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CHANGE IN SPECIES COMPOSITION AND ITS IMPLICATION ON CLIMATE VARIATION IN BALI STRAIT: CASE STUDY IN 2006 AND 2010

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Abstract: *Sardinella lemuru* is a dominant small pelagic fish (80-90%) caught by purse seiner in Bali Strait, while the remaining 10-20% consist *Decapterus* spp., *Euthynus affinis*, and others. This composition typically varies seasonally, whereas Southeast monsoon season was dominated by *S. lemuru*, while Northwest monsoon season replaced by *Decapterus* spp. and *Euthynus affinis*. Fishing trend in the last 14 years indicated regime shift with the shifting in species composition by a seasonal into the inter-annual due to global climate change, such as El Niño and La Niña. 2006 was indicated a cold period of water temperature, which is triggered by the El Nino and positive Indian Ocean Dipole (pIOD). In this cold period, the *S. lemuru* reached peak of fishing, otherwise this fish disappear when warm period (strong La Niña) in 2010. When *S. lemuru* disappeared during warm period, it was substituted by *Decapterus* spp. Furthermore, as predatory fish of both small pelagic fishes, *Euthynnus affinis* always appear throughout the year. Understanding the species composition trend from seasonal to longer period is important for better strategy to manage fisheries of Bali Bali Strait in climate change era.

Keywords: pelagic fish, El Niño, Regime shift, Bali Strait

INTRODUCTION

Sardinella lemuru or commonly known as Bali Sardinella(Fishbase, 2016; FAO, 2016) fishery is part of a long history of fisheries in East Java and Bali. It is a dominant species which is caught in Bali Strait and reached over 90% of the total catch of pelagic fish in Bali Strait (Merta,1992 a, b; Hendiarti et al., 2005).The last four decadal, Bali Strait's fish catch was fluctuated due to some reasons, for example over exploitation (Pet et al., 1996; Buchary, 2010) and climate variation, such as El Niño Southern Oscillation (ENSO)(Hendiarti et al., 2005; Sartimbul et al., 2010a; Buchary, 2010).Moreover,*S. lemuru* catch declined dramatically in 2010 to 2012, and caused economic lossesas reported in Statistical data report of Fishery Port Muncar (2012).

Even El Nino Southern Oscillationwas nothing important to debate in the few decades ago, particularly for fisheries implication, however, in the last two decades it has apparently impacted and responsible to the fisheries dynamics mainly in the tropical Pacific. For example temporary collapse of the Peruvian anchovy population during strong El Niño years (Barber and Chavesz, 1983; Bertrand et al., 2004), dramatic changes in the distributions of Pacific tuna, mainly the tropical skipjack (*Katsuwonus pelamis*) species (Kimura et al., 1997; Lehodey et al., 2006), shifting migration pattern of Pacific bluefin tuna during strong El Niño year in 1997/1998 (Kitagawa et al., 2006), increasing S. lemuru catch during weak El Niño and combined bypositive Indian Ocean Dipole (IOD+) 2006in Bali Strait (Sartimbul et al., 2010a) and Southern East

Java (Sartimbul et al. (2010b) and Sambah et al. (2012).

Those researches were revealed that climate change was a triggered climatic phenomenon event as ENSOand IOD in 2006/2007 causedincreasingupwelling intensity and impact to the*S.lemuru* catch in both regions. Furthermore Hendiarti et al. (2004) pointed out that the upwelling caused increasing of primary productivity during Southeast monsoon season. Furthermore, Susanto and Marra (2005) added that the anomalies in the Southeastern Monsoon season caused strong El Niño events in 1997/1998 and was followed by La Nina in relation with Indian Ocean Dipole (IOD), which led to increased productivity of chlorophyll-a in the coast of Java (including Bali) and southern Sumatra.

During 1990s, it is well known that ENSO occurred in 1997/1998 and possibly influence marine organisms all over the world, e.g. the Japan Sea (Chiba and Saino, 2003), while it is also reported that there could be a regime shift around 1998 in the North Pacific (Minobe, 2002; Tian et al., 2004). The dynamics of water temperature and fisheries production in Bali Strait may have been connected with such climate events on both decadal and inter-decadal scales. In addition, recent warming may also give a serious effect on the fisheries production in near future (Tian et al., 2004; Drinkwater, 2005).

It should be taking into account that the period of occurring is becoming shorter recently; from pentadecadal to bidecadal, from decadal to interdecadal, from interdecadal to annual, and so on. For example Minobe (1999) explained that all three climatic regimes in the 20th century (1920th, 1940th, and 1970th) have involved simultaneous phase reversals between pentadecadal (a period of about 50 years) and bidecadal (a period of about 17 years) oscillation.

In this paper, we discuss the change of species composition of coastal fisheries of Bali Strait due to dynamics of sea surface temperature(SST) and chlorophyll-a (Chl-a) abundance in recent climate change era. Combination of in situ and long period of satellite data of SST, Chl-a, and catch per unit effort (CpUE) of fisheries catch might provide a good tool for understanding the species composition trend from seasonal to longer period for better strategy to manage fisheries of Bali Strait in the uncertainty climate era.

DATA AND METHODS

This research was conducted in Bali Strait at 114°26'00"-115°10'00"E and 08°09'00"-08°50'00"S (Fig. 1) as indicated as fishing ground of the Bali Strait.There are three sampling sites representing the region of fishing ground in the Bali Strait, i.e. 114° 26'11"E and 08°27'14"S (St.1), 114°34'43"E and 08° 37'08"S (St.2), 114°40'11" and 08°42'18"S (St. 3) (Fig. 1). In situ and satellite data were used in the current research. In situ sea surface temperature (SST) and chlorophyll-a (Chl-a) data were collected in February-April 2016. Lack of in situ data were substituted by 15-year (2001-2015) of monthly SST and Chl-a obtained from Satellite Aqua MODIS (www.modis.gsfc.nasa.gov). Furthermore, the fish catch data were obtained from Fishing Port Muncar, Banyuwangi. All data were analyzed their trends and anomalies using MS Excel and SeaSurfer 11.0.

In addition to the data of SST and chl-a of Bali, the data catch per unit effort (CpUE) was used in this study. CpUE technique was used to determine the capture rate value of fishing effort based on the division of the total catch (catch) the fishing effort (effort). The common formula used as follows:

$$CpUE_i = \frac{Ci}{fi}$$

Where, C_i: Catch to$_i$ (tonnes), fi: Efforts to $_i$ (trip), and $CpUE_i$: Catch per Unit Effort (kg/trip).

In order to see the deviation trend of data, anomaly technique was used. It is to know how big the data deviated, as following formula:

Anomaly= x-$\bar{x}$

Where: x: value in day a

$\bar{x}$: Average of valuein the same day within whole month or year

Since there were difference ranges in data value, the standardization was used. It is a common method used to standardize wholedatato facilitate the making and reading graphs clearly (Sartimbul et al. , 2006) . The equation used is:

$$Z = \frac{xi - \bar{x}}{s}$$

Where:

z : Standardization

xi : value

$\bar{x}$: Average

s : Standard deviation

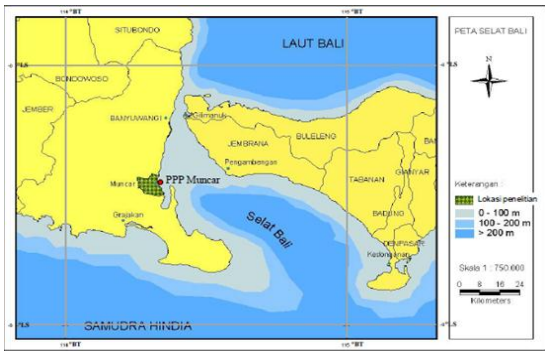

Figure 1Bali Strait is very unique. Semi enclosed strait, allowed water mass exchange from Pacific to Indian Ocean. The shallow and deep sea floor of Bali Strait has provided a good fishing and spawning ground of S. lemuru. Muncar Fishing Port is the main fish landing port of Bali Strait (green).

RESULT AND DISCUSSION

Total fish catch

There were three dominant species captured by purse seiner in Bali Strait, i.e. *S. lemuru, Decapterus spp, Euthynnus affinis,* and others. The total catch of 14-year of purse seiner data collected from Muncar fishing port showed that the maximum catch found in 2007, while minimum ones in 2011 (Figure 2).

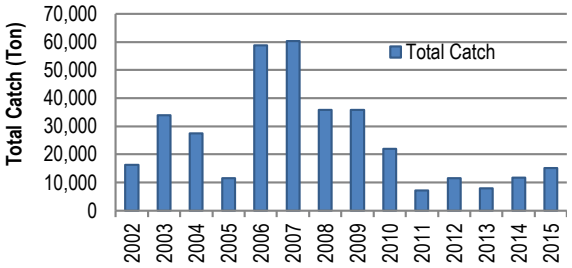

Figure 2 Fourteen-year of total catch of fish landed at Muncar Fishing Port. The maximum catch found in 2007, while minimum catch in 2011.

Based on the inter-annual variation of fish catch (Figure 3), the trend of fish catch of Bali Strait were mainly developed by S. lemuru, as clearly seen in the percentage of dominant fish caught in Bali Strait (Figure 3).

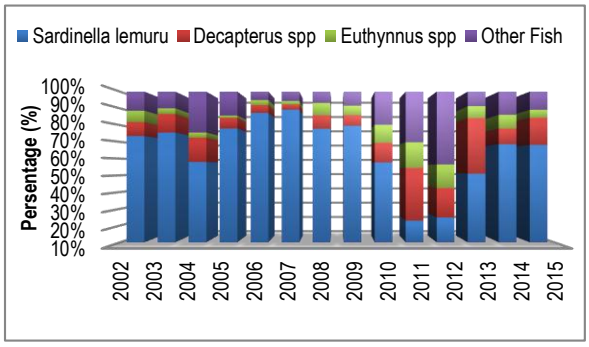

Figure 3 Inter-annual of catch composition and its percentage. *Sardinella lemuru* was dominant for the whole research year except for 2011 and 2012.

Sardinella lemuru dominated in the whole year except for 2011 and 2012. During peak year (2006-2007), there was nearly 80-90% of fish catch was S. lemuru. On the contrary, there was only 10-20% of S. lemuru caught in desert year. The steep declining of S. lemuru catch were started in the end of 2010 andcontinued to the end 2012. Longer period of desert year has apparently giving negative impact on the fishermen in the Bali Strait due to economic loses, because S. lemuru was usually used as raw material of many fisheries industries in Bali Strait as revealed by Pet et al (1996).

Seasonal variation of Sardinella lemuru, Sea surface Temperature and Chlorophyll-a

Sardinella lemuru catch were vary seasonally. Minimum catch occurs in transition-2 (inter-monsoon: Mar, Apr, May) and maximum catch in transition-1 (inter-monsoon: Sep, Oct, Nop) (Figure 4). This seasonal trend was also revealed by previous researchers, for example Buchary (2010) and Sartimbul et al. (2010).

Seasonal pattern of S. lemuru has relation to dynamics of SST and chl-a pattern in different time lag. Seasonal variation of SST showed that maximum temperature occurs on Feb-Mar, while minimum temperature found on Aug-Sep (Figure 5). During Southeast monsoon (Jun, Jul, Aug), wind prevails from Australia to south Indonesia then generates increasing upwelling intensity in South Indonesia (including Bali Strait).

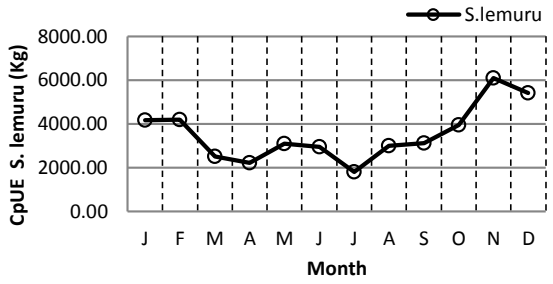

Figure 4 Seasonal trend of Catch per Unit Effort of S. lemuru based on 14-year data. Maximum catch of S. lemuru were found in transition-1 (Inter-monsoon: S, O, N)) season.

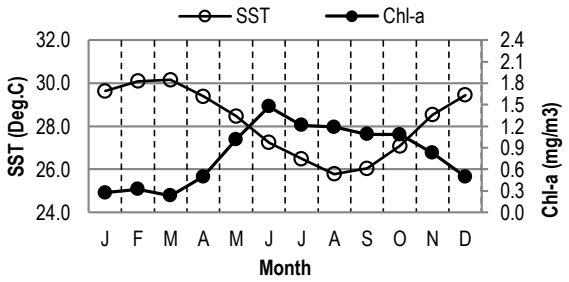

Figure 5 Seasonal variation of sea surface temperatureand chlorophyll-a in Bali Strait. Highest temperature occurs in Feb-Mar, while minimum temperature in Aug-Sep vice versa for chlorophyll-a.

During this season, primarily production of South Java increases. Indeed Bali Strait reaches the blooming season of plankton, that represented by increasing chl-a concentration. During Southeast monsoon season, chl-a concentration was the highest and continued to transition-2 season. However, lowest chl-a concentration occur in northwest monsoon season. During this season, there is no intensity of upwelling at Bali Strait. It is common that there is negative correlation between Sea surface temperature and chl-a as, and has a month lagged shown in Figure 5.

Sea surface temperature gives impact on chl-a about 1 month lagged (Larink and Westehide, 2006) while 3-month lagged on S. lemuru caused of grazing process (Sartimbul et al, 2010).

Inter-annual variation of fish catch, Sea Surface Temperature, and Chlorophyll-a

Temperature is the first physical parameter of sea water. Figure 6 showed the seasonal and annual variation of sea surface temperature.

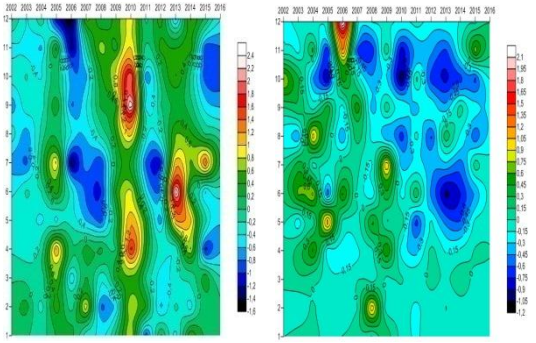

Figure 6 Seasonal and annual Sea Surface Temperature (left panel) and Chlorophyll-a (right panel).

However, current trend showed that *S.lemuru* did not only vary seasonally but also inter-annually (Fig. 7).SST declines in 2006 from June December. Decreasing temperature in 2006 coincided with El Nino and modified by positive Indian Ocean Dipole (Sartimbul et al., 2010).

During El Nino event, trade wind weakening and let eastern Pacific sea surface temperature kept stagnant. It has implication to the declining of water temperature about 1.5°C below normal year due to

upwelling intensity at western Pacific including Bali Strait.

On the contrary, in 2010 was indicated as La Nina (NOAA, 2016). Water temperature increased about 2.4°C above normal year.

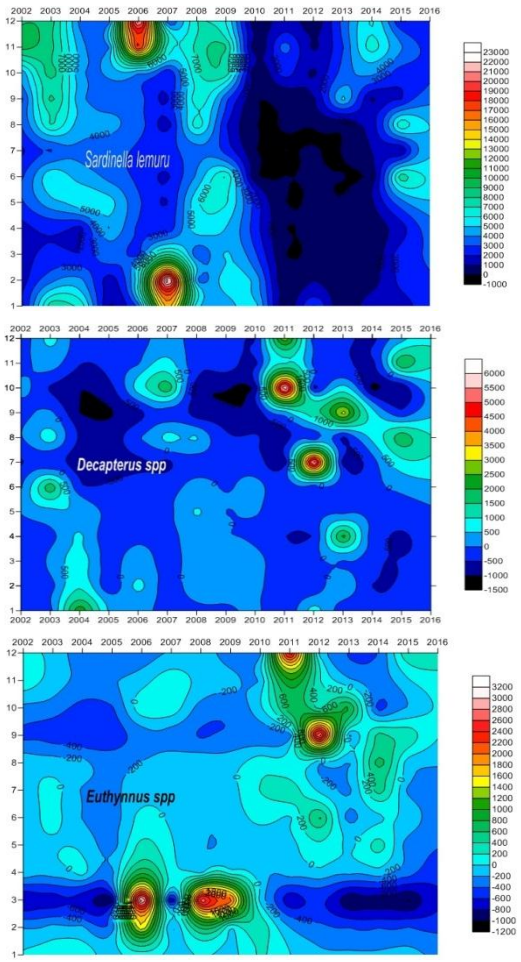

Figure 7 Time series of CpuE anomaly for three dominant species which landed at Muncar Fishing Port. S. lemuru catch reached peak year in the end of 2006 and continued in early 2007.

Increasing water temperature has begin on May to December and continued on June 2013.

Standardization method in Figure 8 were used to obtain the difference value be standard and provided easy reading of graph.

In Figure 8, sea surface temperature dropped in 2006 lead to strong upwelling in Bali Strait. Strong upwelling produced high productivity that represented by high concentration of Chl-a. As the result, after 3 month lagged, catch of S. lemuru dramatically increased as shown by Figure 8. The higher intensity of upwelling activity in the 2006 well known due to El Nino and positive IOD phenomenon (Sartimbul et al., 2010).

On the contrary, during La Nina even (2010), SST increased in Bali Strait. There were no upwelling throughout the year and prolong to 2011. As the result chl-a concentration dropped moreover for catch of S. lemuru in Bali Strait. There was reported that S. lemuru was disappeared from 2010 to 2011, and causes economic loses for Bali Strait.

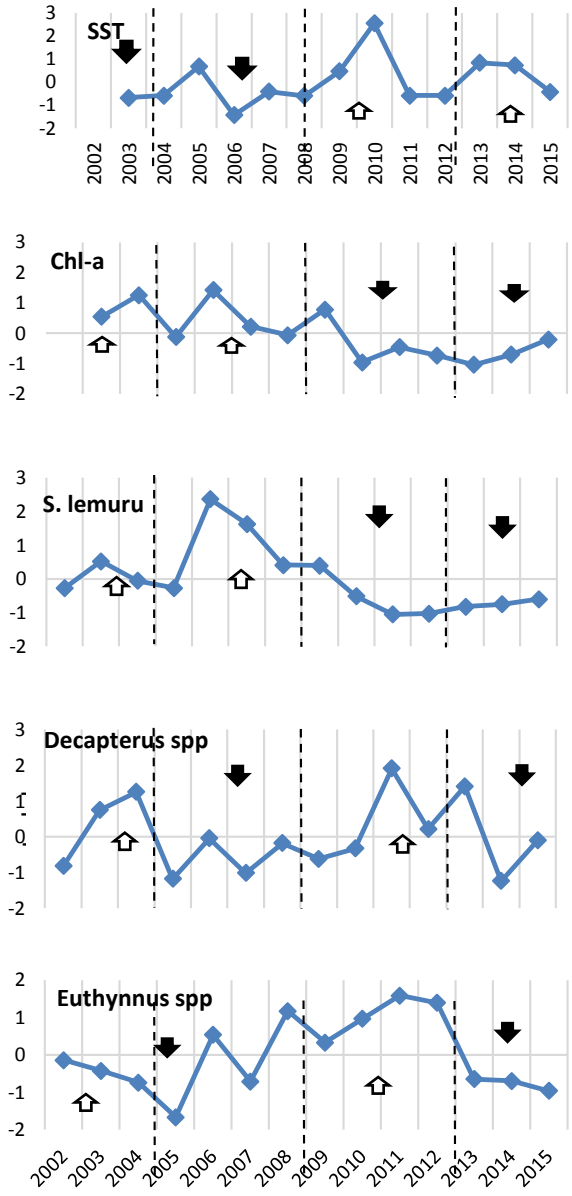

Figure 8 Standardization of SST, chl-a, and S.lemuru, Decapterus spp, and Euthynus affinis.

As a function of resilience of environment, disappearance of one species usually will be substituted by other species (Mann and lazier, 2006; Bakun, 1996). Similarly, loses of S. lemuru therefore it has been substituted by Decapterus spp to handle his function in the food web of marine ecosystem of Bali Strait. Furthermore, as higher tropic level than *S. lemuru and Decapterus spp., Euthynnus affinis* always appeared in every season or annually due to food availability for this species.

CONCLUSION

Sardinella lemuru is important species for coastal economic development in Bali Strait. Repeatedlylosses of *S. lemuru*fishery of Bali Straitare important as reminder tool for government to manage *S. lemuru* fishery in Bali Strait wisely and properly for the future sustainable fishery.

Sea surface temperature, chlorophyll-a and climate index have provided good tool for fish abundance prediction in Bali Strait.

Understanding the change in species composition due to climate variability in Bali Strait has provided information for fisheries manager to manage fisheries sustainable.

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PRODUCTION OF MICROBIAL SILAGES FROM ANIMAL WASTES AS FISHMEAL REPLACER IN THE AQUACULTURE DIETS

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ABSTRACT

The experiment was carried out to produce and assess the storage quality of microbial silages from three animal wastes namely fish offal (FO), chicken gut (CG) and shrimp head (SH) using yoghurt as dietary fishmeal (FM) replacer for aquaculture. There was prevalent relationship between decreasing of pH and silage process that was analyzed by One-Way ANOVA as the alpha level ($p<0.05$). Ensilage process was completed within 14-21 days and three liquefied silages of desired pH level (<4.5) were obtained. The crude protein content found was highest in shrimp head silage (31%) followed by chicken gut silage (30%) and fish offal silage (16%) while highest crude fat was found in fish offal silage (69%) followed by shrimp head silage (53%) and chicken gut silage (43%) respectively. Total amount of essential amino acids (EAA) in chicken gut silage was found highest (9.33 $g.100^{-1}$ protein) followed by shrimp head silage (8.91 $g.100^{-1}$ protein) and fish offal silage (4.70 $g.100^{-1}$ protein) respectively. During 90 days storage, pH was found to be stabilized below 4.5, no change in color and smell and no sign of putrefaction in the produced silages were seen. The results of proximate composition including EAA reveal that produced silages have potentials to be used as protein supplement in aquafeed.

Keywords: Microbial Silage, Animal Wastes, Fishmeal, Ensilage, Liquefied, EAA

INTRODUCTION

Fishmeal the most reliable [1, 2] source of dietary protein [3] and rich with amino acid profile, essential fatty acids and vitamins content [4, 5, 6]. Nutritional value of fish feed largely depends on the quality of protein of the ingredients used in the formulation of feed [7, 8, 9]. However, the price of FM has risen undoubtedly at an alarming rate due to its inconsistent supply and increasing demand in animal and fish feed industries [10, 11, 3, 12] compelled to identify other alternative protein sources [13, 12, 14] to reduce cost and improve cost effective aqua feed [15, 16].Various less expensive experiment tested with plant origin [17, 18, 19, 20, 21, 22] in the formulation of feed for fish but were fruitless [23]. From the failure, particular concentration given to the use of animal waste products as eco-friendly system called silage started by [24] in 1980s and tested successfully in feeds for fish species such as Chinook salmon [25, 26], silver Sea bream [27], rainbow trout [28, 29], red drum [30, 31], gilthead Sea bream [32, 33], Indian major carp [34], Australian snapper [35], Australian silver perch [36, 37], Nile tilapia [38], sunshine bass [39], grouper [40], and mirror carp [3]. Terrestrial animal by-products are considered as alternative protein sources because of their high protein content, low carbohydrate content, optimal amino acid profiles and lack of anti-nutritional factors [9, 23] and also close in nutritional value to natural feed [41, 42, 43]. Moreover, the use of fish processing waste reduce the cost of producing fish feed by approximately 15 to 20% [44, 45, 46, 47] and 75% fish meal can be replaced without any compromise on the growth and nutritive value of the fish [9, 48] even though the amount of fishmeal replacement possible

is species specific [26, 49, 50, 51, 28, 52, 53, 38, 33, 39, 54, 55). Besides nutritional quality, use of animal waste is also constrained by high moisture, indigestible particles, microbial contaminants etc. [56, 57]. Animal waste like fish offal, shrimp head and chicken gut have great nutritional value [58] and fish offal is most promising ingredient due to its moderate level of protein and high level of lipid, the later being capable of rendering protein sparing-effect [59]. Nutritional quality improves by fermentation and digestibility of the ingredients [60] as environment friendly, cost effective biotechnological tool in fish feed formulation [61, 62, 63] but this may create hyper amino acidemic effects in most of the stomach less fish species leading to ultimate loss of consumed protein and reduction of growth performance due to increasing digestibility and rapid absorption of dietary protein in the form of free amino acid (FAA) [64]. The present experiment was conducted to produce silage from the selected animal wastes as the means of fish meal replacement and to assess proximate composition of raw animal wastes and ultimately to evaluate the storage quality of silage.

MATERIALS AND METHODS

Selection, Collection and Preservation of Animal Wastes

In the present research, fish offal and chicken gut were collected from local retail fish market and poultry shop while shrimp heads were collected from a processing factory of Chittagong city. After collection, these raw materials were brought to the nutrition and feed technology laboratory of Institute of Marine Sciences and Fisheries, University of Chittagong in insulated ice box and kept in freezer (at -20^0C) until processing.

Preparation of Microbial Silage

Fish offal, chicken gut and shrimp head were mixed separately with molasses as fermentable carbohydrate and yogurt as inoculum for Lactobacillus bacteria for anaerobic fermentation at ambient temperature (25-300C). For silage mixture, 70% of each raw material was mixed with 15% molasses, 15% yogurt and 2.5% ascorbic acid (as mould and yeast inhibitor). At first, the frozen raw wastes were thawed at room temperature, washed thoroughly with clean water and then minced by using an electrical mincer (National MK-G20NR). Each of the minced raw materials was mixed properly with other ingredients in a plastic bowl and then packed in separate polyethylene bags. The poly bags were then placed in three air tight plastic containers and incubated at ambient temperature. The mixtures were swirled regularly to let the lactic acid (produced by bacteria) blend well with the fermenting mixtures. After completion of ensilage process, the three produced silages were stored for 90 days.

Proximate composition and amino acid analysis

Proximate composition of the selected animal wastes and produced silages in terms of crude protein, crude fat, ash and moisture content were determined according to the methods given in AOAC (1990). Moisture content was determined by oven-drying at 1050C for 24 hour, lipid content by extracting the residue with petroleum ether for 8 hour, crude protein (total nitrogen) by Kjeldahl method and ash content by ignition at 5500C for 24 hour.Amino acid compositions of the three microbial silages were determined by using amino acid analyzer (Shimadzu, Japan) in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. For separation and detection of the amino acids, ion exchange chromatography and post-column derivatization method were used respectively.

Observation of pH and other organoleptic parameters

The initial pH values of the raw materials and changes in pH during ensilage [65] and storage were determined by diluting 10g of the sample in 10 ml deionized water by an electronic Bench Top pH meter (Hannah pH 209). A portion of each silage mixture (10-12g per container) was distributed in 4-5 separate air tight small plastic containers (packed in small poly bag) to facilitate monitoring of pH through ensilage process without opening the main silage containers and thus maintaining anaerobic condition for fermentation.Besides, changes in liquefaction, color and smell during ensilage and storage were also observed.

Bacteriological Methods

Since lactic acid bacteria are mainly responsible for fermentation, so it was of interest to know which species were involved in the fermentation of the selected animal wastes. Therefore, lactic acid bacteria were isolated from the produced silages and yoghurt sample using MRS (de Man Rogosa and Sharpe, Oxoid Ltd.) media and the isolates were identified according to their colony morphology, gram staining, motility and biochemical tests namely catalase test, oxidase test, citrate utilization test and indole test [66].

RESULTS AND DISCUSSION

Fish Meal is the most important source of protein that has made it ahigh demand ingredient [67], consequently price has risen from about US$ 600 per metric ton in 2005 to about US$ 2000 per metric ton in first quarter of 2010 [68]. For meeting the increasing demand, several researches had been conducted on mechanically deboned poultry meat residue (MDM) [32, 29, 39], Grouper [40], feather meal [69, 70, 71], soybean meal [72, 73], yeast [74, 75, 76, 77, 78], oil seed (Refstieet al. 2000), and algae (Broun 1980; Appler H.N 1983; Zeinhom 2004; El-Hindawyet al. 2006; Tartielet al. 2008) in various fish species diet (Gallagher and LaDouceur 1995; Negreet al. 2001; Emreet al. 2003; Wang et al. 2005) to find out an alternative sources of protein As compared to FM, animal wastes contains a marginally lower concentration of one or more amino acids essential for fish, including methionine +cystine, lysine, and phenylalanine [75, 89, 33, 55].

Production of microbial silage

The pH of fish offal and chicken gut declined to 3.99 and 3.60 from initial pH 5.40 and 4.95 respectively on day 3 (Table 1). There was significant relationship between decreasing of pH and silage process that was analyzed by one-way analysis of variance (ANOVA) as the significance level ($p<0.05$). Along with decrease in pH, gradual liquefaction of the two raw materials started on day 3 and completed on day 14. In addition, the color of fish offal changed from initial grey to black greenish and color of chicken gut from light brown to brown respectively. At the beginning, smell of the two raw wastes was characteristic of fish and chicken which changed into pungent malty or fermented aroma. Development of mould or insect infestation was not visible in the ensiled products and pH stabled below 3.90 till day 14.

Table 1: Changes in Different Parameters of Fish Offal (FO) and Chicken Gut (CG) During Microbial Ensilation

Day	pH		Liquefaction		Color		Smell		Remark
	FO	CG	FO	CG	FO	CG	FO	CG	
0	5.40	4.95	Thick	Thick	Grey	Light brown	Fish odor	Chicken odor	Ensilage process
3	3.99	3.60	Semi liquid	Semi liquid	Same	Same	Fermented aroma pungent kerosene like	Fermented aroma pungent	Ensilage process, no mould or insects
7	3.97	3.80	Almost liquefied	Almost liquefied	Black greenish	Brown	Same	Same	Ensilage process, no mould or insect
14	3.95	3.83	Liquid silage	Liquid silage	Same	Same	Same	Same	Fish offal and Chicken gut fermented into silage

In case of shrimp head, sharp decline of pH value from initial 6.87 to 4.87 was observed on day 3 and gradually decreased to 4.31 on day 21 (Table 2). The minced shrimp head started to liquefy on day 3 and completed on day 21 with undigested portion as residue at the bottom. Liquefaction was accompanied with the change in color of shrimp head from initial brown to bright brown on day 7 and remained same till end of ensilage. Besides, the characteristic smell of shrimp head turned into fermented pleasant aroma by day 7. Spoilage or growth of mould was not visible and pH remained stable <4.5 through ensilage. Considering pH, liquefaction, color, smell and absence of spoilage, it was confirmed that fish offal, chicken gut were ensiled by day 14 and shrimp head by day 21 and thus successfully converted into liquid silages.

Table 2: Changes in Different Parameters of Shrimp Head (SH) during Microbial Ensilation

Day	pH	Liquefaction	Color	Smell	Remark
	SH	SH	SH	SH	
0	6.87	Thick	Brown	Shrimp Odor	Ensilage Process
3	4.87	Semi liquid	Same	Same	Ensilage Process, no mould or insects
7	4.39	Almost liquefied	Bright brown	Fermented aroma pleasant	Ensilage process, no mould or insects
14	4.30	Almost liquefied	Same	Same	Ensilage process, no mould or insects
21	4.31	Liquid silage and residue	Same	Same	Shrimp head fermented into silage

[90] prepared fermented silage from fish by-products (head, skin, fins and viscera) by mixing minced fish by-products (60%) with orange peel (30%) as filler, molasses (5%) and yoghurt (5%) and incubated at 30-38^{0}C temperature. In addition, potassium sorbate solution (1%) was used as antimicrobial agent. The ensilage process completed after 30 days and a liquid silage of pH of 4.5 was obtained having brownish color and strong fish odor. It can be seen that ensilage took more time than the present study which may happen as the rate of liquefaction depends on the activity of digestive enzymes, pH, temperature and proximate composition of raw materials [91]. In addition, amount of molasses and yoghurt used in silage mixture were lower than the present experiment which may have influence on ensilage time. Though the quantities of carbohydrates used are quite variable, [41] recommended at least 10% addition of molasses to produce a stable silage and in the present experiment, 15% molasses was used for fermentation. [59]fermented fish offal (viscera of cultured carp) with mustard oil cake, rice bran, microbial suspension (10^8 cell.ml^{-1}), molasses and water anaerobically at 27-30^{0}C. Completion of fermentation was indicated by the generation of a characteristic pleasant aroma on 12th day for 25% fish offal mixture and on 18th day for 30% fish offal mixture respectively which is comparable with the result of present study. They found the pH of the fermented mixture gradually decreased from initial value of 8.0-8.2 to 4.4-4.6 respectively which differs

from the pH of fish offal silage obtained in the present study. However, since initial pH of raw materials varies, the pH drop will depend on this value [92]. According to [93], desired pH of successful fermentation ranges from 4.0-4.5 which matches with the present study as pH of fish offal reduced below 4 within 72 hours and remained so till ensilage. [94]reported that fermentation of poultry intestine was accelerated by higher temperature and a microbiologically sterile silage of pH 4.2 was obtained in 24, 6 and 5 hour in samples fermented at 26±2°C, 37°C, both without starter culture and at 37°C with starter culture, respectively. So, it can be said that higher incubation temperature and inoculation of 5% pre-fermented silage as starter culture differing from the present experiment may have influenced the fermentation or pH reduction. In another investigation, [94] obtained poultry intestine silage by fermenting with no less than 10% molasses at ambient temperature (26 ± 2°C) for 6 days whereas in the present study ensilage took 14 days to complete. In fact, standardization of fermentation process is difficult to achieve even though same substrate is used due to differences in combination and proportion of substrate and variations in fermentation conditions [95].[96]fermented shrimp wastes (20 kg) comprising heads of different *Penaeous* species with suspension of *Lactobacillus plantarum* (50 ml/kg) and sugar cane molasses (150 ml/kg) and obtained liquid silage after 14 days of ensilage. The pH value of the silage dropped from initial 7.10 to 4.39 on 14th day which is comparable with the present experiment where ensilage of shrimp head completed after 21 days and pH value declined from initial 6.87 to 4.31. It should be noted that this drop of pH is within the recommended value for successful silage fermentation [97] and also supported by the reports of [98 and 99].

Isolation and identification of lactic acid bacteria from microbial silages and yoghurt

After anaerobic incubation of inoculated MRS agar plates at 37^0C for 72 hours, all four isolates produced cream colored, circular, convex, shiny, moist colonies with smooth edge which is similar to the morphological characteristics of *Lactobacillusspp.* In microscopic view, the cells were found gram positive (i.e. retaining purple color) rod or coccus bacteria. No spreading of colony was found after incubation (in MRS broth with agar powder) in anaerobic condition for 72 hours and confirmed as non-motile. All the isolates were found negative in catalase, oxidase citrate and indole test confirming the biochemical characteristics of *Lactobacillus* bacteria. Considering the morphological and biochemical characteristics, the four strains (isolates) were identified as *Lactobacillus* bacteria. and the results are shown in Table 3.

Table 3: Morphological and biochemical characteristics of four isolates: fish offal silage

Test parameters	Results			
	Isolate -1 FOS	Isolate 2- CGS	Isolate 3- SHS	Isolate 4-Yoghurt
Macroscopic single colony morphology	Cream colored, convex, circular	Cream colored, convex, circular	Cream colored, convex, circular	Cream colored, convex, circular
Motility	-	-	-	-
Gram stain	+	+	+	+
Microscopic cell morphology	Medium rod shaped	Small, circular	Short rod	Short rod
Catalase test	-	-	-	-
Oxidase test	-	-	-	-
Simmon's citrate slant test	-	-	-	-
Indole test	-	-	-	-

(+ = positive result in gram staining, motility and biochemical reactions; - = negative result in gram staining, motility and biochemical reactions)

Isolated *Lactobacillus* strains were preserved in T_1N_1 media for species level identification in future by DNA sequencing method.

Proximate composition of selected animal wastes and produced microbial silages

The proximate compositions of selected animal wastes (i.e. fish offal, chicken gut and shrimp head) showed that crude protein (CP) content was highest in shrimp head (70%) followed by chicken gut (47%) and fish offal (32%) (Fig 1). [100] found the CP rate of silage as 14.9%, while [45] reported a CP rate of 18.20% for a silage made of a whole pearl mullet. [101] found a CL rate of 14.84 %, [102] reported a value of 12.20%, while a CL rate of 3.44% was found by [45] for silage made of whole pearl mullet. While highest crude fat (CF) was found in fish offal (30%) followed by shrimp head (20%) and chicken gut (9%) respectively. Highest ash content was found in shrimp head (20%) and lowest in fish offal (4%). In the present study, all three raw materials were found to have good percentage of protein i.e. >30% and on the basis of proximate composition, chicken gut seems to be more promising as feed ingredient.

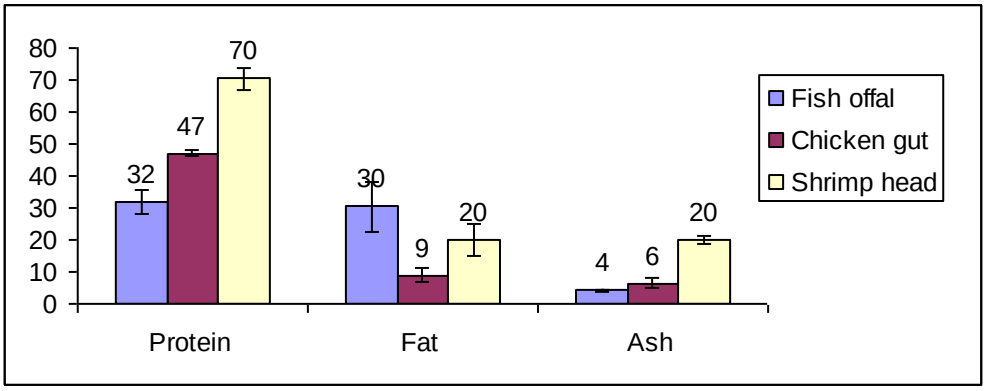

Figure 1: Proximate composition of the raw animal wastes (g. 100^{-1} Dry matter)

The CP of fish offal (32%) obtained from the present study is within the range of CP of carp viscera (31.5% - 38.9%) as reported by [59]. In addition, 30% crude fat of fish offal found in the present experiment is lower but compares favorably with the fat content of carp viscera i.e. 40.6% - 43.8% on a dry matter basis. The protein of chicken gut (47%) found in the present study is much higher than the protein content of 12.4% obtained from the poultry intestines reported by Shaw *et al.*(1998). However, apart from protein, fat (9%), ash (6%) and moisture content (73%) of chicken gut The CP of fish offal (32%) obtained from the present study is within the range of CP of carp viscera (31.5% - 38.9%) as reported by [59]. In addition, 30% crude fat of fish offal found in the present experiment is lower but compares favorably with the fat content of carp viscera i.e. 40.6% - 43.8% on a dry matter basis. The protein of chicken gut (47%) found in the present study is much higher than the protein content of 12.4% obtained from the poultry intestines reported by Shaw *et al.*(1998). However, apart from protein, fat (9%), ash (6%) and moisture content (73%) of chicken gut found in the present experiment are comparable with the fat (12.4%), ash (1.7%) and moisture (75.8%) content of poultry intestines [94]. The CP (70%) of shrimp head found in the present study showed that this waste product is rich in protein. The shrimp head CP of present findings is higher than the CP of 54.8% and 49.47% reported by [103], and [104] respectively. These differences in values may be attributable to the type of shrimp wastes (single or mixed species, only head or mixture of head and shell wastes etc.), sources and also freshness of the waste materials. However, ash content of shrimp head (20%) found in the present study is comparable with the findings of [103] and [104] as 27.4%, and 18.39 respectively. The proximate compositions of three microbial silages. The CP content found was highest

in shrimp head silage (31%) followed by chicken gut silage (30%) and fish offal silage (16%) while highest CF content was found in fish offal silage (69%) followed by shrimp head silage (53%) and chicken gut silage (43%).

In the present study, all three silages were found to have good percentage of protein i.e. ≥30% except fish offal silage (Fig 2). The proximate composition of chicken gut silage (CP 30%, CF 43% and ash 5%) in present experiment is comparable with the findings of [105] and [106]in poultry offal silage (CP 28.1%, CF 48.6% and ash 4.41%) and in broiler offal silage rice bran mixture (CP 20±0.7%, CF 30±0.8% and ash 5.1±1.2 %) respectively.

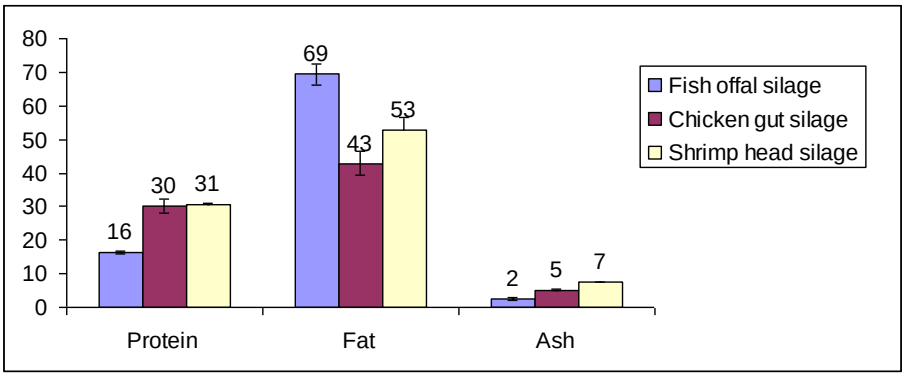

Figure 2: Proximate composition of the microbial silages (g. 100^{-1} Dry matter)

In general, composition of fish silage is very similar to that of the material from which it is made [107]. In the present study, the CP and CF content of raw animal wastes and their resultant silages showed similar trend but CP of fish offal, chicken gut and shrimp head were found to reduce considerably in their respective silages. On the other hand, the CF content of fish offal, chicken gut and shrimp head were found to increase considerably in their resultant silages. This reduction of protein content in the produced silages is comparable with the findings of [59, 108], where the CP of carp viscera ranged between 31.5% - 38.9% and in its silage between 19.2% - 20.7%. Further, [99] recorded lower protein content in tilapia silage (42.35±2.50%) than in the raw material i.e. minced tilapia (62.85±3.40%). The lower protein content of tilapia silage was justified as due to the addition of molasses and slight dilution effect by acid produced [109]. Similar result also reported by [103] for shrimp head silage showing lower CP content (45.6%) than the fresh material (54.8%).In the present study, the higher fat content of produced silages than their raw materials is supported by [103] for shrimp head (CF 4.8% in silage and 2.7% in fresh material). In addition, [99] reported higher fat content of tilapia silage (10.63±1.53%) than that of minced tilapia (7.08± %). The higher fat content of silage could be related to extraction of lactic acid along with ether during fat determination as lactic acid is reported to be soluble in ether [110].

Amino acid composition of the microbial silages

Amino acid (AA) compositions of the three microbial silages are presented in Table 4. The essential amino acid (EAA) content of chicken gut silage showed relatively high (>1%) for isoleucine, lysine, methionine and threonine except for arginine, histidine, leucine and valine which are below 1%. Tryptophan and phenylalanine are not shown in the table as the analysis method used in this study is limited for these two particular essential amino acids. Higher content of three EEA's i.e. isoleucine, lysine and threonine were found in shrimp head silage while in fish offal silage, only lysine was found considerably high. In terms of total amount of EAA, chicken gut silage was found highest (9.33 $g.100^{-1}$ protein) followed by shrimp head silage (8.91 $g.100^{-1}$ protein) and fish offal silage (4.70 $g.100^{-1}$ protein) respectively.

Table 4: Amino acid compositions (EAA, g. 100^{-1} protein) of three microbial silages

Amino acids	Fish offal silage	Chicken gut silage	Shrimp head silage
Arginine	0.18	0.35	0.37
Histidine	0.14	0.37	0.46
Isoleucine	0.41	1.63	1.08
Leucine	0.71	0.45	0.49
Lysine	2.07	2.98	2.96
Methionine	0.40	1.03	0.95
Threonine	0.69	1.80	1.76
Valine	0.10	0.72	0.84
Tyrosine[1]	0.92	1.37	1.52
Aspartic acid[1]	1.63	2.24	2.41
Serine[1]	0.26	0.71	0.82
Glutamic acid[1]	0.96	0.85	1.31
Glycine[1]	1.06	1.34	1.16
Alanine[1]	0.49	1.28	1.25
Total EAA	4.70	9.33	8.91

[1]Non-essential amino acids

In the present study, fish offal silage was found to cover all the indispensable amino acids except for tryptophan and phenylalanine (could not be detected by the analytical method used); however, the values of EAA (and also total amount of EAA) obtained are much lower than those of fermented fish by-product silage (FFS) reported by [90]. The values of EAA found for shrimp head silage in the present experiment are comparable to the findings of [96] except for arginine and leucine. The EAA values obtained for chicken gut silage in the present experiment are similar to the values of citric-acid-ensiled poultry viscera silage meal (PVSM) except for arginine, leucine and valine [111]. A limitation in tryptophan was recorded in PVSM which could not be compared with the present finding.

Storage quality of microbial silages

After completion of ensilage process, the produced silages were stored for 90 days at ambient temperature (25-30^{0}C). During storage period, slight fluctuations in the pH value of the three silages were observed (Table 5). At the end of 90 days, the pH of fish offal silage, chicken gut silage and shrimp head silage were found as 4.0, 3.70 and 4.28 respectively. No change in color and smell of the produced silages and no signs of putrefaction/spoilage were seen.

Table 5: Storage quality of fish offal silage (FOS) and chicken gut silage (CGS) shrimp head silage (SHS) after ensilation

Storage Day	pH			Color			Smell			Putrefaction (insect/mould)
	FOS	CGS	SH	FOS	CGS	SH	FOS	CGS	SH	
0	3.95	3.83	4.31	Black greenish	Brown	Bright brow	Fermented aroma	Fermented aroma	Fermented aroma	Not visible

						n	pungent kerosene like	pungent	pleasant	
15	3.96	3.76	4.29	Same	Same	Same	Same	Same	Same	Same
30	4.01	3.76	4.25	Black greenish	Brown	Same	Same	Same	Same	Same
45	3.95	3.63	4.26	Same	Same	Same	Same	Same	Same	Same
60	3.89	3.74	4.22	Same	Same	Same	Same	Same	Same	Same
75	4.01	3.71	4.31	Same	Same	Same	Same	Same	Same	Same
90	4.00	3.70	4.28	Black greenish	Brown	Bright brown	Pungent Kerosene like	Pungent	Pleasant	Not visible

The rapid and sharp decline of pH at the beginning of ensilation and changes in pH values through ensilation and storage of the three silages are shown in Fig 3. Despite little variations, the pH remained almost stable through the storage period.

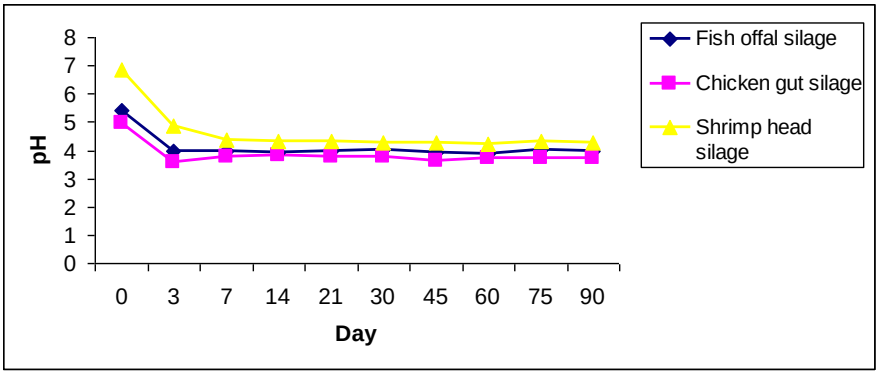

Figure 3: Changes in pH value of the microbial silages during ensilation and storage

Generally, the quality of silage depends on the quality of the raw materials. The fresher the raw materials used for silage preparation, the better the quality of the end product [103]. At present, there are no accepted chemical or biological quality parameters for silage but increase in pH was suggested as a good indication of quality deterioration in fish silage [112]. In the present study, stability of pH below 4.5, absence of microbial spoilage and no visible changes in color or smell throughout the storage indicated that quality of the three silages were preserved up to 90 days. These results are in agreement with fermented silages of short bodied mackerel (short bodied mackerel) stored for 180 days by [113]. According to Tatterson& Windsor (2001), silage at correct acidity can be stored at room temperature up to 6 months without putrefaction and any significant loss of nutrients. The changes mainly occur during storage are solubilization of protein and increase in free fatty acids content.

CONCLUSIONS

From the results obtained in the present study, it can be concluded that silages can be successfully prepared by microbial fermentation of fish offal, chicken gut and shrimp head using yoghurt as a

source of *Lactobacillus*. The crude protein (CP) and crude fat (CF) content of the raw animal wastes and their resultant silages showed similar trend but considerable reduction in CP and increase in CF in their respective silages were found, which is in agreement with the process of ensilage. In terms of total amount of essential amino acids, chicken gut silage was found highest followed by shrimp head

silage and fish offal silage respectively. Stability of pH below 4.5, absence of microbial spoilage and no visible changes in color or smell throughout the storage indicated that quality of the three silages were preserved up to 90 days.

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APPENDICES

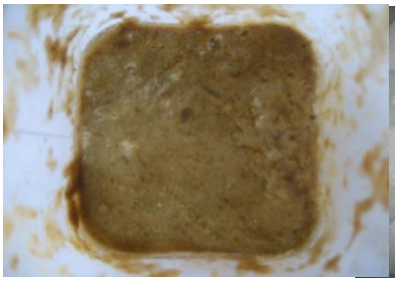
Appendix 1: Photographs

Shrimp head

Chicken gut

Fish offal

Minced shrimp head



Minced chicken gut

Minced fish offal

Protein digestion

Fat extraction

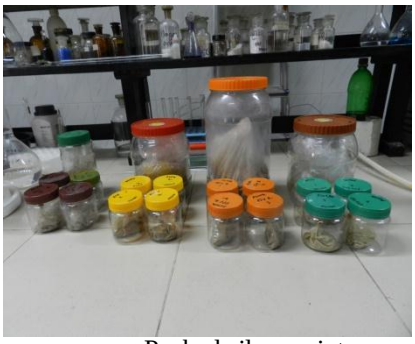
Mincing chicken gut

Fish offal silage

Chicken gut silage

Shrimp head silage

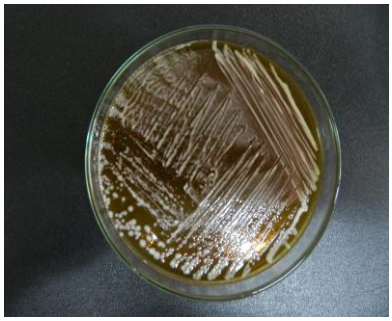
Appendix 2: Photographs



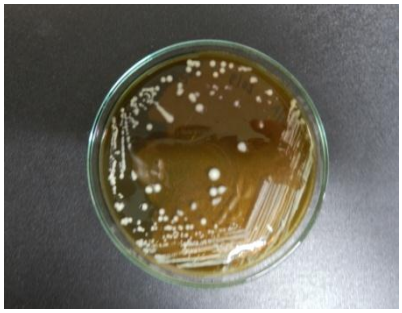
Packed silage mixtures

Fish offal silage (Isolate-1)

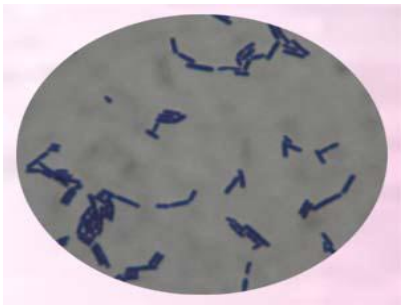
Chicken gut silage (Isolate-2)



Shrimp head silage (Isolate-3)



Yoghurt (Isolate-4)



Microscopic view after gram staining