Sudan University of Science and Technology

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Bacterial Contamination In Commercial Poultry Feed In Khartoum State

التلوث البكتيري في علائق الدواجن التجارية في ولاية الخرطوم

By

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قال تعالى

(وَمَا أُوتِيتُم مِّن الْعِلْمِ إِلاَّ قَلِيلاً)

صدق الله العظيم

الإسراء : 85

DEDICATION

To my mother and father.

To my sisters and brother.

I dedicate this work.

ACKNOWLEDGEMENT

First and foremost, my thanks to the most beneficent and merciful, the Almighty Allah, for giving me the power and health to complete this work. Next, I would like to express my special appreciation to my supervisor Professor Intisar Yousif Turki for her help, suggestions, advice and follow up throughout this study.

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Abstract

This study was conducted in order to determine and investigate the bacterial contamination of commercial poultry feeds marketed in Khartoum state. A Total of (45) samples of three different commercial poultry feed namely, pre starter for chicks, broiler finisher and layer finisher obtained from five localities in Khartoum state namely (Sharg Alneel , Khartoum , Bahri , Omdurman and Jabel Awliaa) were examined for their microbiological quality using microbiological and analytical methods. The mean total bacterial counts vary within the samples of the three types of poultry feeds while statistical analysis revealed no significant difference between mean of the three type of poultry feeds. Statistical analysis also revealed a high significant difference in the mean of total bacterial counts for the five localities ($P \leq 0.01$), and the highest mean of total bacterial counts found in Jabel Aweliaa locality was (5.71) $\log_{10}$ CFU\cm, while the least in Bahri locality was (4.57) $\log_{10}$ CFU\cm. Three genera of bacteria were isolated and their percentage occurrence was Escherichia coli (60%), salmonella spp (53.33%) and staphylococcus aureus (26.66%). Proximate composition showed a different variation in moisture, ash, fat, crude fiber and protein content among the experimental samples obtained from Bahri and Jebel Aweliaa localities. Approximate analysis indicated a deterioration in nutritive value specifically protein percent for samples collected from Jabel Aweliaa locality which showed highest bacterial contamination in it. The obtained results indicated, the tested samples of commercial poultry feeds marketed in Khartoum State contain contaminants.

Abstract

أجريت هذه الدراسة بهدف التحقق من التلوث البكتيري في أعلاف الدواجن التجارية التي تباع في أسواق ولاية الخرطوم . خمسة وأربعون عينة لثلاثة أنواع من أعلاف الدواجن التجارية تسمى : قبل البادي للكتاكيت ، ناهي لاحم و ناهي بياض ، تم جمعها من أسواق خمسة محليات في ولاية الخرطوم وهي (شرق النيل ، الخرطوم ، بحري ، أمدرمان ، جبل أولياء).

متوسط العدد الكلي للبكتريا مختلف بين عينات أعلاف الدواجن الثلاثة بينما أظهر التحليل الإحصائي أنه لا يوجد فرق معنوي كبير في متوسط العدد الكلي للبكتريا للثلاثة أنواع لأعلاف الدواجن . أيضا أظهر التحليل الإحصائي أنه يوجد فرق معنوي عالي في متوسط العدد الكلي للبكتريا بين عينات أعلاف المحليات الخمسة ($P\leq0.01$) ، وأكبر متوسط للعدد الكلي للبكتريا وجد في محلية جبل أولياء بمتوسط (5.71) بينما أقل متوسط للعد الكلي للبكتريا وجد في محلية بحري بمتوسط (4.57) . ثلاثة أجناس من البكتريا تم عزلها من الأنواع الثلاثة لأعلاف الدواجن وظهرت نسبها كالأتي الاشريكية القولونية (60%) ، السالمونيلا (53.33%) و المكورات العنقودية الذهبية بنسبة (26.66%) .

التحليل التقريبي للمواد الغذائية أوضح النسب المئوية للرطوبة ، الرماد ، الألياف ، الدهون والبروتين لعينات أعلاف الدواجن التي جمعها من محليتي بحري وجبل أولياء . حيث أظهرت النتيجة انه حدث تدهور في قيمة المواد الغذائية خصوصا نسبة البروتين في العينات التي تم جمعها من محلية جبل أولياء التي ظهرت بها أعلى نسبة تلوث بكتيري .

أوضحت النتائج المتحصل عليها أن عينات أعلاف الدواجن التجارية المختبرة المسوقة في ولاية الخرطوم ملوثه بالبكتيريا.

CHAPTER ONE

INTRODUCTION

The term ‘poultry’ used in agriculture generally refers to all domesticated birds kept for egg laying or meat production. Poultry comes from the French word poul, which was derived from Latin word Pullus meaning small animals. Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat (Raloff, 2003).

Feed for poultry production are composed largely of grains such as corn, wheat or barley, oil seeds, cake or meal originating mainly from oil producing seeds such as soybeans, sunflower seeds, peanuts, cotton seed and protein products of animal origin such as fish meal, meat and bone meal, slaughter house offal’s and feather meals (Bale et al.,2002). Since these feeds are expected to be the sole sources of nutrition of the birds, they usually contain essential mineral and vitamin additives (Dhand et al.,1998).

Feed is the single most expensive factor in raising poultry industry or production representing 75-80% of the total production expenses depending on the geographic location, season and country. (Mojtaba et.al.,2002).

Livestock (poultry) get infected when pathogenic organism passes to the susceptible animal through feeding (Barnes et al., 2003). Reports by Gill and Best (1998) and Ruff (1992) have listed animal feed as one of the sources of micro-organisms to animals. Specifically, some of the additives have been incriminated amongst the principal sources of bacteria of public health concern.

Micro-flora can be decreased on poultry feed through nutritional changes, physical damage and other factors. Microbial contamination of poultry feed is a significant potential pathway for entry of pathogens into human food supply, and at present there is no comprehensive program that addresses it in the Sudan Food Safety Program (Maciorowski et.al., 2009).

Various types of farmed animal diseases such as diarrhoeal diseases like bacillary dysentery, amoebic dysentery, fowl cholerae, Salmonellosis, staphylococcosis, colibacillosis, erysipelas and listeriosis have been traced to the contamination of animal feed (Healing and Greenwood, 1991). Several incidents have been reported in which human illness was traced back to contaminated animal feed. A semi-quantitative risk assessment of human health impact of Salmonella contamination of soybean feed products . (Hald et.al 2006).

There is a major threat to humanity and it comes from the very food we eat a terrible consequence of our modern farming systems. Some diseases that infect animals can also be passed on to humans. These are known as zoonotic diseases. Zoonotic diseases are a major global threat to public health and animal welfare. Animal products contaminated with bacteria such as Salmonella, Campylobacter and E. coli are responsible for large numbers of foodborne human infections, which can be fatal. (Micheal, 2013).

Objectives:

1. To determine and investigate bacterial contamination of Poultry feed in Khartoum State.
2. Isolation and Identification of the major bacteria from the Poultry feed.

CHAPTER TWO

2: LITERATURE REVIEW

2.1. Introduction for poultry production in Sudan

2.1.1. Poultry population in Sudan

Out of a total population of 45.3 million chickens in Sudan the conventional sector comprises around 30 million from which the annual meat and egg production is 20.1 million birds and 900 million eggs, respectively (Sulieman 1996). Desai (1962) classified the indigenous breed (Baladi) into three types that include large Baladi, bare-neck and Betwil. The large Baladi is the most common type and distributed all over the country.

2.1.2. Poultry industry in Sudan

Poultry keeping in Sudan is an old practice, where the domestic fowl has been kept for generations in villages and backyards of dwellings to supply both eggs and meat for own consumption. Recently, with increase in demand for poultry products, poultry production has witnessed an increasing intensification resulting in commercial poultry farming concentrated in Khartoum State, the capital of Sudan and in the peripheries of some other big cities. Other parts of the country depend on the governmental poultry units and small-scale farms (Sharabeen, 1996). Commercial Poultry production in Sudan is divided into three farming system

- Open System
- Semi Closed system
- Closed system

Khartoum State produce almost 90% of Sudan's poultry production (Mohamed, 2014).

Ninety six percent of the commercial poultry production is located in Khartoum State of Sudan. This could be explained by the continuous urbanization from rural areas to the cities, implying a future rising market demand in this area. However, Sudan remains the Arab country with the lowest intake of poultry meat per capita a year. In 2005, the intake of commercially bread poultry was 0, 77 kg of meat per capita which can be compared to Egypt with 9 kilos per year or Saudi Arabia with a yearly intake of 39 kg of poultry meat per capita (Freiji, 2008).

The low intake of poultry in Sudan can be explained by the price of meat. Traditionally, the price of red meat from sheep and cattle has been low, but during the last decade, a rise has been noted. In the past, poultry meat production has been dependent on the importation of production inputs such as feed, vaccines and parent stock (Freiji, 2008).

As the industry is growing and the agribusinesses establish themselves, the agribusinesses tend to be able to produce chicken more efficiently. For Sudanese poultry producers, the cost of environmental regulation systems and feed are the two major expenditures affecting the producers' final profit. Feed cost itself stands for 50-70% of the producers' total costs. Depending on the production system used, air condition can be the second largest expense (Emmam and Hassan, 2010).

According to Mohamed (2014), Poultry production in Sudan may be groped as follows:

- High cost of nutrition
- High percentage of wastage due to quality of raw materials, bio security, mismanagement, long production cycle and high mortality (arrival/ slaughter).
- Electricity/ fuel
- Raw material quality
- Hard currency for imports.

2.2. Importance of poultry products for human consumption

Poultry provide humans with companionship, food and fiber in the form of eggs, meat and feathers. Many people love to raise and show chickens and other poultry species at fairs and other poultry shows. Others just love to raise them for backyard pets and for fresh eggs every day. There is a large commercial chicken industry that provides us with eggs and meat. This reflects that consumption based, in turn, on consumer preference for these high-quality products and the relatively low price because of high efficiency of production; hence the safety of poultry products is a prominent quality issue (Mead et.al., 1993).

2.3. Poultry Feed

Feed for poultry production are composed largely of grains such as corn, wheat or barley, oil seeds, cake meal (originating mainly from oil producing seeds such as soybeans), sunflower seeds, peanuts, cotton seed and protein products of animal origin such as fish meal, meat and bone meal, slaughter house offal's and feather meals (Bale et al.,2002). Since these feeds are expected to be the sole sources of nutrition of the birds, they usually contain essential mineral and vitamin additives

(Dhand et al.,1998). However, there are variations in nutrient requirements for different farm animals, but the level of dietary energy and associated nutrient should be high enough to allow expression of animals potentials under certain environmental circumstances within the economic limitations (Wilson, 1990).

2.4. Feed Quality

Quality livestock feed is necessary for the maintenance of physiological functions and animal defense systems against diseases and parasites. Traditionally, feed quality has been specified on basis of the nutritional value of every individual feed component (Fink-Gremmels, 2004). The quality of ingredients used for feed production is very important because what birds eat can affect flock quality and the wholesomeness of the flock's meat and eggs. Most raw ingredients are grown, harvested, processed and transported by some- one outside the poultry industry. Therefore, the ingredient quality control component of a poultry operation's feed mill is an important first step in preventing contamination of birds on the farm (Mojtaba et.al.,2002).

Livestock feed quality may however be affected by various microorganisms such as bacteria and fungi growing in different parts of the world. Most fungal contaminants in stored feed materials usually arise from infestations that began in the field, although some can directly infest storage grains as well when conditions are right (Vieira, 2003; Mabbett, 2003).

Reports by Gill and Best (1998) and Ruff (1992) have listed animal feed as one of the sources of micro organisms to animals. Specifically, some of the additives have been incriminated amongst the principal sources of bacteria of public health concern (Ogbulie and Okpokwasili

1998). Various types of farmed animal diseases such as diarrhoeal diseases like bacillary dysentery, amoebic dysentery, fowl cholera, Salmonellosis, staphylococcosis, colibacillosis, erysipelas and listeriosis. have been traced to the contamination of animal feed (Healing and Greenwood, 1991). Livestock (poultry) get infected when pathogenic organism passes to the susceptible animal through feeding (Barnes et.al., 2003).

2.5. Source of contamination

2.5.1. Ingredient contamination

The greatest isolation was found among animal proteins and by–products of animal origin such as bone meal, meat meal, fishmeal and vegetable proteins, particularly sesame and groundnut cakes (Sakazaki, 2000). The lower the number of organisms per gram in a particular feed ingredient has a lower rate of contamination and possesses a lower risk of causing infection.

The whole process of minimizing the risk of contamination must start with selection of a suitable raw material. A program for frequent inspection and cleaning is necessary, and must be employed to prevent the growth of potential harmful micro-organisms into previously safe ingredients (Sakazaki, 2000).

2.5.2. Storage (temperature and humidity)

Heat treatment was found to be very efficient in sterilization of feed ingredients such as bone meal, fish meal, meat meal and vegetable by-products; it decreases the total viable count or bacterial load of feed. Bacteria are affected by heat treatment according to the degree of

temperature used, holding time, pressure, moisture, and type of bacteria.

At a temperature of 60^{o}C and less, the total viable count typically in the order of 1.0x10^{6} colony forming unit (cfu) per gram of sample, where most of the dangerous bacteria were not killed and is considered dangerous or unsafe. At 80^{o}C the total viable count decreases and is typically be in the order 1.0x10^{5} cfu per gram of sample or lesser (Quadri and Deyoe, 1998). At a temperature of 100 ^{o}C and over, the range of the total viable count decreases and will be in the order 1.0x10^{3} - 1.0x10^{2} and at a temperature of 120 ^{o}C the total viable count is nil (Quadri and Deyoe, 1998). Additionally, Salmonella present in feed ingredients may multiply during storage. Data obtained from the questionnaires compiled by Sauli et al. (2005)

2.5.3. Handling and transportation

Contamination of feed and feed mills has a number of sources which include handling and transportation of ingredients and finished feeds, which can be a risk if we couldn't mange them on the way that considers safety of feed and feed ingredients and during feed manufacture processes (grinding, mixing, packaging.), handling might increase the contamination as noticed by Kashiwazki (1999).

2.6. Bacteria contaminating poultry feeds

Poultry feed component of plants and animal origin are commonly contaminated with microorganisms, mostly bacteria and fungi and\or insects. However, the number and types of microorganism and insects vary depending on the function of materials, location of the origin, climatic conditions encountered, harvesting, processing, storage and

transport technologies employed and packing materials. Other microorganisms that have been implicated as contaminants of poultry feeds include Eschericha coli, Evewinia herbicola, Salmonella spp, Listeria spp, Enterococcus faecalis, Aspergillus flaves, A. parasiticus. Penicillum spp, and Fusarium spp. (D' Mello, 2006).

The type of feed processing and storage conditions can all be factors that influence the population levels and types of micro-organisms present. It has long been known that infectious agents can be transmitted to animals through contaminated feed, as example, workers in UK demonstrated that non-Typhi serotype of S. enterica could be transmitted to chicks through feed contamination by faeces of infected rodents (Wilson, 1948).

Poultry feed may contain diverse micro-flora that is acquired from multiple environmental sources including dust, soil and insects. Poultry feed material may be inoculated with pathogens at any time during growing, harvesting, processing and storage of feed (Watkins et.al., 2003).

Micro-flora growth is dependent on moisture contents of feed materials, however; the majority of micro-organisms must exercise various strategies to survive until there is sufficient moisture to support microbial activities. Micro-flora can be decreased on poultry feed through nutritional changes, physical damage and other factors. Microbial contamination of poultry feed is a significant potential pathway for entry of pathogens into human food supply, and at present there is no comprehensive program that addresses it in the Sudan Food Safety Program (Maciorowski et.al., 2009). Large quantities of feeds destined for animals and increasing quantities of dehydrated pet foods

are in international trade. Feeds and pet foods have constituents of vegetable and animal origin, both of which can be contaminated with many kinds of microorganisms or their metabolites, including those of animal or human health significance. Although the absence of other pathogens in feeds cannot be guaranteed, the main organisms of concern are salmonellae (ICMSF, 1978).

2.6.1. Escherichia coli

Escherichia coli is worldwide in distribution and many strains are part of normal flora of the intestinal tract of humans and animals (Carter and Wise, 2004). E. coli are always found in the gastrointestinal tract of birds and disseminated widely in feces; therefore, birds are continuously exposed through contaminated feces, water, dust and the environment. Colibacillosis, a syndrome caused by Escherichia coli, causes elevated morbidity and mortality leading to economic losses on a farm especially around the peak of egg production and throughout the late lay period. Colibacillosis is a common cause of sporadic death in both layers and breeders, but can cause sudden increased mortality levels in a flock (Charlton, 2006). Inflammation of the oviduct (salpingitis) caused by E. coli infection results in decreased egg production and sporadic mortality, and it is one of the most common causes of mortality in commercial layer and breeder (Nolan et al., 2013).

2.6.2. Staphylococcus aureus

Staphylococcus aureus is non-motile, non-spore-forming, aerobic and facultative anaerobic bacteria. During recent years there have been several reports on the isolation of methicillin resistant Staphylococcus aureus from poultry farms or slaughter houses, carcasses, or feed of poultry origin. (Persoons et.al., 2009; Lim et.al., 2010).

2.6.3. Salmonella

Salmonella is rod-shaped, Gram-negative, non-spore forming. Salmonella is one of the most important foodborne zoonotic pathogens, with significant health and economic impact in both humans and animals (Voetsch et al. 2004). Salmonella incidence from feed ingredients of plant origins including the major cereal grains used in feed production have become recognized as sources of Salmonella contamination. Early studies were already able to isolate a wide variety of serovars from several seeds and cereal grains including peanut meal, sunflower meal, bran meal, barley, corn sorghum, and wheat (MacKenzie and Bains, 1976). Animal feed is a potential source of Salmonella infection. Crump et.al. (2002) report, several cases in which the Salmonella strains found in human food have been traced back to animal feed. Pelleted and mash poultry feeds have long been recognized as vectors for Salmonella contamination of commercial poultry production systems. A study conducted in the southern United States found that 8.8% of mash feed samples and 4.2% of pelleted feed samples were contaminated with Salmonella (Threlfall et.al. 2003). Contamination of feed ingredients during storage and transportation can occur through wild animals (rodents, birds) or pets (e.g. dogs), or be a consequence of cross-contamination from previous batches of ingredients, e.g. due to insufficient disinfection or inadequate drying of storage rooms or vehicles after cleaning (Hald et.al. 2012) . Fish meal also has the potential for the spread of Salmonella, although it seems to be less contaminated than other animal derived protein feed. Animals ingesting feeds contaminated with Salmonella may contract clinical disease or subclinical infections and excrete salmonellae for long periods. This fact has become important with the current increase in world trade of feeds,

and fish meal and meat-bone meal have been responsible for the spread of certain Salmonella serotypes, such as Salmonella agona, world-wide. (Clark et.al. 1973).

CHAPTER THREE

3: MATERIALS AND METHODS

3.1. Study area

This study was carried out in Khartoum State, where geographic and agro climatic characteristics were similar. The farmers purchase their poultry feeds from local markets distributed in Khartoum State localities (table 1).

3.2. Sample collection:

Duplicate samples of three commercial poultry feed include : pre starter for chicks (PS) , layer finisher (LF) and broiler finisher (BF) were collected from markets of 5 localities of Khartoum State (Sharq Alneel , Khartoum , Omdurman , Bahri and Jabel Awliaa).

Table (1): Distribution of types of poultry feed samples collected from different localities:

Localities	Type of feed		
	Pre starter	Broiler finisher	Layer finisher
Sharq Alneel	3	3	3
Khartoum	3	3	3
Omdurman	3	3	3
Bahri	3	3	3
Jabel Awleiaa	3	3	3
Total	15	15	15

3.3. Collection methods :

For collection of samples , sterile plastic bottle were used . A sample size of (1) kilo gram was taken of each feed . The sample were delivered to the laboratory and processed as soon as possible .

3.4. Sterilization of materials

The glassware and the wire loops were properly washed, air dried, wrapped with kraft paper and sterilized in hot air oven at 180^{o}C for 2 hours.

3.4.1. Disinfection

Alcohol (70%) was used to disinfect working benches in the laboratory and media preparation room.

3.5. Preparation of media used

The media were aseptically prepared when necessary according to the manufacturer's instructions on the labels of the media and autoclaved at 121^{o}C for 15 min.

3.6. Culture media

All media used in this study were purchased.

3.6.1. Solid media

3.6.1.1. Nutrient Agar (Oxoid CM3)

Used for the cultivation of microbes supporting growth of wide range of non-fastidious organism, it can grow a variety types of bacteria.

3.6.1.2. Xylose-Lysine Deoxycholate Agar (XLD Agar)

Xylose-Lysine Deoxycholate Agar (XLD Agar) is a selective medium recommended for the isolation and enumeration of Salmonella species.

3.6.1.3. EMB Agar M317

EMB Agar (Eosin Methylene Blue Agar) is recommended for the isolation and differentiation of gram negative enteric bacteria from clinical and nonclinical specimens.

3.6.1.4. Mannitol Salt Agar (7143)

Mannitol Salt Agar is used for the isolation of Staphylococci.

3.6.2. Liquid media

3.6.2.1. Nutrient Broth (Oxoid CM1)

Supports the growth of a great variety of microorganisms.

3.7. Culture of samples and isolation methods

One gram of each feed sample, after being mixed, was put into a test tube containing 10 ml of nutrient broth and incubated overnight at 37^{o}C. After incubation period, the broth culture was sub-cultural on Mannitol Salt Agar , Eosin Methylene Blue Agar and Xylose Lysine Deoxycholate Agar by the four quadrant streaking method. Inoculated plates were incubated at 37^{o}C for 24 h .

3.8. Gram's stain reagents

Firm (smear) of each of the isolates were prepared by picking a small portion of microbial growth from the plates with the aid of a sterilized wire loops into a drop of sterile distilled water on glass slides and after making the smear, the slides were heat fixed by carefully passing them over a benzin burner flame.

The heat fixed smears were stained with crystal violet for 60 second, washed off with water and drained, then flooded with Lugol's iodine for about 60 second and washed off gently with water and drained. The samples/slides were rinsed with 50- 50 alcohol – acetone for 3 second and were rinsed with water and drained. The slides were then counter stained with Safranin for 1 min (60 s) after then, the stains were washed off with water. The slides were air dried. Immersion oil was dropped on the smears and examined under the oil immersion objective of the microscope.

3.9. Biochemical testing

The following biochemical tests were conducted and performed according to Barrow and Feltham (1993).

3.10. Bacterial viable count

The bacterial count was done according to Harrigan and McCane (1967).

3.10.1. Preparation of the dilutions

One germ of feed sample was added to test tube containing 9 ml sterile normal saline were prepared, by sterile tip on micropipette transferred 1 ml of dilution labeled as the first dilution.

Ten-fold serial dilutions of poultry feed samples were prepared. Four test tubes containing 9 ml sterile normal saline were prepared. A micropipette with sterile tip was held vertically and introduced not more than 3 cm below the surface of the feed sample and then 1 ml was taken to the first tube of the dilution series without touching the diluting fluid, the tip was discarded and the tube was labeled as the first dilution tube (10^{-1}). A fresh sterile tip was used to mix the contents of the first dilution and 1 ml of first dilution was transferred to the second tube of dilution series without touching the dilution fluid, the tip was discarded and the tube was labeled as the second dilution tube (10^{-2}). Further dilution of 10^{-3}, 10^{-4} and 10^{-5}. were prepared similarly.

3.10.2. Preparation of the plates

The surfaces of the Nutrient Agar plates were dried for half an hour at 37^{o}C. A fresh sterile tip was used to mix the contents of each dilution by sucking up and down ten times, then 0.02 ml of each dilution for 10^{-4} and 10^{-5} was withdrawn and transferred to Nutrient Agar and evenly distributed on the surface using a sterile glass rod. Two replicates of each dilution were made. The plates were labeled by the number of the dilution, and incubated at 37^{o}C for 24 h.

3.10.3. Colony count

Colonies of microorganisms that developed on the plates after incubation period were counted according to Miles and Misra surface colony count (Miles and Misra, 1938). An average colony count from the two replicates of each dilution.

3.11. Proximate analysis of feed sample

The proximate analysis of each of commercial feed samples was carried out according to procedures of AOAC (1995). For ash, moisture, crude fiber, fat and protein content.

3.11.1. Determination of Moisture Content :

Plastic dishes were washed thoroughly dried in the oven and placed in the desiccators to cool after which they were weighed. Samples were collected from the formulated diets and were added into the weighed dishes. Then, the weight of the dish and un-dried samples were weighed in duplicate. The samples in the dishes were dried in the oven at 70-80◦C for 2hrs and at 100-135°C for the next 4 hrs, till the o weight become constant. The samples were cooled in the desiccator and the dry weight of the sample and the dish was taken. Lastly, the moisture content was calculated as described in the equation 1 while % Total dry matter was calculated as 100 - % Moisture.

$$\% \text{ Moisture} = \frac{(w2-w3)}{(w2-w1)} \times 100$$

Where: W_1 = Initial weight of the empty dish,

W_2 = weight of the dish + un-dried sample,

W_3 = final weight of dish +dried sample.

3.11.2. Determination of Ash Content:

Two to five grams (2-5 g) dry samples were weighed accurately into a dish. The samples were charred on a heater inside a fume cupboard, to drive off most of the smoke. The samples were then transferred into a pre-heated muffle furnace at 550°C and were left at this

temperature for 2hrs (Until white or light gray ash resulted). When the residues were black in colour, there were moistened with a small amount of water to dissolve salts and dried in the oven once again and the ashing process repeated. They were then cooled in the desiccators, reweighed and the results were calculated as follows:

$$\% \text{ Ash} = \frac{Weight\ of\ Ash}{(Weight\ of\ Original\ Sample)} \times 100$$

$$\text{i.e } \% \text{ Ash} = \frac{(w3-w1)}{(w2-w1)} \times 100$$

where: W_1 = weight of empty dish,

W_2 = weight of the dish + un-ashed sample,

W_3 = weight of the dish + Ash.

3.11.3. Determination of % Crude Fiber:

Two gram of the materials were deflated with petroleum ether. The samples were boiled in a test tube for 30mins with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution. The mixtures were filtered and residue washed with boiling water severally to get rid of acid components. The residues were then transferred to a beaker and boiled for 30mins with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residues were filtered through a thin but close pad of washed and ignited asbestos in a crucible. They were dried in the oven and weighed. They were then incinerated, cooled and reweighed.

% Crude Fiber = The Lost in weight after incineration×100.

3.11.4. Determination of Fat Content

Two hundred and fifty millitre (250) mls clean boiling flasks were dried in an oven at 105 - 110°C for about 30 mins. The flasks were transferred into desiccators and allow to cool. Two (2) g of the samples were accurately weighed into the labeled thimbles. The boiling flasks were then filled with about 300ml of petroleum ether (Boiling point 40-60°C). The extraction thimble was lightly plugged with cotton wool. The soxhlet apparatus was assembled and allowed to reflux for about 6hrs. The thimble was removed with care and petroleum ether was collected in the top container of the set-up and drained into a container for re-use. The collected ether was removed and dried at 105-110°C for 1hr, until the flask was almost free of petroleum ether. It was then transferred from the oven into desiccators and allowed to cool.

$$\% \text{ Fat} = \frac{Weight\ of\ the\ Fat}{Weight\ of\ Origimal\ Sample} \times 100$$

3.11.5. Determination of Crude-Protein:

Two grams of the feed samples were weighed and added into a Kjeldahl flask. 5 g of anhydrous $NaSO_4$ was added to the feed sample. This was followed with addition of 1 g $CuSO_4$ and a tablet 4 of Kjeldahl catalyst. Into the mixture, 25ml conc. sulphuric acid and glass beads were introduced (Glass beads prevent bumping during heating). The mixture was heated gently in a fume cupboard, the heating was then increased with occasional shaking, till solution assumed a green colour. It was then cooled in a dessicator and all the black particles that settled at the mouth and neck of the flask was washed with distilled water. The mixture was reheated gently at first until the green colour disappeared and then allowed to cool. After cooling, the particles were rewashed into a

250 ml volumetric flask and the mark was made up with distilled water. This was followed by distillation, using Markham distillation apparatus. The sample was steamed through the Markham distillation apparatus for about 15minutes. Under the condenser, 100ml conical flask containing 5ml of boric indicator was placed, such that the condenser tip was under the liquid. 5ml of the digest was pipetted into the body of the apparatus via the small funnel aperture, washed down with distilled water followed by 5ml of 60% NaOH solution. This was steamed through for about 5-7 minutes to collect enough ammonium sulphate. The receiving flask was then removed and washed down the tip of the condenser into the flask. This was followed by the removal of the condensed water. The solution was titrated into the receiving flask using N/100 (0.01N) hydrochloric acid and the nitrogen content was calculated and hence the protein content of the sample.

3.12. Statistical analysis

The data were analyzed with SPSS software (Statistical Package for the Social Sciences) version 16.0.

All bacterial counts were converted to $\log_{10}$ CFU\cm^{-2} for analysis and Factorial design was performed, statistical significant was set at value of ($P \leq 0.01$).

CHAPTER FOUR

RESULTS

Table 2: Occurrence of Bacterial Isolates Contaminating Brands of Poultry Feeds (%):

Isolates	PS N=10 n(%)	BF N=10 n(%)	LF N=10 n(%)	Percentage overall N-30 n(%)
Escherichia coli	2(20)	8(80)	8(80)	18(60)
Salmonella spp	4(40)	6(60)	6(60)	16(53.33)
Staphylococcus aureus	2(20)	5(50)	1(10)	8(26.66)

KEY: PS = Pre Starter BF = Broiler Finisher

LF = Layer Finisher N = Number of samples

n = Number of isolates.

Table 2 shows the bacterial isolates recovered from the three brands of poultry feeds and their respective percentage occurrence . The overall sample specific percentage occurrence of the isolates revealed that Escherichia coli (60%), occurred more than salmonella spp (53.33%), and staphylococcus aureus (26.66%).

Table 3: Biochemical properties of isolated bacteria :

	Escherichia coli	Salmonella spp	Staphylococcus aureus
Gram reaction	GN	GN	GP
Cultural characteristics	Small rods (round convex colonies	Small rods	Cocci in cluster(yellow and smooth on mannitol salt agar)
Motility	+	+	-
Catalase	+	+	+
Oxidase	-	-	-
Coagulase	ND	ND	+
Indole	+	-	-
Methyl-Red	+	+	+
Voges proskaver	-	-	+
Citrate utilization	-	-	-
H2S production	+	-	-
Nitrate reduction	+	+	+
Urea hydrolysis	-	-	+
Glucose	A/G	A/G	A
Sucrose	-	-	A
Lactose	A	-	A
Maltose	A	A	A
Mannitol	A	A	A

KEY: + = Positive - = Negative

GP = Gram Positive ND = Not Done

GN = Gram Negative A = Acid production

A/G = Acid and Gas production

Identification criteria involve cultural and biochemical tests.

Table 4: Mean of viable Total bacterial count ($\log_{10}$ CFU\cm) for three types of poultry feed:

Locality	Feed type	10^{-4}	10^{-5}
		M±SD	M±SD
Sharg Alneel	Pre starter	4.99±0.13	5.81±0.29
	Broiler finisher	4.90±0.17	6.04±0.14
	Layer finisher	5.02±0.10	5.75±0.08
Khartoum	Pre starter	4.89±0.11	5.69±0.09
	Broiler finisher	5.11±0.10	5.86±0.09
	Layer finisher	4.96±0.12	5.79±0.10
Bahri	Pre starter	4.65±0.16	5.10±0.17
	Broiler finisher	4.59±0.26	5.26±0.24
	Layer finisher	4.46±0.15	5.10±0.17
Omdurman	Pre starter	5.21±0.94	5.65±0.16
	Broiler finisher	5.10±0.18	5.30±0.00
	Layer finisher	5.08±0.12	5.86±0.09
Jabel Awliaa	Pre starter	4.67±0.19	5.59±0.26
	Broiler finisher	4.61±0.15	5.36±0.10
	Layer finisher	4.84±0.06	5.67±0.06
Significant		N.S	N.S

NS: No significant difference.

No significant difference in mean total viable counts of bacteria of the three type of feeds was detected.

Table 5: Mean of viable Total bacterial count ($\log_{10}$ CFU\cm) for five localities of Khartoum State:

Locality	10^{-4}	10^{-5}

	M±SE	M±SE
Sharg Alneel	4.97±0.05[b]	5.87±0.05[a]
Khartoum	4.99±0.05[b]	5.78±0.05[a]
Bahri	4.57±0.05[d]	5.15±0.05[c]
Omdurman	5.13±0.05[a]	5.60±0.05[b]
Jabel Awliaa	5.71±0.05[c]	5.54±0.05[b]
Sig	**	**

**: Highly significant difference (P≤0.01).

There was high significant difference in viable counts of five localities of Khartoum State (P≤0.01), highest mean of viable count in jabel awliaa locality (5.71±0.05) while the least in bahri locality (4.57±0.05) .

Table 6: Proximate composition of the poultry feed samples(%):

Proximate composition (%)						
Locality	Feed type	MC	AC	FC	CF	CP
Bahri	PS	8	6.09	5	2.97	18.90
	BF	9	10.83	1.98	6.93	18.75
	LF	7.33	6.18	3.96	6.44	19.60
Jabel awliaa	PS	7	9	5.02	2.98	19.08
	BF	8.5	8.33	3..39	4.85	9.98
	LF	9	8.67	1.97	6.40	9.28

Key: MC= Moisture content AC=Ash content FC= Fat content

CF= Crude Fiber CP= Protein content.

PS= pre starter BF= Broiler finisher LF= Layer finisher.

The considerable percentage of ash, fat, crude fiber and protein content, for the samples were collected form Bahri and Jabel Awelia localities . The results show a deterioration in the nutritive value, specifically protein percent for samples collected from Jabel Aweliaa locality.

CHAPTER FIVE

DISCUSSION

The obtained results revealed that three major bacterial genera were isolated from poultry feeds samples analyzed, where by the time factor did not affect the bacterial isolates in the feeds. Animal feeds have been listed as one of the sources of microbes of farmed animals and poultry. Thus the high bacterials obtained may indicate a potential hazard to the animal. The high occurrence of bacterial species of public health concern may indicate obvious health hazard in terms of direct consumption of bacteriological contaminated feed or their toxins by farmed animal (Frazier and Westhoff, 1978). The source of these organisms may vary extensively. The bacterial genera may have originated from nitrogenous waste products used in compounding animal feeds such as dung and chicken excreta as reported by Ogbulie (1995). On the other hand, the presence of Escherichia coli and Salmonella species may suggest fecal as well as environmental contamination. Some of these organisms are well known pathogens of birds and farmed animals (Mallinson, 1984). For instance, E. coli is implicated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis whereas Salmonella and Staphylococcus aureus are capable of producing acute and chronic infections in all or most types of birds and animals (Mallinson, 1984). The presence of these bacteria in poultry feeds, suggests that the feeds contain sufficient nutrients for the growth of these organisms and the activities of these organisms on the feed under study may cause degradation thereby reducing the nutrients that would have been wholly available for the poultry to feed on. This is in accordance with the report of Aganaga et.al; (2000). These bacteria may probably have originated from the raw materials from which the feeds

are being produced. (Arotupin and Akinyosoye, 2001). D'Mello (2006) reported microbial contamination of poultry feeds of plant and animal origin are associated with climatic conditions, harvesting, processing, storage and transport technologies employed. However, package and packaging materials, environment and handling circumstances, including the nature and extent of the quality control measures greatly influenced the source and degree of contamination. (Dessie,1996; Hancock et al; 1998).

With the high colonization of bacteria in poultry feeds, good manufacturing practice, handling and retailing methods need to be improved to enhance the microbiological quality of these products, and thus health hazards by consumption such products minimized to remarkable extent.

CHAPTER SIX

Conclusion

This study revealed high microbial counts and the presence of pathogenic bacteria in different poultry feeds investigated.

Recommendation

1. Reflect the level of bio security and hygienic practices in the production, handling and storing of the feeds.
2. Incorporation of feed additives into poultry feeds that would prevent microbial contamination should be encouraged.
3. Findings emphasize the need of constant quality assessment of these commercial foods on sale order to maintain the production of microbiology stable poultry feeds and poultry products for human consumption.

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