Sudan University of Science and Technology

College of Graduate Studies

Assessment of Aflatoxin Level in Commercial Layers Feed and Producers Awareness on its Negative Effects in Khartoum State

تقييم مستوى الأ فلاتوكسين في العلف التجاري للدجاج البياض ومدى وعي المنتجين بالأثار السالبة للافلاتوكسين في ولاية الخرطوم

A Thesis Submitted for Fulfillment of the Requirements for Master Degree in Animal Production

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(2021)

DEDICATION

To the soul of my father , to dear mother , to the beloved husband ,to siblings, to my dear children Tartiel ,Ahmed , Tibian and Tanziel ,this work is dedicated .

ACKNOWLEDGEMENT

I thank Allah Almighty before and after. I wish to express my sincere gratitude to my supervisor professor Osama Elsheikh Yassin for his patience ,guidance and encouragement through the study .

Thanks to Dr. Bader H.Eljack for his encouragement and assistance all through .

Thanks also are expressed to the staff of the Sudanese Standard Metrology Organization Laboratories in Khartoum specially Dr . Noha .

Acknowledgement is expressed to the Ministry of Animal Resource for providing financial assistance .

Thanks for analysis of data carried by Dr .Amani H. Dho Albat

Thanks to Adil A. Ali and Adil Alabeed for their help in collecting the samples

Thanks to Engineer Ezzeldin Abdelrahman for his assistance.

Acknowledgement is expressed to Detaasi Laboratory .

Abstract

A study was conducted during the period July –November 2018 to assess the level of contamination of commercial layer feed by aflatoxin in Khartoum State and also to study the awareness of the producers on aflatoxin hazards on the poultry industry and human health .25 commercial layer's feed samples(10 from Khartoum locality poultry farms , 10 from Khartoum North and 5 from Omdurman) were randomly selected and used in the study .A questionnaire was also designed to collect information on management, biosecurity and level of awareness of the producers on aflatoxin and its negative impact on poultry industry and human health .Data of the feed samples were statistically analyzed by ANOVA and LSD Test while the data of the farm management was done by simple percentage . The main findings, most of the producers were specialized in poultry solely and 84% of them just in table eggs production .The majority used closed housing system , 68% raised over 4000 birds in all-in –all -out production system . 44% of the farm managers were veterinarians and 40% Animal Production specialist and had well knowledge on the effect of aflatoxin contamination on poultry production and human health . The majority of producer kept production and health records. 64% from producers stored feed for one week. On biosecurity side most farms were fenced , use gate antiseptics and keep acceptable distances between farms. 80% of producers studied added anti-aflatoxin in the feed while 20% in drinking water. For the aflatoxin calibration (56%) was done by veterinarians . 80% of producers have lack of information source while (56%) did not check feed for aflatoxin contamination due to high cost of the analysis test. determination of aflatoxin level was carried out using (Afla Test) in Sudanese Standard Metrology Organization (SSMO)Laboratories in Khartoum. The result showed that, the aflatoxin level was

1.76PPb for Khartoum ,4.66PPb for Khartoum North and 1.26PPb for Omdurman .The mean aggregate of aflatoxin level in Khartoum State was found to be 2.6PPb.The result showed significant difference($P \leq 0.05$) between ,Khartoum North and Khartoum and Khartoum North and Omdurman while no significant difference observed between Khartoum and Omdurman .The study concluded that the level of aflatoxin in commercial layer's feed is less than 20PPb which is safe margin for layers in Khartoum State as out lined by SSMO in (2015).

الملخص

تمت الدراسة في الفترة يوليو –نوفمبر 2018 لتقييم نسبة تلوث علف دجاج البيض التجاري بالافلاتوكسين في ولاية الخرطوم و لتقييم المستوى مدى معرفة المربين بالاثار السالبة للافلاتوكسين على الدواجن وتم اختبار 25مزرعة عشوائياً(10مزارع من محلية الخرطوم و10 من محلية بحري و5 من محلية ام درمان) وتم جمع عينات العلف وتصميم استبيان مجرب لجمع المعلومات عن الادارة والامن الحيوي والمستوى المعرفي لمخاطر الافلاتوكسين على صناعة الدواجن وصحة الانسان وتم تحليل المعلومات للعينات العلفية بطريقة تحليل التباين واختبار المقارنات لاستخدام اختبار اقل فرق معنوي (LSD) .وتم تحليل المعلومات عن الادارة بالنسب المئوية البسيطة وكانت اهم النتائج التي توصلت الها الدراسة ان معظم المنتجين يعملون في مجال الدواجن التخصصي ومنهم 84% في انتاج بيض المائدة فقط و يلجأون للمساكن المغلقة وان 68% يربون اكثر من 4000 طائر في الدفعة الواحدة بنظام(ادخل الكل واخراج الكل) ومعظم مديري المزارع اطباء بيطريين 44% و40 من اختصاصي كليات الانتاج الحيواني ولديهم معرفة بالاثار السالبة للافلاتوكسين على انتاج الدواجن وصحة الانسان ومعظم المنتجين يحتفظون بسجلات انتاجية وصحية ويخزنون العلف لفترة اسبوع او اقل وفي جانب الامن الحيوي 92%الوحدات المدروسة مسورة ويستعملون المطهرات في الابواب ويحتفظون لمسافة كافية بين المزارع و80% من المنتجين يستعملون مضادات الافلاتوكسين في العلف و20%يتم استخدامه في الماء ويتم تحديد جرعات الافلاتوكسين بواسطة اطباء بيطريين بنسبة 56%ورغم أن المنتجين أوضحوا معرفتهم عن بالافلاتوكسين إلان 80% ليس لديهم مصادر للمعرفة ونسبة 56% لايفحصون أو يتابعون نسبة الافلاتوكسين في العلف وعزي بعضهم ذلك لاستعمال مضادات الافلاتوكسين والتكلفة العالية للفحص.يتم تحديد مستوى الافلاتوكسين في العلف بواسطة

(AflaTest)في معامل الهيئة السودانية للمواصفات والمقاييس .اظهرت النتائج أن مستوى الافلاتوكسين في المتوسط في ولاية الخرطوم PPb 2.6.وكان المتوسط للمحليات 1.76 محلية الخرطوم4.66 الخرطوم بحري و1.26PPb في محلية امدرمان واشار التحليل الاحصائي الي وجود فروقات معنوية ($P\leq0.05$) بين الخرطوم والخرطوم بحري وبين الخرطوم بحري وامدرمان الا انه اشار الي عدم وجود فروقات معنوية بين الخرطوم وامدرمان وختم البحث ان مستوى الافلاتوكسين في علف الدجاج البيض التجاري بولاية الخرطوم اقل من20PPb وهو في حدود الهامش السلمي للدواجن حسب ماهو وارد في المواصفات والمقاييس (عام 2015) .

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CHAPTER ONE

1.0 INTRODUCTION

Mycotoxins are secondary metabolites produced by several fungal species, toxic to humans, animals and plants. Their ingestion, inhalation or dermal absorption may cause different diseases and even death(Ramos et al ,2011).Aflatoxins (AF) are mycotoxins that are produced by various Aspergillus species including A. flavus, A.parasiticus and A. nominus. As secondary metabolites of these fungi,(Anjum,et al, 2012). AF may contaminate a variety of food and feedstuffs, especially corn, peanuts and cotton seed. Chemically, AF are difuranocoumarin compounds and include aflatoxin B1 , B2 , G1 , G2 , M1 and M2 depending on their structures. Aflatoxin M1 and M2 , however, mainly occur in milk (small quantities of M1 have been reported in eggs) as metabolites of B1 and B2 , respectively. Among the known AF, B1 is most commonly encountered and considered the most toxic (Yunus et al, 2011). These fungi are capable of growing and contaminating the grains and cereals at any time before and after harvesting, during storage, transportation and processing of feed ingredients and feeds formulation. The spores of the fungi remain dormant but when the level of moisture is more than 12 per cent with a temperature of 25-35°C, humidity of 80 per cent and adequate aeration initiate their growth. Mycotoxins have adverse effect on both health and productivity in almost all species of domestic animals including poultry. In general, mycotoxicosis results in reduced feed intake, diminished feed conversion, decrease in production and subsequently increased susceptibility to various infections depending upon the type of toxins ingested (Xue et al., 2010).

The incorporation of various ingredients of plant origin into poultry feed mixtures increases the risk of contamination by fungi and their toxic metabolites(Bryden,2012,Rodrigues,et al 2011). Aflatoxins are a major concern to poultry production and health because of the serious economic losses [Bryden, 2012,Oguz,.2012].

Study importance :

Aflatoxin has negative effects on human health and the poultry industry but few studies have been conducted in the Sudan to determine their maximum limits in raw feed material and processed feed and the awareness of farmers on the negative effects of aflatoxins.

Research problem :

Many cases of aflatoxicosis were reported in the Sudan, so mycotoxin accumulation in poultry feed and the hazardous effect on human and poultry health should be investigated to stick to the acceptable standards stated by SSMO.

Research Objectives:

Main Objective:

The main objective of this research is to assess aflatoxin level in poultry feed and to determine the level of contamination of poultry feed in Khartoum State.

Specific objectives:

- To compare the levels of aflatoxin in layers feed with the Sudanese and international standards.
- Provide information for extension agents to advise producers to avoid the negative effects of mycotoxin.

- To assess the knowledge level of mycotoxins and specially aflatoxin by the producers ..

Study justifications :-

- Aflatoxin is a major contaminant and feed hazard in poultry and Poultry farmers do not periodically assess aflatoxin level in their feed .
- Many farm owners lack knowledge on aflatoxin as an animal and human hazard .
- Authorities do not follow aflatoxin level in poultry feed and no regulations or legislations are implemented.

Study hypothesis:

Layers feed in Khartoum State are contaminated by aflatoxins and farmers have no information's about the negative effect of aflatoxins.

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CHAPTER TWO

2:0 LITERATURE REVIEW

2.1 AFLATOXINS :-

There are about 200 species of moulds in the world of which, 16 species of Aspergillus are dangerous to humans by causing disease and infection (Dagenais and Keller 2009). Aspergillus species are worldwide distributed probably because they produce numerous airborne conidia which easily spread by air movements and insects (Hedayati et al 2007). Aflatoxin producing fungi utilize the nutrients present in the ingredients for their metabolism and propagation, and thereby reduce the nutritional quality of the feed ingredients (Akande et al., 2006).

Recently aflatoxin has been one of the most important global concerns regarding contamination of food products [Selim.2010]. There are four major aflatoxins, namely B1, B2, G1, and G2. B1 usually being the aflatoxin of the highest concentration in contaminating feed and food. Aflatoxins are a major concern to poultry production and health because of their serious economic losses [Bryden,2012 ,Oguz,2012]. Aflatoxin B1, is the most potent natural carcinogenic known [Bryden,2007] and may pass to poultry products, such as meat or eggs at very low levels [Bryden, 2012]. Aflatoxins are liver toxins and especially B1, were recognized as inhibitors of nucleic acid and protein synthesis in animals, B1 was identified to be the most toxic and most prevalent compound, followed by G1, B2 and G2 with decreasing toxicity (Murphy et al., 2006).(beside affecting the health of birds and consumers, aflatoxins also cause economic losses in poultry industry.) The Council for Agriculture Science and Technology recorded an annual crop losses of $932 million due to mycotoxin contamination and additional losses of

$466 million in efforts to reduce contamination (Richard and Payne, 2003). The Food and Agricultural Organization (FAO,2004) estimated that 25% of the world's crops are affected by mycotoxins, of which the most notorious were aflatoxins. Aflatoxin losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency (Vincelli et al., 1995)). 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).

2.2.Favorable condition for Fungal growth :-

. Fungi need 24 to 35°C temperature and humidity over 75% for growing and aflatoxin production (Williams et al., 2004) Food storage without suitable condition and humidity has caused absorption of Aspergillous infection and aflatoxin production (Hell et al., 20003; Turner et al., 2005). It is noted that mycotoxins cause more harm and have serious effects on humans due to which it is emphasized in many countries to ascertain maximum tolerated level (MTL) regarding mycotoxins toxicity in foods and feeds (FAO, 2004). Aflatoxins are frequently found in foods grown and manufactured in Africa because of various parameters including excessive heat, high humidity, lack of aeration in the stores, and insect and rodent damage resulting in the proliferation and spread of fungal spores. Thus strategies to minimize quantitative and qualitative postharvest losses have been developed (Groopman and Kensler, 2008). when the soil humidity is below the normal level before harvest, it increases the number of A.flavus spores in the air resulting in more fungal infection, and thus high level of aflatoxin accumulation. During end processing and packaging, storage of animal-derived food, "cold chain" transfer or pollution of the packing material could also lead to the A. flavus infection and aflatoxin contamination (Duan et al., 2009). Aflatoxins are abundantly found in hot and humid countries. many favorable conditions prolonged drying period, high temperature, stress, and drought conditions are some of the highly favorable conditions. (Firdousa and Ejaz, 2012).Aflatoxin contamination has also shown seasonal variation . the highest contamination being detected in Summer where aflatoxin was detected in(78.95%)of samples followed by Autumn (66.67%) , while in Winter the least contamination prevailed (47.3%) This is consistent with view that the production of aflatoxin increase when climatic condition such as high temperature and high relative humidity prevail (Elzupir ,et .al ,2009)

2.3.Effects of Aflatoxin on humans health :-

The total number of people exposed to uncontrolled aflatoxins each 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 which mainly targets liver and kidney causing toxicity and carcinogenicity (Ayub and Sachan, 1997). Moreover, aflatoxins have been linked to the immune suppression (Turner et al., 2005). The presence of aflatoxins in egg is a potential threat to the health of the consumer. growing children are more sensitive than adults, as egg is one of their main sources of nutrients(International Agency for Research on Cancer .IARC, , 1993). Aflatoxicosis is primarily a hepatic disease: as a result, its main target organ in humans and other mammals is the liver. (Alpers et al. 2002). After a person eat aflatoxin contaminated food, it may cause fever, abdominal pain, vomiting, more seriously splenohepatomegalia, hepatalgia, skin mucous membrane stained yellow, ascites, edema of lower limbs and dysfunction of liver after 2~3 weeks, the cardiac dilatation, pulmonary edema, coma, spasm may also occur (Xiao and Xing, 2003).

2.4.Effect of Aflatoxins on Layers performance:-

Poultry industry suffers greater economic losses due to the greater susceptibility of the species in comparison with other animals to the toxin apart from continuing intermittent occurrences in feeds (Thapa, 2008). When chicken is fed with aflatoxin contaminated feed, the liver, kidneys, immune system and thus the performance of birds will be affected. Aflatoxin toxicity is related to biochemistry, hematology, reproduction and pathological changes (Ortatatli and Oguz, 2001). Good quality of poultry feed plays the most important role in the poultry production as its share is 70%. quality feed and resistant strain of chicks can lead to greater production and more profit for the poultry farmer. It is suggested that use of chicks resistant to aflatoxicosis will help in minimizing problem of poor growth rate and poor feed conversion ,which perhaps are the two most important factors in poultry production (Dhanasekaran, et ,al (2009) .In the Sultanate of Oman(Tchana et al.2010) reported the presence of Aflatoxin in eggs collected from a poultry farm .Aflatoxins influence the metabolism of poultry, reducing the activity of enzymes that digest starch, proteins, lipids, and nucleic acids, decrease blood protein, total cholesterol, and urea, and increase the activity of serum enzymes that indicate liver damage (Aravind et al., 2003). The main manifestations of chronic aflatoxicosis in layers are reduced egg production and weight, and increase in liver fat levels (Rosmaninho et al., 2001). The economic loss in the poultry industry due to aflatoxicosis is estimated to run to millions of dollars (Raju et al., 2005)In laying hens, aflatoxin consumption is associated with reduction in egg production, egg weight and yolk weight as well as changes in yolk colour, shell weight and shell integrity (Zaghini ,et ,al 2005).

Aflatoxin at a level of 20 ppm when fed for 7 days resulted in impaired egg production by reducing liver synthesis and transport of yolk precursors (Garlich et al., 1973) . Laying hens fed 1 ppm level of aflatoxin had significantly lower egg production whereas feed efficiency was adversely affected at 2 ppm level of aflatoxin (Iqbal et al., 1983). There was significant decrease in egg production and egg weight in laying hens fed a diet containing 3,310 μg of AFB1 and 1,680 μg AFB2 per kg for a period of 28 days by third and fourth week, respectively (Wolzak et al., 1985). The transfer of the toxin into meat and eggs is influenced by the toxin level as well as the period of exposure to toxin (Jacobson and Wiseman, 1974; Lotzsch et al., 1977). Iqbal et al. (1983).

2.5 Methods to reduce toxic effect of aflatoxins :-

Lots of antiseptic methods have been created to wash out food toxicant and reducing infectious-production toxic signs. These are biological, physical and chemical methods.

2.5.1Physical methods :- .

Aflatoxin concentration should be reduced by food drying for two days (Gowda et al. 2009). . The utilization of mycotoxin-binding adsorbent before applying this technique for routine use, is essential to establish that the adsorbent does not remove essential nutrients from the diet (Manafi., 2009).

Moreover, the use of surface absorbent such as bentonite and hydrated sodium calcium aluminosilicate in contaminated feed has proven to be effective in reducing the bioavailability of aflatoxin in animals . It has been shown that Calcium Montmorillonite is a safety absorbent for humans (Wang et al. 2005).

2.5.2 Chemical methods:-

Chemically, aflatoxins can be washed out with calcium hydroxide, mono methyl amine, ammonia and ozone. Among all chemicals, massive ammonia was used for cottonseed meal, peanut meal and sunflower. But the main forms of using chemical substances have fallen back to the risk of animal health (Galvano et al. 2001).

2.5.3 Biological methods :-

Flavoubacterium urantiatum can delete aflatoxin B1 from liquid medium and its application in the peanut production as biological parser. In recent years, lactic acid bacteria have been studied as an in vitro-field aflatoxin liquidator (Diarra et al. 2005). certain species and strains of yeasts have been observed to detoxify mycotoxins through its degradation (Cooney, 1980).

2.6 Permissible level of Aflatoxin :-

To control the presence of aflatoxins in foods, many countries established maximum tolerated concentrations through legislation (Moss. (1996). As a general rule, growing poultry should not receive more than 20 ppb aflatoxin in the diet. However, feeding levels lower than 20 ppb may still reduce their resistance to disease, decrease their ability to withstand stress and bruising and generally make them unthrifty. Laying hens generally can tolerate higher levels than young birds, but levels should still be less than 50 ppb (Jones et al., 1994). The current United States Food and Drugs Authority (FDA) administration action guideline is 20 µg/kg total aflatoxins in products intended for animal feeds (Schweitzer et al 2001) Aflatoxin contamination in feed may cause reduction of immune response in chicken, thus they become vulnerable to several diseases (Dhanasekaran et al., 2009). At the international level, the Codex Alimentarius Commission, through its committee on Food Additive and Contaminants and relevant commodity committees was considering the establishment of international guideline levels for various mycotoxins based on risk assessment performed by Joint FAO/WHO experts(Codex,2002). Many countries have passed legislation stating maximum tolerance levels for aflatoxins, which vary from 1–50 µg/kg (Van Egmond 1989).

2.7 Aflatoxins in Poultry Feed and Raw Feed Ingredients in Sudan:-

The groundnuts from central Sudan, irrigated region ,are free from aflatoxin . however, groundnuts from rain fed western region have variable level of aflatoxin contamination this because they are subjected to drought stress. Damage pods were highly contaminated with A.flavus and accumulated large amount of aflatoxin .intact pods have lower fungal contamination and almost free of aflatoxin. Groundnut products from Khartoum North market have higher aflatoxin than Khartoum and Omdurman . (Elamin et al .1988)Moderate level of aflatoxin were detected in peanut cake 7 -10 µg / Kg. the predominant types were B1 followed by AFG1 ,AFB2, AFG2 (Younis and Malak 2003).Elzupir et ,al.(2009) determined the aflatoxin in animal feed ration in both raw materials and manufactured rations in Khartoum State ,they reported that 64.29% of feed samples were contaminated with aflatoxin at average concentration of 130.63 ppb. The manufactured ration showed highest percent of aflatoxin (87.5 %) with concentration range (54.41 - 579.87 µg/kg). Mursal (2009)reported that feed samples from poultry farms in Khartoum State were found positive for aflatoxin which varied between 10 -97 ppb . Jean et ,al (2013) reported that the concentration of aflatoxin in layers feed average 6.6µg/kg) 2 to 23 µg/kg for layer feed. Rehab et al (2018) reported that aflatoxin concentration in layers feed in Khartoum state ranged (7.6 -18.3) ppb which was within the acceptable ranges stated by NRC (1994) and SSMO (2015). Salah Eldeen et, al (2012) reported that the percentage of aflatoxins contamination of animal feed was about 60% of the tested samples with a range of total traces (< 0.5) – 125 µg kg-1and a mean 19.8µg kg. .

CHAPTER THREE

3.0 MATERIALS and METHODS

3.1 Research area

The research area was Khartoum State which lies between longitudes 31,5 and 34.45 degree east and latitudes 15.8 and 16.45 north and is bordered by seven states. These are the Nile River Nile , Northern Kordufan ,Kassala ,Gedarif , Gezira and White Nile States .Most of these states located in the semi-desert climatic zone, while the northern parts are located in the desert zone. The climate is hot to warm and rainy in summer and warm to cold in winter. Temperatures range during summer between 25- 40 from April to June and 20 – 35 from July to October and 15 -25 between November to March.(Khartoum State government,2017)

3.2.Feed samples Collection and Analysis:-

According to the records of Ministry of Agriculture and Animal Resource and Irrigation-Khartoum State (2016) only 78 mixed poultry farms were operating in Khartoum State,32 in Khartoum locality ,29 in Khartoum North and 17 in Omdurman. about 30%of these farms were randomly selected for the study comprising 10 farms from Khartoum Locality , 10 from Khartoum North locality and 5 from Omdurman Locality.

One kg of feed sample was randomly collected in sterile bags from 5 sacks of stored feed from each farm .Samples were kept at -20 □ until analysis. The samples were transported to Sudanese Standard Metrology Organization (SSMO) Laboratories for analysis .

3.2.1.Aflatoxin Determination procedure :-

AflaTest from VICAM was used for aflatoxins detection. The samples were ground,50 gram of ground sample was mixed with 5 gram of Sodium Chloride(NaCl) and 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 and ,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, to remove 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 .Ten ml of filtered diluted extract was filtered through microfiber filter paper directly into glass syringe barrel. Ten ml filtered diluted extract was passed through AflaTest column,(it bound 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 air 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- 2 drop per second until air come through the column . Glass cuvette was placed under the column and 1ml 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 .One 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

3.3. Questionnaire procedure

Questionnaire was structured and designed ,using the same farm samples above ,to collected information on management, biosecurity ,anti aflatoxin use and the level of information on the negative effects of aflatoxin on human health ,the poultry industry and production .The questionnaire was pre- tested in 10 farms before the final data collection.

3.4 Statistical Analysis

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) ,while data on farm management were calculated by simple percentage.

CHAPTER FOUR

4.0 RESULTS

The results of the information collected by the questionnaire from the laying hens' farms and the results of the feed samples that were examined for aflatoxin contamination are presented in the following tables and figures.

4.1 Personal Characteristics:

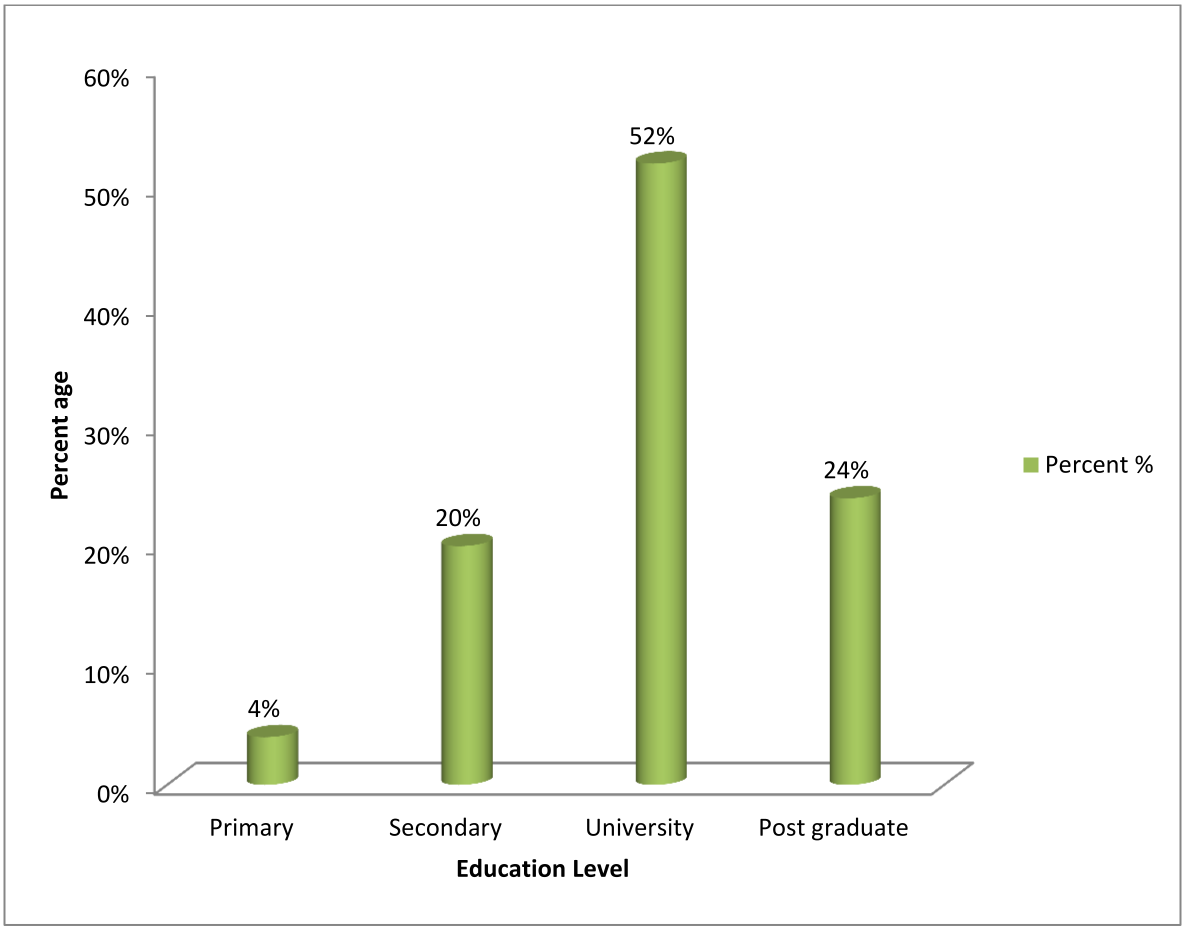
4.1.1 Tab (1) Age range of farm owners

Age/year	Frequency	Percent (%)
20 – 30	2	8
30 – 40	6	24
more than 50	17	68
Total	25	100

The majority of the farm owners (68%) were more than 50 years old, followed by (24%) 30 – 40 years old .

4. 1.2 Fig (1)

Educational level of the farm owners



76% of farm owners were university graduates

4.2 .Management and production

4.2.1 .Tab(2)

Specialization of Farming System

Production system	Frequency	Percent (%)
Poultry	18	72
Mixed(poultry and vegetables)	7	28
Total	25	100

The majority of respondents (72%) specialized in poultry production.

4.2.2.Tab(3).

Type of production.

Type of production	Frequency	Percent (%)
Commercial chicks	1	4
Table eggs	21	84
Table eggs and broilers	3	12
Total	25	100

The majority of respondents (84%) worked in table eggs production .

4.2.3.Tab(4)

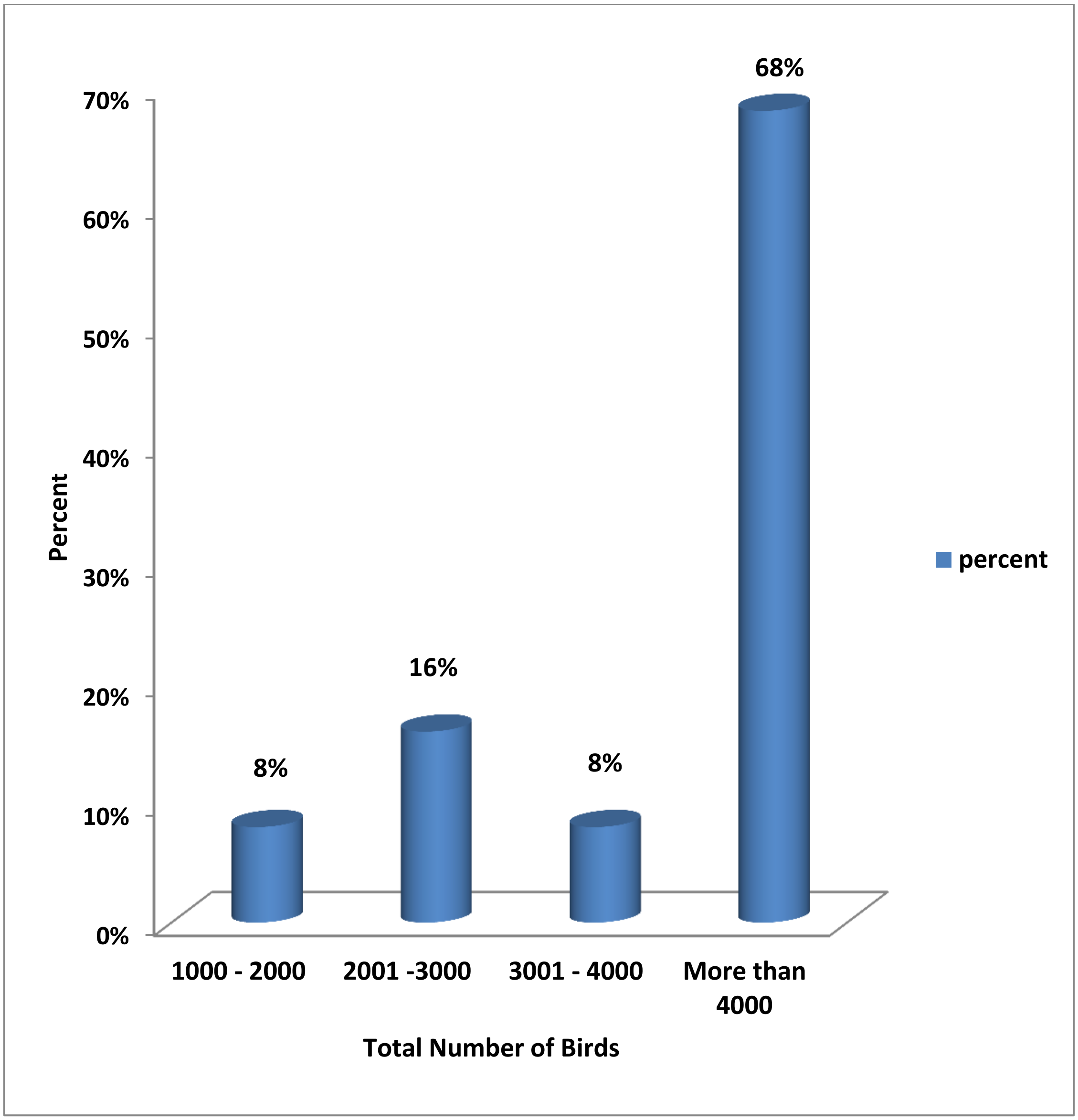
Type of housing system

	Frequency	Percent (%)
Open	1	4
Closed	13	52
Semi closed	11	44
Total	25	100

(52%) of respondents used closed system and 44% used semi-closed system.

4.2.4.Fig (2)

Number of birds/ farm



The majority of respondents (68%) had more than 4000 birds

4.2.5 .Tab(5)

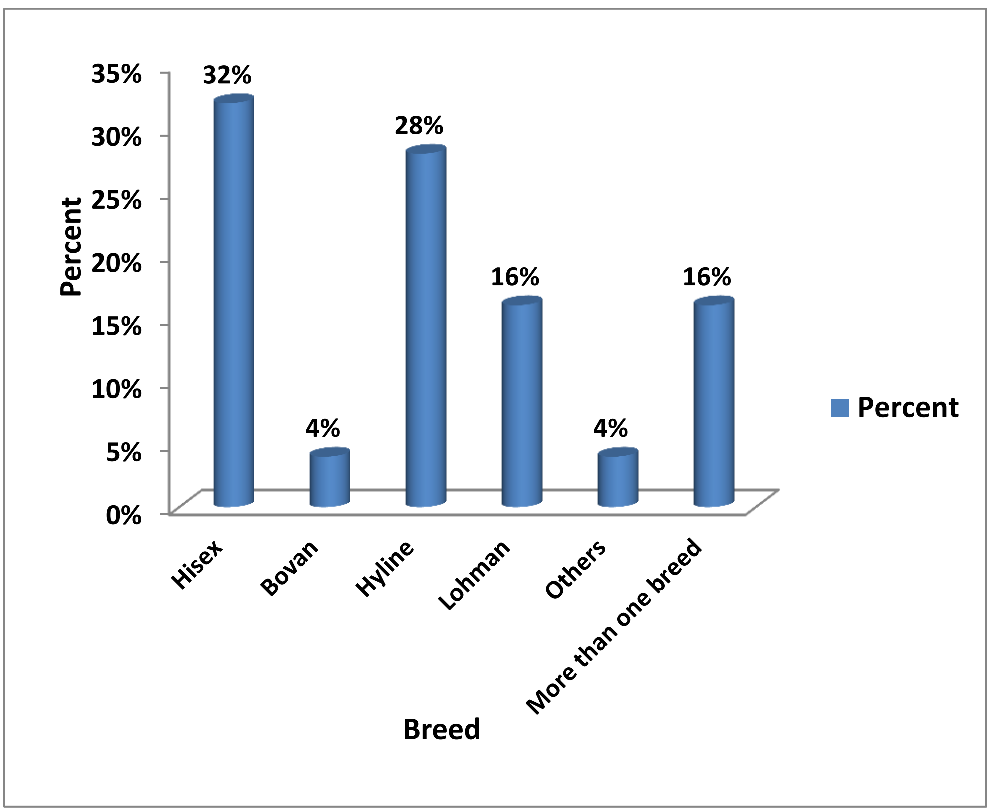
Age of birds

Age	Frequency	Percent (%)
Same -age	16	64
Multi-age	9	36
Total	25	100

64% raised one age birds

4.2.6.Fig (3)

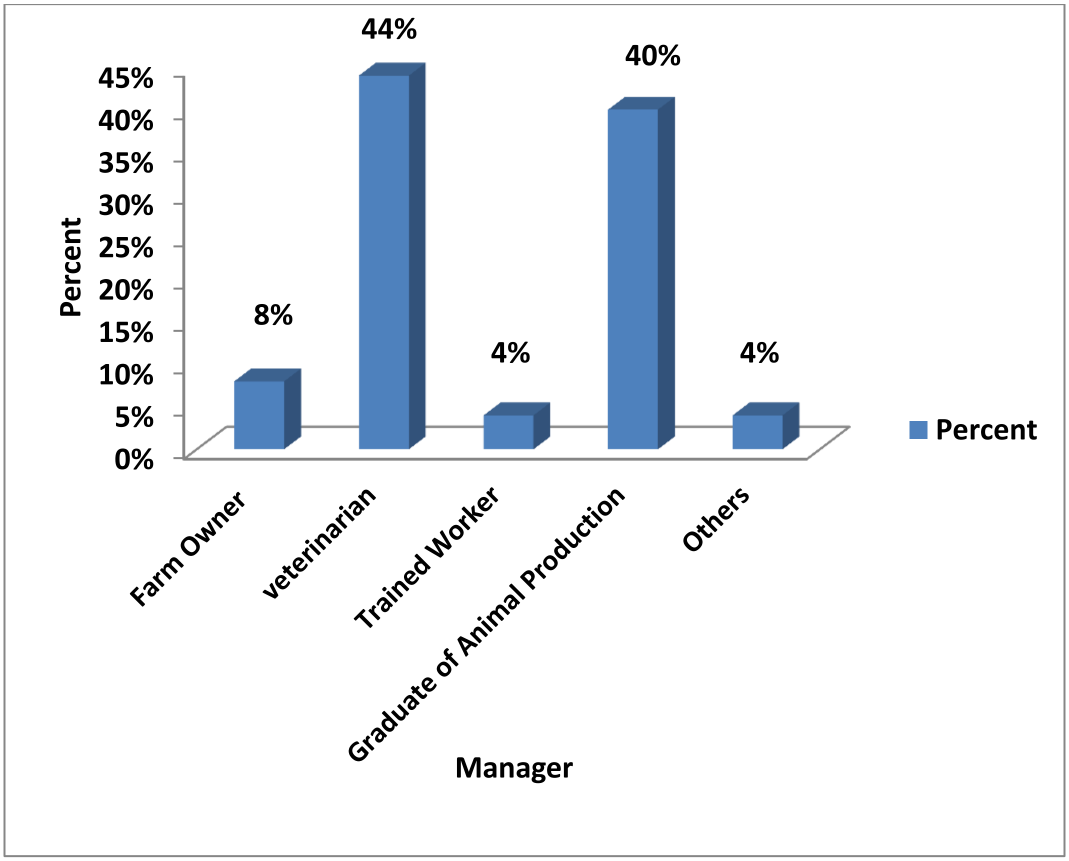
Type of commercial hybrid strains



Hi sex and Hyline were the most raised commercial layers hybrid strains

4.2.7. Fig(4)

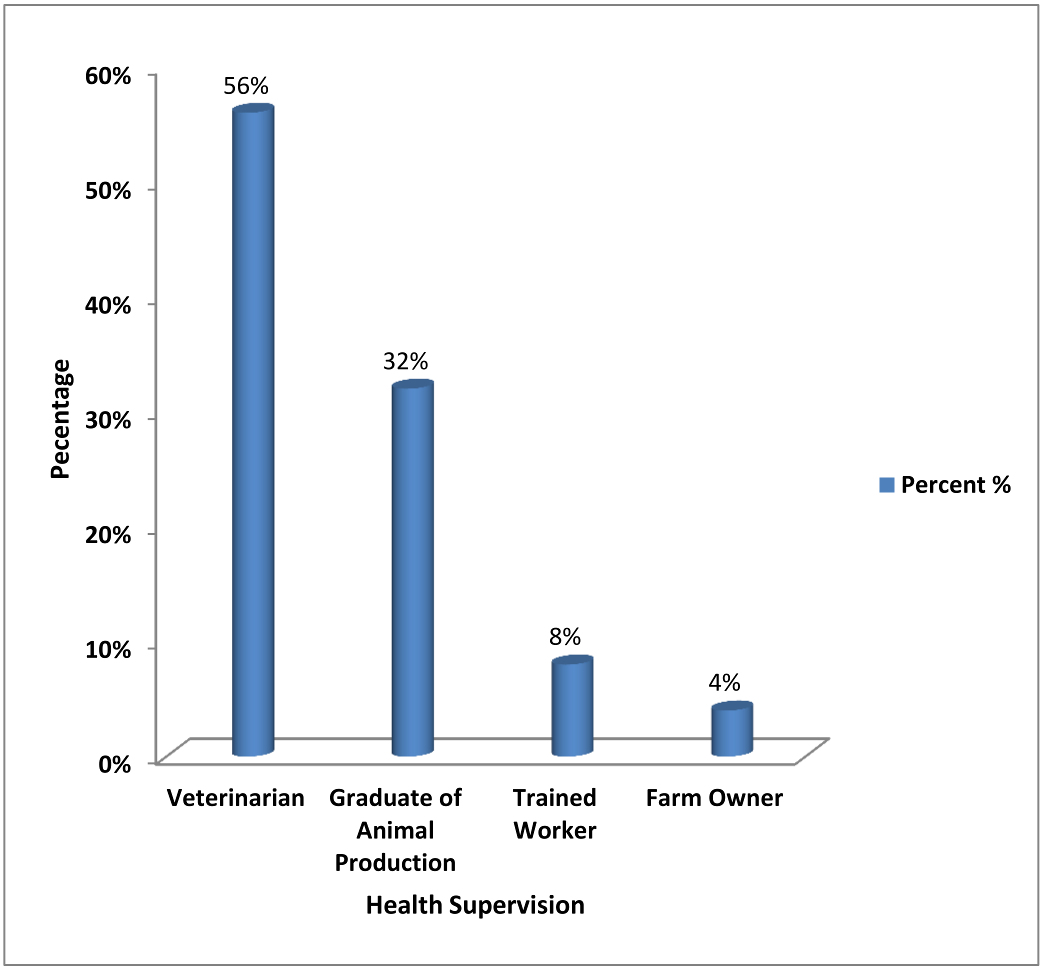
Farm Manager



The majority of managers (44%) veterinarian and 40% Animal Production specialist.

4.2.8.Fig(5)

Health supervision



56% of respondents for health supervision were veterinarians .

4.2.9.Tab (6)

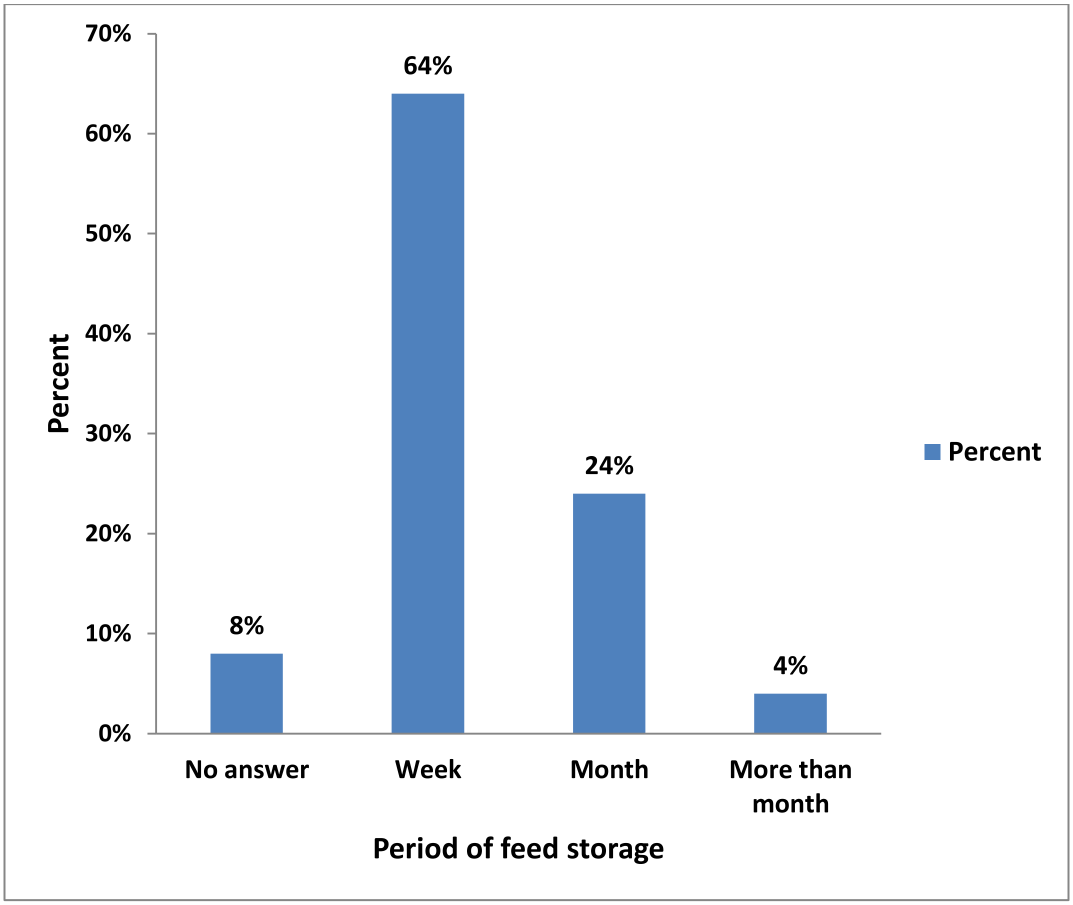
Type of records kept

	Frequency	Percent (%)
Production records	8	32
Health records	1	4
Management records	2	8
All records found	14	56
Total	25	100

56% of respondents had all type of records.

4.2.10 .Fig(6)

Feed storage duration period



The majority of (64%) of respondents stored feed for one week .

4.3.Biosecurity:-

4.3.1.Table (7)

Fence around the farm

	Frequency	Percent (%)
Yes	23	92
No	2	8
Total	25	100

The majority (92%) of respondents had a fence around the farm.

4.3.2.Table .(8)

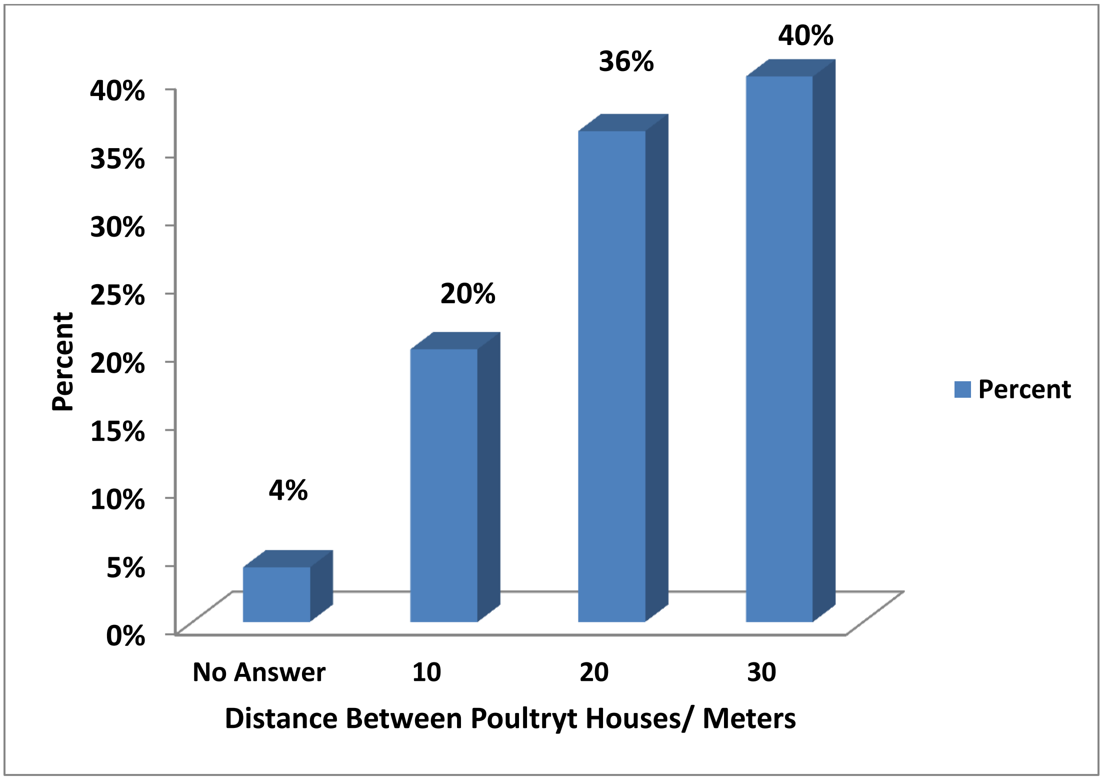
Disinfection for vehicles at farm gate

	Frequency	Percent (%)
Yes	19	76
No	6	24
Total	25	100

The majority(76%) of respondents used disinfectiont for vehicle at farm gate.

4.3.3 Fig.(7)

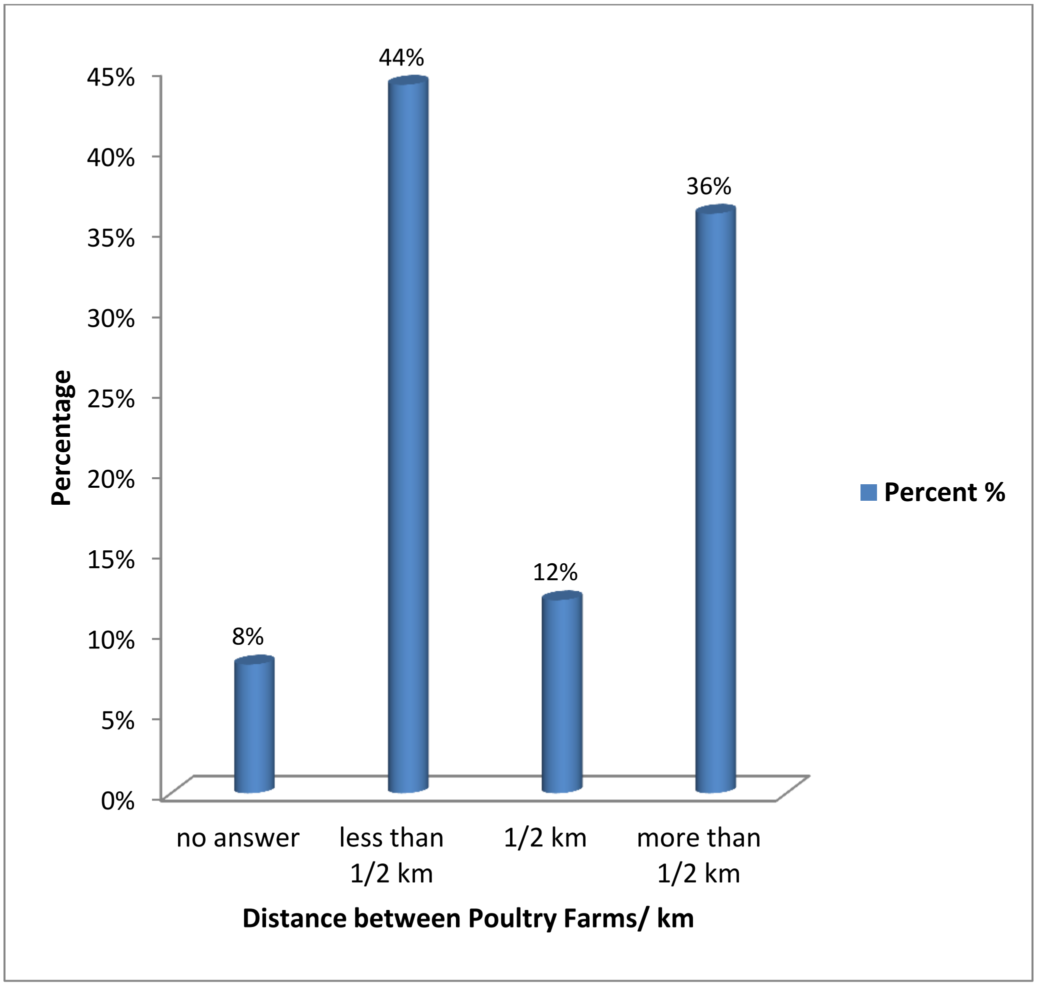
Distance between poultry houses (m)



40% of distance between the farm units was(30 meters) 36% of distance was(20 meters) ,20% of distance was(10 meters), and 4% did not respond

4.3.4.Fig(8)

Distance between Poultry farms/ km



(44%) of the respondents, the distance between their farms and other farms less than 500 meter, 36% is more than 500 meter, 12% is 500 meter, and 8% did not answer .

4.4.Aflatoxin status:-

4.4.1.Tab (9).

Source of information about aflatoxin hazards

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

80% no extension work about anti Aflatoxin.

4.4.2.Tab.(10)

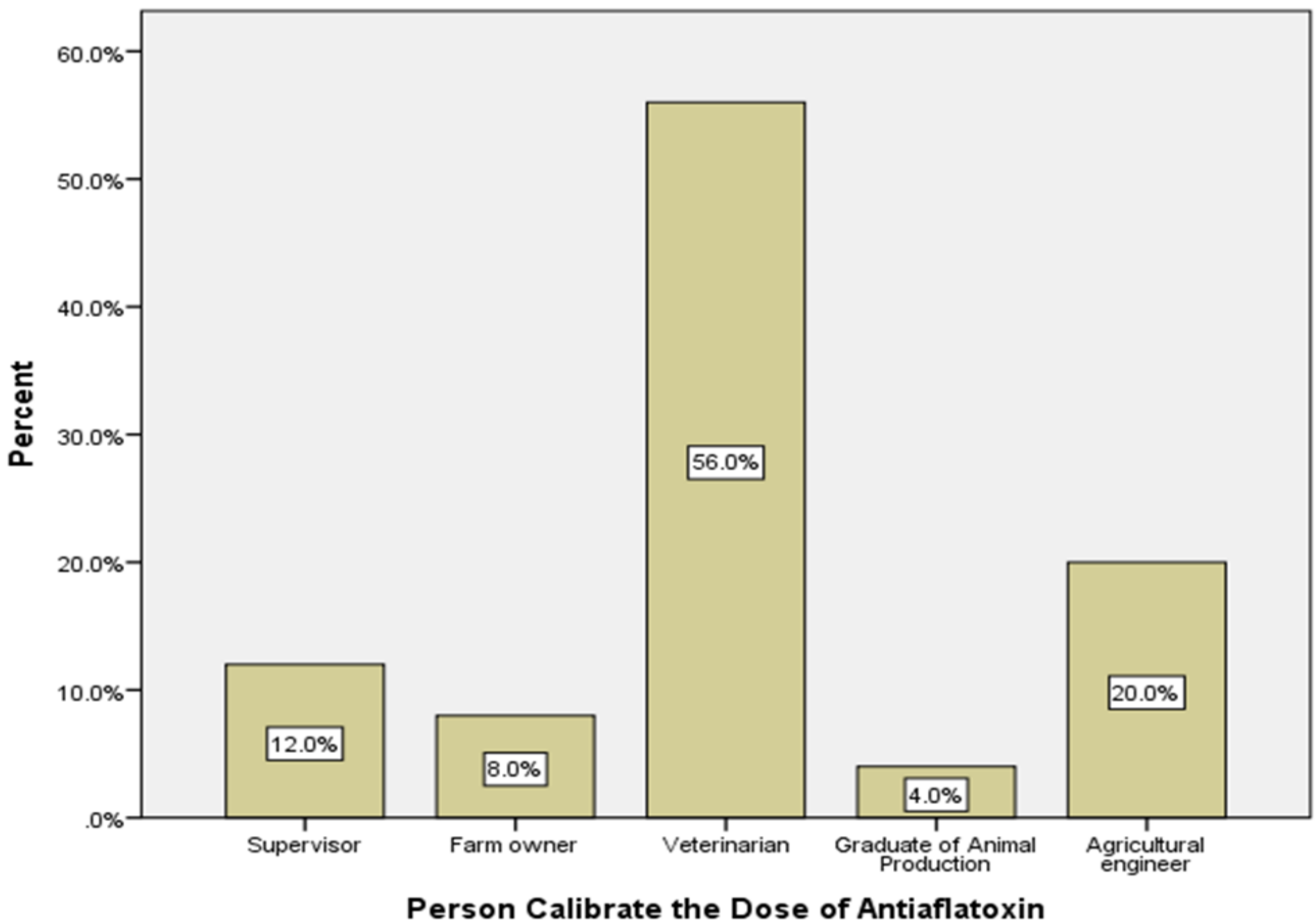
Supplementation of antitoxins

	Frequency	Percent (%)
Water	5	20
feed	20	80
Total	25	100

80%of respondents used antitoxin through feed

4.4.3.Fig.(9)

Calibration of aflatoxin binder dose



44 % of persons who calibrated the dose of anti toxin in farms were not veterinarian.

4.4.4.Table(11)

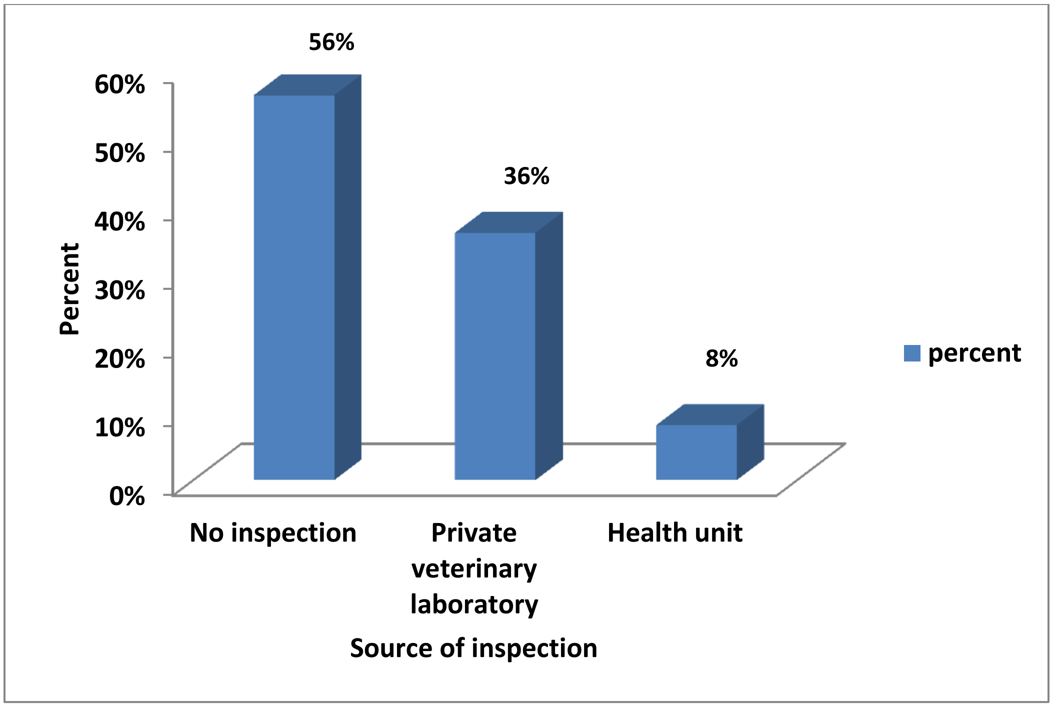
Knowledge on the effect of aflatoxin and health hazards

	Frequency	Percent (%)
Yes	20	80
No	5	20
Total	25	100

80% of respondents knew that the aflatoxin was harmful to human.

4.4.5.Fig(10)

Inspection of Aflatoxin



56% of respondents did not inspect feed for aflatoxin contamination

4.4.6 .Tab (12)

Role of SSMO on aflatoxin information

	Frequency	Percent (%)
Yes	3	12
No	22	88
Total	25	100

The majority (88%) of respondents did not recevied any information from SSMO about aflatoxin hazard .

4.5.1 .Tab.(13)

Multiple Comparison Test (Least Significant Test (LSD)) for Aflatoxin test results between localities

(I) Locality	(J) Locality	Mean Difference (I – J)	Sig.
Khartoum North	Khartoum	2.8	0.045
Khartoum North	Um Dorman	3.6	0.039
Khartoum	Um Dorman	0.7	0.655

Statistical analysis using multiple comparison test (LSD) : The mean difference in Aflatoxin test result was significant between Khartoum North and Um Dorman (0.039 < 0.05), was significant between Khartoum and Khartoum North (0.045<0.05) and was not significant between Khartoum and Um Dorman at significant level ($p \leq 0.05$)

CHAPTER FIVE

5.0 DISCUSSION

5.1.Personal Characteristics:-

The results showed that the majority of farm owners (68%) were above 50 years of age and (%76) were university graduates which indicates high experience and background on the business which demands patience, endurance and good follow-up .

5.2 Management and Production:-

Seventy two percentage of the respondents were solely specialized in poultry production and 84% of them in table egg production. For housing system 52% used closed housing system and 44% semi –closed which lead for better flock management . A total of 68% raised more than 4000 birds in one batch, 64% of them follow All-in – All -out system of rearing. The commercial hybrids raised in these farms were High sex and Hy-line as many farm owners claim that these two hybrids are more adaptable to Sudan conditions.

Most of the farm mangers were veterinarian, 44% supervised by animal production specialist which indicated reasonable farm management level. This result in accordance with the finding of Sirdar (2012) who choosing the most adapted hybrid goes in line with Sirdar (2012) and Alsraf (2015) .The study showed that 56% of the farm owners kept all types of records. A total of 64% of the farm owners stored feed for one week or less, which decreased the possibility of aflatoxin contamination, in addition to the use of closed system in poultry farming which avails better management and health control this result agreed with Askora et,al (2016) .

5.3. Biosecurity Measures :-

The study showed that more than 92% of the farms were fenced for protection against predators, rodents and disease transmitting agents .For antiseptics application 76% used gates with antiseptics, 96% of farm units were located at 10 to more meters apart and the distance between each farms was not less than 500 meters, this finding agreed with Sirdar (2012)and Osman(2008) . Health supervision was followed by veterinarians (56%) and 32% by animal production specialist .

5.4.Farmers Awareness :-

80% of the Farm owners reflected good background about the harmful effect of aflatoxin on animal and human health, in spite of that 56% did not inspect poultry feed for aflatoxin contamination .This may be due to the fact that all the studied farms used Anti-toxins in feed (80%) or water (20%), in addition to the high cost of tests used and/or the absence of follow up and limited inspection by government authorities represented by SSMO, 88% of the farmers stated that no information were provided by SSMO. For Aflatoxin calibration 56% performed by veterinarians and 44% by other specialist.

5.5.Aflatoxin determination:-

The averages of the study findings were 1.76 ppb ,4.66 and 1.26 ppb for Khartoum , Khartoum North and Omdurman Localities respectively .These are in agreement with the result obtained by Rodrigues et al (2011) in Northern , Southern and Central Europe for the period 2009 -2011 in finished poultry feed and who reported Zero to 3ppb . The obtained results show that the total average value of aflatoxin in Khartoum State was 2.6ppb which was less than that (6.6 ppb) found by Jean et al.(2013). This figure is still less than the recommendation stated by SSMO (20 ppb). Rehab et al.(2018) stated 7.6 -18.3 ppb for Khartoum State which agreed with the findings of the current study while Mursal (2009) and Elzupir (2009) noted 10 -97 and 54.41 -579.87ppb respectively in animal feed in Khartoum State. The results of the study are the least compared to the previous studies and indicated the safety margin for Khartoum State poultry feeds.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:-

1-The evaluation of commercial layer feed contamination by aflatoxin in Khartoum State poultry farms revealed acceptable results for the State .

2-The Results were within the standards stated by the Sudanese Standard Meteorological Organization.

3-Khartoum North locality showed the highest level of aflatoxin contamination.

4-Weak farmers awareness about aflatoxin hazards

5- Frequent analysis of layer feed for antitoxins detection was not performed.

6.2 Recommendations:-

- Study on aflatoxin in poultry feed raw materials in Khartoum State should be conducted .
- Extension services should be provided for poultry farmers
- Further study is needed to explain the high level of aflatoxin in Khartoum North poultry feed compared to other localities
- Provision of facilities for aflatoxin testing and assessment

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APPENDICES

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير في الانتاج الحيواني

<u>**استبيان لدراسة مدى وعي المنتجين بالأثارالسالبة للأفلاتوكسين**</u>

1- اسم صاحب المزرعة

2-عمر صاحب المزرعة 20- 30 ( ) 30-40( )اكثر من50 ( )

3-المحلية

4- المنطقة

5-المستوى التعليمي: امي( ) اساس( ) ثانوي( ) جامعي( ) فوق الجامعي( )

6-نظام الانتاج: متخصص دواجن() مختلط دواجن نباتي() مختلط دواجن حيواني()

7-نوع التخصص الدواجن : كتاكيت تجارية ( )بيض مائدة ( )ففرخ لاحم ( ) بيض مائدة ولاحم ( )

8- نظام الاسكان مفتوح ( ) مغلق ( ) شبه مغلق ( )

9-عدد الطيور الكلي بالوحدة : 1000-2000دجاجة( ) 2001-3000( )
3001-4000( )أكثر من 4000( )

10-أعمار القطعان الحالية : عمر واحد ( ) متعدد الأعمار( )

11-نوع الهجين التجاري :هايسكس( ) بوفان ( ) هايلاين( ) لوهمان( ) -
اخرى( ) أكثر من هجين( )

12-الاشراف الاداري الكلي للمزرعة : صاحب المزرعة ( ) طبيب بيطري ( )
عامل مدرب ( ) خريج انتاج حيواني ( ) مهندس زراعي ( ) أخرى ( )

13-كمية العلف المخزن يكفي لمدة : أسبوع ( ) شهر ( )أكثر من شهر ( )

14-هل هنالك عمل إرشادي عن مضادات الافلاتوكسين يصلكم من الوحدة الإدارية
:المحلية ( ) الوزارة المعنية ( ) لايوجد( )

15-انواع السجلات الموجودة بالمزرعة :سجلات انتاج ( ) سجلات صحية ( )
سجلات مالية ( ) سجلات إدارية ( ) جميع السجلات ( ) لا توجد سجلات ( )

16-طريقة او إستعمال مضادات الافلاتوكسين :الماء( ) العلف( ) طرق أخرى ( )

17-تحديد الجرعة وطريقة استعمال مضاد الافلاتوكسين: المشرف ( ) صاحب المزرعة ( ) طبيب بيطري ( ) صيدلي ( ) مهندس زراعي ( ) خريج انتاج حيواني() عامل مدرب ( ) أخرى ( )

18-هل تعلم أن الافلاتوكسين يضر الانسان عن طريق الدجاج ومنتجاته:

نعم ( ) لا ( )

19- اذا كانت الإ جابة نعم اين يتم الفحص ؟

20-هل يتم تحليل العلف لفحص الافلاتوكسين دورياً؟

نعم ( ) لا( )

21-هل يوجد سور حول المزرعة؟ : نعم ( ) لا( )

22-هل توجد بوبة مخصصة لدخول الزوار؟ نعم ( ) لا( )

23- هل نظام المطهر للمركبات للدخول للمزرعة مطبق؟

نعم ( ) لا( )

24هل نظام الامن الحيوي للزوار مطبق؟

نعم ( ) لا ( )

25- المسافة بين كل حظيرة و الاخرى : 10 متر ( ) 20متر( ) 30متر( )

26- المسافة بين كل مزرعة وأخرى: أقل من نصف كيلو ( ) نصف كيلو ( ) أكثر من نصف كيلو ( )

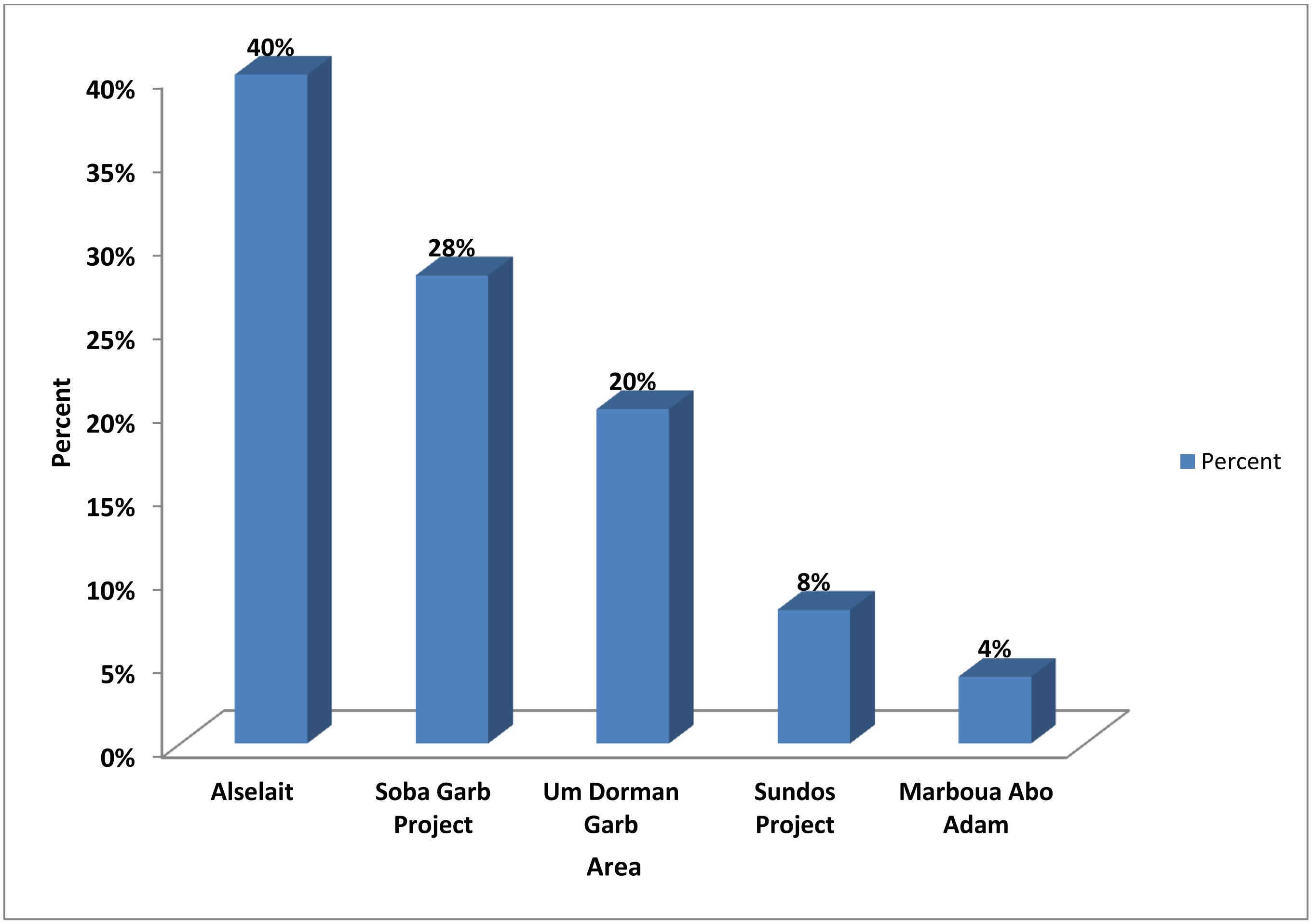
27- هل تصلكم نشرات المواصفات التي تصدرها هيئة المواصفات والمقاييس السودانية عن اضرار الافلاتوكسين ؟

نعم( ) لا( )

Khartoum Map

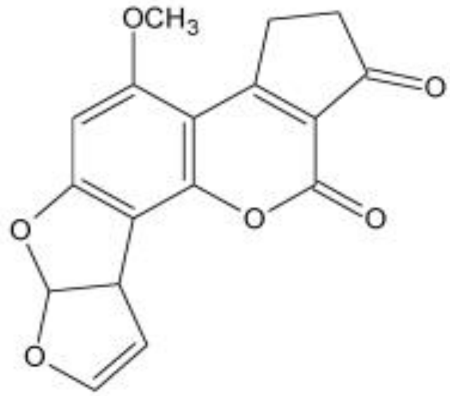
Fluorometer for measuring aflatoxin

Respondents distribution and area with in the locality

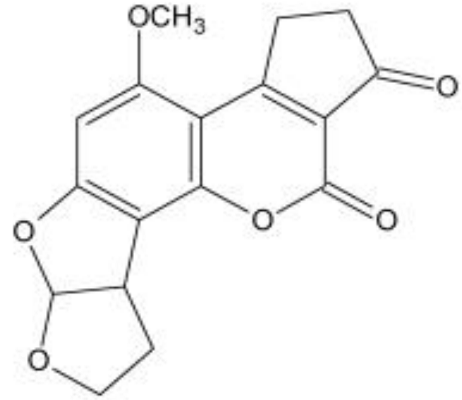


The respondents number in Alselait region were (40%) and in Maroua AboAdam were (4%) and Soba Garb project were (28%),Sundos project were (8%) and Um Durman Garb were 5 (20%).

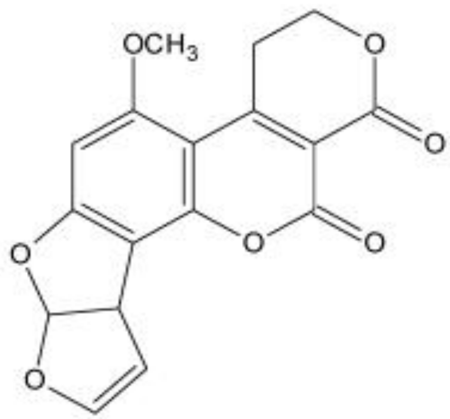
Chemical structure of Aflatoxin



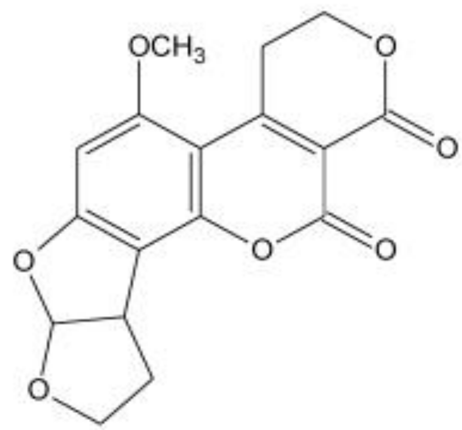
Aflatoxin B_1



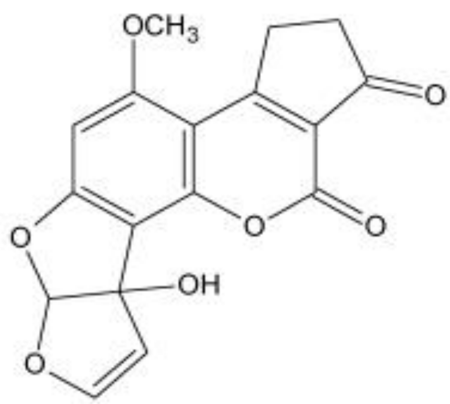
Aflatoxin B_2



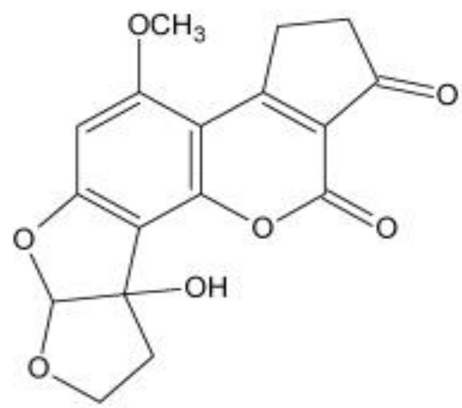
Aflatoxin G_1



Aflatoxin G_2



Aflatoxin M_1



Aflatoxin M_2

Test report results for aflatoxin

Khartoum North Locality Sample Test Report

Sample type: layers feed

Sample No	Lab. Environmental Conditions		Laboratory Sampling No	Date		Test result				
	Temperature	**Humidity %**		**Sampling receiving to lab.**	**Test date**	**Test**	**Test method**	**result**	**Measure unit**	**Standard unit**
1	25.9C°	51	A 18ح 8/29	30/8/2018	04/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	3.00	ppb	Not more than 20
2	26.4C°	55	B 18ح 7/19	25/07/2018	29/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	2.2	ppb	Not more than 20
3	26.4C°	55	C 18ح 7/19	25/07/2018	29/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20
4	25.9C°	51	E 18ح 8/29	30/8/2018	04/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	4.5	ppb	Not more than 20
5	25.0C°	59	E 18ح 7/19	25/07/2018	26/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	2.9	ppb	Not more than 20
6	26.4C°	55	F 18ح 7/19	25/07/2018	29/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	14	ppb	Not more than 20
7	26.4C°	55	G 18ح 7/19	25/07/2018	29/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	11	ppb	Not more than 20
8	25.9C°	51	T 18ح 7/19	30/8/2018	04/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	2.4	ppb	Not more than 20
9	28.2C°	57	K 18ح 8/29	30/8/2018	03/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	6.6	ppb	Not more than 20
10	28.2C°	57	L 18ح 8/29	30/8/2018	03/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20

Note: Detection limit 1 ppb- B.D.L = below detection limit - The average of aflatoxin = 4.66 ppb

Khartoum Locality Sample Test Report

Sample type: layers feed

Sample No	Lab. Environmental Conditions		Laboratory Sampling No	Date		Test result				
	Temperature	Humidity %		Sampling receiving to lab.	Test date	Test	Test method	result	Measure unit	Standard unit
11	25.0C°	59	A ^{18}ح 7/19	25/07/2018	26/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20
12	25.0C°	59	H ^{18}ح 7/19	25/07/2018	26/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	2.8	ppb	Not more than 20
13	25.0C°	59	I ^{18}ح 7/19	25/07/2018	26/07/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20
14	27.2 C°	52	F ^{18}ح 8/29	30/8/2018	02/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	2.9	ppb	Not more than 20
15	27.2 C°	52	D ^{18}ح 8/29	30/8/2018	02/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20
16	28.2C°	57	I ^{18}ح 8/29	30/8/2018	03/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	4.2	ppb	Not more than 20
17	28.2C°	57	M ^{18}ح 8/29	30/8/2018	03/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20
18	28.2C°	57	H ^{18}ح 8/29	30/8/2018	03/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	2.9	ppb	Not more than 20
19	27.2 C°	52	C ^{18}ح 8/29	30/8/2018	02/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	4.8	ppb	Not more than 20
20	27.2 C°	52	B ^{18}ح 8/29	30/8/2018	02/09/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20

Note: Detection limit 1 ppb -B.D.L = below detection limit - The average of aflatoxin = 1.76 ppb

Um Dorman Locality Sample Test Report

Sample type: layers feed

Sample No	Lab. Environmental Conditions		Laboratory Sampling No	Date		Test result				
	Temperature	Humidity %		Sampling receiving to lab.	Test date	Test	Test method	result	Measure unit	Standard unit
21	28.2C°	36	A ^{18}ح 10/7	29/10/2018	30/10/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	B.D.L	ppb	Not more than 20
22	28.2C°	36	**B** ^{18}ح 10/7	29/10/2018	30/10/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	1.2	ppb	Not more than 20
23	24.4C°	27	A ^{18}ح 11/2	04/11/2018	05/11/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	2.6	ppb	Not more than 20
24	24.4C°	27	B ^{18}ح 11/2	04/11/2018	06/11/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	1.1	ppb	Not more than 20
25	24.4C°	27	C ^{18}ح 11/2	04/11/2018	06/11/2018	Aflatoxin	Flourometer Vicam Series 4 Manual	1.4	ppb	Not more than 20

Note: Detection limit 1 ppb-B.D.L = below detection limit-The average of aflatoxin = 1.26 ppb