Detection and Detoxification of Aflatoxin B1 from Fish Feedstuff Using Microwave and Ozone Gas

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Received in:8 /October /2017, Accepted in24/ December /2017

Abstract

The current study was designed to investigate the occurrence of aflatoxin B1 in thirty two samples of fish feedstuff were collected randomly from some Iraqi local markets using ELISA technique. Aflatoxin B1 was detected in thirty samples and the concentration of toxin ranged from 50 ppb to 1000 ppb.

Microwave and ozone were used for detoxification of aflatoxin B1 from sample with highest concentration (1000 ppb), two degree of temperature and two times (50°C and 100°C for 5 minute and 10 minute to each degree) of microwave, also two doses and two times (2 g and 4 g for 5 minute and 10 minute to each dose) of ozone gas were used.

Degradation of aflatoxin B1 by microwave has been found to cause a significant ($P \leq 0.05$) decrease of aflatoxin B1, Moreover, the concentration of aflatoxin B1 was dependent on temperature degrees and exposure time, also sample subjected to ozone gas caused a significant ($P \leq 0.05$) decrease in aflatoxin B1 contents and the concentration of aflatoxin B1 was dependent on doses and times of exposure. Results showed that ozone gas was more effective in aflatoxin B1 reduction when compared with microwave.

Key words: Aflatoxin B1, Fish feedstuff, Detoxification, Microwave, Ozone gas

Introduction

As other animals fish are required to nutrition for their growth, reproduction and all physiological functions, and it is very necessary to intake of proteins, minerals, vitamins, growth factors and energy every day, for all kinds and categories of fish, cereals are necessary as source of energy and leguminous as protein feedstuffs and they are considered as the main part of feed (up to 90%) [1].

In the aquaculture industry fish feedstuff is the major cost item and constitutes 40–50% of the total production costs in intensive culture systems [2].

Fungal spores can be contaminating the feed during processing, particularly when grains are ground and the feed pelleted [3].

The processing methods, feed storage practice, environmental temperatures >27 C°, humidity levels >62%, and moisture levels in the feed >14% are some of the factors that can increase fungal growth in feed, and this may result in production of mycotoxin [4].

The exposure of fish to mycotoxigenic fungi can be reduce their growth rate, damage the liver, reduce immune responsiveness, increase mortality, and lead to a steady and gradual decline in quality of reared fish stock, posing serious challenges to aquaculture development [5].

Mycotoxins are toxic secondary fungal metabolites produced by mycotoxigenic fungi, mainly of the genera *Aspergillus*, *Fusarium* and *Penicillium* and have been identified as a worldwide food and feed safety issue [6], some of this mycotoxins include Aflatoxins, Ochratoxins , Citrinin , Patulin, and *Fusarium* toxins [7].

Aflatoxins are a group of fungal metabolites produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. The four major naturally produced aflatoxins are known as B1, B2, G1, and G2. B and G refer to the blue and green fluorescent colors produced under UV light, while the Subscript numbers 1 and 2 indicate major and minor compounds, respectively [8].

Continuous studies regarding the monitoring of aflatoxin B1 in finished feed to be used as animal feed are being performed worldwide [9, 10], However, there is little information on feed contaminated with aflatoxin B1 intended for fish feed.

The main aim of the present study was to detection and detoxification of aflatoxin B1 from finished feed intended for fish using microwave and ozone Gas.

Materials and Methods

Samples collection:

Thirty two sample of locally fish feedstuff were randomly collected from some Iraqi local markets in different regions of baghdad governorate including (Al- adhamya, Al- shulaa, Abu ghraib, AL- zaafrania and Al- doura) in addition to Al kut and Al basrah governorates from January to April (2015), two kilogram of each sample was placed in plastic bags and stored at 5°C until analysis.

Detection of aflatoxin B1 in fish feedstuff extracts by Enzyme Linked Immune Sorbent Assay (ELISA) technique:

Detection of aflatoxin B1 by ELISA technique was performed using ELISA kit which supplied by Shenzhen Lvshiyuan Biotechnology company, the extracted samples, aflatoxin B1 enzyme conjugate and aflatoxin B1 Antibody working solution were mixed and added to micro well. On removal of non-specific reactants, substrate (A & B) were added, and the

micro wells measured optically using microplate reader at 450 nm for yellow color or at 650 nm for unstopped blue color to determine the OD value.

Determination of aflatoxin B1 concentration:

The concentration % of aflatoxin B1 in test samples was calculated using aflatoxin B1 standard curve according to the following equation [11]:

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\ \%$$

B = the average OD value of the sample or the standard solution.
B0 = the average OD value of the 0 ng/ml standard solution.

Detoxification of aflatoxin B1 from tested samples:

The first method used for detoxification was performed by microwave radiation in oven (type NM3850DGS, Capacity of 1000 W, Co, Ltd, Korea), two gram of contaminated sample (S8) with highest concentration (1000 ppb) chosen (in three replicates)and exposed to microwave (temperatures used were 50° C and 100° C for 5 and 10 minute respectively).

The other method was performed using ozone gas (wall mound incorporated ozonizer, Local manufacturing),(two gram of contaminated samples with highest concentration (1000 ppb) chosen (in three replicates) , and exposed to ozone gas (doses used were 2g and 4g of ozone gas for 5 and 10 minute respectively)[12].

Statistical analysis:

All analytical determinations were performed at least in triplicate using SPSS program v 11.5. Values of different parameters were expressed as the mean ± standard error using student T-test. A difference of $P \leq 0.05$ were considered statistically significant [13].

Results

Detection of aflatoxin B1 in fish feedstuff samples using Enzyme Linked Immune Sorbent Assay (ELISA) technique:

Thirty two samples of locally stored fish feedstuff were analyzed for aflatoxin B1 content (quantitatively) using ELISA kit.

Result showed that thirty samples of fish feedstuff were contained aflatoxin B1, and the concentration ranged from 50 ppb to 1000 ppb, table (1).

Detoxification of aflatoxin B1 using microwave and ozone gas

Detoxification of aflatoxin B1 was conducted using one sample (S8) with highest concentration (1000 ppb), two methods were used for this purpose, the first one was microwave with temperature 50°C (times used were 5 minute and 10 minute) and 100°C (times used were 30 and 60 minute), table (2).

Results of the present study observed that using of microwave for detoxification of aflatoxin B1 from tested sample was effective, and can reduce the concentration of aflatoxin B1 significantly ($P \leq 0.05$), results showed that using of 50° C temperature for 5 minute could reduce the concentration significantly ($P \leq 0.05$) from 1000 ppb to 675 ppb and for 10 minute could reduce the concentration significantly ($P \leq 0.05$) from 1000 ppb to 430 ppb, and using 100°C temperature for 5 minute can reduce the concentration of aflatoxin B1 significantly ($P \leq 0.05$) from 1000 ppb to 212 ppb and for 10 minute at the same temperature can reduce the concentration of aflatoxin B1 significantly ($P \leq 0.05$) from 1000 ppb to 108 ppb.

The other method used in this study for detoxification of aflatoxin B1 from tested sample was ozone gas, results revealed that using of ozone gas was significantly (P≤ 0.05) very effective for reduction of aflatoxin B1 from tested sample, two doses of ozone gas were used at differents times, the first dose was 2 g for 5 minute can reduce the concentration of aflatoxin B1 significantly (P≤ 0.05) from 1000 ppb to 200 ppb and for 10 minute can reduce the concentration of aflatoxin B1 significantly (P≤ 0.05) from 1000 ppb to 105 ppb.

The other dose of ozone was 4 g, using this dose for 5 minute was effective for reduction of aflatoxin B1 from tested sample significantly (P≤ 0.05) from 1000 ppb to 53 ppb and for 10 minute can reduce the concentration of aflatoxin B1 significantly (P≤ 0.05) from 1000 ppb to 25 ppb.

Discussion

Aflatoxin B1 is the main type of aflatoxins which produce mainly by *A.flavus* and *A. parasiticus,* it is considered as a potent carcinogen for human and animals [14], Aflatoxin B1 is an essential contaminant of foods including corn, cotton seeds, peanuts and other grains in addition to animals feeds [15, 16].

In the present study aflatoxin B1 was detected in thirty two samples of fish feedstuff which collected from some Iraqi local market using (ELISA) technique and results showed the presence of aflatoxin B1 in thirty samples and the concentrations of aflatoxin B1 in contaminated samples ranged from 50 ppb-1000 ppb. [17] revealed that aflatoxin B1 was occurred with fumonisin in 13 of 24 fish feed samples using LC–MS/MS technique, and the concentration of aflatoxin B1 ranged from 2 ppb to 806 ppb, Whereas [18] showed the presence of aflatoxin B1 in 42 of 43 sample of fish feedstuff and the concentration ranged from 0.00- 0.05 ppm, results by [19] showed presence of aflatoxin B1 in most of fish feed samples using HPLC technique and the concentration ranged from 0.06 ppb - 212.18 ppb, while [20] showed that aflatoxins was detected in all collected fish feedstuff samples (28 sample) using ELISA technique and the median concentration was 2.82 ppb, results observed by [21] showed presence of aflatoxin B1 in 55% of tested fish feed stuff samples (60 sample) using TLC technique, the levels detectable but not quantifiable for the technique used.

Under specific environmental conditions toxigenic fungi produce aflatoxin B1, foods stored under high moisture/humidity (>14%) at warm temperatures (>20°C) and/or inadequately dried can potentially become contaminated. Warm (air temperature of 24 C°–35 C°) and humid (moisture content of substrate between 25% and 35%) conditions lead to extensive mold growth and aflatoxin B1 production [22]

Twenty ppb is the action level of aflatoxin B1 in animals feed and feed ingredients which determined by U. S Food and Drug Administration (FDA) [23].

According to FDA´s action levels and when comparing with our results, we can conclude that all of contaminated fish feedstuff collected samples are disqualify for consumption due to the concentrations of aflatoxin B1 in contaminated samples were exceeded the allowable limit.

The main physical methods using to reduce the level of aflatoxin B1 from contaminated food and feed include: radiation, heating, adsorption from solution and cleaning [24].

Radiation can be divided in to two type, the first type is ionizing radiation (such as gamma ray, X ray), this type of radiation has ability to produce changes and increasing hazardous molecular with little or without temperature increasing, while the other type of radiation is non- ionizing radiation (such as microwaves, visible light, radio waves and infrared waves) with sufficient intensity that leads to a rise in temperature and the molecular changes are not hazardous to man [24].

As showed in our results, microwave was reduced the concentration of aflatoxin B1from 1000 ppb to 675 ppb at 50° C for 5 minute to 430 for 10 minute, while using of 100° C can be decrease the concentration from 1000 ppb to 212 ppb for 5 minute and to 108 ppb for 10 minute, while [25] revealed that using of microwave at temperature 100° C can reduce the concentration of aflatoxin B1 from 894 ppb to 880 ppb, 800 ppb, 780 ppb and 690 ppb for 2, 4, 8 and 10 minute respectively, and reduce the concentration from 395 ppb to 387 ppb, 380 ppb, 320 ppb and 310 ppb for 2, 4, 8 and 10 minute respectively, and from 192.1 ppb to 180 ppb, 160 ppb, 140 ppb and 130 ppb for 2, 4, 8 and 10 minute respectively from chick feed contaminated with aflatoxin B1. [26] showed that using of microwave at 92° C for 5 minute can reduce the concentration of aflatoxin B1 from (5 ppb to 0 ppb) , (183.2 ppb to 46.7 ppb) , (175 ppb to 7 ppb), (116.6 ppb to 8 ppb), (5 ppb to 0 ppb), (5 ppb to 0 ppb) and (23.3 ppb to 0 ppb) from Salted peanut, Peanut Toffee Grade I, Peanut Toffee Grade II, Peanut slab Grade I, Peanut slab Grade II, Peanut slab Sugar/Bleached and Peanut Respectively.

Ozonation is an oxidation method has been developed for the detoxification of aflatoxins in foods [27], ozone considered as a powerful oxidant, and able to react across the 8, 9 double bond of the furan ring of aflatoxin through electrophilic attack, causing the formation of primary ozonides followed by rearrangement into monozonide derivatives such as aldehydes, ketones and organic acids [24]. Our results showed that using of ozone can decrease the concentration of aflatoxin B1 from 1000 ppb to 200 ppb at dose 2 g for 5 minute and to 105 ppb for 10 minute, while using of 4 g dose of ozone gas can reduce the concentration from 1000 ppb to 53 ppb for 5 minute and to 25 ppb for 10 minute. Results by [28] showed 20 ppb and 40 ppb of ozone for 5 minute can reduce the concentration of aflatoxin B1 from 90 ppb to 70 and 40 ppb respectively from wheat grains, while [29] revealed that 6 mg of O3/L and 50 mg of O3/L can reduce the percentage of aflatoxin B1 65.8% and 89.4% respectively in peanut. [30] observed that using of O3 at 60 µmol/mol for 180 minute can decrease aflatoxin B1 to 96.6% from wheat grains. Results by [31] showed that 50 mg of O3/L for 60 minute can reduce aflatoxin B1 from corn flour to 78.7%.

From all that mention above we can conclude that using of microwave and ozone gas considered as a suitable methods for degradation of aflatoxin B1from fish feedstuff contaminated sample, and when the temperature and time of exposure to microwave increase the effect of toxin reduction will be increase, also when the dose and time of exposure to ozone gas increase this lead to increasing of detoxification effect. In addition to that our results showed that ozone was more effective method in detoxification of aflatoxin B1 than microwave.

Table (1): Detection of aflatoxin B1in stored fish feedstuff samples using ELISA technique.

Fish feedstuff samples	Aflatoxin B1 concentration / ppb
S1	150
S2	125
S3	150
S4	200
S5	75
S6	300
S7	111
S8	1000
S8	600
S10	325
S11	850
S12	75
S13	100
S14	125
S15	75
S16	100
S17	75
S18	100
S19	75
S20	125
S21	100
S22	80
S23	225
S24	225
S25	125
S26	50
S27	125
S28	0
S29	0
S30	100
S31	67
S32	200

Table (2): Detoxification of aflatoxin B1 from contaminated fish feedstuff sample (S8) with concentration (1000 ppb) using microwave

Temperature (°C)	Time of exposure (min)	Concentration of aflatoxin B1(ppb) (Each value expressed as Mean ± Standard Error (SE) of three replicates
50	5	675*
50	10	430*
100	5	212*
100	10	108*

* = Significant (P≤ 0.05)

Table (3): Detoxification of aflatoxin B1 from contaminated fish feedstuff sample (S8) with concentration (1000 ppb) using ozone gas

Doses of ozone gas (g)	Time of exposure (min)	Concentration of aflatoxin B1(ppb) (Each value expressed as Mean ± Standard Error (SE) of three replicates
2	5	200*
2	10	105*
4	5	53*
4	10	25*

* = Significant (P ≤ 0.05)

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