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SOME MYCOTOXINS CONTAMINATION IN ANIMAL FEED AND FEEDSTUFF INGREDIENTS IN DUHOK PROVINCE

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ABSTRACT

Fungi produce different types of mycotoxin under improper storage conditions and during crops growing period which cause chronic or acute animal toxicities. Aflatoxin B1, Ochratoxin A and T-2 toxin are mycotoxins that pose a threat to human and animal health. This study was carried out to determine AFB1, OTA and T-2 levels in animal feed and feedstuffs materials used in animal feed factories and livestock enterprises throughout Duhok province. The samples that used for analysis (35 mixed feeds and 54 feedstuffs materials) collected from different feed factories and enterprises. Mycotoxins concentrations were quantified by using immunoassay (ELISA). Results showed that positive samples for AFB1 found in 7 (7.87%), OTA were 21 (23.60%) and T-2 were 8 (8.99%) from 89 samples analysed, whereas negative samples found for AFB1 were 82 (92.13%), OTA were 68 (76.40%) and T-2 were 81 (91.01).

KEYWORDS: Animal mixed feed: Animal feedstuffs: mycotoxin: Aflatoxin B1: Ochratoxin A: T-2 toxin.

1. INTRODUCTION

Mycotoxins are secondary metabolites with low molecular weight produced by some fungi strains such as *Fusarinm, Pencillium* and, *Aspergillus*, which develop during crops production in field and invade food under improper storage conditions with high amount of temperature and humidity (Iheshiulor *et al*., 2011). Over the world, there are more than 100 fungi species that may invade plants and produce mycotoxins (Sultana and Hanif, 2009). Crops infections develop during various production stage by mycotoxin and mold production at end stage of growth in the field, during crops harvesting and storage (Martins *et al*., 2007). Mycotoxins implicate regularly in their toxic syndromes in both humans and animals (charoenpornsook and Kavisarasai, 2006 and Wayne, 2007). By the intensity of mycotoxins synergetic effect and toxic properties, they are considered as health risk to the consumers with contaminated feed and food (Yiannikoiuis and jonany, 2002; Omede, 2008).

Mycotoxins have been found in all parts of the world and detected in different commodities of food and considered as the most dangerous contaminants crops production and animal feed (Okoli *et al*., 2006a,b; Okoli *et al*., 2007 a,b). Researches showed that there is no parts of the world escape from mycotoxin effects and according to Lawlor and Lynch, (2005) and Okoli *et al*., (2006b), they estimated that mycotoxins are effect more than 25% of the crops production around the world per year. They are produced only in the aerobic environments (Ratcliff, 2002).Their negative effects have been well recognized on animal production and health when animals farmed intensively such as cattle, swine and poultry enterprises as a consequence of the consumption of high amount diet with of oilseeds and cereals (charoenpornsook and Kavisarasai, 2006). Inhibition of protein synthesis occur by many types of mycotoxins such as Aflatoxin B1 (AFB1), Ochratoxin A (OTA) and T-2 toxin (T-2) (charoenpornsook and Kavisarasai, 2006). This inhibition may be the secondary mechanism involve in their immunotoxic activity. They may effect selectively on many target organs, damage membranes or intervenes with macromolecular functions and synthesis. (Sharma, 1993).

Mycotoxins formation in nature was considered a global problem, however, in certain geographical areas of the world, some mycotoxins are produced more readily than other (Ratcliff, 2002; Lawlor and Lynch, 2005). The Mycotoxins problem does not end in animal feed or detraction its performance may become accumulated in animal products (e.g; meat, egg

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and milk) by which transmit to human food and pose a threat to human health (Akande *et al*., 2006). Therefore, the current experiment was designed to study the contamination of some mycotoxins in animal feed and feedstuff ingredients in Duhok province.

2. MATERIALS AND METHODS

2.1. FEED AND FEEDSTUFFS SAMPLES

Over this study, 89 samples (35 mixed feeds and 54 feedstuffs samples) were collected from different livestock enterprises and animal feed factories during the summer season in July and August 2019 in Duhok province. Samples were gathered according to a method determined by Ergun *et al*., (2007). Feed samples consisted of sheep (n=17), cattle (n=8) and poultry (n=10) rations, while feedstuffs samples include corn (n=14), wheat (n=7), barley (n=11), sunflower seed (n=3), chick pea (n=2), sorghum (n=3), soybean meal (n=7) and wheat bran (n=7). The samples taken from storage at the enterprises were kept at +4°C in the refrigerator and analyzed within one week.

2.2. BIOCHEMICAL ANALYSES

Levels of mycotoxins AFB1, OTA and T-2 in feed samples were determined by ELISA device (BioTek ELx800 Absorbance Microplate Reader, USA) using the commercial kit (BIO SCIENTIFIC, Art No: 1055-04 for AFB1, 1036-02 OTA and 1037-01 for T-2) via the competitive inhibition enzyme immunoassay method based on the manufacturer's procedure. Limit of detection for AFB1, OTA and T-2 in feed were 0.4ppb, 0.15 ppb and 10 ppb respectively as the described method according to Elisa kit procedure (BIO scientific crop, 2016). Weighed out 5 g of the each ground and 25 mL of 70% Methanol were added and vortex vigorously for 3 minutes, Centrifuge the samples for 10 minutes at 4,000 x g at room temperature (21 °C). About 50 µl taken from each pre-processed sample was added to the wells. They were incubated for 30 minutes at room temperature and in a dark place after adding by adding 100 µl of conjugate enzyme. Then 100 µl TBM substrate was added and incubated for 15 minutes and by adding 100 µl of Stop Buffer to stop the enzyme reaction then the plates were Read on a plate reader with 450 nm wavelength (Gumus *et al*., 2018).

2.3. STATISTICAL ANALYSIS

SPSS 20.00 statistical software program used to estimate mean, standard deviation, minimum and maximum values of mycotoxin data (SPSS Chicago, 2011).

3. RESULT AND DISCUSSION

The mixed feed and feedstuffs ingredient samples with mean ± Sx, minimum and maximum values for mycotoxins; AFB1, OTA and T-2 are given in Table 1.

Table (1): Levels of mean, minimum and maximum of Aflatoxin B1, Ochratoxin A and T-2 toxin in mixed feed and feedstuffs ingredients (ppb).

Feeds	n	Aflatoxin B1			Ochratoxin A			T-2 toxin		
		X ± Sx	Min	Max	X ± Sx	Min	Max	X ± Sx	Min	Max
Mixed Feeds										
Sheep Feed	17	0.080±0.029	0.00	0.46	0.092±0.012	0.00	0.18	1.953±0.651	0.0	11.4
Cattle Feed	8	0.085±0.060	0.00	0.49	0.055±0.019	0.00	0.17	3.69±1.12	1.9	11.0
Poultry Feed	10	0.113±0.044	0.00	0.49	0.111±0.025	0.02	0.2	2.84±1.59	0.0	12.4
Feedstuffs										
Corn	14	0.134±0.042	0.00	0.48	0.073±0.018	0.00	0.18	2.66±1.05	0.0	15.2
Wheat	7	0.056±0.026	0.00	0.16	0.032±0.011	0.00	0.08	3.26±1.25	0.0	10.45
Barley	11	0.093±0.040	0.00	0.42	0.116±0.021	0.00	0.19	2.30±0.94	0.0	11.00
Sun flower seed	3	0.350±0.153	0.05	0.55	0.046±0.018	0.02	0.08	4.60±3.60	0.0	11.70
Chick pea	2	0.270±0.220	0.05	0.49	0.114±0.094	0.02	0.208	1.04±1.04	0.0	2.08
Sorghum	3	0.087±0.059	0.00	0.20	0.068±0.012	0.04	0.084	1.13±0.61	0.0	2.10
Soybean meal	7	0.019±0.008	0.00	0.04	0.086±0.023	0.02	0.168	0.80±0.52	0.0	2.90
Wheat bran	7	0.060±0.016	0.00	0.12	0.053±0.023	0.00	0.18	1.29±0.49	0.0	2.70

X± Sx: refer to mean ± Standard error, Min: minimum, Max: maximum.

3.1. AFLATOXIN B1

In the present study, AFB1 contamination was found to be 5.71% in animal mixed feed and 9.26% in feed ingredients. Seventeen samples of sheep feed out of total 89 different feed and feed ingredients are analysed an overall all samples were found within no AFB1 contamination (Table 2). Cattle feed samples were observed 12.5% and for poultry feed were found 10.0 % within limited detection range.

For feed ingredients, the AFB1 detected contamination rate were 14.29 %, 0.0 %, 9.01 %, 66.67 %, 50.0 %, 0.0 %, 0.0 % and 0.0% for each corn, wheat, barley, sunflower seed, chick pea, sorghum, soybean meal and wheat bran respectively. Similarly, Hashemi (2016), studied 359 different feed and feedstuffs and found contaminated samples with AFB1 at different percentage ratios including; cattle feed 12.50%, Wheat bran 10%, corn 35.71%, barley 0.0%, cotton seed meal 0.0%, soybean meal 20%, wheat flour 0.0% and meat powder 0.0%.

Patterson and Roberts, (1980), reported during a survey study that conducted over a period of 13 years on the detection AFB1 taken 740 samples of animal mixed feeds, including dairy feeds, 27% of the feed samples were contaminated with toxin, at the higher level of more than 30 µg/kg. Whereas Rodrigues *et al*., (2011) studied on 324 grain, feed and feedstuff ingredients in the Middle East and Africa and found the high percentage of AFB1 detection among the analysed samples, including maize, wheat, poultry mixed feed, pig feed, grass silage, wheat bran, soybean meal and other feedstuffs, the percentage of positive samples were; 93%, 82%, 100%, 100%, 99%, 19%, 24% and 38% respectively. In accordance with the result demonstrated in a study conducted on maize, the AFB1 content was found in a percent of 77% (Sauza *et al.*, 2013). Martins *et al*., (2007), mentioned that AFB1 monitoring by the feed industry, beside awareness to some of breeders is quite recommended and beneficial, since AFB1 cause animal's health disorders and the contaminated milk with this mycotoxin can be a serious hazard for human health.

Table 2: Aflatoxin B1, ochratoxin A and T-2 toxin levels in mixed feeds and feedstuffs ingredients.

Feeds	n	Aflatoxin B1				Ochratoxin A				T-2 toxin			
		Undetected		Detected		Undetected		Detected		Undetected		Detected	
		n	%	n	%	n	%	n	%	n	%	n	%
Mixed Feeds													
Sheep Feed	17	17	100	0	0.0	14	82.35	3	17.65	16	94.12	1	5.88
Cattle Feed	8	7	87.5	1	12.5	7	87.5	1	12.5	7	87.5	1	12.5
Poultry Feed	10	9	90.0	1	10.0	6	60.0	4	40.0	8	80.0	2	20.0
Feedstuffs													
Corn	14	12	85.71	2	14.29	11	78.57	3	21.43	13	92.86	1	7.14
Wheat	7	7	100.0	0	0.0	7	100.0	0	0.0	6	85.71	1	14.29
Barley	11	10	90.91	1	9.01	5	45.45	6	54.55	10	90.91	1	9.01
Sun flower seed	3	2	33.33	1	66.67	3	100.0	0	0.0	2	66.67	1	33.33
Chick pea	2	1	50.0	1	50.0	1	50.0	1	50.0	2	100.0	0	0.0
Sorghum	3	3	100.0	0	0.0	3	100.0	0	0.0	3	100.0	0	0.0
Soybean meal	7	7	100.0	0	0.0	5	71.43	2	28.57	7	100.0	0	0.0
Wheat bran	7	7	100.0	0	0.0	6	85.71	1	14.29	7	100.0	0	0.0
Mixed Feeds	35	33	94.29	2	5.71	27	77.14	8	22.86	31	88.57	4	11.43
Feedstuffs	54	49	90.74	5	9.26	41	75.93	13	24.07	50	92.59	4	7.41
Total	89	82	92.13	7	7.87	68	76.40	21	23.60	81	91.01	8	8.99

3.2. OCHRATOXIN A

The percentage and numbers of detected and undetected OTA in mixed feed and feedstuff ingredients are shown in Table 1. The results showed that OTA ratio was 24.07 % in feedstuffs ingredients and 22.86 % in mixed feeds, while these value are lower than limited safety range for OTA in feeds, it may cause animal health problems and loss its production when consume feeds for a long period time

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(Moura *et al*., 2004). The results of study conducted in Turkey showed that the ratio of OTA was 47.83% in feedstuffs materials, while it was 66.67% in mixed feeds (Yıldız, 2009). In another study, the ratio of OTA found to be 74.58% in mixed feeds and 66.67% in feedstuffs materials (Gumus *et al*., 2018). Araguás *et al*., (2005), analysed 72 different cereal crops samples, found 9% of the samples were contaminated with OTA and only 2 samples have the value above the allowed limit range. Similarly Vega *et al*., (2009) studied 91 crop grain and demonstrate that only 1 sample of OTA had toxin above the allowed value. In the other study it was found only 2 samples from 56 had OTA value more than limit value (Altintas *et al*.2011).

Current study showed the samples taken from sheep, poultry and cattle feed have OTA 17.65%, 12.5% and 40.0% respectively above the limited of detection (Table 1). Yıldız, (2009), concluded in a survey study that 60.00% of cattle feed samples contaminated with OTA above limited detection value. While Rosa *et al*., (2008) showed that 25% for finished cow´s feed had positive OTA, 31% corn samples and 22% for barley samples. Sonal & Oruc, (2000) was reported that 100% of the poultry feed samples taken from poultry enterprises around Bursa province had OTA value above the detection limit, while in our study there was only 40.0 % of poultry feed contaminated with OTA. However, it is well known that the likelihood a value of only one mycotoxin being found with very low amount in the mixed feed and feedstuff, but it is known that many types of mycotoxins produced by the same species of fungi and simply infest animal feed (Hussein & Brasel, 2001). In our results, we found that 0.0%, 28.57 %and 14.29% of samples contaminated with OTA of feedstuffs ingredient for sunflower seed, soybean meal and wheat bran respectively, while Gumus *et al*., (2018) reported that OTA was higher than allowed limited value in sunflower meal (80.00%); soybean meal (66.66%) and wheat bran (60.00%). The studied results by Kaya *et al*., (1990) are highly quite when compared to our results, which may be due to the some factors such as regional differences, climate conditions, and humidity of the atmosphere.

3.3. T-2 TOXIN

The T-2 numbers performed tests on mixed feed and feedstuffs samples, number of the respective positive and negative ratios were summarized in Table 1. The frequency of positive samples for T-2 was 11.43% and 7.41% in mixed feed and feedstuff ingredients, respectively. These results were lower than that analysed by Souza *et al*., (2013), and demonstrate that complete feed and feedstuffs ingredients had 98.0% and 100% T-2 contamination, respectively. In our study, the incidence of T-2 were 5.88% for sheep feed, 12.5% for cattle feed and 20.0% for poultry feed, while for feedstuff ingredients such as corn, wheat, barley, sunflower seeds, chick pea, sorghum, soybean meal and wheat bran were; 7.14%, 14.29%, 9.01%, 33.33%, 0.0%, 0.0%, 0.0% and0.0%, respectively. However, Rodrigues *et al*., (2011), reported that corn contaminated with T-2 at ratio of 94.8%, wheat 94.9%, and barley 95.3%. While Marijani, *et al*., (2019) showed feedstuff samples had t-2 such as corn, soybean meal and cotton seed at the rate of 3.4%, 16.7% and 4.9%, respectively. Kocasari *et al*., (2013), by using ELISA method analysed 180 cattle and lamb feeds and reported that T-2 toxin were found in samples 85 (47.2 %).

A wide range of T-2 toxicity can cause: loss of body weight, diarrhoea, pests in liver and digestive system disorders (Li *et al*., 2011). Especially in poultry it reduce egg yield production, decrease hatching egg and de-feathering are some symptoms of chronic exposure to high dose of T-2 (Diaz *et al*., 1994; Wyatt *et al*., 1975). In addition, T-2 causes oral cavity ulcers which can be also the factor that depress animal feed intake (Wyatt *et al*., 1973).

4. CONCLUSION

This study revealed that AFB1, OTA and T-2 may be found in majority of the complete feed and feedstuffs ingredient in animal farms of the Duhok province. Mycotoxins concentration of AFB1 OTA and T-2 in proportions of mixed feed (5.71%, 22.86% and 11.43%) and feedstuff ingredients (9.24% 24.07% and 7.41%) samples had higher values than limited of detection. Therefore, there should be regulate a program for monitoring the mycotoxins in animal feed and feedstuffs commodities and destined for animal consumption feed that produced locally or imported to prevent animal from mycotoxins toxicity and also their formation and transportation to animal products.

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پیسبونا ئالفێن گیانەوەرا وکەرەستێن دەستپێکی بو چێکرنا ئالفی ب چەند جورێن ژەهرێن کیفکویی

پوختە

ئەڤ ڤەکولینە هاتە ریکخستن بو دیارکرنا هەبونا ژەهرێن دئێنە بەرهەم ئینان ژ لایێ کیفکوییێن گەشێ دکەن لسەر ئالفێ گیانەوەرا و کەرەستێن دەستپێکی بو چێکرنا ئالفی، ئەوێ دبنە ئەگەرێ ژەهرەبونا دوم درێژ یا گیانەوەرا، ژەهرێن ئەفلاتوکسین B1 و ئوکراتوکسین A و T-2توکسین ئەگەرێن مەترسینە لسەر تەندروستیا گیانەوەران، 89 سامپلێن ئالفی هاتنە وەرگرتن (35 ئالف و54 کەرەستێن دەستپێکی بو چێکرنا ئالفی) ژ پروژێن خودانکرنا گیانەوەرا وکارگەهێن چێکرنا ئالفی لسەرانسەر پاریزگەها دهوکێ، ریژا هەبونا ژەهران هاتە پیڤان ب کارئینانا ئامیرێ (ELISA)، داتایێن پوزەتیڤ هاتنە بەرچاڤکرن ییێن ژەهرا ئەفلاتوکسین B1 پوزەتیڤ دەرچووین 7 سامپل بون ب ریژا 7.87% و ژەهرا ئوکراتوکسین A 21 سامپل بون ب ریژا 23.60% و ژەهرا T-2توکسین 8 سامپل بون ب ریژا 8.99% ژ سەرجەمێ 89 سامپلان بو هەر جورە ژەهرەکێ ئەوێن هاتینە شلوڤەکرن، سەرجەمێ سامپلێن نیگەتیڤ دەرچووین بو ئەفلاتوکسین B1 82 سامپل بون ب ریژا 92.13% و ژەهرا ئوکراتوکسین A 68 سامپل بون ب ریژا 76.40% و ژەهرا T-2توکسین 81 سامپل بون ب ریژا 91.01%.

پەیڤێن کلیل: ئالفێ گیانەوەرا، کەرەستێن دەستپێکی بو چێکرنا ئالفی، ژەهرێن کیفکوی: ژەهرا ئەفلاتوکسین B1، ژەهرا ئوکراتوکسین A، ژەهرا T-2توکسین.

تلوث بعض السموم الفطرية في علف الحيوانات ومكونات الاولية لتكوين العلائق

الخلاصة

تنتج الفطريات أنواعًا مختلفة من السموم الفطرية في ظل ظروف التخزين غير مناسبة وأثناء فترة زراعة المحاصيل التي تسبب سمية مزمنة أو حادة للحيوانات. الأفلاتوكسينB1 والاكراتوكسين A و-T 2توكسين هي السموم الفطرية التي تشكل تهديدًا لصحة الإنسان والحيوان. أجريت هذه الدراسة لتحديد مستويات الأفلاتوكسينB1 والاكراتوكسين A و2-T تـوكسين في مواد الاولية لتكوين الأعلاف والأعلاف الجاهزة في مصانع التكوين لأعلاف الحيوانات ومشاريع الثروة الحيوانية في محافظة دهوك. تم جمع العينات المستخدمة للتحليل (35 علف مخلوط و 54 مادة اولية للأعلاف) من مصانع ومشاريع الحيوانات مختلفة. تم قياس تراكيز السموم الفطرية باستخدام جهاز الطيفي (ELISA). أظهرت النتائج أن العينات الإيجابية لافلاتوكسينB1 وجدت في 7عينات (7.87٪) و الاكراتوكسين A كانت 21 عينات (23.60٪) و T-2توكسين كانت 8 عينات (8.99٪) من مجموع 89 عينة تم تحليلها ، بينما العينات السلبية منها لافلاتوكسينB1 كانت 82 (92.13٪) ، و لاكراتوكسينA كانت 68 (76.40٪) ول T-2 توكسين كان 81 (91.01).

الكلمات الدالة: **علف الحيوانات، مواد الاولية لتكوين الأعلاف، السموم الفطرية: الأفلاتوكسينB1، اكراتوكسين A، توكسين T-2.**

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