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Evaluation of Biogenic Amine Contents of Iranian Kilka Fish Meal

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Abstract

The present study was conducted to evaluate the biogenic amine contents of Iranian Kilka fish meal (KFM). A total of 10 composed samples of KFM were provided during two months sampling period from rendering units of two industrial fish meal plants in Gilan province. The biogenic amine contents including tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine for each composed samples of KFM were measured by reversed phase high performance liquid chromatography equipped with C_{18} Luna column. The data were analyzed in a completely randomized design. Each sample was examined in 6 replication. The results of this study indicated that the biogenic amine contents including tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine had highly significant differences ($P<0.01$) among the KFM samples and their average values were 19.6, 18.3, 116.8, 145.9, 64.7, 76.4, 60.2 and 52.1 microgram per gram, respectively. The average values for biogenic amine contents of the KFM samples were highly significantly lower than those of their maximum limited allowances ($P<0.01$). According to the results of this experiment, it seems that KFM can be used in poultry diets without any concern.

1. Introduction

Although fish meal is the best animal protein source for poultry (Leeson and Summers, 2001, 2005) but one of the most important problems regarding to fish meal utilization in poultry diets is its contamination to biogenic amines (Keirs and Bennett, 1993; Friday *et al.*, 1999: Barnes *et al.*, 2001; Jaw *et al.*, 2012; Ruiz-Capillas *et al.*, 2015). Biogenic amines are naturally occurring low molecular weight organic bases characterized by the presence of an amine group (Tamim *et al.*, 2002; Tamim and Doerr, 2003; Fusi *et al.*, 2004). These substances are found at low levels in all organisms such as plants, animals and microorganisms (Tamim *et al.*, 2002). These compounds in small amounts are necessary for cellular growth, development and differentiation (Bardócz *et al.*, 1995; Sousadias and Smith, 1995; Mogridge *et al.*, 1996; Smith *et al.*, 1996; Phuntsok *et al.*, 1998). Also, biogenic amines contribute in protein, DNA and RNA synthesis (Mogridge *et al.*, 1996; Fusi *et al.*, 2004). In poultry, biogenic amines are present in all cells specially lung cells (Eaton and Fedde, 1978, 1980) and regulate the activity of poultry immune system (McCorkle and Taylor, 1993) and enhanced hypothalamic biogenic amine levels in broiler chicks showing advanced sexual maturation (Davison and Kuenzel, 1991).

Biogenic amines are produced by decarboxylation of amino acids (Friday *et al.*, 1999; Tamim *et al.*, 2002; Tamim and Doerr, 2003; Jaw *et al.*, 2012; Figueiredo

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et al., 2014; Wójcik *et al.*, 2024) or by amination and transamination of aldehydes and ketones (Friday *et al.*, 1999; Wójcik *et al.*, 2024). The most important biogenic amines include putrescine, cadaverine, spermine, spermidine, histamine, tryptamine, β-phenylethylamine, tyramine, dopamine, phenethylamine, ethanolamine, cysteamine, aminopropanol, β-alanine, gama-aminobutylate (GABA), serotonin and 5-adenosylmethioninamine (Sander *et al.*, 1996; Phuntsok *et al.*, 1998; Tamim *et al.*, 2002; Tamim and Doerr, 2003). Biogenic amines are found in silages such as corn silage and alfalfa silage (Phuntsok *et al.*, 1998; Fusi *et al.*, 2004), ruminal bacteria (Phuntsok *et al.*, 1998), protein sources (Phuntsok *et al.*, 1998), spoiled meat products (Tamim and Doerr, 2003), fermented poultry carcasses (Sander *et al.*, 1996), egg (Figueiredo, *et al.*, 2014) and animal protein sources such as fish meal, meat and bone meal and poultry by-product meal (Keirs *et al.*, 1994; Tamim *et al.*, 2002; Tamim and Doerr, 2003). Putrescine is derived from decarboxylation of arginine *via* ornithine and acts as a precursor of spermine and spermidine. Cadaverine is produced from lysine decarboxylation, spermine and spermidine by degradation of putrescine, histamine from decarboxylation of histidine, tryptamine *via* decarboxylation of tryptophan, β-phenylethylamine from decarboxylation of phenylalanine, tyramine by decarboxylation of tyrosine, dopamine from dopa degradation, phenethylamine from degradation of phenylethylamine, ethanolamine by decarboxylation of serine, cysteamine from decarboxylation of cysteine, aminopropanol by decarboxylation of threonine, β-alanine *via* decarboxylation of aspartic acid, gama-aminobutylate (GABA) from decarboxylation of glutamic acid, serotonin by decarboxylation of 5-hydroxytryptophan and 5-adenosylmethioninamine from degradation of 5-adenosylmethionine (Keirs and Bennett, 1993). Currently, biogenic amines are used as indicators of foods such as egg, meat and meat products freshness and hygienic quality (Tamim and Doerr, 2003; Fraqueza *et al.*, 2012; Hutarova *et al.*, 2013; Figueiredo, *et al.*, 2014; Wójcik *et al.*, 2024) and high concentrations of different biogenic amines have long been used as indicators of spoilage (Tamim *et al.*, 2002).

The use of animal by- product meals such as fish meal, poultry by- product meal, meat and bone meal, blood meal and feather meal in poultry diets allows the opportunity for the presence of biogenic amines. The toxicity of high levels of biogenic amines resulting from the presence of spoiled animal by- products has been suggested as a causative factor for reduction in poultry performance and a condition named "necrotic cellular debris" (Barnes *et al.*, 2001). The consumption of high amounts of biogenic amines in poultry resulted in reduced feed intake, reduction of growth and performance, reduced feed efficiency, the occurrence of proventricular lesion, gizzard erosion, gizzard and intestinal lesions, intestinal epithelial cells necrosis, increasing feed passage rate and thus transmission of undigested feed from gastrointestinal tract and presence of too much mucosa in upper parts of intestine (Harry *et al.*, 1975; Harry and Tucker, 1976; Keirs and Bennett, 1993; Keirs *et al.*, 1994; Friday *et al.*, 1999; Barnes *et al.*, 2001; Tamim *et al.*, 2002; Tamim and Doerr, 2003).

In Iran, Kilka fish (*Clupeonella engrauliformis*) is obtained from the Caspian sea in Golestan, Mazandaran and Gilan provinces. The most part of the catch is processed into fish meal by different processing methods. Now, there are 9 rendering plants in Iran that produce Kilka fish meal by processing whole Kilka fish, which is mostly used in poultry and cold water fish diets. Although some studies have been conducted on biogenic amine of fish meal in other countries (Harry *et al.*, 1975; Jaw *et al.*, 2012; Ruiz-Capillas *et al.*, 2015), but the biogenic amine contents of Kilka fish meal produced in Iran has not been described quantitatively. Due to lack of information, this study was carried out to determine the biogenic amine contents of Iranian Kilka fish meal.

2. Materials and Methods

The KFM samples were provided during two months sampling period from rendering units of two industrial fish meal plants in Gilan province. The samples collected in each six days were mixed together and 10 composed samples were prepared so that samples #1, #2, #3, #4 and #5 were taken from plant A and samples #6, #7, #8, #9 and #10 were taken from plant B. The raw material source of all KFM samples was whole Kilka fish. After grinding and mixing the samples, all samples were stored at -20°C until further analysis.

The biogenic amine contents of the KFM samples were determined by reversed phase high performance liquid chromatography equipped with C_{18} Luna column as described by Tamim *et al.* (2002). Briefly, perchloric acid was used to extract the biogenic amines from a 30 gram of ground-up sample. The extract was then made alkaline by saturated sodium bicarbonate solution. Amines in extract were then dansylated with dansyl chloride and extracted from the aqueous solution by diethyl ether. The dansylated biogenic amines were analyzed by reversed-phase high performance liquid chromatography using a Shimadzu 6-A system with a C_{18} Luna column. Water and methanol in a gradient elution program were used to elute the amines, which were detected by a fluorescence detector at an excitation of 365 nm and emission of 520 nm. Each sample was examined in six replicates. The data were analyzed in a completely randomized design using the GLM procedure of SAS (SAS, 1999). Comparison of means was conducted by Duncan's multiple range test. Comparison of the average of biogenic amine contents of the KFM samples with their maximum limited allowances was conducted using two-sided t-test (Zar, 1996).

3. Results and Discussion

The biogenic amine contents of each KFM samples are shown in Table 1. The tryptamine content showed significant differences (P<0.01) among the KFM samples and its average value was 19.6 μg/g and varied between 10.7 to 29.7 μg/g. The phenylethylamine content had significant differences (P<0.01) among the KFM samples and its average value was 18.3 μg/g and varied between 9.5 to 25.6 μg/g. The putrescine content showed significant differences (P<0.01) among the KFM samples and its average value was 116.8 μg/g and varied between 76.4 to 168.7 μg/g. The cadaverine content had significant differences (P<0.01) among the KFM samples and its average value was 145.9 μg/g and varied between 86.5 to 188.4 μg/g. The histamine content showed significant differences (P<0.01) among the KFM samples and its average value was 64.7 μg/g and varied between 41.8 to 85.5 μg/g. The tyramine content had significant differences (P<0.01) among the KFM samples and its average value was 76.4 μg/g and varied between 68.2 to 91.2 μg/g. The spermidine content showed significant differences (P<0.01) among the KFM samples and its average value was 60.2 μg/g and varied between 49.2 to 71.6 μg/g. The spermine content had significant differences (P<0.01) among the KFM samples and its average value was 52.1 μg/g and varied between 41.2 to 61.3 μg/g. The highest levels of biogenic amines were observed for cadaverine and putrescine, respectively. The reason for these results is that most biogenic amines are derived from degradation of essential amino acids by bacterial activity (Tamim *et al.*, 2002; Tamim and Doerr, 2003; Jaw *et al.*, 2012; Wójcik *et al.*, 2024). Because the highest levels of essential amino acids in fish meal are for lysine and arginine (NRC, 1994), it is logical that the highest levels of biogenic amines are also achieved for cadaverine and putrescine. The average values for biogenic amine contents of the KFM samples were significantly (P<0.01) lower than those of their maximum limited allowances. Ruiz-Capillas *et al.* (2015) assessed the biogenic amine levels in one sample of Capelin fish meal and found that the cadaverine, putrescine and histamine levels in Capelin fish meal were 208, 164 and 46 μg/g, respectively. They also reported that the biogenic amines that presented the highest levels in Capelin fish meal were cadaverine followed by putrescine, and biogenic amine levels in the fish meal studied were low when compared to the toxic levels observed in these kinds of products which are in agreement with the results obtained in the present study. It has been reported that the biogenic amine contents are low during the first 24 to 36 hour of raw material storage prior to rendering and increase rapidly thereafter so if raw materials are processed in short time after collection, the risk of product contamination with biogenic amines is not too high (Tamim and Doerr, 2003). Because the raw material used for production of KFM samples in this experiment processed within maximum 8 hours after collection, their biogenic amine contents were significantly (P<0.01) lower than those of their maximum limited allowances. According to the results of this study, it seems that KFM can be used in poultry diets without any concern.

Table 1
The biogenic amine contents (μg/g) of Kilka fish meal

Sample No.	1	2	3	4	5	6	7	8	9	10	Average	Maximum Limited Allowance[1]
Tryptamine	11.3^{h}	21.6^{d}	24.5^{c}	29.7^{a}	15.8^{f}	22.1^{d}	18.5^{e}	27.2^{b}	10.7^{h}	14.6^{g}	19.6	46**
Phenylethylamine	10.1^{ef}	19.7^{c}	24.2^{b}	25.6^{a}	13.4^{d}	23.9^{b}	20.5^{c}	25.3^{a}	9.5^{f}	10.8^{e}	18.3	28**
Putrescine	87.6^{g}	144.9^{c}	168.7^{a}	153.2^{b}	76.4^{h}	121.8^{e}	117.4^{e}	129.2^{d}	92.3^{f}	76.5^{h}	116.8	285**
Cadaverine	125.9^{e}	179.6^{b}	188.4^{a}	166.1^{cd}	119.3^{e}	167.6^{cd}	160.3^{d}	171.6^{c}	86.5^{g}	93.7^{f}	145.9	363**
Histamine	50.9^{f}	78.7^{b}	85.5^{a}	73.4^{c}	55.8^{e}	79.7^{b}	66.1^{d}	71.4^{c}	43.7^{g}	41.8^{g}	64.7	144**
Tyramine	68.2^{e}	88.5^{a}	91.2^{a}	77.9^{bc}	71.7^{de}	81.4^{b}	69.3^{e}	74.9^{cd}	69.5^{e}	71.4^{de}	76.4	118**
Spermidine	54.3^{f}	71.6^{a}	67.2^{bc}	68.4^{b}	51.3^{g}	64.1^{d}	58.6^{e}	65.2^{cd}	52.1^{fg}	49.2^{g}	60.2	172**
Spermine	48.4^{f}	61.3^{a}	55.9^{cd}	53.2^{e}	44.1^{g}	57.6^{bc}	54.8^{de}	58.9^{ab}	45.6^{g}	41.2^{h}	52.1	164**

[1] The asterisk shows highly significant difference between the average value for KFM and maximum limited allowance value (P<0.01).
$^{a-h}$ In each row, means with different superscripts are highly significantly different (P<0.01).

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