Yemeni Journal of Agiculture and Veterinary Sciences
ISSN Online: 2789-1593, Print: 2311-7788
YJAVS- 24–05-02-03
DOI: https://doi.org/10.70022/yjavs.v5i2.2240
Available online at: http://journal.tu.edu.ye/index.php/yjavs/index

Original Article

Determination of mycotoxins level in poultry feeds at Dhamar Governorate, Yemen

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KEYWORDS

Determination, ELISA, Dhamar Governorate, Mycotoxins, Poultry feed, Yemen

Article`s history

Received

26th June, 2024

Accepted

15th October, 2024

ABSTRACT

Mycotoxins contamination in feed is a global safety concern. It induces significant economic losses to the poultry industry and poses substantial hazards to human health. The present study aimed to determine the levels of mycotoxin contamination in formulated and raw materials feeds used to grow broilers chicken in Dhamar Governorate, Yemen. A total of 36 samples were randomly collected from variety types of poultry feed used in poultry farms at Dhamar governorate. Methanol was used as organic solvents for mycotoxin extraction from solid feed samples. Quantitative, rapid ELISA test kits were used to detect levels of mycotoxins. The results revealed that, Aflatoxins, T-2 toxins, Ochratoxins A, and Zeralenone were detected in 36.11%, 83.33%, 22.22%, and 100% of the tested samples, with contamination levels of 0.37, 21.67, 0.8, and 14.04 ppb, respectively. The highest levels of aflatoxins and Ochratoxins were found in Ordinary Feed-I (1.00 and 3.47 ppb) and Ordinary feed-II (1.07 and 4.9 ppb) respectively. Similarly, the highest level of Zearalenone was detected in Ordinary feed-I (19.87 ppb). The highest levels of T-2 toxin were detected in the Primitive concentrate (49.23 ppb) and Final Concentrate (49.47 ppb). Mycotoxins were detected at relatively lower levels in the other feed types tested. Statistical analysis showed a significant difference *(P* value < 0.05) in mycotoxin levels between feed types. Ordinary Feeds were more contaminated compared to raw ingredient feeds. These findings highlight the threat posed by mycotoxins to poultry and public health in Dhamar governorate and point to the need to implement intervention measures to reduce these risks. Further studies are required to determine the factors associated with mycotoxins contamination in poultry feeds.

INTRODCTION

Mycotoxins, a diverse group of toxic secondary metabolites produced by filamentous fungi, pose detrimental effects on human and animal health. These toxins can enter human and animal bodies either directly, through contaminating Agricultural products or ready-to-eat items, or indirectly, through the consumption of products derived from animals and poultry feed on contaminated materials (Adanyi et al., 2018). Mycotoxins produce a variety of diseases, collectively called “mycotoxicoses,” directly or in combination with other primary stressors such as pathogens (Raju and Devegowda, 2000). These diseases are exhibited by symptoms and lesions, which can be used to clinically diagnose the presence of mycotoxins although these symptoms are not just straightforward. When Aflatoxins (AF) and Ochratoxins (OTs) are co-contaminants of poultry feed, they interact in a synergistic manner. When AF and T-2 toxin are co-contaminants of poultry feed, the T-2 toxin prevent the major effects of AF. This reduces the ability to diagnose aflatoxicosis in the field (Huff et al., 1988).

Recent literature has implicated physiological and immunological effects of mycotoxins at lower and more common levels of contamination. As many of the mycotoxins and their metabolites inhibit protein synthesis, tissues with high levels of protein synthesis and turnover, such as those within the gastrointestinal tract (GIT) can be particularly susceptible to their toxic effects. In particular, the GIT is repeatedly exposed to mycotoxins at concentrations likely higher than other organ systems (Grenier and Osoald. 2011). However, it has been clearly demonstrated that some cells of poultry bodies (such as immune, intestinal, and hepatic cells) are predominantly affected by mycotoxins

(Grenier and Applegate, 2013).

Mycotoxins do not possess immunogenic properties, meaning they are not able to induce an immune response unlike pathogens. But they do interfere with the signaling pathways that are responsible for cell growth or death (apoptosis) (Murugesan et al., 2015). Mycotoxins are typically produced by filamentous fungi, especially those belonging to the genera *Aspergillus, Penicillium, Alternaria, Fusarium*, and *Claviceps spp,* which are the main producers of mycotoxins (Sforza et al., 2006).

Approximately 300 to 400 mycotoxins have been identified and reported so far by the Council for Agricultural Science and Technology and others (CAST, 2003; Schollenberger et al., 2007; Pinotti et al., 2016). While hundreds of mycotoxins have been identified, only a few, including Aflatoxins (AFs), Fumonisins (FMs), Ochratoxins (OTs), Trichothecenes (TRCs), and Zearalenone (ZEN), are considered major safety and economic concerns (FAO and WHO., 2007; Smith et al., 2016; Santos et al., 2019). T-2 toxin is the most toxic fungal secondary metabolite produced by different *Fusarium spp,* (Chen et al., 2020). Moreover, T-2 is the most common cause of poisoning that results from the consumption of contaminated cereal-based food and feed reported among humans and animals (Milicevic et al., 2010). T-2 toxin was identified as a significant threat to human and animal health (Nayakwadi et al., 2020). T-2 has different toxic effects depending on the dosage, age, and ways of exposure (oral, dermal, and aerosol).

Generally, observed acute toxicological effects are feed refusal, vomiting, hemorrhages, stomach necrosis, and dermatitis (Garai et al., 2020). Aflatoxins, in particular, are notorious for their carcinogenic properties and are classified as human carcinogens (group I) by the International Agency for Research on Cancer (IARC, 2012). It is produced by *Aspergillus spp*. and includes four forms: AFB1, AFB2, AFG1, and AFG2 (Awika, 2011). Similarly, OTs, FMs, and Sterygmatocistin have been classified as possible human carcinogens in Group 2B (IARC, 1994). On the other hand, some mycotoxins, such as Zearalenone, exhibit estrogenic activity, leading to hyperestrogenism, sterility, and abortions in affected animals (da Rocha et al., 2014). Although not very prevalent, HT-2 and T-2 toxins are the most toxic TRCs (Streit et al., 2012; Groopman et al., 2013; Marin et al., 2013; Kovalsky et al., 2016). Where they are linked to specific syndromes in farm livestock and poultry (Caloni and Cortinovis., 2010).

In livestock and poultry, the consumption of mycotoxin-contaminated feed results in substantial economic losses, manifesting as impaired growth, decreased productivity, and compromised reproductive efficiency. It causes liver and kidney damage and immunosuppression (Bentvihok et al., 2002; Richard, 2007; Marroquin-Cardona et al 2014). Moreover, simultaneous exposure to multiple mycotoxins can lead to synergistic effects, exacerbating the negative impacts on animal performance and health (Streit et al., 2013).

Consequently, regulatory bodies worldwide have established permissible limits for mycotoxin levels in feed to mitigate these risks (FAO, 2004). For instance, the maximum permitted levels according to the European Union (EU) regulations are 5-20 µg/kg for AFB1, 100–500 µg/kg for zearalenone, and 50–100 µg/kg for OTs, depending on the feed materials (ECCR, 2006). Despite the regulatory measures in place, studies have highlighted the pervasive presence of mycotoxins in poultry feed and its raw ingredients, with some samples exceeding permissible limits (Kosicki et al., 2016; Arroyo-Manzanares et al., 2019). Therefore, continuous monitoring and control of mycotoxin contamination in animal feed are imperative to safeguard animal and human health and maintain low toxin levels in the food chain (EUC, 2015).

In Yemen, data on mycotoxin contamination of poultry feeds are scarce. A study published in 2018 assessed mycotoxin levels in poultry rations from four governorates in Yemen, including Sana'a, Taiz, Ibb, and Dhamar. The highest contamination with aflatoxins (42.5 ppb) was found in rations from Taiz and Dhamar. The study highlights significant fungal contamination and mycotoxin levels in poultry feed, which could impact the poultry industry economically in Yemen (Algabr et al., 2018). In 2022, the poultry sector in Yemen faced significant obstacles, with data indicating a negative growth rate (FAOSTAT, 2024), largely due to exposure to infections and mycotoxins (Anonymous, 2022). This study aimed to assess the contamination levels of mycotoxins in broiler poultry feed and its raw materials in Dhamar Governorate, Yemen.

MATERIALS AND METHODS

Study area and Sampling

This study was conducted in Dhamar Governorate; Samples were collected at the end of the summer season in 2023. Thirty-six feed samples were randomly collected from twelve types of poultry feed and raw materials used in different broiler rearing stages. 100g

were collected for each sample. These samples were gathered from feed manufacturing companies and feed stores in poultry farms of Dhamar Governorate. The feed samples represented four poultry feed categories including: Starter feed, grower feed, finisher feed and raw materials. All the samples were collected with a sterile spoon, placed inside sterile plastic bags, and labeled with the necessary information data. Samples were then stored at 4°C until sent to the laboratory for mycotoxins analysis. The first group of feed samples from 1- 9 were contain Soybean, Corn, Broiler concentrate and supplementation with different concentrations; Whereas; the second group of feed samples from 10-12 were contain yellow corn, white corn and soy-bean as presented in Table 1.

Laboratory analysis and quantification of mycotoxins

The mycotoxin content or level in the feeds was determined by the ToxinFast® ELISA kit (Meizheng Bio-Tech Company, Beijing, China). Four ELISA test kits were used to determine levels of the mycotoxin in poultry feeds. The test was performed at the laboratory of Al-Sanabany Company, Sana'a, Yemen. The test was performed following the manufacturer's instructions. Briefly, samples of raw materials were ground. Mycotoxins were extracted from each sample using the following procedure: 20g of each sample was mixed with 100 ml of 70% methanol solvent (ratio 1:5 w/v) in sterile tubes. The mixture was shaken, centrifuged, and then filtrated with filter paper. Filtrates were collected in sterile tubes and diluted in deionized water at a ratio of 1:1 v/v). The extracted, filtered samples were cleaned by add deionized water. Diluted filtrates samples were run in ELISA in triplicate, and placed in a microwell strip holder (ELISA microplate) to tested with the ELISA test kits as indicated by the manufacturer instructions. The optical density/absorbance value (OD) was measured at 450 nm by ELISA spectrophotometer. The amount of mycotoxins in the feeds samples were calculates automatically by Microwell Reader (NEOGEN ® Stat-Fax 4700, SKU No. 9303. USA).

Statistical analysis

Microsoft Excel was used for data manipulation. A two-way ANOVA was used to determine descriptive statistics, interaction, and generation of graphics using Graph Pad Prism 8.4.2 software. Results were presented as mean ± SD. A *P value* of <0.05 was used as a statistically significant difference.

RESULTS

The contamination level of mycotoxins in poultry feeds in Dhamar governorate are presented in Figure 1. As shown, four toxins were identified, namely, Aflatoxins, T-2 toxins, Ochratoxins, and Zeralenone. The levels of contamination with these mycotoxins were 0.37, 21.67, 0.8, and 14.04 ppb respectively.

Aflatoxins were present in 36.11% of the tested samples. The highest level of aflatoxins. contamination was in Ordinary Feed-I one and Ordinary feed-II, the mean aflatoxin level in parts per billion was highest was 1.00 and 1.07ppb respectively. Very low quantities of aflatoxins with mean range between 0.33 to 0.73ppb were also detected in Levantine corn, Soya bean, Final non-granular, Primitive concentrate, and Final concentrate. Other feed varieties, including Primitive granular, Final granular, Primitive local feed, White corn, and Primitive non-granular, do not contain any measurable quantities of aflatoxins.

T-2 toxin was present in 83.33% of the tested samples. The mean of T-2 toxin levels in the Primitive concentrate and Final concentrate were the highest, with mean values as 49.23 and 49.47ppb respectively. The feed samples labeled "Ordinary feed-I, "Primitive local feed", "Soya bean," "Final granular," and "Levantine corn" exhibited notable amounts of **T-2 toxin with mean value as** 19.1-22.5 ppb. The amounts **of T-2 toxin** in Ordinary feed-II (a grower mash feed II - Soybean, Corn, Broiler concentrate and Supplementation)**,** Primitive granular, Final non-granular, and Primitive non-granular are rather low, ranging between 10.67 to 15.20 ppb. however, white corn was not contaminated with T-2 toxins.

Ochratoxins were detected in 22.22% of the tested samples. Only Ordinary feed-I and Ordinary feed-II were exhibited low quantities of Ochratoxin, with mean concentration value as 3.47 and 4.9 ppb), respectively.

Zeralenone was detected in all the tested samples. The zearalenone contamination level in Ordinary feed-I (a grower mash feed. I - Soybean, Corn, Broiler concentrate and Supplementation) was the highest, with mean value as 19.87 ppb, whereas; notable amounts of zearalenone were detected in Primitive granular, Final granular, Primitive local feed, Soya bean, Final non-granular, and Primitive non-granular, with range values between 13.3 to 16.1ppb. Furthermore,

the results of this study revealed that, the samples of White corn, Primitive concentrate, Final concentrate, and Levantine corn exhibited the low amount of contamination with means ranging between 10.5 to 12.4 ppb.

The statistical analysis of the present study findings revealed notable differences in the levels of contamination among various feed types and different mycotoxins types as depicted in Table 2. The tested 12 feed types showed a significant difference in mycotoxin contamination (F value = 19.58 and P value < 0.0001). The type of feed accounts for 9.135% of the total variance. On the other hand, the type of the mycotoxin (n = 4) showed a significant difference in their distribution among feed types (F value = 467.0 and P value < 0.0001). The type of mycotoxin was the main factor affecting variance, where it accounts for 59.41% of the total variance. Additionally, there was a significant interaction between the types of feed and the types of mycotoxins (F value = 19.57 and P value < 0.0001), implying that the impact of feed type on the levels of mycotoxins contamination differs depending on the specific type of mycotoxins. The interaction accounts for 27.39% of the total variance.

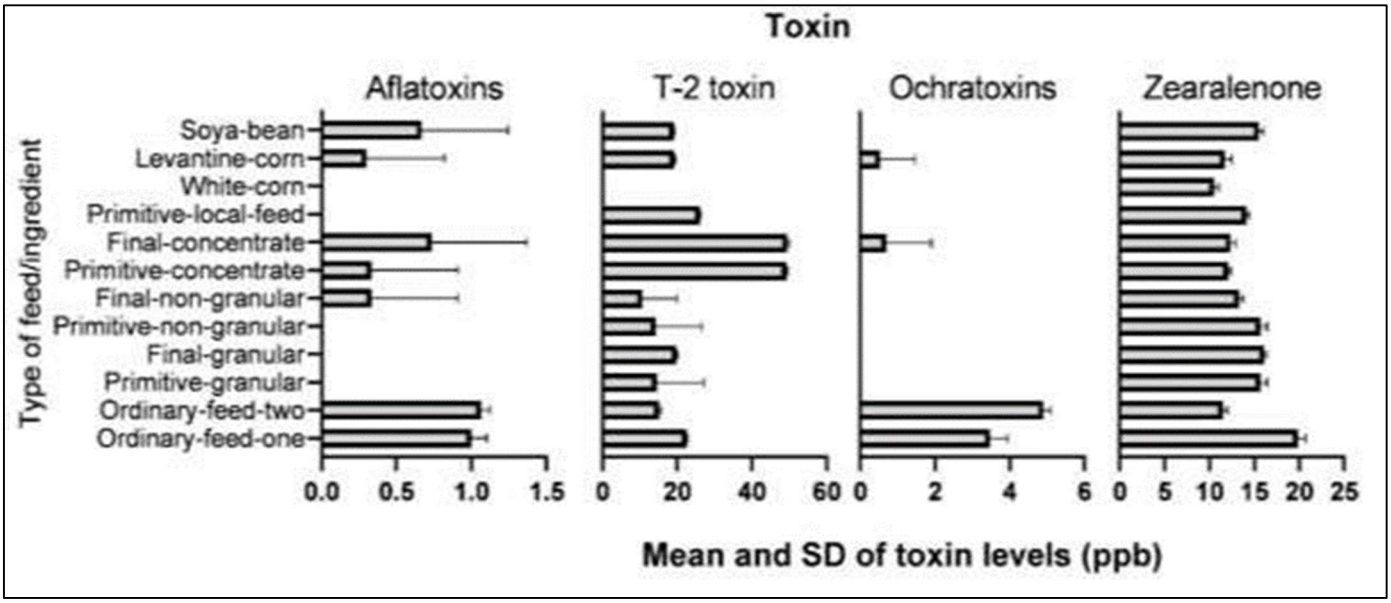

Figure 1. Contamination level (ppb) of mycotoxins in poultry feed and raw materials used on growing broilers in Dhamar governorate

Table 1. Type, Categories and composition of poultry feed tested in this study

NO.	Feed`s type	Categories	Cp(%) & Me(K/Kg)	Age of chicks
1	Primitive concentrate	A starter concentrate feed	CP 44,0 , ME 2230	1-3 weeks
2	Primitive local feed	A locally starter feed	CP 8.9, ME 3350	1-3 weeks
3	Primitive granular	A starter pelleted feed	CP 9.0, ME 3350	1-3 weeks
4	Primitive non granular	A starter mash feed	CP 22.8, ME 3150	1-3 weeks
5	Final non granular	A grower mash feed.	CP 18.3, ME3250	3-6 weeks
6	Ordinary feed -I	A grower mash feed. I	CP 23.0, ME 3180	3-6 weeks
7	Ordinary feed -II	A grower mash feed. II	CP 18.5, ME3260	3-6 weeks
8	Final granular	A finisher pelleted feed	CP 23.2, ME 3230	6wk upwards
9	Final concentrate	A finisher concentrate feed	CP 17.9, ME 3155	6wk upwards
10	Levantine corn	Feed raw material	CP 23.4, ME 3320	All ages
11	White corn	Feed raw material	CP 20.2, ME 3205	All ages
12	Soy-bean	Feed raw material	CP 20.0, ME 3200	All ages

CP = crude protein (%), ME = metabolizable energy (K Calorie/Kg), Wk= Week

Table 2. Results of the two-way ANOVA analysis on contamination level of Mycotoxins in different Poultry Feeds tested

Sources of variation	SS	DF	MS	F (DFn, DFd)	P value	Attributed Variance %
Interaction	5435	33	164.7	F (33, 96) = 19.57	P<0.0001	27.39%
Type of feed	1813	11	164.8	F (11, 96) = 19.58	P<0.0001	9.135%
Type of mycotoxin	11790	3	3930	F (3, 96) = 467.0	P<0.0001	59.41%
Residual	807.9	96	8.416			

SS= Sum of Square DF= Degree of Freedom MS= Mean Squares F= F value

DISCUSSION

The poultry sector is a vital sector in the Yemeni economy (UNDP, 2020), and it has experienced challenges in the past few years, including broiler poultry in Dhamar Governorate. The country's annual poultry production (live birds) in the year 2022 dropped by 1.91% compared to the year 2021 (FAOSTAT, 2024). Non-compliance with vaccination programs and inappropriate storage of poultry feeds were accused for exposure to infections and mycotoxins (Anonymous, 2022).

In this study, the contamination level of feed samples with aflatoxins and T-2 toxins were as follows: (0.37 and 21.67 ppb), respectively. The levels were below than the levels established by the safe feeding levels for mycotoxins in poultry feed, the recommendations of European Community concerning complete feeding stuffs and European Union regulatory levels (a) and established guidelines (b) on mycotoxins in feed stuffs for broilers i.e 20 and 250 ppb (ECCR, 2006; ECCR, 2013). The mean concentration levels of contamination of feed samples with Ochratoxins was as 0.8 ppb; this level also was below than level established by the safe feeding levels for mycotoxins in poultry feed, the recommendations of Food and Agriculture Organization, the acceptable recommended level is 100 ppb (FAO, 2004). Moreover, the results of this study also displayed that, the mean concentration of zearalenone in feed samples was 14.04 ppb, these findings are lower than level established by the safe feeding levels for mycotoxins in broilers and recommendations used by the OISC i.e. 500 ppb (MPFC, 2011; ECCR, 2006). The results of present study on aflatoxins levels in poultry feeds are in agreements with findings of previous reports studies in Dhamar governorate (Algabr et al., 2018).

The contamination of the food and feed ingredients with aflatoxins in current study was in line with several previous reports on aflatoxin contamination of cereals, nuts, legumes, oilseeds, and their products (Ezekiel et al., 2012; Ezekiel et al., 2013; Adetunji et al., 2014; Egbontan et al., 2017; Oyedele et al., 2017;). The most frequently found toxin in soybeans was aflatoxins, which are produced by *Aspergillus spp*. Similarly, High level of aflatoxins was reported from other countries, including Cameroon, India, Nigeria, South Africa and Cuba (Oluwafemi et al., 2009; Njobeh et al., 2012; Abia et al., 2013; Kehinde et al., 2014; Kotinagu et al., 2015; Ochieng et al., 2021). For example, the aflatoxins level in poultry feed reported from Nigeria and Cuba were 198 ppb and 5.0 ppb (Escobar and Regueiro, 2002) respectively.

In present study, the aflatoxins concentration in poultry feed was 0.37ppb, these findings are higher than that reported in broiler feed (0.104 ppb) from the Czech Republic (Mikula et al., 2020) and lower than findings reported from Pakistan i.e. 5 to 89.9 ppb by Fareed et al., (2014). The concentration of Ochratoxins was 0.8 ppb, these results are higher than (0.380 ppb) reported from the Czech Republic (Mikula et al., 2020) and lower than findings reported from Pakistan (22.5 to 85ppb) by Sherazi et al., (2015). In Serbia, Krnjaja et al., (2014) reported the Ochratoxins in chicken feeds as 34.4 ppb, and in laying hen feeds as 43.89 ppb. The concentrations of T-2 toxins and zearalenone were as 21.67 and 14.04 ppb respectively, these results are lower than findings of Mikula et al. (2020) who reported the level of

contamination as 40 and 343 ppb respectively in Czech Republic.

The discrepancy in mycotoxins contamination level in poultry feeds reported among above studies and present study could be attributed to several of factors such as: climatic factors, agricultural, processing practices (handling and storage) of raw materials and formulation utilized during the compounding of the feed (Gutleb et al., 2015). Moreover, many workers (CAST, 2003; Golob, 2007; Daghir, 2008; Waliyar et al., 2009; Köppen et al., 2010; Schmidt-Heydt et al., 2011; Rodriguez-Carrasco et al., 2013; Dzuman *et al.*, 2014; Pereira *et al.*, 2014; Xie *et al.*, 2016; Smith et al., 2016; Anfossi *et al.*, 2016; Marroquín-Cardona et al., 2014; Garbaba et al. 2018) suggested that, the occurrence of mycotoxins varies with seasons, poor storage (the main factor that may encourage fungal growth and mycotoxin production for both local and imported poultry feeds), environmental and weather factors such as temperature, rain, PH and analysis techniques or assay for determination of mycotoxins. Furthermore, It has been reported that poultry feed components, primarily imported to Yemen from abroad, that may could be carried mycotoxins contamination from their origin to country (Algabr et al., 2018).

CONCLUSIONS

The present results showed that all feed types and raw ingredients tested contained relatively low levels, and there were differences in the level and types of mycotoxins between the feed manufacturing companies, with the exception of zearalenone, which is found in all types of feed and exceeds the permitted limit. The co-occurrence of multiple mycotoxins may enhance overall toxicity due to synergistic effects, reduce profitability for farmers, and possibly affect final consumers. There is a need to minimize mycotoxin contamination in broiler feeds, these may be adapted through the proper storage of feeds and ingredients, regular monitoring of mycotoxins in poultry feeds, implementing good agricultural practices, and exploring less contaminated crop alternatives.

ACKNOWLEDGMENTS

The authors are grateful to owners of poultry feed factories and poultry farms in Dhamar Governorate for their assistance extended to us during collection of samples. The authors would also like to express their gratitude to Al-Sanabani Agriculture and Poultry Company, Sana`a and all the workers especially Dr. Mohammed Dahim, the laboratory expert, for help offered during conducting laboratory tests.

CONTRIBUTION OF AUTHORS

HAG designed the study, conducted experiments, wrote the manuscript. Authors, AAA, FAB and SMAA, collection and contributed in testing of specimen and analysis of data. All authors have seen and approved this version of the manuscript.

FUNDING

The authors received no direct funding for this research.

COMPETING INTERESTS

The authors declare no competing interests.

DATA AVAILABILITY

All data generated and analyzed during this study are included in this published article.

ETHICS CONSIDERATIONs &Approval

The study was approved by Faculty of Veterinary Medicine, Thamar University, Yemen.

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تحديد مدى تلوث أعلاف الدواجن بالسموم الفطرية في محافظة ذمار، اليمن

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الملخص

يعد تلوث علاف الدواجن بالسموم الفطرية مصدر قلق عالمي للصحة العامة، إذا إنه يتسبب في خسائر اقتصادية كبيرة في صناعة الدواجن، ويشكل مخاطر كبيرة على صحة الإنسان. هدفت الدراسة الحالية إلى تحديد مستويات التلوث بالسموم الفطرية في الأعلاف المصنعة والمواد الخام المستخدمة في تربية الدجاج اللاحم في محافظة ذمار، اليمن. تم جمع ما مجموعه 36 عينة عشوائيا من أنواع مختلفة من أعلاف الدواجن المستخدمة في مزارع الدواجن في محافظة ذمار. تم استخدام الميثانول كمذيب عضوي لاستخلاص السموم الفطرية من عينات الأعلاف المستهدفة في الدراسة. تم استخدام اختبارات وتقنية **ELIZA**للكشف عن مستويات السموم الفطرية العلائق/ الاعلاف المستهدفة. تم التعرف على اربعة سموم فطرية هي: Aflatoxins, T-2 toxins, Ochratoxins A, and Zeralenone بنسبة36.11 ٪ و 83.33 ٪ و 22.22 ٪ و 100 ٪ من العينات المفحوصة، وكانت متوسطات مستويات التلوث بالسموم المذكورة هي: 0.37 و 21.67 و 0.8 و 14.04 جزء في البليون على التوالي **.** كشفت النتائج أيضا ان أعلى مستويات aflatoxins and Ochratoxins كانت في العلف Ordinary feed-I (1.00 و3.47 جزء في البليون) والأعلاف Ordinary feed-II (1.07 و 4.9 جزء في البليون) على التوالي .وبالمثل، كانت عن أعلى مستوى Zearalenone في العليقة Ordinary feed-I (19.87 جزء في البليون) . كما وأوضحت النتائج ان أعلى مستويات T-2 toxin في العليقة المركزة البادئة 49.23(جزء في البليون) و العليقة المركزة النهائية (49.47 جزء في البليون) . بينما كانت مستويات التلوث أقل نسبيا في أنواع الأعلاف الأخرى التي تم اختبارها .أظهر التحليل الإحصائي ان هناك فروقا معنوية ذات دلالة احصائية (P< 0.05) بين متوسطات و مستويات السموم الفطرية في أنواع الأعلاف التي تم دراستها. كانت الأعلاف العادية أكثر تلوثا مقارنة بأعلاف ذات المكونات الخامة. سلطت النتائج هذه الدراسة الضوء على التهديد الذي تشكله السموم الفطرية على الدواجن والصحة العامة في محافظة ذمار واكشفت عن ضرورة اتخاذ التدابير الخاصة للحد من هذه المخاطر .كما واوصت بأجراء المزيد من الدراسات لتحديد العوامل المرتبطة بتلوث السموم الفطرية في أعلاف الدواجن.

الكلمات المفتاحية: أعلاف الدواجن، السموم الفطرية، الإليزا، تحديد، محافظة ذمار، اليمن.

To cite this article: Golah AA, Al-Kubati AA, Badi FA. AlMaswari SMA. 2024. Determination of Mycotoxins Level in Poultry Feeds in Dhamar Governorate, Yemen. Yemeni Journal of Agriculture and Veterinary Sciences; 5(2): 1-11.