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Assessment of Aflatoxin level in Commercial Layer feed in Khartoum State and the Farmers' awareness of Aflatoxin negative Effects

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Abstract

A study was conducted during the period July – Nov. 2018 to assess aflatoxin contamination in commercial layer feed and farmer knowledge on aflatoxin hazards. Feed was collected from 25farms (4 companies and 21 farmers) in Khartoum State and from the same farms data were collected using questionnaire as research tool. The main findings of questionnaire showed that 72% of the farmers studied were solely specialized in poultry of whom 84% in table egg production .Most of them used improved housing conditions, raised more than 4000 birds per flock in all in –all out system, used antiseptics and stored feed for one week or less .On aflatoxin knowledge most of farms managers were veterinarian or animal Production graduates, who had some information on aflatoxin hazards . Dose calibration conducted by veterinarians (56%) but a total of (56%) do not inspect feed for aflatoxin contamination attributed to use of anti aflatoxin and high cost .The average of aflatoxin feed content was 2.6 ppb for Kh. State in a range up to 14ppb . Mean aflatoxin content was 1.76 ppb for Kh. Locality, 4.66 ppb for Kh. North State .and 1.26ppb for Omdurman. Over all level was less than 20 ppb within the accepted safety standard and was the lowest among the previous studies findings.

Keywords: Mycotoxins, contamination, hazard,- absorption

Introduction

Presently aflatoxin has been one of the most important global concern regarding Contamination of food products (Selim , 2010) .Aflatoxins are major concern to the poultry industry because of serious economic losses it causes (Oguz, 2012, Bryden,2012). Groundnut meal is used commercially as the main source of protein for poultry in Sudan, it has anti nutritional properties and highly susceptible for aflatoxin contamination (Ali et al., 2011). Its cultivation is mostly confined to the tropical, subtropical, and warm temperate (zones) countries (F.A.O.2006). Aflatoxins are the major mycotoxins that are most commonly associated with groundnuts (Dohlman, 2003). Ground nut cake infested with Aspergillus sp., which will produce aflatoxins under favorable conditions (Adebesin et al.2004).

As general rule growing poultry should not receive more than 20 ppb (parts per billions) as it may reduce their resistance to disease, the ability to with stand stress and bruising and generally makes them unthrifty. laying hens can generally tolerate higher level than young birds , but the level should still be less than 50ppb (Jones et al 1994) Favorable condition for Aflatoxin growth are 24-35℃ and 75% humidity (Willams et al .,2004) .Bad storage conditions particularly humidity

and temperature for feed and feed ingredients resulted in absorption of Aspergillus infection and aflatoxin production (Hell et al.,2003).

Aflatoxin affects human health and the total number of people exposed to uncontrolled aflatoxin every year is very high and is calculated to be around five billion in all over the world (Strosnider *et al.,* 2006). Aflatoxins are highly toxic substances and mainly target the liver and kidneys (Alpers *et al.,* 2002) and are also linked to immune suppression (Turner *et al.,* 2005).

As for poultry the industry suffers great economic losses due to greater susceptibility of poultry compared with other animals to the toxin apart from continuing intermittent occurrences in feeds (Thapa, 2008).

Aflatoxin consumption in layer hens is associated with reduction in egg production, reduction egg yolk weight, change in yolk colour, reduced in shell quality (Rosmaninho et al., 2001).

The objective of this study is to assess level of contamination by aflatoxin in commercial layer feed in Khartoum State and producer's awareness on aflatoxin hazards.

Materials and methods

This study was conducted in Khartoum State to assess level of aflatoxin contamination of commercial layer feed on the farm level and to assess the level of information of producers on aflatoxin. From the 78 operating farms (Ministry of Agriculture and Animal Resource , record ,2016) comprising 32 in Khartoum Locality, 29 in Khartoum North and 17 in Omdurman were used for the study .A sample of 30% from each Locality was randomly selected using the paper ballot system .As such ten farms were selected from Khartoum (Kh) ,10 from Khartoum North (Kh .N) and 5 from Omdurman (0md.) .From each farm , of the sample, one kg of commercial layer feed was randomly collected from 5 randomly selected bags during period July – November, (2018).Samples were kept at -20℃ (in the Sudan Standards Metrology Organization SSMO)Laboratories before the analysis .

Samples analysis procedure

Afla test was used for analysis of the samples, in the Sudanese Standard Metrology Organization laboratories in Khartoum. Afla Test from VICAM is the only Aflatoxin test that produces precise numerical results The samples were ground ,50 grams of ground sample with 5 gram of Sodium Chloride (Na Cl) were placed in blender jar, 100 ml of methanol: water (80:20) was added to the jar ,it was covered and blended in high speed for one minute. The cover was removed from the jar ,the extract was poured into fluted filter paper to separate the sample extract solution from the coarse particulate sample solid and the filtrate was collected in a clean container .The second filtration step was gravity filtration of the extract through microfiber filter. This removed any precipitates in the extract and assures that the extract would pass easily through the affinity column Micro Filtration was performed just prior to affinity chromatography ,a small funnel was placed in the top outlet of syringe barrel ,microfiber was placed gently into small funnel by pressing the filter into funnel with index finger .ten ml of filtered extract was poured into a clean vessel then was diluted with 40 ml of purified water and mixed well.10 ml of filtered diluted extract was filtered through microfiber filter paper directly into glass syringe barrel .10 ml of filtered diluted extract was passed through AflaTest column(it bind with specific antibodies to aflatoxin at this stage ,the aflatoxin bound to the anti-body in the column) at rate of about 1 drop per second until come through the column. Then 10 ml of purified water was passed to rid immune affinity column of impurities and this was done twice through the column at rate of 1- 2drop per second until air come through the column . Glass cuvette was placed under the column and one ml of

HPLC grade methanol into glass syringe barrel. The column was eluted at a rate of one drop per second or slower by passing the methanol through the column then the sample was collected in the glass cuvette.1 ml of Afla test developer solution was added to the eluate in the cuvette. The eluate was then mixed well and was placed in calibrated FLuoro meter. The aflatoxin concentration was read after 60 seconds .The tests were done at 26.4℃ average temperature and 46.9 average humidity.

Collected data were subjected to analysis of variance (ANOVA) and the Least Significant Difference (LSD) test used to assess the significant differences among dietary treatments means. Statistical analysis was carried out according to Snedecor and Cochran (1980). For the knowledge assessment, a pre –tested questionnaire was structured and designed using the same farm sample. Data and information was collected on management, biosecurity ,anti aflatoxin use and effect of aflatoxin on human health . The data collected was tabulated and analyzed by simple percentage.

Results and Discussion

On personal characteristics the study showed that the majority of farm owners (68%) were above 50 years of age and (76%) university graduates which indicates better understanding and knowledge on poultry which demand patience ,endurance and knowledge follow .up. On farm specialization (72%) solely specialized in poultry and of whom (84%) in table egg production .Closed and semi –closed housing system were predominate for better flock management as was stated by (Askora et al ., 2016). A total of (68%) raised more than 4000 birds in one flock and of whom (64%) went for all in –all out practice. Among the commercial hybrids raised Hisex and hyline were rated as most adapted to the Sudan conditions though no adaptability studies were made in the Sudan. Results agree with (Sirdar et al,2012), (Alwali, 2015).Most farm managers were veterinarians(44%) followed by Animal Production College graduates (40%) who reflects positively effect on farm management and most farms kept financial technical and health records at (56%) rate .Most farms stored feed for one week or less which reduces possibility of feed contamination .For biosecurity measures (92%) were fenced farms, (76%) used antiseptics on gates ,farm units more than 100 meters apart and distance more than 500meters between farms which agrees with Sirdar et al,(2012),Osman (2008).As for aflatoxin status as many as (80%)of the producers indicated no source of information as is shown in table (1) following

Table (1) Source of information on aflatoxin

	Frequency	Percent (%)
Locality	3	12
Relevant ministries	2	8
Not found	20	80
Total	25	100

Also 80% stated no extension work about anti Aflatoxin field wise.

In spite of that (80%) of the farm owners stated knowledge on the harmful effects of Aflatoxin on both human health and poultry industry. Again in spite of that (56%) of the farm owners did not inspect poultry feed and/ or feed ingredient for Aflatoxin contamination .Reasons given were that most farm owners (80%) used mycotoxin binders in feed and (20%) in water, the high cost of testing and absence of both extension and inspecting authorities an example of which was that (88%) of the farm owners did not receive any information from (SSMO).

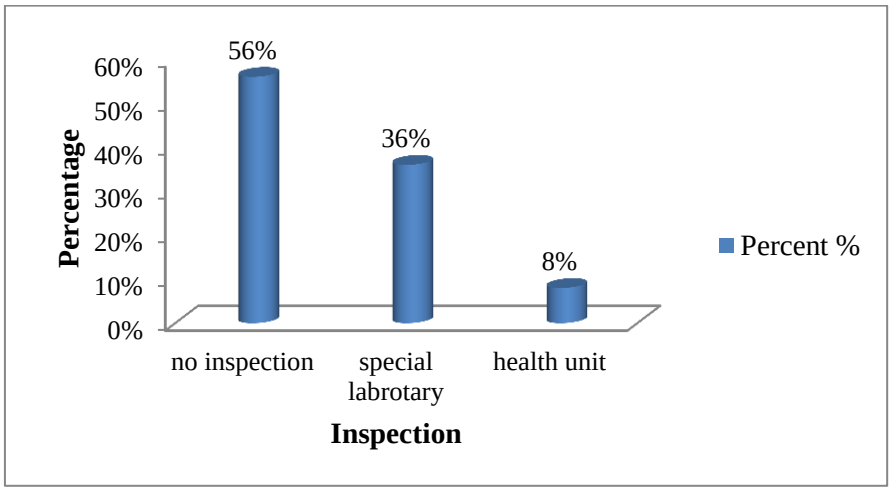

Figure (1): Source of inspection of Aflatoxin

56% of respondents did not inspect feed for aflatoxin

For dose calibration **(Fig. 2)** shows that it was by veterinarians (56%) and others at (44%) of non-medically specialties which poses a safety margin question

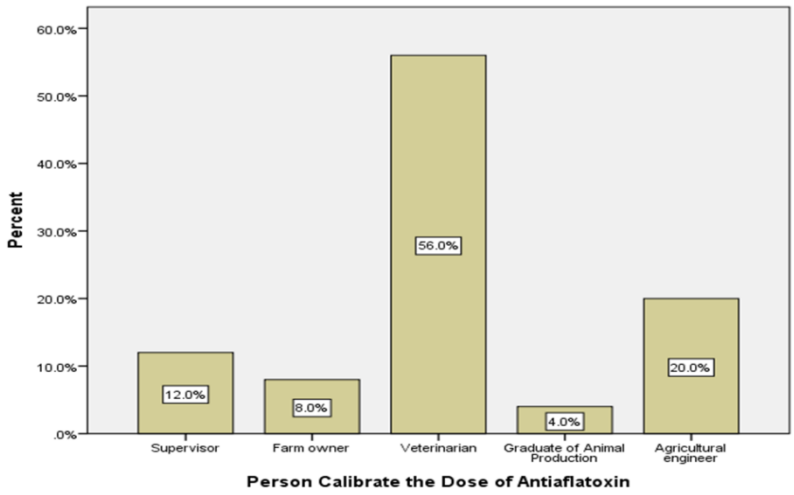

Figure (2): Calibration of aflatoxin dose

44 % of persons calibrated the dose of anti aflatoxin in farms of layers were not veterinarians.

The study results showed that from the samples tested that the range was up to 14 ppb for Khartoum State with average of 2.6ppb . The averages for each locality was 1.76 , 4.66 and 1.26 ppb for each of Kh ,Kh .N and Omd. Localities respectively .Kh .N. had highest average and range point which agrees with Elamin *et al*, (1988).

Table (2) Analysis of variance (ANOVA) for Aflatoxin contamination in Kh State

	Sum of Square	D F	Mean Square	F	Sig
Between Groups	58.99o	2	29.495	3.314	.055
Within Groups	195.792	22	8.900		
Total	254.782	24			

The mean difference in aflatoxin test results between the three localities showed no significance at ($p \leq 0.05$).

Table (3) Least Significant Difference (LSD)test for aflatoxin contamination among the Localities

(I)Locality	(J)Locality	Mean Difference (I - J)	Sig
Khartoum North	Khartoum	2.8	0.045
Khartoum North	Omdurman	3.6	0.039
Khartoum	Omdurman	0.7	0.655

Results showed significant difference between Kh .N .and Kh and between Kh. Average of aflatoxin results of 0.0 and 3ppb were reported in Northern and Southern and central Europe between 2009 -2011 in finished poultry feed (Rodrigues et al. ,2011) .The average of aflatoxin in layer feed in Kh . State. was 2.6 ppb while it was 6.6 in Cameroon ,(Jean et al .,(2013).The range of aflatoxin in this study was 0.0 - 14 ppb while Zein, et al.,((2019) reported 7.6 - 18 ppb for the State .Mursal (2009) reported results as high as 10 -97 ppb while Elzupir,et al, (2009) reported 54.4 -579.9 ppb. Zain, ,(2011) in a study of evaluation of poultry feed quality in Kh .S. using six feed samples reported 4 above 20ppb and 2 below (10 -19 ppm).They attributed the variation to type of ground nut and sorghum used . The study concludes that the rate of aflatoxin contamination, for Kh .S. lies within the standard limit of 20ppb which may be attributed to short storage periods , quality of raw materials and probably improved management condit-ions. As for knowledge on aflatoxin health hazards farmers need more information and more extension and authorities follow –up. The study recommended more investigation on the causes of differences between the Localities specially Kh. North.

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تقييم نسب الأفلاتوكسين في العلف التجاري للدجاج البياض ومدى وعي الامنتجين بالأثار السالب للأفلاتوكسين في ولاية الخرطوم

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المستخلص

تمت الدراسة في الفترة يوليو - نوفمبر 2018 لتحديد نسبة التلوث بالأفلاتوكسين في علف الدجاج البياض التجاري بولاية الخرطوم و تقييم مدى المام المربين بمخاطر الافلاتوكسين و أظهرت النتائج أن 72% من المزارع قيد البحث كانت لتربية الدجاج فقط ومنها 84% دجاج بياض. معظم المربين اتجهولنظام المساكن الحديثة حيث ويربى اكثر من 4000 طائر في القطيع الواحد بنظام ادخال الكل وإخراج الكل ويستعملون المطهرات ويخزنون العلف لفترة أسبوع أوأقل وفي جانب المعرفة عن مضار الافلاتوكسين واستعمال مضادات السموم الفطرية معظم مديري المزارع كانوا من الاطباءالبيطريين او خريجي كليات النتاج الحيواني ولهم المام جيد بهذا الامر وبالنسبة لتحديد جرعة مضادات الافلاتوكسين نجد أن معظم الكوادر من الأطباءالبيطريين(56%) الا ان نسبة 56% من المربين لا يقومون بفحص وتحديد كميات الافلاتوكسين في العلف لاستعمال مضادات الافلاتوكسين ولارتفاع تكلفة الفحص . متوسط احتواء الاعلاف من الافلاتوكسين كان 2.6 جزء من البليون لولاية الخرطوم في مدى تروح الي 14 جزء من البليون اما المتوسط في المحليات كان 1.76 جزء من البليون للخرطوم و4.66 للخرطوم بحري و1.26 لام درمان والمستوى الكلي كان اقل من 20 جزء من البليون الرقم المقبول للامان وكان متوسط الدراسة الاقل بين نتائج الدراسات السابقة.