***IDENTIFICATION AND QUANTIFICATION OF MICROBES IN CHICKEN EGGS (UNFERTILISED) SOILED WITH FEACAL DROPPINGS***

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, BOWEN UNIVERSITY, IWO, OSUN STATE, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER DEGREE IN ANIMAL SCIENCE.

APRIL 2014.

**DECLARATION**

I hereby declare that this thesis was written by me and is a record of my own research work. It has not been presented before in any application for any degree. References made to published literature have been duly acknowledged.

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**DEDICATION**

This work is dedicated to my Creator, the source of my living. Also to my amiable parents; Hon.S.O. and Dns.F.O.Olaniregun.

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**ABSTRACT**

The effect of poultry feacal soilage on the microbial counts in the yolk and albumen of table eggs was studied alongside the antibiotic resistance pattern of *Staphylococcus aureus* and *Shigella* Species obtained from the internal egg contents. Fresh table eggs were soaked in feacal poultry droppings for 0 hour (Control- no soaking), 12 hours,24 hours, 48 hours and 72 hours, respectively and microbial counts in the internal contents of the eggs enumerated on nutrient agar for aerobic bacterial count, Bairl Parker Agar for *Staphylococcus aureus* count, Eosin Methylene Blue agar for *Enterobacteriaceae* counts, *Salmonella – Shigella* agar for *Salmonella- Shigella*  counts, and Potato Dextrose agar for Fungal and Mould counts. Generally, a highly significant (P<0.001) increase in aerobic plate counts, *Staphylococcus aureus, Enterobacteriaceae* count, *Salmonella-Shigella* count and fungal soiling. Suggesting increased microbial penetration through the shell membrane into the internal content of the table eggs. Identified microorganisms associated with soiled internal content of table eggs included *Staphylococcus aureus, Serratia* species, *Klebsiella* species, *Bacillus brevis, Shigella* species *Edwardsiella tarda, Salmonella* species, *Bacillis pumitus, Citrobacter* species and *Kluyvera* species, *Edwardsiella* species, *Cedecea* species, *Escherichia coli.* Multiple antibiotics resistance to ceftazidin, cloxacillin and erythromycin was observed to occur in *S. aureus* at 48 hours while shigella species were resistant to Gentamicin at 48 hours and therefore table eggs should be collected frequently from the cage and kept cool always to prevent bacterial growth and further studies should be carried out on the detrimental effect of these organism on human health.

**CHAPTER ONE**

1. **INTRODUCTION AND LITERATURE REVIEW**
	1. **INTRODUCTION**

**1.2. Poultry Production in Nigeria**

Agriculture continues to be the most important sectorof the Nigerian economy in terms of provision of employment (Adeyemo and Onikoyi, 2012), and also the challenges of food insecurity and hunger in Nigeria especially have continued to receive attention from experts and government (Emaikwu, Chikwendu, & Sanni, 2011; FAO, 2003). The small scale poultry production represents one of the few opportunities for saving, investment and security against risks. It accounts for approximately 90% of total poultry production (Branckaert, 1999).

Poultry is one of the major sources of animal protein in Nigeria and also a mean of bridging the gap between the level of animal protein consumption and the expected level (Ogundipe and Sanni, 2002) and the sector is experiencing faster growth (Abubakar, Bello, Tukur,& Basir, 2011).The fast growth could have been triggered by a drive to attain the Food and Agriculture Organisation recommended protein consumption target of 35gm/caput perday as proffered by Adebayo and Adeola (2005).

Poultry makes a significant contribution to human nutrition and economic sustenance both in developed and developing countries. It provide substantial amount of high quality protein in form of meat and table eggs (Abubakar *et al*., 2002). The productive value of poultry production rank second in husbandry industry 15 to17% after pig production (75 to 76%) (FAO 2008). And second best to milk in terms of nutritive value and the most economical produced animal protein (FAO, 2008).

Poultry refers to all birds of economic value to man as source of meat, egg and fibre. The poultry industry provides raw materials such as wool, hides, and skin for the development of local industries using them to produce items such as clothing, shoes, jackets, rugs for human use (Okunmadewa, 1999),for the production of vaccine, mattress and offer employment to many people.

Poultry eggs have many attributes that make them to be preferred to other animal protein sources. They are inexpensive, easy to cook, eat and digest. Nutritionally, the egg is very rich, it is low in calories and the protein in egg is such of a quality that it is used as standard against which other proteins are compared.

Poultry meat and eggs offer considerable potential for meeting human needs for dietary animal supply (Folorunsho and Onibi, 2005).

**1.3.** **Economic Importance of Poultry in Nigeria**

Nigeria has the highest number of poultry farm in Africa, with a flock population of about 104,247,960 representing (58%) of the total livestock resources (Adeyemo and Onikoyi 2012).Egg is a popularly consumed animal protein unrestricted by any religion/culture. (Adoyi, 2013). It is recorded that the poultry industry contributes to about 25% to the nation’s GDP in 1995 and increase to 27% in 1999.

Nigeria’s livestock resource include 13,885,813 cattle; 34,453,724 goats; 22,092, 02 sheep; 3,406,381 pigs and 104,247,960 poultry (Adeyemo and Onikoyi, 2012) while FAO (2010) recorded 72,400,856 as the total population for chicken in Nigeria.

According to FAO(2010), Nigeria presently produces about 650,000 tons of eggs yearly and a parallel record from Poultry Association of Nigeria indicates that Nigeria presently produces above 1.25million tones of eggs per year.

The poultry industry in Nigeria has gone through series of developmental stages in the last fortyyears. The industry hadwitnesses’ tremendous progress in all area (Nmadu, Iwuajoku, & Jiya, 2012)

Poultry occupies a unique position in the Nigerian livestock production. It is important to know the fact that poultry is relatively free from the many pathological, ecological and economical, technicalconstraint that affect the commercial production of other breeds and classes of livestock (Adoyi, 2013). The economic and nutritional importance of poultry cannot be overemphasized as poultry provides man with companionship, and fibre (feathers), food in terms of egg and meat, provide direct and indirect employment to suppliers of products and services such as grain farmers, feed mill operators as well as those products used to support poultry production and marketing activities.

**1.4. Problems Facing Poultry Production In Nigeria**

One of the most common problems being faced in the poultry production sector in Nigeria is disease outbreak e.g. Newcastle, Marek, Avian pox, Gumboro etc. The important parasites of poultry include; bugs, flies helminthes etc. (Olaniregun, 2010).

In spite of all the attention given to agriculture by expert and government, due to fast growing population, Nigeria is threatened with the problem of food insecurity and poverty where average Nigerian still consumes far less animal protein, industry is still growing due to hydra-headed problems and per capita income which is low leading to a consumption of less than 9gram of protein per capita per day as compared to over 50grams per capita per day in North America and Europe (Grigg,1995), some countries even in the developing world are already considering novel approaches to meat production such as in-vitro meat production (Sachan *et al.,2012)*but in Nigeria animal production is facing numerous challenge with certain factors militating against successful animal production like lack of capital, high cost of feeding, animal diseases, access to veterinary services, poor level of education of farmers, the role of the government, Urbanization, market and storage facilities, inadequate extension services, inadequate manpower, transportation,Inadequate basic infrastructure, etc.(Pwaveno,2013).

**1.5. The Production System forCommercial Poultry Production**

The three systems of production adopted for livestock include; Extensive system, Intensive system and Semi-intensive system each with its own advantages and disadvantages.

Extensive system involves little or no care for the chicken, the system is faced with many challenges including diseases, predators and inadequate feed (Kitalyi, 1998).On the other hand the system is very cheap with little labour requirement and the chickens have access to green pastures and are able to exercise themselves, however it is not suitable for commercial purposes. (Olaniregun, 2010).

In the intensive system, the birds are confined to the house entirely with no access to land outside, battery cage system and deep litter methods are most common. The birds are less exposed to disease organism since there is no practically contact between them and their droppings. This is the best system for commercial production.

According to United Egg producers (UEP) about 95% of commercial egg production in the United States is from modern cage housing with other 5% coming from cage-free system. Caged housing includes conventional cage and furnished cages. Conventional cages are the most common form of housing for layer birds in the world’s table egg industry as they are designed to manage animal health and egg production even though it has been currently and legally banned for use in European Union from 2012 (Anon, 1999) and other developed countries of the world from the welfare point of view (Pritchard, 2012).The type of production system may influence egg qualities and also determine the degree of contamination. The battery cage system do not expose the birds to high contaminant and less predisposed to infection because the exposure to litter ammonia and mutual contact is minimal. However Battery cage system is capitally intensive and because of its high cost of maintenance in case of leakages of the battery cages, the eggs are seldom prone to breaking be it mechanical or automation and also fall in to poultry droppings (feaces).

Deep liter system, which is also an intensive form of housing chickens, only differs to battery cage system with litter material spread on the floor instead of cages. The litter converts poultry dropping to a drier material which is easier to remove and the frequency of removing the dropping is reduced. Under a good management whereby the litter material is constantly mixed with the droppings, the pathogens present are spread out.

In a poorly managed system, the litter may be wet producing pungent odour of ammonia that may affect the bird and even increase disease risk by acting on a favourable medium for the development of pathogen that can resist the destructive effect of ammonia.

Semi intensive of housing chicken combine the features of intensive and extensive system, the birds are provided with shelters but still allowed to move within an enclosed area.

 **1.6 Objectives and Aim**

The objectives of this work are therefore,

To investigate the effect of duration of soilage of table eggs with poultry droppings on;

* The quantity of micro-organisms present
* Type of microbial contaminants
* To determine the resistivity pattern of these microbes to antibacterial agents.

**1.7 Justification**

Table eggs are produced by hens housed inside cages, while some may be on deep-litter and free-range systems. The probability of contact with feacal droppings from poultry birds on to the eggs cannot be totally ruled out. Table eggs contact with poultry feaces may range from few hours to several days depending on when these eggs are picked or gathered by handlers. Information is rarely available on internal microbial content of the egg which leads to the need for this research.

Feacal droppings from poultry birds are sample effluents of intestinal bacterial organism present within these birds. The persistence of eggs within the dropping overtime may present opportunities for the spread of antibiotic- resistance organisms particularly from the feacal droppings through the egg’s shell membrane into the egg’s internal environment.

Furthermore, it is thought that the close association of shells of table eggs with poultry droppings overtime, may also probably facilitate the emergence and spread of antibiotic-resistant organisms particularly from the feacal droppings in to the internal egg content and consequently among poultry birds with possible transmission to human.

Therefore, a cause for human health risk concern on the possible spread of antimicrobial resistance from enteric zoonotic bacterial of food animals to the human population may be created, as recommended by Olaniregun (2010).

**1.8. LITERATURE REVIEW**

**1.9. Structure of Egg**

Laying hens are kept to produce eggs intended for human consumption, the egg is an important product derived from keeping poultry. The size of eggs varies from flock to flock and is generally heavier than 58g(Oluyemi and Robert, 2000). The egg is oval in shape and is mostly of smooth texture, the color is usually white, brown or tinted light brown, the shell is about 0.33mm thick with 7500pores through which micro-organism can enter the egg and cause spoilage (Smith, 1990).



**Fig 1.1.** Schematic drawing of the egg and its components (Stadelman, 1995).

Egg is primarily a storage nutrient for the chick embryo. The size of the eggs varies from flock to flock and is heavier than 58g in temperate area but lower in the tropic (Oluyemi and Robert, 2000). The egg is oval in shape, and is mostly of smooth texture.

**Albumen:** Albumen is the liquid part of a raw egg that surrounds the yolk, practically transparent when raw and white when cooked. Eggs possess some antimicrobial defense mechanisms such as its organization in the albumenous sac and the viscosity of its protein(Duguid and North, 1991).

**Yolk:** The yolk is easily identified by its bright yellow colour both raw and cooked, it is surrounded by a thin elastic transparent membrane floating on top of the yolk with white spot called blastodisc.

**Shell:** The shell is about 0.33mm thick and has over 7500pores through which microorganism may enter the egg and cause spoilage (Smith, 1990). The shell acquire infection from all surfaces with which it makes contact and the extent of infection is directly related to the under humid conditions (RH>98), the cuticle can be colonized and digest by *Pseudomonas* spp (Board, Loseby, & Miles, 1979).

**1.9.1 The formation of the hen’s egg**

The hen reproductive system is a very complex system that can produce an egg in 26.5 hours. The formation of an egg occurs in the ovary and oviduct. The first step is the ovulation of the yolk (with associated ovum) from the left ovary in to the left oviduct. The right ovary and oviduct do not develop in the commercial laying hen (Robert, 2004).

The largest follicle is the one to be ovulated to produce an egg while the ovary will normally produce one matured yolk on a 24hours light/dark cycle (Shur-gain, 2005).

The oviduct consists of six regions (Solomon 1991; Robert and Brackpool, 1994) from ovary to the cloacae. They are:

1. Infidibulum or funnel which receives the oocyte after it has been shed by the ovary.

2. The magnum or albumen secreting region.

3. The isthmus, which forms the shell membranes.

4. The tubular shell gland where the calcification of the shell begins.

5. The shell gland pouch (uterus) where the bulk of shell growth occurs.

6. The vagina. The follicular membrane ruptures and releases the follicle at the time of ovulation just about 30minutes after the hen has laid.

**1.9.2 The formation of yolk**

The yolk is captured by the infidibulum where the developing egg remains for about 15minutes (Robert, 2004), Meanwhile, yolk development (ovogenesis) begins 10 to 12 days preceding ovulation(Solomon, 1991). Yolk component are then formed in the liver and transported via blood in to the ovary.

**1.9.3 The formation of albumen**

The egg moves in to the magnum (normally about 30cm long), (Koen, 2006), where it remains for about 3hours while the egg white (albumen) protein are produced and majority of albumen is formed, formation of protein takes about 1-2 days. The albumen in magnum is in a concentrated form and represents only half of the volume of albumen. Additional fluid (water along with glucose and electrolytes) is added to the albumen in the shell gland pouch to produce the final volume of the albumen.

The ovum moves through the magnum via peristaltic action (Robert and Brackpool, 1994).The albumen has several formation which include a nutritional source for a developing embryo , a cushion to protect the yolk against mechanical injury ,a bactericide to protect infection and as a template for the deposition of the shell membrane (Robert, 2004; Shur-gain,2005).

**1.9.4 Formation of the shell membrane**

The developing eggs passes in to the isthmus where there is a rapid development of inner and outer shell membrane around the albumen. The shell membranes are formed at some 3 to 4 hours after ovulation.

The isthmus, which is narrower than magnum has a thick circular layer of muscle and is approximately 10cm long (Robert and Brackpool, 1994).

**1.9.5 Formation of the Shell**

After the shell membrane is formed, the egg enters the tubular shell gland where there is transfer of calcium of salts onto the membrane fibre and spends 18hours to 21hours, then the egg passes into the shell gland pouch where two process occur simultaneously. Firstly, there is a slow calcification for almost the first 4hours with the main event to occur during which the albumen takes up electrolytes (salt and glucose) and water through a process called plumping. (Robert, 2004; Shur-gain, 2005; Koen 2006).

The formation of the mammillary cones commences and is thought to be also the stimulus for the rapid phase of calcification to begin. The hen’s calcium requirements are highest at this stage of egg formation. The bulk of the real shell formation now takes place and the egg spends about 20hours in the shell gland pouch and the process of plumping is completed (Koen, 2006).

Once shell is completely formed, a protective coating called the cuticle is laid down over the shell. The laying of the egg is induced by hormonal contraction of the uterus. The egg passes through the vagina and exits via the cloacae.

**1.9.6. Factors That Affect Egg Quality and Shelf Life**

The quality of egg depends on several factors such as genetic base (Akbar *et al*., 1983) and is classified into external and internal. The External qualities are Egg weight, Egg Shape, Shell quality (shell thickness, shell weight shell density, shell colour) (USDA,2000; Hussain, 2011) cleanliness, shape, texture and soundness (King, 2012) and the internal quality involves functional, aesthetic and microbiological properties of egg yolk and albumen (King, 2012). The proportions of component for fresh egg are 32% yolk, 58% albumen and 10% shell. According to King (2012), the unacceptability of egg mostly depend on the external quality with 10% while 1% is due to internal quality defects in which these defects lower the shelf life of egg and also increase egg breakage, hatchability and consumer appealing.

**1.9.7. Factors Affecting Internal Qualities of Table Eggs and Shelf –Life**

Internal qualities of table eggs refers to egg white (albumen) cleanliness and viscosity, size of the air cell, yolk shape and yolk strength and involves functional, aesthetic and microbiological properties of the egg yolk and albumen (King, 2012). The proportions of components for fresh table egg are 32% yolk, 58% albumen and 10%shell (Encyclopedia Britannica, 2012). The albumen is the clear liquid contained within the egg and the yolk is the yellow spherical part of an egg that is surrounded by the albumen.Therefore, as soon as the egg is laid, the internal quality begins to decline.

Some the factors that affect internal qualities of table eggs as described by Stadleman (1977­), as the characteristic of an egg that affect its acceptability to the consumer.

Factors affecting the internal qualities of egg are storage, Hen age and strain, nutrition, induced moult and disease.

**1.9.7.1. Storage:** Storage time and temperature have been shown to affect degree of egg mottling.Jones *et al.,* (1990), stated that as the internal temperature of the egg increases above 70C, the protein structures of the thick albumen and vitelline membrane breakdown faster. The quality of egg decreases due to poor storage condition (Long storage period, high temperature and presence of strongly scented material in egg store)(King, 2012).

**1.9.7.2. Induced Moult:**The albumen quality in the older hens has been shown to improve following an induce moult (Tona, Bmelis, De-Ketelaere, Braham, & Decuypene, 2002).

**1.9.7.3. Nutrition:** Many report have been given that albumen quality can decrease with increasing dietary protein and amino acid content (Hammershoj and Kjaer, 1999) The excess of riboflavin in the diet cause egg white turn green. Esonu (2006), said ‘the absence of xanthophylls in the diet will lead to pale yolk and inclusion of more than 5% cottonseed in the diet will result in olive or salmon colored yolk’., also the anticcocidal drug, Nicarbazin when fed at concentration of 0.005% or greater in the diet and deworming drugs, such as Piperazine and dilbutyltin dialaurate have been reported to cause yolk molting (Jacqueline, Richard, & Mather, 2011). The inclusion of more than 5% cottonseed meal in a layer diet will result in olive or salmon coloured yolks (Beyer,2005).Both inadequate mixing of the diet as well as excessive mixing of the diet will result in a heterogenous feed, and subsequent variation in the amount of pigment consumed by each hen will result in the yolk colour not being uniform while pale yolk can result from any factor (worms,coccidiosis) which prevents the absorption of pigments from the diet or the deposition of the pigment in the yolk (Complete Fedd Solution, 2006).

**1.9.7.4. Microbial Contaminants:** Bacteria and fungi contamination occur due to feacal contamination, improper washing procedures, high storage temperature and humidity, long storage period and infection of hen’s oviduct.Meat and blood spot in addition to aesthetic and ethical problem may also increase the risk of infection such as salmonella (Smith and Musgrove, 2008).

**1.9.7.5. Age and Strain of Hen:** As hen ages,the production of spot tends to increase(Bustany and Elwinger, 1987), Haugh unit decrease with increasing bird age value, with decreasing by around 1.5 to 2 units (Coutts and Wilson,1990).Strain of hen has also been shown to play a role in albumen consistency, with some strains producing eggs with thin albumen(Curtis, Gardner, & Mellor, 1985).

**1.9.7.6. Disease:**Evidence has shown that infectious bronchitis impairs the synthesis of albumen protein in the magnum (Buthler, Curtis, Pearson & McDougall, 1972)

**1.10. Shelf –Life And Quality Of Table Egg**

Shelf life of eggs can be affected by the storage medium, absence of cracks on the shell and weather condition ((Alabi,O.M.,Olaniregun B.C., Aderemi F.A. and Oludapo E.O, 2013)

**1.10.1. Shell Thickness:** The amount and thickness of the egg shell have been found to be related to egg shell strength, shell thickness is determined by the amount of time it spends in the shell gland and the rate of calcium deposition during shell formation, therefore if the egg spends a short period in the shell gland, the less the thickness (Neospark, 2012). Reduction in shell quality lowers egg shelf-life, hatchability and increases breakages. Shell thickness has significant effect on moisture loss during storage and incubation (Bennett, 1992).

**1.10.2. White Egg (Albumen):** Egg albumen is the clear liquid contained within an egg. Its purpose is to protect the egg yolk and provide additional nutrition for the embryo. the development of watery whites is chiefly due to increasing age of the egg. When an egg broken on to a flat surface has a watery white, it indicates that the egg is stale (King, 2012). Egg white is the only portion of egg that is capable to develop into achiola if the egg is fertilized before it is laid. It is rich in proteins and contains almost no fat, unlike the yolk.

**1.10.3. Yolk Quality:** Yolk quality is related to its appearance, texture, firmness, and smell, the yolk of a freshly laid egg is round and firm (Jacob, Miles, & Mather, 2000; Olaniregun, 2010) As an egg ages, the yolk takes up water from the albumen which in turn dilutes blood spot and makes them look like meat spot.(King,2012). Primary determinant of yolk colour is the plant pigment (Xanthophyll) which could be added to the layer’s feed naturally or synthetic, however the higher inclusion or in correct ratio of pigment can lead to orange-red yolks (Coutts and Wilson, 1990).

**1.10.4. White Ropey Things (Chalazae):** is the chord that anchor or keep the yolk in the middle of the egg, it is more noticeable in fresh egg and generally disappear when the egg is cooked (Lee, Lee, Lee & Jeong, 1996).

* 1. **Egg Contamination And Preservation**

It is hypothesized that bacterial contamination of the egg content could result from the penetration of the shell by bacterial deposited on the surface of the egg after it has been laid which could be refers to has horizontal infection route (Schonfeld, 1995). Contaminated environment in which the hen laid its eggs such as nest box, the hatchery environment or the hatchery truck, can lead to outer shell contamination.(Gantois*et al*, 2009), The increasing number of micro-organism on the egg shell consequently increase the risk of microbial egg shell penetration and egg content contamination (De Reu *et al.*, 2006). The presence of chicken manure and other moist organic materials facilitate the survival and growth of some micro-organism. Aside the horizontal route of bacterial infection of eggs, egg contamination can also occur through vertical or transversal route (Bruce and Drysdale, 1994), therefore contaminant are capable of passing through.

In a study carried out by Humphrey, Whitehead, Gawler, Henley, & Rowe, (1991) suggest that internal egg contamination is more likely to occur during formation of the egg rather than by penetration through the shell.

In other words, Eggs can be contaminated during its formation before laying by colonization of the reproductive organ; which has been shown to be a consequence of systemic spread of airborne infections (Baskerville *et al*., 1992; Leach *et al*., 1999) and was even observed by Leach *et al*., (1999) that the contamination rate of eggs was much higher following an aerosol challenge of the laying hens than following an oral challenge. Also colonization of the ovary, it is been said that yolk contamination is more often associated with vitelline membrane (Gast and Beard,1990; Gast and Holt,2001).Howard *et al*. (2005) believes that the penetration of immature follicles has practical implications because it can lead to contamination of egg after maturation and can cause continuous transovarian infection of eggs throughout the reproductive cycle because high level of nutrients are available to bacteria invading ovarian follicles (Gantois,*et al*., 2009). However follicle colonization is not believed to be an important source of egg contamination. (Gantois *et al*., 2009).

Vaginal colonization is another site of egg contamination, Gantois*et al*., (2009) studies show high egg contamination rate after intravaginal infection, indicating the high risk of contamination (primarily eggshell contamination) as the egg passes through a heavily colonized vagina.

Colonization of isthmus and magnum can result in contamination of eggshell membranes (Bichler, Kabambi, Nagaraja, & Halvorson, 1996; Miyamoto*et al.,* 1997; Okamura*et al*., 2001a, b). Among the most common of these harmful bacteria is *Salmonella* species which is most often spread through poultry, eggs and eggs product etc. (MicrosoftEncarta, 2009) or that were not properly refrigerated.

Preservation of egg is on the concept of minimizing the losses of carbon dioxide which from the starting point of deteriorative change minimizing losses is achieved largely by physical techniques (Olaniregun, 2010). Oiling of eggs within 24 hours of lay is very effective in slowing down reduction in albumen quality though it does not replace the need for cool storage (Jacqueline *et al*., 2011).

**1.11.1** **Egg Storage**

Egg handling and storage practices have a significant impact on the quality of eggs, Throughout the ages there are different ways ofstoring eggs, Bahrouz, (2005) says that there is minimal or no growth of microorganism when eggs are refrigerated at 40Oiling of eggs can also help to reduce CO2 losses and thus help maintain internal egg quality (Beyer, 2005; Koelkebeck, 1999; Coutts and Wilson, 1990) but is not a substitute for cool storage (Jacob et al., 2000).

* + 1. **Egg Contamination The Risk Of Human Health**

Microbial contamination of egg has important outcome to the poultry industry and illness from contamination is a source of a serious public health challenge around the world (Hajieh *et al.,* 2011).The significance of these disease in human vary from mild symptoms to life threatening situation(Kaneko,Hayashidani, & Ohtomo, 1999).The egg and its product as an important component source of necessary nutrients and a major food within the human diet, inspite of the antimicrobial factors, it can still be infected with different bacteria. Some bacteria have been identified as causes of food borne diseases such as Salmonella, *Escherichia coli*, *Listeria monocyttogens*, c.jejuni e.t.c. *Listeria monocytogens* can cause illness in pregnant women, babies and people with reduced immunity and febrile gastroenteritis in healthy people. (Esteban, Oporto, Aduriz, Juste, & Hurtado, 2008).

*Escherichia coli* is one of the most common bacteria which cause diarrhea especially in children, the elderly, infants and those with impaired immune systems may have a more severe illness (Hajieh*et al.*, 2011).In view of this, the infection may spread from the intestine to the blood stream and then to other part of body and can lead to death unless the person is treated promptly with antibiotics.

One of the most important factors constituting a risk to consumer health is contamination of egg with salmonella, which is closely correlated with the spread of these bacteria among poultry.Salmonella can be inside of the completely normal – appearing eggs, and if the eggs are eaten raw or undercooked, the bacterium can cause illness (Davies and Breslin, 2003).

* + - 1. **Reduction Of Contaminant In Eggs**

In other to reduce the risk of externally contaminated eggs in food chain, the need to rapidly remove any feacal contamination. However, intensive control measures in the United States, such as examining eggs for cracks, and washing and disinfecting eggs have not eliminated egg contamination (Braden, 2006). Egg should be collected regularly, reducing the time they are exposed to higher environmental temperature and contaminant and also stored at temperature of 7 to 130C and humidity of 50 to 60%.

Bacterial and fungal contamination of eggs, resulting from faecal contamination of the egg, can be prevented by good management practices, including regular replacement of nesting materials or good cage maintenance as appropriate (Beyer, 2005; Coutts and Wilson, 1990)

Proper handling and storage of eggs following collection will minimize the opportunity for bacterial or fungal contamination. However, improper washing procedures, high storage temperatures and humidity will increase the incidence of bacterial of fungal contamination (Coutts and Wilson, 1990)

Careful attention should be paid to feed source, as Salmonella spp. can be transmitted through the feed

**1.12 Mechanism Of Microbial Contamination Of Intact Eggs**

Microorganism (bacteria) infects the eggs by either vertical transmission of the egg within the ovary or horizontal Transmission through trans-shell contamination (Kinde *et al.*, 2000, FAO, 2002, Daviesand Breslin,2003; Van Immerseel*et al.*, 2005)

**1.12.1 Transovarian Or Vertical Transmission**

Vertical transmission is considered to be a direct deposition of micro-organism especially salmonella species in to the follicle/ yolk while still present and or attached to the ovary. Vertical transmission can originate from infection of the ovaries of a laying hen via systemic infection through an oral infection (EFSA, 2005) or from an ascending infection from the contaminated cloaca to the vagina and lower region of the oviduct (Keller, Benson, Krotec, & Eckroade, 1995). Many studies have shown that poultry can become infected after oral inoculation without showing clinical sigh (EFSA, 2005) however after intramuscular infection, birds may become ill (Desmidt, Ducatelle, & Haesebrouck, 1997).

The salmonella enteriditis infection of the yolk was only occasionally found and more frequently present on the yolk membrane (Gast and Holt, 2001) but Barrow and Lovell (1991) found ovaries infected with salmonella species but could not isolate this bacterium from the egg content. Sequential feacal shedding of these bacterial can occur with potential risk to the forming of egg.

**1.12.2. Horizontal Transmission**

Horizontal transmission can occur both before (internal) and after (external) shell formation. The microorganisms penetrate through the eggshell (Koen,2006), Infection of the egg contents can occur from the moment of ovulation onwards until consumption (EFSA,2005), and this cover the whole area from detachment of mature follicles ,to the point where the egg is collected and further to include trans- shell penetration. Trans - shell movement of bacteria can occur under the appropriate condition of temperature, humidity etc. in spite of a number of defense mechanism to limit the effects of such an event.

During the journey of the ovum from the ovary to the cloacae there are many possibilities for infection since bacteria from the cloacae area can enter the oviduct and move toward the upper part of the magnum. The egg passes through highly contaminated cloaca area at the moment of laying which is illustrated by visible feacal contamination on the shell, the content will contact and a negative pressure is been established inside the egg thereby moving contaminants through the shell (Padron, 1990).

 This contamination will deposit at the shell membranes andpose a future threat to the egg (EFSA,2005).Grijspeerdt *et al.*,( 2004) show in a growth model how such an infection can develop inside an egg. However the egg presents a complex series of defensive barrier to the contaminating organisms and although microbes may successfully penetrate theshell of the egg (Bruce and Drysdale, 1994).

 The likelihood of trans- shell penetration increases with the length of time that the eggs are in contactwith contaminating materials (EFSA, 2005). However, in all, there is no indication that *salmonella* species can move more effective through eggshells and the underlying membranes that other competing feacal organisms (Humphrey, 1994).

**1.12.3 Other Factors Affecting Horizontal Transmission**

The extrinsic factors that can affect the penetration of micro-organism through horizontal transmission include; Temperature differential, Moisture and the presence of bacterial contamination and feaces.

**1.12.3.1 Temperature Differential**

One of the main factors governing microbial contamination of egg is temperature differential which is at the moment of laying from the point of lay as the warm egg cools, there is a negative pressure (Berrang *et al*., 1999) that is been created down the pore which may results in drawing contamination bacterial of the shell through the pores (Bruce and Drysdale, 1994).

**1.12.3.2 Moisture**

According to some of these authors(Berrang, Cox, Frank, & Buhr, 1999) who reported that moisture is needed to allow penetration and it is well established that penetration will be greatly enhanced in circumstances where in addition to moisture a positive temperature differential is present which can cause the content of egg to contract and even draw any water present through the open pores (Board and Halls, 1973; Berrang *et al*., 1999).

**1.12.3.3 Presence Of Bacterial Contamination And Feaces**

It is generally accepted that the egg is most susceptible to contamination at the point of lay, following that the microbiological status of the environment in to which the newly laid eggs are deposited has a great influence on the incidence of contamination in eggs. As it is said by Bruce and Drysdale (1994) that eggs laid into a heavily contaminated environment suffer more bacterial spoilage than those laid in clean environment.

The level of these contamination ranges from 103 -105 Cfu aerobic bacterial per egg in a clean condition and 107 – 109 Cfu in a dirty condition (Koen, 2006).The presence of feacal materials on deep litter waste thereby appears to increase contamination which cannot be attributed solely to increased moisture level (Koen, 2006). Therefore feacal or other soiling materials may contain substances which reduces the surface tension of any moisture present which increase the rate of bacterial penetration (McKenney and Allison, 1997) the feaces or other soiling material may contribute some chemical like iron which interferes with the natural defense mechanisms of egg thereby allowing bacteria to establish more easily in to the egg once penetration has taken place. (Bruce and Drysdale,1994).

**CHAPTER TWO**

**2.0 MATERIALS AND METHODS**

**2.1 Source and collection of Samples of Materials**

Five trays (150pieces) of farm fresh table eggs were collected from the poultry unit housed using battery cage system of the Teaching and Research farm of Bowen University, Iwo.

Poultry droppings (semi–solid) from the pit under the batterycages were obtained from the same poultry unit and collected into four(4) sterilized plastic bowls.

**2.1.1 Treatment Groups and Experimental Design**

The farm fresh table eggs were randomly allotted into five (5) treatment groups in a complete randomized design [CRD] of 30 pieces per treatment as expressed in Figure 2.1. The sums of 15 pieces of table eggs were used in each treatment group.

Treatment [T1] consisted of unsoaked eggs at 0 hour, and served as the control while treatments T2, T3, T4, and T5 were soaked in poultry droppings contained in sterilized plastic bowls for 12 hours, 24 hours, 48 hours and 72 hours, respectively.

Treatment(T2),30 pieces in poultry droppings Soaked For 12 Hours

Treatment(T3), 30 pieces Soaked in poultry droppings for 24 Hours

Treatment(T4), 30 pieces Soaked in poultry droppings for 48 Hours

Treatment (T5), 30 pieces Soaked in poultry droppings for 72 Hours

5 Trays of freshly laid Table Eggs (150 pieces)

Treatment (T1), 30 pieces of Unsoaked Eggs for 0 Hour

**Fig 2.1**. **Formulation of Treatment groups for Sampled Table Eggs**

**2.1.2. Isolation of microbial species**

Microbial Samplings was done on each treatmentat 0 hour, 12 hours, 24 hours, 48 hours and 72 hours, respectively. At each sampling time, five (5) table eggs from each of the treatment groups described in section 2.1.1 were surface-sterilized using the method of Arathy*et al.* (2009). The outer surface of each egg shell was swabbed with cotton wool soaked with ethanol in order to avoid more penetration of contaminants. The surface-sterilized eggs were then allowed to air- dry for 30 seconds to ensure that the alcohol treatment had evaporated.

The shells were opened around the air- sac region with sterilized scissors. The albumen and the yolk with intact vitelline membrane were then drained and transferred, respectively, into separate sterile beakers. The yolks as well as the albumen from five-egg samples in each treatment group were pooled together, separately, to form one yolk and albumen sample. The separate yolk and albumen samples were then thoroughly mixed together using a sterile glass-rod.

A sterilized swab stick was inserted in to the separately pooled samples of yolk and albumen and then in to 10ml of sterile maximum recovery diluent [MRD] contained in test-tubes and their contents incubated at 37°C for 24 hours in an incubator. After incubation, the yolk-treated MRD was serially diluted with sterile MRDs and 0.1ml of the final dilution plated using the pour–plate technique into different solid media. Similarly, 0.1ml of the MRD treated albumen was also pour-plated in to different solid media. The experimental procedure was repeated in triplicates.

The solid media used for enumeration were Nutrient Agar for Aerobic bacterial counts; Baird Parker Agar for *Staphylococcus aureus* count, Eosin-ethylene Blue Agar for *Enterobacteriaceae* count enumeration, Salmonella-Shigella Agar for *Salmonella*-*Shigella* counts and Potato Dextose Agar for Fungi or yeast and mouldcount.

The plates for bacterial isolation were incubated at 37°C for 24 hours while plates for fungi were incubated at 25°C for 48 hours as described by Olutiola *et al.* (2000). After incubation, the viable cell counts obtained on the different inoculated media plates were converted to microbial load using the formula;

Microbial Load = N/V x R

Where, N = Number of colonies; V = Volume of Inoculum and; R = Dilution factor

The average colony counts obtained from the countable plates were expressed as Colony Forming Units per milliliters (CFU/ml). The mixed cultures obtained after each incubation period were further purified by directly streaking the selected organism on the appropriate media-plate until pure cultures were obtained. Pure cultures were then stored on nutrient agar slants contained in separate sterile McConkey bottles and kept in a refrigerator until needed.

**2.2. Cultural identification of bacterial isolates**

Standard procedures described below were utilized for the characterization and identification of pure bacterial cultures obtained from the study. The probable identities of the isolates were determined usingstandard characteristics as specified by Buchanan and Gibson, (1974) and Farmer *et al*. (1985).

**2.2.1. Gram – staining**

A standard Gram- staining procedure as described by Fawole and Oso (2001) was carried out on each bacterium isolate obtained. A smear of the pure bacterial isolate was prepared by placing a drop of sterile water on a clean grease-free slide and rubbing 16 to 18 hours old butt bacterial colony in it using sterile inoculating loop. This was spread into a thin smear along the slide. The smear was allowed to air –dry and the slide heat –fixed by passing the reverse side over a flame 3 times as described by Fawole and Oso (2001).

The smear was flooded with crystal violet stain for 30 -60 seconds, drained quickly and washed with Gram’s iodine. The iodine solution was allowed to stay on the smear for 60 seconds, drained off and the slide washed gently under the tap. The slide was then washed with 95% ethanol until the slide appeared free of violet stain, rinsed under the tap and flooded with sterile distilled water and blotted dry.

Microscopic examination followed under an oil-immersion lens objective with a magnification strength of 1000. A pink colouration indicated Gram – negative while purple colour indicated Gram – positive reaction.

**2.2.2. Citrate utilization test**

The presence of enzyme citrate and their production as a source of carbon which breaks down citrate to oxalo-acetic acid and acetic acid was demonstrated as described by Arora and Arora (2007). Sterile Simmon Citrate agar slants were prepared in test – tubes. In duplicates, test organisms were streaked on the slants and incubated at 37°C for 48 hour. A change in colour from green to blue indicated a positive reaction.

**2.2.3. Sugar fermentation test**

Sucrose, glucose, Sorbitol, Raffinose, Arabinose, Mannitol and Lactose were separately added into different test tubes at 0.05% concentration together with nutrient broth. Two drops of 0.001% phenol red indicator and an inverted Durham’s tube were added into each broth. These were sterilized in an autoclave at 121°C for 15 minutes. After cooling each of the bacterial isolate was inoculated separately into the medium.

Uninoculated tubes for each sugar were also prepared and these served as control. Both the uninoculated and the inoculated tubes were incubated for three days. Acid production was shown by a change in colour. The production or non-production of gas in the Durham’s tube was also noted.

**2.2.4. Motility test**

Bacterial motility was demonstrated as described by Arora and Arora (2007). A test bacterial colony was picked with the aid of sterilized inoculating needle and stabbed into a sterile semi – solid nutrient agar having 0.4% agar concentration. The medium was contained in a slant. The medium was then incubated at 37°C for 24 hours. Motile organisms swarm away from the line of inoculation into uninoculated area in the medium while non-motile organisms were found only along the line of inoculation.

**2.2.5. Triple sugar iron test**

The production of sugars [Sucrose, dextrose,lactose and Hydrogen sulphide (H2S)] by fermentation was demonstrated as described by Harisha (2007). Sterile Triple sugar iron agar was prepared in test tubes which were autoclaved and allowed to cool and solidify.

Separate test organisms were then pierced in to the butt region and also streaked on the slant region. The tubes were incubated for 24 – 48 hours at 37°C.

**2.2.6. Starch hydrolysis**

This biochemical test detects the production and presence of the starch hydrolytic enzyme, amylase, to act on starch in order to produce maltose. The starch hydrolysis procedure as described by Aneja (2003) was utilized. Starch agar media were prepared in sterile petri – dishes and allowed to solidify. The test organisms were streaked on the agar plates and incubated for 48 hours at 37°C.

A clear zone surrounding the colonies indicated a positive reaction while a dark blue colouration indicated a negative reaction.

**2.2.7. Indole test**

The production of the enzymes tryptophanase in a test organism with the conversion of the amino acid tryptophan into molecules of indole was demonstrated as described by Harisha (2007). Ten milliliters of sterile peptone water was dispensed into two separate test- tubes. One test tube served as control while the other test- tube was inoculated with the test organism and incubated at 37°C for 72 hours. Then after the incubation, Kovac’s reagent of 0.5ml [dimethyl-aminobenzaldehyde] was added to 5ml of the culture, mixed properly and allowed to settle.

The formation of deep red colour indicated a positive reaction while a negative reaction gave no colour change.

**2.2.8. Catalase test**

Presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide in a test organism was demonstrated as described by Fawole and Oso (2001). A thick emulsion of each isolate was made on a clean glass slide using saline water. A loopful of 3% hydrogen peroxide was later added to the thick emulsion.

Effervescence caused by the liberation of oxygen as gas bubble indicated the production of catalase.

**2.2.9. Methyl red**

The presence and production of large amount of organic acid like formic, acetic, lactic and succinic [end products] from glucose was demonstrated as described by Harisha (2007).

The test organism was inoculated into test-tube of sterile peptone water and the culture was incubated at 37°C for 48 hours. 5 drops of methyl red solution were added and the colour examined. The formation of red colour indicated a positive reaction.

**Voges-Proskauer test**

Presence and production of the neutral products from glucose was demonstrated as described by Aneja (2003). A tube containing sterile peptone water was inoculated with the organism and incubated at 37°C for 5 days. Whereby 1ml of the inoculums was pipette into a test- tube 0.5m of 6% alpha- naphtol solution and 0.5ml potassium hydroxide was added to the test- tube after which the test – tube was shaken and allowed to stand for 5 minutes.

The formation of red/ pink colour indicated a positive reaction.

**2.2.10. Casein hydrolysis**

The ability of micro- organism to utilize casein as a source of nitrogen and produce protease enzyme which breaks down the casein was demonstrated as described by Olutiola *et al.,* (2000). A sterile milk agar (nutrient agar plus 10% skim milk) was prepared and autoclaved at 105kgcm-2 for 15 minutes. This was then dispensed into a sterile petri –dishes and allowed to cool.

Test organisms were then inoculated by streaking once across the surface and the plate incubated at 370C for 48 hours. Indication of casein hydrolysis gave a clear zone around the streaked location.

**2.3. Identification Of Yeast**

Standard Morphological and Physiological/biochemical test were utilized in the identification of the isolated yeast cells to species level. The obtained yeast isolate were grown on potato dextrose agar (PDA) for determination of their colony morphologies. Wet mounts of the yeast isolates were prepared on clean slides and stained with crystal violet solution and observed under x40 objective and oil immersion lens of compound microscopes. The shape and presence or absence of spore i.e. whether they existed singly, paired or aggregated in large clumps were noted as described by Chanchaichaorivat, Ruenwongsa, & Panijpan, (2007).

The physical/biochemical characteristics of each yeast isolate was determined by detecting the ability of the isolate to ferment certain sugars semi- anaerobically and to assimilate a variety of carbon compounds as a major source of carbon source.

**2.4. Antimicrobial susceptibility**

Antimicrobial susceptibility test was carried on *Staphylococcus aureus* and *Shigella* species obtained from the eggs soaked in poultry droppings for 0 hour, 12 hours, 24 hours, 48 hours and 72 hours. Separately, a 16 -24 hour old pure colony of each test organism obtained at the different time interval was inoculated on sterile nutrient agar plates and the inoculum uniformly spread over the surface with sterile cotton swab. Antimicrobial disc were then placed carefully on the surface of each plates using a pair of forceps. The plates were incubated at 37°C for 24 hours. The diameters of inhibition zones were determined using a transparent ruler.

The *S. aureus* isolates were tested using Gram-Positive multi-facet antibiotics disc containing; Ceftazidine (30ug), Cefuroxime (30ug), gentamycin (10ug), Ceftriaxime (30ug), Erythomycin (5ug, Cloxacillin (5ug),Ofloxacin (5ug) and Augmentin (30ug), while the*Shigella* species were tested with a Gram- negative multi-facet antibiotic disc containing Ceftazidine (30ug), Cefuroxime (30ug), gentamycin, Cefiximine (5ug), Ofloxacin (5ug), Augmentin(30ug), Nitrofurontion (300ug) and Ciprofloxacin (5ug ) bacteria.

**2.5. Statistical analysis of data**

The experimental data obtained were subjected to statistical analysis using the Genstat Release Discovery Edition, version 2009 (VSN, International Limited). The one-way analysis of variance (ANOVA) for utilized for all analyses except when comparing the effect of two factors on a measured parameter during which a two-way ANOVA was used. Significant differences were assessed at *P<0.05* Least significant difference (LSD) was used to separate the obtained mean differences.

**Experimental Model**

Yij = N + I +j +ij +e

N = General mean.

I = Effect of Length of soilage on type of microbes in theegg.

J = Effect of Length of soilage on thepopulation of each microbes in the egg.

Ij = Interactive effect of length of soilage on type and population of organism.

E = Standard error.

**CHAPTER THREE**

**3.0 RESULTS**

**3.1** **Microbial counts in the egg content**

**3.1.1** **Aerobic plate count**

Figure 3.1 showed the changes in the aerobic plate count of internal content of table eggs soaked in poultry droppings. Generally, it was observed that the aerobic plate count in the albumen gradually increased with time from an initial mean of 3.77 Log CFU/ml at the onset of the soaking process to a maximum of 5.43 Log CFU/ml at 48hours. However, at 72 hours the aerobic plate count had dropped to 4.74 logCFU/ml. statistically, the obtained mean aerobic plate counts were highly significant (P<0.01) at 12, 24,48, and 72 hours respectively has compared to the control, both in the yolk and the albumen. (Appendix 1).

Aerobic plate counts in the yolk also increased from an onset mean of 6.62 Log CFU/ml to a maximum of 7.36 log CFU/ml at 48 hours. Statistically, the obtained mean aerobic plate counts were significant (*P<0.05*) at 0 hour and 12 hours.

Statistically, the aerobic plate count obtained in the yolk was highly significantly (*P<0.01*) higher than those in the albumen for all the treatments. Furthermore, time had a highly significant effect on the aerobic plate count obtained. The interaction between time and egg part had a significant effect(*P <0.05)* on the internal aerobic plate count.

**3.1.2.** ***Staphylococcus aureus* count**

Figure 3.2 showed the changes in the *Staphylococcus aureus* plate count of internal content of table eggs soaked in poultry droppings. In the albumen, the *S.aureus* plate count steadily increased with time from an initial mean of 0.00 Log CFU/ml at the onset of soaking process to a maximum of 4.61 CFU/ml at 72 hours. At 24 hours, the *S. aureus* count decreased down to 4.17 log CFU/ml at 48 hours. Statistically, the obtained mean *S. aureus* plate counts were highly significant (P<0.01) at 12 hours (Appendix 2).

In yolk the mean *S aureus* plate count rose from an initial count of 6.83 logCFU/ml at 0hour to a maximum of 6.88 Log CFU/ml at 12 hours. Afterwards, the count fell and rose to 6.47 LogCFU/ml and 6.53 Log CFU/ml, respectively. A final mean declined count of the 6.32 log CFU/ml was obtained at 72 hours. Statistically, the obtained mean *S. aureus* counts obtained were not significantly (p<0.05) difference at the different periods studied.

Statistically, the staphylococcus plate count in the albumen is highly significant. Furthermore, time had a highly significant effect on the staphylococcus aureus count. The interaction between the time and egg content is also highly (p<0.01) significant.

**Fig 3.1**.Changes in mean aerobic plate counts of internal content of eggs soiled with poultry droppings

**Fig 3.2.** Changes in mean of *Stapylococcus aureus* plate count of internal contents of egg soiled with poultry droppings

**3.1.3. *Enterobacteriaceace* Count**

Figure 3.3 showed the changes in the *Enterobacteriaceace* count of internal content of table eggs soiled with poultry dropping. In the albumen, the *Enterobacteriaceace* count slowly escalated from an initial mean of 3.46 Log CFU/ml at the beginning of the soaking process to a maximum of 5.68 Log CFU/ml at 48 hours. At 72 hours, the *Enterobacteriaceace* count had declined to 5.13 log CFU/ml. statistically, the attained mean *Enterobacteriaceace* plate counts were highly significant(P<0.01) at 0 hour, 12 hours and 48 hours (Appendix 3).

Enterobacteriaceace plate count inside the yolk also progressed from the commencement mean count of 5.77 Log CFU/ml to the highest value of 7.52 Log CFU/ml at 72hours. Statistically, the achieved mean *enterobacteriaceace* plate counts were highly significant (P<0.01) at 0 hour, 12 hours and 24 hours.

Statistically, the *enterobacteriaceace* plate count in the yolk was highly significantly more than those in the albumen. Furthermore, time had a highly significant effect on the *enterobacteriaceace* count obtained. The interaction between the time and egg part had a significant (P<0.05) effect on the internal content of *enterobacteriaceace* plate count.

**3.1.4.. *Salmonella* –*Shigella* Plate Count**

Figure 4.Showed the variation in the *salmonella-shigella* plate count of internal content of tables eggs soaked in poultry dropping. It was detected that the salmonella and *shigella* in the albumen progressively increased with time from an initial mean of 0.00Log CFU/ml at the onset of the soaking process to a maximum of 5.42 Log CFU/ml at 72 hours. Meanwhile at 48 hours there is a decline in count before the count increases at 72 hours again. Statistically, the mean *salmonella* and s*higella* plate counts were significant (P<0.05) at 0 hour, 12 hours and 24 hours. (Appendix 4).

The mean *salmonella* and *shigella* plate count within yolk also rose from an onset mean count of 0.00 Log CFU/ml to a maximum of 6.95 Log CFU/ml at 48 hours. Nevertheless at 72 hours, the *salmonella* and *shigella* plate counts were highly significant (P<0.005) at 0 hour, 12 hours.

Statistically, the *salmonellashigella* counts obtained in the yolk was highly significant (p<0.005) than in the albumen. Furthermore, time had a significant (P<0.05) effect on the plate counts obtained. The interaction between time and egg part had a significant (P>0.05) effect on *salmonella* and *shigella* counts obtained.

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|  |

Fig 3.3. Changes in mean of *Enterobacteriaceace* plate count of internal content of egg soiled with poultry droppings

Fig 3.4. Changes in mean of *Salmonella – Shigella* plate count of internal content of egg soiled with poultry droppings

**3.1.5. Fungi Plate Count**

Figure 5 showed the differences in the fungi plate count of internal content of table eggs soiled with poultry droppings. In the albumen, the fungi plate, count slowly with time from an initial mean of 3.90Log CFU/ml at inception of the soaking activity to a peak of 5.31 log CFU/ml at 72 hours. Between 24 hours and 48 hours the fungicount, begin to drop to 3.99Log CFU/ml and 1.57 log CFU/ml respectively. Statistically, the acquired mean of fungi plate counts were highly significant (p<0.005) especially at 0 hour, 12 hours, 24 hours and 72 hours. (Appendix 5).

The fungi plate count in the yolk also increased from an onset mean count of 6.66 Log CFU/ml to a maximum of 7.12 log CFU/ml at 48 hours. Meanwhile at 72 hours, the fungi plate count had dropped to 7.12 log CFU/ml. The obtained mean fungi plate count were significant (p<0.05) at 24 hours, 48 hours and 72 hours.

The yeast in the albumen and yolk is highly significant (p<0.005). More so, time had a highly significant effect on the yeast plate count attained. And the interaction between time and egg content had highly significant effect also on the internal yeast plate counts.

Fig 3.5. Changes in mean of fungi plate count of internal content of egg soiled with poultry droppings.

**3.2. Properties and probable identities of bacterial isolates.**

The biochemical properties and probable identities of the bacteria isolates obtained from the yolk and albumen contents of table egg s soiled in poultry feacal droppings are expressed in Table 1.Two groups of bacterial isolates were identified based on Gram staining and biochemical test. The first were the Gram-positive rods which were identified as *Staphylococcus aureus*, *Bacillus substilis* and *B. brevis*. The second were the Gram- negative rods which were identified as *shigella* species, *Edwardsiella tarda*, *Escherichia* coli, *Salmonella* species, *Klebsiella* species, *Citrobacter* species, *Kluyvera* species, *cedecea* species and *Serretia* species.

**3.3. Frequency of Identification of the obtained bacteria Isolates**

The distribution of the obtained bacterial isolates during the soaking process is shown in Table 3; at 0 hour *Bacillus pumitus*, *Klebsiella* species, *Citrobacter* species, *Kluyvera* species and *serratia* species were discovered in albumen of the egg. *Shigella* species, *staphylococcusaureus, Edwardsiella tarda, Bacilluspumitus salmonella* species and *Serretia* species were more frequently isolated than the other bacterial organisms at different soaking interval of albumen of the table eggs. In the yolk, *Staphylococcus aureus*, *Bacillus pumitus, Edwardsiella tarda*, *Edwardsiella* species*, bacillus brevis*, *Klebsiella* species, *citrobacter* species, *Kluyvera* species and *Serratia* species were isolated in the yolk at 0 hour, while *Shigella* species, *Staphylococcus aureus,Bacillus pumitus, klebsiella* species were frequently found.

**Table 3.1.** **Properties and probable identities of bacterial isolates**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S/N** | **ISOLATE** | **GRAM STAINING** | **CASEIN HYDROLYSIS** | **TRIPLE SUGAR IRON** | **SCA** | **STARCH TEST** | **INDOLE TEST** | **METHYL RED** | **MOTILITY TEST** | **CATALASE TEST** | **V.P.TEST** | **GLUCOSE** | **SUCROSE** | **MANNITOL** | **XYLOSE** | **RAFFINOSE** | **SORBITOL** | **PROBABLE ORGANISM** |
| 1 | A1 |  - |  |  - |  |  - |  +  |  - |  - |  + |  |  - |  - |  - |  - |  - |  - |  - | *Shigella*species |
| 2 | A2 |  - |  |  - |  |  + |  + |  + |  - |  + |  + |  + |  - |  - |  - |  |  - |  - | *Shigella* species |
| 3 | A3 | + |  |  - |  |  + |  - |  - |  - |  + |  + |  + |  + |  + |  + |  + |  +  |  + | Staphylococus aureus |
| 4 | A4 |  - |  |  + |  |  + |  + |  + |  - |  + |  - |  - |  + |  + |  + |  + |  + |  + | *Edwardsiella* tarda |
| 5 | A5 |  - |  |  - |  |  + |  - |  - |  - |  + |  + |  + |  + |  + |  + |  - |  + |  - | *Edwardsiella* tarda |
| 6 | A6 |  - |  |  + |  |  + |  + |  - |  - |  + |  - |  - |  - |  - |  - |  - |  - |  - | *Shigella* species |
| 7 | A7 |  - |  |  |  |  - |  + |  - |  - |  + |  - |  + |  - |  - |  - |  - |  - |  - | *Shigella* species |
| 8 | A8 |  - |  |  - |  |  + |  - |  + |  - |  + |  + |  - |  + |  + |  + |  + |  +  |  + | *Escherichia* coli |
| 9 | A9 | + |  |  - |  |  + |  - |  + |  - |  + |  - |  + |  + |  + |  + |  + |  +  |  + | *Bacillus* substilli |
| 10 | A10 | + |  |  + |  |  + |  - |  - |  - |  + |  + |  - |  + |  + |  + |  + |  +  |  + | *Bacillus* brevis |
| 11 | A11 | - |  |  + |  |  + |  - |  + |  - |  + |  + |  + |  + |  + |  + |  + |  +  |  + | Salmonella species |
| 12 | A12 |  - |  |  - |  |  + |  - |  + |  - |  + |  +  |  - |  + |  + |  + |  + |  +  |  + | *Klebsiella* species |
| 13 | A13 |  - |  |  - |  |  + |  - |  + |  - |  + |  + |  + |  + |  + |  + |  + |  +  |  + | Citrobater |
| 14 | A14 |  - |  |  - |  |  + |  + |  - |  - |  + |  - |  - |  + |  + |  + |  + |  +  |  + | *Kluyvera* species |
| 15 | A15 |  - |  | - |  |  + |  - |  + |  - |  + |  - |  - |  + |  + |  + |  + |  +  | + | *Cedecea* species |
| 16 | A16 |  - |  |  - |  |  + |  - |  - |  - |  + |  - |  - |  + |  + |  + |  - |  +  |  + | *Serratia*marcescens |

**Source:** Derived from the Experiment

**Table 3.2. Frequency of Identification of the Obtained Bacteria Isolates**

Species Soiling Time (Hours) No of Isolate

Albumen 0 12 24 48 72

*Shigella* species - + + + + 4

*Staphylococcus aureus* - + + + + 4

*Bacillis pumitus*  + - - + + 3

*Cedecea* species - + - - + 2

*Edwardsiella tarda* - + + + + 4

*Edwardsiella* species - + + - + 3

*Escherichia coli* - + - + - 2

*Bacillis brevis* - + + + + 4

*Klebsiella* species + - - + + 3

*Citrobacter* species + - - + + 3

*Serratia* species + + + + + 5

*Salmonella* species - + + + + 4

*Kluyvera* species + - - + + 3

**Total**  **44**

**Yolk**

*Shigella* species - + + + + 4

*Staphylococcus aureus* + + + + + 5

*Bacillis pumitus* + + - + + 4

*Cedecea* species - - + + + 3

*Edwardsiella* species + - + - + 3

*Edwardsiella tarda* + - - + + 3

*Escherichia coli*  - - - + + 2

*Bacillis brevis* + - + - + 3

*Klebsiella* species + + + + + 5

*Citrobacter* species + - + + + 4

*Serratia* species + - + + + 3

*Salmonella* species - + + + - 3

*Kluyvera* species + + - - + 3

**Total** **47**

**Note**: All sign + indicate the presence of the organism.

**3.4. Pooled Antibiotic Behavioural Pattern of *Staphylococcus aureus* in Table Eggs**

Within the study time of 72 hours, it was observed that there was a 20% development of resistance to Ceftazidine, Cloxacillin and Erythromycin and 10% development of resistance toAugmentin and Cefuroxime (Fig 3.8)

**3.5. Pooled Antibiotic Behavioural Pattern of *Shigella* species in Table Eggs**

Within the study time of 72 hours, it was observed that there was a 20% development of resistance to Gentamicin and 10% development of resistance to Augmentin, Ceftazidine, Cefuroxime, Cefiximine and Ofloxacin (Fig 3.11)

**Fig3.6. Susceptibility pattern of *Staphylococcusaureus* in the albumen of table eggs**

**Fig 3.7: Susceptibility pattern of *Staphylococcusaureus* in the yolk of table eggs**

**Fig 3.8. Resistivity Development of *Staphylococcusaureus* in table eggs to antibiotics**

**Fig 3. 9. Susceptibility pattern of *Shigella* species in the albumen of table eggs to antibiotics**

**Fig 3.10. Susceptibility pattern of *Shigella* species in the yolk of table eggs to antibiotics**

**Fig 3.11**. Resistivity Development on *Shigella* species in table eggs to antibiotics

**CHAPTER FOUR**

**4.0.** **DISSCUSSION**

4.1 **Microbial counts in table eggs**

The significant aerobic plate counts of 3.77 log CFU/ml, 5.34 log CFU/ml and 6.62 log CFU/ml, 7.23 log CFU/ml obtained in the albumen and yolk at 0 hour and 12 hours, respectively, seem not only to reflect the fact that the internal contents of table eggs are never a microorganism free zone despite the possession of intact shell covering to provide a degree of physical protection from the external environment, but also that the albumen and yolk may also provide the required nutrient to support organisms present in them, as earlier reported by Stepien-Pysniak (2010).

It has been asserted that the internal contents of eggs can be contaminated during the process of egg formation via such sources of penetration through the eggshell from the colonized gut or from contaminated feaces during or after oviposition (horizontal transmission), or by direct contamination of the yolk, albumen before oviposition, originating from the infection of reproductive organ (Vertical transmission) (Okamura *et al*., 2001; Messens, Grijspeerdt, & Herman, 2005; De Reu *et al*., 2006, Gantois *et al*., 2009).

It is believed that bacterial penetration from the feacal droppings in which the table eggs were soiled in, through the egg shell membrane, into the internal egg content, could have contributed to the significant increase in aerobic plate count result obtained at 12 hours in both the yolk and albumen.

Furthermore, it is envisaged that the presence, strength and influence of the antimicrobial, lysozyme, in the albumen could have greatly inhibited the *Staphylococcusaureus* counts such that the organisms could not have found the albumen medium supportive for growth and thus produced the mean counts of 0.00 log CFU/ml observed at 0 hour. Obi and Igbokwe (2009) have expressed that the antimicrobial lysozyme is present in the albumen of table eggs and is effective against Gram positive bacteria. Egg albumen is also known to contain avidin, an antimicrobial which forms a complex with biotin (a vitamin necessary for the growth of microbes) thus slowing the growth of broad spectrum bacteria (Obi and Igbokwe, 2009). The possible movement *S. aureus,* from the feacal droppings through the shell membrane to the albumen, could have accounted for the subsequent rise in counts to 4.57 log CFU/ml observed at 12 hours in the albumen. The peak rise in *S. aureus* counts to 4.61 log CFU/ml at 72 hours in the albumen could indicate not only a surge in the growth and influx of the organism from the outside environment, but also a decline in the strength of the antimicrobial agents present in egg at curtailing these organisms.

Generally, Gram-negative were observed to increase in both yolk and albumen with time from 5.77 log CFU/ml to 7.52 log CFU/ml and 3.46 log CFU/ml to 5.68 log CFU/ml, respectively. This may reflect not only as an influx and growth of Gram negative organism from the outside environment via the egg shell membrane, but also an ability to be able to tolerate antimicrobial activity which could have been posed by the antimicrobial agent present in the internal egg contents. Arathy *et al.* (2009) have articulated that contaminants of Enterobacteriaceae in table eggs are usually derived from trans-shell contaminations and those bacteria are translocated from the outside environment to the inner surface. Also, Board and Tranter (1995) have stipulated that the internal properties of eggs favour the survival and growth of contaminating Gram negative organism.

The low growth in Salmonella species count obtained at 0 hour in both albumen and yolk of the table eggs studied may be attributed to the antibiotic treatment given to the layers by the farm management prior to table egg collection. Nevertheless, the surge in growth in both albumen and yolk at every subsequent time interval studied reflect in cushion of this organism from the outside environment via the egg shell membrane. De Reu *et al.* (2008) have mentioned that Salmonella species can survive the attacks of antimicrobial molecules contained inside hen’s eggs. This may explain the general increase in Salmonella-Shigella counts obtained with time.

The mean fungi count of 3.90 log CFU/ml and 666 log CFU/ml were observed to be present in the albumen and yolk respectively at 0 hour, this indicate that fungi organisms may be present through horizontal transmission while the subsequent increase in discount value with time is a reflection of vertical transmission.

Generally, more counts of microorganisms were obtained in the yolk as compared to the albumen, Braun and Felhaber, (1995); Chen, Clarke, & Griffiths,(1996) have also observed that invasion of yolk was much more rapid than in other part of egg. Similarly Gantois *et al.*,(2009) reported that penetration through the vitelline membrane provides an opportunity for extensive bacterial multiplication inside the yolk. Stępień-Pyśniak (2010) also mentioned that many nutrient substances present in eggs can create an excellent environment for the development of bacterial micro flora.Duguid and North,(1991), reported that transovarian result in yolk infection.In addition Bahrouz (2005), says when bacteria reach yolk they vegetate well as particularly anaerobes in infertile eggs and leads to the explanation that yolk of infertile may supply appropriate condition for growth of anaerobes that reaches yolk.

4.2. **Micro-organism associated with soiled table eggs**

*Staphylococcus aureus,Serratia* species, *Klebsiella* species, *Bacillis brevis, Shigella* species *Edwardsiella tarda, Salmonella* species, *Bacillis pumitus, Citrobacter* species and *Kluyvera* species, *Edwardsiella* species, *Cedecea* species, *Escherichia coli* were isolated from the eggs that were soaked inside the poultry droppings.Baccilus substilis, *S.aureus, Serratia* species, *Salmonella* species have been reportedly isolated by (Obi and Igbokwe, 2009) in a freshly laid table eggs. Arathy*et al.,* (2009) has also reported the isolation of *Klebsiella* species, *Citrobacter* Species, *Cedecea* Species and *Escherichiacoli* from table eggs.

**4.3. Antibiotic resistance pattern in *Staphylococcus aureus***

There is a multi – occurrence of antibiotic resistance in S*. aureus* at 48 hours to Augmentin, Ceftazidin, Cloxacillin and Erythromycin in the albumen. Generally, multi antibiotic resistance to Ceftazidine, Cloxacillin and Erythromycin in the internal content of the egg. This suggest that egg should be picked at most 24 hours after laying in order to reduce development of multi- antibiotic resistance in table eggs of poultry birds. The use of antibiotics is a major factor in emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine (Tollefson and Flynn, 2002). Also resistance to antibiotics can either be naturally occurring for a particular organism/drug combination or acquired resistance, where mis-use of antimicrobials result in a population being exposed to an environment in which organisms that have genes conferring resistance (either spontaneously mutated or through DNA transfer from other resistant cells) have been able to flourish and spread.(Hemen *et al.,*2012). Antibiotic selection pressure for resistance bacteria in poultry is high and it has been established that the feacal flora contain a relatively high proportion of resistant bacteria (Van de Bogaard and Stobberingh, 1999)

**CHAPTER FIVE**

**5.1. CONCLUSION AND RECOMMENDATION**

The study has shown that when table eggs are soiled in poultry droppings, there is a significant increase in aerobic plate count of with time, 3.77 log CFU/ml,5.34 log CFU/ml and 6.62 log CFU/ml,7.23 log CFU/ml respectively, obtained in the albumen and yolk at 0 hour and 12 hours.

Gram – negative bacterial were also observed to increase in both the yolk and albumen with time from 5.77 log CFU/ml to 7.52 log CFU/ml and 3.46 log CFU/ml to 5.68 log CFU/ml respectively. The increase reflect microbial penetration through the shell membrane in to the internal content of the table eggs.

Micro-organism that has been associated with soiled table eggs include *Staphylococcus aureus, Serratia* species, *Klebsiella* species, *Bacillus brevis, Shigella* species *Edwardsiella tarda, Salmonella* species, *Bacillis pumitus, Citrobacter* species and *Kluyvera* species, *Edwardsiella* species, *Cedecea* species, *Escherichia coli.* Which the consumption of any eggs infected with these micro-organism will leads to one or more ailment like abdominal pain, followed by bloody diarrhoea, as described by Riley *et al.* (1983). Multiple antibiotic resistanceswere observed to occur in *Staphylococcus aureus.*

Eggs should always be kept cold to prevent bacterial growth.

Further studies should be carried out to characterize these identified organism obtained to identify their detrimental effect on human health in time of their pathogenicity.

Cages to be kept clean always, Eggs to be collected from the trays of the cages frequently and Good hygiene must be maintained at the poultry house and egg store room.

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**APPENDIX**

**APPENDIX 1**.**EFFECT OF TIME ON MEAN AEROBIC PLATE COUNT ON INTERNAL CONTENT OF TABLE EGG SOILED WITH POULTRY DROPPINGS.**

**NUTRIENT AGAR**

**Internal content of egg**

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

TIME YOLK ALBUMEN

0 6.62b 3.78bc

12 7.23a 5.34a

24 7.17a 5.37a

48 7.36a 5.43a

72 7.29a 4.74b

Fpr 0.025 <0.001

LSD 0.4663 0.4052

\*Values followed by different superscript letters on the same column are highly significantly different (P<0.005)

**APPENDIX 2**. **EFFECT OF TIME ON MEAN *STAPHYLOCOCCUS AUREUS* PLATE COUNT OF INTERNAL CONTENT OF TABLE EGG SOILED WITH POULTRY DROPPINGS**

**BAIRL PARKER AGAR**

**Internal content of egg**

TIME YOLK ALBUMEN

0 6.83a 0.00b

12 6.88a 4.57a

24 6.47a 4.29a

48 6.53a 4.17a

72 6.32a 4.61a

Fpr 0.393 <0.001

LSD 0.683 0.2485

\*Values followed by different superscript letters on the same column are highly significantly different (P<0.005) and significantly different (P<0.05).

**APPENDIX3.EFFECT OF TIMEON MEAN *ENTEROBACTERIACEACE*PLATE COUNT OF INTERNAL CONTENT OF TABLE EGG SOILED WITH POULTRY DROPPINGS**.

**EMB AGAR**

**Internal content of egg**

TIME YOLK ALBUMEN

0 5.77b 3.46c

12 5.83bc 4.59b

24 6.81b 5.37a

48 7.22a 5.68a

72 7.52a 5.13a

Fpr <0.001 <0.001

LSD 0.4102 0.5570

\*Values followed by different superscript letters on the same column are highly significantly different (P<0.005)

**APPENDIX 4**. **EFFECT OF TIME ON MEAN *SALMONELLA SHIGELLA* PLATE COUNT INTERNAL CONTENT OF TABLE EGG SOILED WITH POULTRY DROPPINGS.**

**SALMONELLA SHIGELLA AGAR**

**Internal content of egg**

TIME YOLK ALBUMEN

0 0.00bc 0.00bc

12 2.18b 1.60b

24 6.73a 5.22a

48 6.95a 4.85a

72 6.84a 5.42a

Fpr <0.001 <0.001

LSD 1.856 1.382

\*Values followed by different superscript letters on the same column are highly significantly different (P<0.005)

**APPENDIX 5**.**EFFECT OF TIME ON MEAN FUNGAL PLATE COUNT OF INTERNAL CONTENT OF TABLE EGG SOILED WITH POULTRY DROPPINGS.**

**POTATO DEXTROSE AGAR.**

**Internal content of egg**

TIME YOLK ALBUMEN

0 6.66c 3.90ba

12 6.76b 4.63a

24 6.98ab 3.99a

48 7.19a 1.57c

72 7.19a 5.31a

Fpr 0.002 <0.001

LSD 0.2951 1.355

\*Values followed by different superscript letters on the same column are highly significantly different (P<0.005)

NUTRIENT AGAR

**APPENDIX 6. ANOVA TABLE OF INTERACTIVE EFFECT OF EGGPART AND TIME ON THE MEAN AEROBIC PLATE COUNT C0NTENT OF EGGS SOILED WITH POULTRY DROPPINGS.**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Source of Variation df SS MS Fpr

TIME 4 11.2902 2.8226 <0.001

EGGPART 1 72.6191 72.6191 <0.001

TIME.EGGPART 4 2.6150 0.6538 0.002

RESIDUAL 50 6.7488 0.1350

TOTAL 59 93.2732

TABLE OF MEANS:

**PARAMETER LSD­­­­­­­­­­­­­­­­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

EGGPART ALBUMEN YOLK

4.931b 7.131a 0.1905

TIME 0.00 12.00 24.00 48.00 72.00

 5.201a 6.282a 6.267ab 6.395ab 6.011c 0.3013

EGGPART.TIME 0.00 12.00 24.00 48.00 72.00

ALBUMEN 3.779cd 5.339bc 5.370bc 5.431bc 4.738cd

YOLK 6.623 7.165a 7.360a 7.285a 0.4260

GRAND MEAN: 6.031

\*Values followed by different superscript letters on the same row are significantly different (P<0.05) or highly significantly different (P<0.005)

B.P AGAR

**APPENDIX 7. ANOVA TABLE OF INTERACTIVE EFFECT OF EGGPART AND TIME ON MEAN INTERNAL C0NTENT OF EGG SOILED WITH POULTRYDROPPINGS.**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

 Source of Variation df SS MS Fpr

TIME 4 42.0685 10.5171 <0.001

EGGPART 1 141.9689 141.9689 <0.001

TIME.EGGPART 4 53.6352 13.4088 <0.001

RESIDUAL 50 12.8916 0.2578

TOTAL 59 250.5642

TABLE OF MEANS

PARAMETER LSD

TIME 0.00 12.00 24.00 48.00 72.00

 3.415b 5.730a 5.383a 5.348a 5.464a 0.4164

EGGPART ALBUMEN YOLK

 3.530b 6.606a 0.2633

TIME.EGGPART 0.00 12.00 24.00 48.00 72.00

ALBUMEN 0.00c 4.577b 4.292b 4.169b 4.612b

YOLK 6.830a  6.884a 6.474a 6.526a 6.317a 0.5888

GRAND MEAN 5.068

\*Values followed by different superscript letters on the same row are significantly different (P<0.05) or highly significantly different (P<0.005)

EMB AGAR

**APPENDIX 8. ANOVA TABLE OF INTERACTIVE EFFECT OF EGGPART AND TIME ON MEAN INTERNAL C0NTENT OF EGG SOILED WITH POULTRY DROPPINGS.**

Source of Variation df SS MS Fpr

TIME 4 30.2616 7.5654 <0.001

EGGPART 1 47.9547 47.9547 <0.001

TIME.EGGPART 4 3.3188 0.8297 0.002

RESIDUAL 50 8.4618 0.1692

TOTAL 59 89.9969

TABLE OF MEANS

PARAMETER LSD

TIME 0.00 12.00 24.00 48.00 72.00

 4.611d 5.208c 6.089ab  6.451a 6.321a 0.3373

EGGPART ALBUMEN YOLK

 4.842b 6.630a 0.2133

TIME.EGGPART 0.00 12.00 24.00 48.00 72.00

ALBUMEN 3.456bcd  4.583bc 5.368ab 5.677b 5.125bc

YOLK 5.766b 5.832b 6.809ab 7.224a 7.516a 0.4771

GRAND MEAN 5.736

\*Values followed by different superscript letters on the same row are significantly different (P<0.05) or highly significantly different (P<0.005)

SHIGELLA

**APPENDIX 9. ANOVA TABLE OF INTERACTIVE EFFECT OF EGGPART AND TIME ON MEAN INTERNAL C0NTENT OF EGG SOILED WITH POULTRY DROPPINGS.**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Source of Variation df SS MS Fpr

TIME 4 390.019 97.505 <0.001

EGGPART 1 18.883 18.883 0.003

TIME.EGGPART 4 8.252 2.063 0.372

RESIDUAL 50 94.692 1.894

TOTAL 59 511.846

TABLE OF MEAN

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

PARAMETER LSD

TIME 0.00 12.00 24.00 48.00 72.00

 0.00c 1.89b 5.97a 5.90a 6.13a 1.128

EGGPART ALBUMEN YOLK

 3.42b 4.54a 0.714

TIME.EGGPART 0.00 12.00 24.00 48.00 72.00

ALBUMEN 0.00bc 1.60b 5.22ab 4.85ab 5.42a

YOLK 0.00bc 2.18b 6.73a 6.95a 6.84a 1.596

GRAND MEAN 3.98

\*Values followed by different superscript letters on the same row are significantly different (P<0.05) or highly significantly different (P<0.005)

PDA

**APPENDIX 10:ANOVA TABLE OF INTERACTIVE EFFECT OF EGGPART AND TIME ON MEAN INTERNAL C0NTENT OF EGG SOILED WITH POULTRY DROPPINGS.**

Source of Variation df SS MS Fpr

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

TIME 4 22.4469 5.6117 <0.001

EGGPART 1 141.8457 141.8457 <0.001

TIME,EGGPART 4 26.8364 6.7091 <0.001

RESIDUAL 50 34.0258 0.6805

TOTAL 59 225.1547

TABLE OF MEANS

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

PARAMETER LSD

TIME 0.00 12.00 24.00 48.00 72.00

 5.28bc 5.70b 5.49b 4.38d 6.25a 0.676

EGGPART ALBUMEN YOLK

 3.88b 6.96a 0.428

TIME.EGGPART 0.00 12.00 24.00 48.00 72.00

ALBUMEN 3.90bc 4.63b 3.99bc 1.57bd 5.31b

YOLK 6.66a 6.76a 6.98a 7.19a 0.957

GRAND MEAN 5.42

\*Values followed by different superscript letters on the same row are significantly different (P<0.05) or highly significantly different (P<0.005)