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Nutritive Value of Fish Waste Silage Meal

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The Fish Waste Silage Meal (FWSM) was prepared using fish offals, *Lactobacillus plantarum* culture (10^8 cfu/ml), jaggery and De-Oiled Rice Bran (DORB). Fish waste silage (FWS) was prepared by incubating minced and autoclaved fish offal slurry with 10 percent jaggery and 5 percent *Lactobacillus plantarum* culture (10^8 cfu/ml) in an anaerobic condition for 14 days at room temperature (37°C) with occasional mixing. After the fermentation period, the FWS was mixed with DORB (Slurry: DORB, 7:1) uniformly and sun-dried for 3-4 days for the production of FWSM, 0.72 kg FWSM was produced from 1.0 kg of FWS. The FWSM was analysed for its nutritive value, the FWSM contains 10.8% moisture, 27.5% crude protein (CP), 4.97% crude fiber, 14.01% ether extract and 17.09% total ash. Since FWSM is prepared from inedible parts of the fish; FWSM was screened for major pathogenic microorganisms' *viz., E. coli*, *Salmonella* and *Clostridium* and found negative. Further, FWSM is analyzed for five common mycotoxins (Aflatoxin (B_1, B_2, G_1 and G_2), Ochratoxin, T-2 toxin, Citrinin and Zearalenone) and found negative. The transformation of fish

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waste into FWSM not only produces a highly nutritious end product but also offers an alternative and beneficial means of utilization. The FWSM contains 27.5% CP, which is almost equal to the 50% CP value of soybean meal, thus it can be used as a feed ingredient in animal feed preparation.

Keywords: Fish; meal; offals; silage; waste.

1. INTRODUCTION

Feed is a major component in animal farming since it accounts for 60–80% of production costs. The quality and degree of incorporation of the proteinaceous feed ingredient in the diet determine the feed cost per kilogram. Ozyurt *et al*. (2017) mentioned that the primary constraint in animal husbandry is the lack of protein, which is mostly because it is expensive and scarce.

Kim and Ester (2001) reported that animal-derived proteins have shown great promise, while plant-based protein sources often lack some important amino acids (Vidotti *et al*., 2003). Hauzoukim *et al*. (2021) mentioned that animal protein sources have certain advantages over vegetable proteins, such as balanced amino acid composition and low levels of anti-nutritional substances which have the potential to interfere with the digestion and absorption of other nutrients.

Utilizing fish waste as an alternate protein source is one of the options. Making use of the offals obtained from fish processing facilities has the possibility of substituting the high-protein supplements. The traditional method of fishmeal production that has been used for many years in India has resulted in rising feed costs over time because the process is complex and requires a large amount of energy, capital, and manpower. This can be replaced by "FISH WASTE SILAGE," which is a simple and affordable substitute. Encouraging the conversion of fish waste into silage using an inexpensive technique such as lactic acid fermentation may help address the issue of limited protein sources for animal feed.

Zynudheen *et al.* (2017) suggested that fish ensiling has been proposed as an alternative method to enhance the utilization of fish waste and fishery by-products as a high-quality source of protein for animal feeding. Fish silage is a liquified product made from whole fish or parts of fish through acid preservation (Tatterson & Windsor, 1974), fermentation of lactic acid bacteria, or both (Raa *et al*., 1982), which improves the action of enzymes in the fish to break down tissues and limits the growth of pathogenic bacteria. Vazquez *et al.* (2011) reported that using acids (especially organic acids) is more expensive and mimics the bio-conversions that lactic acid bacteria (LAB) fermentation could carry out.

Fish waste ensiling is a classical technique for preserving organic matter against deterioration and can be utilized as a probiotic ingredient for poultry feeding (Hammoumi *et al.,* 1998). Tropea *et al.* (2021) proposed that, among the various microbes used, lactic acid bacteria have an advantage over other microbes for fermentation because they are generally recognized as safe. Further, the final fermented product is low in spoilage microorganisms and high in beneficial microorganisms, indicating a safe and nutritious final substrate enhanced with added value. Furthermore, the products of *Lactobacillus* fermentation have been reported to have additional beneficial effects on the intestines of various aquatic animals (anti-microbial properties, anti-oxidative properties), making them ideal for food/feed applications. Indeed, they are ideal for use as probiotic aquaculture feeds as they adapt well to the gut environment of both aquatic and domestic animals. Hence, the present study aims to evaluate the nutritive value and safety of fish waste silage meal as an alternate protein source for animal feed preparations.

2. MATERIALS AND METHODS

2.1 Preparation of Fish Waste Silage (FWS)

The *Lactobacillus plantarum* (MTCC 2621) culture for the preparation of FWS was obtained as a freeze-dried culture from Microbial Type Cell Culture (MTCC), Chandigarh and it was revived using deMan, Rogosa, Sharpe (MRS) broth. The desired quantity of 10^8 colony-forming units (cfu/ml) was obtained with serial dilution in physiological saline (0.9%) and cultured in MRS Agar.

Fig. 1a. Fish waste

Fig. 1b. Fish waste - cooked

Fig. 1c. Fish waste - slurry

Fig. 1d. Autoclaved – FW slurry

Fig. 1e. Addition of 10% jaggery

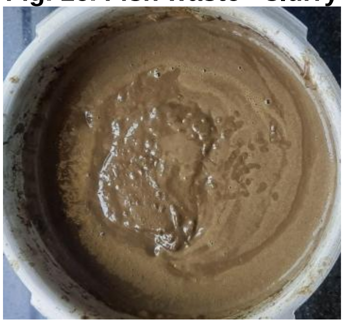

Fig. 1f. Addition of 5% of *Lactobacillus plantarum* culture containing 10^8cfu/ml

Fig. 1g. Culture and Jaggery mixed FW slurry

Fig. 1h. Anaerobic fermentation of FW slurry for 14 days

Fig. 1i. Fermented FW slurry

Fig. 1j. Mixing of DORB (Slurry: DORB, 7:1)

Fig. 1k. Sun drying of FWSM

Fig. 1l. Final FWSM after sun drying

Fig. 1. Fish waste silage and fish waste silage meal preparation steps

Fish wastes for silage production were procured from the local fish market, Orathanadu, Thanjavur. It primarily consists of the head, tail, fins, scales, viscera, gut contents and body trimmings of various fishes like Sardine, Mackerel, Common carp, Tilapia, Red snapper, Murrel, Silver scabbard and Silver pomfret.

To proceed with the preparation of silage (FWS), fish offals were ground and autoclaved (121ºC under 15 lbs) for 15 minutes to make a slurry and allowed to cool down to room temperature. The cooled slurry was transferred into a plastic container and 10% jaggery and 5% *Lactobacillus plantarum* culture containing 10^8cfu/ml were added and mixed uniformly and sealed air tightly to facilitate the anaerobic fermentation for 14 days at room temperature (37°C) with occasional mixing of contents. After 14 days of anaerobic fermentation, the fermented silage was opened and pH content of the silage was measured using a pH meter (Model 101, deluxe pH meter) and strips. The entire procedure was adopted for the preparation of five different FWSs at different durations.

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2.2 Preparation of Fish Waste Silage Meal (FWSM)

After the fermentation period, the FWS was mixed with DORB (Slurry: DORB, 7:1) uniformly and sun-dried for 3-4 days for the production of FWSM, the dried material was ground to get a homogenized particle size and then stored in an air-tight container for the future use. The entire procedure adopted for the preparation of five different FWSMs at different durations is given in Fig.1.

2.3 pH of FWS

The pH content of the FWS was estimated using pH meter (Model 101, deluxe pH meter) and strips.

2.4 Microbial and Mycotoxin Analysis of FWS and FWSM

The presence of pathogenic microorganisms was done by streaking FWSM liquid (prepared by adding 1 g FWSM in 9 ml of sterile peptone water) on sterile Eosin Methylene Blue (EMB) agar, Salmonella Shigella (SS) agar and MRS agar for the identification of *Escherichia coli, Salmonella,* and *Lactobacillus* respectively and inoculated in Robertson Cooked Meat (RCM) broth for *Clostridium* (Hassan & Heath, 1986).

The FWSM samples were sent to AFAQAL, Namakkal for mycotoxin ((Aflatoxin (B_1, B_2, G_1 and G_2), Ochratoxin, T-2 toxin, Citrinin and Zearalenone) analysis.

2.5 Nutritive value of FWSM

The proximate composition of FWSM was analysed as per the guidelines of AOAC (2000). For the estimation of crude protein, ether extract and crude fibre, the following instruments were used Kel Plus, SOCS PLUS and Fibertec (Model No. 1030), respectively. The yield of silage was calculated by the difference (before and after drying) in weight.

2.6 Statistical Analysis

Each silage was considered as an experimental unit for data analysis. All the data obtained from the study were subjected to statistical analysis (one way ANOVA). All the data were analyzed using Statistical Package SPSS (version 20.0).

3. RESULTS AND DISCUSSION

The consistency of the silages varies from liquid to semisolid and it appears to be light brown to dark brown in colour with acceptable odour. An average 0.72 kg FWSM was produced from 1.0 kg of FWS. The nutritive value, pH, microbial and mycotoxin levels of produced FWSM were presented in Table 1. The average pH content of the silage is 4.0±0.16, the silage is quite acidic, which guarantees fermentation and it is essential for the preservation and inhibition of the growth of undesirable microorganisms during the ensiling process (Raa *et al*., 1982). Similar to the current finding, several authors have also reported that the pH content of silage ranges from 3.2 - 4.7 (Kjos *et al.,*1999; Collazos & Guio, 2007; Gullu *et al.,* 2015;). The changes in the protein fraction indicate that variations in the liquefaction process can be influenced by low pH which enhances the action of endogenous proteases enzyme (Alwan *et al*., 1993).

The moisture content in FWSM samples ranges from 9.79 to 13.47% (avg. 10.80%). A similar kind of finding was reported by Yathavamoorthi *et al.* (2015), who reported that the moisture content of co-dried silage with various filler materials varies between 3 to 10 percent, this indicates the effective moisture removal (Ologhobo *et al.,* 1988; Babu *et al.,* 2005; Vijayan *et al.,* 2009). Low moisture content is essential for preserving the silage and preventing the growth of spoilage microorganisms and facilitates easy handling, transportation and storage (Vijayan *et al*., 2009).

Table 1. Chemical composition (% on DM basis) of FWSM

Attributes (%)	FWSM
Moisture	10.80 ± 0.39
Dry matter	89.20 ± 0.39
Organic matter	82.91 ± 0.96
Crude protein	27.57 ± 0.90
Ether Extract	14.01 ± 1.50
Crude fiber	4.97 ± 0.65
Total ash	17.09 ± 0.96
Acid insoluble ash	2.13 ± 0.09
Nitrogen free extract	36.36 ± 1.54
Gross energy (kcal/kg)	5804.81 ± 229.11

Table 2. Microbial and mycotoxin analysis of fish waste silage meal (FWSM)

Microorganism	Indication
Escherichia coli	Nil
Salmonella	Nil
Clostridium	Nil
Mycotoxins	
Aflatoxin (B_1, B_2, G_1 and G_2)	Nil
Ochratoxin	Nil
T-2 toxin	Nil
Citrinin	Nil
Zearalenone	Nil

The total ash content in the FWSM samples varies from 13.17 to 22.28% (avg. 17.09%). The total ash content of co-dried silage with various filler materials varies between 5 to 15 percent (Babu *et al.,* 2005; Vijayan *et al.,* 2009; Yathavamoorthi *et al.,* 2015). The high bone content in the raw materials (Alwan *et al*., 1993; Hammoumi *et al*., 1998) or contaminants in the carbohydrate source used for fermentation (Ali & Sahu, 2002; Pagarkar *et al*., 2006) could be the cause of the higher ash content. The total ash content of co-dried silage with various filler materials varies between 5 to 15 percent (Babu *et al.,* 2005; Vijayan *et al.,* 2009; Yathavamoorthi *et al.,* 2015).

The average crude protein content of the FWSM samples was measured as 24.42 to 31.15% (avg. 27.57%). Compared to the current finding, a wider CP range (26-60%) was reported (Vijayan *et al.,* 2009; Yathavamoorthi *et al.,* 2015) in the co-dried fish silage, this higher CP level could be due to the use of whole fish for silage preparation, in the present study we used fish offals for the silage preparation.

The crude fibre content of the samples ranges from 2.5 to 8.25% (avg. 4.97%). Although the fiber content of fish waste was relatively low (Hammoumi *et al*., 1998; Vijayan *et al*., 2009), the fiber content of DORB (13–15%), which was used as a co-dried material to obtain the final dried product in the present study, maybe the cause of the rise in fibre content of the FWSM.

The ether extract content in the FWSM samples varies from 8.29% to 19.72%. The lipid content of fish meal was lower than the fish silages because of cooking and subsequent pressing, which might remove a certain amount of lipid along with the stick water. The lipid content of fermented silage was higher than acid silage and fish meal may be due to extraction of lactic acid as well along with fats during ether extraction (Ali & Sahu, 2002). In contrast, Babu *et al*. (2005) documented that the fat content of fermented silage was significantly lower (1%), than the acid silage (3-5%) which may be due to the effective trapping of oil within the microbial silage, which is facilitated by the strong binding capacity of the added polysaccharide. Here the increase in fat content may be attributed to the variation in crude fat levels of fish used as raw materials and the efficiency of the defatting process during production as proposed by Johnsen and Skrede (1981). Vidotti *et al*. (2002) also mentioned that the nutritional composition of silage can be easily influenced by the composition of raw materials used for production.

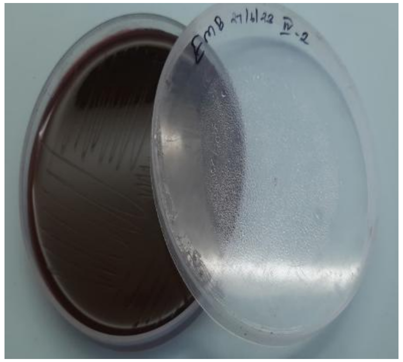

Fig. 2a. *E. coli* negative on EMB agar

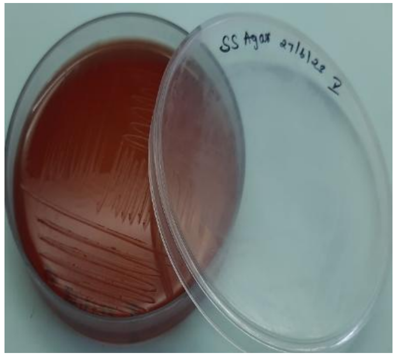

Fig. 2b. *Salmonella* negative on SS agar

Fig. 2c. *Clostridium* negative on RCM broth

Fig. 2d. Lactobacillus positive on MRS agar

Fig. 2. Microbial analysis of fish waste silage meal (FWSM)

The average NFE content of the FWSM is 36.36%, this value was higher than the value reported by Pagarkar *et al*. (2006). Pagarkar *et al*. (2006) estimated the NFE content of bio-fermented silage was higher (10.36%) in comparison with 4% sulfuric acid (7.2%) and 4% organic acid (8.1%). The higher NFE content in the present study could be due to the addition of DORB for co-drying.

The average GE content of the FWSM is 5804.81± 229.11 kcal/kg. Similarly, Gullu *et al.* (2015) calculated the GE content of different silages prepared such as whole pearl millet silage, silage made from the fish processing industry and silage mixture (30% whole pearl millet silage and 70% processing wastes silage) and found that the GE contents were 4805 kcal/kg, 5034 kcal/kg and 5312 kcal/kg, respectively. The GE content of co-dried silage produced with various proportions of maize, cassava, groundnut meal and wheat offal ranges between 4000-4200 kcal/kg (Ologhobo *et al*., 1988).

Since FWSM is prepared from inedible parts of the fish; FWSM was screened for major pathogenic microorganisms' *viz., E. coli*, *Salmonella* and *Clostridium* and found negative. Further, FWSM is analyzed for five common mycotoxins (Aflatoxin (B_1, B_2, G_1 and G_2), Ochratoxin, T-2 toxin, Citrinin and Zearalenone) and found negative (Table 2).

The nutritional composition of Fish Waste Silage Meal (FWSM) shows variability across different samples. Since the fish waste used for the preparation of FWSM is related to different types of fishes, the nutritive value of invidivial species varies, which may be a reason for the variation in the nutritive content of different silages. Each sample offers unique nutritional characteristics, and the selection of FWSM should be based on the specific dietary requirements of the target animal species. Regular monitoring and analysis of FWSM will ensure that they meet the nutritional needs of the intended livestock or aquaculture species, promoting optimal growth and overall health (Candido et al., 2017; Ennouali et al., 2006).

4. CONCLUSION

In conclusion, fish waste silage meal (FWSM) production presents a promising and sustainable solution for utilizing discarded fish by-products. The FWSM contains 27.5% CP, which is almost equal to the 50% CP value of soyabean meal, thus it can be used as a feed ingredient in animal feed preparation. This innovative technique not only aids in waste management but also contributes to a circular economy by converting fish waste into valuable resources of animal feed. Through a well-structured process that involves cooking, autoclaving, fermentation, and sun drying, fish waste is transformed into a nutrient-rich silage with excellent nutritional value. The incorporation of jaggery and *Lactobacillus plantarum* culture during fermentation ensures a safe and hygienic final product, free from harmful bacteria and toxins. The nutritional analysis of the fish waste silage reveals a high protein content, making it a valuable feed supplement for animals.

This eco-friendly approach aligns with the principles of sustainability and resource conservation, reducing the environmental burden associated with fish processing industries. Further research and development in this area can unlock even greater benefits, making fish waste silage a vital component of a more sustainable and environmentally conscious future.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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