHB. Among essential amino acids, arginine, histidine, phenylalanine and threonine contents reduced significantly in both groups, while leucine and lysine contents reduced significantly in WFSHB and valine content reduced significantly only in LFSHB.

Table 3. Proximate composition* of pre-fermented and fermented slaughter house blood.

Parameters (percent dry matter)	Pre-fermented SHB	LFSHB	WFSHB
Crude protein	46.72±0.04[a]	46.18±0.04[b]	46.02±0.11[c]
Crude lipid	2.09±0.03[a]	2.14±0.02[a]	2.13±0.03[a]
Crude fibre	1.16±0.02[a]	0.94±0.03[b]	0.81±0.01[c]
Moisture	13.14±0.18[b]	14.52±0.04[a]	14.68±0.04[a]
Ash	4.53±0.05[a]	4.33±0.03[b]	4.28±0.13[b]
Nitrogen free extract	29.34±0.12[b]	29.66±0.23[a]	29.88±0.11[a]
Free amino acid	0.13±0.04[a]	0.73±0.03[b]	1.16±0.02[c]
Gross energy (kJ g^{-1})	10.53±0.02[a]	10.31±0.01[b]	10.27±0.01[c]
Essential amino acids			
Arginine	2.62 ± 0.03[a]	2.37 ± 0.01[b]	2.32 ± 0.02[b]
Histidine	1.65 ± 0.02[a]	1.44 ± 0.02[b]	1.46 ± 0.02[b]
Isoleucine	0.71 ± 0.03[a]	0.7 ± 0.02[a]	0.74 ± 0.01[a]
Leucine	6.43 ± 0.03[a]	6.42 ± 0.03[a]	6.34 ± 0.03[b]
Lysine	4.65 ± 0.07[a]	4.66 ± 0.05[a]	4.5 ± 0.03[b]
Methionine	0.93 ± 0.05[a]	0.92 ± 0.005[a]	0.93 ± 0.03[a]
Phenylalanine	3.02 ± 0.04[a]	2.93 ± 0.03[b]	2.81 ± 0.04[c]
Threonine	2.22 ± 0.02[a]	2.19 ± 0.01[ab]	2.16 ± 0.01[b]
Valine	2.2 ± 0.03[a]	2.14 ± 0.03[b]	2.2 ± 0.02[a]
Non essential amino acids			
Alanine	4.11 ± 0.05[a]	3.95 ± 0.01[b]	3.95 ± 0.03[b]
Aspartic acid	5.58 ± 0.05[a]	5.43 ± 0.07[b]	5.5 ± 0.02[b]
Cystine	0.32 ±0.01[b]	0.4 ± 0.01[a]	0.37 ± 0.02[a]
Glutamic acid	4.77 ± 0.07[a]	4.17 ± 0.04[b]	4.15 ± 0.02[b]
Glycine	0.95 ± 0.03[a]	0.85 ± 0.01[b]	0.91 ± 0.02[a]
Proline	1.21 ±0.01[b]	1.29 ± 0.01[a]	1.23 ± 0.04[b]
Serine	0.94 ± 0.03[a]	0.93 ± 0.02[a]	0.97 ± 0.01[a]
Tyrosine	0.35 ±0.02[a]	0.24 ± 0.02[b]	0.24 ± 0.02[b]

*Data are mean of three replications. Dissimilar superscripts in a row indicate significant difference (P<0.05).

4. Discussion

4.1. Effects on Microbiological Profile

The pre-fermented SHB exhibited a high degree of contamination by hazardous microorganisms, like faecal coliform bacteria, *Staphylococcus* and *Clostridium.* The presence of such microorganisms in drinking water or food is of great concern for public health (Prescott *et al*., 2002). Irrespective of treatments, LAB became the dominant microorganisms at the end of fermentation, an obvious outcome of successful fermentation process (Faid *et al*., 1994; Burns and Bagley, 1996). Acid tolerant yeast also grew in the medium as observed during the fermentation of other organic wastes (Oude Elferink *et al*., 2000), but failed to inhibit the growth of LAB. Results of the present study indicate that fermentation by whey or *L. acidophilus* could substantially reduce the population of *Staphylococcus*, *Clostridium* and coliform bacteria. Faecal coliforms were totally removed from all groups of fermented products, while in LFSHB there was no trace of any kind of coliform. The increase in the population of LAB and subsequent production of organic acids created an acidic environment during fermentation, which probably resulted in conditions unfavorable to the hazardous microorganisms mentioned above (Nwad *et al*., 2008). El Jalila (2001) also observed a net decrease in the number of the hazardous microorganisms (enterobacteria, enterococci, *Clostridium*, *Salmonella*) during fermentation of poultry wastes manure with *Lactobacillus plantarum* and *Pediococcus acidilactici* as inoculum. Ndaw *et al*. (2008) did not either observe any growth of *Staphylococcus aureus*, *Salmonella* sp. and sulfite-reducing *Clostridium* after two weeks of fermentation of the fish *Sardina pilchardus* with *Lactobacillus delbrueckii*.

A prolonged exposure of non-fermented product to open air also showed aerobically unstable condition, because moulds and yeast grew moderately in them. Such a microbial growth does not only reduce the nutritional value of the product, but it also increases the risk of the proliferation of potential pathogenic and undesirable microorganisms (Driehuis and Oude Elferink, 2000). Yeast is the principal spoiling organism that oxidizes preserving acids, increases pH and, finally, results in the growth of moulds (Woolford, 1990; Pahlow *et al*., 2003). Such growth of moulds and yeast reduces the feed value and the palatability of the fermented products and produces a negative effect on animal health (Oude Elferink *et al*., 2000). In the present study, both of the fermented products showed an aerobically stable condition due to the inhibition of the growth of yeast and mould and, thus, indicated enhanced shelf life of the SHB after fermentation. Shelf life of the fermented products depends, to a large extent, on WSC content left after fermentation, because WSC acts as a substrate for growth of moulds and yeast when exposed to air. Accumulation of LA and AA in sufficient quantity in both the fermented products probably produced antagonistic effects on the growth of moulds and yeast (Moon, 1983) rendering aerobic stability to the two fermented products. Danner *et al*. (2003) observed that, during ensilaging, the main factor behind the aerobic stability of the fermented products was the accumulation of AA. Driehuis *et al.* (2001) also observed a similar aerobic stability of the product after the fermentation of grass silage.

4.2. Effects on Biochemical Parameters

The desired level of pH indicative of completion of fermentation is 4.0 to 4.2. Within this pH range the fermented products are safe from spoilage and fit for the use as feed stuff for animals (Lee *et al*., 2004; Yashoda *et al*., 2001). Fermentation of SHB was completed within 4 days by both the inoculums. LA and AA are the major metabolites responsible for the reduction of pH in a fermentation mixture. In the present study, LA was generated in a higher quantity than AA because of the capacity of LAB to produce LA as the major metabolite (Piard and Desmazeaud, 1991). This is also a normal phenomenon in silage fermentation process (Kung and

Shaver, 2001). While comparing with the fermentation of fish offal (FO) by *L. acidophilus* and whey (Samaddar and Kaviraj, 2014), the present study revealed less amount of LA generation during fermentation, even though the amount of AA generated and the amount of WSC reduced were similar in the fermentation of FO and SHB. In the fermentation of FO, *L. acidophilus* was found to complete fermentation within 3 days as compared to 5 days by whey. In the present study fermentation was completed within 4 days in both the inoculums. LABs are known to require complex nitrogenous sources for their metabolism during fermentation (Narayanan *et al*., 2004). FO appeared, as a better source of nitrogen for *L. acidophilus* than SHB, while SNB served as a better source of nitrogen than FO for the complex group of fermentation microorganisms supplied by whey. Whey, being a by-product of dairy industry, is expected to carry homo-fermentative group of LAB (Moreira *et al*., 2000; Ng *et al*., 2011). Since AA was also detected in all fermentation mixtures, the presence of some hetero-fermentative LAB or other AA producing microorganism in these mixtures could not be ruled out. A probable source of these microorganisms was either SHB or molasses. The accumulation of AA in a relatively higher amount in WFSHB, as compared to LFSHB, is probably helpful to protect spoilage under aerobic condition (Filya, 2003; Kung *et al*., 2003). There was no significant difference in WSC content between the two fermentation groups, indicating that the carbohydrate utilization rate was the same in these two groups.

Significant variations were observed in the proximate compositions between the pre-fermentation mixture and the fermented SHB except crude lipid and ash content. The crude protein level reduced significantly in all fermented products, as compared to the pre-fermented category, but the reduction was restricted up to 1.15 % in LFSHB and 1.49 % in WFSHB. Reduction in the level of crude protein in the fermented products was probably due to the utilization of a part of the protein by microorganisms as a nutrient source in their metabolism through extracellular proteolytic activity. As a result, there was a breakdown of a part of the protein along with the increase in the Free Amino Acid (FAA) level in both fermented products (Savijoki *et al*., 2006; Haq and Mukhtar, 2006). The breakdown of the protein and the subsequent increase in FAA contents increase the digestibility of the food ingredient and the excess FAA can also act as potential chemo-attractants for fish (Ramírez-Ramírez *et al*., 2008; Lian *et al*., 2005). Among the essential amino acids, the reduction of arginine, histidine, phenylalanine and threonine contents in both groups indicated their need for the microorganisms irrespective of the inoculums used, while leucine and lysine reduced significantly in WFSHB and valin reduced significantly in LFSHB, indicating selective requirements of these amino acids by different groups of microorganisms grown from these two inoculums. However, only a slight variation was observed in the level of crude protein and essential amino acids between the two fermented products and both products contained adequate amount of crude protein and essential amino acids required for formulating balanced aquaculture feed. A significant reduction of crude fiber level of the SHB mixture due to fermentation is another advantage of using fermented SHB as feed ingredient. The decrease in crude fiber level in the fermented products was due to cellulolytic microbial action during fermentation (Shi *et al*., 2006). El Jalil *et al.* (2001) also observed a depletion of crude protein and crude fiber level along with unchanged crude lipid value, as observed in the present study, during fermentation of poultry wastes manure with *L. plantarum* and *Pediococcus acidolactici* as inoculum.

5. Conclusion

Fermented Slaughterhouse Blood (SHB) showed an excellent protein quality with the availability of some essential amino acids like, arginine, leucine, lysine, phenylalanine, threonine and valine, essential for a product to establish itself in aquaculture feed industry as a good quality protein supplement. Fermentation renders a microbiological safety, an enhanced shelf life and a minimum loss of nutrients (especially crude protein and essential amino acids). Besides, commercially available *L.acidophilus* as fermentation inoculums, whey, a byproduct of milk industry and an easily available source of LAB, appeared to make technology a more eco-friendly and feasible one to the small scale aquaculturists.

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References

Agbedi OT, Outbusin SO and Ogunleye FO. 2009. Effect of different levels of substitution of fishmeal with blood meal in pelleted feeds on catfish *Clarias gariepinus* (Burchell 1822) culture in net cages. *Eur J Sci Res*., **31**: 6-10.

Aladetohun NF and Sogbesan OA. 2013. Utilization of blood meal as a protein ingredient from animal waste product in the diet of *Oreochromis niloticus. Int J Fish Aquacult*., **5(9)**:234-237.

Bergleiter S. 2011. **Organic Aquaculture – From a "Nice Niche" to the "Whole Cake" to Increase the Organic Share of the World Aquaculture**. EAF special publication, pp 114-115.

Burns JC and Bagley CP. 1996. Cool-season grasses for pasture. In: Moser L.E., Buxton D.R., Casler M.D. (eds.), **Cool Season Forage Grasses**. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison pp. 321–355.

Cappuccino J and Sherman N. 2007. **Microbiology: a Laboratory Manual**. Pearson Education, New Delhi, p 14.

Danner H, Holzer M, Mayrhuber E and Braun R. 2003. Acetic acid increases stability of silage under aerobic conditions. *Appl Environ Microbiol*., **69(1)**:562.

De Silva SS and Anderson TA. 2009. **Fish Nutrition in Aquaculture.** Springer International Edition, Indian reprint, New Delhi.

Driehuis F and Oude Elferink SJWH. 2000. The impact of quality of silage on animal health and food safety: a review. *Vet Q*., **22**: 212–217.

Driehuis F, Oude Elferink SJWH, Van Wikselaar PG. 2001. Fermentation characteristics and aerobic stability of grass silage

inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. *Grass Forage Sci*., **56**: 330-343.

Dremetsika AV, Siskos PA and Bakeas EB. 2005. Determination of formic and acetic acid in the interior atmosphere of display cases and cabinets in Athens museums by teverse phase high performance liquid chromatography. *Indoor Built Environ*., **14**: 51–58.

El Jalila MH, Faid M and Elyachioui M. 2001. A biotechnological process for treatment and recycling poultry wastes manure as a feed ingredient. *Biomass Bioenerg*., **21**: 301–309.

Faid M, Karani H, Elmarrakchi A and Achkari-Begdouri A. 1994. A biotechnological process for the valorization of fish waste. *Bioresour Technol*., **49**: 237–241.

Fearon J, Mensah SB and Boateng V. 2014. Abattoir operations, waste generation and management in the Tamale metropolis: Case study of the Tamale slaughterhouse. *J. Public Health Epidemiol*., **6 (11)**:14-19.

Filya I. 2003. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. *J Dairy Sci*., **86**: 3575–3581.

Gomez KA and Gomez AA. 1984. **Statistical Procedures for Agricultural Research**. John Wiley and Sons.

Haq IU and Mukhtar H. 2006. Biosynthesis of protease from *Lactobacillus paracasei*: kinetic analysis of fermentation parameters. *Indian J Biochem Biophys*., **43**: 377–381.

Helrich W. 1990. **Official Methods of Analyses. Association of Official Analytical Chemists**. Washington DC. USA.

Jayathilakan K., Sultana K., Radhakrishna K. and Bawa A. S. (2012) Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *J Food Sci Tech*., **49(3)**: 278–293.

Kader A, Shunsuke K, Manabu I, Saichiro Y, Mahbuba B, Nguyen B, Gao J and Laining A. 2012. Can fermented soybean meal and squid by-product blend be used as fishmeal replacements for Japanese flounder (*Paralichthys olivaceus*). *Aquacult Res*., **43**: 1427–1438.

Kung L and Shaver R. 2001. **Interpretation and use of silage fermentation analyses reports**. In: Focus on forage, Forage management fact sheet, University of Wisconsin, Madison, USA, Vol. 3, No. 13. Available via http:// http://www.uwex.edu/ces/crops/uwforage/Fermentaion.html. Accessed 21 Sept, 2013.

Kung L, Taylor CC Jr, Lynch MP and Neylon JM. 2003. The effect of treating alfalfa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. *J Dairy Sci*., **86**: 243–336.

Kwanyuen P and Burton JW. 2010. A Modified amino acid analysis using pitc derivatization for soybeans with accurate determination of cysteine and half-cystine. *J Am Oil Chem Soc*., **87**: 127–132.

Lee KS, Lee KY, Oh CS, Lee DG, Kim YJ. 2004. Effect of aeration for the probiotic feed production from food wastes by *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*. *J Kowrec*., **11(4)**: 114– 119.

Lian P.Z., Lee C.M. and Park E. (2005) Characterization of squid-processing by-product hydrolysate and its potential as aquaculture feed ingredient. *J Agric Food Chem*., **53**: 5587–5592.

McHugh J, Dumont SN, Julie P, Nguyen AL, Levesque S and Carrier A. 2006. New and simple HPLC method for the determination of lactic acid content in ciprofloxacin injection. *J Liq Chromatogr Related Technol*., **29**: 1905– 1916.

Moore S and Stein WH. 1948. Photometric ninhydrin method for use in the chromatography of amino acid. *J Biol Chem*., **176**: 367-388.

Moon NJ. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *J Appl Bacteriol*., **55**: 453–460

Moreira M, Abraham A and De' Antoni G. 2000. Technological properties of milks fermented with thermophilic lactic acid bacteria at suboptimal temperature. *J Dairy Sci*., **83**: 395–400.

Murphy PT, Moore KJ, Richard TL, and Bern CP. 2007. Enzyme enhanced solid- state fermentation of kenaf core fiber for storage and pretreatment. *Bioresour Technol.*, **98**: 3106-3111.

Narayanan N, Roychoudhury PK and Srivastava A. 2004. L (+) lactic acid fermentation and its product polymerization. *Electron J Biotechn*., **2**: 0717-3458.

Ng E, Yeung M and Tong P. 2011. Effect of starter cultures on the survival of *Lactobacillus acidophilus*. *Int J Food Microbiol*., **145(1)**: 169–175.

Nwad AD, Faid M, Bouseta A, Zinedine. 2008. Effects of controlled lactic acid fermentation on the microbiological and chemical quality of Moroccan sardines (*Sardina pilchardus*). *Int J Agr Biol*., **10(1)**: 21-27.

Oude Elferink SJWH, Driehuis F, Gottschal JC and Spoelstra SF. 2000. Silage fermentation process and their manipulation, in: Mannetje L. (eds.), **Silage Making in the Tropics with Particular Emphasis on Smallholders.** Proceedings of the FAO electronic conference on tropical silage, FAO, Rome, pp. 17-30.

Pahlow G, Muck RE, Driehuis F, Oude Elferink SJWH, Spoelstra SF and Madison HJH. 2003. Microbiology of ensiling, in: Buxton D.R., Muck R.E. (eds.), **Silage Science and Technology**. American Society of Agronomy, Madison, WI, USA, pp. 31-93.

Prescott LM, Harley JP and Klein DA. 2002. **Microbiology**, 5th edition. McGraw-Hill, London, UK.

Piard JC and Desmazeaud M .1991. Inhibiting factors produced by lactic acid bacteria: 1. Oxygen metabolites and catabolism end products. *Lait*., **71**: 525- 541.

Pyar H, Peh KK and Liong M. 2011. Inhibitory effects of metabolites from probiotics *Lactobacillus acidophilus* strains on growth of pathogenic bacteria. *J Pharmacol Toxicol*., **6(5)**: 533-540.

Ramírez-Ramírez JC, Huerta S, Arias L, Prado A. and Shirai K. 2008. Utilization of fisheries by-catch and processing wastes for lactic acid fermented silage and evaluation of degree of protein hydrolysis and in vitro digestibility. *Rev Mex Ing Quim*., **7:** 195-204.

Ribeiro RA, Ozóório RODA, Batista SMG , Pereira-Filho M , Ono EA and Roubach R. 2011. Use of spray-dried blood meal as an alternative protein source in Pirarucu (*Arapaima gigas*) diets. *J Appl Aquacult*., **23(3):**238-249.

Samaddar A, Kaviraj A. 2014. Processing of fish offal waste through fermentation utilizing whey as inoculum. *Int J Recycl Org Waste Agricult*., **3**: 45.

Savijoki K, Ingmer H and Varmanen P. 2006. Proteolytic systems of lactic acid bacteria. *Appl* Microbiol *Biotechnol*., **71**: 394–406.

Shi J.G., Zeng G.M., Yuan X.Z., Dai F., Liu J. and Wu X.H. (2006) The stimulatory effects of surfactants on composting of wastes rich with cellulose. *World J Microb Biot*., **22**: 1121-1127.

Woolford MK. 1990. The detrimental effects pf air on silage. *J Appl Bacteriol*., **68**: 101-116.

Yashoda KP, Sachindra NM, Rao D and Mahendrakar NS. 2001. Changes in microbial population during fermentation of silk worm pupae. *Sericologia*., **41(2)**: 253-261.

Zhu H, Gong G, Wang J, Wu X, xue M, Niu C, Guo L and Yu Y. 2011. Replacement of fishmeal with blend of rendered animal protein in diets for Siberian sturgeon (*Acipenser baerii* Brandt), results in performance equal to fish meal fed fish. *Aquacult Nutr*., **17**: 389-395.