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Nutrient Content and Fatty Acid Composition of Twaite Shad Fish Silage

Rifat TEZEL*
Muğla Sıtkı Koçman University, Faculty of Fisheries, Department of Aquaculture, Muğla, Türkiye

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*: https://orcid.org/0000-0002-0870-7049

***Corresponding author's:**
Rifat TEZEL
Muğla Sıtkı Koçman University, Faculty of Fisheries, Department of Aquaculture, Muğla, Türkiye
✉: rifattezel@mu.edu.tr

Abstract: The recycling of fish which cannot be used for human consumption and fish processing industry wastes into feed raw materials is an environmentally friendly method that ensures the efficient and sustainable use of resources. In this study, fish silage was prepared from twaite shad fish, which could not been utilised as human food, by using formic acid application and the nutrient content and fatty acid composition of the produced silages were investigated. The dry matter values of the silages varied between 26.14% and 26.63%, crude protein values between 73.79% and 78.55%, and fat values between 5.09% and 5.46%. It was determined that the amount of crude protein decreased in silages produced by using low doses of formic acid ($p<0.05$). Total mono unsaturated fatty acid (MUFA) value of the silages varied between 18.56% and 19.63% and total poly unsaturated fatty acid (PUFA) value varied between 24.74% and 26.35%. Oleic acid (C18:1n9) was the dominant MUFA with an amount ranging from 12.91% to 14.27% and Docosahexaenoic acid (DHA, C22:6n3) was the dominant PUFA with an amount ranging from 16.39% to 17.54%. ΣSFA, ΣMUFA, ΣPUFA, Σn3 and Σn6 values were statistically similar in silages with different acid ratios ($p>0.05$). Especially crude protein and PUFA content of the produced silages showed that twaite shad fish silage can be utilised in fish feed. The use of fish silage with valuable nutrient content as feed raw material will provide both economic gain and contribute to the protection of the environment.

Keywords: Aquaculture, environment, fish nutrition, formic acid silage, nutritional value, poly unsaturated fatty acids.

Tirsi Balığı Silajının Besin İçeriği ve Yağ Asidi Kompozisyonu

***Sorumlu yazar:**
Rifat TEZEL
Muğla Sıtkı Koçman Üniversitesi Kötekli Kampüsü, Su Ürünleri Fakültesi, 48000, Menteşe/Muğla, Türkiye
✉: rifattezel@mu.edu.tr

Öz: İnsan tüketimine sunulamamış balıkların ve su ürünleri işleme sanayi atıklarının, balık silajı olarak yem hammaddesine dönüştürülmesi, kaynakların etkin ve sürdürülebilir kullanımını sağlayan çevre dostu bir yöntemdir. Bu çalışmada, insan gıdası olarak değerlendirilememiş tirsi balıklarından, formik asit uygulamasıyla balık silajı üretimi yapılmış ve üretilen silajın besin içeriği ve yağ asidi kompozisyonu incelenmiştir. Silajların kuru madde içeriği %26,14 ile %26,63 arasında, ham protein miktarı %73,79 ile %78,55 arasında, yağ miktarı ise %5,09 ile %5,46 arasında değişim göstermiştir. Düşük dozda formik asit kullanılarak üretilen silajlarda ham protein miktarının düştüğü belirlenmiştir ($p<0,05$). Silajların toplam tekli doymamış yağ asidi (MUFA) değeri %18,56 ile %19,63 arasında, toplam çoklu doymamış yağ asidi (PUFA) değeri ise %24,74 ile %26,35 arasında değişim göstermiştir. Oleik asit (C18:1n9) %12,91 ile %14,27 arasında değişen miktarıyla baskın MUFA, DHA (C22:6n3) ise %16,39 ile %17,54 arasında değişen miktarıyla baskın PUFA olarak tespit edilmiştir. Farklı asit oranlarına sahip silajlarda ΣSFA, ΣMUFA, ΣPUFA, Σn3 ve Σn6 değerlerinin istatistiksel olarak benzer olduğu görülmüştür ($p>0,05$). Üretilen silajların özellikle ham protein ve PUFA içeriği, tirsi balığı silajının balık yemlerinde kullanılabileceğini göstermiştir. Değerli besin içeriğine sahip balık silajının yem hammaddesi olarak kullanılması, hem ekonomik kazanç sağlayacak hem de çevrenin korunmasına katkıda bulunacaktır.

Anahtar kelimeler: Balık besleme, besin değeri, çevre, çoklu doymamış yağ asitleri, formik asit silajı, su ürünleri yetiştiriciliği.

INTRODUCTION

Nowadays, fulfilling the food need in a sustainable manner that arising as a result of the increasing world population is one of the prioritised subject. Effective and sustainable use of existing and potential resources is a priority in all areas (Güllü et al., 2015). Due to the limited natural resources, the food production sector has to utilise its resources sustainably with maximum benefit. Aquaculture sector, which is one of the leading sectors in meeting the food demand, plays a critical role in ensuring the nutrition of the global population (Ahmed et al., 2019).

The increase in the aquaculture production has led to demand for fish meal and fish oil. Fish meal (FM) is an expensive raw material that increases the feed prices in aquaculture sector (Parisi et al., 2020). Fishmeal and fish oil obtained from pelagic fish is traditionally used feed ingredients used in feed for farmed fish (Olsen & Toppe, 2017). These pelagic fish used for fish meal and fish oil production is generally suitable for human consumption. The use of these fish, which can be used directly as human food, as raw material in feed production is a controversial issue. Furthermore, fishing pressures on natural fish populations are increasing due to the need for fish meal (Ahmed et al., 2019). Therefore, developing novel feed raw materials that can be an alternative to fish meal and fish oil will contribute to the reduction of fishing pressure on natural fish stocks and the sustainable utilisation of limited natural fish populations. Many researchers have stated that the use of fish silage as feed raw material in aquaculture feeds provides economic gains with its high nutritional values (Güllü et al., 2015).

The wastes resulting from industrial processing of aquaculture products are utilised for environmental protection and production of new products (Ramasubburayan et al., 2013). However, the use of unmarketable or non-consumable fish in producing silage will provide both economic values and environmental sustainability. Methodologies to use these materials as a protein source for human consumption are necessary and urgent. Several studies are being conducted with this objective; in the meantime these materials are commonly used in animal product food (Vidotti et al., 2003).

Fish silage is an excellent protein product with high biological value for animal feeding, which can be produced from dead fish, sub-utilized species, by-products from marine fishing, commercial fish waste and industrial residues. These are considered low quality raw materials, that if not used may cause environmental, health, and economical problems (Vidotti et al., 2003). Fish silage can be described as a stable semi-liquid product that produced from fish by-products and the liquefaction occurs by the impact of naturally present proteolytic enzymes in the fish and this process is accelerated by reducing pH below 4.5 (Alwan et al., 1993). In silage production, the pH is reduced below 4.5 and therefore the development of deterioration factors and pathogenic bacteria in fish mince is eliminated due to the low pH (Özkütük & Özyurt, 2022).

Producing fish silage from fisheries industrial processing wastes is a cost-effective method. Silage producing process does not require expensive investments, energy and labour costs and resulting in an ingredient of high biological value that has high levels of protein and energy (Olsen & Toppe, 2017; Özyurt et al., 2019; Santana et al., 2024). In this method, silage is produced from fishery wastes that are not suitable for human consumption or fish that have lost their consumption properties and the produced fish silage is used as feed raw material in feed industry. There are two main methods currently using in fish silage production. The first one is using fermentation becterias and the other one is using acid in fish silage production (Güllü vd., 2015). Due to the fact that there is no need for neutralisation of the silage that produced, it is the best option to use formic acid in silage production (Ramasubburayan vd., 2013). Using formic acid in the silage may also contribute to the improved well-being and growth of the farmed animals and fish. Short-chain organic acids like formic acid, are among the candidates that may be used as growth promotors in feeds (Olsen & Toppe, 2017).

In the regions where small-scale fishing activities are carried out, the captured fish are usually sold locally in fisheries cooperatives or small-scale fish markets. In case the fishermen catch more than fish that can be sold locally, the caught products are usually transported to the fish wholesale centres and offered for sale. However, in cases where the amount of products to be sold in wholesale fish market is financially inadequate and the value to be obtained as a result of this sale cannot fulfil the trasportation and labour costs, the fished fish products are not sent to wholesale fish markets. In this case, some of the caught fish can be sold locally, while the rest of the fish is discarded. As a result, a product with valuable nutritional content that can actually be consumed by humans turns into garbage that can cause environmental problems. In addition, a significant amount of discard fish are also caught as a result of fishing activities (Kaykaç et al., 2020; Cerim et al., 2022). Although they are target species, sometimes some of them are discarded as a result of deformation on fish due to parasites and other reasons (Kaykaç et al., 2020). The discard fish that can be returned to the nature are immediately released back to the sea by the fishermen. The dead discard species are removed from the nets and either disposed into the sea or disposed into the garbage containers of the municipalities at the piers. Although discarded fish species are not preferred for human consumption, they have significant nutritional content. In fact, fish that have economic value but cannot be offered for sale because they cannot meet the marketing costs, discarded fish species that are not preferred for human consumption and fish processing wastes that arising locally can cause significant environmental risks (Özkütük & Özyurt, 2022). In the era of Blue Growth, in which environmental sensitivity and sustainable growth are aimed, awareness for utilizing these wastes as feed ingredient resourses there has been increasing (Olsen & Toppe, 2017).

The present study was aimed to produce feed raw material by using acid silage production technique from twaite shad fish which were caught and could not be sold in small-scale fishing activities. The nutrient composition and fatty acid profile of fish silages produced by using formic acid at different ratios were investigated.

MATERIAL AND METHOD

Silage Preparation: Within the scope of the study, 12 kg twaite shad fish (*Alosa fallax*, Lacepède, 1803) samples were provided from a fisherman in Çeşme District of İzmir Province. Samples were randomly selected from fish that could not be offered for sale and would be discarded. The samples were brought to Muğla Sıtkı Koçman University Faculty of Fisheries and stored at -18 ˚C until silage was made. Before making silage, fish samples were minced by using a meat grinder with 8 mm diameter holes. The fish mince were then divided into 9 containers, each container containing 1.3 kg of fish mince sample. Each container was randomly selected and allocated into 3 different groups with 3 replicates.

Acid silage was prepared by acidifying the fish mince in each container with three different concentrations (2.5%, 3% and 3.5% (w/v)) of formic acid. The pH value of the silage in each container was monitored with a portable pH meter (Sartorius, PT series). The prepared silages were stored at ambient temperature (20-25 ˚C). Silage in each container were stirred daily until ripening.

After the 30-day storage period, samples were taken from each container. The proximate composition and fatty acid composition analyses of the samples were carried out.

Proximate Composition Analyses: The proximate composition of silage samples were analysed in triplicate. The lipid content of fish silages were analysed according to the Bligh and Dyer, (1959) method. The moisture and ash content of fish silages were determined according to AOAC, (1990) methods. The crude protein of samples were analysed according to AOAC, (2006) method. The proximate composition of the silages was calculated in terms of dry matter considering the moisture and the dry matter content.

Fatty acids analysis: The methyl esters of lipid obtained from the samples were prepared by transmethylation using gas chromatography-flame ionizing detector (GC-FID) according to the method described by Ichihara et al., (1996). 25 mg of extracted oil was dissolved in 2 ml isooctane, and then 4 ml of 2 M KOH (in methanol) was added. Then, the tube was vortexed for 2 min at ambient temperature. Separation into methyl esters was performed in triplicate for each sample. The samples were centrifuged at 4000 rpm for 10 minutes, after that the isooctane layer was taken for gas chromatography (GC) analysis.

The fatty acid methyl esters were analyzed by using a gas chromatography (Agilent Technologies model 7820 equipped with a flame ionization detector (FID) and fitted with an HP-88 capillary column (60 m × 0.25 mm × 0.25 μm thickness)). Helium was used as the carrier gas at a constant pressure of 16 psi. Injection port was maintained at 220ºC, and the sample was injected in split mode with a split ratio of 50:1. During the analysis, detector temperature was 280ºC. Column temperature was started at 175ºC, and then programmed at 3ºC/min to 220ºC, ramped at 1°C/min to 220ºC, and held for 10 min. Identification of fatty acids was carried out by comparing sample fatty acids methyl esters (FAME) peak relative retention times with those obtained for Supelco standards (Supelco 37 Compounds FAME mix 10 mg/l in CH2 Cl2–47,885 U, Supelco 1819–1 Ampule FAME mix C4-C24). The results were expressed as percentage of total fatty acid methyl esters (ISO, 1990).

Statistical analysis: The values obtained were determined as mean ± standard deviation (SD) of triplicate groups. Shapiro-Wilk normality test and Levene's test were used to analyse the normality and homogeneity of variance of the data, respectively. Differences in the proximate composition of the groups were statistically analysed by using a Tukey Post-Hoc test following one-way analysis of variance (ANOVA) and a value of 0.05 was assumed as significant for p value. All statistical analysis conducted via using the software SPSS version 22.

RESULTS AND DISCUSSION

In the present study the pH value of fish silages produced via application 2.5%, 3.0% ve 3.5% formic acid was respectively found as 4.33±0.04, 4.12±0.02 ve 3.99±0.03 after 30 days (Figure 1). After the first week of the study, it was observed that the pH value decreased to approximately 4 and remained at this level throughout the study. Liquefaction with formic acid can be carried out in the pH range 4-4.5 due to the antiseptic properties of formic acid (Raeesi et al., 2023). Although the ripening period of the silage varies depending on the characteristics of the raw material used, it was observed that the silage ripening process was completed within 10-12 days, similar to the literature (Güllü et al.,2015; Özkütük & Özyurt, 2022). According to the observations made during this period, it was observed that the fish mince, which initially contained solid pieces, was transformed into a more fluid, homogeneous product over time (Figure 2). In addition, in all groups stored at ambient temperature (20-25 ˚C), it was noted that the odour was an acceptable pleasant malt odour (Güllü et al., 2015).

Ramasubburayan et al., (2013) was found the pH value of grouper *Epinephelus malabaricus* processing waste silages that prepared via 2%, 2.5% ve 3% formic acid application as 3.98±0.40, 3.60±0.30 and 3.42±0.04 respectively, at the end of 30 days. The pH value of silages were found higher than that was found by Ramasubburayan et al., (2013). It is considered that this difference is due to the characteristics of the raw material used in silage production.

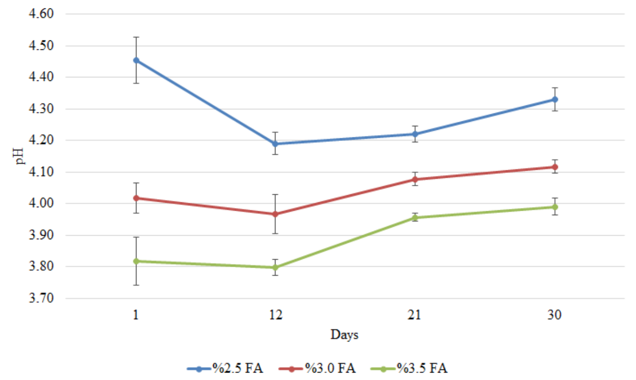

Figure 1. pH values of the silages produced.

Figure 2. Sample image of twaite shad fish silage before and after ripening.

In the present study, the dry matter content of the produced silages were found to be approximately 26% (Table 1). Goddard and Al-Yahyai, (2001) was found that the dry matter content of silages between 21.7-26.8% that were prepared from sardine fish (*Sardinella longiceps*) by using the mixture of formic acid and propionic acid. Ramasubburayan et al., (2013) was found the dry matter content of processing waste silage at the end of 30 days was between 9.33% and 19.14%. The dry matter content of silage may vary depending on the method applied. In addition, water evaporation occurring during the silage production process is also effective on the dry matter content. Ramasubburayan et al., (2013) reported that, there was a significant decrease in the dry matter content of the 2% formic acid silage, unlike the low amount of reduction in 2.5% and 3% formic acid silages at the end of the 30-day silage production process. They stated that this may be due to hydrolysis of the proteins used as a result of enzyme or microorganism activity. In the present study, there was no statistically significant difference in the dry matter content of the groups.

In this study, the crude protein content of silages were found between 73.79±1.09% and 78.55±1.05% (Table 1). The protein values of the silages were statistically different ($p<0.05$). It was noticed that the protein level of produced silages was varied depending on the amount of acid used. In this study, this situation may be occurred as a result of protein hydrolysis (Ramasubburayan et al., 2013). It was seen that the amount of acid applied is very important for protein hydrolysis. In the literature, the protein contents of the produced silages from different raw materials were reported as 66.9-72.5% by Goddard and Al-Yahyai, (2001), 36.06-38.40% by Ramasubburayan et al., (2013), 13.3% by Santana et al., (2023) and 24% by Santana et al., (2024). It is an evident that the amount of protein can vary significantly depending on the raw material from which the silage is produced. Considering that the produced silages will be used as a substitute for fish meal, it would be appropriate to compare the protein content with the protein values of fish meal. Turan et al., (2007) reported the crude protein value in anchovy meal as 74-76%, Guo et al., (2020) reported the protein value in anchovy meal as 68.3%, Soto et al., (2023) reported the crude protein value in anchovy (*Engraulis ringens*) meal as 68.1%, Bayraklı, (2023) reported the protein value in salmon by-products meal as 65.22%. As a consequence of the comparison of the protein value of the produced silage with the literature reports, it was seen that this product can be utilised as a substitute partly for fish meal.

Table 1. Proximate composition of fish silages.

	2.5% FA Silage	3.0% FA Silage	3.5% FA Silage
Dry matter (%)	26.26±0.40^{a}	26.14±0.60^{a}	26.63±0.22^{a}
Protein (%) DM	73.79±1.09^{a}	77.87±1.90^{b}	78.55±1.05^{b}
Lipid (%) DM	5.16±0.51^{a}	5.46±0.23^{a}	5.09±0.14^{a}
Ash (%) DM	17.35±0.10^{a}	15.80±1.36^{a}	17.16±1.04^{a}

DM: Dry matter, FA: Formic acid, Each value is given as the mean ± standard deviation of three replicate samples, Values with different letters in the same row indicate significant differences between the groups ($P<0.05$).

The lipid amounts of silages were found between 5.09±0.14% and 5.46±0.23% in this present study (Table 1). In previous studies, the lipid amount of silages produced by using different raw materials was found as 11.5% by Goddard and Al-Yahyai, (2001), 10.66-12.24% by Ramasubburayan et al., (2013), 69.9% by Santana et al., (2023) and 24% by Santana et al., (2024). The lipid amounts of fish meal that produced from different raw materials were reported as 8-9% by Turan et al., (2007), 8.53% by Guo et al., (2020), 9.28% by Bayraklı, (2023) and 8.7% by Soto et al., (2023). In the present study, lipid content of silages was lower than previous studies. It is thought that the lipid value is lower than previous studies due to seasonal conditions, nutritional characteristics and various other factors (Metin et al., 2022).

In this study, the ash content of silages were between 17.35±0.10 and 15.80±1.36 (Table 1). The ash content of formic acid silages did not show any statistical difference between the groups (p>0.05). The crude ash refers to the total inorganic matter in a raw material. The crude ash values determined in fish silages are due to the head, scales, bones and similar parts in fish (Özkütük & Özyurt, 2022). In the present study, although some difference was observed between the ash amounts of silages produced by using acid at different ratios, this difference was not statistically significant. This indicates that the amount of acid used in the present study had no effect on the inorganic matter composition of silage.

In the present study, total saturated fatty acids (ΣSFA) values of the produced silages were found between 50.24% and 52.40% (Table 2). In previous studies, ΣSFA values of acid silages produced using different raw materials were reported as 40.3% by Özyurt et al., (2016), and 43.6% by Santana et al., (2023). Turan et al., (2007) reported that ΣSFA value in anchovy meal was found as 33%. Similar to the studies in the literature, the predominant saturated fatty acids found in the silages was palmitic acid (C16:0) (Tezel et al., 2016; Özyurt et al., 2016).

In this research, total mono unsaturated fatty acid (ΣMUFA) values were found between 18.56% and 19.63% (Table 2). In previous studies, ΣMUFA values were reported as 24.4% in Klunzinger's ponyfish silage by Özyurt et al., (2016) and 20-21% in anchovy meal by Turan et al., (2007). Oleic acid (C18:1n9) was found to be the dominant mono unsaturated fatty acid in silages. The obtained data shows that ΣMUFA values are in compliance with the literature.

Table 2. Fatty acid composition of fish silages.

Fatty Acids	**2.5% FA Silage**	**3.0% FA Silage**	**3.5% FA Silage**
C6:0	1.27±0.11^{b}	1.00±0.05^{a}	1.32±0.02^{b}
C8:0	0.46±0.08^{a}	0.35±0.06^{a}	0.62±0.01^{b}
C14:0	5.52±0.02^{a}	5.56±0.10^{a}	5.39±0.39^{a}
C16:0	29.38±0.44^{a}	28.61±0.65^{a}	29.20±0.24^{a}
C17:0	1.68±0.01^{b}	1.67±0.04^{b}	1.57±0.02^{a}
C18:0	12.42±0.55^{a}	12.08±0.36^{a}	13.38±0.64^{a}
C24:0	1.00±0.05^{a}	0.97±0.06^{a}	0.93±0.00^{a}
ΣSFA	**51.72±1.09^{a}**	**50.24±1.30^{a}**	**52.40±0.01^{a}**
C14:1	1.48±0.00^{a}	1.52±0.04^{a}	1.45±0.09^{a}
C16:1	4.17±0.02^{a}	4.06±0.08^{a}	3.92±0.28^{a}
C18:1n9	12.91±0.01^{a}	13.89±0.54^{ab}	14.27±0.53^{b}
ΣMUFA	**18.56±0.01^{a}**	**19.47±0.42^{a}**	**19.63±0.90^{a}**
C18:2n6	2.40±0.13^{a}	2.40±0.07^{a}	2.27±0.09^{a}
C20:3n3	1.59±0.10^{ab}	1.46±0.05^{a}	1.63±0.01^{b}
C20:5n3	4.81±0.22^{b}	4.81±0.04^{b}	4.44±0.04^{a}
C22:6n3	17.54±0.66^{a}	17.40±0.51^{a}	16.39±1.12^{a}
ΣPUFA	**26.35±1.10^{a}**	**26.07±0.52^{a}**	**24.74±1.18^{a}**
Undefined	3.37±0.01^{a}	4.22±1.40^{a}	3.24±0.28^{a}
Σn3	**23.94±0.97^{a}**	**23.67±0.59^{a}**	**22.47±1.08^{a}**
Σn6	**2.40±0.13^{a}**	**2.40±0.07^{a}**	**2.27±0.09^{a}**
n3/n6	**9.96±0.12^{a}**	**9.86±0.51^{a}**	**9.90±0.08^{a}**

C6:0 Caproic acid, C8:0 Caprylic acid, C14:0 Myristic acid, C16:0 Palmitic acid, C17:0 Heptadecanoic acid, C18:0 Stearic acid, C24:0 Lignoseric acid, ΣSFA Total saturated fatty acids, C14:1 Myristoleic acid, C16:1 Palmitoleic acid, C18:1n9 Oleic acid, ΣMUFA Total mono unsaturated fatty acids, C18:2n6 Linoleic acid, C20:3n3 Eicosatrienoic acid, C20:5n3 Eicosapentaenoic acid, C22:6n3 Docosahexaenoic acid, ΣPUFA Total poly unsaturated fatty acids, Σn3 Total n3 fatty acids, Σn6 Total n6 fatty acids. Each value is given as the mean ± standard deviation of three replicate samples, Values with different letters in the same row indicate significant differences between the groups (P <0.05).

Fish oils are rich sources of poly unsaturated fatty acids (PUFA). Metin et al., (2022) reported that the ΣPUFA content was 40.34% in the flesh of sea bass that produced in aquaculture conditions. This indicates that fish in aquaculture should be fed with feeds with high PUFA values. The ΣPUFA values of the silages produced in this study were found between 24.74% and 26.35% (Table 2). In the literature, ΣPUFA value was reported by Tezel et al., (2016) as 24-26% for whole pearl mullet and silages produced from processing wastes of this fish, by Özyurt et al., (2016) as 14.3% for Klunzinger's ponyfish silage, by Santana et al. (2023) as 10.9% for silage produced from fish processing wastes, by Turan et al., (2007) as 31-32% for anchovy meal. Docosahexaenoic acid (DHA, C22:6n3) was the dominant PUFA in fish silages. Özyurt et al., (2016) reported the ratio of n3:n6 fatty acids as 10.5 for formic acid silage. Similar results were obtained in the present study. It was observed that the ΣSFA, ΣMUFA, ΣPUFA, Σn3 and Σn6 values in silages with different acid ratios were statistically similar (p>0.05). In the study, it was observed that the amount of formic acid used in silage production did not have a significant effect on the fatty acid profile except some parameters.

In terms of nutrition, fish meat is a very important foodstuff due to the high-quality protein and fatty acid values (Metin et al., 2022). Fish has always been considered as an important nutritional food and has always maintained an important position in the common diet recommendations and animal feed rations. This is mainly due to their high protein content as well as being an important source of poly unsaturated fatty acids (Özkütük & Özyurt, 2022). In the present study, it is shown that especially the crude protein and PUFA content of the produced silage is a suitable feed raw material that can be utilised in fish feeds.

In conclusion making silage from the fish that have economic value but cannot be offered for sale because they cannot meet the marketing costs, discarded fish species that are not preferred for human consumption and fish processing wastes is very important. The utilisation of these products with valuable nutritional content will both create economic gains and also contribute to the protection of the environment. In regions where small-scale fishing activities are carried out, it is very important to utilise these wastes with the participation of cooperatives or local administrations.

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