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Microbiological and Quality Assessment of Commonly Used Fish Diets from Basrah, Iraq

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ABSTRACT

This research was conducted to determine the microbial and quality assessment of some fish diets commonly used in Basrah Governorate. Samples weighing 1kg each were collected in December 2023 from various areas in the Basrah Governorate, randomly selected to represent the conditions of the sampled sources. The pour plate method was used for microbial counting, and the concentrations of biogenic amines were monitored using HPLC (High-Performance Liquid Chromatography). The quality characteristics measured included pH, TVN, FFA and TBA. The results indicated that the fish diet samples had a pH ranging from 6.53 to 6.95, with volatile nitrogen bases measured between 17 and 18.22mg nitrogen/100g fish. The free fatty acids values ranged from 0.27 to 0.84%, while the results for malondialdehyde (TBA) varied between 1.53 and 3.28mg malondialdehyde/kg fish. The diets contained 18 amino acids in a balanced composition of essential and non-essential amino acids, with varying profiles among all treatments. The microbial count results showed variability in numbers depending on diet type. The highest recorded total bacteria count was 200cfu/ g. The counts for protein-degrading bacteria, fat-degrading bacteria, *Staphylococcus*, coliforms and fungi were 90, 6, 3, 2, and 5cfu/ g, respectively. Histamine concentrations were measured between 0.547 and 1.582mg/ kg. In conclusion, the result confirmed the validity of the examined diets for feeding cultured fish in Basrah, Iraq. However, the study demonstrated the necessity of monitoring and evaluating the qualitative parameters of fish diets and detecting the levels of chemical, microbial, and histamine indicators to maintain fish health and support their growth.

INTRODUCTION

Proper nutrition is a crucial factor for the success of aquaculture by following a comprehensive dietary regimen that meets the needs of the cultivated fish species through the use of functional ingredients with immune-boosting properties in diet, which can enhance fish health, growth performance and disease resistance **(Singh *et al.*, 2021)**. Development of aquafeed industry relies primarily on the improvement of diets and the selection of high-quality raw materials to obtain balanced, high-quality diets that contain all the necessary nutrients for optimal growth and good health **(FAO, 2020)**. Fish diets are susceptible to spoilage and deterioration due to microorganisms and autolytic enzymes. These undesirable changes usually occur during storage period due to increased temperatures up to 25ºC and humidity exceeding 10% **(Marijani *et al*., 2017)**. The extent of contamination varies with geographical location, handling methods, storage periods and site cleanliness, which reduces the nutritional value of feed materials due to the production of mycotoxins by some fungi present in the diet, as well as the formation of biogenic amines and the generation of undesirable odors from diets fish in Basrah, Iraq **(Al-Noor *et al*., 2023a)**. Since fish diets contain high levels of nutrients such as proteins and fats, they are prone to breakdown due to oxidation and autolysis, resulting in many acids and volatile compounds that could adversely affect fish health leading to stunted growth phenomenon **(Olayiwola & Adedokun, 2015)**. **Craig and Helfrich (2018)** indicated that diets should not be stored for more than 100 days and should be carefully inspected before use in fish feeding to avoid diseases and reduced survival rates. The immune system of fish can be depressed when they are fed contaminated diets containing fungi, bacteria, or toxic metabolites **(FAO, 2019)**. Nutritional value could be significantly reduced because of such contamination which lead also to substantial losses in important vitamins like C, E and thiamine with progression of storage, leading to decreased growth rates and making fish more susceptible to diseases **(Fallah *et al*., 2014)**. These negative effects result in profit losses, slow growth, reduced weight and wastage of food quantities provided to reach market weight, in addition to treatment costs **(Bryden *et al*., 2012)**. Protecting diets from spoilage involves maintaining the quality of raw animal and plant feed ingredients, as well as optimal storage conditions for feed materials **(Diyie *et al*., 2024)**. Given the nutritional and economic importance of fish diets, this research was conducted to evaluate fish diets quality by applying microbial and qualitative tests and to diagnose the potential causes of spoilage and deterioration in diets used in fish farming to mitigate spoilage, prevent economic loss and to assess their impact on diet quality and fish nutrition.

MATERIALS AND METHODS

Sample collection

The study inspected five types of locally produced fish diets obtained from various regions in Basrah Governorate, southern Iraq. Each sample weighed 1kg and was randomly selected to represent the condition of the sampled source. The diets were in the form of pellets and were of the sinking type. Diet samples collected during December 2023 were transported into polyethylene bags to the laboratory at the Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah for further laboratory testing.

Estimation of chemical composition

Moisture contents were assessed by oven drying at 105°C, ash percentage was calculated in a muffle furnace at 525°C, protein contents were estimated using Kjeldahl, lipid content was determined using Soxhlet extraction according to the method described in the study of **Egan *et al*. (1988)**. The carbohydrate percentage was calculated mathematically according to **AOAC (2000)**.

Estimation of amino acids

Amino acid profiles of prepared fish meal samples was 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 chemical indicators

Estimation of pH value

A pH meter was used to measure pH values according to the method described by **Wong *et al*. (1991)**.

Estimation of total volatile nitrogen TVNB

Based on **Egan *et al*. (1988)** method, the collected distillate was titrated with sulfuric acid of 0.1 normality until light pink color appeared instead of green-blue. The volume of acid used in titration was multiplied by 14 to obtain the TVNB (Total volatile nitrogen base) in milligrams nitrogen per 100 grams of fish meat according to the following formula: **100g fish = ml 0.1N $H2SO4$ × 14⁄ TVNB mg N**

Estimation of thiobarbituric acid (TBA)

The applied method to estimate the thiobarbituric acid was adopted from **Egan *et al*. (1988)**, by using TBA reagent and the final solution measured spectrophotometer at a wavelength of 538 nanometers. The TBA number was calculated using the following formula:

$$7.8 \times \text{Absorbance} = \text{TBA mg malonaldehyed/kg fish}$$

Estimation of acid value (AV)

The calculation of free fatty acids was conducted according to **Wong *et al*. (1991)** based on oleic acid using the following formula:

$$\text{FFA} = \frac{\text{Volume of NaOH in milliliter} \times \text{Molarity} \times 28.2}{\text{Weight of the Sample (gm)}}$$

Estimation of microbial counts

Microbial counts for the samples were estimated by preparing decimal dilutions. The pour plate method was then used, transferring 1ml from 10^{-3} and 10^{-6} dilutions into sterile Petri dishes and adding a nutrient medium at 45°C. Moreover, nutrient agar with 10% skim milk was used to grow protein-degrading bacteria and Tween 80 was added to isolate fat-degrading bacteria. MacConkey Agar was used to isolate total coliform bacteria, *Staphylococcus* bacteria were isolated using Staph 110 medium and fungi were isolated using Potato Dextrose Agar according to the method outlined by **Andrews (1992)**.

Detection and determination of biogenic amines

High Performance Liquid Chromatography (HPLC) technique was applied to detect and quantify biogenic amine (histamine) levels providing the conditions mentioned by **Moret and Conte (1996)**. Samples of 10µl of standard biogenic amines as well as 10µl each studied sample were examined. The HPLC device was used in the laboratories of the Ministry of Industry and Minerals/Baghdad, Iraq, and the reverse-phase column ODS2 C18 with dimensions of 6.4 × 250mm and the H-Plex-Hi column type were used. The detection was performed at a wavelength of 245nm. Separation was carried out using a mobile phase consisting of a mixture of 5:5 H2O: Acetonitrile: H2O (v:v), at a temperature of 40°C and a flow rate of 1mL/ minute. The concentration of biogenic amines was estimated using the following equation:

Biogenic amine conc. Mg/kg. = conc. of standard X (area of amine area / area of sample)

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 was 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

Examined diets chemical composition is shown in Table (1). The results illustrated the chemical composition variability between the studied diets. Highest recorded moisture content was 8.36% for diet T5, while the lowest was 6.32% in diet T2 compared to the others T1, T3 and T4 which had moisture contents of 8.32, 7.47, and 6.64%, respectively. Regarding protein content, diet T3 had the highest protein value at 31.33%, differing from the other treatments T1, T2, T4, and T5, which had protein values of 29.25, 30.88, 29.42, and 30.14%, respectively. Simultaneously, the highest fat level was found in diet T4, with a percentage of 5.77%, differing from treatments T1, T2, T3, and T5, which had fat percentages of 3.18, 4.52, 5.44, and 4.29%, respectively. All treatments showed varying levels of ash content depending on diet type with values ranging from 10.71, 8.12, 7.75, 9.11, and 7.46% for diets T1, T2, T3, T4, and T5, respectively. The results for carbohydrate values indicated that the lowest content was attributed to diet T3 at 48.01%, differing from the other treatments with carbohydrate values of 48.54, 50.16, 49.06, and 49.74% for diets T1, T2, T4, and T5, respectively.

Table 1. Proximate composition (%) in examined fish diets from Basrah, Iraq

Nutrient (%)	T1	T2	T3	T4	T5
Moisture	8.32	6.32	7.47	6.64	8.36
Crude protein	29.25	30.88	31.33	29.42	30.14
Crude lipid	3.18	4.52	5.44	5.77	4.29
Ash	10.71	8.12	7.75	9.11	7.46
Carbohydrate	48.54	50.16	48.01	49.06	49.74

The results of the chemical evidence presented in Table (2) show variability depending on the type of studied diets. The results indicated variability in pH values across the treatments, with the highest pH value being 6.95 in diet T1, while diet T2 had the lowest pH value at 6.53. The pH values for diets T3, T4, and T5 were 6.91, 6.74, and 6.88, respectively. No significant differences ($P > 0.05$) appeared among study treatments. Regarding the volatile nitrogenous bases in the different diets, the results showed obvious variations among the treatments, with the highest ratio being in treatment T1 at 18.22mg nitrogen/100g fish, followed by treatment T3 at 17.7mg nitrogen/100g fish, with a significant difference ($P < 0.05$) from the other treatments. The lowest values for volatile nitrogenous bases were found in diet T2 averaging 17mg nitrogen/100g fish, while the other treatments had values of 17.11mg nitrogen/100g fish for T4 and 17.2mg nitrogen/100g fish for T5, respectively. The results showed variability in the average values of free fatty acids across the different diets with the highest values found in samples T1 at an average of 0.84%, followed by diet T5 at 0.61%. The lowest average of free fatty acids was 0.27% for diet T2, followed by diet T4 at 0.33%, while the free fatty acid percentage in diet T3 was 0.57%. Significant differences ($P < 0.05$) were evident among all treatments. The results indicated that the highest level of thiobarbituric acid was in T1 at an average of 3.28mg malondialdehyde/kg fish, followed by diet T3 at 2.95mg malondialdehyde/kg fish. Diet T2 had the lowest average thiobarbituric acid value at 1.53mg malondialdehyde/kg fish, with the other treatments varying in values to reach 1.86 and 2.56mg malondialdehyde/kg fish for diets T4 and T5, respectively. Statistical analysis revealed significant differences ($P < 0.05$) among all treatments.

Table 2. Chemical indicators in examined fish diets from Basrah, Iraq

	T1	T2	T3	T4	T5
pH	6.95a ±0.01	6.53a ±0.05	6.91a ±0.02	6.74a ±0.07	6.88a ±0.01
TVN	18.22bc ±0.40	17a ±0.00	17.7ab ±0.070	17.11a ±0.200	17.2a ±0.030
FFA	0.84c ±0.024	0.27a ±0.033	0.57b ±0.033	0.33a ±0.016	0.61b ±0.033
TBA	3.28c ±0.05	1.53a ±0.09	2.95bc ± 0.03	1.86a ±0.05	2.56b ±0.04

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

Table 3. Amino acid profiles in examined fish diets from Basrah, Iraq

Amino acid		T1	T2	T3	T4	T5
EAA	Histidine	1.62	2.036	0.97	1.57	1.81
	Isoleucine	3.39	3.87	3	3.2	3.77
	Leucine	5.55	5.536	4.37	5.52	5.63
	Lysine	5.77	6.18	5.87	5.95	6.26
	Methionine	1.45	1.55	1.19	1.41	1.45
	Phenylalanine	3.13	3.72	3.83	3.3	3.61
	Arginine	4.11	4.49	4.39	4.09	4.39
	Threonine	3.15	3.67	4	3.43	3.73
	Valine	4.21	3.91	4.66	3.54	3.94
∑EAA		**32.38**	**34.96**	**32.28**	**32.01**	**34.59**
NEAA	Aspartic acid	6.54	6.62	7.77	6.6	6.68
	Glutamic acid	7.403	8.02	8.76	7.59	7.95
	Serine	2.88	3.09	3.46	2.9	2.97
	Glycine	3.63	4.13	4.18	3.75	4.23
	Alanine	4.03	4.41	4.51	4.16	4.34
	Proline	2.53	3.2	3.09	2.54	2.92
	Tyrosine	2.66	2.84	3.06	2.65	2.75
	Cysteine	0.88	1.14	0.69	0.88	1.19
	Trptophan	0.84	1.37	2.32	0.86	1.37
∑NEAA		**31.39**	**34.82**	**37.84**	**31.93**	**34.4**

* EAA, Essential amino acids; NEAA, Non-essential amino acids.

The results in Table (3) present the amino acid analysis using HPLC for the examined diets, indicating that the diets contained 18 essential and non-essential amino acids in a balanced composition and with varying proportions across all treatments. The current results showed that the amino acid glutamic acid was present at the highest level in diet T3, at 8.76µg/ 100µg protein, as well as in all diets, with values of 7.40, 8.02, 7.59, and 7.95µg/ 100µg protein for diets T1, T2, T4, and T5, respectively. Conversely, diets T2, T3, and T5 had the lowest levels of the amino acid cysteine, at 1.14, 0.69, and 1.19µg/ 100µg protein, respectively. The lowest levels were recorded for the amino acid tryptophan, with values of 0.84 and 0.86µg/ 100µg protein in diets T1 and T4. Regarding essential amino acids, their proportion was higher in diet T2 at 34.96%, while the highest levels of non-essential amino acids were found in diet T3 at 37.84µg/ 100µg protein.

The results presented in Figs. (1, 2, 3, 4, 5, and 6) show the microbial counts for the examined diets. The findings indicated clear variations in microbial counts based on diet type. Highest values for total bacteria were recorded for treatment T1, which reached 260cfu/ g, followed by treatment T3 at 200cfu/ g, while the counts for treatments T2, T4, and T5 were 180, 170, and 184cfu/ g, respectively. As for protein-degrading bacteria, diet T1 had the highest count at 110cfu/ g, followed by treatments T3 and T5, each with 90cfu/ g. Treatment T2 had a count of 70cfu/ g, while diet T4 recorded the lowest count for protein-degrading bacteria at an average of 60cfu/ g. Additionally, a variation in the counts of fat-degrading bacteria was observed among the treatments, with the highest counts in treatments T1 and T3 at 6cfu/ g. This value decreased to 4cfu/ g in treatments T2 and T4, which represented the lowest counts compared to treatment T5, which had 5cfu/ g. Regarding the number of *Staphylococcus* colonies, there was close counts between the studied treatments, with 3cfu/ g for diets T1 and T3, decreasing to 2cfu/ g for diets T2, T4, and T5. The coliform bacteria counts were recorded at 2cfu/ g for diets T1 and T3, while the counts for diets T2, T4, and T5 were 1cfu/ g. The results also indicated differences in fungal counts among the studied diets, with diets T1 and T3 having the highest values at 5cfu/ g, which decreased to 2cfu/ g in diet T2, while the counts were 3 and 4cfu/ g for diets T4 and T5, respectively.

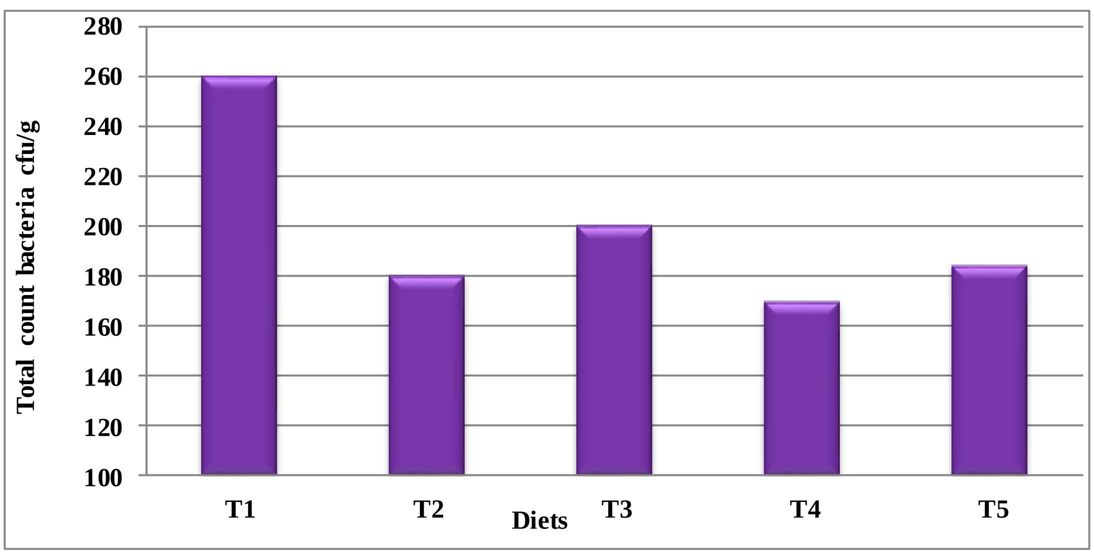

Fig. 1. Total bacterial counts in examined fish diets from Basrah, Iraq

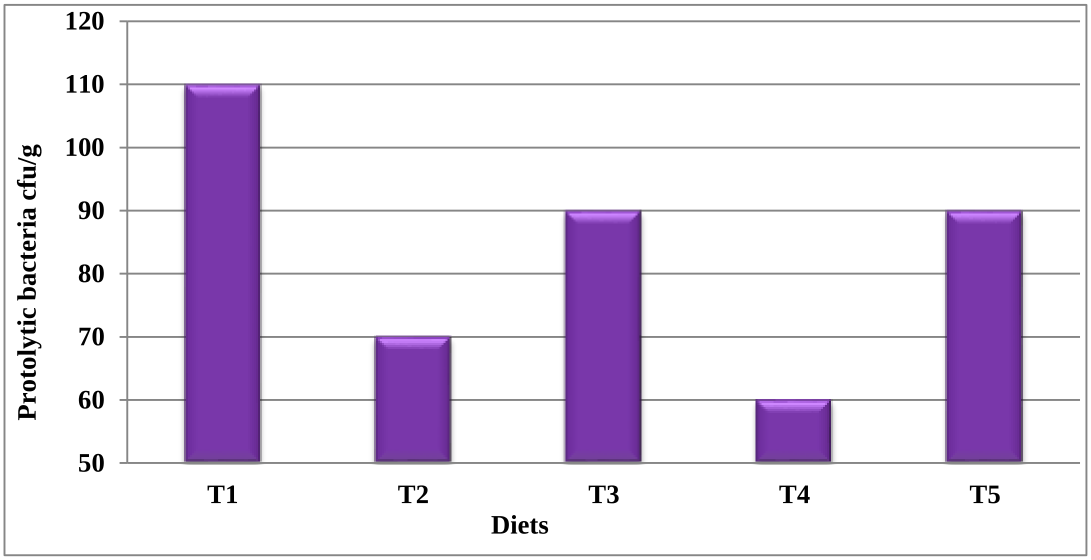

Fig. 2. Proteolytic bacteria in examined fish diets from Basrah, iraq

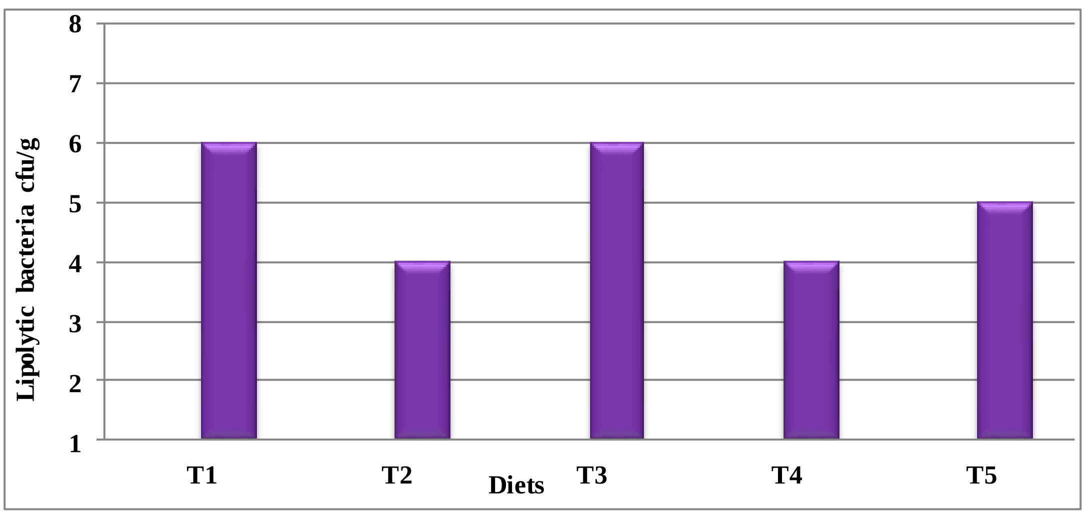

Fig. 3. Lipolytic bacteria in examined fish diets from Basrah, Iraq

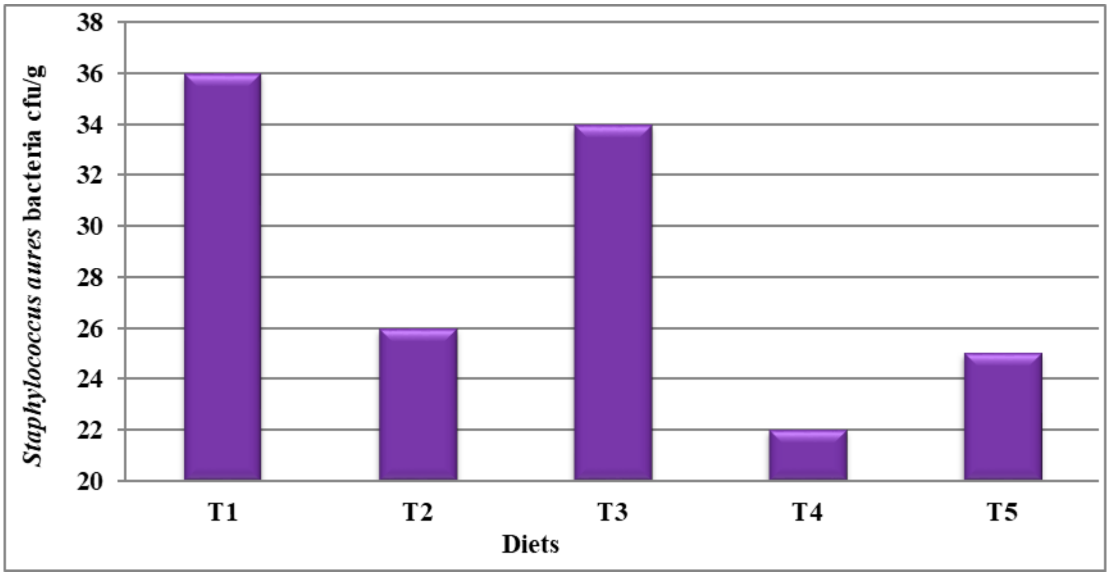

Fig. 4. *Staphylococcus aureus* bacteria in examined fish diets from Basrah, Iraq

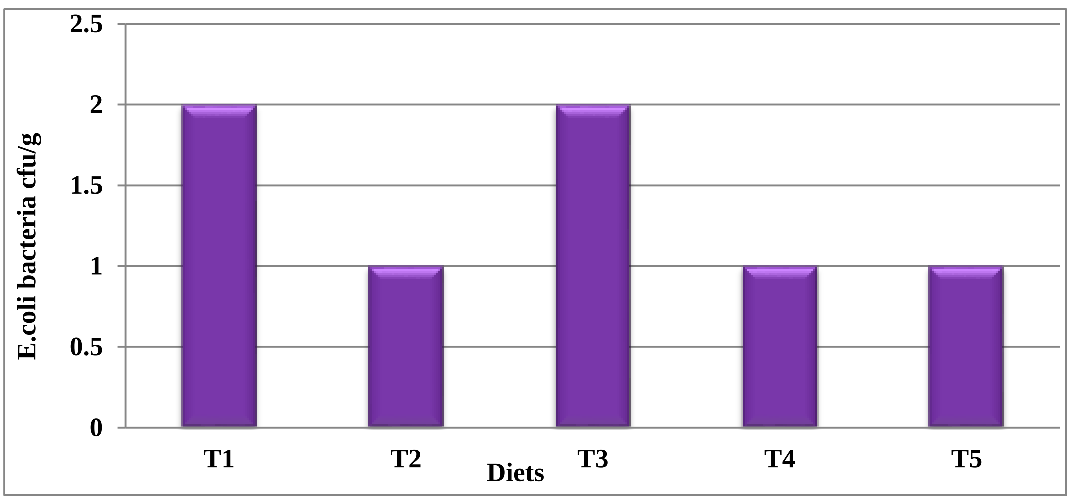

Fig. 5. *E. coli* bacteria in examined fish diets from Basrah, Iraq

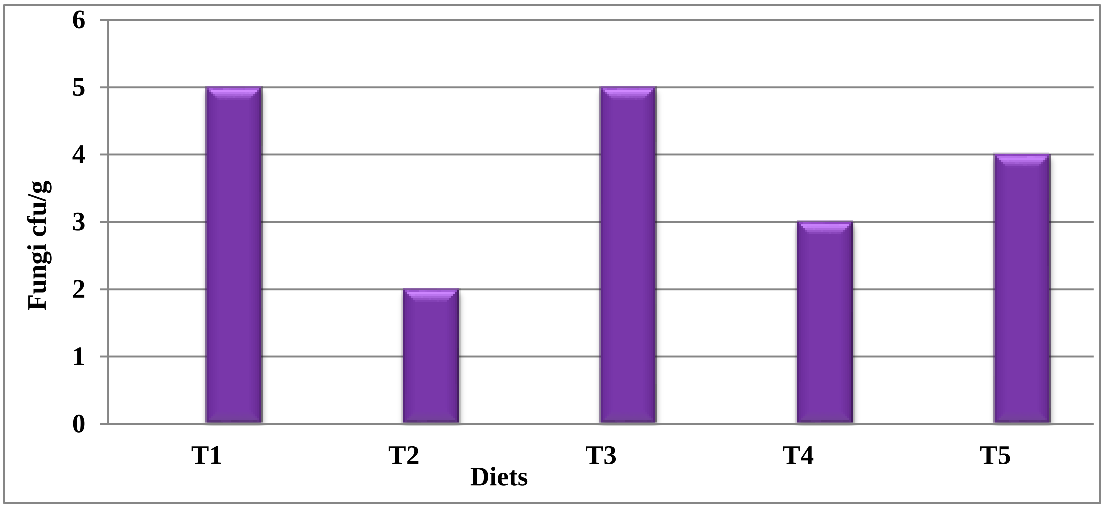

Fig. 6. Fungi in examined fish diets from Basrah, Iraq

The results shown in Fig. (7) illustrate the concentration of biogenic amines (histamine) isolated from the studied fish diets. A noticeable variation in amine concentrations was obvious among the studied samples, with the lowest histamine concentration recorded in diet T2 at 0.547mg/ kg and the highest was found in diet T1 at 1.582mg/ kg. The concentrations in the other samples were 1.203, 0.836, and 0.977mg/ kg for diets T3, T4, and T5, respectively.

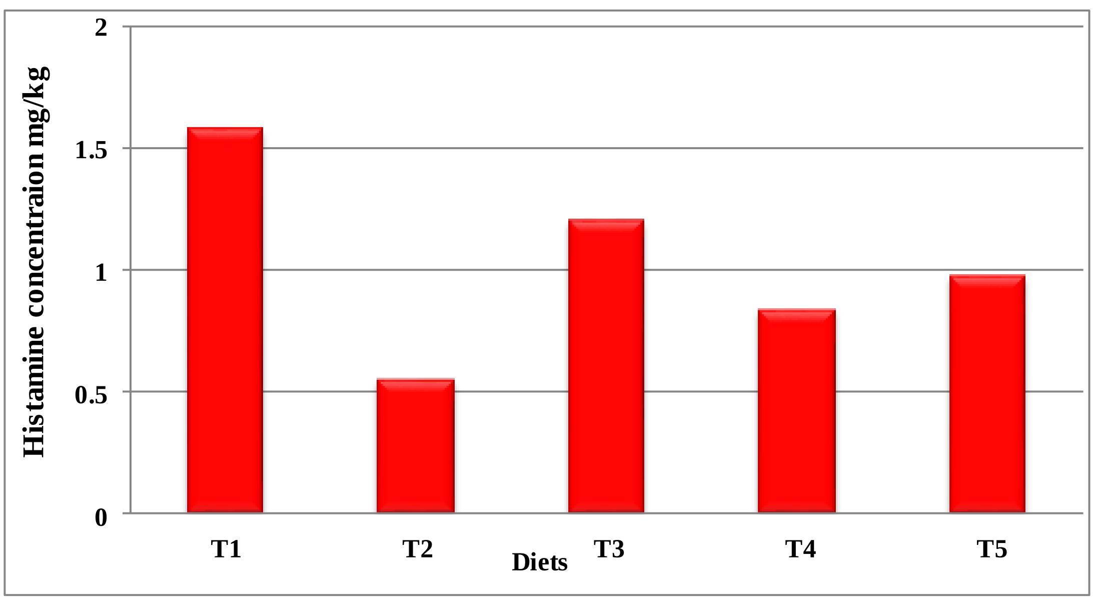

Fig. 7. Histamine concentrations in examined fish diets from Basrah, Iraq

DISCUSSION

Diets chemical composition and additives are considered to be important factors that have a significant impact on the chemical composition of fish bodies **(Craig & Helfrich, 2017)**. **Richard *et al*. (2021)** confirmed that the presence of essential nutrients necessary for growth and energy production, particularly proteins, fats, carbohydrates, and vitamins, is ideal and balanced in fish diet, such as amino acids, fatty acids, natural additives, and probiotics, which enhance the nutritional value of the diets, positively impacting fish growth and body chemical composition **(Luo *et al*., 2021)**. **Aydin and Gumus (2013)** indicated that moisture content in diets ranged from 8.63 to 7.61%. Regarding protein values, they were at 30.17%, which is consistent with the findings of **Taher *et al*. (2022)**, who noted that protein content reached 30.11% for diets prepared for feeding the grass carp (*Ctenopharyngodon idella*). **Ayuba and Iorkohol (2013)** observed a clear difference in the chemical composition of various examined diets, which was also confirmed by **Al-Tameemi (2015)** in his previous study in which he evaluated five different types of diets for fish feeding. The values evaluated are similar to those reported by **Amtul and Amna (2012)**. The differences in the chemical composition of diets are consistent with the study by **Rhema and Al-Noor (2022)**, who found a clear variation in the chemical compositions due to differences in the components of manufactured diets. Additionally, **Jassim *et al*. (2024)** found differences in protein, fat, and other components of the diet depending on the preparation method. **Abdulwahab *et al*. (2023)** also noted differences in the chemical composition of various types of manufactured fish diets, indicating that the variation could largely depend on the type of raw materials used in the manufacturing process.

The role of microorganisms in breaking down proteins into amino acids and the release of nitrogen which leads to ammonia accumulation as a result of amino acid degradation could be a possible reason for raising pH values **(Souza *et al*., 2019)**. The pH values were an appropriate indicator for assessing diet quality taking into the account that the differences between current treatments were not significant. The hydrolytic degradation of proteins caused by proteolytic enzymes secreted by protein-degrading bacteria could be one possible reason for the increased total volatile nitrogen **(Duarte *et al*., 2020)**. The levels of volatile nitrogenous bases are affected by different manufacturing methods, storage durations, temperatures and variations in raw materials. According to the standard specification ES 3439:2005, the permissible values for volatile nitrogenous bases are 30mg nitrogen/100g fish. The increase in FFA ratios may be attributed to increased occurrence of hydrolytic rancidity caused by bacterial enzymes **(Mehrabi *et al*., 2021)**. The increase in the value of thiobarbituric acid is attributed to the occurrence of autoxidation and aldehydes and ketones production **(Tingting *et al*., 2012)**. **AL-Kuraieef *et al*. (2022)** emphasized the necessity of assessing the qualitative and chemical quality of fish and its products by measuring volatile nitrogenous substances, the formation of biogenic amines, and fat oxidation to determine quality indicators during storage. Measuring TBA is crucial for determining the level of autoxidation and undesirable rancid odors. The standard specification ES 3439:2005 indicates that the upper limit for TBA values does not exceed 4.5mg malondialdehyde/kg fish **(Nazemroaya *et al*., 2011)**.

Balanced amino acids ranging from 25 - 30% make fish proteins well-structured and nutritionally valuable as it should contain both essential and non-essential amino acids, which depend on nutrition and seasonal variations, and thus fish can have high nutritional and economic value **(Ghaly *et al*., 2013)**. Amino acid profiles in fish diets could significantly vary according to the type and method of preparing fish meal and protein concentrates used in diet formulation, which reflects on the levels of protein, fats, vitamins, and minerals **(Al-Noor *et al*., 2023b)**. Amino acid variations could be based on prepared meal types which has been previously confirmed by many researchers**.** **Hendalia *et al*. (2019)** showed that amino acid profiles in the prepared meals varied according to the processing method, indicating that processed meals contained a complete set of essential amino acids with arginine, methionine, valine, and tryptophan being the highest. These findings align with a later study by **Jeyasanta and Patterson (2020)** on the amino acid composition of fish meal prepared from different raw materials. The authors found variability in proportions with high levels of the amino acids alanine, glutamic acid, aspartic acid, arginine, and methionine compared to other amino acids. The current research are consistent with several previous studies that demonstrated clear variations in the proportions and quantities of amino acids in manufactured fish diets and their impact on fish growth depending on the source of preparation and the method used **(Prado *et al*., 2016; Ween *et al*., 2017)**. **Osibona *et al*. (2009)** confirmed that essential amino acids can be obtained in a balanced manner and in abundant quantities through fish consumption of protein-rich foods or dietary supplements and probiotics. Amino acids could play a significant role as a source of energy, protein building and the regulation of metabolic pathways, especially essential amino acids which cannot be endogenously synthesized and must be exogenously obtained through nutrition **(Hamidoghli *et al*., 2018)**. **Nekoubin and Sudagar (2012)** emphasized that a good and balanced diet could play a significant role for the success of aquaculture, positively impacting growth and production. Successful aquaculture relies on nutritionally balanced diets that provide all necessary nutrients for vital functions, growth, and optimal health **(Kord *et al*., 2021)**. Additionally, dietary supplements contribute to and support growth rates, improve digestibility, increase disease resistance and immunity, and reduce stress and mortality **(Lund, 2021)**.

The variation in microbial counts among the diets could be attributed to the presence of certain natural or synthetic additives that inhibit microbial growth at different levels depending on the source of the formulated diet **(Genskowsky *et al*., 2015)**. This was confirmed by **Pezeshk *et al*. (2015)**, who found that the use of natural preservatives reduced microbial activity in various fish diets. These findings corroborate with the results of **Hussain *et al.* (2021)**, who showed that the use of natural oils could reduce oxidation and inhibit microbial enzymes that degrade fats and proteins in fish products. Additionally, the level of microbial contamination in raw materials used for diet manufacturing can vary depending on inadequate storage conditions, resulting from poor lighting, ventilation, and humidity which enhance the growth and spread of fungi **(Lee & Ryu, 2017)**. Several studies have documented the existence of fungi in fish diets and their ingredients in varying proportions. For instance, *Mucor* sp., *Penicillium*, and *Eurotium* were isolated from fish diets and their components by **Greco *et al*. (2015)**. **Marijani *et al*. (2017)** studied the existence of fungal species in various fish diets according to the type of diet and their storage method. **Eskola *et al*. (2019)** conducted a review study on field crop contamination with an emphasis on fungi due to their adverse effects on the health of humans and animals by producing mycotoxins **(Hassan *et al*., 2014)**. Environmental conditions, including high temperature and humidity during storage, increase the likelihood of these microorganisms growing in food products **(Lee & Ryu, 2017)**. The current study aligns with the results of **Actis *et al*. (2001)**, who confirmed the contamination of fish diets by various types of Gram-negative rod and cocci bacteria at varying levels, leading to contamination of the diets with pathogens affecting public health. Consequently, diseases and poisoning can occur in humans consuming contaminated fish products **(Mitiku *et al*., 2023)**. **Ghaly *et al*. (2010)** reported a waste and loss of fish up to 30% due to microbial activity, while **Olayiwola and Adedokun (2015)** identified 28 distinct bacterial isolates from certain fish diets, noting the presence of *Bacillus* sp. at 33%, *Staphylococcus* sp. and *Streptococcus* sp. at 13% each, and *Proteus* sp. at 6%, with *Klebsiella* sp. and *Pseudomonas* sp. at 7%. They indicated that inappropriate storage conditions promote the survival and multiplication of microorganisms in diets, producing harmful toxic substances for fish. **Diyie *et al*. (2024)** revealed the presence of various bacterial and fungal contaminants in fish diet types, reporting *Streptococcus iniae*, *Streptococcus agalactiae*, and *Staphylococcus aureus* at 30%, with fungal contamination at 70%. Thus, diet contamination can be considered a route for disease entry into aquaculture systems, causing significant economic losses in fish farming **(Verner Jeffreys *et al*., 2017)**. **CDC (2013)** reported that fish account for approximately 24% of foodborne illness outbreaks and 6% of all food poisoning cases.

Biogenic amines is defined as chemical compounds that can form in fish meals and animal protein concentrates resulting from the biological degradation of proteins present in these products **(Park *et al*., 2010)**. The significant difference in histamine levels in the diets could be related to several factors like processing method, quality of raw material, storage, transport, manufacturing and the variation in amino acids quantity and quality among the concentrates and fish meal **(Özdestan & Üren, 2010)**. Lower levels of biogenic amines could be good indicator of high quality in fish meal, animal protein concentrates and manufactured diets **(Mundheim *et al*., 2004)**. The increase in the levels of biogenic amines is linked to the presence of certain bacteria in fish and protein concentrates, as well as the enzymes they produce, which are responsible for the rise in biogenic amines, adversely affecting their levels in the diets **(Naila *et al*., 2012)**. **Jasour *et al*. (2018)** showed the influence of biogenic amines on fish growth due to the low essential amino acids resulting from microbial degradation during storage, which in turn adversely affects fish growth parameters. Such a result concurs with that of **Tapia-Salazar *et al*. (2004)**, who discovered a reduction in fish growth performance when fish were fed diets containing biogenic amines formed during storage due to microbial action. Similarly, **Kordiovská *et al*. (2006)** found elevation of biogenic amine concentrations with higher temperatures and longer storage periods. The results of this study are consistent with those of **Jaw *et al*. (2012)**, who reported varying levels of biogenic amines ranging from 1.4 to 9.12mg/ 100g in 40 samples of fish diet, fish meal, and protein concentrates due to five species of histamine-producing bacteria. **Kennedy *et al*. (2004)** recorded histamine levels exceeding 20mg/ 100g in 11 out of 25 samples of fish meal, indicating that biogenic amines primarily form through the decarboxylation of specific free amino acids by decarboxylase enzymes released by foodborne bacterial species, as confirmed by **Tsai *et al*. (2005)**. However, high concentrations of histamine and biogenic amines in diets and their ingredients could be toxic and pose risks, leading to numerous adverse effects on fish health, including lowered growth rates, reduced diet intake, lower immunity and weight loss **(Lumsden *et al*., 2002)**.

CONCLUSION

In conclusion, the sustainability of the aquafeed production sector and its ability to continue and remain economically competitive while generating profits seriously depends on ensuring the quality and safety of these products, their ability to achieve optimal fish growth, and maintaining the health and well-being of the fish. The practices of manufacturing aquafeeds, preparing its components, and storing the ingredients and produced diet all require continuous monitoring and inspection to ensure adherence to the highest required quality standards. Fish diets with higher quality are crucial for fish growth, health and the overall success and sustainability of fish farming activities.

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