Chapter one
INTRODUCTION

1-1 Introduction:-

Poultry is an important source of meat, worldwide, and the consumption of poultry products is still increasing. According to Pearson and Dutson (1997) and De Haan *et al.* (2001) this is due to increasing human population, religion (most world religions do not prohibit the consumption of poultry meat), the western beauty image (low fat percentage in poultry meat) and women working outdoors in the western world (poultry meat is fast and easy to cook).

Broiler breeds are selected for their rapid growth: nowadays, broiler chicks are growing from 50 g at day of hatch to more than 2 kg within 6 weeks. This genetic selection in combination with production in industrialized systems has fulfilled the increasing worldwide demand for poultry meat: over the last 40 years growth rate of broilers has more than doubled (Julian,1998). This rapid growth, however, poses these birds for high demands. The greater part of ingested energy is invested in growth, so Rauw *et al.* (1998) concluded that broilers, selected for high production efficiency, seem to be more at risk for welfare and health problems. Among other reasons, the rapid growth and high flock density might explain the frequent occurrence of a variety of health problems in broilers (Barlett, 1988). In this context, infectious diseases are important in broiler industry due to increased mortality, growth retardation, and the curative and preventive use of antibiotics and chemotherapeutics. Moreover, economic losses may result from loss of uniformity of the flock and condemnations in the slaughterhouse (Goren, 1991; Vandemaele *et al.*, 2002; McKissick, 2006).

In the Sudan ,poultry products on commercial basis was commenced in 1979 by Sudanese Kwaiti Poultry Production Company .Poultry product as Commodity is highly demanded by the domestic people and the public of the neighbouring countries especially the Arabs . The progressive market demand for poultry meat and table eggs encouraged investors to establish additionats large scale poultry production projects .In 1980 , the preceding commercial poultry projects achieved great stride in making poultry products available to consumers at favorable prices . In 1990 , however, several constrains have projected and jeopardized most of the accomplished successes (El-Amin ,1990) .

The rapid expansion of poultry production in the Sudan in recent years has Stimulated many workers to study many of major diseases of poultry which cause severe economic losses.

Gram-negative bacteria particularly *Escherichia coli* is one of the most Important causes of economic losses for broiler industry (Gross,1991;Yogartnam1995)

1.2. OBJECTIVES :-

The present work was carried out :

1-To isolate and identify of the aerobic Gram-negative bacteria from respiratory tract of diseased chickens in Khartoum North .

2-To assess the effect of the identified Gram –negative bacteria on broiler performance (growth rate ,feed intake , feed conversion ratio , weight gain carcass quality and mortality) . in Khartoum North.

3- To determine the hematological changes of experimentally infected birds by the most common isolated Gram negative bacteria .

Chapter two

LITERATURE REVIEW

2-1Bacteria:-

The Bacteria (*singular*: bacterium) are a large group of unicellular microorganism. Typically a few micrometers in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals. The name derives from the Greek, *baktērion*, meaning "small staff". Bacteria are ubiquitous in every habitat on earth, growing in soil, acid hot spring , radioactive waste, water, and deep in the Earth crust, as well as in organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cell in a gram of soil and a million bacterial cells in a millilitre of fresh water; in all, there are approximately five nonllion (5×10^{30}) bacteria on Earth, forming much of the world's biomass. Bacteria are vital in recycling nutrients, with many important steps in nutrient cycles depending on these organisms, such as the fixation of nitrogen from the atmosphere and putrefaction. However, most bacteria have not been characterized, and only about half of the phyla of bacteria have species that can be cultured in the laboratory. The study of bacteria is known as bacteriology, a branch of microbiology. Bacteria single cells have neither a membrane-bounded nucleus nor other membrane organelles like mitochondria and chloroplast(Cavalier,2006).

2.2. Gram Negative Bacteria:-

Gram-negative bacteria (or negibacteria) are bacteria that do not retain crystal violet dye in the Gram stain protocol . In a Gram stain test, a counterstain (commonly safranin) is added after the crystal violet, coloring all Gram-negative bacteria with a red or pink color. The test itself is useful in classifying two distinct types of bacteria based on the

structural differences of their bacterial cells walls. Gram-positive bacteria will retain the crystal violet dye when washed in a decolorizing solution (Salton and Kim ,1996) .

The pathogenic capability of Gram-negative bacteria is often associated with certain components of Gram-negative cell walls, in particular, the liposaccharide layer (also known as LPS or endotoxin layer (Salton and Kim ,1996) .

2.3.Classification of Gram- negative Bacteria:-

According to Madigan and Martinko (2005) .The *Proteobacteria* are a major group (phylum) of bacteria. They include a wide variety of pathogens, such as *Escherichia*, *Salmonella*, *Vibrio*, *Helicobacter*, and many other notable genera. Others are free-living, and include many of the bacteria responsible for nitrogen fixation. The group is defined primarily in terms of ribosomal RNA (rRNA) sequences, and is named for the Greek god Proteus (also the name of a bacterial genus within the Proteobacteria), who could change his shape, because of the great diversity of forms found in this group.

All Proteobacteria are Gram-negative, with an outer membrane mainly composed of lipopolysaccharides. Many move about using flagella, but some are non-motile or rely on bacterial gliding. The last include the myxobacteria, a unique group of bacteria that can aggregate to form multicellular fruiting bodies. There is also a wide variety in the types of metabolism. Most members are facultatively or obligately anaerobic and heterotrophic, but there are numerous exceptions. A variety of genera, which are not closely related to each other, convert energy from light through photosynthesis. These are called purple bacteria, referring to their mostly reddish pigmentation) Madigan and Martinko 2005).

2-4 Sections/Classes of Protteobacteria

The *Proteobacteria* are divided into five sections, referred to by the Greekletters alpha through epsilon, again based on rRNA sequences. These are often treated as classes. Although it has been suggested previously that the *Gammaproteobacteria* are paraphyletic to the *Betaproteobacteria*, recent molecular data suggests that this is not so. The divisions of the proteobacteria were once regarded as subclasses (e.g. α-subclass of the *Proteobacteria*), but are now regarded as classes (e.g. the *Alphaproteobacteria*) and should be styled in italics as one word

) Madigan and Martinko 2005).

Alphaproteobacteria **2-4-1**

The *Alphaproteobacteria* comprise most phototrophic genera, but also several genera metabolising C1-compounds (e.g. *Methylobacterium* spp.), symbionts of plants (e.g. *Rhizobium* spp.) and animals, and a group of pathogens, the Rickettsiaceae. Moreover the precursors of the mitochondria of eukaryotic cells are thought to have originated from *Rickettsia* spp) Madigan and Martinko 2005).

Betaproteobacteria **2-4-2**

The *Betaproteobacteria* consist of several groups of aerobic or facultative bacteria which are often highly versatile in their degradation capacities, but also contain chemolithotrophic genera (e.g. the ammonia-oxidising genus *Nitrosomonas*) and some phototrophs (members of the genera *Rhodocyclus* and *Rubrivivax*). *Betaproteobacteria* play an role in nitrogen fixation in various types of plants, oxidizing ammonium to produce nitrite- an important chemical for plant function. Many of them are found in environmental samples, such as waste water or soil. Pathogenic species within this class are the Neisseriaceae (Gonrrhea and meningitis) and edispecies of the genus *Burkholderia*) Madigan and Martinko 2005).

Gammaproteobacteria 2-4-3

The *Gammaproteobacteria* comprises several medically and scientifically important groups of bacteria, such as the *enterobactericeae* , *Vbirionaceae* and *pseudomonadaceae*. A number of important pathogens belongs to this class, e.g. Sallmonella spp. (enteritis and typhoid fever), *Yersinia pesti* (plague), *Virio cholerae* (cholera),*Pseudomonas aeruginosa* (lung infections in hospitalized or cystic fibrosis patients), and *Escherchia coli* (food poisoning) Madigan .(and Martinko (2005

Deltaproteobacteria 2-4-4

The *Deltaproteobacteria* comprises a branch of predominantly aerobic genera, the fruiting-body-forming *Myxobacteria*, and a branch of strictly anaerobic genera, which contains most of the known sulfate- (*Desulfovibrio*, *Desulfobacter*, *Desulfococcus*, *Desulfonema*, etc.) and sulfur-reducing bacteria (e.g. *Desulfuromonas* spp.) alongside several other anaerobic bacteria with different physiology (e.g. ferric iron-reducing *Geobacter spp.* and syntrophic *Pelobacter* and *Syntrophus* spp. .(Madigan and Martinko (2005

Epsilonproteobacteria 2-4-5

The *Epsilonproteobacteria* consists few known genera, mainly the curved to spirilloid *Wolinella* spp., *Helicobacter* spp., and *Compylobacter* spp. Most of the known species inhabit the digestive tract of animals and serve as symbionts (*Wolinella* spp. In cow) or pathogen (*Helicobacter* spp. in the stomach, *Campylobacter* spp. in the duodenum). There have also been numerous environmental sequences of *Epsilonproteobacteria* onmental samples. Membrecovered from hydrothermal vents and cold seep habitats Madigan and Martinko (2005).

2-5 Enterobacteriaceae

The Enterobacteriaceae are a large family of bacteria, including many of the more familiar pathogens, such as *Salmonella* and *Escherichia coli* . Genetic studies place them among the Proteobacteria, and they are given their own order (Enterobacteriales), though this is sometimes taken to include some related envirers of the Enterobacteriaceae are ros shapeg, and are typically 1-5 μm in length. Like other Proteobacteria they have Gram-negative stains, and they are facultative anserobes, fermenting sugars to produce lactic acid and various other end products. Most also reduce nitrate to nitrite, although exceptions exist (e.g. *Photorhabdus*). Unlike most similar bacteria, Enterobacteriaceae generally lack cytochrome C oxide , although there are exceptions (e.g *Plesiomonas)*. Most have many flagella used to move about, but a few genera are non-motile. They are non-spore forming, and except for *Shigella* strains they are catalase-positive.

Many members of this family are a normal part of the gutflora found in the intestine of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. *Escherichia coli*, better known as *E. coli*, is one of the most important model organism, and its genetics and biochemistry have been closely studied. Most members of Enterobacteriaceae have peritrichous Type I fimbriae involved in the adhesion of the bacterial cells to their hosts.

2-6 Characteristic of Gram-negative bacteria :-

According to Gibbson and Murray (1978) the characteristics of Gram-negative bacteria include :-

1-Cytoplasmic membrane .

2-Thin peptidoglycan layer (which is much thinner than in Gram –postive bacteria.

3-Outer membrane containingliposaccharide (LPS, which consists of lipidA, core polysaccharide, andOantigen) outside the peptidoglycan layer.

4-Porins exist in the outer membrane, which act like pores for particular molecules.

5-There is a space between the layers of peptidoglycan and the secondary cell membrane called the periplasmic space.

6-The S-layer is directly attached to the outer membrane, rather than the peptidoglycan.

7-If present, flagella have four supporting rings instead of two.

8-No teichoic acids or lipoteichoic acids are present.

9-Lioproteins are attached to the polysaccharide backbone.

10-Most of them contain Braun s lipoprotein, which serves as a link between the outer membrane and the peptidoglycan chain by a covalent bound.

11-Most do not sporulate (Coxiella burnetii , which produces spore-like structures, is a notable exception) .

2-7 Isolation of Gram –negative Bacteria from respiratory tract of Chickens :-

Khogali (1970) isolated six *Haemophilus gallinarum* , ten isolates of *Escherichia coli* and six isolates of *Staphylococcus albus* were isolated from respiratory system of chickens .

El-galii, (1992)isolated different positive and gram –negative bacteria from nasal cavity ,infra-orbital sinuses, tracheas and lung

which *included : Staphylococcus epidermidis , staphylococcus aures ,Micrococcus varians , Micrococcus roseus, Corynebacterium murium ,Bacillus cereus ,Bacillus firmus,Bacillus megaterium, Bacillus pantothenticus ,Bacillus brevis ,pseudomonas aeuroginos, pseudomonas*

diminuta , pseudomonas putida , pseudomonas fluorescen. Escherichia coli and Yersinai spp.

Murthy *et al*, (2008). isolated several micro-organisms involved in in respiratory diseases complex which included : *Ornithobacterium rhinotracheale, Pasteurella multocida , Haemophilus paragallinarum and Escherichia coli .*

E coli has been isolated from outbreak of respiratory diseases Chu, (1958) and from different sites of the respiratory tract of chickens .

Elnasri, (1997) isolate *E coli* from infra orbital sinus and treacha.

Secondary infection commonly occurs as complication of *Mycoplasma gllisepticum* nfection (Zahida, 2004) . *E coli* infection caused by a single agent occur rarely . *E coli* often infected respiratory tract of bird concurrently with various combination of infectious bronchitis viruses ,Newcastle viruses including vaccine strain,Mycoplasma Transmission can be through inhalation ,contamination of drink water or feed contamination (Zahida,2004).

E coli with respiratory infection in chickens has also reported by ELsukhon *et al* ,(2002) . with tracheitis ,exudative pleuritis and pericardits.

2-8 Common Gram-negative bacteria isolated from respiratory tract of chickens :-

2-8-1 *Escherichia coli* :-

2-8-1-1 Characteristics of *Eschericha coli* :-

Escherichia coli is Gram-negative ,facultative anaerobic and non – sporulating. Cells are typically rod-shaped and are about 2micrometeres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 (μm) .

E.coli is part of digestive tract of chickens. In general *E. coli* is of low pathogenicity for chickens (Hofstad *et al*., 1978; Nakamura *et al*., 1992), but pathogenic avian strains of *E. coli* (APEC) are identified, which belong to a small range of serotypes including O78:K80, O1:K1 and O2:K1 (Hofstad *et al*., 1978; Wray *et al*., 1996; Mead and Griffin, 1998).

Pathogenic *E.coli* differ from non pathogenic strain by the presence of virulence factors organized in clusters in the chromosome (Puente and finlay 2001). Some serotype cause specific diseases in poultry Known as colibacillosis ,which is a complex syndrome characterized by multiple organs lesions with air sacculitis and associated pericarditis , other cause diseases under certain condition and act as secondary invaders (Buxton and Fraser ,1977) .

2-8-1-2 Diseases causes by *Escherichia coli* :-

Colibacillosis :-

According to Barnes *et al.* (2003), colibacillosis in chickens refers to anylcalized or systemic infection caused entirely or partly by *Escherichia coli* . Colibacillosis is an infectious disease caused by the bacterium *Escherichia coli*, or *E. coli* as it is commonly known, and is seen in poultry flocks worldwide. *E. coli* can cause an infection under the skin, known as cellulitis, and is commonly associated with respiratory disease in birds, which in severe cases leads to septicaemia and death. Avian colibacillosis primarily affects broiler chickens between the ages of 4 and 6 weeks and is responsible for a significant proportion of the mortality found in poultry flocks. This mortality, treatment of the disease and decreased feed conversion efficiency result in significant

costs to the poultry industry(Moulin and Fairbrother 1999).

colibacillosis mainly results in respiratory infections (airsacculitis) and peritonitis/ pericarditis (Gross, 1961; Goren, 1978; Pourbaksh *et al.*, 1997).

Avian pathogenic *Escherichia coli* (APEC) are *E. coli* strain that can cause disease in birds of various ages : septicaemia, chronic respiratory disease, vitellus infection, salpyngitidis, peritonitis, chronic skin infections, osteomyelitis, swollen head syndrome (Mainil *et al*,2002).

Infection with APEC generally begins as a localized infection of the air sacs commonly referred to as airsacculitis or the air sac disease which in turn may spread to other internal organs resulting in systemic infection. This initial infection generally occurs in 4–9- week-old broiler chickens and in laying hens at the peak of egg production that takes place around week 30 (Bames *et al* ,1999).

Escherichia can cause disease such of air sac disease , salpingitis , omphalitis , alone or in combination with other pathogenic (Viruses and mycoplasma) .Colibacillosis is a secondary infection caused by inhalation, particularly in young broilers and poults. The severity of this septicaemic disease is due to a combination of factors as the virulence, exposure to erogenic infection, the *E. coli* strains involved and the intensity of the predisposing alteration of the respiratory epithelium by viruses.Mycoplasmas and/or CO2, NH3 and dust in the atmosphere. Despite preventive vaccination against several predisposing viral infections and the successful eradication of M. gallisepticum in the Netherlands, colibacillosis continues to be an important disease in the broiler industry. Treatment is confronted with a number of serious problems: high costs when treating flocks with the correct (relatively

high) dosage for a sufficiently long period, resistance to bacterial drugs and drug residues. Despite negligible resorption from the gastrointestinal tract, very satisfactory therapeutic results were obtained by oral treatment, even against in vitro resistant *E. coli* strains (Landman. and Cornelissen,2006).

2-8-1-3 Experimental infection of the respiratory system of broiler by *Escherichia coli* :-

In experimental infections with *Escherichia coli* (E. coli), serotype 026K60 was pathogenic only during the first week of age, whereas serotype 078K80 caused lesions during the first 3 weeks. Natural resistance against *E.coli* occurred after 1 and 3 weeks respectively. Chicks were much more susceptible to intra-tracheal than to oral inoculation. The disease could be produced in newly hatched chicks only with large oral doses. It is considered likely that natural infection occurs by the airborne route, and that most field infections are secondary to infections of the respiratory tract of by virulent viruses. Adequate ventilation, especially during the last half of the fattening period, is therefore recommended as a possible prophylactic measure (Gorena, 1978).

Another study determined optimal conditions for experimental reproduction of colibacillosis by aerosol administration of avian pathogen *Escherichia coli* to 2- to 4 wk –old broiler chickens . the basic model for reproducing disease was intra nasal administration of approximately 10 mean embryo infectious dose of infectious bronchitis virus (IBV)followed by aerosol administration of an O2 or an O78 strain of *E.coli* in a Horsfall unit (100ml of a suspension of 10 colony – forming units\ml over 40 min) . Score were assigned to groups of infected chickens on the basis of death ; frequency and severity of lesions in the

air sac,liver and heart;and the recovery challenge *E.coli* 6 days post-E.coli infection . An inteval of 4 days between the IB V and *E.coli* challenge was best whether the chicken received the IBV at 8 or 20 days of age , Typically ,50%- 80%of the chickens developed airsacculitis and 0-29 %of the chicken developed pericarditis or perihepatitis ,with little or no mortality .*Escherichia coli* alone resulted in no death and 0-20% air sacculitis ,but these percentage increased from 0 to 5%and 52% to 60%when the *E.coli* aersol administered through a cone shaped chamber .Administration of IBV alone failed to induce lesion .Recovery of the challenge E.coli did not correlate well with lesions. On the basis of these data ,administration of IBV to 20- days-old chickens followed by 4 days later by exposure to an avian pathogenic *E .coli* reproduce avian colibacllosis with low mortality , high percentage of airsacculitis ,and low percentage of septicemic lesions characterstic of the condition seen in the natural disease(Glyles t*e al*,1999).

In order study the dynamics of avian colibacillosis, commercial broiler chickens were inoculated with a pathogenic *Escherichia coli* strain (01:K1:H7) into the left caudal thoracic air sac. Chickens were euthanatized at different times from 3 to 48 hr postinoculation and examined for bacterialcounts and macroscopic and microscopic lesions. The E. coli strain colonized the air sacs, lungs, and trachea and was recovered from blood and all tested extrarespiratory organs of inoculated birds. A gradual increase in bacterial counts in the trachea, lungs, air sacs, and liver was observed from 3 to 12 hr. Clinical signs and macroscopic lesions of colibacillosis were observed in all inoculated birds. Moderate to severe lesions of airsacculitis, pericarditis, perihepatitis, and splenic hypertrophy were observed. Microscopically, inflammatory cell infiltration, serious to fibrinous exudate, and cellular debris on serosal surfaces were present in the liver, spleen, and air sacs. In air sacs,

heterophils were present in low numbers perivascularly 2hr after inoculation and became more numerous by 24 hr postinoculation.

Ultrastructurally, epithelial cells in the air sacs and in air capillary regions of the lung were swollen and vacuolated beginning at 3 hr postinoculation. Bacteria were adherent to and present within the epithelial cells at 3 hr postinoculation and were also seen in phagocytic cells and, rarely, in the connective tissue of these organs at 24 hr postinoculation. These results indicate that both air sacs and lungs can be the portal of entry for E. coli into the systemic circulation, probably via damaged epithelium (Pourbakhls et al ,1997).

2-8-1-4 Effect of *Escherichia coli* on broiler production:-

Day-old chicks may become infected via the yolk sac, but in older chicks the infection is considered to be mainly airborne. Young broiler chickens up to three weeks of age are highly susceptible to the disease, but chickens of four weeks and older are considered quite resistant to primary colibacillosis (Goren, 1978). However, various risk factors may increase the susceptibility of broilers to colibacillosis, e.g. respiratory viruses and environmental factors like dust and high concentrations of NH3 and CO2. In addition, the *E. coli* concentration in the air of the broiler house is an important factor. In general this concentration is highest in the second half of the production period due to increased production of droppings; and especially in winter due to limited ventilation. Airborne dust particles in broiler houses can contain 105-106 *E. coli* bacteria/g and these bacteria may persist for long periods (Harry and

Hemsley, 1965). As a consequence, colibacillosis most frequently occurs in the second half of the production period in flocks reared in the cold season.

Colibacillosis is mainly treated with antibiotics, but the use of these drugs is costly and the period in which broilers can be treated is limited because of the withdrawal period. Moreover, treatment often does not result in sufficient recovery before slaughter (Goren, 1991). So, colibacillosis is an often reported observation in slaughterhouses, and responsible for a considerable part of condemnations at processing (Yogaratnam, 1995).

2-8-1-5 Clinical signs of broilers chickens infected by *E.coli* :

Avian pathogenic *Escherichia coli (*APEC), the causal organism of *E. coli* infections of poultry, are responsible for significant morbidity and mortality in the poultry industry worldwide (Ewers *et al*,2003).

A controlled experimental *Escherichia coli* infection was developed in broiler chicks. Infection with *E. coli* significantly reduced feed intake, altered growth of the whole body, eviscerated carcass, skeletal muscles, heart and liver. Organ weight and/or the proportions of organs within the body were affected. Although the infected chicks showed no viable bacteria at day 12 after infection, chicks did not reach the same body weight as controls by day 30 after infection(Tian and Baracosa1989).

Broilers suffering from colibacillosis are depressed, show respiratory distress and growth retardation. Mortality usually remains below 5%, but morbidity often reaches more than 50% (Wray *et al.*, 1996; Vandekerchove *et al.*, 2004).

The chickens inoculated with *E.coli* developed rough feather and loss of appetite .The majority of the broiler chickens suffered from diarrhea by

10 h post inoculation (pi) and 23 pi ,some birds had died . (Shen *et al* , 2002).

The respiratory tract disease syndromes that occur in birds associated *Escherichia coli* infection, acute colisepticemia, characterized by hyperemic and with swollen viscera, tended to occur in young birds. Subacute fibrinopurulent serositis involving air sacs and pericardium was more common in older birds. Chronic granulomatous pneumonitis was not seen as flock epornitics but as chronic disease in birds dying in small numbers some time after one of the previously mentioned forms of the disease (Chevlle and Arp,1985).

Zaki,(2012) reporetd that under *E coli* infection there is a significant decrease on body weight at 7, 15, 30 days and mortality rate is increase in the 1" week after infection, signs of infection appears in the form of depression, loss of body weight, bloody diarrhea and ascites .

Clinically, the birds affected with colibacillosis sneezed, coughed, and were depressed (Nakamura *et al*, 1992).

2-8-1-6 Hematological parameters of broiler Chickens Infected by *Escherichia coli* :-

A significant decrease in RBcs count Hb concentrations PCV, MCV, MCH, MCHC after experimental infection of broiler by E.coli . Also TLC such decrease was very highly significant on days 30 of infection ($P< 0.01$)(Zaki *et al*, 2012).

2-8-2 *Citrobacter freundii* :-

Citrobacter .is a genus of Gram –negative coli form bacteria in the enterobacteriaceae family .Citrobacter freundii can use citrate as a sole carbon source and has the ability to convert tryptophan to indole , ferment lactose, and use malonate .Lipsky et al (1980).

Citrobacter freundii are long –rod shaped bacteria .typically 1-5 m in length Most *freundii* are surrounded by many flagella used to move about, but a few are not motile . It habitat includes the environment (soil , water, sewage) ,food and intestinal tract of animals and human (wang et *al* ,2000).

Citrobacter freundii is responsible for a number of significant opportunistic infection It is known to be the cause of nosocomial infections of the respiratory tract blood, urinary tract and several other sites (Whales *et al*, 2007) .

A study conducted by Chanq ,(2000) to determine the presence of Salmonella spp. In raw broilers and shell eggs in Korea. In total, 135 dozen shell eggs and 27 raw broilers were tested. None of the egg yolks were found to contain Salmonella organisms but *Escherichia coli, Escherichia hermanii*, and *Citrobacter freundii* were isolated from egg .

2-8-*3 Haemophilus paragallinarium*

Heterogenous group of small Gram-negative bacteria ,aerobic bacilli ,non

spore forming (Gordan ang Joradn ,1982).

Haemophilus paragallinarium requiring enriched media for culturing and growth , the organism is classified according to the X factor (hemin) and V factor (nicotinamide adenine dinucleotide) (Eliot and Lewis ,1934) also Narita *et al*,(1978) and Black and Reid (1982) confirmed this finding .Two species are named : *Haemophilus gallinarium* and *Haemophilus paragallinarium* ,these two species are identical in growth charcterstic and ability to produce disease(Rinter ,1979).

De Bleich (1932). was the first one who isolated causative agent of infectious coryza and named and name the organism *H coryza gallinarium*

Infectious croyza is an acute or chronic disease of upper respiratory tract of poultry caused by Haemophilius group of bacteria .

Bacteriological examination of infectious coryza infected chickens in Sudan show the disease is caused by *H gallinarium* (Shigidi,1971).

The is usually transmitted through drinking water contaminated with infectious nasal exudates Page , (1962).infection may also occur by air-borne infected dust or droplet.

2-8-4 *Pseudomonas spp* :-

Gram-negative , rod shape , motile ,aerobic and non spore forming .The

organism is distributed widely in nature and found in soil and water .

Pseudomonas spp are associated with infection in man and animal (Marchent and Packer ,1967). is not only responsible for embryonic mortality but also for motality of chickens and heavy losses of broiler (Valadae ,1961; Saad *et al*,1981 ; Andreev *et al* 1982 and Bapat *et al* , 1985) .

2-9 Broiler performance :-

There are several measures that can be used to evaluate the performance of a flock of broilers – growth rate, days

to market, mortality, and feed efficiency. Feed is typically the most costly expense in broiler production. As a result, feed efficiency is typically the primary tool by which a flock is evaluated. In North America, feed efficiency is calculated by dividing feed intake by weight gain, resulting in typical values around 1.8 for 42 day old broilers. Thus the lower the number (referred to as Feed Conversion Ratio – FCR) the more efficient the flock was in using the feed supplied. In some European countries, however, feed efficiency is calculated as weight gain divided by feed intake, and a corresponding value would be 0.56. For Europe, therefore, higher numbers represent a more efficient feed conversion .

2-10 Factor affecting broiler performance :--

Many factors affect both growth rate and feed intake, and thus affect feed efficiency, the single largest factor affecting feed efficiency is :

2-10-1 Energy level of the feed

Several years ago high energy feeds were given - examples 3,000 kcal/kg (1361 kcal/lb) in the starter; up to 3,200- 3,300 kcal/kg (1452-1497 kcal/lb) in the finisher. Now, because of the cost of energy-rich

feedstuffs, as well as other management problems, much lower energy values are typically used in formulating all diets of a feeding program. Broilers are also being grown to a wide variety different market weights. Feed efficiency declines as broilers get older so it can not be compared with different age flocks.

2-10-2 House temperature

Probably the most important non-dietary factor influencing feed conversion is the ambient temperature of the poultry house. Chickens are homeotherms (warm-blooded) meaning they maintain a relatively constant body temperature regardless of the environmental temperature. Broilers perform best when there is minimal variation in house temperature over a 24 hour period of time. There is a trade off between energy provided by feed or fuel, and the most economical temperature will depend on the relative prices of the two.

In a cool environment, broilers will eat more feed but many of the calories they obtain from this feed will be used to sustain normal body temperature. When the calories are used for warmth, they are not converted to meat. Optimum temperatures allow the broilers to convert nutrients into growth rather than using the calories for temperature regulation. The ideal environmental temperatures for promoting feed conversion will be provided by your service personnel. At high environmental temperatures, broilers consume less feed, and convert this feed less efficiently. The biological cooling mechanisms that birds use during hot weather (panting, etc.) require energy, just as the warming mechanisms do during cool weather.

2-10-3 Litter quality

Litter conditions significantly influence broiler performance and, ultimately, the profits of growers and integrators.

Litter is defined as the combination of bedding material, excreta, feathers, wasted feed, and wasted water.

2-10-4 Feed wastage and feed deprivation

Placing too much feed in the chick feeders results in feed wastage and contributes to an inferior feed conversion. To prevent excessive loss of feed, add small quantities of feed to the feeder lids by running the automatic feeders frequently for short periods. This will stimulate the chicks to eat more often. Also, this will encourage the chicks to feed from the automatic feeding equipment quickly.

Feed deprivation can occur during the growing period and contribute to an inferior feed conversion. This often occurs the first time the automatic feeding system is raised. Be careful not to raise the feeders too early and/or too high during the production cycle.

Early feed deprivation will result in uneven growth, causing poor

uniformity.

2-10-5 Diseases and culling

The general health of a flock influences feed conversions. Sick broilers do not perform well. administration can adversely affect weight gain and feed conversion. Eliminate, as early in the grow-out as possible, broilers that have no chance of making it to market.

Obviously an unhealthy broiler is likely to have poor feed efficiency. The main reason for this is that feed intake is reduced, and so again proportionally more feed is directed

towards maintenance. With enteric diseases there can be more subtle changes in feed utilization because various parasites and microbes can reduce the efficiency of digestion and absorption of nutrients. A broiler with sub-clinical coccidiosis is not likely to absorb nutrients with optimum efficiency, because the oocytes will destroy some of the cells lining the cut. More recently the phenomenon of so-called ‘feed-passage’ has been observed in broilers. Undigested feed particles are seen in the excreta, and so consequently feed efficiency will be affected. The exact cause of this problem is unknown, but is most likely the consequences of a microbial challenge.

2-10-6 Human factors

The relationship between human behavior, broiler fear levels and productivity indicate potential that exists to improve productivity by reducing fear levels in broiler chickens. The behavioral response of broilers to humans was used as a measure of broilers’ fear of humans. A significant positive correction was observed between speed of movement and first week mortality, which would appear to be mediated by broiler fear levels. This would indicate that very young broilers may be susceptible to stressors such as the stockperson’s speed of movement. However, this susceptibility may be reduced as the broilers grow and become habituated to stockperson behavior

Chapter Three

MATERIALS AND METHODS

3-1 Isolation and identification of aerobic gram –negative bacteria from respiratory tracts and internal organs of chickens with respiratory system disorder :-

3-1-1 Study Area and duration of the experiment :-

This study was undertaken in three areas of Khartoum North , Poultry Market (Bahary and Kuku market) ,East of the Nile Poultry farms and Central of Khartoum North poultry farms (Shambat and Samrab). The study was conducted during May2009- August 2010

3- 1-2 Sampling:-

3-1-2-1 Source of samples:-

During this study 720 different samples were collected from 120 sick chickens with clinical symptom of respiratory tract diseases .These symptom included mucoid and serous nasal discharge ,sneezing ,lacrimation and facial swelling . The samples were collected from common breeds raised in Khartoum North (foreign strains including both sexes at different ages).

The majority of birds (80 birds) were obtained from the poultry farms for meat production in Khartoum north, while other birds (40 birds) were taken from poultry market .

3-1-3 Specimens For Bacteriological Examination :-

720 samples were taken from 120 chickens .The samples were taken from trachea , lung , liver , spleen , intestine , and reproductive system under sterile aseptic conditions.

3-1-4 Method of sterilization ;-

Sterilization was carried according to the method of Merchant and Packer (1967).

3-1-4-1 Dry Heat :

3-1-4-1-1 Hot air oven :

Hot air oven sterilization was used for the clean glass ware that were wrapped in papers or in stainless steel cans. The temperature and time of exposure were 160 C^{o} for 1 hour.

3-1-4-1-2 Red heat :

This method was used for sterilization of wire loops ,straight wires , point of tissue forceps and searing spatula.

3-1-4-1-3 Flaming :

This method was used for sterilization of the cotton plugged tubes opening, scalpels and glass slide .It was done by exposing the object to flame for 5-50 seconds .

-1-4-2 Moist heat :\

3-1-4-2-1 Autoclaving :

Autoclaving was used for sterilization of cultural media and material that could not withstand the dry heat at 115^{o} C (15 Ib/inch) for 15 minutes .

3-1-5 Preparation of Media :-

3-1-5-1 Sugar Media

Those media were prepared as followed 900 ml of the base medium was adjusted to the pH 7.1 .10 ml Andrad' s indicator was added .The mixture was sterilized by autoclaving at 115^{o} C for 15 minutes . ten gr ams of the appropriate sugar were dissolved in 90 ml of distilled water and sterilized by autoclaving . The sugar solution was added a aseptically to the

above base medium and mixed thoroughly .The medium was tubed in five ml amount (Barrow and Feltham 1993).

3-1-5-2 Selenite Broth :-

The broth was prepared by dissolving the ingredients Barrow and Feltham (1993) in the water with gentle heat. The pH was adjusted to 7.2. The mixture were sterilized at 121° C for 30 minutes. Sodium hydrogen selenite , 40 % solution was sterilized by filtration and store at 40° C. 1 ml 40 % selenite solution was added to the 99 ml of broth , mixed and distributed into 4 ml sterile test tubes .

3-1-5-3 Nutrient Broth :-

The ingredient were product of Oxoid dehydrate d nutrient broth ; 13 grams were added to 1000 ml of distilled water .The medium was thoroughly mixed ,the pH adjusted to 7.2 and sterilized by autoclaving at 121° C for 20 minutes .

3-1-5-4 Methyl Red and Voges – Proskaure Media :-

These media were prepared according to Barrow and Feltham (1993) . Five grams of peptone and five grams of potassium hydrogen phosphate were dissolved in 100 ml distilled water and steamed until solid were dissolved . The media were adjusted to PH 7.4 .Five grams of glucose per 1000 ml were added to the media then the media were distributed into tubes and sterilized by autoclaving at 115° C for 15 minutes.

3-1-5-5 Peptone water :-

The medium was prepared by dissolving 10g peptone and 5 g sodium Chloride in 1000 ml of distilled water .The mixture was distributed in 5ml volumes into clean bottles and sterile bottles and sterilized by autoclaving at 121 °C for 15 minutes.

3-1-5-6 Nutrient agar slant :-

This was prepared by adding 28 grams of nutrient agar to 1 L of distilled water and dissolved by boiling .The pH was adjusted to 7.4 .The prepared medium was distributed in 10 ml volume into clean bottles , sterilized by autoclaving at 121^{o} C and left to solidify in inclined position.

3-1-5-7 Glucose-phosphate medium (MR-VP)test medium :-

Five grams peptone and 5grams phosphate buffer were added to 1 L distilled water ,then dissolved by steaming and filtered .The PH was adjusted to 7.5 , 5 gram of glucose was added and then well mixed .The complete medium was distributed into clean test tube in 10 ml amount. The medium was sterilized by autoclaving at 115^{o} C (15 Ib/inch) for 15 minutes.

3-1-5-8 Nutrient Agar :-

This was prepared by adding 28 grams of nutrient agar to 1 l of distilled water and dissolved by boiling .the pH was adjusted to 7.4 and then sterilized by autoclaving at 115 C (15 Ib/inch) for 15 minutes .The prepared medium was distributed in 20 ml volume into sterile petri dishes .The poured plate were allowed to solidify on flat surface .

3-1-5-9 Blood Agar :-

This was prepared according to Barrow and Feltham(1993) by suspending 40 grams of blood agar base in 100 ml distilled water and dissolved by boiling .The mixture was sterilized by autoclaving at 115^{o} C (15 Ib/inch) for 15 minutes and cooled down to about 50^{o} C , then defibrinated sheep blood was added a aseptically to make a final concentration of 10 % . The prepared medium was mixed and distributed in 20 ml volumes into sterile petri dishes .The poured plate were allowed to solidify on leveled surface .

3-1-5-10 : MacConkey' sAgar :-

Fifty two gram of MacConky s agar were dissolved in one liter of distilled water .The pH was adjusted to7.4 sterilized by autoclaving at121^{o} C (15/inch^{2}) for 15 minutes and then distributed in 20 volume into sterile dishes .The poured dishes were allowed to solidify on flat surface .

3-1-5-11 Haugh Lefson's medium O/F medium :-

This medium consists of pepton powder , 5grams of sodium chloride ,0.3 gram of dipotassium hydrogen phosphate (K_2HPO_3) and 3 gram of agar .Those solids were dissolved in 1oo ml distilled water .The pH was adjusted to 7.1 and the indicator bromocresol purple was added . The complete medium was distributed aseptically into 10 volumes sterile test tubes sealed with cotton plugs .

3-1-5-12 Simmon' Citrate medium ;-

The medium contains sodium ammonium , ammonium di-hydrogen phosphate ,magnesium sulphate ,sodium chloride ,bromothymol blue as indicator and agar .The medium was obtained from Oxoid(Ltd).It was prepared according to Barrow and Feltham(1993) by dissolving 17 grams powder in 1L distilled water .The prepared medium was distributed in 10 ml volumes into clean bottles , sterilized by autoclaving at 121^{o} C(15/inch$^{2)}$ for 15 minute and left to solidify in inclined position.

3-1-5-13 Urea agar media:-

This medium was obtained from Oxoid (Ltd) . It contains peptone water ,dexterose , disodium phosphate , sodium chloride , potassium dihydrogen phosphate , a gar and phenol red . It was prepared according to manfacture instruction by adding 2.4 grams to 95 ml of distilled water and dissolved by boiling .The prepared medium was sterilized by autoclaving at 121^{o} C and then 5 ml of sterilized 40% urea solution (Oxiod SR2) were added under aseptic condition .The medium was

distributed in 5 ml volume into sterile bottles and left to solidify in inclined position .

3-1-6 Diluents :-

3-1-6-1 Physiological Saline :-

The solution was prepared as described by Barrow and Feltham (1993) . To prepare the diluents 0.85 gram of sodium Chloride (Na Cl) was added to 100 ml distilled water , mixed , dissolved and sterilized at 121 C and under pressure 15 pounds per square inch for 15 minutes .

3-1-7 Reagents:-

3-1-7-1 Alpha-naphthol solution :-

Alpha-naphthol is a product of British Drug House (BDH) ; London. This reagent was prepared as 5% aqueous solution for Voges Prokauer (VP) test

3-1-7-2 Potassium hydroxide :-

This was used for Voges Prokauer test and prepared as 40% aqueous solution

3-1-7-3 Hydrogen peroxide :-

This reagent was obtained from Agropharm Limited Buckingham.It was prepared as 3% aqueous solution and it was used for catalse test .

3-1-7-4 Methyl red :-

This was prepared by dissolving 0.04 g methyl red in 40 ml ethanol .The volume was made to 100 ml with distilled water .It was used for Methyl red test (MR).

3-1-7-5 Tetra methyl-P-phenl diamine dihydrochloride :-

This was obtained from Hopkin and William ; London .It was prepared in aconcentration of 3% aqueous solution and was used for oxidaze test .

3-1-7-6 Nitrate test reagent:-

Nitrate test reagent was consisting of two solution which were prepared according to Barrow and Feltham9 1993) .solution A was composed of 0.33% sulphanilic acid dissolved by gentle heating in 5-N acetic acid solution .B was composed of 0.6% dimethyl amine-alpha-naphthylamine dissolved by gentle heating in 5 N-acetic acid . It was used for nitrate reduction test .

3-1-7-7 Kovac's reagent:

This reagent is composed of para-dimethyl aminobenzaldehyde , amylalcohol. The reagent contains p-dimethyl amino-benzaldhyde amyl alcohol and concentrated hydrochloric acid.

It was prepared as described by Barrow and Feltham (1993). by dissolving the aldehyde in alcohol by heating in water bath ,it was then cooled and acid was added carefully .The reagent was protected from light and stored at 4 C for later use in indole test .

3-1-8 Indicators:-

3-1-8-1 Andrade, s indicators:-

It consists of 5 g of acid fuchin, 1L distilled water and 150 ml N Na OH. The acid fuchin was dissolved in distilled water and then the alkali solution was added and mixed . The solution was allowed to stand at room temperature for 24 hour with frequent shaking until the color changed from red to brown.

3-1-8-2 Bromothymol blue :-

Bromorhymol blue was obtained from BDH. The solution was prepared by dissolving 0.2 g of Bromorhymol blue powder in 100 ml distilled water .

3-1-8-3 Phenol red:-

Phenol red was obtained from Hopkin and Willium Ltd; London, It was prepared as 0.2% aqueoua solution

3-1-8-4 Lead Acetate :-

Filter paper strips , 4-5 mm wide and 50-60 mm long were impregnated in lead acetate saturated solution and then dried .It was used for hydrogen sulphide test .

3-1-9 Sampling and culture :-

Sterile cotton wool swabs were used for taking samples from inside trachea and intestine t of recently slaughter chickens . Lung ,liver , spleen and reproductive system were incised by scalpel and the samples were taken by rubbing the swabs in side these organ .

3-1-10 Primary culturing :-

The swabs from sick chicken were inoculated on nutrient agar and incubated aerobically over night at 37 C^{o} for 24 hours .The plates were observed for bacterial growth , isolated colony was picked and sub culturing on Mackonkey and blood agar.Asingle colony with similar characteristics was picked observed .smear were made from each for preparation of smear and stained by Gram's stain for examination of staining and morphological characters of the isolate using bright light microscope .The cultural characteristics of the isolate were confirm by inoculating the pure colonies on nutrient agar and nutrient broth test .Biochemical tests were performed to confirm species of the bacteria.

3-1-11 Purification and preservation of cultures :-

of Purification of cultures was made by sub culturing part of typical well separated colony on nutrient agar .

The process was repeated several times .The purity of the culture was checked by examining stained smear . Pure culture was then inoculated into nutrient agar slant medium and incubated over night at 37 C ° .The pure culture was then stored at 4 C ° for studying cultures and bio-c chemical characteristics isolates.

3-1-12 .Microscopic examination :-

Smears were made from each types of colony on primary cultures and from purified colonies.

They fixed by heating and stained by Gram s stain method as described by Barrow and Feltham (1993).and examined microscopically under oil immersion lens .The smear was examined for cell morphology, cells arrangement and stained reaction .

3-1-13 Staining Technique:-

Clean microscopically slide, normal saline , bacteriological loop and Bunsen burner were used .

A part of an isolated colony was emulsified in a drop of saline the centre of the slide and spread thinly , left to dry and fixed by passing 2-3 times over the flame . the smear were stained as follow :-

I. Crystal violet solution was applied for two minutes.
II. The smear was washed with distilled water.
III. Lugol, s iodine solution was applied for one minutes.
IV. The smear was then wash with distilled water.
V. The smear was decolorized by acetone for ten seconds.
VI. The smear was then washed with distilled water.

VII. Diluted carbolfuchsin solution was applied for one minute.

VIII. The smear was washed with distilled water, dried and examined microscopically .

Gram–negative bacteria appeared red to pink in color, while Gram positive bacteria appeared deep violet (Carter *et al*, 1986).

3-1-14 Identification of bacteria:-

The purified isolated bacteria were identified according to criteria outlined by Barrow and Feltham (1993). This included stained reaction , cell morphology ,growth conditions, colonial characteristic on different media and biochemical characteristic .

3-1-15 Bio-chemical method for identification of isolated bacteria :- All biochemical tests were performed as described by Barrow and Feltham (1993) .

They included :-

3-1-15-1 Catalase test :- A drop of 5 % hydrogen peroxide was placed on a cleaned microscopic slide and a colony of tested culture on nutrient agar was picked by glass rod and added to the drop of H_2 O_2 .positive reaction was indicated by evolution of gas (air bubble) .

3-1-15-2 Oxidase test :-

Strip of filter paper was soaked in 1% solution of tetra methyl-p-phenylene di hydrochloride and dried in hot air oven and then paced on clean glass slide by sterile forceps .Afresh test culture put on the filter paper strip. If purple color developed with 5 -10 second ,the reaction was considered positive .

3-1-15-3 The oxidation and fermentation (OF) test :-

Duplicated tubes of Haugh and Liefson' s medium were inoculated by stabbing with straight wire .One of the tube was sealed by a layer of sterile soft paraffin oil to protect it from air , both inoculated tubes were incubated at 37 C^0 and examined daily for a period of fourteen days .Yellow color in open tube indicated oxidative reaction , yellow color in both tubes indicated fermentative reaction .Blue color in the open tubeand green in the sealed tnbe indicated production of alkali .

3-1-15-4 Nitrate reduction test :-

Nitrate broth was inoculated lightly and incubated for up to five days and 1 ml of reagent (A) sulphanilic acid was added followed by 1 ml of reagent (B)α- naphthylene amine. A deep red color indicated that nitrate has been reduced .To the tubes not showing a red color within 5 minutes ,powder zinc was added and allowed to stained .Red color indicated negative reaction .

3-1-15-5 Urease activity test:-

Urease test medium of christensen was inoculated with the isolated bacteria under test ,incubated at 37° C and examined for up to seven days .Urea hydrolysis was indicated by the change of the medium to pink color .Blue color indicated positive reaction.

3-1-15-6 Hydrogen sulphide production :-

A tube of peptone water was inoculated by tested organism and lead a cetate paper was inserted between the cotton plug and the examined daily for a week . Blacken of paper indicated H_2S production

3-1-15-7 Citrate utilization test

A single streak was done over the surface of the slope of Simmon' s citrate medium , then incubated at 37° C and examined daily up to seven days . Blue color indicated positive reaction .

3-1-15-8 Indole production test :-

Pepton water was inoculated by isolate to be tested and incubated at 37 C° for 48 hours . One drop of Kovac' s reagent was added to the the medium . The presence of indole was indicated by development of pink color .

3-1-15-9 Methyl red (MR) test :-

The test culture as inoculated into glucose phosphate medium and then incubated at 37 C ° for 48 hour . Two drops of Methyl red reagent were added and shaken well .Red color indicated positive reaction ,yellow or orange color indicated negative reaction .

3-1-15-10 Vogues-Proskaur (V.P) test :-

The test culture as inoculated into glucose phosphate medium and then incubated at 37 C ° for 48 hour . One ml of culture medium was transferred aseptically into sterile test tube and then 0.6 ml of 5 %alpha-naphthol solution was added , followed by 0.2 of 40 % KOH aqueous solution .The test tube was shaken well and kept at slant position for 1 hour . Positive reaction was indicated by strong red color .

3-1-15-11 Test for sugar break down:-

Carbohydrated medium was inoculated with test culture then incubated at 37 C° and examined daily for 7 days . The acid production was indicated by change in color to pink and gas production was indicated by presence of empty space in Durham ' s tube .

3-1-15-12 Motility test:-

Motility medium was inoculated by stabbing with straight wire into the centre of the Cragie tube and then incubated at 37 C° for 24 hour . The

organism was considered motile if there was turbidity in the medium in and outside the Cragie tube while the growth of non motile organism confined inside Cragie tube .

3-2 Effect of isolated Gram-negative bacteria [*Ecoli* and *Citrobacter frendii*] on broiler performance ,carcass quality and blood picture :-

3-2-1 Experimental Site, duration and housing :

The experiment was conducted in the premises of College of Veterinary Medicine ,during the period 15 December 2010 to25 January 2011,where the average range was between 28^{o} C to 38^{o} C.

The experimental birds were accommodated in an open sided , deep litter constricted of iron posts ,corrugated iron sheets roofing, wire netting sides and concrete floor. The long axis of the house was located in east – west facing the direction of the winds foe efficient natural ventilation .The house was partitioned into equal size pens of 1 square meter each , The dividing partitions were made of wire netting walls .

The pen was cleaned and disinfected by 5% formalin .A four inch layer of wood shaving litter material was laid on each pen .60 watts incandescent light bulbs was used to provide continuous lighting and warmth through- out the experimental period .

3-2-2 Dietary ingredient and experimental diets :-

The ingredient included sorghum , ground nut meal , sesame meal ,super concentrate oyster shell , bone meal and salt were purchase from kuku Market .

On basis of analyzed chemical composition of ingredient (Ellis,2000) , iso-caloric and iso-nitrogenous. Diet was formulated to meet the requirements recommended by National Research Council (NRC,1984)

are shown in Table (1) . The calculated and the determined chemical composition of the experimental diet are shown in tables (2) and (3) respectively .

3-2-3 Chemical composition of the experimental diet :-

The chemical analysis of the experimental diets was carried out in accordance of standard method of Association of Official Analytical Chemists (A.O.A.C.,2000) .The metabolizable energy was calculated on the basis of the chemical composition using the equation of(Lodhi *et. al* 1976) that:

ME = 1.549+0.0102CP+0.0275EE+0.0148NFE-0.0034CF .

ME : Metabolizable energy . CP : Crude protein , EE :Ether extract . NFE: Nitrogen free extract , CF : Crude fiber

3-2-4 Experimental birds :-

A total of 130 one day old un- sexed commercial broilers chicks(Lohmann) were purchased from Arab Company for Live stock Development (ACLID).The birds were transported at night to College of Veterinary Medicine and Animal Production (Kuku) .

3-2-5 Experimental Design:-

The experiment was conducted under complete randomized design. Each of the three treatment(Group A: control Group ,B: infected with *Escherichia coli* and group C infected with *Citrobacter freundii*) was randomly imposed to birds in 18 experimental pens so that each treatment was replicated 6 times and in each replicates 7 birds were used

3-2-6 Husbandry and Management procedure :-

Immediately after the birds arrival, the chicks were a accommodated and brooded over night in a prepared pen . In the next morning, chicks were weighed, under weighed, over weighed and morbid chicks were

discarded. The remaining chicks were randomly selected, weighed and assigned into three treatment group .Each treatment group was divided further into 6 replicate of 7 birds each (18 experimental pens , 7 chicks /pen). Broiler chickens were allowed to consume feed and water ad libitum during the 49 days trial period .

3-2-6-1 Broiler performance :-

Production performance (weight gain, feed intake and Feed conversion ratio) were measured on weekly basis for 7 weeks.

3-2-6-1-1Feed intake:-

Daily feed offered was recorded and refused feed was weighed at the end of each week to determined weekly feed consumption.

3-2-6-1-2 Weight gain :-

Weight gain was calculated by weighting the broiler chickens at the begins and the end of each weeks and by subtracting weight gain of each weeks was determined

3-2-6-1-3 Feed Conversion ratio:-

Data of weight gain and feed intake was used to calculate feed conversion ratio (FCR).

$$FCR = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

3-2-6-1-4Mortality : - Mortality was recorded when occurred.

Table (1) :-Ingredients of the Formulated Experimental diet (Kg):-

Ingredients	Kg
Sorghum	65
Groundnut meal	16
Sesame meal	12
Super concentrate*	5
Oyster shell	0.7
Bone meal	1
Salt	0.3
Total	**100**

*Broiler super concentrate ; 1600 K.Cal , 35.5% Crude protein , 10% Calcium ,4.1% Phosphorus ,12% Lysine ,4.5%Methionine .

Table (2): Calculated Chemical Composition of the Experimental Diet

Constituents	
ME(Mj/Kg)	13.00
Crude protein%	22.0%
Crude fiber%	4.1%
Lysine%	1.2%
Methionine%	o.5%
Calcium%	1.25%
Phosphorus %	0.49%

Table (3) Determined Chemical Composition of the Experimental Diet :-

Component	
Dry matter %	93.22%
Crude protein %	22.68%
Ether extract %	4.41%
Crude fiber %	6.50%
Ash %	7.40%
Nitrogen free extract (NFE)	52.23
Metabolizable energy (MJ/Kg)	12.60

3-2-7 Preparation of bacterial inoculums size :

E.coli and *Citrobacter freundii* as the most common Gram negative bacteria were selected for experimental infection .

Pure colony of identified bacteia *Escherichia coli* and *Citrobacter freundii* were cultured in nutrient agar at 37° C for 24 hours ,then swabs were taken from the agar and immersed in test tubes containing 10 ml sterile normal saline and 10 fold dilution were prepared (Qunin *et al*, 2000).A total of 100µ (0.1ml) was taken from final dilution and poured in sterile Petri-dishes and then (15-20) ml of sterile nutrient agar solution (NA)were added to the Petri dishes content . Mixing was done by shaking the Petri-dishes. The content were left to solidify before being incubated at 37ºC for 24to48 hours for colony count . The average value from each duplicate from the same dilution was taken .The colonies were calculated using the following formula:

Colony count ={(Average value ×10× Dilution factor)/10})CFU/cm ^{2} (10 refers to the area used for counting ,CFU /cm^{2} refer to colony forming unit per square centimeter.

At 28 days of age birds on treatment B were inoculated with *Escherichia coli* treatment C was inoculated with *Citrobacter freundii* oculo-nasally (one droplet of 0.2 ml per bird in each eye and nostril) of inoculums . The concentration was5.7 × 10^{9} CFu (Colony forming unit) *E. coli/*ml and the concentration was 4 × 10^{9} *C freundii* /ml.

3-2-8 Carcass preparation :-

At the end of the 7 weeks experimental period ,the birds were fasten for 16 hours and then three birds from each treatment were randomly selected , weighed , leg banded , slaughtered and de-feathered by using boiling water. Head and legs were removed and weighed .Then complete evisceration of the carcasses was fulfilled by a posterior-ventral cut .

The weight of the hot carcasses and internal visceral organs (heart , kidney , liver, gastro-intestinal tract, spleen ,bursa ,internal fat) were registered .All carcasses were then chilled in an air chilling refrigerator for 24 hours at 4 C^{o} .The weights of the cold carcasses were then determined .The dressing out percentage of the prepared carcasses was calculated . After separation of subcutaneous tissues from the skin , the subcutaneous fat was determined and the ratio of the internal to subcutaneous fat was calculated .Finally the carcasses were de-boned and meat to bone ratio was determined (Preston and William,1973).

3-2-9 Blood analysis :-

Hematological indices measured in the study were total erythrocytes count (TEC) , total leukocyte count (TLC), hemoglobin concentration (Hb) and pack cell volume (PCV).

3-2-9-1 Collection of blood samples :-

Blood samples were collected from the wing vein of chickens by using 5 ml .disposable syringes .

The skin was first dampened with 70% alcohol to disinfect the area .The needle was inserted opposite to the direction of the blood flow and the blood was drawn into the syringe barrel . The blood was then transferred

into labeled vial contain Anti-coagulant ethylene di-amine tetra acetic

acid (EDTA) and immediately used for the above mentioned indices determination.

3-2-9-2 Erythrocyte profile

3-2-9-2-1 Total Erythrocytes Count (TEC):

The number of erythrocytes count was determined according to Jain , (1986) blood was sucked up to the marked indicated above the pipette pulp, then let to set for 2-3 minutes ,two drops were dripped out . one drop was dripped on the two sides of chamber and let to set in a covered place away from air draft . Then red cells were count using 40 * objective lens of bright field microscope.

The counting area in the chamber ,consist of primary nine squares .The central primary squares were used for the erythrocytes count and it present 25 secondary squares ,each of which is subdivided into 16 tertiary squares.of All erythrocytes in five of the secondary squares (four corners and the central square) were enumerated for counting cells .

Cells touching top and left center were count and cells toughing bottom and right central lines were not counted.

Cells from 5 squares were added and the sum of them was multiplied by 10.000 .The calculation of the erythrocytes count was based on the following

Erythrocytes count ($\times 10^6$/ml) = Nx200=5x10.000

-Where N is the sum of counted cells .

3-2-9-2 -2 Haemoglobin(Hb)concentration :-

Haemoglobin concentration was determined by a cyanomethemoglobin

method as described by (Van Kampen and Zijlstra ,1961).

Calculation $[Hb]\ (g/dL) = \frac{\text{OD of sample}}{\text{OD of standard}} \times 15$

3-2-9-2-3 Packed cell volume (PCV): Blood Heamatocrit (HCt):

The method described by Jain , (1986). was adopted for the determination of PCV .

The Packed Cell Volume was measured by using plain capillary tubes (Umedic, Germany). The capillary tubes (7.5mm×1.0mm) were filled with fresh blood to three fourth ,and one end was sealed , The sealed capillary tubes were centrifuged for 5 minutes at 12ooo r.p.m .in a micro-haematocrit centrifuge. A special reader was used to determined Blood heamtocrit as a percentage of whole blood .

3-2-9-2-4 Calculation of MCV , MCH , MCHC :

MCV : Mean Corpuscular Volume was calculated by the formula :

$$\textbf{MCV} = \frac{\text{PCV } 10}{\text{RBCs } 10}$$

MCH : Mean Corpuscular Haemoglobin concentration was calculated by the formula

$$\textbf{MCH} = \frac{\text{Hbc} \times 10}{\text{RBCs}}$$

MCHC : Mean corpuscular Haemoglobin concentration was calculated by the formula :

$$\text{MCHC} = \frac{\text{Hbc} \times 10}{\text{PCV}}$$

3-2-9-3 Leukocyte profile

3-2-9-3-1 Total Leukocyte Count (TLC):

The TLC was performed in an improved Neubauer heamocytometer (Hawksly and Sons, Ltd England) by using Turk ,s fluid (glacial acetic acid 1,ml 1% aqueous gentian violet 1ml ,and distilled water up to 200 ml). The pipette was filled with filled with fresh blood to 0.5 marks and then filled with diluting fluid to the 11mark on the stem distal to the bulb .The dilution of blood obtained was 1:20 .The heamocytometer and a cover slip were cleaned and the cover was pressed on the surface of the heamocytometer .The diluted blood was mixed thoroughly and the counting chamber was filled carefully Then the cells were allowed to settle .Using the light microscope (Olympus Optical Co , Ltd , Japan) under low power (×10) objective, the number of leukocytes was counted in each of 4 large corner squares . The TLC was obtained by multiplying the number of cells counted by both dilution factor and volume factor. Each large squares has an area of $1mm^2$ and a depth of 0.1 mm, giving a volume of $0.1mm^3$ .Since four large squares were used for counting , the total volume was $0.4mm^3$ .As the total volume used in counts is $1mm^3$ this volume should be multiplied by 2.5.

Calculation:

TLC ($10^3/\mu l$) =N ×1/0.4 ×20 = N ×2.5× 20 = N × 50

Where: N = Number of cells counted in the 4 squares

3-2-10 Statistical analysis:-

The data obtained were subjected to analysis of Varince as shown by (Steel and Torri ,1960). Duncans multiple range test used to assess significance of difference between treatment means as described by (Gomez and Gomez 1984).

Chapter Four

RESULTS

4.1 Isolation and identification of aerobic gram –negative bacteria from respiratory tracts and internal organs of sick chickens:-

4.1.1 Taxonomic identification of Gram negative bacteria isolated :-

Seven hundred and twenty samples were collected from 120 broiler chickens from different organs (Lung, trachea. spleen, liver ,intestine and reproductive system) .Two hundred and fifty three(35.14%) samples showed positive growth while the best samples (19.72%) were found negative .Three hundred and twenty five(45.14%) were Gram-negative bacteria .

Bacterial isolates are shown in tables (4) to(9) .

Table (4) shows the primary tests for identification of the Gram negative –bacteria ,while the biochemical tests for identification of Gram negative bacteria are shown in tables (5) to (9) .

Results of the isolated Gram negative bacteria from broiler chicken with respiratory signs are shown in table (10) .A according to the results of the growth characteristics, colony morphology, biochemical reactions and carbohydrate fermentation and reaction patterns Gram negative bacteria isolates were :

Escherichia coli 187 isolates (57.54%) ,Citrobacter freundii 47 (14.46%), Haemophlus paragallinarium 44(13.5%), , pseudomonas diminuta 26 (8.00%) and pseudomonas Aeruginosa 21 (6.46%).

Escherichia coli was the highest load of bacteria in different organ of the chickens.

Gram –positive bacteria isolated from respiratory tract of infected chickens were: *Staphylococcus aures* , *Staphylococcus gallinarium* , *Bacillus cereus, Sterptococcus lentus* .

Table (4): Primary tests for identification of the isolated Gram- negative bacteria

Test Bacteria	*E coli*	*Pseudomonase spp*	*C.freundii*	*Hoemophilus spp*
Shape	Rod	Rod	Rod	Rod/Sphere
Aerobic growth	+	+	+	+
Catalase test	+	+	+	+
Oxidase test	+	+	+	+
Glucose	_	+	_	_
O.F	F	O	F	N.T

N.T : not tested by used method

+ =Strain are positive

_ =Strain are negative

F = Fermentative

O = Oxidative

Table (5): Biochemical identification of the isolated *Escherichia coli*

Test	Reaction
Oxidase	_
Catalase	+
Indole production	+
Urease	_
MR test	+
VP test	_
Nitrate reduction	+
Growth on MacConkey's agar	+
Fermentation of Fructose	+
Lactose	+
Maltose	+
Galactose	+
Glucose	+
Sucrose	_
H2 S production	_
Citrate	_
Motility	_

Table (6): Biochemical identification of the isolated *Citrobacter freundii*

Test	Reaction
Oxidase	–
Catalase	+
Indole production	+
Urease	–
MR test	+
VP test	+
Nitrate reduction	+
Growth on MacConkey's agar	+
Fermentation of Fructose	+
Lactose	+
Maltose	+
Galactose	–
Sucrose	+
H2 S production	+
Citrate	+
Motility	+

Table (7): Biochemical identification of the isolated Pseudomonas *diminuta*

Test	Reaction
Oxidase	+

Catalase	+
Urease	+
Nitrate reduction	+
Fermentation of Fructose	–
Lactose	–
Maltose	–
Xylose	–
Glucose	–
Sucrose	–
H2 S production	–
Motility	+

Table (8): Biochemical identification of the isolated *Pseudomonas areuginosa*

Test	Reaction
Oxidase	+
Catalase	+
Urease	–
Nitrate reduction	+
Fermentation of Fructose	+
Lactose	–
Maltose	–

Xylose	+
Glucose	+
Sucrose	–
H2 S production	–
Motility	+

Table (9) Biochemical identification of the isolated *Haemophlus paragallinarium*

Test	Reaction
Oxidase	–
Catalase	–
Indole production	–
Urease	–
MR test	–
VP test	–
Nitrate reduction	+
Growth on MacConkey's agar	–
Fermentation of Fructose	+
Lactose	–
Maltose	+
Galactose	–
Glucose	+
Sucrose	+
H2 S production	–
Citrate	–

Motility _

Table (10) :Bacterial isolates from the different organs of broiler chickens with respiratory manifestation

Bacteria	***Escherchia coli***	***Citrobacter freundii***	***Pseudomonase diminuta***	***Pseudomonas aeruginosa***	***Haemophlus paragllnalium***
Trachea	140	47	26	21	44
Lung	-	-	-	-	-
Liver	19	-	-	-	-
Spleen	-	-	-	-	-
Intestine	28	-	-	-	-
Reproductive system	-	-	-	-	-
No of isolate	187	47	26	21	44
%	57.54	14.46	8.00	6.46	13.54

4-2.Infection of the broiler chicken with the isolated Gram negative bacteria (Escherichia *coli* and *Citrobacter freundii*) to determine its effect on performance and carcass quality :-

4-2-1Clinical signs

Clinical signs were noticed after 24 hours post experimental infection of broiler chicks with *Escherichia coli* and *Citrobacter freundii.*

Birds in group B (infected with *E coli*) showed :weakness , decreased feed intake , light breathing with impact movement of the chest ,ruffled feather , gasping ,nasal mucous discharge and diarrhea .

The clinical signs of group C (infected with *Citrobacter freundii*) were : decreased feed intake , nasal discharge and weakness .

4.2.2 Weekly feed intake data :-

Table (11) shows the effect of experimental infection of broiler by *Escherichia coli* and *citrobacter frenudii* .

The obtained result indicated that birds which were infected with *Escherichia coli* consumed less feed during the fifth, sixes, and seven Weeks of the experiment (432 grams,414 grams and 518 grams) respectively .

Group C (infected with *Citrobacter freundii*) also showed remarkable decreased in feed intake during the last three weeks(444 grams, 426 grams and 530grams) respectively .

4.2.3 Weekly body weight gain data :-

The effect experimental infection of broiler chicks with *Escherichia coli* and *Citrobacter freundii* on weekly body weight gain are shown in table (12).

The result revealed that body weight gain the three treatments increased until the fourth weeks. After experimental infection group B (infected with *E coli*) showed a decreased of body weight gain . The average weight gain were 216 grams, 195 grams and 263 grams respectively in fifth, sixes, and seven weeks of experiment .

Citrobacter freundii infected group (group C) also showed decreased in weight gain throughout the last three weeks, which were (231grams , 211grams and 266 grams) respectively .

4.2.4 Data of weekly conversion ratio :-

Table (13) showed the effect of experimental infection by bacteria and the control (free from bacteria). The presented data depicted that up to the fourth week of the experimental period there was better feed utilization efficiency in all groups.

In the three last weeks the least feed utilization efficiency was recorded by group B(infected with *Escherichia coli*) in last three weeks and were : 2.00 , 2.01 and 1.97 respectively.

The Feed utilization efficiency of (group C) infected with *Citrobacter Freundii* was better than group B (infected with *Escherichia coli*) during last three weeks and were : 1.92, 2.01 and 1.99 respectively .

4.2.5 Summary of Overall Bird Performance :-

The overall result of seven weeks old broiler chicks that were infected by *Escherichia coli* and *Cirtobacter freundii* are shown in table (14) .The obtained result indicated that by the end of 7^{th} week ,either feed intake ,total weight gain or overall mortality rate of group B (infected by *E coli*) and group C (infected by *C freundii*) birds were significantly affected . Group A excelled the infected groups in providing the highest body weight gain (1904.05 grams) and livability , the overall feed

conversion ratio of (group A) control was1.74 and found to be best among the infected groups . The least feed utilization efficiency was obtained by group (infected with *Escherichia coli*) and was 1.90 .

Table 11 : Effect of experimental infection of broiler by *Escherichia coli* and *Citrobacter frenudii* on weekly feed intake (g/bird)

Age in weeks	Group A Control feed intake (g/bird)	Group B inoculated by *E*.coli feed intake (g/bird)	Group C inoculated by *C freundii* feed intake (g/bird)
Week 1	108.93±0.54	106.76±0.65	106.46± 0.63
Week 2	286.66±4.70	285.39±4.08	284.19 ±3.74
Week 3	377.88±8.20	374.58±10.43	373.28±9.16
Week 4	550.10 ±8.10	545.03±7.84	547.69±7.36
Week 5	650.74^{a}.± 5.80	432.40^{b}±7.59	444.20^{b}±6.73
Week 6	746.41^{a}± 3.50	414.73^{b}±4.25	426.09^{b}±4.47
Week 7	655.74^{a}± 8.70	518.60^{b}±6.63	530.28^{b}±6.40

Values followed by similar letters within a row are not significantly different at **P<0.05**

Table 12: Effect of experimental infection of broiler by by *Escherichia coli* and *Citrobacter frenudii* on weeky weight gain (g/bird)

Age in weeks	Group A Control Weight gain (g/bird)	Group B inoculated by *E*.coli Weight gain (g/bird)	Group C inoculated by *C freundii* Weight gain (g/bird)
Week 1	**60.185±0.8**	**57.75±1.2**	**56.81±0.8**
Week 2	**162.88± 5.1**	**159.30±4.9**	**159.60±5.6**
Week 3	**231.81± 9.5**	**213.83±5.4**	**217.91±0.3**
Week 4	**314.18±6.0**	**300.84±4.6**	**301.81±2.7**
Week 5	**327.93^{a}±4.o**	**216.50^{b}±4.2**	**231.69^{b}±0.8**
Week 6	**434.30_{a}±5.5**	**195.60^{b}±4.8**	**211.99^{b}±2.4**
Week 7	**368.33^{a}± 9.0**	**263.25^{b}± 3.6**	**266.47^{b}±4.1**

Values followed by similar letters within a row are not significantly different at **P<0.05**

Table 13 :Effect of experimental infection of broiler by by *Escherichia coli* and *Citrobacter frenudii* on weekly feed conversion ratio (gm feed /gm gain)

Age in weeks	Group A Control	Group B inoculated by *E*.coli	Group C inoculated by *C freundii*
Week 1	**1.81±0.017**	**1.85±0.035**	**1.87± 0.026**
Week 2	**1.76±0.039**	**1.79±0.031**	**1.78 ±0.055**
Week 3	**1.63± 0.037**	**1.75±0.022**	**1.71± 0.045**
Week 4	**1.75± 0.019**	**1.81±0.024**	**1.82± 0.010**
Week 5	**1.73^{a}± 0.020**	**2.00^{b}±0.021**	**1.92^{b}± 0.028**
Week 6	**1.72 ^{a}± 0.025**	**2.01^{b}±0.026**	**2.01^{b}± 0.019**
Week 7	**1.78 ^{a}± 0.026**	**1.97^{b}±0.021**	**1.99^{b}± 0.014**

Values followed by similar letters within a row are not significantly different at **P<0.05**

Table 14 : Overall performance of 7 weeks old broiler chicks infected experimentally by *Escherichia coli* and *Citrobacter frenudii*

Parameters	Group A Control	Group B inoculated by *E.coli*	Group C Inoculated by*C freundii*	SEM
Total Feed intake g/bird	3376.46[a]	2680.15[b]	2709.53[b]	± 2.32
Weight gain g/bird	1904.05[a]	1407.60[b]	1449.05[b]	± 1.16
Feed conversion ratio	1.74[a]	1.90[b]	1.87[b]	± 0.07
Mortality%	0.00	19.05[b]	11.91[b]	± 0.33

Values followed by similar letters within a row are not significantly different at **P<0.05**

4.2.6 Carcass and non Carcass characteristic Data

Table (15) shows the effect of experimental infection of broiler chickens with *E coli* and *C freundii* on carcass characteristics, the obtained results indicated that there was cleared significant difference between the control group and the infected groups . The highest live weights was obtained by the control group (1847.03grams) .

Table (16) shows the effect of experimental infection by *E coli* and *C freundii* on non carcass characteristic .There was no significant difference between the control group and the infected groups but the infected birds had slightly higher weights of the head ,liver and heart

Table (15): The effect of experimental infection of broilers by *Escherichia coli* and *Citrobacter freundii* on carcass characterstic

Parameters	**Group A Control**	**Group B inoculated by E.coli**	**Group C inoculated C freundii**	SEM
Live weight (g)	1847.03^{a}	1517.47^{b}	1525.27^{b}	± 0.12

Eviscerated weight (g)	1163.23[a]	956.01[b]	960.92[b]	± o.12
Dressing out %	62.98[a]	63.00[a]	63.00[a]	± 2.27
Meat to bone ratio	2.08`[a]	1.72[b]	1.73[b]	± 0.18
Meat to fat ratio	7.74[a]	5.23[b]	5.30[b]	± 0.72

Values followed by similar letters within a row are not significantly different at **P<0.05**

SEM : Standard error of the mean

Table 16: The effect of experimental infection of broiler with *Escherichia coli* and *Citrobacter freundii* on non carcass component of broiler chicks

Parameter	**Group A Control**	**Group B inoculated by by *E.coli***	**Group C inoculated *C freundii***	SEM
G.I.T%	10.12[a]	10.18[a]	10.09[a]	± 1.21
Liver%	2.18[a]	3.48[a]	3.68[a]	± o.33
Kidney%	0.37[a]	0.32[a]	o.34[a]	± o.44
Heart%	0.37[a]	0.48[a]	O.52[a]	± o.23
Head%	2.97[a]	3.92[b]	4.95[b]	± o.51
Shank%	4.72[a]	4.56[a]	4.84[a]	±o.58

Values followed by similar letters within a row are not significantly different at **P<0.05**

SEM : Standard error of the mean

4.2.7. Hematological parameters of broilers chickens experimentally infected with *Escherichia coli* :-

Hematological values of broilers chickens experimentally infected with *Escherichia coli* are shown in table (17).

Based on the analysis of variance (ANOVA) ,the haematological parameters TEC(Total erythrocytes count),TLC(total leukocyte count), hemoglobin concentration (Hb) and packed cell volume (PCV) of the three groups are significantly different at 30 days of production period (two days after experimental infection by *E coli* and *C freundii*).

The haematological parameters are significantly (P>0.05) different Between treatment A (control) and the treatments infected group with Gram - negative bacteria .There were increased in total leukocyte counts in B (infected by *E coli*) 11.21 $\times 10^2$ mm and group C (infected by C freundii)11.83 $\times 10^2$ mm. The values of Total erythrocytes counts , hemoglobin concentration and pack cell volume were decreased after infection in both infected groups .

Table17: Hematological values of normal and experimentally infected broiler Chick with E *coli* and *citrobacter freundii:-*

Parameter	Group A(control)	Group B infected by *Citrobacter freundii*	Group C infected by *E . coli*
RBCs	3.93±0.05[a]	2.20±0.13[b]	1.99±0.04[b]
x10^{6}mm			
HB(g/dl)	8.70±0.16[a]	6.49±0.29[b]	6.28±0.78[b]
PCV%	28.90± 0.43[a]	21.10±0.17[b]	21.80 ±0.11[b]
MCV	73.50±1.73[a]	95.90±2.00[b]	109.50±2.20[b]
MCH Pg	22.13±0.30[a]	29.50±0.71[b]	31.55±0.11[b]
MCHC%µl	30.10±0.25[a]	30.75±0.23[a]	28.80±0.16b
WBCs	9.93±0.03[a]	11.21±0 .01[b]	11.83± 0.24[b]
x10^{6}mm			

Values followed by similar letters within a row are not significantly different at **P<0.05**

SEM : Standard error of the mean

Chapter Five

DISCUSSION

Respiratory infection is the most serious disease affecting poultry and cause heavy economic of losses in the poultry industry world wide .

Diseases of the respiratory tract are caused by a wide range of pathogens of bacterial , viral, mycoplasmal and fungal origin They play a significant role in death and losses in poultry industry .Any respiratory diseases has a direct negative impact on commercial parameter of poultry industry live weight gain ,egg production and liveabilitywhich cause considerable losses.

Several micro organism of the genus *Pasteurella* (*P. multocida, P.gallinarum, P. haemolytica* and *P. anatipestifer), Bordetella* (*B. avium*) and *Haemophilus* (*H. paragallinarum*) were involved in respiratory diseases complex (Hafez, 2002). *Escherichia coli* associated with respiratory infection in chickens has also been reported (EL-Sukhon et al., 2002).

This study was undertaken to isolate Gram-negative bacteria from the respiratory system of broiler and to determined its effect on broiler performance and carcass characteristics and also to determined the haematological change by common identified organism (*E coli* and *Citrobacter freundii*) . Gram-negative bacteria isolated from chickens showed typical respiratory signs' included : *Escherichia coli* 178 isolate (57.54%) , *haemophlus paragallinarium* 44 isolate(13.5%) , *pseudomonas diminuta* 26(8.00%) *and pseudomonas Aeruginosa* 21(6.46) and *Citrobacter freundii* 47 (14.46 %) .

In this study *Escherichia coli* was isolated from trachea of infected chickens .Several authors reported isolation of *Escherichia coli* from respiratory tract of infected chickens (Chu ,1958; Khoagali , 1970 ;Elgalli,1992 ; Elnasri ,1997and Murthy ,*et al* 2008). Also other study conifirm isolation of *E.coli* from lung (Hofstad *et al* ,1978). Zahida ,

(2004) drscribed the respiratory tract as the primary route of invasion of *Escherichia coli* .

In the present study *Escherichia coli* was isolated from several organs: (140 were from the trachea, 19 were from liver and 28 from intestine)

Suggesting that systemic spread might occur. These findings accord the results of Murthy *et al* ,(2008) .

In this study *Escherichia coli* inoculated group (group B) chicken suffered from weakness ,lack of appetite ,ruffled feather , mucous discharge from the nostril and from diarrhea post inoculation . After 24 hours birds had died and this confirms with the finding of (Nakamura , (1992); Tian and Baracosa ,(1989); Wray *et al*,(1996); Vandekerckhove *et al*, (2oo4) ; Shen *et al* ,2002 and Zaki ,(2012).

Feed intake is economically important to broiler industry because feed is the mostexpensiver item in poultry production .There are many factor affect feed intake of chickens and hence determine the efficiency of poultry production ,disease is one of them. Clinical and subclinical infections can have a significant negative impact on the performance of broiler flocks (Morel *et al*,2001).

The results of this study showed that among the first four weeks of broiler trial period there was no Significant difference in feed intake among the three groups. After experimental infection of broiler chicks with *Escherichia coli*(group B) there were a significant differences between control group and inoculated groups. Group B (inoculated by E*scherichia coli*)showed a high decrease in feed intake in fifth ,sixes and seven weeks of trial period (432.40, 414.73 and 518.60grams) in contrast to control groups(650.74,746.41, 655.74 grams) respectively .

The Results of this study also showed that there is a highly significant

difference in weight gain after infection between the control group and Group B (inoculated groups *Escherichia coli)*.

Group B which inoculated with *E coli* showeded the lowest body weight gain in the last three weeks of the experiment because experimental infection with *E .coli* decreased feed efficiency which lead to decreased in weight gain and this is in accordance with the finding of (Tian and Baracosa ; 1989 Moulin and Fairbrother,1999).

Feed conversion ratio (FCR) is a measure of how well a flock converts feed intake into live weight and provides an indicator of management performance, and also profit at any given feed cost. As feed costs represent 60-70% of the total cost of broiler production, the efficient conversion of feed into live weight is essential for profitability, and small changes in FCR at any given feed price can have a substantial impact on financial margins also). High mortality, especially late mortality, results in a significant increase in FCR. The dead birds consumed a significant amount of feed but do not contribute to final flock live weight (Zoons *et a*l,1991).

The result of the study revealed that Feed utilization efficiency of group B(Infected with *E coli*) was much lower than the control groups in week six and seven, and this is due growth retardation In group B and this is in agreement with ((Moulin and Fairbrother,1999).

On the basis of obtained overall birds performance its clear that after inoculation of group(B) with *E.coli* growth rate ,feed consumption rate ,feed conversion were statistically different from group (A) control. At the end of the experiment group B which infected with *E coli* had the lower weight gain(1407.60 grams) ,lower feed

intake(2680.15grams) , lower feed utilization efficiency and higher mortality rate (19.05 %) and this is in agree with finding of (Wray *et al*,1996 and Vandekerckhove *et al*, 2004).

In t this study pathological lesion were , pericarditis, perihepatitis and enlarged hyperemic spleen , tracheitis and accumulation of of fibrinn the surface of the lungs the some result were found on (Shen *et al* ,2002 and Asher *et al* ,2008).

The present study shows a significant decrease in RBCs Count , and Hb concentration and PCV In the affected birds indicates anemia of microcytic hyoochromic . This result is in accordance with (Zaki et al , 2012).

Citrobacter freundii associated with nosocomial was reported by Whales *et al*,(2007).In this study *Citrobacter freundii* was isolated from trachea broiler chicks (47 1solates) .

The results of this study showed that feed intake on broiler chicks inoculated with *Citrobacter freundii* was significantly different from the control , feed intake in the last three weeks of the experiment was:444.20,426.09 and 530.28 grams respectively where as feed intake the control group in that weeks was :650.74,746.41and 655.7 grams.

In this study the results of weight gain revealed that ,after experimental infection of group C with *Citrobacter freundii* there was clearly decreased in weighted gain in the last three weeks (231.69, 211.99and 266.41grams)respectively.

Citrobacter freundii infected group showed better feed utilization efficiency in compared with *Escherichia coli* infected group. Also the mortality rate of group C (infected with *Citrobacter freundii*) is better than *Escherichia coli* infected group. Mortality rate was 11.91% in *Citrobacter freundii* inoculated group and 19.05% in Escherichia *coli* inoculated group.

Clinical signs of group C (infected with *Citrobacter freundii)* in this study was decreased feed intake, weakness and nasal discharge .The pathological finding was slighted congestion of the lung and that was in confirmed with finding in group B (infected with *Escherichia coli*)

The end of this study revealed that Gram- negative bacteria constrain poultry industry in Khartoum North in particular is *Escherichia coli* .

CONCLUSION

The results of the present study demonstrated that :

1- Both gram-positive and gram negative bacterial pathogen were isolated from respiratory tract of infected chickens.

2- Gram-negative bacteria isolated are : *Escherichia coli ,Citrobacter freundii , Haemophlus paragallinarium , pseudomonas diminuta and pseudomonas Aeruginosa*

3- Broiler chickens experimentally infected with *Escherichia coli and Citrobacter freundii* showed significant decrease in feed intake and weight gain while feed *conversion* ratio and mortality rate were increased 4- Carcass and non carcass characteristics were not affected by experimental infection of broiler by either with *Escherichia coli nor* with *Citrobacter freundii*

5- Mycoplasma, and other Pathogen than bacteria could be a cause of respiratory tract infection of chickens as 19.72% of sample collected from infected chickens did not show bacterial growth .

6- Respiratory tract due to bacterial infection could be an important constrain in poultry industry in Khartoum north.

RECOMMENDATIOM

From results and discussion of this study, the following recommendation are suggested ..

(1) The high prevalence of *Ecoli* associated with respiratory infection of chickens needs further study .

(2) The further studies should be considered to minimized the spread of bacterial respiratory diseases .

(3) The role of the other pathogens such as mycoplasma , *C freundii* viruses and other Gram-negative bacteria should be studies .

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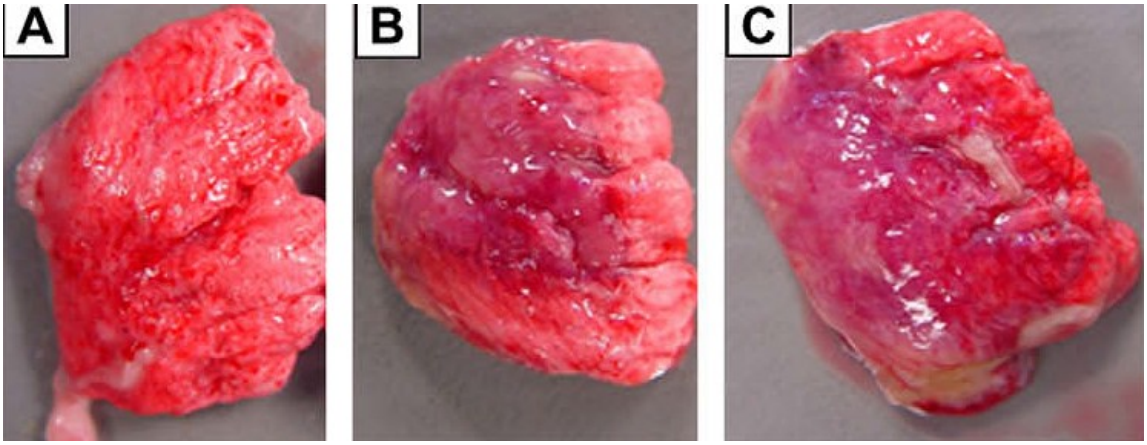
Appendix 1:Number and percentage of Gram-negative bacteria isolates from respiratory tract of chickens

Appendix 2: Effect of experimental infection of broiler by *Escherichia coli* and C*itrobacter frenudii* on weekly feed intake (g/bird)

Appendix 3: Effect of experimental infection of broiler by *Escherichia coli* and C*itrobacter frenudii* on weekly weight gain (g/bird)

Appendix 4: Effect of experimental infection of broiler by ***Escherichia coli* and *Citrobacter frenuddii* on weekly feed conversion ratio (gm feed / gm gain)**

Appendix 5: lung of broiler chicken infected with *E coli* and C *frenudii*

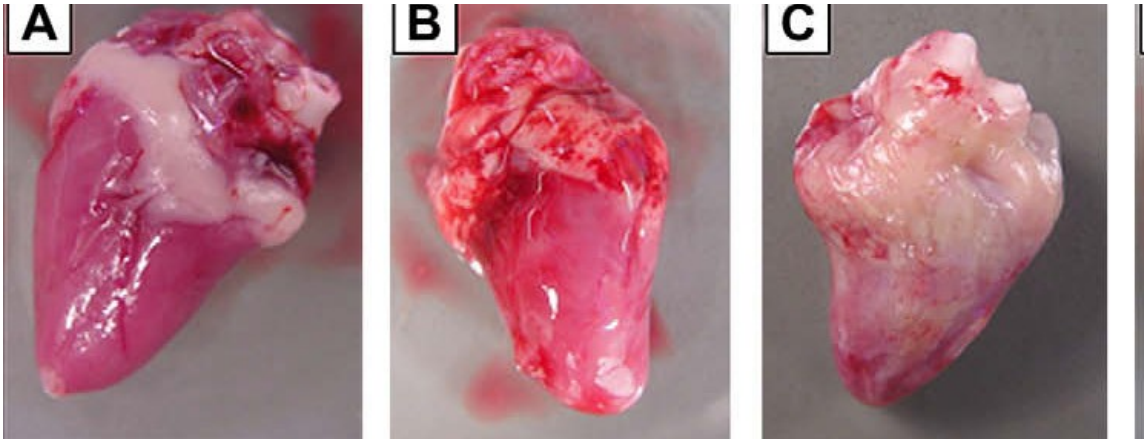


A: Control

B: Infected with *Escherichia .coli*

C: Infected with *Citrobacter freundii*

Appendix 6 : Heart of broiler chicken infected with *E coli* and C *frenudi*



A: Control

B: Infected with Escherichia .coli

C: Infected with *Citrobacter freundii*

Figure:7 Liver of broiler chicken infected with *E coli*

Liver with a greenish colour and is mottled and multiple small necrotic foci.