

## Detection and Levels of Some Mycotoxins and Biogenic Amines in Fish Diets and Feed Ingredients from Basrah, Iraq

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### ABSTRACT

The present research was conducted with the aim of isolating and diagnosing mycotoxigenic fungi in fish feed and some of its components (soybean meal, yellow corn, wheat flour, barley flour and wheat bran), as well as determining the levels of biogenic amines contamination in fishmeal and animal protein concentrate. Samples were randomly collected from various locations in Basra province, with 1kg per sample, in October 2022 to represent the sample taken. The ability of some isolated fungi to produce aflatoxins was detected using three culture media (potato dextrose agar, yeast extract agar, and coconut agar) and three temperature levels (25, 30 and 35°C). Results showed that six types of fungi were isolated from the grain and fish feed samples. *Aspergillus niger* was the most frequent fungal species found in the studied samples, with a frequency rate of 22.7% to 34%. The fungal species *Alternaria alternata* ranked second in frequency rate, ranging between 11.16% and 20.96% in the studied samples. The remaining fungi, including *Rhizopus*, *Mucor*, *Penicillium* spp. and *Aspergillus flavus* had lower frequency rates than the two previous fungi. Some of the fungal species had the ability to produce aflatoxins at a temperature of 35°C, especially *A. flavus* and *A. niger* on the YES and PDA culture media. The production ability of the remaining fungi was low and varied. The results showed that the concentration of amines varied among the different samples. Histamine had the lowest concentration in the animal protein concentrate at 1.382mg/ 100g, while its concentration was higher in fish meal at 2.836mg/100g. Cadaverine had a lower concentration than histamine, with 0.384mg/100g in animal protein concentrate and increasing up to 0.778mg/100g in fish meal. It was concluded that monitoring fish feed and its components is necessary to detect levels of fungi, mycotoxins and various amines in order to maintain fish health.

### INTRODUCTION

The success of fish farming depends largely on developing feeds, selecting and blending the suitable raw materials to achieve balanced diets, as it has a crucial impact on fish biological activity, growth and optimal health (Enyidi *et al.*, 2017). Fish feeds are typically manufactured from animal-based ingredients (slaughterhouse waste, fish and

crustacean waste and blood meal) and plant-based materials, including grains, oilseeds and their by-products. These ingredients may become contaminated with various fungi, bacteria and auto-enzymes during storage or blending (Bryden, 2012), which can reduce feed quality due to the production of an unstable feed odor or toxic secretions of some fungi in the feed that produces mycotoxins in addition to the formation of harmful biogenic amines (Eskola *et al.*, 2019; Feddern *et al.*, 2019). Aflatoxins are formed during storage when the temperature rises to 25°C and humidity reaches more than 10%, and the extent of contamination varies by geographic location, trading practices and storage methods (Marijani *et al.*, 2017). Aflatoxins and ochratoxins, produced by various fungi, are among the most potent toxins known and can cause serious pathology at concentrations as low as less than 10ppm (Embaby *et al.*, 2015). The reason for this is that they are heat-resistant to a degree that makes them difficult to destroy by traditional thermal treatments used in diet manufacturing, and the second reason is that they spread quickly from fungal colonies to feed. Therefore, removing fungal-infected parts of feed does not completely eliminate mycotoxins formed in these feeds, and thus, fungal growth on these feeds should be entirely avoided (Lević *et al.*, 2013). Aflatoxins in feed are produced by the fungi *A. flavus* and *A. parasiticus*, and the most important types of aflatoxins are B1, B2, G1 and G2 (Barbosa *et al.*, 2013).

Biogenic amines are organic compounds with low molecular weights that are mainly formed by decarboxylation of amino acids or by amination or transamination reactions of aldehydes and ketones. They are often created through natural metabolic activity in animals, plants and microorganisms (Özdestan & Üren, 2010). The most common biogenic amines found in foods and feeds are histamine, tyramine, putrescine, cadaverine, spermine and spermidine (Park *et al.*, 2010). Biogenic amines can affect animal and human health, and their levels increase as a result of uncontrolled microbial growth or food contamination. High levels of biogenic amines can cause different kinds of poisoning (Karovičova & Kohajdova, 2005). Moreover, they are also considered as serious carcinogens because they can form nitrosamine compounds, which are known to be carcinogenic either through certain enzymatic reactions of some microorganisms or through some manufacturing processes involving nitrates and nitrites (Kim *et al.*, 2009). (Barbosa *et al.*, 2013) reported that, fish immune systems are weakened when they are fed with diets contaminated with mycotoxins, biogenic amines or toxic metabolites, resulting in a decrease in nutritional value and loss of vitamins C, E, and thiamine over the storage time. In addition, growth rates decrease significantly, making fish more susceptible to diseases. These detrimental effects often continue unobservable by fish culturists, leading to slow growth and weight loss and a costly increase in diet amount of needed to reach market weight, as well as increased treatment costs (Bryden *et al.*, 2012).

Feed contamination with mycotoxins and biogenic amines is one of the most important problems facing the feed industry, especially in developing countries due to the

lack of modern technologies for harvesting, drying and storage of grain crops, which are widely used as raw materials for feed production. This increases the likelihood of contamination by various fungi and bacteria (Oliveira, 2009). Therefore, the aim of this study was to detect and determine the levels of fungi, mycotoxins and biogenic amines in the main raw materials used in fish feed formulation, as well as in feed itself, to ensure the safety of the diets used in aquaculture from these hazardous contaminants that can seriously affect the health of both humans and animals.

## MATERIALS AND METHODS

### Sampling

Samples of locally made fish feed were collected in addition to some of their components including soybean meal, yellow corn, wheat flour, barley flour, and wheat bran from different areas in Basrah governorate, Iraq. Each sample was 1kg in weight, and they were randomly collected to represent the status of sampled batch. The samples were collected in October 2022 and placed in tightly sealed polyethylene bags. Upon arrival to the laboratories at the Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, samples were appropriately prepared for testing and analysis.

### Moisture and protein content

Sample percentage moisture contents were assessed by oven drying at 105°C, protein contents were estimated using Kjeldahl according to the method described in the study of Egan *et al.* (1988).

### Isolation and identification of fungi

Serial dilution method was used to isolate fungi by taking 1g of each sample and adding it to 9mL of sterile 0.1% peptone water solution; they were mixed well to prepare the first dilution (10-1), followed by preparing the remaining decimal dilutions up to 10-6. Then, the pour plate method was used by transferring 1mL of each dilution (10-3 and 10-6) separately onto sterilized petri dishes containing potato dextrose agar (PDA) medium, with three plates for each dilution. Plates were incubated at 25°C for 5 days until the fungal colonies appeared clearly on the surface of the medium (Andrews, 1992). Colonies were identified based on their morphological features (shape, color and texture of the colony) as well as their microscopic characteristics (nature of hyphae and conidia and their connections) using taxonomic keys described in Booth (1977), Domsch *et al.* (1980) and Pitt and Hocking (1997). The percentage frequency of each fungal species was calculated according to Tseng *et al.* (1995), using the following equation:

$$\text{Fungus frequency (\%)} = (\text{Fungus isolate no.} / \text{Total isolate no.}) \times 100$$

### Detection of aflatoxin production capability

The method described by Satio and Machida (1999) was followed using three different media: potato dextrose agar (PDA), yeast extract agar (YEA), and coconut agar (COA) and three different incubation temperatures (25, 30 and 35°C). Three replicates

were prepared for each fungal strain. The pure fungal colonies were inoculated onto the media and incubated for 7-14 days at 25°C. Later, the plates were inverted, and 0.2ml of 25% ammonia was added to plate lid. The plates were then incubated at 25, 30 and 35°C, and observed daily for any color change in grown fungal colonies. The results were recorded based on the color change of the colony to pink-red or orange-yellow, with different color intensities, indicating the ability of the fungus to produce aflatoxin.

- : No fluorescence (no capability to produce aflatoxin)

+ : Weak fluorescence, colony size 1-6cm<sup>2</sup> (low capability to produce aflatoxin)

++ : Moderate fluorescence, colony size 7-12cm<sup>2</sup> (medium capability to produce aflatoxin)

+++ : High fluorescence, colony size 13-18cm<sup>2</sup> (high capability to produce aflatoxin)

++++ : Very high fluorescence, colony size  $\geq$  19cm<sup>2</sup> (very high capability to produce aflatoxin)

The size of the fluorescent colony was measured by extrapolate onto transparent graph paper.

#### **Detection and determination of biogenic amines**

High performance liquid chromatography (HPLC) technique was applied to detect and quantify biogenic amine (histamine, cadaverine) levels providing the conditions mentioned by **Moret and Conte (1996)**. Samples of 10 $\mu$ l of standard biogenic amines and 10 $\mu$ l of each studied sample were examined. The HPLC device was used at the laboratories of the Ministry of Industry and Minerals / Baghdad, Iraq and the reverse-phase column ODS2 C18 with dimensions (6.4  $\times$  250) mm 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 H<sub>2</sub>O: Acetonitrile: H<sub>2</sub>O (v:v), at a temperature of 40°C and a flow rate of 1 mL/minute. The concentration of biogenic amines was estimated using the following equation:

Biogenic amine conc. mg/100g = conc. of standard  $\times$  (area of amine area / area of sample)

#### **Preparation of biogenic amine standards**

Biogenic amine standards were prepared at a concentration of 5.0mg/ ml in 5% TCA for each standard. A volume of 200 $\mu$ l was withdrawn for both the standard amine and the sample and transferred into a sterile glass tube. Then, 0.5ml of saturated NaHCO<sub>3</sub> was added, and the mixture was stirred. Subsequently, 1ml of the Chloride Dansyl reagent (0.1mg in 10ml of acetone) was added, and the mixture was vigorously mixed. The mixture was then placed in a water bath at 70°C for 10 minutes. The mixture was extracted three times by adding 5ml of diethyl ether, and the upper solvent layer was collected each time in another covered glass tube. The diethyl ether layer was then concentrated by air flow in a water bath at 35°C to evaporate the remaining solvent. Finally, the sample was diluted with 1ml of methanol, and the standard amines were

injected at a volume of 10 $\mu$ l into an HPLC device according to the method of **Moret and Conte (1996)**.

#### **Extraction of biogenic amines**

Biogenic amines were extracted according to the method described by **Moret and Conte (1996)**. Samples weighing 25g were mixed with 125ml of 5% TCA for 3 minutes, and then the mixture was filtered using a Whatman No. 1 filter paper. A volume of 10ml of the extract was transferred to a glass vial with a cover, and 4ml of NaCl and 1ml of 50% NaOH were added. The extraction was carried out using 5ml of n-butanol:chloroform (1:1) and mixed vigorously for 2 minutes, followed by centrifugation of the mixture at 3000rpm for 10 minutes. The mixture was then left to settle to obtain two layers, and the upper liquid layer was drawn off with a sterilized syringe into a 50ml separation funnel, while the lower layer was discarded. A volume of 15ml of n-heptane was added to the separation funnel, and the contents were well mixed. The extraction was repeated in triplicate using 1ml of 0.2 N HCl, and the contents of the separation funnel were mixed to obtain two layers. The clear lower layer was collected in a glass vial with a cover containing the biogenic amines, and the upper layer was discarded. The glass vial was placed in a water bath at a temperature of 95°C and left to dry with the help of an air stream. Then, the process of forming dansylated derivatives was carried out by adding 0.5ml of saturated NaHCO<sub>3</sub> solution, followed by the gradual addition of 1ml of Dansyl Chloride reagent while stirring vigorously. The mixture was then placed in a water bath at a temperature of 70°C for 10 minutes. The mixture was extracted three times by adding 5ml of diethyl ether, and the upper solvent layer was collected each time in another glass vial with a cover. The diethyl ether layer was then concentrated using an air stream and in a water bath at a temperature of 35°C, and the sample was finally diluted with 1ml of methanol and injected in a volume of 10 $\mu$ l into HPLC device.

## **RESULTS AND DISCUSSION**

The results of estimating the moisture content of grains are presented in Table (1). Values show variations in the percentage of different grains' samples, ranging from 5.6 in wheat bran to 12.4 in wheat flour, with averages of 9.8 for barley flour, followed by yellow corn, soybean meal with averages of 7.6 and 6.2%, respectively. The natural moisture content of each grain type could be related to differences in the climatic conditions of the crop cultivation sites, as well as differences in harvesting methods, storage periods and the degree of crop maturity at harvest (**Chulze, 2010**). These moisture contents fall within the allowable range for proper grain storage, which averaged 13 $\pm$ 2% (**Felicia, 2006**). The results coincide with those of **Rajkumar and Selvakulasingam (2019)**, who examined the chemical composition of 17 types of grains. **Abdulrahman and Omoniyi (2016)** also observed differences in moisture content in their study on some field crops, indicating that low-moisture grains are more suitable for

storage. Additionally, our results agree with the variation in moisture levels in grains found in recent studies of **Sulaiman *et al.* (2020)** and **Saidu *et al.* (2021)**. **Amadi and Adeniyi (2009)** stated previously that storing crops in unsuitable locations and improper storage conditions, such as poor flooring and poorly sealed rooms leads to increased humidity in the storage environment, which in turn affects the moisture content of stored grains and increases the spread of various types of bacteria and fungi. This increases the risk of contamination of grains with various fungal toxins, especially with increased humidity (**Pitt & Miller, 2017**).

**Table 1.** Moisture content of studied grains

Sample	% Moisture <sup>*</sup>
Soybean meal	6.21±0.42
Yellow corn	7.68±0.51
Wheat flour	12.43±0.43
Barley flour	9.84±0.62
Wheat bran	5.63±0.39

\*Each value is an average of triplicate

Table (2) displays protein content in fish meal and animal protein concentrate samples, which represented 67.34% and 62.55%, respectively. The reason for this variation in protein levels can be attributed to several factors, including the type and the original protein content of raw material, affecting the protein percentage in the final product. Moreover, differences in preparation and manufacturing methods of protein concentrates and meals can also affect their protein levels. These results are consistent with those of **Al-Hassoon *et al.* (2021)**, who recently detected variation in protein values for protein concentrates depending on the preparation method. The difference in protein percentage between protein concentrates and fish meal is also consistent with several previous studies, including the work of **Ojutiku *et al.* (2009)** found that the protein percentage in dried fish meal varied from 59.8% to 62.5% and that of **EL-Husseiny *et al.* (2018)**, who studied the chemical composition of protein concentrates and. Moreover, the present findings concur with those of **Kari *et al.* (2022)** with respect to the evaluation of the chemical composition and protein content of anchovy fish meal. While, **Lee *et al.* (2016)** studied the protein percentage in fish protein concentrates and recorded values ranging from 72.3% to 77.3%. Furthermore, The current results match the most recent study of **Lo *et al.* (2023)** about various types of protein concentrates, as well as many other previous studies, viz., **Moutinho *et al.* (2017)**, **Hossain *et al.* (2018)** and **Costa *et al.* (2019)**.

**Table 2.** Protein contents in studied fish meal and animal protein concentrate

Sample	% protein*
Fish meal	67.34±3.42
Animal protein concentrate	62.55±3.76

\*Each value is an average of triplicate

The results in Table (3) show moisture and protein contents in the examined fish diet. The moisture content was 6.11%, which is similar to the finding of **Amtul and Amna (2012)** who reported moisture contents in the range of 8.63-7.61%. For the protein contents, it averaged 30.17%, which matches the results of Al-Tameemi (2015) who evaluated five different types of commercial fish diets. **Ayuba and Iorkohol (2013)** found significant variation in the chemical composition of the studied diets, and their results are consistent with those of **Taher et al. (2022)**, where the protein content was 30.11% for diets prepared for grass carp *Ctenopharyngodon idella*.

**Table 3.** Moisture and protein content averages of examined fish diet

Sample	% Moisture*	% Protein*
Fish diet	6.11	30.17

\*Each value is an average of triplicate

The results of isolating fungi from grain and fish feed samples in Table (4) indicate variation among the samples, where six types of fungi were recorded in grain and fish feed samples. The fungus *A. niger* was the most frequent species recorded in the examined samples, and this could be attributed to its ability to tolerate harsh environmental variations of moisture and temperature, as well as its air disseminating spores, which can be easily transferred through different locations (**Marijani et al., 2017**). Meanwhile, the fungus *A. alternata* ranked the second in terms of frequency between the samples studied, with a percentage ranging from 11.16 to 20.96%. This species is a well-known storage fungus and has a high enzymatic capacity to break down and utilize the components of surrounding medium, enabling it to exist in various grain and feed stores; it is also known for its ability to produce dangerous mycotoxins such as alternariol, which can significantly reduce the storage life of feeds and grains (**Greco et al., 2015**). Other fungi genera which had lower frequencies include *Rhizopus*, *Mucor*, *P. spp.* and *A. flavus*, which are known for producing hazardous mycotoxins such as aflatoxin (**Barbosa et al., 2013**).

Several studies have indicated that important crops such as corn, barley, peanuts, rice and others are prone to contamination by fungi, particularly from the genera *Aspergillus*, *Fusarium* and *Penicillium* which have the capability to produce dangerous mycotoxins (**Hassan et al., 2014**). This could be attributed to the fact that the majority of fungi present are storage fungi due to poor storage conditions resulting from inadequate lighting, ventilation and high humidity, which facilitate the growth and spread of these

fungi (Lee & Ryu, 2017). Field crops are considered the most suitable materials for the growth of various fungi, especially those producing mycotoxins, such as aflatoxins, fumonisins, ochratoxins, and others, which cannot be completely eliminated from crops (Carrido *et al.*, 2012). Other studies have also shown that fish diets and their basic ingredients are susceptible to infection by various fungi and contamination with mycotoxins (Greco *et al.*, 2015). Environmental conditions such as high temperatures and humidity during storage increase the probability of the growth of these genera in food materials, and Daniel *et al.* (2011) attributed contamination by mycotoxins to the high moisture content of stored crops after harvest, as well as contamination during preparation and manufacture of animal feeds (Van der Fels-Klerx *et al.*, 2016). In addition, the highly competitive ability of these fungi is due to their possession of wide-ranging enzymatic systems that enable them to exploit a wide range of food materials and their high speed of growth and production of reproductive units, as well as their strong antagonism capacity toward other coexisting fungi (Bennett & Klich, 2003).

Previous studies have shown the presence of fungi in fish diets and their raw materials, including Greco *et al.* (2015) who isolated *Mucor* sp., *Penicillium* and *Eurotium* from fish feed and its ingredients as well as Marijani *et al.* (2017) who identified different fungal species with varying frequencies in 52 samples of local and imported fish diets and their components, including *A. flavus*, *A. tamarii*, *A. niger*, *M. velutinosus*, *Phoma* sp., *E. rubrum*, and *P. chrysogenum*. Eskola *et al.* (2019) reviewed the contamination of crops with fungi of concern due to their harmful effects on human and animal health. Furthermore, Abdual-shahid *et al.* (2013) identified five genera of storage fungi, mostly belonging to genera *Aspergillus*, *Mucor*, *Alternaria* and *Penicillium* which were associated with isolated fish feed from 15 diet samples used for fish farming.

**Table 4.** Occurrence (%) of fungi associated with examined grains and fish diet

Sample	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Penicillium</i> spp.	<i>Alternaria alternata</i>	<i>Mucor</i>	<i>Rhizopus</i>	Sum
Fish diet	34	1	6.7	11.16	3.89	9.44	66.19
Soybean meal	22.7	18.4	7.2	20.34	16.12	11.10	95.86
Yellow corn	43	19.2	17.5	20.96	3.94	13.19	117.79
Wheat flour	7.21	4.4	3.74	11.32	9.11	2.18	37.96
Barley flour	15.1	8.5	13.73	13.87	7.69	7.88	66.77
Wheat bran	13.9	9.44	9.80	11.97	5.25	11.35	61.71
Average	22.65	10.15	9.77	14.93	7.66	9.19	74.35



Regarding the ability of fungi to produce aflatoxins, the results in Table (5) show the ability of some fungal species, especially *A. flavus* to produce high levels of aflatoxins at a temperature of 35°C. This fungal species produced the largest quantity of aflatoxin in all three tested media at this temperature, and to a lesser extent, at lower temperatures. On the other hand, *A. niger* showed moderate ability to produce aflatoxin in laboratory isolates at 30 and 35°C on YES and PDA media, without producing any on the COA medium. The production ability of other fungi was low and varied, while only observed at 35°C on the studied media. The high protein and energy contents in feeds and their components is believed to be the cause of fungal contamination and toxicity. Fungal toxins are one of the dangerous environmental pollutants affecting the health and growth parameters of fish, causing significant economic losses to fish farms (**Magouz et al., 2018**). Specific detection of fungal toxins is important as it indicates the level of degradation of stored grains, their possible hazards and the health consequences of consuming these crops by humans or farm animals (**Bryden, 2007**). The current results are consistent with those of **Marijani et al. (2017)** who estimated fungal toxins in fish diet and their components and identified 14 types of fungal toxins, with AF, FB, OTA, DON and ZEA being the most widespread toxins.

In the same context, **Eskola et al. (2019)** postulated that, crop contamination by fungal toxins reaches up to 25% globally and some of the most common toxins are AFs, DON, ZEN, FBs, T-2, HT-2, and OTA. Moreover, **Sanchis et al. (1993)** reported infection of stored barley samples with several fungi, most commonly *A. flavus*, *P. spp.* and *A. alternata*, of which 156 of 176 isolates of *A. alternata* were found to produce the mycotoxin tenuazonic acid, while 6% of 190 isolates of *A. flavus* were found to produce aflatoxins. **Shihab et al. (1998)** found that, all samples of studied corn were infected with the fungus *F. moniliforme*, and 33% of them produced FB1. Additionally, **Shekhany et al. (2001)** identified various fungal genera on wheat grains, including *A. spp.*, *P. spp.*, *A. spp.*, *Drechslera spp.*, *F. spp.* and *Rhizoctonia spp.* at varying rates. While, **Abdualshahid et al. (2013)** detected the mycotoxins deoxynivalenol and zearalenone in eight samples of fish diets, and **Hassan et al. (2014)** isolated the mycotoxin aflatoxin B1 from fungi associated with animal feed and corn, of which *A. flavus*, *A. niger*, *F. spp.*, *R. spp.* and *P. spp.* were observed as storage fungi associated with grains.

The results in Figs. (1, 2) illustrate that the concentrations of the biogenic amines histamine and cadaverine isolated from fish meal and animal protein concentrate were clearly varied between the examined samples. Histamine concentration was lower in the animal protein concentrate at 1.382mg/100g, while it was higher in fish meal at 2.836mg/100g. As cadaverine concentration, it decreased in the samples but to a lesser extent compared to histamine, with 0.384mg/100g in the animal protein concentrate while increasing to 0.778mg/100g in fish meal. Biogenic amines are chemical compounds that could be formed in fish meal and animal protein concentrate due to the biological breakdown of proteins present in these animal products (**Park et al., 2010**). The reason for the variation in the quantity of histamine and cadaverine in these products could be largely attributed to factors such as the manufacturing process, the quality of the raw

material used, storage, transportation, processing and the variation in the quantity and quality of amino acids between the two materials (Özdestan & Üren, 2010). A low content of biogenic amines is considered an indicator of high-quality fish meal and protein concentrates (Mundheim *et al.*, 2004), while an increased concentration can be attributed to the presence of bacteria and enzymes (Naila *et al.*, 2012).

**Table 5.** Detection of fungal aflatoxin production capability

Fungi	COA media			PDA media			YES media		
	Temperature °C			Temperature °C			Temperature °C		
	35	30	25	35	30	25	35	30	25
<i>Aspergillus niger</i>	-	-	-	**	*	-	++	+	-
<i>Aspergillus flavus</i>	+++	++	+	***	**	*	+++	++	+
<i>Penicillium spp.</i>	+	-	-	-	-	-	+	-	-
<i>Mucor</i>	-	-	-	*	*	*	+	-	-
<i>Rhizopus</i>	-	-	-	*	*	*	+	-	-
<i>Alternaria alternata</i>	*	-	-	-	-	-	*	*	*

+ Light rose

Low producibility

\* Light orange

++ Moderate rose

Moderate producibility

\*\* Moderate orange

+++ Dark rose

High producibility

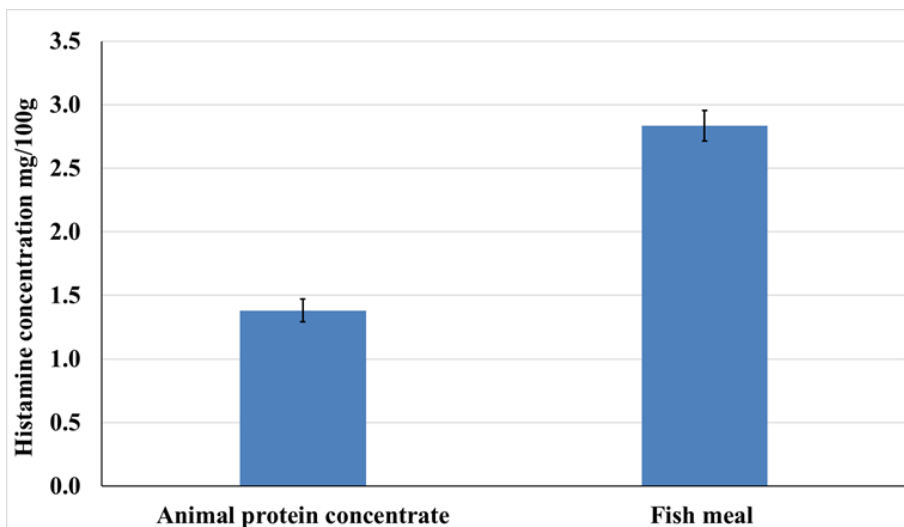
\*\*\* Dark orange

- No production

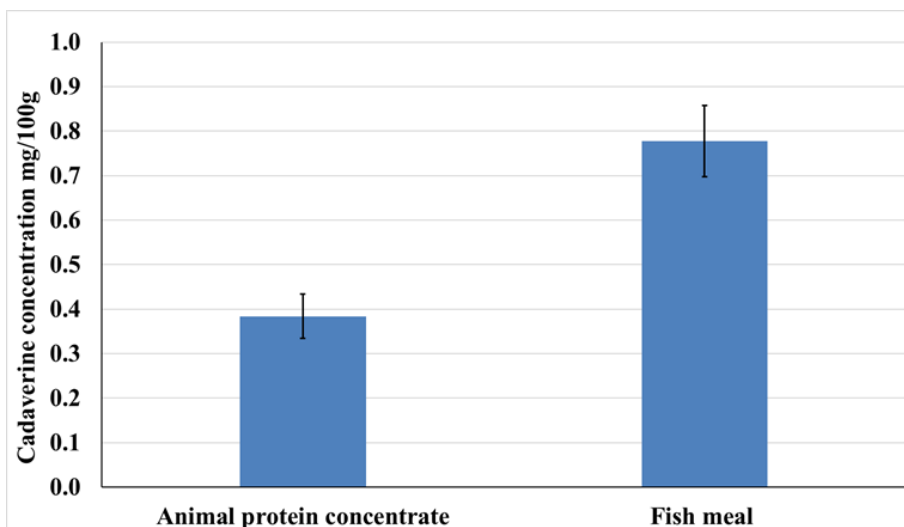
In his study, Pike (1991) mentioned that fish meal made from spoiled sardines contained 83mg/100g of histamine, while the meal made from fresh sardines contained less than 0.3mg/100g of histamine. This explains the high levels of biogenic amines found in protein concentrates and animal feeds' samples due to the use of poor-quality raw materials, as well as poor production, storage and transportation conditions. It is noteworthy that, many bacterial species have histidine and decarboxylase that are capable of producing histamine, including *Morganella morganii*, *Raoultella planticola*, *Enterobacter aerogenes*, and the *Bacillus* genus (Bjornsdottir *et al.*, 2009; Jaw *et al.*, 2012). In a related context, Jasour *et al.* (2018) noted the impact of biogenic amines on fish growth due to a deficiency in basic amino acids resulting from bacterial degradation during storage, which negatively affects fish growth rates. This is consistent with what was also found in the study of Tapia-Salazar *et al.* (2004) regarding lower growth performance in fish fed diets containing biogenic amines formed during storage due to bacterial activity. Kordiovská *et al.* (2006) arrived to the same conclusion that the concentration of biogenic amines increases with higher temperatures and longer storage periods.

Additionally, the results of the current study coincide with the finding of Jaw *et al.* (2012) who found different concentrations of biogenic amines ranging from 1.4-9.12mg/100g in 40 samples of fish diet, fish meal and protein concentrates produced by

five genera of histamine-producing bacteria. In addition, **Kennedy *et al.* (2004)** observed levels of histamine exceeding 20mg/100g in 11 out of 25 samples of fish meal, stating that biogenic amines are mainly formed by the decarboxylation of specific free amino acids by external decarboxylases released by microbial species associated with seafood, a phenomenon which was also confirmed by **Tsai *et al.* (2005)**. As reported in the investigation of **Macan *et al.* (2000)**, histamine was present at a level of 50-510mg/100g in fish meal. However, the high levels of histamine and biogenic amines in diet and raw materials are considered toxic posing a serious threat and causing many negative effects on fish health, such as decreased growth rates and feed consumption in addition to immunity and weight loss (**Lumsden, 2002**).



**Figure 1. Histamine concentrations in fish meal and animal protein concentrate**



**Figure 2. Cadaverine concentrations in fish meal and animal protein concentrate**

## CONCLUSION

In conclusion, raw materials and ingredients intended to manufacture fish diet must be carefully selected in order to obtain high quality final product. Procuring these raw materials from reputable suppliers who are known by credibility and professional competence is crucial to guarantee an optimal fish diet. High quality fish diets are vital condition for fish health, growth and eventually successful fish farming projects.

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