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Utilization of Poultry Slaughterhouse Waste Silage as a Protein Source in Diets of the Common Carp (*Cyprinus carpio* L.) Fingerlings

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ABSTRACT

The current study aimed to prepare silage from poultry slaughterhouse waste using an organic acid fermentation method. The chemical composition of the silage was assessed, with results showing a moisture content of 9.21%, protein content of 59.71%, fat content of 12.81%, ash content of 12.63% and an NFE (nitrogen-free extract) of 5.64%. Amino acid analysis revealed that the silage contained 18 amino acids in a balanced composition of essential and non-essential amino acids, with varying concentrations. Glutamic acid was the most abundant amino acid, measuring 7.11mg per 100mg of protein. Additionally, the study determined the quantity and quality of total fatty acids in the poultry waste silage, identifying 19 fatty acids with varying levels depending on the sample type. Palmitic acid had the highest concentration at 24.1µl per 100µl of oil. Four experimental diets were formulated, three of which included varying levels of silage as a partial substitute for fishmeal: T1 (25% substitution), T2 (50% substitution), and T3 (75% substitution), while the final treatment, C, served as a control with no added silage. The results indicated that T2 diet outperformed the other treatments in terms of growth and nutritional parameters, including final weight, total weight gain, daily growth rate, relative and specific growth rates, feed conversion ratio, protein efficiency ratio, protein intake and overall digestibility. The study demonstrated that poultry slaughterhouse waste silage could be used as a partial replacement for fishmeal, up to 50%, in common carp (*Cyprinus carpio* L.) diets without any adverse effects on nutritional and growth performance parameters.

INTRODUCTION

Aquaculture is an important agricultural sector for producing protein-rich foods, supplying nearly half of the fish consumed globally **(Khanum *et al*., 2022)**. It constitutes about 46% of the total global fish supply **(Ochokwu *et al*., 2014)**. Global per capita fish consumption was doubled from 9kg/ year in the 1960s to 20.2kg/ year in 2020 **(FAO, 2022)**. Therefore, it is crucial to develop the aquaculture sector by ensuring the success factors, improving feeding practices, and managing them efficiently to positively impact growth rates, production, feed conversion efficiency, protein utilization, and feed digestibility, ultimately resulting in good economic returns **(Kord *et al*., 2021)**. The expected future growth in aquaculture will increase the pressure on the main feed components, especially animal protein sources **(Tacon & Metian, 2015)**. The sustainability of aquaculture heavily relies on feed production which constitutes 50-60% of production costs **(Taher, 2020)**. Fish meal is the preferred animal protein source in aquafeeds due to its high palatability, balanced essential amino acids, fatty acids, vitamins, minerals, and other growth stimulants **(Qiu *et al*., 2023)**. However, the limited fish meal supply and increasing demand in aquaculture, livestock, and poultry feeds have driven up its cost, making it challenging to rely solely on fish meal as an animal protein source **(AL-Noor *et al*., 2023)**. The aquaculture industry alone uses approximately 87% of the global fish meal production **(FAO, 2020)**. Thus, finding alternatives to fish meal is essential, and numerous attempts have been made to partially replace it with other, less expensive animal protein sources to reduce feed production costs **(Najim & Al-Tameemi, 2023)**. Poultry slaughter produces significant waste, including internal organs, feet, heads, and feathers, which make up about 20% of the bird's live weight **(Sionkowska *et al*., 2011)**. Disposing of this waste poses a significant challenge due to high economic costs, as it often involves landfilling or incineration, leading to environmental damage and air pollution **(Koul, 2022)**. Due to the availability of poultry slaughter waste in large quantities and its lack of utilization, it has become an important environmental issue that must be addressed **(Prasanthi *et al*., 2016)**. Therefore, it is essential to employ the best chemical, biological, and physical methods to convert this waste into useful materials for industrial, agricultural, and food applications **(Vineis *et al*., 2019)**. Poultry waste is particularly valuable due to its high digestibility, rich protein content, essential amino acids and certain vitamins **(Orisasona, 2018)**. A key goal in formulating fish feed is to achieve a nutritional balance while using the minimum amount of fish meal necessary to meet the essential amino acid requirements for fish growth and reproduction while reducing feed costs **(Abdulwahab *et al*., 2023)**. This study aimed to utilize poultry slaughterhouse waste, a readily available and inexpensive agricultural byproduct, as a partial protein substitute for fish meal in the diet of common carp. This approach has economic benefits, enhances sustainable fish production and reduces environmental pollution by recycling poultry waste, which would otherwise accumulate in soil causing environmental contamination and increasing health risks for humans and animals.

MATERIALS AND METHODS

Raw materials for silage preparation

In this study, poultry slaughter by-products (heads, feet, and internal organs) were used as the primary raw material for silage preparation. These by-products were obtained from local markets in Basrah Governorate, southern Iraq as residuals from the processing and cleaning of chicken carcasses. After transporting the waste to the laboratory, it was thoroughly washed with water to remove blood and adhered materials, then cut into small pieces and mixed well. A random representative sample was taken from the waste for chemical analysis, while the remainder was placed in polyethylene bags and stored frozen (-12°C ± 2) until silage preparation.

Preparation of poultry slaughter waste silage

The silage was prepared following the method of **Al-Kanaani (2014)**. Poultry slaughter waste (heads, feet, and internal organs) was cut into small pieces using sharp knives and thoroughly washed with tap water to remove impurities. Acid silage fermentation was used by taking 1000 grams of the chopped waste and mixing it with 100ml of 5% acetic acid, which contained 3% citric acid dissolved in it, along with 50 grams of date pulp (as a carbohydrate source) and 50ml of distilled water. The contents were mixed thoroughly for 15 minutes and transferred into 2000-gram capacity polyethylene bags, which were tightly sealed, allowing for some headspace for the expansion of the mixture and gases produced during fermentation. The samples were placed in tightly sealed plastic containers to ensure successful fermentation and incubated at 40–45°C for 5–7 days, with daily stirring and shaking for 20 minutes to maintain uniform internal temperature distribution. After the completion of fermentation process, the formed fat layer was removed. The resulting product was then concentrated using a rotary evaporator at 70°C for 2 hours. It was dried in a conventional oven at 55°C and stored in plastic bottles in a refrigerator for analysis and future use.

Estimation of chemical composition

Moisture content was determined by oven drying at 105°C. Ash percentage was calculated using a muffle furnace at 525°C. Protein content was estimated using the Kjeldahl method, while lipid content was determined via Soxhlet extraction following the method outlined by **Egan *et al.* (1988)**. The carbohydrate percentage was calculated mathematically according to **AOAC (2000)**.

Estimation of amino acids

Amino acid profiles of prepared silage and fish meal samples were determined according to **Vidotti *et al*. (2003)**. An ion exchange column and post-column ninhydrin derivatization were used for analysis, utilizing the Visible-UV Detector -6 Av uv -Spd Shimadzu in an automatic analysis system. High-performance Liquid Chromatography (HPLC) equipment, under the supervision of the Ministry of Science and Technology in Baghdad, Iraq, was employed for this purpose.

Estimation of total fatty acids

The total fatty acid content in the oils extracted from silage and fish meal samples was analyzed using the method described by **Abdulkadir *et al*. (2010)**. The oils were examined using Gas Chromatography-Mass Spectrometry (GC-MS), a comprehensive spectral analysis technique, at the laboratories of the Chemistry Department, the Ministry of Science and Technology, Baghdad, Iraq.

Feed formulation

After determining the proportions of the primary feed ingredients used to formulate the experimental fish diets as shown in Table (1), the feed materials were finely ground and passed through a 2mm sieve. These ingredients were then thoroughly mixed according to the calculated proportions to ensure uniformity. Four experimental diets were prepared: three of these contained different replacement levels of the prepared silage as a substitute for fish meal as follows: T1) 25% replacement, T2) 50% replacement, and T3) 75% replacement, while the final treatment was left without silage addition as a control (C). Afterward, approximately 100ml of boiling water was added to every 250 grams of the mixture. Upon thorough mixing, the temperature of the mixture was raised to 80°C and was then allowed to cool before adding vitamins and minerals. The feed dough was shaped into pellets using a Braun® meat grinder with 4mm diameter holes. The pelleted feed was then air-dried in the laboratory for 48 hours, with frequent turning to ensure complete moisture removal. Finally, the manufactured feed was stored in 2kg plastic containers that were placed in a refrigerator until use.

Table 1. Formulation ingredients (%) of experimental diets

Ingredient	C	T1	T2	T3
Fish meal	30	22.5	15	7.5
Silage	0	7.5	15	22.5
Soybean meal	20	22	24	26
Barley flour	16	16	16	16
Yellow corn meal	18	18	18	18
Wheat bran	11	10	9	8
Vegetable oil	3	2	1	0
premix	2	2	2	2
	100	**100**	**100**	**100**

Fish and experimental system

A total of 150 common carp (*Cyprinus carpio* L.), weighing between 10.13 and 18.14 grams, were obtained in January 2024 from the fish ponds of the Aquaculture Unit in Al-Hartha District, affiliated with the College of Agriculture, University of Basrah. The experimental fish-rearing system was designed as a closed recirculating system in the Fish Culture Laboratory, Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah. At the beginning of the experiment, the fish were randomly distributed at a rate of ten fish per tank (20L.). The fish were acclimated to experimental conditions for ten days, during which they were fed a standard diet.

Feeding experiment

Fish growth

The feeding trial was conducted from February 20, 2024, to May 5, 2024. During this period, the fish were fed with the formulated diets containing silage, as well as a control diet, at a feeding rate of 3% of their body weight throughout the trial (feeding was done daily). The fish were fed twice a day, with the first meal at 9:00 AM and the second meal at 2:00 PM. This feeding rate was adjusted every 15 days based on the fish's weight to determine the appropriate feed amount. Additionally, 30% of the water was replaced every 14 days to maintain water quality in the closed recirculating system used for the feeding and growth trial. The total weight gain (TWG) and daily weight gain (DWG) were calculated following **Sevier *et al.* (2000)**. On the other hand, the relative growth rate (RGR) and specific growth rate (SGR) were determined according to the method described by **Jobling (1993)**. Furthermore, values for the food conversion ratio (FCR), food conversion efficiency (FCE), protein intake (PI), and protein efficiency ratio (PER), were calculated using the method applied by **Tacon (1990)** as follows:

TWG (g/fish) = Final weight – Initial weight

DWG (g/fish/day) = TWG / time (day)

Relative (RGR) and specific (SGR) growth rates were calculated as described by

RGR (%) = TWG / Initial wt. X 100

SGR (%/day) = (ln final wt. – ln Initial wt.) / time (day) X 100

FCR = Consumed feed (g) / TWG (g)

PI (g/fish) = Consumed feed (g) X Feed protein content (%)

PER (%) = TWG / PI

Feed apparent digestibility

To measure total apparent digestibility (TADC) and nutrient apparent digestibility (NADC) coefficients, the indirect method described by **Talbot (1985)** was applied using chromium oxide Cr_2O_3 as a marker. The marker content in experimental diets and collected fish feces was assessed by measuring absorbance spectrophotometrically at 350nm as follows:

TADC (%) = 100 – [100 x (% marker in feed) / (%marker in feces)

NADC = 100 – [100 x {(% marker in feed) / (%marker in feces)}/ {(% marker in feces) / (%marker in feed)}]

Statistical analysis

The growth experiment was designed according to the complete randomized design (CRD) with four treatments, each with three replications. The same statistical analysis approach was applied for other studied feeding and growth parameters. The significant differences between treatment means were determined using the least significant difference (LSD) test. All statistical analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS) version 26.0.

RESULTS

Table (2) shows the chemical composition of poultry waste, silage, and fish meal under study. The moisture content in the waste was 11.84%, with a protein content of 56.22%. The fat and ash contents were 13.69 and 12.87%, respectively, while the NFE (Nitrogen-Free Extract) value was 5.38%. Regarding the prepared silage, the moisture content was 9.21% with a protein content of 59.71%. The fat content was 12.81%, ash 12.63%, and the NFE value was 5.64%. As for the fish meal, the nutrient composition percentages were 5.56% for moisture, 67.89% for protein, 10.13% for fat, 11.42% for ash, and 5.8% for NFE.

Table 2. Proximate composition (%) of poultry slaughterhouse waste, silage and fish meal

	Moisture	**protein**	**lipid**	**Ash**	**NFE**
Poultry slaughterhouse waste	11.84	56.22	13.69	12.87	5.38
Silage	9.21	59.71	12.81	12.63	5.64
Fish meal	5.56	67.89	10.13	11.42	5.8

Table (3) and Figs. (1, 2) show the analysis of amino acids via HPLC for poultry slaughterhouse waste silage and fish meal. The results indicate that both silage and fish meal contain 18 amino acids in a balanced composition of essential and non-essential amino acids, with varying proportions across all treatments. For the essential amino acids, there were 10 in total. The highest value was for the amino acid lysine, averaging 5.69mg/ 100mg protein in fish meal, while leucine had the highest concentration in poultry slaughterhouse silage, reaching 4.48mg/ 100mg protein. The amino acid tryptophan showed low levels across all samples, measuring 0.73 and 0.71mg/ 100mg protein for fish meal and silage, respectively. On the other hand, there were 8 non-essential amino acids, with glutamic acid presenting the highest values at 7.31 and 7.11mg/ 100mg protein for fish meal and silage, respectively. The lowest non-essential amino acid values were for cysteine, measuring 1.53 and 1.09mg/ 100mg protein for fish meal and poultry slaughterhouse silage, respectively. Overall, the total essential amino acids were 34.75 and 25.65mg/ 100mg protein, while the non-essential amino acids were 32.83 and 29.32mg/ 100mg protein for fish meal and poultry slaughterhouse silage, respectively. The presence and concentration of other amino acids varied depending on the sample type.

Using gas chromatography-mass spectrometry (GC-MS), the total quantity and types of fatty acids in fish meal and poultry gizzard silage were determined. The results, presented in Table (4) and Figs. (3, 4, and 5), revealed the presence of 19 different fatty acids with varying concentrations depending on the sample type. For saturated fatty acids, their quantities reached 29.6 and 44.95µl/ 100µl oil, with high concentrations of palmitic acid at 17.31 and 24.1µl/ 100µl oil for fish meal and poultry gizzard silage, respectively. As for monounsaturated fatty acids, the quantities varied, with the highest amount in fish meal at 34.63µl/ 100µl oil, compared to 22.52µl/ 100µl oil in poultry gizzard silage. Oleic acid was the predominant monounsaturated fatty acid, reaching 19.1 and 18.2µl/ 100µl oil in fish meal and silage, respectively, compared to other monounsaturated fatty acids. Additionally, polyunsaturated fatty acids were higher in fish meal at 35.90µl/ 100µl oil, compared to 32.53µl/ 100µl oil in silage. Linoleic acid had the highest values among polyunsaturated fatty acids, at 16.5 and 14.9µl/ 100µl oil for fish meal and poultry gizzard silage, respectively. The results also indicated clear variations in the composition and percentages of the remaining fatty acids between fish meal and poultry gizzard silage.

Table 3. Amino acid profiles (µg/ 100µg protein) of fish meal and prepared silage

Amino Acid			**Fish meal**	**Silage**
Essential Amino Acids (EAA)	Arginine	Arg	4.69	3.96
	Histidine	His	2.31	1.21
	Isoleucine	Iso	3.98	2.67
	Leucine	Leu	5.33	4.48
	Lysine	Lys	5.69	2.49
	Methionine	Met	2.78	1.59
	Phenyl alanine	Phe	3.01	2.56
	Threonine	Thr	2.98	2.51
	Tryptophan	Try	0.73	0.71

	Valine	Val	3.25	3.47
	Σ EAA		**34.75**	**25.65**
Non-Essential Amino Acids (NEAA)	Glycine	Gly	5.12	5.66
	Glutamine	Glu	7.31	7.11
	Proline	Pro	3.17	3.02
	Serine	Ser	3.85	2.45
	Cysteine	Cys	1.53	1.09
	Aspartate	Asp	6.03	4.98
	Tyrosine	Tyr	2.14	1.76
	Alanine	Ala	3.68	3.25
	Σ NEAA		**32.83**	**29.32**
TAA			67.58	54.97
TEAA/TAA%			51.42	46.66
TEAA/TNEAA%			105.8	87.48

EAA, Essential Amino Acids; NEAA, Non-Essential Amino Acids

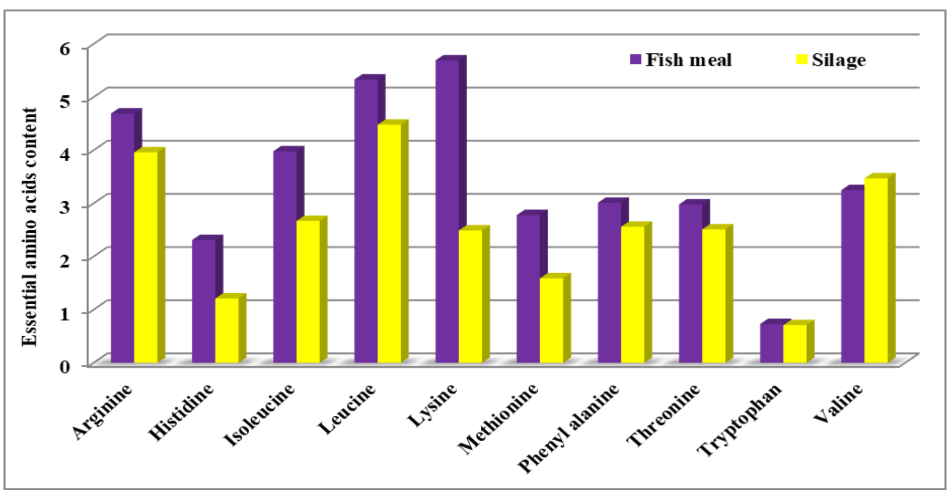

Fig. 1. Proportions and composition of essential amino acids in fish meal and prepared silage

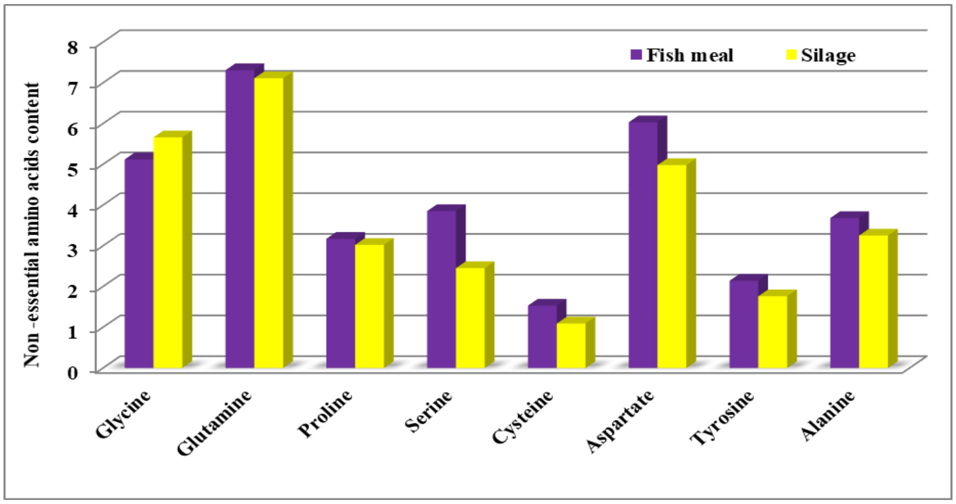

Fig. 2. Proportions and composition of Nonessential amino acids in fish meal and prepared silage

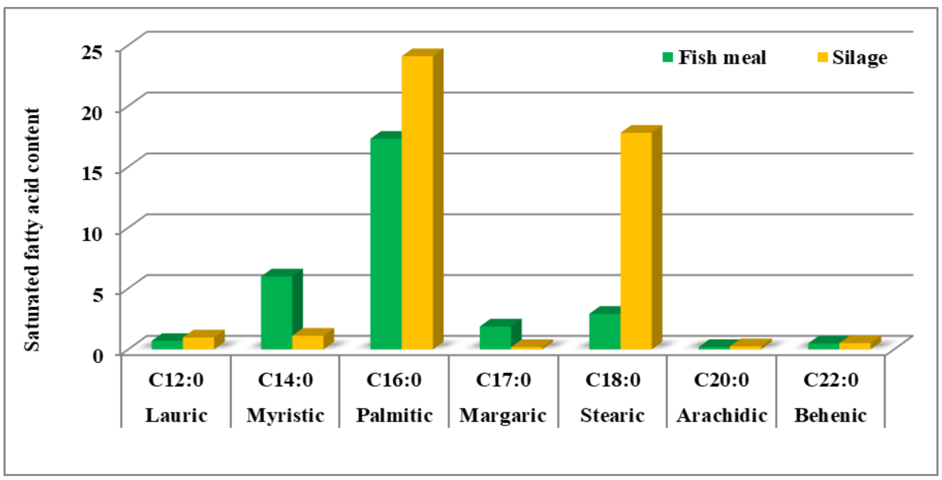

Fig. 3. Saturated fatty acids (µl/ 100µl oil) of fish meal and prepared silage

Table 4. Fatty acid profiles (µl/ 100µl oil) of fish meal and prepared silage

Fatty acid			Fish meal	Silage
Saturated fatty acids (SFA)	Lauric acid	C12:0	0.69	0.98
	Myristic acid	C14:0	5.99	1.12
	Palmitic acid	C16:0	17.31	24.1
	Margaric acid	C17:0	1.88	0.19
	Stearic acid	C18:0	2.92	17.8

	Arachidic acid	C20:0	0.19	0.25
	Behenic acid	C22:0	0.48	0.51
	ΣSFA		**29.46**	**44.95**
Monounsaturated fatty acids (MUFA)	Myristoleic	C14:1 w5	0.51	0.17
	Palmitolenic	C16:1 w7	7.79	1.86
	Ginkgolic acid	C17:1 w7	2.11	0.10
	Oleic acid	C18:1 w9	19.1	18.2
	Gadoleic acid	C20:1 w9	2.05	0.10
	Nervonic acid	C24:1 w9	3.07	2.09
	ΣMUFA		**34.63**	**22.52**
Polyunsaturated fatty acids (PUFA)	Linoleic acid	C18:2 w6	16.5	14.9
	α-linolenic acid	C18:3 w3	6.89	1.35
	Eicosatrienoic acid	C20:3 w3	2.97	0.58
	Arachidonic acid	C20:4 w6	2.07	13.7
	Eicosapentaenoic acid (EPA)	C20:5 w3	4.28	0.17
	Docosapentaenoic acid (DHA)	C22:6 w3	3.19	1.83
	ΣPUFA		**35.90**	**32.53**

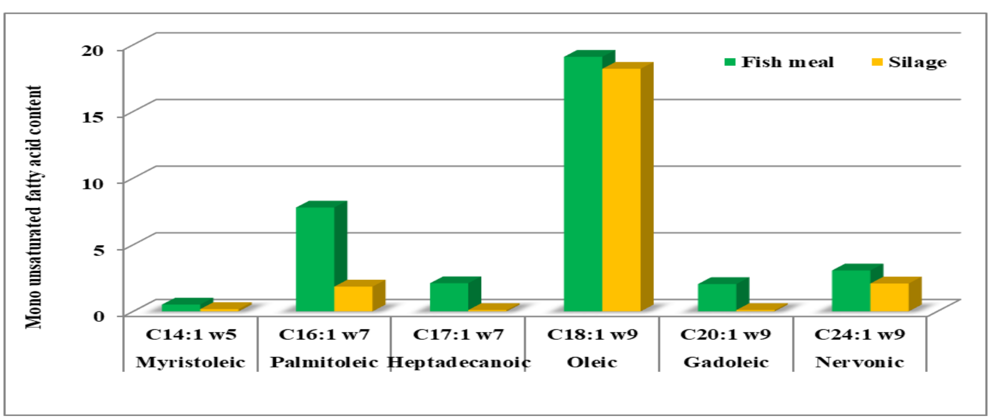

Fig. 4. Monounsaturated fatty acids (µl/ 100µl oil) of fish meal and prepared silage

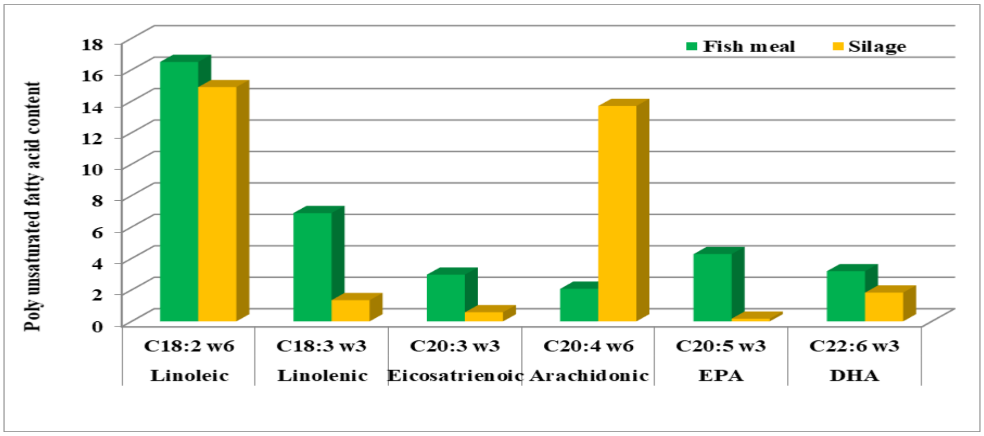

Fig. 5. Polyunsaturated fatty acids (µl/ 100 µl oil) of fish meal and prepared silage

The results in Table (5) show a variation in the chemical composition among the different types of formulated diets under study. The highest moisture content was found in diet T2 at 9.71%, while the lowest was in diet T1 at 7.76%, compared to the moisture contents of diets C and T3, which were 7.85 and 8.49%, respectively. For protein content, the highest value was recorded in diet T2 at 32.52%, while diets C, T1, and T3 showed protein contents of 30.31, 30.25, and 31.47%, respectively. In terms of fat, diet T3 had the highest value at 8.36%, differing from diets C, T1, and T2, which had fat percentages of 7.05, 6.67, and 7.51%, respectively. All diets showed varying levels of ash content depending on the type, with the lowest value in diet C at 6.25%, while diets T1, T2, and T3 had ash contents of 11.53, 11.95, and 12.75%, respectively. Carbohydrate values also varied among the diets, with contents of 48.53, 43.79, 38.31%, and 38.93% for diets C, T1, T2, and T3, respectively, in the same order.

Table 5. Proximate composition of experimental fish diets

	Moisture	**Ash**	**Crude lipid**	**Crude protein**	**Carbohydrate**
C	7.85	6.25	7.05	30.31	48.53
T1	7.76	11.53	6.67	30.25	43.79
T2	9.71	11.95	7.51	32.52	38.31
T3	8.49	12.75	8.36	31.47	38.93

Table (6) shows the initial weight (g), final weight (g), total weight gain (g), daily growth rate, relative growth, and specific growth rate. The results recorded the highest values for final weight, total weight gain, daily growth rate, relative growth, and specific growth rate in diet T2, reaching 47.24g, 33.91g, 0.43g/ day, 13.28%, and 1.63, respectively, which differed significantly ($P \leq 0.05$) from the other treatments. Meanwhile, growth values varied among treatments C, T1, and T3, with statistical analysis indicating no significant differences ($P > 0.05$) between them. The best feed conversion ratio (FCR) was achieved with diet T2, which recorded a value of 2.08, showing a significant difference ($P < 0.05$) compared to the other treatments, which had FCR values of 2.38, 2.36, and 2.5 for diets C, T1, and T3, respectively. Fish fed on diet T2 also showed the highest feed conversion efficiency at 60.33, compared to the other treatments, which recorded values of 48.77, 50.82, and 43.58, respectively. Statistical analysis indicated significant differences ($P \leq 0.05$) between treatment T2 and the other treatments. The results also showed that the highest protein efficiency ratio was in diet T2, reaching 1.86, while the lowest was in treatment T3 at 1.39. The remaining values were 1.61 and 1.68 for diets C and T1, respectively, with statistical analysis confirming significant differences ($P \leq 0.05$) in protein efficiency ratio between treatment T2 and the other treatments. Regarding the percentage of protein intake, the highest value was in diet T2 at 1829.85%, followed by diet T3 at 1615.83%, compared to diets C and T1, which recorded values of 1593.30 and 1592.84%, respectively. Statistical analysis indicated a significant difference ($P \leq 0.05$) between diet T2 compared to diets C and T1 and a similar performance to diet T3. For the amount of protein intake, statistical analysis showed significant differences ($P \leq 0.05$) between diet T2, which had the highest value at 66.22g, and the other diets, which recorded values of 60.91, 60.52, and 60.56 for diets C, T1, and T3, respectively, with no significant differences ($P > 0.05$) among them. The results demonstrated that using silage in diets T1, T2, and T3 led to good growth compared to the control diet (C), which had no additives. Diet T2 outperformed all other diets in growth criteria, indicating the successful use of poultry gizzard silage as a protein source in formulating diets for juvenile common carp. Diet T3 showed the lowest weight gain, but overall, diet T2 proved to be the most effective.

Table 6. Feeding and growth parameters of experimental fish

Parameter	**Treatment**			
	C	**T1**	**T2**	**T3**
Initial weight (g)	13.33±0.06 a	13.38±0.09a	13.32±0.12a	13.42±0.1a
Final weight (g)	39.02±2.89a	40.13±1.09a	47.24±4.75b	35.91±4.37a
Weight gain (g)	25.69±2.86 a	26.76±1.07a	33.91±4.63b	22.46±4.34a
RGR (%)	2.56±0.28a	2.66±0.10a	13.28±0.43b	9.71±0.43a
DGR/day	0.34±0.38a	0.34±0.14a	0.43±0.6b	0.34±0.38a

SGR (%/day)	1.43±0.95a	1.43±0.31a	1.63±0.13b	1.39±0.16a
FCR	2.38 ±0.16	2.36±0.04	2.08±0.17	2.5±0.30
FCE	48.77±3.67a	50.82±0.32a	60.33±6.40b	43.58±5.48a
PER	1.61±0.12a	1.68±0.01a	1.86±0.2b	1.39±0.17a
PIR	1593.30±60.81	1592.84±56.76	1829.85±57.33	1615.83±112.18
FI/g	60.91±2.84	60.52±2.30	66.22±3.34	60.56±4.8

*Different letters within one row indicate the presence of significant differences at the level ($P< 0.05$).

The results in Table (7) show the values of the apparent digestibility coefficient (ADC) for the total diet and individual nutrients in the formulated diets during the study period, highlighting the impact of replacing different levels of poultry slaughterhouse waste silage in the diets of juvenile common carp. Data in Table (7) indicate that the highest ADC value was 86.88% for diet T2 (50% replacement level), while the lowest was 79.23% for diet C (0% replacement level). The digestibility values for diet T1 (25% replacement level) and diet T3 (75% replacement level) were 81.82 and 83.6%, respectively. Statistical analysis showed significant differences ($P \leq 0.05$) among all treatments. In terms of protein, fat, ash, and carbohydrate digestibility, treatment T2 had the highest values, followed by T3, then T1, and finally C. Statistical analysis indicated significant differences ($P \leq 0.05$) among all treatments, except for carbohydrate digestibility, where no significant differences ($P>0.05$) were observed among the treatments.

Table 8. Apparent digestibility coefficients of major nutrients in experimental diets

	Total apparent digestibility	**Protein digestibility**	**Lipid digestibility**	**Ash digestibility**	**Carbohydrate digestibility**
C	79.23±0.46a	97.12±0.02a	82.38±0.18a	41.94±0.04a	99.75±0.66a
T1	81.82±0.01b	97.24±0.08a	89.78±0.22b	72.15±0.14b	99.74±0.29a
T2	86.88±0.11c	98.02±0.12b	94.60±0.21c	84.78±0.17c	99.77±0.03a
T3	83.6±0.65d	97.55±0.26c	92.11±0.23d	83.13±0.12d	99.75±0.74a

*Different letters within one column indicate the presence of significant differences at the level ($P< 0.05$).

DISCUSSION

It was reported that the chemical composition of poultry by-products varies depending on their source. This was confirmed by **Dawood and Najim (2022)**, who found that poultry slaughterhouse by-product meal used in the growth of common carp fingerlings (*Cyprinus carpio* L.) had a moisture content of 12.53%, protein content of 62.74%, fat content of 12.33%, and ash content of 12.41%. This result is consistent with that of **EL-Husseiny *et al*. (2018)**, who used poultry by-products to feed the African catfish (*Clarias gariepinus*), obtaining values of 61.5% protein, 12.36% fat, and 5.16% moisture in the by-products. **Watson (2006)** highlighted that the quality of poultry slaughterhouse by-product meal and its nutrient content depends largely on the quality of raw materials and the manufacturing process, which in turn affects growth performance and the apparent digestibility of protein when using these by-products as a feed ingredient for fish **(Shapawi *et al*., 2007)**. In a study on the nutritional properties and cost of poultry by-product meal in African catfish (*Clarias gariepinus*) diets, **Mamoon *et al*. (2019)** reported a chemical composition of 47.1% protein, 6.50% moisture, 13.31% fat, and 7.70% ash, which was lower than the current study's results. Additionally, **Nandakumar (2013)** found a protein content of 53.54% in protein concentrates prepared from poultry gizzard by-products, while **Taheri *et al*. (2013)** noted a protein content of 20.85% in protein hydrolysates, which was lower than the protein content in the present study. **Taheri *et al*. (2013)** also reported moisture at 66.90%, fat at 7.86%, and ash at 10.62%, with moisture being higher but fat and ash lower than in the current study. **Watson (2006)** emphasized that the quality of poultry by-product meal varies significantly depending on raw material quality and processing methods, which influences growth metrics and nutrient digestibility **(Shapawi *et al.*, 2007)**. Fishmeal composition also varies due to numerous factors, such as the type of fish used and processing methods **(Al-Noor *et al*., 2023)**. Among these factors, fishing season, capture location, fish maturity, size, and diet, all of which affect protein, fat, vitamin, and mineral levels in the fishmeal **(Dale, 2001)**. The current results are consistent with the study of **Al-Hassoon *et al*. (2021)**, who found variation in fishmeal composition depending on preparation methods, with protein levels ranging from 82.33 to 84.25%, fat from 6.05 to 7.12%, ash from 3.41 to 6.67%, and moisture from 3.78 to 4.13%. Studies by **Moghaddam *et al*. (2007)**, **Rostagno *et al*. (2011)** and **Al-Dalawi (2018)** also demonstrated variation in fishmeal composition depending on raw material and preparation methods. **Hossain *et al*. (2016)** compared the chemical composition of fifteen types of fishmeal, finding protein values between 31.3 and 61.2%, fat between 0.8 and 23.5%, and ash between 13.3 and 36.7%. Similarly, **Hendalia *et al*. (2019)** found that fishmeal prepared from fish waste exhibited protein content between 43.77 and 45.81%, depending on preparation methods. **Khan *et al*. (2012)** studied fishmeal made from nine fish sources and their waste, finding protein values between 37.43 and 66.57%, fat from 9.9 to 29.2%, ash from 12.7 to 28.2%, and gross energy from 4118 to 4883kcal/ g. In this respect, **Jeyasanta and Patterson (2020)** noted significant variation in the chemical composition of fishmeal, with moisture ranging from 5.80 to 16.54%, protein from 32.95 to 69.75%, fat from 4.83 to 9.9%, and ash from 11.48 to 14.68%. Correspondingly, **Jassim *et al*. (2024)** reported values of 5.26% moisture, 68.38% protein, 8.90% fat, and 17.46% ash in fishmeal, with ash content being higher than in the current study. **Biswas *et al*. (2022)** also presented comparable results, with crude protein, fat, and ash contents at 73.90, 8.28, and 16.40%, respectively.

The current results for amino acid estimation in poultry waste silage were comparable to those obtained by **Gumus and Aydin (2013)** in their study on the impact of secondary poultry slaughter waste on growth performance and fatty acid composition. They found that 50% of fish meal could be replaced with poultry slaughter waste meal in common carp fingerling diets, without the need to add amino acids. According to the **NRC (2011)**, the protein concentration in this study contained all essential amino acids required by fish, fulfilling all amino acid requirements without additional supplementation. **Osibona *et al*. (2009)** affirmed that essential amino acids could be sufficiently and abundantly provided through protein-rich diets or supplements and probiotics for fish, where amino acids play a crucial role as energy sources, protein builders, and regulators of metabolic pathways, particularly essential amino acids, which cannot be synthesized by the body and must be obtained through diet **(Hamidoghli *et al*., 2018)**. It is noteworthy that differences in amino acid availability may largely depend on processing methods and extraction technology, as well as the varying component amounts in the raw material **(Shapawi *et al*., 2007)**. The amino acid profiles varied across different fish meal types, attributed to the type of fish used and the method of processing **(Hossain *et al*., 2016)** Various researchers have highlighted these amino acid profile differences; **Hendalia *et al*. (2019)** demonstrated that amino acid composition in fish waste meal varied by preparation method, containing a full range of essential amino acids, with high levels of arginine and methionine, along with elevated valine and tryptophan content. These results align with those of **Jeyasanta and Patterson (2020)**, who observed significant variability in amino acid ratios across fish meals prepared from different raw materials, with alanine, glutamic acid, aspartic acid, arginine, and methionine showing elevated levels compared to other amino acids. Similarly, **Ween *et al*. (2017)** identified 12 essential amino acids crucial for growth and energy production when analyzing amino acids in two types of fish meal, highlighting lysine's importance due to its limited presence in plant protein sources. The current results align with multiple previous studies indicating clear differences in amino acid ratios and quantities and their influence on growth depending on the source and preparation method **(Cho and Kim, 2011; Ghaly *et al*., 2013; Prado *et al*., 2016)**. **Al-Noor *et al*. (2023)** identified 18 amino acids with varying ratios in different fish meal types, with glutamic acid showing the highest levels across all samples and tryptophan consistently low, similar to findings in our study. Balanced amino acids ranging between 25 and 50% contribute to fish proteins' high-quality composition and nutritional value, which is essential for fish to contain both essential and non-essential amino acids. These amino acids depend on diet and seasonal changes, making fish a highly nutritious and economically valuable food source **(Ghaly *et al*., 2013)**.

Some researchers have attributed the variability in fatty acid composition to differences in chemical composition, which are influenced by environmental factors, diet, sexual maturity, season, as well as variations in extraction methods and oil composition **(Jobling *et al*., 2002; Al-Kanaani, 2014)**. This aligns with **Lee *et al*. (2017)**, who found differences in fatty acid values across protein sources. Similar results were observed by **Jeyasanta and Patterson (2020)**, who identified variations in fatty acid ratios and compositions in two types of fish meal, with palmitic, oleic, and palmitoleic acids being dominant, while other fatty acids were present in varying proportions. **Ghaly *et al*. (2013)** and **Ido and Kaneta (2020)** also noted that fish meal contained high and varied levels of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) among different types.

The variability in chemical composition of the formulated diets aligns with numerous studies. For example, **Amtul and Amna (2012)** examined grass carp (*Ctenopharyngodon idella*) diets by replacing fish meal with poultry slaughter waste (chicken intestines). **Gumus and Aydin (2013)** found that the moisture content in the diets ranged from 7.61 to 8.63%, and ash values were similar, ranging between 11.23 and 11.42%. The protein content was close to 30.17%, consistent with the current study results. **Emre *et al*. (2003)** reported ash percentages between 6.48 and 8.25%, which were comparable to those recorded in the current study. **Dawood and Najim (2022)** observed similar results upon using various levels of protein concentrate from poultry waste. **Ayuba and Iorkohol (2013)** identified a clear variability in the chemical composition of the tested diets, which was also confirmed by **Al-Tameemi (2015)** when evaluating five different diets for fish feeding. **Tabinda and Butt (2012)** found protein percentages ranging from 42.40 to 49.65%, which were higher than those recorded in the current study. Similar protein content of 30.11% was also observed in **Taher *et al*. (2022)** for grass carp (*Ctenopharyngodon idella*) diets. **Jassim *et al*. (2024)** reported differences in protein, fat, and other diet components depending on preparation methods, which matches **Abdulwahab *et al*. (2023)** in their study on the chemical composition of various formulated fish diets, indicated that the variability is influenced by the type of raw materials used in the manufacturing process.

Weight and overall weight gain parameters, as well as growth rate, are essential and widely used criteria to evaluate feed quality and various feed additives, as they reflect the final outcome that is critical for achieving optimal results from high-quality feed sources **(Hepher, 1988)**. Numerous studies have examined the impact of various dietary components to establish and update guidelines for their use in fish nutrition, based on the specific nutritional needs they provide for the cultured species. Notable studies have explored their effects on growth, biochemical blood parameters, fish resistance to environmental stress, enhanced immunity, disease resistance, and production efficiency at minimal cost **(Bob-Manuel & Alfred-Ockiya, 2011)**. The results indicated that treatments containing poultry waste silage significantly outperformed the control, likely due to poultry slaughter by-product meal being rich in protein, fats, minerals, and a valuable source of essential amino acids, giving it high nutritional value **(Gindaba *et al*., 2019)**. These findings corroborate with those of **Siddik *et al*. (2019)**, who used 75 and 100% bio-processed poultry slaughter by-products in *Lates calcarifer* juvenile diets, observing no growth differences when fish meal was replaced with poultry slaughter by-product meal. The superior growth parameters of fish fed diets with poultry waste protein silage, as a partial fish meal substitute concur with studies such as **El-Husseiny *et al*. (2018**), where the specific growth rate ranged from 1.86 to 2.46g/ day when poultry by-products were used in African catfish diets. Similarly, **Sabbagh *et al*. (2019)** demonstrated that complete replacement of fish meal with poultry by-product meal in *Sparus aurata* diets did not affect fish growth or quality, suggesting that poultry by-products can be a valuable and sustainable raw material for fish feed. The current study showed that all growth parameters remained favorable at up to 50% replacement of fish meal with poultry slaughter waste silage. **Dawson *et al*. (2018)** reported significant differences in feed conversion ratio (FCR) and protein efficiency ratio (PER) between fish fed 100% fish meal and those with varying replacement levels. **Srour *et al*. (2016)** found that replacing fish meal with poultry slaughter by-product meal was acceptable in *Dicentrarchus labrax* diets up to 60%, with an optimal replacement at 40%, preserving survival rates and showing best FCR, PER, and PPV values at 60% replacement. **Dawood and Najim (2022)** also observed superior growth parameters with replacement diets compared to the control when using various levels of poultry waste protein concentrate. **Al-Habib and Al-Bassam (2011)** reported that improved nutrient digestibility is due to higher protein and fat digestibility in diets, while **Mohammed *et al*. (2013)** postulated that enhanced digestibility leads to better growth and survival rates. **Hernandez *et al*. (2014)** noted that nutrient digestibility values for *Lutjanus guttatus* decreased with higher fish meal replacement by poultry by-products, with significant differences attributed to lower amino acid content, particularly lysine and methionine, as replacement levels increased. **Zhou *et al*. (2011)** found high energy digestibility coefficients (ADC) of approximately 90.5% when studying secondary poultry by-products. **Dawson *et al*. (2018)** showed that protein and fat digestibility coefficients were favorable at all replacement levels, with protein ranging from 82 to 84% and fat from 90 to 92%. **Sugiura *et al*. (2000)** observed that raw materials with high bone and ash content reduce protein digestibility coefficients. In the current study, the apparent protein digestibility coefficient was relatively high, especially for treatment T2, indicating the high quality of the silage used, with protein digestibility reaching 98.02%. This high digestibility suggests that the protein in poultry slaughter waste silage is comparable to fish meal protein, making it a viable feed component for common carp (*Cyprinus carpio*). These results align with **Takakuwa *et al*. (2006)**, who used 40% poultry by-product meal with or without amino acid supplementation. **Yones and Metwali (2015)** noted that protein digestibility ratios increased with replacement levels, ranging between 94.3 and 94.6%, with high fat digestibility across treatments, comparable to fish meal-based diets. Similarly, **Shapawi *et al*. (2007)** found no differences in fat digestibility between the control diet and diets with 75 and 100% fish meal replacement in *Humpback Grouper* feed. The apparent carbohydrate digestibility coefficient was also high in this study, along with a higher ash digestibility coefficient than the control, with significant differences between treatments at a 0.05 significance level ($P \leq 0.05$), similar to **Al-Bassam (2020)** in his study on enzyme- and acid-treated protein-based diets for common carp.

CONCLUSION

In conclusion, this study confirmed experimentally that utilizing poultry waste silage as a protein source in the formulation of the common carp fingerling diets, at various replacement levels (25, 50, and 75%) as a partial substitute for fish meal, is successful, viable and sustainable option. The replacement process showed no adverse effects on feed and growth performance parameters, with the 50% replacement level outperforming all other diets across all studied parameters.

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