



An approach to converting raw animal waste to fish feed formulation: a case study for sustainable industrial waste management using acid silage methods

M Abdullah Al^{a,s}, A Akhtar^{*a,†}, M N A Sikder^a, M W Alam^b & H Zamal^c

^aFaculty of Marine Sciences and Fisheries, Institute of Marine Sciences, University of Chittagong, Chittagong – 4331, Bangladesh

^bFaculty of Marine Sciences and Fisheries, Department of Oceanography, University of Chittagong, Chittagong – 4331, Bangladesh

^cFaculty of Marine Sciences and Fisheries, Department of Fisheries, University of Chittagong, Chittagong – 4331, Bangladesh

[E-mail: [†]aysha_imsfcu@yahoo.com; ^smamun.imsfcu16@gmail.com]

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Acid silage is a convenient method for converting raw poultry wastes (i.e., chicken offal) to fish feed ingredients. To investigate the potentiality of chicken offal for fish feed formulation; combination of two acids were used in the trail (90-days). The proximate compositions of raw offal contained 37.22 % moisture, 37.24 % protein, 18.80 % fat, 19.04 % ash and 62.78 % dry matter; ensiled offal contained 25.02 % moisture, 47.31 % protein, 13.79 % fat, 13.45 % ash and 74.98 % dry matter; and post storage offal contained 23.05 % moisture, 44.33 % protein, 13.10 % fat, 12.75 % ash and 76.95 % dry matter. It took 13 days to convert raw offal into final product that was confirmed by physical observation in necked eyes (i.e., raw smell converted into pungent acidic; thick solid form liquefied; raw pink color converted to bright brownish and absence of microorganisms). No significant difference was observed during trail and storage period for all components as moisture, protein, fat, ash and dry matter. The pH value was found to be stable at 1.90 in 90-days storage period. These results suggest that chicken offal could be potentially used for aqua feed formulation that would be a cost effective means for industrial waste management.

[**Keywords:** Acid silage, Chicken offal, Feed formulation, Microbial activity, Waste management]

Introduction

Preservation techniques of biodegradable waste materials by addition of several acids are known as acid silages or acid ensilage¹⁻⁵. Acid ensilage using poultry by-product has been used in many fish farming industries as feed ingredients with low cost investment⁶⁻⁹. Investigations have reported that chemicals like propionic acid, formic acid, sulfuric acid and hydrochloric acid are used for controlling microorganisms at lower pH level¹⁰⁻¹². However, several other acids such as benzoic acid, propionic acid, sorbic acid and common salt have also been used for long time preservation in feed industries^{13-15,19,20}. These preservatives have significant roles to prevent/retard chemical and biological deterioration of foods. Previous studies have reported that using either single or combination of phosphoric acid, citric acid, sulfuric acid and hydrochloric acid can reduce pH drastically (4.3 to 1.0) to obtain sterile materials⁹⁻¹⁵.

Feed is one of the major inputs in aquaculture^{19,20}. The success of fish farming depends primarily on the provision of adequate nutritionally balanced feed which is acceptable to the fish^{19,20}. Fishmeal is the main animal protein source in aqua diet formulation

which is quite expensive and in short supply^{19,20}. Alternatively, squilla, shrimp/prawn, meat and bone, hydrolyzed feather, flashings and blood, dried fish and chicken viscera have been tried in diets replacing fishmeal either partially or fully. Although, these are not sufficient to meet the growing demands of fish raising industries^{21,22,28}. However, in recent years, application of acid of ensiled products in aquatic feeds are increasing to minimize feed cost^{22,23}. Agro-based rural business (i.e., fish/poultry farms) are well established and produces huge by products; and only a small quantity of these by products are used either sun-dried or direct throw in animal feeds²¹⁻²³. In this regards, considerable amount are discarded in open places or dumped in the river which cause serious health hazards including environmental pollution²³. Therefore, an optimized acid ensilage need to be developed in the laboratory to prepare pathogens free silage product^{22,23}. Studies have reported that broiler offal processed by lactic fermentation contained reduced numbers of pathogens²³⁻²⁵. Since microorganisms are relatively inactive in ensilage than raw materials, it can ensure potentially high nutritive value which is suitable for feeding and more

digestible for fishes²²⁻²⁵. The pH level in feeds plays an important role for determining the survival and growth of microorganisms during feed formulation including processing, storing and distribution²⁴. In modern food preservation, low pH level below 4 is prerequisite to resist microbial growth, reduces microbial heat resistance and food poisoning²⁶⁻²⁸.

In the present study, a 90-days experimental trial was conducted to convert raw chicken offal to acid silage product using a combination of 99 % acetic acid and 99 % sulfuric acid with 1.5:1.5 ratio. The objectives of the research were: (1) to observe the changes in physical characteristic of raw materials during silage production and 90-days storage period; (2) to assess the proximate composition of raw, ensilage and storage product in order to recommend the feasibility of chicken offal silage in fish feed industries.

Materials and Methods

Sample collection and processing

Raw samples were collected from poultry shops available in Bahaddarhat local market, Chittagong, Bangladesh and immediately preserved in icebox (-20 °C).The preserved samples transported to the laboratory for biochemical assessment in the Faculty of Marine Sciences and Fisheries, University of Chittagong, Bangladesh.

After freezing (1-day), samples were thawed and minced through a mincer (supper mincer made in Japan with 3.50 mm sieve), then minced samples used for silage preparation. The detail experimental working flow was depicted in Figure 1. The proximate

composition analyses were done by method AOAC (Association of Official Analytical Chemists)⁴.

Preparation of silage

A required amount of sample was separated for proximate composition analysis of raw materials. The initial pH of the sample was recorded using a digital pH meter after adjusting temperature.

In brief, 100 g of minced sample was taken in each glass jar. Acetic acid 1.5 ml and sulfuric acid 1.5 ml with a total volume of 3 ml (1.5 + 1.5 ratio) acids were added from a beaker through a pipette in the samples. A total of 3 replicated experiments were setup for each sample during the study for avoiding experimental error and observed significant differences among the replicates. The initial pH value of the raw material was found 6.80. The addition of acid stopped when pH value reached at 3.98 and samples were continuously being mixed using glass rod to avoid untreated offal pockets. Routine monitoring (i.e., physical properties, mixing and smelling) and pH were done every day during trial and acid was added when necessary (if the pH value > 4).

Data analysis

The proximate composition such as moisture, fat, ash and dry matter were measured by following AOAC methods using formulas below:

$$\% \text{ of moisture} = \{(\text{Weight of original sample} - \text{Weight of dried sample}) / \text{Weight of original sample}\} \times 100 \dots (1)$$

$$\% \text{ of ash} = (\text{Weight of ash} \div \text{Weight of sample}) \times 100 \dots (2)$$

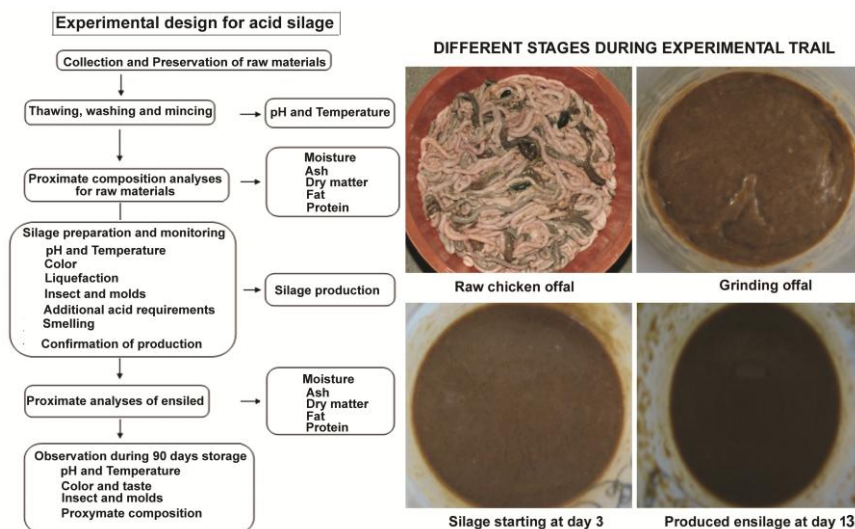


Fig. 1 — A brief description of experimental procedure and different stages during silage production.

% of Nitrogen = $\{(\text{Volume of HCl} \times \text{N. of HCl} \times 14) \div \text{Weight of sample}\} \times 100$... (3)

% of Crude Protein = % of Nitrogen \times Conversion factor (Conversion factors for animals and plants origin are 6.25 & 5.90, respectively) ... (4)

% of crude fat = $\{\text{Corrected weight of fat} \div \text{Weight of sample}\} \times 100$... (5)

% of dry matter = $100 - \% \text{ of moisture}$... (6)

[Protein and fat were determined by dry matter basis]

Results

Routine monitoring of pH level

A daily routine was performed to check smell, color, insect/mold and liquefaction observed *in situ* with necked eyes. This routine was followed once a day during production period (0 to 13 days) and twice each week during storage period. The physical characteristics change during silage production is summarized in Table 1. The pH value on day 3 slightly increased at 4.10. Therefore, additional 5 ml of acids mixture were needed to reduce pH value below 4. The final pH value of samples gained at 1.90 after addition of total 8 ml of acids (3 + 5 ml) during the trial. The temporal variation in pH change is shown in Figure 2. Production of chicken offal ensilage was confirmed by liquefaction that was started on day 3 and finished on day 13 (Table 1 & Fig. 2). It took a total of 8 ml of acids and 13 days for silage production.

Proximate composition of raw and ensilage

The proximate compositions of raw chicken offal contained 37.22 % moisture, 37.24 % protein, 18.80 % fat, 19.04 % ash and 62.78 % dry matter while produced ensilage contained 25.02 % moisture, 47.31 % protein, 13.79 % fat, 13.45 % ash and 74.98 % dry matter (Table 2 & Fig. 3).

Variation in proximate composition of raw, ensilage and 90-days storage

Significant variations in proximate composition of chicken offal ensilage among the three forms are shown in Table 3. After silage production, the ensilage jar was kept for 90-days for observing significant changes (if any) of composition. The proximate composition in storage ensilage contained 23.05 % moisture, 44.33 % protein, 13.10 % fat, 12.75 % ash and 76.95 % dry matter (Fig. 4).

Routine monitoring of physical characteristics of the storage ensilage

The changes in physical characteristics during 90-days storage period of ensilage is summarized in Table 4. During storage there was no significant physical changes observed except a little fluctuation of pH values (Fig. 5).

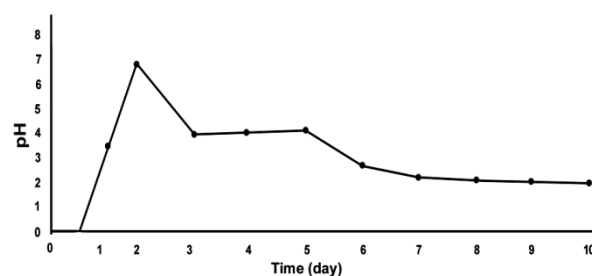


Fig. 2 — Temporal change of pH value during silage production

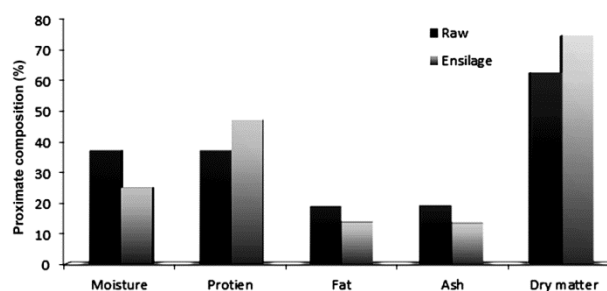


Fig. 3 — Proximate composition of raw and produced ensilage chicken offal

Table 1 — Routine monitoring of raw chicken offal during acid silage production

Day	pH	Liquefaction	Color	Smell	Remarks
0	6.80-3.98	Thick	Brownish	Chicken offal flavor	Silage Processing
3	3.76	Semi liquid	Same	Mild acid flavor	Silage starting
10	1.98	liquid	Light Brown	Pungent acid flavor	Silage produced

Table 2 — Proximate composition of raw chicken offal

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Dry matter (%)
Raw Chicken offal	37.22	37.24	18.80	19.04	62.78
Produced ensilage	25.02	47.31	13.79	13.45	74.98

Table 3 — Variation in proximate composition of raw, ensilage and storage

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Dry matter (%)
Raw offal	37.22	37.24	18.80	19.04	62.78
Ensilage	25.02	47.31	13.79	13.45	74.98
Storage	23.05	44.33	13.10	12.75	76.95

Table 4 — Physical characteristics changes during 90 days storage period of ensilage

Time (Day)	pH	Liquefaction	Color	Smell	Remarks
Day 16	1.98	Liquid	Brownish	Pungent acidic	Ensilage
Day 19	1.98	same	Same	Same	Same
Day 21	1.93	same	Same	Same	Same
Day 24	2.02	same	Same	Same	Same
Day 27	1.90	same	Same	Same	Same
Day 30	1.90	same	Same	Same	Same
Day 37	1.90	same	Same	Same	Same
Day 44	1.90	same	Same	Same	Same
Day 51	1.90	same	Same	Same	Same
Day 58	1.90	same	Same	Same	Same
Day 65	1.92	same	Same	Same	Same
Day 72	1.93	same	Same	Same	Same
Day 79	1.90	same	Same	Same	Same
Day 86	1.90	same	Same	Same	Same
Day 90	1.90	same	Same	Same	Same

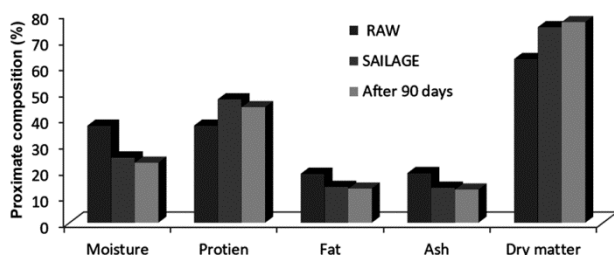


Fig. 4 — Variation in proximate composition in raw, ensilage and storage ensilage

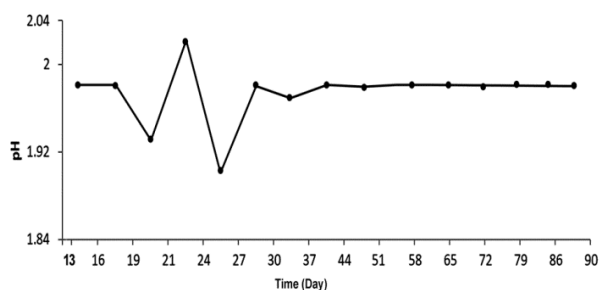


Fig. 5 — Changing of pH value during (90 days) storage period of ensilage

Discussion

Industrial waste management now is one of the crucial issue for pollution free and healthy environment^{1-5,9,16}. Therefore, we hypothesize that silage production using different acids would be a cost effective technique for converting raw poultry waste to aquatic diet formulation for agro-based

industries. The output of this technique would lead to reduce environmental degradation and provide fish feed ingredient within low cost investment.

In this study, proximate composition of chicken offal contained high level of protein up to 37.24 ~ 47.31 %. Significant change was observed for fat contents that were reduced evidently from raw to ensilage. Acid silage production using chicken offal are highly nutritive and cost effective due to its higher protein value^{7,8,10,11} which is comparable to present results of ensilage contained around 47.31 % protein. Thus, these results suggest that higher protein and low fat contents of poultry waste may have potentials to convert into aquatic diet formulation.

Based on the present findings, produced ensilage showed that moisture and fat contents reduced significantly than in the raw offal, which is essential to control rancidity of feedstuffs and protect from microorganism during long time storage/preservation. Investigations have reported that breakdown of organic substrates in feedstuffs by microbes depends on fat and moisture contents¹⁵⁻¹⁷. However, the fat and moisture contents after ensilage production showed significantly lower than raw materials. Thus, these findings simply prove that ensilage of poultry offal using acids might increase the efficiency of aquatic diets by reducing fat and moisture contents.

In this study, mold or fungal attack was not visible during ensilage production through naked eyes that might be due to stable and low pH level. For long time preservation microbial growth, microbial heat resistance and food poisoning organisms are well controlled at lower pH level have reported in several studies^{1-7,26-28}. Therefore, present findings are consistent with other researches that shows lower pH level controlled the microbial growth and increased storage quality of the ensilage.

In summary, poultry offal has high protein value and it would be applicable in aquatic diet formulation. Our laboratory trial revealed that produced ensilage retained adequate nutritional value that might be prospective low cost protein ingredient for aquaculture feed. More so, application of acids for silage production using poultry by product will protect environmental hazards biological wasters. However, more researches are recommended with details elaboration with feeding trail to justify this conclusion.

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Conflict of Interest

The authors declared that there are no conflicts of interest from financial authority for this research.

Author Contributions

MAA conceived the idea with the help of HZ, and AA and designed the laboratory experiment. MAA, performed laboratory experiment including data analysis and wrote first draft of manuscript with the help of MWA and MNAZS while HZ and AA monitored the experimental workflow, data analysis and revised the manuscript. All authors have revised and corrected the final version of the manuscript.

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