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Prevalence of Aflatoxin B_1 in Feedstuffs in Northern Iran

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Abstract: Mycotoxins are fungal toxins of which aflatoxins are the most important. By consuming aflatoxin B_1 (AFB_1) by milk-cattle, AFM_1 is transferred through milk and products to human. Due to its carcinogenic effects on liver, immunosuppression and growth, it is potentially dangerous to humans. In summer 2008 and winter 2009, 384 feedstuff samples (such as concentrated, beetroot refuse, wheat bran and cottonseed refuse) were collected at cattle farms located in villages in Mazandaran province, northern Iran. In every season 192 samples and of each feedstuff 48 samples were selected. AFB_1 were tested by competitive ELISA. In summer, of feedstuff taken from Sari, Babol, Chalos and Tonekabon 22.9, 31.3, 25 and 29.2% were positive (more than 5 μg/kg AFB_1), respectively. In general, of 192 samples, 52(27.1%) were positive (max=11, min=1, mean=3.83). Whereas in winter, 39.6, 45.8, 47.9 and 33.3% were positive, respectively and in general, of 192 samples, 82(42.7%) were positive(max=14, min=0.4, mean=5.09). In northern Iran due to favorable aflatoxigenic moulds growth, especially in cold seasons, some measures should be taken in production, processing and storage of feedstuffs. There was a significant relationship between AFB_1 contamination level and kind food and seasons applying statistical test.

Key words: Aflatoxin B_1 · Mazandaran · Feedstuff · ELISA

INTRODUCTION

Aflatoxins are structurally related to a group of toxic compounds found in most plant products such as wheat, peanut, copra, Soya, maize and rice. These toxigenic fungi contaminate food products in different phases of production and processing, especially in suitable heat and moist conditions [1, 2]. Main Aflatoxins are B_1, B_2, G_1 and G_2. They are generally produced by special strains of *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*. They are dihydrofuran moiety metabolic or tetrahydrofuran which is bound to coumarin ring [3]. *Aspergillus flavus* merely produces AFB, while other species produce both B and G [4]. They are grown during feed and food growth, harvest and storage. When animals consume contaminated feedstuff with AFB_1, the toxin is metabolized in the liver and execrated as AFM_1 via milk, urine and feces [5, 6]. AFM_1 is bound to milk protein portion, especially to casein [6,7]. Aflatoxins are acute toxic compounds, immunosuppressive, mutagen, tratogen and carcinogen. International Agency for Research on Cancer (IARC) of WHO introduced Aflatoxins B_1 and M_1 as primary and secondary groups of carcinogenic compounds, respectively [8]. Their main target organ for toxicity and carcinogenicity is liver. Although mutagenic and carcinogenic level of AFM_1 is lower than AFB_1, its genotoxic activity is high [9]. AFM_1 resists against pasteurization and autoclave heat and other food producing and processing steps and there is no known effect to decrease these toxins [5, 10]. European committee considered the permissible level of AFB_1 in feedstuff 5μg/kg and in foodstuff 2 μg/kg [1, 11, 12]. If mycotoxin consumption is ≥70 mg in cattle it will go beyond the standard in raw milk accepted by most countries [13]. Many researchers have reported linear relationship between AFM_1 in milk and AFB_1 in feedstuff consumed by animals [14].

Baydar *et al.* [15] reported the level of AFB_1 between 0.03-1.61 μg/kg in foodstuff and feedstuff in 2005. Thieu *et al.* [16] in 2007 reported the level 7.5 μg/kg

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in concentrated feedstuff in southern Vietnam. Charoenpornsook and Kavisarasia [17] isolated AFB_1 by mean= 7.56 µg/kg in 23 samples of feedstuff (92%) out of 25 in Thailand in 2006. Kangeth *et al.* [18] isolated AFB_1 in 69 samples (98.6%) of feedstuff out of 70 ones in Nairobi, Kenya in 2007. Oruc *et al.* [19] measured AFB_1 in 19 animal maize samples collected from different regions in Turkey and 7 other imported American samples in 2006. The first contained contamination between 0.01 to 32.30 by mean 10.94 µg/kg. The second group contained contamination between 0.90 to 1.50 by mean 0.78 µg/kg. Binder *et al.* [20] studied, 2753 feedstuff samples at European, Mediterranean and Asian supermarkets for mycotoxins during 2007. 33% of Asian feedstuffs were positive which mostly contained Deoxynivalenol, Zeralenone, fumonisin and Aflatoxin[20]. In 2004-2005, Ghali *et al.* [21] tested 209 samples (spices, dried fruit, corn, wheat and barley) for aflatoxins at Tunisian supermarkets. Aflatoxins and AFB_1 contamination were 50.5 and 37%, respectively. Toteja *et al.* [22] determined AFB_1 in wheat ≤ 5 µg/kg in 40.3% of samples in India. High concentration of 30 µg/kg (Indian limit) was observed in 16% of samples. Tam *et al.* [23] observed more than 0.1 µg/kg AFB_1 in 4 and 1% in breakfast corn and children corn respectively in Canada (European standard limit for AFB_1 in children corn, is 0.1 µg/kg). As feedstuff is the crucial food resource for animals, their fungal contaminations play a crucial role in endangering animal and human health. Due to the carcinogenic and immune-suppressive effects of Aflatoxin and its health deteriorating effects in animal and human, conducting such a study namely Aflatoxin B_1 determination in feedstuff in the region looks necessary. The aim of this study was to investigate the occurrence of AFB_1 in feedstuff in Mazandaran province, northern Iran, whereas to the best of our knowledge there are no available data about detection in this area.

MATERIALS AND METHODS

This study was conducted to determine Aflatoxin B_1 (AFB_1) in summer 2008 and winter 2009, 384 feedstuff samples (such as concentrated feedstuff, beetroot refuse, wheat bran and cottonseed refuse). Samples were collected from cattle farms located at villages in Mazandaran province, northern Iran. In every season 192 samples and of each feedstuff, 48 samples were selected. Aflatoxin B_1 contamination was measured by competitive ELISA by the RIDASCRIN kit. Samples were put in light-proof and sterilized plastic bags and frozen in -20°C. First, 5 grams of each sample was added to 25ml of methanol 70% and was shaken for 3 minutes. The obtained extract was purified by Vatman No.1; then, 1 ml of it was diluted by 1 ml distilled sterilized water. After that, 50 Microlitre of it was poured into every micropipette ELISA well. The solid phase in plastic micro-wells was coated with anti-Aflatoxin B_1 antibodies. At first, with pipettes, standard samples and conjugated solutions and anti-Aflatoxin antibodies, were added to each well. During the first incubation, free AFB_1 molecules and enzyme conjugation competed for anti-AFB_1 antibodies of the solid phase. Any unbound enzyme conjugation was removed by washing. Using substrate in wells, its red color was converted into blue and by using the stop solution its blue color was changed into yellow. AFB_1 concentration and absorbance was read by ELISA Reader at 450nm. Data were analyzed by ANOVA utilizing SPSS software package. The results were analyzed by comparing them with standard limits [19, 24].

RESULTS

In summer, of feedstuff taken from Sari, Babol, Chalos and Tonekabon 22.9, 31.3, 25 and 29.2% were positive (more than 5 µg/kg AFB_1), respectively. In general, of 192 samples, 52 (27.1%) were positive. In other words, 27.1% samples were above the limit of European community regulations (5 µg/kg). Maximum concentration of AFB_1 was 11 µg/kg and minimum was 1 µg/kg (mean 3.83). Of each feedstuff, 48 samples were selected, 15 samples (31.3%) were concentrated, 10 samples (20.8%) were beetroot refuse, 4 samples (8.3%) were wheat bran and 23 samples (47.9%) were cottonseed refuse. In winter, 39.6% of samples in Sari, 45.8% in Babol, 47.9% in Chalos and 33.3% in Tonekabon were positive. In general, of 192 samples, 82 (42.7%) were positive. In other words, 42.7% samples were above the limit of European community regulations (5 µg/kg). Maximum concentration of AFB1 was 14 µg/kg and minimum was 0.4 µg/kg (mean = 5.09). Of each feedstuff, 48 samples were selected, 26 samples (54.2%) were concentrated animal food, 18 samples (37.5%) were beetroot refuse, 5 samples (10.4%) were wheat bran and 33 samples (68.8%) were cottonseed refuse (Table 1, 2). The highest contamination was observed in winter and the lowest contamination was observed in summer and autumn. There was a significant relationship between AFB_1 contamination level and kind food and seasons applying statistical test (p<0/05).

Table 1: Distribution of AFB_1 contamination in feedstuff samples in summer 2008 and winter 2009, in Mazandaran province, Iran

Seasons/ Samples		Total number	More than 5 µg/kg * N	%	Mean±S.E.	S.D.	Max	Min
Summer	Concentrated	48	15	31.3	4.27±0.35	2.43	11	1
	Beetroot refuse	48	10	20.8	3.45±0.35	2.39	9	1.01
	Wheat bran	48	4	8.3	2.48±0.23	1.56	8	1.03
	Cottonseed refuse	48	23	47.9	5.13±0.37	2.54	9	1
Winter	Concentrated	48	26	54.2	5.74±0.51	3.53	14	1
	Beetroot refuse	48	18	37.5	4.6±0.43	2.96	12	1
	Wheat bran	48	5	10.4	2.85±0.24	1.70	9	0.4
	Cottonseed refuse	48	33	68.8	5.16±0.49	3.40	14	2

S.E= Standard Error of Mean, S.D=Standard Deviation, N=Number, µg/kg =Microgram/kilogram

*European community regulations= 5 µg/kg

Table 2: Distribution of AFB_1 contamination in feedstuff samples by varies month in Mazandaran province, Iran

Years/ Months		Total number	More than 5 µg/kg N	%	Mean±S.E.	S.D.	Max	Min
Summer								
2008	Jul	64	21	32.8	4.16±0.33	2.61	9	1
	Aug	64	14	21.9	3.60±0.28	2.61	9	1.01
	Sept	64	17	26.6	3.74±0.31	2.47	11	1
Winter								
2009	Jan	64	30	46.9	5.51±0.42	3.39	13	1
	Feb	64	25	39.1	4.81±0.42	3.39	14	0.4
	Mar	64	27	42.2	4.93±0.41	3.31	14	1

DISCUSSION

Although it is difficult to prevent Aflatoxin formation in feed prior to harvesting due to high heat and moisture, it is possible to attain favorable result by correct storage [1]. Decreasing fungal growth and AFB_1 formation in feedstuff is essential since it is consumed by both human and animals. Storka *et al.* [25] reported AFB_1 contamination in cattle feed 1.2-3.6 µg/kg in some areas in Belgium, which was lower than European standard limit (5µg/kg). Decastelli *et al.* [26] reported the contamination 8.1% in 541 feedstuff samples (corn and concentrated feedstuff) in Italy in 2004, but it was zero in 75 samples in 2005. Oruc *et al.* [19] measured AFB_1 in animal maize samples in Turkey and imported American samples. The contamination was 0.01 - 32.30 by mean 10.94 µg/kg and 0.90 - 1.50 by mean 0.78 µg/kg, respectively. Whereas, in our study in summer and winter, 27.1 and 42.8% were positive, respectively. Aflatoxin potential risks to humans using milk and during products, especially in liver cancer have been proved [27]. As epidemiological studies in Iran on AFM_1 contamination in milk and dairy products have less been conducted, then it is essential to be placed on the government agenda, hence regulations to reduce contamination to aflatoxigenic moulds in feedstuff. To increase milk quality it is necessary for feedstuff to be without AFB_1 contamination [28]. Results of this study showed that AFB_1 contamination level in feedstuff is high. It is a serious public health problem. As a result, milk and dairy products should be controlled regularly at least twice a year; furthermore, keeping low AFB_1 level in feedstuff is of importance. To reach the goal, feedstuff should be kept away from probable contamination. In northern Iran, due to favorite aflatoxigenic moulds growth, especially in cold seasons, some measures should be taken in production, processing and storage of feedstuff. In any case, the product may be contaminated and it will jeopardize animal and human health. It would be feasible to pass some regulations regarding to decrease aflatoxigenic moulds in feedstuff. Therefore, the following recommendations are given: 1. Using unmolded feed stuff 2. Preventing contamination in production, storage and consumption 3. Harvesting On-time and preventing damage to the product 4. Cultivating and producing of feed stuff strains resistant to mould growth, or toxic formation 5. Applying chemical measures to stop fungal growth in feed stuff 6. Drying of feed stuff suitably before storage 7. Providing suitable physical condition in storage 8. Stopping prolonged storage of feed stuff in unsuitable conditions 9. Determining standard regulations for Aflatoxin in feed stuff 10. Measuring AFB_1 in suspected feed stuff products in production and shopping centers 11. Controlling constantly on imported feed stuff in ports 12. Establishing laboratories to detect toxins in the capital of provinces 13. Demanding producers and officials care to achieve Aflatoxin standard level in feed stuff.

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