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Effect of Poultry Breed and Management on Egg Characteristics and IgY Production and its Effect on Bacterial Growth and Broiler Performance

تأثير سلالة الدواجن والإدارة علي خصائص البيض(IgY) وأثره علي الجراثيم وأداء الدجاج اللاحم

A thesis submitted of the requirements for the degree of PhD in Poultry Production

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Dedication

To my beloved father Elshiekh who taught me right from wrong?

To my lovely mother Fatima Gaho for her endless giving

To my wife Guaireia

To my Brothers

To my sisters

To my widely family

To my dears and wonderful friends

With deep love and respect

ABDELMOHSIN, E. M.

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Abstract

Three experiments were conducted in this study. Experiment one aimed to study the effect of poultry breed (Hisex, Sudanese Baladi) and management system (Semi closed and Traditional) on some external and internal egg characteristics. The studied external and internal egg characteristics are (egg weight, egg volume, surface area, shell weight, shell thickness, shell index and specific gravity) and (albumen height, albumen weight, Haugh unit, yolk weight, yolk volume and yolk-albumen (%)) respectively. A total of 204 eggs were collected from Hisex birds which are kept under semi closed and traditional system and from Sudanese Baladi raised under traditional system (68 eggs each). Surface area, shell weight and specific gravity were affected by both breed and management system. While egg weight, egg volume and shell thickness were significantly affect by breed, on the other hand shell index was only affected by management system. Internal egg characteristics (albumen height, albumen weight, Haugh unit, yolk weight, and yolk- albumen %) were significantly affected by breed and management system. Yolk volume was significantly affected by breed. Generally the Hisex breed showed higher values of the external and internal egg characteristic compared to Sudanese Baladi. The external egg characteristics of the eggs produced under semi closed system showed higher values compared to those raised under traditional system. On the contrary the internal egg characteristics of the birds raised under semi closed system showed lower values compared to those raised under tradition al system. Experiment two aimed to study the effect of poultry breed and management system on the production of immunoglobulin yolk (IgY) and it's antibacterial effect. In the present study, a total of 24 eggs were collected from Hisex birds which are kept under semi closed and traditional system and from Sudanese Baladi raised under traditional

system (8 eggs each). The IgY was separated from egg yolk by using modified polyethylene glycol (PEG) precipitation method. The effect of IgY on three bacterial spp. (E. coli, Staphylococcus and Pseudomonas) was studied. The results revealed that, the breed had significant effect on egg weight, yolk volume, IgY concentrate (mg/g-egg) and (mg/ml-yolk), on the other hand the management system had significant effect on egg weight and (IgY) concentrate (mg/g-egg). Generally, the growth of all bacterial spp. studied (E. coli, Staphylococcus and Pseudomonas) was inhibited irrespective of the breed and management system. Experiment three was conducted to study the effect of feeding different levels of egg yolk powder (0.0, 0.2 and 0.4%) on broiler performance. Ninety chicks (Ross) were randomly distributed into 15 replicate (5/treatment) of 6 chicks each. The result showed a significant effect of feeding different levels egg yolk powder on broiler chicken performance during the starter period (feed intake, body weight, weight gain and feed conversion rate). While during the finisher period a significant effect was only obtained in feed intake and feed conversion rate. Birds fed higher levels of egg yolk powder showed higher performance.

المستخلص

هذه الدراسة اشتملت على ثلاثة تجارب، التجربة الأولي تهدف الي دراسة أثر سلالة الدواجن (الهايسكس والبلدي السوداني) ونظام الإدارة (شبه المغلق والبلدي) علي بعض خصائص البيض الخارجية والداخلية. الخصائص الخارجية والداخلية للبيض التي شملتها الدراسة هي (وزن البيضة، حجم البيض، محيط البيضة، وزن القشرة، سمك القشرة، مؤشر القشرة و الثقل النوعي) و(ارتفاع البياض، وزن البياض، وحدة الهوف، وزن الصفار، حجم الصفار، نسبة الصفار الي البياض) علي التوالي. جمعت (204) بيضة لهذه الدراسة من سلالة الهايسكس تم تربيتها تحت نظام شبه المغلق و نظام تقليدي ، والبلدي السوداني تحت نظام تقليدي (64 بيضة لكل). تاثر كل من مساحة سطح البيضة، وزن القشرة، الثقل النوعي بالسلالة ونظام الإدارة في حين ان وزن البيضة ، حجم البيضة وسمك القشرة تأثرت بالسلالة فقط. بالمقابل مؤشر القشرة تأثر بنظام الإدارة فقط. الخصائص الداخلية (ارتفاع البياض، وزن البياض، وحدة الهوف، وزن الصفار) تأثرت جميعها بالسلالة ونظام الإدارة. حجم الصفار ونسبة الصفار تأثرا بالسلالة فقط. عموما سلالة الهايسكس كانت الأعلي قيما لخصائص البيض الخارجية والداخلية مقارنة مع البلدي السوداني. التجربة الثانية هدفت لدراسة أثر سلالة الدواجن ونظام الإدارة علي إنتاجية الأجسام المضادة (IgY) واثرها علي البكتيريا. استخدم في هذه الدراسة عدد 24 بيضة جمعت من طيور الهايسكس التي تمت رعايتها تحت النظام شبه المغلق و التقليدي و طيور البلدي السوداني التي تمت رعايتها تحت النظام التقليدي (8 بيضات لكل). تم فصل الIgY من صفار البيض باستخدام طريقة البولي اثيلين المحورة. و لمعرفة أثر الأجسام المضادة علي الباكتيريا تم استخدام ثلاثة انواع من الباكتيريا (الإيكولاي، الإستافيلوكوكس والسودومونص). اظهرت النتائج ان للسلالة أثر علي وزن البيضة، حجم البيضة، تركيز الأجسام المضادة (مليجرام/بيضة) و(ملي جرام/مل/ (صفار). ومن ناحية آخرى فان نظام الإدارة له أثر علي وزن البيضة وتركيز الأجسام المضادة (ملي جرام جرام/بيضة). عموما، فان نمو انواع الباكتيريا المدروسة (الإيكولاي، الإستافيلوكوكس والسودومونص) توقف بغض النظر عن السلالة ونظام التربية. التجربة الثالثة تهدف الي دراسة أثر تعليف مستويات مختلفة من بدرة صفار البيض (0.0، 0.2 و 0.4) كبديل للمضاداة الحيوية علي أداء الدجاج اللاحم. أستخدم عدد 90 كتكوت (روس) تم توزيعه عشوائيا الى 15 مكرر (5 لكل معاملة) وكل واحد يحتوي علي 6 كتاكيت. اظهرت النتائج أثر معنوي لبدرة صفار البيض علي أداء الدجاج

اللاحم أثناء فترة البادي (إستهلاك العلف، وزن الجسم، عائد الوزن ومعدل التحول الغذائي) بينما كان إستهلاك العلف ومعدل التحول الغذائي مختلف معنويا اثناء فترة النهائي. الطيور التي تم تغذيتها علي أعلي مستوي من بدرة صفار البيض كانت الافضل اداءا من حيث الانتاجية.

CHAPTER I

INTROTUTION

1.1 Introduction

Animal protein in sufficient and balanced levels is considered necessary for human health (Uluocak *et al.*, 1995). Among the protein sources, Poultry products are generally acceptable to people all over the world and provides an excellent source of protein. Poultry are good converters of feed into useable protein in meat and eggs. The production cost is low relative to other types of livestock and the return on investment is fast, thus farmers need just a small amount of capital to start poultry, also poultry species have significant place due to the fact that they have short generation interval, high prolificacy, fast growth rate and ease of raising. Poultry products such as meat and eggs are amongst the most nutritious foods and eggs are rated with milk as one of the best balanced protein foods rich in iron (Fe) and vitamins (Oluyemi and Roberts, 2000).Egg industry worldwide, the production of eggs which are of good egg external characteristic and good internal characteristic is critical to the economic viability of the industry, problems with egg characteristics currently cost the industry many of millions of dollars per year. Egg characteristic is the general term which refers to general standards which define both external and internal egg quality such as egg weight, shell weight, shell thickens, surface area albumen weight, Haugh unit and yolk weight, (Oluyemi and Roberts., 2000), egg weight, shell weight, shell thickness, weight of egg yolk and albumen are the important egg traits influencing egg characteristic (Khurshid *et al*. 2003). Therefore, it is of great importance to understand the factors that affect external and internal egg characteristic. Genetic differences in eggshell formation characteristics exist between species, and

between breeds, strains and families within the species (Buss, 1982), also there are many factors effecting egg characteristics such as move from conventional cages to either an enriched cage or a non-cage system may affect the safety or characteristic of the eggs laid by hens raised in this new environment. So monitoring and evaluation of external and internal egg characteristic of chicken is important in production economy. Little research has been done to examine the influence of selection on eggshell structure and shape in egg production stocks.

Avian eggs are the largest source of major nutrient which consist of various type of proteins, fats, vitamins, growth factors and minerals that are required for the developing embryo, also significant number of defense factors for protection against many viral and bacterial infection chicken egg yolk contain antibodies called immunoglobulin, IgY is incorporated from circulating blood into developing ovarian follicles, and accumulates in oocyte cytoplasm as egg yolk. Immunoglobulin is called IgY because it is present in the egg yolk and due to the differences in protein nature compared to that of the mammalian Ig (Chalghoum, *et al*.2009), (IgY) was the major antibody present in birds, playing similar role like mammalian antibody (IgG) (Kowalczyk, *et al*. 1985). Chicken egg yolk has been considered as ideal source of immunoglobulin (IgY) and IgY is understood to be the predominant antibody in egg yolk (Hamal,*etal*.2006). Egg yolk contains massive amount of immunoglobulin Y (IgY), the functional equivalent to mammalian IgG, which plays a central role in the protection of the newly hatched chick against infectious diseases (Kowalczyk, *et al*.1985).From an animal welfare point of view, chickens are an attractive alternative to mammals as antibody producers because large quantities of antibodies can be produced from the egg yolk making restraint from the blood sampling obsolete techniques to the benefit of the animals used for this purpose (Schade, *et al*.1996). Chicken have potential to be use to

complete the spectrum of animal used for antibody production (Kumaran, 2016). There is increasing interest in the use of chicken egg yolk for polyclonal antibody production due to economic reasons, and have been applied successfully for scientific, diagnostic, prophylactic, therapeutic purposes and therapy against bacteria (Schade *et al*. 1997., Sarker *et al*. 2001., Tini *et al*. 2002 and Amaral *et al.* 2002). To obtain non specific IgY antibodies against an antigen of interest, the production and amount of IgY produced from chicken egg yolk can be affected by different factors, such as methods of preparing IgY, yolk extract, breed and climatic condition (Groos and Siegeel.1990.Carlander and Larsson. 2001). IgY concentration in the egg yolk of chickens has been measured by many investigators, but the reported IgY concentrations have varied from to mg/g yolk (Hamal, *et al*.2006). It seems likely that the scattering of the yolk IgY concentration data is caused by multiple reasons including differences in strains of chickens (Gross and Siegel.1990) , one of the main reasons is that the methods of preparing IgY yolk extract differed among the investigators. A Several methods were used for purifying IgY such as: water dilution method (Akita and Nakai,1992), polyethylene glycol (PEG) (Jensenius, *et al*.1981., Polson *et al*.1985), dextran sulphate and sodium sulphate (Jensenius, *et al*.1981), dextran blue (Bizhanov *et al*.2000), ammonium sulphate (Jensenius, *et al*.1981., Svendsen, *et al*. 1995) caprylic acid(Svendsen, *et al.* 1995), and sodium citrate (Akita and Nakai,1993)the methods of purification of IgY concentration based on the strategy of separation of proteins from lipoproteins and the rest of the yolk lipids (Scader *et al*., 1996., Kitaguchik *et al*., 2008). The first objective of this experiment was to study the effect of breed and management system on IgY concentration in egg yolk. Second objective to study antibacterial effect of IgY. Poultry depended upon two primary management tools: vaccination and antibiotics (cook, 2004). Since the discovery of antibiotics,

they have played a substantial role in the advancement and prosperity of the poultry industry. Antibiotics have been supplemented in animal feed at sub-therapeutic doses to improve growth and feed conversion efficiency (cook, 2004). One controversial aspect of the antibiotic resistance issue is whether routine feeding of antibiotics to farm animals contributes to the increase of antibiotic-resistant bacterial strains. With the increase in regulations regarding the use of antibiotic growth promoters and the rise in consumer demand for poultry products from 'Raised Without Antibiotics' or 'No Antibiotics Ever' flocks, European countries have banned the routine usage of antibiotics in chicken feeds (Pingel , 2003), the European commission decided to ban all commonly feed antibiotics, and there exists a great need for the development of antibiotic alternatives that can help improve performance and maintain optimal health of food animals (Gaddet, *et al*.2017). The various alternatives available to increase animal productivity and help poultry perform to their genetic potential under existing commercial conditions include Probiotic, prebiotics, symbiotic, organic acids, enzymes, phylogenies, antimicrobial peptides, hyper immune egg antibodies, bacteriophages, clay, and metals (Gaddet, *et al*. 2017). Avian eggs are a vehicle for reproduction, they are also a staple food within the human diet and have a natural balance of essential nutrients (De Ketelaere, *et al.,* 2004 and Anton *et al*., 2006), egg play an important role in the human diet and nutrition as an affordable nutrient-rich food commodity that contains highly digestible proteins, lipids, minerals, and vitamins (Fisinin *et al*. 2008). Chicken egg yolk antibody (IgY) has received special attention because it can be easily produced in a high quantity and is both feasible and safe (Gassmann *et al*.1990). The residual yolk is internalized into the abdominal cavity of chicks, and may supply the nutritional needs of chicks for a short period of time after hatch (Noy & Sklan, 2001). Dried egg powder can be fed to large flocks, and eggs

antibodies improve feed conversion ratio and growth rates (Cook,2004) .The population of beneficial microorganisms, such as lactic-acid bacteria and the total bacteria, increased with the inclusion of egg powder in the starter diet, and the Eggs contain bioactive components and lysozyme, which may act as antibiotics against undesirable microorganisms (Spark, 2006., Anton, *et al.* 2006). Esmailzadeh, *et al*. (2016) reported that egg powder is highly digestible and may be efficiently utilized by young chickens, It was concluded that the egg powder inclusion at the level of 40 g/kg in the starter diet improves the performance and intestinal health of broiler chickens. The quest for alternative products or approaches has intensified .A great deal of research has focused the poultry industry is the most dynamic sector within the global meat business, with the greatest growth reflected in the food global demand increase. Chickens is the most common sources of poultry meat, Consumer preference also has been changing in many developed countries, characterized by greater demand for low-calorie foods and changes in lifestyle. The objective of this study is to investigate the effects different level of dried eggs yolk powder (0%, 0.2% and 0.4%) inclusion into broiler diet on feed intake, body weight, weight gain and feed conversion rate of broiler chickens.

General Objective:

Study the effect of breed and management system on IgY production.

Specific objective:

To study the Effect of breed and management system on

1- Some external and internal egg quality characteristic.
2- IgY production and it' s effect on bacterial growth.
3- Effect of IgY on broiler performance.

CHAPTOR II

LITERATURE REVIEW

2.1 Poultry breeds

2.1.1 Layer breeds:

According to the nature and color of egg, layer hens are of two types.

White Egg Laying Hens, This type of hens is comparatively smaller in size. Relatively eat less food, and the color of egg shell is white. Isa White, Lehman White, Nike chick, Bab Cock BV-300, Havard White, Hisex White, Sever White, Hi line White, Bovanch White etc. are some popular white egg laying chickens. Brown egg laying hens are relatively larger in size. They eat more foods, compared to white egg layers. Lay bigger eggs than other laying breeds. Egg shell is brown colored. There are many types of brown layer available. Among those Isa Brown, Hi Sex Brown, Sever 579, Lehman Brown, Hi Line Brown, Bab Cock BV-380, Gold Line, Bablona Tetro, Bablona Harko, Havard Brown etc. are very suitable for commercial layer poultry farming. The poultry sector is possibly the fastest growing and most flexible of all livestock sectors. Driven primarily by very strong demand it has expanded, consolidated and globalised over the past 15byears in countries of all income levels. Livestock is fundamental to the livelihoods of about one billion of the world's poorest people. Rural poultry, in particular, is essential for the livelihood of many resource-poor farmers often being the only asset they possess. It makes up about 80 percent of poultry stocks in low-income food-deficit countries and significantly contributes to improving human nutrition, providing food (eggs and meat) with high quality nutrients and micronutrients, generating a small income and savings, especially for women, thus enhancing the capacity to cope with shocks and reducing economic vulnerability,

providing manure for vegetable garden and crop production. The importance of the socio-cultural and religious functions of village poultry production for smallholder livelihoods, beyond its economic or nutritional importance, is also widely recognized. They can produce about one kg of eggs by consuming about 2.25 kg of food during their egg laying period. For the purpose of producing hybrid eggs layer, consider the various characteristics of cock and hen before breeding. There are various types of highly egg productive layer breeds available throughout the world. Different strains of laying hens vary significantly in eggshell quality, egg size and production (Curtis *et al*., 1985). There are also clear differences between modern commercial birds and traditional breeds of laying fowl (Hockings *et al*., 2003).Tyler and Geake (1958) reported that the white shelled eggs from white Leghorns had thicker shells than the brown shelled eggs from Rhode Island Red breed.

2.1.2 Exotic breeds:

Exotic chicken distributed to farmers in different agro-climatic zones are exposed for to various risk factors that predispose for high chicken losses. Furthermore, the existing improper management such as improper nutrition, substandard hygienic standard, lack of appropriate disease prevention and control program are major constraints for exotic-chicken production and these contributed for high mortality rates chickens. Exotic breeds of chicken kept under intensive condition produce around 250 eggs /year/hen with average egg weight of 50-56g. Significant difference was found between the weights of exotic and indigenous breed eggs.

Maintain superiority in the overall quality of an egg, evaluation of external and internal quality traits has became essential in today's production market. The major egg consumption comprises of eggs either laid by exotic or indigenous breed chickens. Eggs from exotic breed are comparatively cheaper in price and high consumption rate when compared with the eggs

from indigenous breed (Hussain *et al*., 2013). Alem. (2014) reported that average egg production per clutch per hen of exotic chicken Rode I land Red was 38.5 and 45.2. White leghorn, Red Island Red and Fayoumi chicken under village household condition produce 173eggs, 185eggs and 144eggs/year/hen respectively (Solomon. (2004) reported that, there was no significant deference between Leghorn and local pullets assigned to household condition with or without supplementation in rate of maturity as measured by age at first egg.

2.1.3 Local breeds:

The total chicken population in the country is estimated at 51 million ,The majority (98%) of these chickens are maintained under traditional system with little or no input for feeding (Mushi *et al*. 2005). The primary objective in feeding poultry is to secure the most economical gains in weight during growth and fattening, and the most economical production of eggs throughout the laying period (Nigussie *et al*., 2003).Production of Baladi chickens is far behind that of foreign breeds (Al-Aqil,1998). Alganesh *et al*., (2003) reported that the egg production potential of local chicken kept under village management conditions, is 30-60 eggs/year/ hen with an average egg weight of 38g A considerable amount of work has been carried out to improve the production in this breed. Najib (1994) showed that average hen day production for Baladi layers may increase up to 44 % when 18 %protein level was used in the ration, while using 16 % deteriorate the production to 37 %.Later. Al-Yousef and Najib (1997) demonstrated that as level of protein increased from 13 to 17 % in Baladi diet, hen-day egg production was increased from 41 % to 48 %.Under household conditions local hens produced 69% of the egg production of Leghorn layers. The egg production of Leghorn and local layers increased by 46% and 15% as a result of supplementation with a daily ration of 60 g/head, respectively the Leghorn layers kept under rural household

conditions were superior in egg production to local layers kept under similar conditions and found to be more responsive to supplemental feeding than local hens (Matawork Milkias, 2016).local breeds have adapted to environmental conditions of the region of their origin and may therefore be suitable in alternative housing systems. There are limited studies in which traditional breeds and commercial hybrids have been compared. Hocking *et al.* (2003) observed that commercial lines displayed greater rates and persistency of lay, and laid larger eggs, which contained more albumen of higher quality than traditional breeds. In contrast, eggshell weight and thickness have not changed over time. Anderson *et al*. (2004) did not find differences in eggshell thickness, percentage or specific gravity between historic strains and commercial laying stock.

2.2Management systems:

The aim of management is to provide the conditions that ensure optimum performance of the birds (Bell and Weaver, 2001).Poultry management involves monitoring poultry health; ensuring that the poultry house is maintained with appropriate brooding, rearing, growing and laying conditions; and ensuring that recommended vaccinations are given and appropriate feeding programmers are used. All poultry houses need some form of ventilation to ensure an adequate supply of oxygen, while removing carbon dioxide, other waste gases and dust. In commercial operations, minimum ventilation is often practiced in colder climates, but not generally in tropical ones (Glatz and Bolla, 2004). Poultry have seasonal and daily biological rhythms, both of which are mediated by light, particularly day length. For day length to exert its controlling effect, there needs to be a dark phase (night) when light levels should be less than 0.5 lux. Day length and Light intensity during the breeder bird’s life has an important role in development of the reproductive system. The difference

in day lengths and light intensities between the rearing and the laying phases is the principal factor responsible for controlling and stimulating ovarian and testicular development (Lewis and Morris, 2006). In developing countries, it is often difficult to achieve optimum performance from birds, owing to less-than optimal housing conditions and lack of quality feed, vaccines and trained staff. Many poultry flocks are kept in controlled environment houses, which can give accurate control over microclimate. Improvements to poultry housing systems in developing countries have focused on providing an environment that satisfies the birds' thermal requirements. Newly hatched birds have a poor ability to control body temperature, and require some form of supplementary heating, particularly in the first few days after hatch. Many developing countries are located in tropical areas where minimal heating is required. Indeed, the emphasis in these countries – particularly for meat chickens – is on keeping the birds cool. The quality of eggs and egg shell can be influenced by different factors such as: breeding system, lighting program, ambient temperature, nutrition, provision of adequate dietary mineral and water quality (Skrbic*et al*. 2006., Vitorovic*et al*., 2002 and Petricevic *et al*. 2017), eggshell quality and interior egg quality traits are affected by modified and conventional cages and deep litter systems (Abrahansson and Tauson.1995).Housing system had significant differences for egg weight; shell thickens and surface area (Clerici, *etal* .2006). Significant higher values for egg surface area and volume were observed on the eggs from aviary housing (Galic, *et al*, 2018). There was no significant difference between cage and ground system in term of the shell thickens, no housing effect was revealed for egg weight, surface area, yolk: albumen ratio (%) and shell index but had significant on shell thickens. Housing system revealed that there was no significant difference in egg weight, shell thickness, specific gravity, Haugh unit (Turker and Alkan.2018).Ledvinka

,*et al.*(2010) revealed House have no significant effect on yolk weight, albumen weight but had significant on Haugh unit.

2.2.1 Semi closed system:

Semi-closed it‘s a mix between the closed and the open systems, and still yet not clearly classified or evaluated. The various chicken management systems in the tropics could be classified into the extensive, semi-intensive and intensive systems (Omoruyi *et al.*1999). Omoruyi *et al*. (1999) explained that the advantages of semi intensive system included proper accommodation, prompt culling of unproductive birds, proper control of diseases and predators, good record keeping and high egg production and the disadvantages included high capital investment, problem of cannibalism and diseases outbreak. Akinsanmi (1994) reported that egg production was highest under the intensive system. He also found that the major problems associated with the raising of exotic breeds of layers commercially were their susceptibility to diseases and sensitivity to feeding and other environmental problems. Production was higher in semi-closed, birds under controlled housing condition showed lower mortality rate than others under traditional housing system, the hens under semi-closed system consumed less feed and give less production than others under closed system(Rayan, *et al.* 2015.,.

2.2. 2. Traditional system:

Adegbola *et al.* (1986) described systems of poultry management in the tropics namely traditional, free range, restricted range and intensive systems. Williamson and Payne (1978) observed that the major advantages of the range rearing system were that the birds acquired part of their diet by scavenging for herbage, seeds and insects and that the birds usually remained healthy, the system exposed birds to predators and unfavorable weather conditions. Adegbola *et al*. (1986) observed that local birds kept under the extensive system for purpose of eggs performed poorly compared

to the exotic breeds. They found that the local hen kept under the extensive system produced about 60 eggs whereas the exotic breed raised intensively produced about 120 eggs a year. When compared with eggs from conventional cages, aviary eggs have been classified as having greater shell thickness, lower shell deformation, greater shell weight, and a greater percentage of shell, Consequently, Hidalgo *et al*. (2008) reported the greatest percentage of shell and shell strength in conventional cage eggs versus all other housing systems, Guesdon and Faure (2004) saw no differences in shell breaking strength between furnished and conventional cages. Free-range eggs had greater shell thickness and stronger shells compared with conventional cages (Hughes, *et al.*1985).Van Den Brand, *et al*. (2004) compared egg quality in hens housed in individual cages and hens housed on range with males. Lay began 3 wk earlier in the caged versus free-range hens. Yolk color was darker in the free-range eggs. In general, the authors found inconsistencies in external and internal quality (as monitored by egg physical and compositional quality) in the free-range eggs.

2.2.3Closed system:

For controlled-environment housing of layers, multi-tier cage systems are common. Most large-scale commercial farms use controlled-environment systems to provide the ideal thermal environment for the birds (Glatz and Bolla, 2004). Birds' performance in controlled-environment sheds is generally superior to that in naturally ventilated houses, as the conditions can be maintained in the birds' thermal comfort zone. Achieving the ideal environment for birds depends on appropriate management of the poultry House. Modern houses are fully automated, with fans linked to sensors to maintain the required environment. Some commercial operators use computerized systems for the remote checking and changing of settings in

houses. Forced-air furnaces and radiant heating are the main methods of providing heat to young chicks (Daghir, 1995).

2.3 Egg characteristics:

The production of eggs which are of good egg shell quality and good internal quality is critical to the economic viability of the industry, egg components depend on egg weight and then their proportions presumably vary too (Johnston and Gous., 2007).One of major factors determining egg quality strain (Williams. 1992).All internal quality traits of the egg were changed at the significant levels depending on the change occurred in the egg weight with respect to the external quality traits of the egg. However, the yolk and shell rates changed opposite to the albumen ratio (Kul and Seker 2004).The weight of eggshell, albumen and the yolk that form the egg as well as their rates affect the amount and price of the product (Altan *et al.*1998). The quality of the eggs is more importance price contributing factor in table and hatching eggs (Stadelman, 1977). Different internal and external egg quality characteristics are high importance in analyzing egg quality (Silversides and Scott, 2001). The egg shell quality has allows been a critical parameters in the egg industry, and has become even more important with the automation of the production system,Farhad Ahmadi and Fariba Rahimi. (2011) reported that egg shell quality and egg internal quality are of major importance to the egg industry worldwide, egg shell quality may be measured as egg size, egg specific gravity and shell color, shell breaking strength, shell deformation (destructive or non-destructive), shell weight, percentage shell, shell thickness and shell ultra structure. New methods emerge from time to time. Egg shell quality may be measured in a number of ways some of these methods necessitate the destruction of egg (Roberts, *et al*. 1995).Eggshell quality can be improved through optimization of genotype, housing system (Ketta and Tumova.2016).Haugh

units were significantly influenced by genotype. The strain effect on yolk:white ratio was not significant (Ahn, *et al.*, 1997). Egg shell quality may be affected by the strain and production system (Ahmadi and Fariba Rahimi, 2011).The content of solids in whole egg is affected byfactors such as the ratio of yolk to white, and the solids content in yolk and white (Washburn, 1979),some internal and external quality traits of the egg were estimated using following formulae on the basis of the aforementioned measures (Marks and Kiney. 1964., Csuka and Lede 1981., Yannakopoulos and Tservenic, Gousi, 1986.,Thompson and Hamilton.1982). The production of good egg shell quality and good internal quality is critical to the economic viability of the industry. So that it is importance to understand the factors that affect egg external and internal quality. Parameters of egg quality vary between strains of hens (Pandey *et al*., 1986., Silverides, *et al.*, 2006), genotype had significant effect on egg weight, egg surface, shell thickens and had significant on yolk weight, albumen weight and Haugh unit (Ledvinka, *et al*. 201 0., Galic *et al*, 2018), solids of whole egg, white, and yolk were significantly affected by the strain of hens (Ahn, *et al.* 1997). Genetic differences in eggshell formation characteristics exist between species, and between breeds, strains and families within the species (Buss, 1982).

2.3.1. External egg characteristic:

2.3.1.1. Egg weight (g):

Egg weight is an important egg trait which influences egg quality as well as grading. It is a parameter which could be determined about the egg without breaking the egg (Wilson and Suarez, 1993).Egg weight is the important egg traits influencing egg quality. The weight of an egg has a direct relation to the weight of the albumen, yolk and shell it contains and this varies significantly with strains (Pandey *et al*., 1986).A progressive increase in egg weight was seen between strains. Anderson, *et al*. (2004)who found

that eggs from the control strains were smaller than current commercial stock. Hanusova, *et al*. (2015), reveal that egg weight was significant affected by breed, while Ptricevic, *et al*. (2017) found that the weight of the eggs did not differ significantly under the influence of genotype, Ledvinka *et al*. (2000)., Baumgartner *et al*. (2007), concluded that egg weight was not affected by housing system. Abou El-Ghar. (2019)reported that the differences between genetic groups were highly significant for egg weight**.**

2.3.1.2. Egg volume (cm^3):

The egg size and its components are influenced by a number of genetic and non-genetic factors (Washburn. 1990).

2.3.1.3. Surface area (cm^2):

Direct estimation of surface area (SA) usually involves a complicated mathematical derivation based on the profile of the egg. Such methods are quite accurate but require numerous measurements of the shell (Carter, 1974., Carter and Jones, 1970). Hence, methods of indirectly estimating surface area from a minimum of simple measurements are of interest. Carter drew attention to the merit of the method based on only length and breadth measurements, because it avoids the obvious difficulty associated with variation in weight loss if the egg is not newly laid Increase in surface area was seen between strains (Anderson, *et al*. 2004), Significant differences among housing systems were observed surface area (Clerici *et al*. 2006).

2.3.1.4. Shell weight (g):

The eggshell is a bio ceramic material in which the mineral ($CaCO_3$ in the polymorphic form of calcite) is intimately associated with an organic matrix (Nys *et al*., 2004).Shell strength, dynamic stiffness, deformation, and thickness are all recognized as parameters for assessing shell quality egg shell quality appears to be independent of egg weight (Roland,

1976).Shell weight did not differ significantly influenced by genotype of laying hens(Ptricevic, *et al*. 2017).Differences between genetic groups were highly significant for shell weight (Abou El-Ghar. 2019**).** The genetic differences between the strains for egg weight were reported by (Carter and Jones, 1970., Arafa *et al*., 1982). Also Nwachukwu *et al*. (2006) found that shell thickness was not significantly differing among different genetic groups of chicken. There were significant differences among the lines for eggshell weight (Alkan, *et al*. 2010).

2.3.1.5. Shell thickens (mm):

Tyler and Geake (1961) have shown that a determination of shell weight per unit surface area is an exceedingly accurate assessment of shell thickness. In fact, they believe shell thickness is probably more reliable as an indication of the mean value of the whole egg shell than the direct measurement itself .Petricevic, *et al*. (2017) revealed that shell thickness was significantly affected by genotype. Jones.(2006) found that hens laying thick shelled eggs retained more dietary calcium than those laying thin-shelled eggs. Although there was no difference in egg production between thick and thin shell layers, both egg and shell weight were greater for the thick shelled eggs Production eggs from conventional cages, aviary eggs have been classified as having greater shell thickness, lower shell deformation, greater shell weight, and a greater percentage of shell and shell strength in conventional cage eggs versus all other housing systems

2.3.1.6. Shell index:

Shell index was significantly affectedly by housing system (Ledvinka *et al*. 2010). Clerici *et al*. (2006) reported that shell index had no significant affected by housing system.

2.3.1.7. Specific gravity (cm^3):

Roland *et al.* (1973a) reported that specific gravity of eggs (ESG) from commercial laying hen varied according to time of oviposition. Roland and

Harms (1974) reported that ESG and egg weight (EW) were related to time of oviposition. However, because EW did not continue to decrease in the afternoon as shell quality increased, these workers suggested that the decrease in EW was not responsible for the change in ESG. Specific gravity of eggs is often used as an indirect measure of their shell strength. No significant differences in specific gravity were seen between the strains (Sekeroglu, *et al*. 2007) reported that housing system had no significant on specific gravity.

2.3.2. Internal egg characteristic

2.3.2.1. Albumen height (mm):

Strain of bird has also been shown to play a role in albumin consistency, with some strains consistently producing eggs with thin albumin Curtis *et al.*(1985). High producing birds tend to lay eggs with relatively lower amounts of thick albumin and, although this can be influenced by selective breeding (Jones, 2006). Albumen height measures the viscosity of the thick albumen. Albumen quality is a measurable trait and it is a function of the height of the inner thick albumen, the Haugh unit is the outcome of this measurement, or more properly the albumen height alone (Cetin *et al.*2002). The albumen has a major influence on the overall interior egg quality. Thinning of the albumen is a sign of albumen loss and this can be seen clearly when a stale egg is broken on a smooth flat surface, albumen quality is influenced by genetic factors and environmental factors such as temperature, humidity, presence of carbon dioxide, pH and storage time, others include nutrition and the hen's age (Roberts and Ball, 2004). Number of nutritional factors have been reported to affect albumen quality within the limits of acceptable commercial practice, albumen quality is largely unaffected by nutrition (Williams, 1992). The albumen quality might be related to the protein source consumed by the laying hen Genotype significantly higher of the albumen height. Albumen quality is

not only an important indicator of egg freshness but also important for the egg breaking industry because albumen and yolk have different functions(Koelkebeck, 1999).

2.3.2.2. Albumen weight (g):

Albumen weight was affected by breeds (Ledvinka *et al*. 2010).Albumen weight significantly affected by breed. found that the differences between genetic groups were highly significant for albumin weight. Abou *et al*. (2009) were reported there were significant differences ($P<0.01$) among genetic groups for albumin weight.

2.3.2.3. Haugh unit:

The Haugh unit is a measure of internal egg quality, which is considered the gold standard for egg quality assessment Haugh(1937),Significant differences in Haugh unit score and other measures of albumen quality have been found to occur between strains, temperature was shown not to influence Haugh unit score Cunningham *et al*. 1960 and Pandey *et al*.1984).Abou El-Ghar. (2019) reported that the different between genetic groups were not significant effect.

2.3.2.4. Yolk weight (g):

The total solids content of egg yolk is generally 50%, and the major constituents of yolk are protein (16%) and lipid (32%). Therefore, eggs with larger yolks will have higher total solids content than those with smaller yolks(Washburn, 1979). Yolk is the major trait which plays a part in increasing egg weight (Singh *et al*. 2009) reported thatHouse have no significant effect on yolk weight but genotype had significant affect on yolk weight. Differences between genetic groups were highly significant effect of yolk weight.

2.3.2.5. Yolk volume (ml):

(Romanoff, 1979) reported that variability in volume of the yolk of the hen's egg is affected by changes in its moisture content, moisture content of the yolk increases the vitelline membrane has a tendency to weaken because of the increased volume of the yolk. However, Fromm (1967) reported that ambient PH was the predominant factoraffecting the strength of the vitelline membrane and shape of the yolk, and that the moisture content of the yolk had secondary role influencing the physical factor of the vitelline membrane.

2.3.2.6. Yolk- albumen (%):

The content of solids in whole egg is affected by factors such as the ratio of yolk to white. The ratio of yolk to white varies widely with the size of eggs (Marion *et al*., 1964).The yolk to albumen (Y:A) ratio was significantly higher on eggs from free range system, This is in accordance with (Dottavio, *et al.* (2005), they found that smaller eggs had higher Y:A ratios than larger eggs. Genotype was affecting yolk weight, albumen weight and their ratio.

2.4. Avian immune system:

The amount of maternal antibody transferred to the egg and the amount taken up by the developing chicks are important parameters that may greatly influence the health and survival of the chicks, Maternal Immunoglobulin Y (IgY) is a major serum antibody in birds, reptiles and amphibians, and it is transferred from serum to egg yolk to confer passive immunity to their embryos and offspring (Nemeth, *et al*.2008). Chicks are susceptible to many pathogens during the first few weeks of age because their immune system is not fully developed; hence, maternal antibodies are the primary means of antigen-specific protection (Hamal, *et al.* 2006). The chicken immune system consists of the bursa of fabric us, which produce

the antibodies (Carlander *et al*. 1999). In chickens, the transfer of I gY from the dam to her offspring takes place in a 2-step process. In the first step, IgY is taken up into the egg yolk by the IgY receptors on the ovarian follicle from the dam's blood, and the second step, IgY is transferred from the egg yolk to the offspring via the embryonic circulation (Cutting and Roth, 1973., Loeken and Roth, 1983).Chicken egg yolk has been considered as an ideal source of immunoglobulin (IgY) is understood to be the predominant antibody in egg yolk (Hamal, *et al*; 2006). Egg yolk contains massive amount of (IgY), the functional equivalent to mammalian IgG, which plays a central role in the protection of the newly hatched chick against infectious diseases (Kowalczyk, *et al*., 1985).The avian egg contain all necessary nutrient and growth factors required for the developing embryo, including antibodies that are transported from the blood of the hen into the egg yolk to provide immunity to the chick, (Kariyawasam *et al*., 2004and Yegani and Korver, 2010).

The consolidation of poultry depended upon two primary management tools: vaccination and antibiotics, which allowed for the prevention of both bacterial and viral infections that could impact poultry, Antibiotics were also found to improve poultry growth and feed efficiency, Hens egg yolk immune globulins IgY have been studied intensively due to their importance, Carlander, *et al*.(2003) found that Laying hens are very efficient producers of antibodies and provide an interesting alternative for large-scale production of specific antibodies, The laying capacity of a hen per year is around 325 eggs, that means a total potential harvest of 20 g total IgY/year based on a mean IgY content of 60 mg total IgY/egg (Pauly,*et al*.,2011), the egg yolk weight greater in hens, is considered to be important factors for the efficient production of IgY(Nakano, *et al*., 1998). High yolk antibodies concentration, over 100 mg of antibodies can be obtained from one egg (Akita *et al*., 1992).So the concentration of IgY in

egg yolk is an important production Parameter (Carlander, *et al*. 2003). Chicken egg yolk antibody (IgY) has received special attention because it can be easily produced in a high quantity and is both feasible and safe (Gassmann *et al*., 1990).

2.5. Egg yolk (antibody) Immunoglobulin:

Laying hens are very efficient producers of antibodies and provide an interesting alternative for large-scale production of specific antibodies. These antibodies also have biochemical advantages over mammalian antibodies (e.g. rabbit antibodies) that can be used to improve immunoassays where antibodies are used. Polyclonal antibodies have traditionally been produced in mammals. These antibodies are purified from blood. Hens, which have been immunized with an antigen, produce specific antibodies against this antigen (Carlander, *et al*.2003). Egg yolk proteins are distributed in two particular parts, the granules and the plasma in which the former are suspended. Granule proteins are composed of a- and b-lipo vitellines(70%), phosvitine (16%) and low-density lipoproteins (12%) (Burley and Cook.(1961). Some of these proteins are very important because of their functional characteristics (Baldwin, 1986). The plasma proteins consist of the a-, b- and g-livetins and low density proteins (McCully *et al.*, 1962). The a- and b-livetins were identified as chicken serum albumin and a2-glycoprotein, respectively (Hatta *et al.*, 1990). The g-livetins are the chicken immune globulins, which are secreted from the blood plasma into the ripening egg follicle (Losch *et al.*, 1986). In fact, egg yolk immune globulins correspond to the blood serum IgG immune globulins and are known as IgY (Leslie and Clem, 1969). The other blood serum immune globulins, IgM and IgA, are found dominantly in egg white (Rose *et al.* 1974).The presence of immune globulins in eggs is an example of passive immunity because these antibodies are derived from the dam and

protect the offspring from various infectious diseases after hatch (Hatta *et al*., 1997). The acquisition of passive immunity in birds was first noted in 1893 when Klemperer showed the transfer of immunity to tetanus toxin from hen to chick (Rose and Orlans, 1981). Leslie and Clem (1969) proposed that chicken IgG be designated as IgY, as it is different from mammalian IgG and forms the main immunoglobulin in chickens (Leslie and Clem 1969., Leslie *et al*. 1971a.,b). IgY is transported from the hen to the embryo via the egg yolk, which, as a result contains high concentrations of this antibody. Other immunoglobulin classes are present in negligible amounts in the egg yolk (Carlander *et al*. 1999) and IgY is not present in the egg white (Rose *et al*. 1974). The amount of IgY deposited in the egg is directly influenced by the circulating levels in the dam (Hamal *et al*. 2006., Kitaguchi *et al*. 2008). As stated earlier, higher levels of antibodies are usually found in egg yolk than serum, although published data in this area of research are not consistent (Rose *et al*. 1974., Kariyawasam *et al*. 2004., Malik *et al*. 2006). A laying hen can produce approximately 300 eggs annually, and each egg yolk volume is approximately 15 ml (Wilkie, 2006). The amounts of IgY in yolk are 20-25 mg/ml (Rose and Orlans, 1981), which would supply over 100 g of antibody per hen per year. There are several reports in the literature indicating that IgY levels in the egg yolk are not always consistent and may vary within and between bird populations. Carlander *et al*. (2001) demonstrated that there was day to day variation in concentration of IgY in eggs produced by individual laying hens although this variability was smaller than what was seen among hens. In another study, it was found that the IgY concentration varies significantly among different genetic lines. Great variations were also observed among individual hens within each strain (Carlander *et al*. 2003). Production parameters may play a role in this regard. Li, *et al*. (1998) demonstrated that egg yolk weight and the percent hen-day production in laying hens

may influence efficiency of IgY production. They compared two lines of laying hens and found that the total content of yolk IgY in the line with higher rate of egg production and larger egg size was greater, although there was no significant difference in the activity of IgY produced by two strains of laying hens. The above-mentioned information indicates that it is possible to increase IgY production by genetic selection within high-producing lines. This could be an important step for large-scale production of egg yolk antibodies (EYA).

2.5.1. Transfer of IgY into egg yolk:

The generation of egg antibodies in the breeding hen that could be passively transferred to the progeny, by way of the yolk, to enhance progeny productivity. Passively transferred antibody to urease was found to enhance progeny growth and feed efficiency(Pimentel *et al*.1991).Maternal antibody can be transferred from hens to the chicks either through the placenta, colostrums, milk or egg (Grindstaff, *et al.* 2003). During egg formation, I g Y in the serum is selectively transferred to the yolk via a receptor on the surface of the yolk membrane specific for IgY translocation (Morrison *et al*. 2002, Tesar *et al*. 2008). Jennifer *et al*.(2010) reported that the Hens deposit large amounts of an antibody called IgY into the egg yolk to transfer passive immunity to the developing chick. Maternal Immunoglobulin Y (IgY) is a major serum antibody in birds, reptiles and amphibians, and it is transferred from serum to egg yolk to confer passive immunity to their embryos and offspring (Nemeth, *et al*. 2008 and Diraviyam, *et al*. 2014). The transfer of IgY from the dam to the offspring takes place in a 2-step process. In the first step, IgY is taken up into the egg yolk by the IgY receptors on the ovarian follicle from the dam's blood (Cutting and Roth, 1973., Lardinois, 2014). In the second step, IgY are transferred from the egg yolk to the offspring via the embryonic circulation (Kowalczyk, *et al.* 1985).The avian egg contain necessary nutrients and

growth factor for developing embryo, including antibodies that transported from blood of hen into egg yolk to provide immunity to the chick (Yegani and Korver 2010).The volume of an ordinary egg yolk is approximately 15 ml, which contains 100 mg I gY antibodies. One hen lays 15 - 20 eggs per month representing an egg yolk volume of approximately 225 - 300 ml or 1500 - 2000 mg IgY. The I g Y concentration in the hen's egg yolk is higher than in the hen's serum in non-immunized animals. It is easy to scale-up the production of yolk antibodies to large quantities, as hens are inexpensive to maintain in comparison with rabbits. The IgY is found in high concentrations in egg yolk. The concentration of I g Y in the yolk, is higher than that in the serum. Agrawal, *et al*. 2016) reported that the weight of egg yolk significantly affects the total protein and IgY concentration, although these levels per unit of volume did not differ and the IgY concentration in hens, higher Values (9.3-11.3 mg/g) were recorded by Ulmer-Franco *et al*.(2012). Agrawal, *et al*.(2016) found that IgY concentration were recorded among different breeds of poultry the IgY concentration ranged from 5.35±0.63-5.83±0.65, 2.3±0.1-2.6±0.2 mg/ml in hen and egg yolk respectively . Nakai *et al.*, (1994) and Gottstein and Hemmeler, (1985), reported that, Chickens store high contents of IgY in the yolk and are considered to be efficient antibody producers one immunized hen produces 298 g of IgY In a period of 6 wk. There were great differences of IgY concentration between individual animals within each line 2.21 mg/ml, 1.95 mg/ml and 1.68 mg/ml , antibody concentrations ranging from (3.66 mg/ml to 8.37 mg/ml).The yield of isolated IgY was varied in the birds 1to 25 mg/g yolk (Cutting and Roth. 1973),Carlander, *et al*.(2003) compared IgY concentrations in egg yolks from two lines, There were great differences between individual animals within each line (2.21 mg/ ml, 1.95 mg/ ml and 1.68 mg /ml). The mean IgY concentration in the yolks from White Leghorn hens with range 1.16-

3.39 mg/ ml ,the mean IgY concentration in the yolks from the SLU-1329 with range1.04-3.21 mg /ml and the lowest mean IgY concentration was found in eggs from the Rhode Island Red hens with range 0.33- 2.97 mg ml also he found the lowest IgY concentration (0.33, 035 and 0.40 mg/ml). The IgY concentration in yolk from eggs of unimmunized birds varied in the range 0.4 – 0.9 mg/ml of egg yolk, Bizanov.(2003) and Hansen, *et al* (1998) reported that the IgY concentration was 0.6 mg/ml of egg yolk. Choudhury Nafisa. (2017) found that the yield of IgY was varied between 206.015 and 392.030 mg / egg and the yield in egg yolk was 16.75± 5.28 gm/ml. Cook and Trott (2010) reported that the IgY yield range from 60-150 mg IgY per egg. Hens can lay approximately 325 eggs per year; this can result in a potential yield of around 20–40 g of IgY per year.

2.5.2 Characteristics of egg yolk (immunoglobulin) antibodies:

Chicken antibodies recognize different epitopes than mammalian antibodies, resulting in a different antibody property (Larrson *et al.*1998and Carlander *et* a*l.* 1999). IgY is composed of two identical heavy (H) and two identical light (L) chains linked by disulfide bridge, and one as a molecular mass of 180 kDa. The light chain of both antibodies consists of one variable domain (VL) and one consist (CL) domain. The heavy chain of IgY consist of one variable domain (VH) and four constant domain (CH1, CH2, CH 3 and CH4) and IgY has a shorter and less flexible hinge region (Warr *et al*. 1995).

2.5.3. Egg yolk (immunoglobulin) IgY Extraction:

For the separation of high purity and intact IgY from egg yolk, lipids and lipoproteins are the major barrier. Brunodemeulenaer and Andreuyghebaert (2001) reported that Isolation and purification methods for immune globulins from hen egg yolk are reviewed. These methods consist of a removal of most of the lipoproteins in order to obtain a water soluble

protein fraction, which can be regarded as an immunoglobulin concentrate. Water dilution methods and the use of particular anionic polysaccharides seem to offer the best IgY recoveries. Moreover both can be applied easily in an industrial environment. Different researchers have proposed several methods for efficient IgY separation, addition of 0.1% of λ-carrageen and was effective in removing lipoproteins from the water extract of egg yolk at pH 5. Asemota, *et al*.(2013) purified IgY from the yolk of avian egg using trichloroacetic acid to separate egg yolk proteins, mainly IgY. Almeida, *et al*. (2008), used PEG 6000 (w/v) and ammonium sulfate to extract IgY from yolk. Commonly and most frequently used procedures involve protein precipitation with ammonium sulphate, dextran sulphate or polyethylene glycol , separation by ion exchange chromatography is also used. Akita and Nakai (1992) compared four different methods of IgY separation, namely polyethylene glycol, dextran sulphate, xanthan gum and water dilution in terms of yield, purity, ease of use, potential scaling up and immune activity of IgY. They have shown that purification methods had no adverse effect on the immune activities of I g Y. However, the yield of IgY may vary depending on the methods. In some cases, depending on the final application, a simple water extract of IgY is sufficient to achieve good results. Practical use of IgY in research and diagnostics is still also limited due to the complex and time-consuming purification steps (Ko KY,*et al* . 2007). Other reasons are lack of laboratory experience with the processing of egg yolk for the extraction of IgY and the methods which are used for the purification of mammalian immune globulins cannot be automatically applied to egg yolk and IgY (Schwarzkopf, *et al*. 1996).There are several methods of IgY isolation depending on the type of starting materials and laboratory facilities (Pauly, *et al*.2011). The methods of IgY extraction consist of two parts. Firstly a water soluble fraction (WSF) is isolated containing the immunoglobulin apart from contaminating proteins, this can

be considered as an immunoglobulin concentrate. A second step consists of the further purification of this concentrate or even the isolation of specific immunoglobulin's to obtain an immunoglobulin isolate, The key of isolating IgY from egg yolk is to remove the water-insoluble components such as lipids and lipoproteins to get water-soluble protein fraction, (Tong, *et al*. 2015). It is obvious that various IgY isolation procedures exist with differing results towards recovery and purity, The purity and yield of chicken IgY can vary greatly from method to method and require extreme optimization for each experiment. Different researchers have proposed several methods for efficient IgY separation, such as: the use of detergents such as SDS, carrageen an, sodium alginate, or xanthan gum, use of solvents such as acetone, chloroform and ethanol, one of extraction IgY methods from egg yolk by means of polyethylene glycol (PEG) precipitation procedure, the purity of the extracted IgY by this method is around 80%, (Pauly *et al*. 2011).

2.5.4 Methods of determination IgY Concentration:

Due to application purification of the immunoglobulin concentrate could be necessary. The concentrate contains other water soluble proteins together with some minor lipids or lipoproteins. Three kinds of separation techniques can be used to eliminate these contaminants: precipitation, chromatography and filtration. The concentration levels used may vary dependent IgY yield and purity using ammonium sulphate. Salt precipitations are generally repeated once or even twice which increases IgY purity drastically. The most successful precipitation method with respect to IgY recovery and purity consists of adding PEG at a 12% level (Akita and Nakai, 1993) as in the method. PEG has moreover the supplementary advantage it can be used at ambient temperatures without any risk for protein denaturation. The use of PEG implies dialysis or gel permeation in order to remove the polymer completely or the use of

chloroform as suggested by Polson (1990). The IgY content of the samples is measured photo metrically at 280 nm and calculated according to the Lamber-Beer law with and extinction coefficient of 1.33 for IgY. The concentration of total serum protein was measured by colorimetric method using Biuret reagent Reinhold and Reiner. (1953), and the results were expressed as g/dl. IgY concentration in serum was estimated using single radial immune diffusion (SRID) Mancini, *et al* (1965). PEG extraction method yield high levels of I gY from egg yolk. The I gY concentration by PEG extraction method yielded almost twice than that of other methods (Amir, *et al*.2000). Raj, *et al*.(2004) reported that Caprylic acid precipitation method gave the highest recovery of IgY and with high purity. Other methods, Organic solvent method (Stegemann. 1984), Caprylic acid method (Bhanushall, *et. all*.1994).

2.5.5. Factors effecting I gY concentration and yield:

The scattering of the yolk IgY concentration data is caused by multiple reasons including differences in strains of chickens, climatic condition (Gross and Siegel.1990., Carlander and Larsson A.,2001), but one of the main reasons is that the methods of preparing IgY yolk extract differed among the investigators (Kitaguchi, *et al*.2008). The egg yolk weight and the percentage hen-day production are considered to be important factors for the efficient production of IgY (Li, *et al*. 1998). The difference in the total protein concentration in different breeds of breeding hen may be due to difference in the genetic makeup as the total serum protein is influenced by breed, age, physiological state, environment and antigen exposure and the levels can be extremely variable (Bell. 1971) reported different levels of IgY/mL of egg yolk among strains, Varied concentration of IgY might be attributed to different techniques used for extraction, purification, and concentration of IgY by different authors, Niranjan *et al*. (2008) and Haunshi *et al*. (2010). Omro, *et al*. (2018) reported that the purity and yield

of chicken IgY can vary greatly from method to method and require extreme optimization for each experiment. Li *et al*. (1988) reported that weight of yolk have direct effect on total protein and IgY levels. Omro, *et al*. (2018) showed that the selection of chicken breed for IgY production is quite important for obtaining high antibody yield. The amount of IgY deposited into the yolk varies depending on several factors, including the age, breed of chicken, and antigen used.

2.5.6 Particularity of I gY:

I g Y is fairly heat stable and most antibody activity remain after 15 min at 70°C. Incubation of IgY at pH above 4 is well tolerated, but at pH 2 and 37°C the activity is rapidly decreased. The rapid activity loss is probably due to conformational changes, as the polypeptide is not broken down as observed by SDS-PAGE. The immunological activity of IgY is not affected by pasteurization at 60°C for 3.5 min. Addition of high concentrations of sucrose stabilizes IgY regarding heat denaturation acid environment as well as high pressure. IgY is phylogenetically distinct from mammalian antibodies and has different properties such as: does not bind bacterial Fe receptors such as Staphylococcal protein A or Streptococci protein G or mammalian Fc receptors (Kronvall, *et all*.1974). Larsson, *et al*.(1993) found that the IgY is very stable under normal condition, at 4c˚can be stored for 10 years, at room temperature for 6 month and at 37c˚ for one month. I gY has been applied successfully in scientific, diagnostic, prophylactic, therapeutic purposes, immunochemical reagents, and in food formulation or supplements due to the stability of IgY under food processing conditions (Raj, *et al*.2004and Schade, *et al*.2006). The Western blot showed two protein bands corresponding to the heavy and light chains of chicken IgY The two chains were identified using Anti-chicken IgY (IgG) (whole molecule) alkaline phosphates-antibody produced in rabbit. The heavy chain with approximately 65 kDa and the light chain with

approximately 27 kDa, which indicates that the purified protein from the egg yolk was the chicken IgY. IgY is relatively stable between pH 4 and pH 11 but displays a rapid reduction in activity above pH 12 (Shimizu *et al*. 1988). At pH 3.5, IgY activity decreases and is almost completely lost at pH 3 because of rapid conformational changes.

2.6 Application and advantage of (egg yolk immunoglobulin) IgY:

Hen's egg yolk immune globulins are currently not used at their full potential; they possess a large number of advantages compared to their mammal analogues. The use of chickens for specific immunoglobulin production is more convenient compared to the use of mammals, because the antibodies are conveniently delivered in an egg and consequently no invasive techniques are necessary to harvest them. Therefore, no bleeding of the animal is necessary which is beneficial for animal welfare (Polson *et al.*1980., Hassl and Aspock, 1988., Svendsen *et al.* 1995).Hen's egg yolk IgY has been extensively applied to many diagnostic, prophylactic and therapeutic uses (Anton *et al*. 2006., Sparks. 2006). The application of IgY medication to humans may be by injection of purified IgY or by encapsulation of an egg yolk concentrate so the IgY is not destroyed by the acidity in the stomach, The egg yolk is a reservoir of antibodies with many proven uses as well as many theoretical applications (Li-Chan, 1998). One of the major strategies to achieve public health in human and domestic animal species is the efficient, noninvasive, and cost-effective production of specific antibodies against various antigens that can be applied in medicine, veterinary medicine, and research (Stockwin and Holmes, 2003) With the increase in regulations regarding the use of antibiotic growth promoters and the rise in consumer demand for poultry products from ‘Raised Without Antibiotics’ or ‘No Antibiotics Ever’ flocks, the quest for alternative products or approaches has intensified, (Gaddet, *et al*. 2017).

The chicken immune system has been studied for many years, and these studies have contributed substantially to the understanding of the fundamental concepts of immunology and the development of different immunoglobulin classes, chickens are an attractive alternative to mammals as antibody producers because large quantities of antibodies from the egg yolk, and eliminates the bleeding process and is thus superior with regards animal welfare (Schade *et al* .1996.,Schade ,*et al.* 1997), also egg yolk antibodies should be an inexpensive way of producing large amounts of specific antibodies, a major advantage of using birds is that the antibodies can be harvested from the egg yolk instead of serum, thus making blood sampling obsolete (Hau and Hendriksen, 2005). The yield of antibodies from eggs is much and larger differences production between breeds than can be achieved from any mammal, (Wala, *et al.* 2018). The quantitative advantage of raising IgY has been presented by (Gottstein and Hemmeler.1985) who showed that the amount of purified IgY produced in one month is 18 times higher than that of I g G produced in rabbit. Another advantage is that conserved mammalian proteins are usually more immunogenic in the phylogenetically distant birds. Poultry have moreover a lower phylogenetically status than mammals and it is therefore desirable to use birds instead of mammals (Svendsen *et al.*, 1995). European centre for the validation of alternative methods (ECVAM), Chicken antibodies do not activate the human complement system and are sometimes a more suitable choice in designing solid-phase immune metric assays than mammalian antibodies (Carlander and Larsson 2001). strongly recommends avian antibodies as alternative to mammalian antibodies. In addition, the antibody productivity of an egg-laying hen is much greater than that of a similar sized mammal. Research exists on the use of egg yolk antibodies as viable alternatives to AGP in improving growth and feed efficiency in poultry (Cook.2004). The use of egg yolk antibodies offers

several advantages. Large quantities of antibodies can be produced in laying hens and non-invasively collected. Their use is environmentally friendly, less toxic and does not select for resistance. Although the existing results seemed encouraging, much more research is needed on using egg antibodies for growth promotion in poultry IgY has been applied successfully in scientific, diagnostic, prophylactic, therapeutic purposes, immunochemical reagents, and in food formulation or supplements due to the stability of IgY under food processing conditions (Raj *et al*. 2004 and Schade *et al*. 2006). Hen's egg yolk IgY has been extensively applied to many diagnostic, prophylactic, and therapeutic uses (Mime and Yoshimasu, 1998). Consequently a better compatibility with modern animal protection regulations is assured. Within this respect it is worthwhile noticing that the ECVAM report recommends the use of chicken antibodies to mammalian antibodies for ethical purposes. Antibody production is more economical because of the higher immunoglobulin production, cheaper housing, the chickens lower susceptibility for diseases and because the production could proceed in commercial egg production units. A supplementary advantage is the evolutionary distance from mammals, which offer the possibility to produce specific antibodies towards for example mammalian antigens (Jensenius *et al.*, 1981). Chickens present a much more economical source of large quantities of specific antibodies (Schade *et al*. 2007). The differences genetic between chickens and mammals make it possible to produce antibodies against highly conserved mammalian proteins, which would not be possible in mammals, and much less antigen is required to produce an efficient immune response. Egg yolk contains only a single class of antibody (IgY) compared with mammalian, which can easily be isolated from the yolk by precipitation techniques and IgY does not activate mammalian complement or interact with mammalian Fc receptors that could mediate an inflammatory response.

2.6.1. Anti-bacterial effect of I g Y:

The inclusion of IgY to control the enteric pathogens has been shown to be effective for the inhibition of several enteric pathogens including: enter pathogenic Escherichia coli in pigs (Girard *et al*., 2006), enterotoxigenic E. coli in early weaned pigs (Owusu-Asiedu *et al*., 2003), *Salmonella* spp. in neonatal calves (Yokoyama *et al*., 1998), Helicobacter pylori in Mongolian gerbils (Nomura *et al*., 2005), Vibrio anguillarumin rainbow trout (Arasteh *et al*., 2004), Candida albicansin mice (Ibrahim *et al*., 2008), and parvovirus in dogs (Van Nguyen *et al*., 2006).The IgY have growth inhibiting effect on *E. coli* 0157:H7 and Pseudomonas aeruginosa (Akita, *et al*.1998., Sunwoo *et al*. 2002). IgY are used successfully in immune his to chemistry for detection of antigens of viral, bacterial, plant and animal origin, scientific, diagnostic, prophylactic and therapeutic purposes, treatment of calves and piglets against Escherichia coli, rotaviruses and corona virus and also to assess the incidence of intestinal parasites in domestic animals And the contamination of foods with toxins or drugs, antibodies have been used to prevent several types of infection in humans and animals (Reilly *et al* .1997., Pichler *et al.* 1998)reported that the antibodies maternally transferred to the progeny decreased ammonia production in the intestinal tract by inhibiting bacterial urease enzyme. The immunoglobulin (I g Y), was reported on by several research groups. The egg yolk is a reservoir of antibodies with many proven uses as well as many theoretical applications (Li-Chan, 1998).I gY have been commonly employed in the prevention and treatment of various enteric diseases in humans and animals (Gadde *et al*., 2015). Egg antibodies used as specific to a variety of different infectious agents such as bacteria (Sugita-Konishi *et al* . 1996), virus (Kuroki *et al* . 1993., Kuroki *et al* .1994) and parasites (Gottstein and Hemmeler, 1985). Oral administration of specific egg yolk

antibodies towards gastrointestinal infections by Escherichia coli (Ikemori *et al.* 1992, OFarrelly *et al.*, 1992), Salmonella enteriditis (Peralta *et al.*, 1994) and murine rotavirus (Bartz *et al.*, 1980) in animal models is already described. Protected against infection by K88+, K99+, and 987P+ E. coli in Pigs (Yokoyama *et al*. 1992), and Reduced rate of *Salmonella*-contaminated eggs in *Salmonella* Enteritidis (SE)-infected chickens, Reduced fecal shedding and cecal colonization in SE-infected broilers, Protected chicks from Infectious bursal disease virus, Protected chicks against avian coccidosis. One of the most successful clinical applications of IgY has been in the prevention of Pseudomonas aeruginosa colonization in the airways of cystic fibrosis (CF) patients. The specific IgY is effective for immunotherapy for long treatment periods without negative side effects. The binding activity of IgY against Salmonella Enteritidis or Salmonella Typhimurium resulted in inhibition of bacterial growth in vitro. This finding suggests that IgY can bind to Salmonella surface components and results in structural and functional changes of these molecules which may subsequently inhibit bacterial growth. In a further study conducted by Wilkie (2006), anti-Salmonella Enteritidis egg yolk antibodies were administered to broilers through feed or oral gavages. These antibodies were able to inhibit pathogen attachment to rat epithelial cells and porcine mucin in vitro. In a follow-up study, egg yolk antibodies were orally administered to day-of-hatch broiler chicks which had been infected with Salmonella Enteritidis, however no significant reduction in intestinal colonization was observed. It was concluded that more work is needed to understand the factors influencing antibody activity in broilers and the intestinal conditions that can antibody efficiency before egg yolk antibodies can be considered a prophylactic strategy to reduce Salmonellosis. The use of whole egg powder containing antibodies as a feed additive may be an alternative way to reduce the rate of Salmonella contamination of eggs.

Gurtler *et al*. (2004) investigated the protective effect of orally-administered egg powder containing antibodies against S. Enteritidis in laying hens.

2.7 Growth promoters:

Antibiotics have been widely used in animal production. Although some are used therapeutically to improve the health and well-being of animals, most were given for prophylactic purposes and to improve growth rate and feed conversion efficiency, A growth promoter removal has led to animal performance problems, feed conversion increases, and a rise in the incidence of certain animal diseases, such as (subclinical) necrotic enteritis (Wierup, 2001; Dibner and Richards, 2005).The impact of phasing out animal growth promoters could be minimized provided that adequate attention is given to the implementation of alternative disease-prevention strategies and management factors, such as alternative husbandry practices in food animal production. Indeed, overall disease and performance problems have been rather limited. Huyghebaert,*et al*.(2011) reported that Livestock performance and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the animal gut, the morphological structure of the intestinal wall and the activity of the immune system. Antimicrobial growth promoters have made a tremendous contribution to profitability in intensive production system. The establishment of a microbial population in the gastrointestinal tract of all warm-blooded animals soon after birth is inevitable. Gut micro flora are a largely unexplored phenomenon, especially regarding the effects of antibiotic growth promoters upon these largely unknown species. It has been hypothesized that gut micro flora decrease nutrient absorption by increasing gastrointestinal tract thickness, the rate of digest a passage, and also increase nutrient requirements of the host by increasing turnover of the

gut mucosa and by competing with the host for a portion of the dietary energy and protein (Ravindran *et al*. 1984)

2.8 Regulations concerning feed additives for animal use:

A zoo technical additive is any additive other than feed material and pre-mixtures used to affect favorably the performance of animals in good health or used to affect favorably the environment. The category of zoo technical additive can be further divided into four functional groups: (1) digestibility enhancers; these are substances which, when fed to animals, increase the digestibility of the diet, through action on target feed materials, (2) gut flora stabilizers these are micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora, (3) substances which favorably affect the environment, (4) other zoo technical additives. Gerard *et al*. (2011) reported that Alternatives for growth promoters are only of practical significance when they improve animal performance at levels comparable to growth promoters. Micro biota modulating and immune modulator compounds have potential and are used as feedstuff of feed additives. Enzymes, acids, pre- and Probiotic and herbs oretheric oils are some examples of product classes which are used as alternatives for A growth promoter.

2.9 Characteristics of growth promoter's alternatives:

alternatives to growth promoters should have the same beneficial effect as growth promoters, The most well-known mechanism to be proposed is that A growth promoters have an antibacterial action that favors performance in different ways: (1) by reducing the incidence and severity of subclinical infections (George,*et al*. 1982., Brennan,*et al*. 2003), (2) by reducing the microbial use of nutrients (Snyderand Wostmann, 1987), (3) by improving absorption of nutrients because of thinning of the intestinal wall, and (4) by reducing the amount of growth-depressing metabolites produced by Gram-positive bacteria (Knarreborg*et al*. 2004).

2.10 Some alternatives for growth promoters and their mode of action:

Scientists do not focus on the wants of the populous but their needs. In fact, in the area of biological science, we have no skills in meeting wants of people. However, we are faced with the balance between the consumer of the product with which our science focuses and with the needs of production to meet both needs and wants, knowing all the while in today's surplus, wants far exceed needs. Consumers will not pay more for chicken meat unless packed in a manner that is to their advantage. Consumers will not pay for health, environment, conservation, welfare, food safety, or any other advocate agenda item. Consumers may expect these items, but only a very small fraction will pay for them. Science must develop food-related technologies that improve human health and safety, promotes conservation, and environmental sustainability. In animal agriculture, novel methods to improve efficiency of meat production must be developed if antibiotic use decreases. Hence, any improvements, which will serve as a replacement for antibiotics in animal feed, must enhance growth, improve feed efficiency, or decrease mortality at no additional cost to the consumer. Natural substances, which prevent the adherence of select micro flora, have been reported as a method of improving animal production efficiency(Fairchild, *et al*. 1999).

2.10.1. Exogenous enzymes:

Non-starch polysaccharides in animal feedstuffs are a complex group of components differing widely in chemical composition, physical properties and physiological activity, many of which have negative effects on growth and performance. Non-starch polysaccharides include hemi celluloses, pectin's and oligosaccharides as well as arabinoxylans and b-glucans (consisting of either a more soluble or a non-soluble fraction).Different cereal types contain variable Non-starch polysaccharides levels with

concomitant differences in chemical composition, making this cereal one with particularly high levels of soluble Non-starch polysaccharides (Choct, 2002).The mechanism by which Non-starch polysaccharides exert their anti-nutritive effects is complex, but their viscous nature is considered a primary cause for their anti-nutritive effect in poultry. This is because the increased bulk and viscosity of the intestinal contents decrease the rate of diffusion of substrates and digestive enzymes and hinder their effective interaction at the mucosal surface (Choct *et al*., 1996). NSPs also induce thickening of the mucous layer on the intestinal mucosa, (Hedemann *et al*., 2009) suggesting that the concentrations of soluble NSPs in wheat are inversely correlated with their metabolisable energy values in broiler chickens (Annison, 1991).

2.10.2 Organic acids:

Organic acids have been shown to have beneficial effects on performance. Organic acids are widely distributed in nature as normal constituents of plants or animal tissues. They are also formed through microbial fermentation of carbohydrates predominantly in the caeca of poultry (Van Der Wielen *et al*.2000).The mechanism of action of organic acids probably reflects their antibacterial nature, such as decreasing the pH of drinking water and reducing the buffering capacity of the feed with subsequent effect on the physiology of the crop and proventriculus (Van Immerseel *et al*. 2006).

2.10.3 Probiotic or direct- fed microbial:

Probiotic have been defined as mono- or mixed cultures of living microorganisms which beneficially affect the host by improving the properties of the indigenous micro biota (Fuller, 1992). The mechanism of action of probiotic as A growth promoter replacers will depend on the nature of the organism. The different bacterial species in the normal micro biota colonizing on the epithelium of the digestive tract or occurring freely

in the gut lumen) of the broiler gut reach a typical equilibrium state after about a week post-hatch, and depends on many factors including location in the gastro-intestinal tract, integrity of the intestinal mucosa and transit time of the chymus (Van Der Wielen *et al*. 2000, 2002., Teirlynck *et al*., 2009).

2.10.4. Prebiotics:

Prebiotics can be defined as non-digestible feed ingredients with selective effects on the intestinal micro biota. Oligosaccharides are the main components and the range is diverse and may be based on any of the hexose monosaccharides, including glucose, fructose, galactose and mannose (Durst, 1996). The mechanism of action of prebiotics as A growth promoter replacers is dependent on the nature of the compound. They are non-digestible feed ingredients that can have a beneficial action because of selective stimulation of the growth or metabolic activity of a limited number of intestinal micro biota species, such as bifid bacteria and Lactobacillus spp. (Gibson and Roberfroid, 1995).

2.11. Effect of growth promoters on broiler performance

The treatment of chemical acid as growth promoter in different level significantly ($P<0.001$) affected feeding pattern of the Baladi birds. It was clear that daily feed intake of the birds were linearly decreased proportional to the increasing levels of arginine (Najib and Basiouni2004).Attention to detail throughout the entire production process can determine final broiler performance and profitability, this involves good management of healthy parent stock, careful hatchery practice and efficient delivery of chicks that are of good quality and uniformity, Chick quality may be influenced at every stage of the process. Livestock performance and feed efficiency are closely interrelated with quantitative and qualitative microbial load of the animal gut, the morphological structure of intestinal wall and activity of the immune system, antimicrobial growth promoters have made a tremendous

contribution of profitability in intensive husbandry (Huyghebaert *et al*.2011).Many of researcher observed the effect of antibiotic (AGP) on improving performance, (Rosen 1995) concluded that inclusion of antibiotic in the diets gave appositive response72% of the time. It was also proposed that the effect of using antibiotic in the poultry industry was 3-5% increase in growth and feed conversion efficiency (Choct. 2000., Dahiya *et al*., 2006). An ideal alternative should have the same beneficial effects of AGP, ensure optimum animal performance, and increase nutrient availability (Huyghebaert *et al*. 2011). Alternative should possess both of these properties in addition to having a positive impact on feed conversion and/or growth (Huyghebaert *et al*. 2011., Seal *et al*., 2013). Several classes of alternatives have been proposed and tested in poultry production, including Probiotic, prebiotics, symbiotic, organic acids, enzymes, phylogenic and metals. Novel alternatives such as hyper immune egg yolk IgY, research exists on the use of egg yolk antibodies as viable alternatives to AGP in improving growth and feed efficiency in poultry (Cook, 2004). Dried egg powder can be improving the growth rate when fed to broiler diet (Cook. 2004., El-Deek and Al-Harthi, 2005). Egg powder inclusion at the level of 40 g/kg in the starter diet improves the performance and intestinal health of broiler chickens (Cook, 2004). The beneficial effects of probiotic supplementation were also reported in laying hens. Kurtoglu *et al*. (2004) showed that hens fed diets supplemented with Probiotic showed increased egg production compared with controls. Lei *et al*. (2013) reported that dietary inclusion of B. licheniformis improved laying performance and egg mass. Miles, *et al.*(2006) reported that The antibiotic was effect on body weight of broilers and feed conversion. Miles, *et al.* (2006) found that the feed intake of broiler will not effected when the birds fed the diet with growth promoter (Bacitracin methylene disalicylate and virginiamycin). Birds given antibiotics were heavier of body weight than

those fed the control diet. Chickens fed virginiamycin growth promoter had better feed conversion (Miles, *et al*.2006).

2.11.1. Feed intake

Peter, *et al*. (2006), reported that feed intake is the major factor that influence both body weight gain and feed conversion rate in broiler chicken, because so many factors can influence feed intake, it is often difficult to correct problem of poor feed intake unless a complete review of feed and management practices is made. Management and flock health issues are usually more likely to cause feed intake problems than dietary factors. Dietary factor that influence feed intake would be common among all flocks is complex rather than individual flock. In contrast, environmental or immunological stresses have the most profound effect on flock- to flock, variation of feed intake. Any management protocol that would alleviate these stressors will improve feed intake (Peter, *et al*.2006). Feeding program is to supply a range of balanced diets that satisfy the nutrient requirements of broilers at all stages of their development and that optimize efficiency and profitability without compromising bird welfare or the environment. During the incubation period, the chicks uses the egg as nutrient supply, so chicks must undergo the physiological transition to obtain their nutrient from the supplied manufactured feed. At this time, the feed intake is at its lowest and nutrient intake requirement are at their higher, the proper dietary nutrient concentration not only be provided but also the right environmental condition to establish and develop good chick appetite. During the grower period the growth phase must supported by adequate nutrient to avoid any reduction in feed intake and growth rate. Broiler fed egg yolk powder on starter diet was improved the feed intake, while feed intake determined during grower and finisher was not affected (Esmailzadeh, *etal*.2020).

2.11.2. Body weight and weight gain:

Cook (2004) reported that hyper immune egg yolk antibodies improved body weight when fed to broiler chickens up to 3 weeks of age. Esmailzadeh, *etal*.2020, reported that egg yolk improved broiler body weight when inclusion in starter diet. The supplementation of 0.15% and 0.30% dried whole processed egg improved body weight and body weight gain (Eldeek, *et al*. 2005). Similar results were shown in a series of trials in which chicks were fed dried egg yolk powder the average improvement in weight gain (Cook .2004). Supplementing feed with egg antibodies for 3 weeks improved the mean weight gain of broilers (Cook, 2001, 2002).

2.11.3. Feed conversion Rate (FCR):

Feed conversion rate significantly improved in broiler due to inclusion of diet egg, reported by Junqueria, *et al*. (1985)., Schmidt, *et al*. (1992) and EL-Deek *et al*. (2005) .Also Cook.(2004) reported that dried egg powder fed to large flocks and eggs antibodies can be improve the feed conversion rate. The broilers fed egg powder better feed conversion ratio (Esmailzadeh, *etal*.2020), supplementing diets with egg powder dose improved the feed conversion efficiency Cook. (2004). Feed contains egg antibodies for 3 weeks improved the mean weight gain of broilers by 5.4% and the FCR by 6.2 points (Cook, 2001, 2002)

CHAPTER III

EXPERIMENT ONE

3.1. Materials and methods:

A total of 204 eggs were collected from Hisex white birds kept under traditional system (n= 68), Sudanese Baladi breed kept under traditional system (n= 68) and Hisex white birds kept under semi close system (n= 68). The eggs were brought to the laboratory and kept at room temperature till the external and internal egg characteristics were determined as follows:

3.1.1. External egg characteristics:

3.1.1.1. Egg weight (g)

All experimental eggs were in dually cleaned , dried and weighted using an digital balance scale at 0.01g sensitivity, weight/(g) take by weighing (204) egg of all group individually used digital balance and the eggs weights recorded.

3.1.1.2. Egg volume (cm^3)

Egg volume was determined by immersing the egg in a graduated container filled with water, the water which was removed from the container equal to the egg volume (Archimedes principle).

3.1.1.3. Shell weight (g)

Each egg was cracked and evacuated from its content then dried and weighted using electrical balance.

3.1.1.4. Shell thickness: (mm)

Shell thickness was determined by using micrometer screw gauge.

3.1.1.5. Specific gravity:

Specific gravity was determined by using the following equation: specific gravity = egg weight (g)/ egg volume (cm^3).

3.1.1.6. Surface area: (cm^2)

Surface area was determined by using the formula reported by Carter (1975).

Surface area = 4.76 X (egg weight /g) $^{0.67}$**.**

3.1.1.7. Shell index:

Shell index was determined by using the following formula: shell weight (g)/ surface area (cm^2) X100 (Sauveur, 1988**).**

3.1.2: Internal egg characteristic:

3.1.2.1. Yolk volume (cm^3):

After cracking the egg and evacuating its content into filter paper to separate the yolk, rolled the yolk on filter paper to be cleaned, penetrate the vitteline membrane, evacuated Yolk volume was determined by poured the yolk into graduate tube and read the top level of the yolk and recorded.

3.1.2.2. Yolk weight: (g)

Yolk weight was determined by weighing empty tube on a digital balance and recoded and weighted the tube with the yolk then subtracted the tube empty weight from the weight of the tube with yolk.

3.1.2.3. Albumen height: (mm)

When the yolk was separated the albumen was poured in flat plate and albumen height was determined by using vernier caliper.

3.1.2.4. Albumen weight: (g)

It was determined as follows: albumen weight= egg weight – (yolk weigh+ Shell weight).

3.1.2.5. Haugh unit:

It was determined by according the formula reported by Tangkere, *et al*.(2001) $100^{*}\log$ (albumen height -egg weight$^{0.37}$+7.61).

3.1.2.6. Yolk – albumen (%)

It was calculated by dividing the yolk weight over the albumen weight multiplied by 100.

3.1.3. Statistical analysis:

The effect of breed and management was statistically determined using independent T test. The SPSS program was used in data analysis.

Table (3.1): Effect of breed and management on some external egg characteristics

Main factor		Egg weight (g)	Egg volume (cm^3)	Surface area (cm^2)	Shell weight (g)	Shell thickness (mm)	Shell index	Specific gravity
Breed[1]	Hisex	52.03 ± 4.0	51.32 ± 4.8	66.95 ± 3.4	7.57 ± 0.9	26.72 ± 3.5	9.18 ± 3.1	1.01 ± 0.0
	Baladi	43.09 ±2.5	40.30 ± 4.9	51.60 ± 1.2	5.33 ± 0.7	24.52 ± 2.7	8.90 ± 2.6	0.93 ± 0.1
	Sig.	**	**	**	**	**	NS	**
Management		52.03 ± 4.0	51.32 ± 4.8	66.95 ± 3.4	7.57 ± 0.9	26.72 ± 3.5	9.18 ± 3.1	1.01 ± 0.0
[2]Traditional								
Semi-closed		53.45 ± 5.6	52.50 ± 6.5	68.96 ± 5.0	8.01 ± 0.9	27.77 ± 3.3	10.06 ±0.8	1.03 ± 0.0
Sig.		NS	NS	*	**	NS	*	*

NS: Not Significant **: Significant differences at (P< 0.001) *: significant differences at (P<0.01) 1: all breed kept under Traditional system. 2: all birds are Hi sex

3.2. Results:

The effect of breed and management on some external egg characteristics have been presented in table (3,1), and those concerning some internal egg characteristics are presented in table (3,2). The result showed significant effect at ($P<0.001$) of breed on egg weight, egg volume, surface area, shell weight, shell thickness and specific gravity, Hisex breed had higher mean values in all external egg characteristic compared to that of Sudanese Baladi birds. The shell index was not affected by breed. On other hand management system (Semi and Traditional) had a significant effect on shell weight, shell index and specific gravity, while egg weight, egg volume and shell thickens were not affected by management system. All internal egg characteristic under study are significantly affected by breed and management system except yolk volume was not affected by management. The results also revealed significant ($P<0.01$) effect of breed on albumen height, albumen weight, Haugh unit, yolk weight, yolk- albumen (%).The yolk weight in Hisex white heaver than that the yolk was the main component of the egg which increases egg weight. The study also presented a significant different of management on albumen height, albumen weight, Haugh unit, yolk weight, yolk- albumen(%),However the management system had not effect significant on yolk volume**.**

Table (3.2): Effect of breed and management on some internal egg characteristics

Main factor	Albumen height (cm)	Albumen weight(g)	Haugh unit	Yolk weight (g)	Yolk volume (ml)	Yolk-albumen (%)
Breed[1] Hisex	7.12 ± 1.3	30.13 ± 2.8	86.21 ± 8.0	14.17 ± 1.6	13.49 ± 1.8	48.77 ± 6.4
Baladi	4.16 ± 0.7	25.53 ±4.1	73.53±10.9	12.81 ± 1.8	12.33 ± 1.8	58.52 ± 6.3
Management[2]						
Traditional	7.12 ± 1.3	30.13 ± 3.6	86.21 ± 8.0	14.17 ± 1.6	13.49 ±1.8	48.77 ± 6.4
Semi close	5.02 ± 1.1	35.32 ± 6.6	71.56± 9.4	13.69 ± 1.4	13.45 ± 1.7	43.58 ± 6.7
Sig.	**	**	**	*	NS	**

NS: Not Significant **: Significant differences at (P< 0.001) *: significant differences at (P<0.01) 1: all breed kept under Traditional system. 2: all birds are Hi sex

3.3. Discussion:

The breed had significant effect on some external egg characteristic, this results agree with that obtained by Zita, *et al*.(2008), Hanusová, *et al*. (2015), Markose, *et al*.(2017),Tumova,*et al*. (2016),Sokotowicz, *et al*. (2018) and Kraus and Zita(2019). Better results were achieved in Hisex white compared to Sudanese Baladi; this might be due to the intensive selection for increasing laying performance of the Hisex breed (Anderson, *et al*.2004). In addition management system in current the study significantly affected the surface area, shell weight, shell index and specific gravity. An obvious effect of management system on surface area, shell weight, shell index and specific gravity was reported by Clerici, *et al*. (2006). In spite of the higher mean values of the egg characteristic of birds kept under semi close system compared to those kept under traditional system the egg weight, egg volume and shell thickness were not significantly affect were observed. In agreement with the present results Sauveur.(1991) and Ledvinka,*et al*.(2010) concluded that egg weight was not affected by housing system. This study showed the external egg characteristic were affected by breed rather than the management this result agree with Clerici, *et al*. (2006)who reported that shell characteristics are not clearly influenced by housing system but seem to be more affected by producer management and other factors such as hen age and strains. Hisex produced larger egg compared with Sudanese Baladi while yolk: albumen% lower in Hisex than Sudanese Baladi the result agree with Ahn,*et al*. (1997) who reported that Larger eggs always have lower yolk: albumen% ratios than smaller eggs in all age group. Generally internal egg characteristic were significant affected by breed and management system, while mean yolk volume was not affected by management. It can be seen that birds having higher egg yolk weight and volume have higher egg

weight and volume respectively. Similar results were obtained by Ledvinka, *et al*. (2010).

3.4 Conclusion:

Generally results of the current study showed that some external and internal egg characteristic was significant affected by breed and management system except shell index not affected by breed whereas shell thickness and yolk volume were not affected by management system.

CHAPTER IV

EXPERIMENT TWO

4.1. Material and method:

4.1.1. Collection of eggs and separation of yolk:

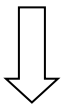
A total of 24 eggs were collected from three groups of hens (30 wks of age). Hisex white bird kept under traditional management system (n=8), Sudanese Baladi bird kept under traditional management system (n=8) and Hisex white bird kept under semi close system (n=8).Each egg was cleaned and weighted individually by using electric balance, carefully cracked and evacuated. The yolk was separated using yolk spoon. Then the yolk was rolled over a filter paper to remove residue of albumen adhered. After yolk was cleaned the vitelline membrane was punctured and yolk was carefully poured into 50ml graduated tube. The yolk volume was recorded.

4.1.2. Isolation of IgY:

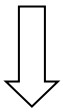
Isolation of (IgY) was done by modified optimizing polyethylene glycol(PEG) 6000 (w/v) precipitation method as shown below. Phosphate buffer saline (PBS) was taken twice of the yolk volume (ml) and mixed with the yolk. Thereafter, 3.5% of (PEG)6000 (w/v) of the total volume was added, vortex well to mixed and rolled on hand for 10 min and centrifuged at 4C˚was used for centrifugation for 30 min at 4500 rpm. The supernatant was poured through a folded filter paper into a new tube, and 8.5% PEG6000 (w/v) was added to the new volume, also the new sample vortex well and rolled on hand for 10 mints and centrifuged again as above. The supernatant was discarded and (PBS) was added to a volume of 10ml. The new volume was mixed with 12% PEG6000 (w/v, 1.2) then

vortex, and rolled on hand for 10min and centrifuged, after that discharged the fluid and the pellet was carefully dissolved in 2ml PBS. Finally stored the IgY extract in 2ml epindorf tubes at -20 C˚.

1part of yolk +2part of PBS + 3.5% Pulverized PEG 6000(w/v) vortex well and rolled on hand for 10min.

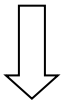


Centrifuge at 4500rpm for 30 min at 4C˚ and supernatant transfer into a new tube.

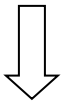


Add 8.5% PEG 6000 (w/v) according to the new volume and centrifuge (as above)

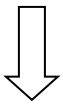
Supernatant discard and dissolve the precipitate in PBS to a final volumeof 10 ml



Add 12% PEG 6000 (w/v) vortex well and rolled hand 10 min and centrifuge as above



Supernatant discard and dissolve the resulting pellet in 2 ml PBS



Stored the IgY extract at – 20C˚

Schematic diagram of the separation of IgY from egg yolk

4.1.2.1 Determination of IgY:

Determination of IgY was done at the faculty of veterinary Medicine, Biochemistry lab. (Khartoum North),using spectrophotometer at 280nm (1:2) dilution with PBS at pH 7.2, one ml of sample put in quvate which inserted into the spectrophotometer and the light pass through the sample

to other side and calculated IgY concentration (mg/g-egg) according to the Lamber-Beer Law with and extinction coefficient of 1.33 for IgY.

The Beer- Lambert's law:

A= ƐXLXC

A: is the absorbance of the solution (no unit)

Ɛ: is the molar absorptive or the molar extinction coefficient (in l/mol.cm).

L: is the distance the light travels through the solution (in cm).

C: is the concentration

4.2. Growth inhibition test:

A total of12 eggs were collected from the three experimental groups of hens (30 wks of age). Hisex white bird kept under traditional management system (n=4), Sudanese Baladi bird kept under traditional management system (n=4) and Hisex white bird kept under semi close system (n=4). each breed. Three dilutions were made by adding different volumes of distilled water to 1ml yolk(1:0.1ml, 1:1ml and 1:10ml).A total of 36 samples were heated in oven at 62˚C for 15 minutes and centrifuged at 4500rpm for 16 minutes, the supernatant was filtered through folded filter paper into 15ml new tubes. Nutrient agar media was prepared in 36 Petri dishes and three wells with 5mm in diameter were made in each Petri dish. E. coli, Staphylococcus and Pseudomonas bacteria were cultured into nutrient agar with spread plate method. 25µl of IgY yolk were poured into the wells and left for two hours at room temperature. Thereafter, the plates were incubated in water bath at 37 C˚ for overnight. After that, the diameter of inhibition zone around the wells was measured in (cm) and compared with the measured size of inhibitory zone manual.

4.3 Statistical analysis:

The effect of IgY on bacterial growth was statistically determined using independent T test. The SPSS program was used in the analysis

4.4. Results:

Table (3.1) showed the effect of breed and management system on the IgY concentration. The highest mean of IgY concentration found in semi close system (75.06±1.5 mg/egg), however when expressing the IgY concentration in mg/g egg the semi closed system showed the lowest value (1.39±0.2 mg/g-egg). The effect of breed on IgY showed that the concentration of IgY (mg/g egg) was significantly higher in Baladi birds compared to Hisex and the reverse is true for IgY (mg/ml yolk). On the other hand the IgY (mg/egg) was not affected by breed. The management system significantly affected the IgY concentration (mg/g – egg).The study reflected the antibacterial effect of IgY on E.coli, Staphylococcus and Pseudomonas (table 3.2).The mean value of the inhibition zone range between (8.00 – 9.40mm). The results showed that E.coli, Staphylococcus and Pseudomonas were not affected by neither breed nor management system.

Table (4.1): Effect of breed and management on IgY concentration in egg yolk.

Main factor		Egg weight (g)	Yolk volume (ml)	IgY con. (mg/egg)	IgY con. (mg/g-egg)	IgY con. (mg/ml- yolk)
Breed[1]	Hisex	53.91±3.9	10.31 ± 1.4	74.29 ±1.5	1.53 ±0.2	6.57 ±0.8
	Baladi	43.09 +2.5	13.20 ± 1.5	73.27 ±1.4	1.71 ±0.1	5.62 ±0.7
	Sig	**	**	NS	*	*
Management[2]	Semi close	53.91 ± 3.9	10.31 ± 1.4	75.06 ±1.5	1.39 ±0.2	7.05 ±0.5
	Traditional	49.11 ± 4.7	11.44 ± 1.2	74.29 ±2.2	1.52 ±0.0	6.57 ±0.8
	Sig	*	NS	NS	*	NS

NS: Not Significant **: Significant differences at (P< 0.001) *: significant differences at (P< 0.01)

1: all breed kept under Traditional system.

2: all birds are Hi sex

Table (4.2): The bacterial growth inhibition zone (mm) as affected by breed and management system

Main factor		E. Coli	Staphylococcus	Pseudomonas
Breed	Hisex	8.75 ± 2.2	8.40 ± 1.1	9.25 ± 1.6
	Baladi	9.00 ± 1.7	9.00 ± 1.4	8.00 ± 2.8
	Sig	NS	NS	NS
Management	Traditional	9.40 ± 1.9	8.40 ± 1.4	8.83 ± 1.8
	Semi close	8.25 ± 2.6	8.00 ± 3.2	9.25 ± 2.1
	Sig	NS	NS	NS

NS: Not Significant :Values mean are inhibition zone measured in mm.

4.5. Dissection:

Experiment two studied the effect of breeds and management on IgY concentration. The result revealed that the range IgY concentration between 1.39 – 75.06. The result obtained showed the varied of IgY concentration was varied between 73.27 -75.29 mg/egg. which is lower than the reported by Rose *et al*. (1974) 100 – 150 mg/egg. When express IgY concentration mg/g-egg the result also lower than 4.0 – 6.8 mg/g-egg which obtained by Choudhury Nafisa. (2017). The result revealed the IgY concentration in mg/ml-yolk was 5.62 – 7.05mg/ml yolk. The result showed higher IgY concentration than 0.4 – 2.21mg/ml yolk obtained by Carlander *et al*.(2010) and lower than 8 -25 mg/ml yolk obtained by Gadde *et al*. (2015). The IgY concentration in the egg yolk has been measured by many investigators. The IgY varied from 1.0 to 25.0 mg/g-yolk (Cutting and Roth, 1973, Carlander, *et al*., 2001 and Hamal, *et al*., 2006). Hansen, *et al*.(1998) and Bizanov, *et al*,(2003) reported that the IgY concentration varied at lower level in the range of (0.4 - 0.9 mg/ml- yolk).The variation of IgY concentration might be due to many factors, strain differences (Kitaguchi, *et al*. 2008), variation between breeds (Carlander, *et al*. 2010), methods of purification (Choudhury Nafisa. 2017), day-to-day variation and between individual laying hens (Carlander, *et al*. 2001).The mean IgY concentration in white Hisex (mg/egg, mg/g-egg and mg/ml- yolk) respectively was (74.29±1.5, 1.53± 0.2 and 6.57± 0.8), the mean IgY concentration in Sudanese Baladi (mg/egg, mg/g-egg and mg/ml- yolk) respectively was (73.27±1.4, 1.71±0.1 and 5.62±0.7), Which represent Intermediate values of the previously reported yolk IgY concentrations. The result showed difference in mean values IgY concentration between the two breed (Carlander *et al*. 2010), the study agreed with Kitaguchi, *et al*. (2008) reported that the Yolk IgY concentration varies among genetic

lines or breeds. The higher and the lower concentration of IgY found in semi close system, this result agree with Gross and Siegel. (1990) reported that the scattering of IgY concentration due to climatic condition. Hisex breed produced lower yolk volume compared to Sudanese Baladi even though Hisex breed produced higher IgY concentration (mg/mlyolk) this maybe due to selective breed. The result revealed a significant effect of breed on yolk IgY concentration (mg/g-egg) and (mg/ml-yolk). The result agree with Carlander, *et al*. (2010) and Hamal *et al*. (2006) reported that there were significant differences in IgY concentration between genotype and two lines. The IgY concentration (mg/g-egg) was significantly affected by management system, The results showed that the IgY had an antibacterial effect against E. coli, Staphylococcus and Pseudomonas. The inhibition zone range from 8.00 to 9.40 mm. Sugita- Konishi, *et al*.(1996) and Hen Kolberg (2017) reported that IgY inhibit bacteria growth. In spite of the significant difference in IgY concentration between the two breeds there was no significant difference due to breed in the inhibition zone.

4.6. Conclusion:

There are several advantages of PEG-precipitation method, such as: eggs are cheap and readily available, antibody in egg yolks can easily avoid bleeding and other invasive techniques normally required to obtain antibodies from animals. The PEG-precipitation method is simple, requires few steps. IgY concentration was varied between breeds. IgY concentration (mg/g- egg) was significantly by Breed and management while IgY concentration (mg/ ml- yolk) significantly affected. Higher concentration of IgY found in Hisex white bird in spite of very low of yolk volume in Sudanese Baladi. IgY inhibits bacteria growth. IgY technology will be expected to play a role in research and opens the door for using IgY antibodies as alternative for antibiotic.

CHAPTER V

EXPERIMENT THREE

5.1. Material and methods:

5.1.1. Chicks and diets.

The experiment was conducted at Poultry farm in Sudan University of Science and Technology. The house was designed as an open sided house, two side south and north closed with wire, the east and west side structure and with higher three and half meter the roof made from zinc, ground is concrete, firstly flame all the ground and sides, clean and disinfected in and out sides by Viru-ceed closed two side after clean and disinfected by Oilskin to prevent the house from contamination, Birds were divided into 3 groups with 5 replication/group and each replicates contained 6 birds, was each cage was supplied with deep litter, feeder and drinker and light system for all house. Inside the house prepared the brooder to receive the chicken at first week, the brooder supplied by (temperature at 33C˚, deep litter, drinkers and feeders) to adapt to the new environment.

A total of (90) Broiler chicks (Ross318) day old purchased from Ommat Company. All chickens treated by antibiotic (Alfa trim) as prevention and multi vitamin (Vitabiomin) for 5 days, chicks was not vaccinated, chicks fed prestarter100gram/chicks from Alshamlal company. All experimental diets were formulated to meet or exceed NRC (1994) nutrient recommendations for broiler chicks. The first week old, chicks weighted as (initial weight) and distributed randomly into three groups each group contain 5 replicate each replicate contain 6 chicks, supplied by water, feed adlib tummy and light 24hour. Egg yolk powder collected from Hisex breed managed in (Traditional and Semi close system) and Sudanese Baladi breed. Two hundred eggs that are not approved for human

consumption collected from three type of breeds, egg cracked and separated the yolk from albumen and other residual of the eggs, then penetrated the vittelline membrane and purred the egg yolk on flat plate and supported to the fan in order to be dry, and levied after dried, then delivered to oven and treated by fresh drying at 60˚C to be ready for using, when yolk prepared, three level of yolk powder (0%, 0.2% and 0.4%) mixed to the chicken diet and fed to the chicken at starter (two weeks) and finisher period (three weeks). Daily weight feed supplied to the birds by electric balance to calculate feed intake, weekly take the body weight through electric balance of all group individually and recorded, weight gain and feed conversion rate also calculated.

5.1.2 Broiler performance parameters:

5.1.2.1. Feed intake: (g)

Feed intake was determined by weighing daily feed fed to the birds used electric sensitive balance, and on second day weighting the residue of feed and subtracted from first weight and recorded.

5.1.2.2. Body weight: (g)

Body weight was measured weekly by weight the birds in each replicate for three group and recorded.

5.1.2.3. Weight gain: (g)

Weight gain was calculated by subtracting the live body weight at the begin of the week from body weight at the begin of next week.

5.1.2.4. Feed conversion rate

Calculated as = feed intake/body weight gain.

5.1.3. Statistical analysis:

The generated data was subjected to analysis of variance using SPSS program.

Table (5.1): Composition of experimental diets contain different levels of egg yolk powder at starter period

Ingredient	Control	Level one 0.2%	Level two0.4%
Sorghum	63.8	63.7	63.6
Ground not cake	27.8	27.7	27.8
Concentrate	5	5	5
DCP	0.4	0.4	0.4
Lime stone	1	1	1
Lysine	0.2	0.2	0.2
Oil	1.5	1.5	1.3
My co toxin	0.2	0.2	0.2
Salt	0.1	0.1	0.1
Yolk	0	0.2	0.4
Total	100	100	100

Table (5.2): Calculated analysis of starter diets powder

Treatment / Nutrients	Control	Level one 0.2	Level two 0.4
ME KJ/Kg	13.26	13.29	13.28
CP%	23.05	23.06	23.16
Ca%	0.83	0.84	0.84
Available Phosphate%	0.65	0.65	0.65
Lysine %	0.89	0.89	0.66
Methionine %	0.60	0.60	0.60

Table (5.3): Calculated analysis of finisher diets contain egg yolk chicken powder

Treatment / Nutrients	Control	Level one 0.2	Level two 0.4
ME KJ/Kg	13.46	13.49	13.52
CP%	20.77	20.81	20.82
Ca%	0.75	0.75	0.76
Available Phosphate%	0.64	0.64	0.64
Lysine %	0.80	.81	0.81
Methionine %	0.60	0.60	0.60

Table (5.4): Composition of experimental diets contain tow different levels of egg yolk powder at finisher period

Ingredient	Control	Level one 0.2%	Level two0.4%
Sorghum	70	69.8	69.7
Ground not cake	21.8	21.8	21.7
Concentrate	5	5	5
DCP	0.4	0.4	0.4
Lime stone	0.8	0.8	0.8
Lysine	0.2	0.2	0.2
Oil	1.5	1.5	1.5
My co toxin	0.2	0.2	0.2
Salt	0.1	0.1	0.1
Yolk	0.	0.2	0.4
Total	100	100	100

5.1.4. Result:

This experiment studied the effect of egg yolk powder (EYP) on broiler performance (appendix 1, 2, 3, 4 and 5).The mean values of initial body weight, feed intake, body weight, weight gain and feed conversion rate during the starter period. During the finisher period feed intake, body weight, weight gain and feed conversion rate range between respectively. Birds fed highest level of EYP 0.4% at starter period showed the highest body weight (413.88g), weight gain (318.61g) and the best feed conversion rate (1.62) compared to the other groups. The results during starter period revealed that feed intake, body weight, weight gain and feed conversion rate were significantly affected by the level of EYP (table, 5.5). The results represented in table (5.6) the effect of EYP on broiler performance during the finisher period. Birds fed higher level represented lower feed intake, feed conversion rate compared with the control and birds fed lower level of EYP. The result showed higher body weight and weight gain on birds fed higher level of EYP. The result revealed that feed intake and feed conversion rate were significant effect by EYP. On other hand body weight and weight gain were not affected. Table 5.7 showed the effect of EYP on broiler overall performance. The result revealed significant low on feed intake the best feed conversion rate the whole period by the group fed high yolk powder.

Table (5.5) Effect of different level of egg yolk powder inclusion on broiler performance(starter period)

Egg yolk powder %	Initial body weight (g)	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
0%	95.83± 1.1	542.50 ± 25.2	393.61± 21.5	297.78 ± 21.8	1.82 ± 0.07
0.2%	95.00± 1.1	506.11± 22.2	366.38± 12.3	271.39± 11.7	1.86 ± 0.11
0.4%	95.20 ± 1.1	524.27± 13.6	413.88 ± 28.4	318.61± 27.9	1.62± 0.18
Significance	NS	*	*	**	*

N= 30 bird per replicate NS= Not significant *= significant at (P<0.05) ** significant at (P<0.001)

Table (5.6) Effect of different level of egg yolk powder inclusion on broiler performance (finisher period)

Egg yolk powder %	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
0%	2044.10± 131.8	1472.80 ± 83.1	1079.20± 74.3	1.89 ± 0.04
0.2%	1970.10 ± 34.88	1378.40 ±10.1	1012.00± 3.0	1.94± 0.03
04%	1830.20 ± 143.3	1468.70± 80.0	1054.80 ± 94.2	1.74± 0.15
Significance	*	NS	NS	*

N= 30 bird per replicate: NS= Not significant: *significant at (P< 0.05)

Table (5.7) Effect of different level of egg yolk powder inclusion on broiler performance (overall)

Egg yolk powder %	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
0%	2586.60 ± 155.7	1472.80 ± 83.1	1377.00 ± 82.8	1.87± 0.01
0.2%	2476.20 ± 23.1	1378.40 ±10.1	1283.40 ± 9.3	1.92± 0.03
0.4%	2354.90 ± 129.7	1468.70 ± 80.0	1373.4 ± 80.9	1.71 ± 0.08
Sig.	*	NS	NS	**

N= 30 bird per replicate: NS= Not significant: *significant at (P< 0.05): **significant at (P< 0.001)

5.1.5. Dissection:

The beneficial effects of feeding broiler chicks with egg by-products, can be included in the diet of newly-hatched chicks as an alternative to antibiotics, due to its high content of antimicrobial proteins, lipids, bioactive nutrient and antibodies, dried egg powder can be fed to large broiler flocks with no negative effects on their performance (El-Deek *et al.* 2011).

The study reveal that broiler fed egg yolk powder at starter and finisher period was improved the performance, and the birds fed higher level showed, lower feed conversion rate and higher body weight and weight gain, the result agree with Esmailzadeh, *et. al.* (2016) reported that the birds fed higher level of EYP at starter period promoted best performance. Also the result agree with him when reported that control birds had lower body weight compared with those fed higher level of egg yolk powder. Similar to this result Mahdavi, *et al.*(2010) reported that The best feed conversion ratio was obtained when the dietary inclusion of at least 0.2% egg yolk antibodies powder continued for 3weeks. The result represented significant effect on feed intake, body weight, weight gain and feed conversion rate at starter period (Esmailzadeh, *et. al.* 2016), Mahdavi, *et al*. (2010) reported that the dietary administration of egg yolk antibodies significant effect of the feed conversion ratio after 2 weeks. Also Cook (2004) reported that the addition of egg yolk antibodies to broiler diet improving growth and feed efficiency in poultry. The results of study showed that egg yolk powder inclusion in the starter diet improved broiler performance this may be due to nutritional value of the egg or duty to increase of Lactic Acid Bacteria, Egg powder is able to supply the required nutrients, and foster the growth of the digestive tract and of other important organs early in life (Jin *et al.*1998., Noy and Sklan.1999., Madsen and Sorensen. 2004., Esmailzadeh, *et. al.*2016)).The result revealed feed intake

significant effect (P< 0.05) by EYP during finishing period the result disagreed with Esmailzadeh, *et. al.* (2016) reported that feed intake determined during grower, finisher and entire experimental period was not significant (P<0.05) among treatment. EYP at 4% showed heaver body weight with no significant during finishing period compared with birds fed 2% of EYP, the result was not agree with El-deek. (2009) reported that dry howl egg significant effect broiler performance at 5% showed heavier body weight at 14, 16 and 20 weeks as compared to the control and 2.5% fed group. The best feed conversion ratios determined in each experimental phase were obtained in the broilers fed 0.4% diet(Esmailzadeh, *et. al.* 2016., El-Deek and Al- Harthi. 2009., Eldeek, *et al*. 2005).

5.1.6 Conclusion:

In conclusion of up to 4% egg yolk powder can be inclusion in a starter broiler diets. EYP up to 4% had improved broiler performance in two phases of production (starter and finisher) more than Birds fed lower level of EYP.

CHAPTER VI

6.1. General Discussion:

Some external and internal characteristic of egg had been significantly affected by Breed (Hisex white and Sudanese Baladi) and management system (semi- closed and traditional).Better results were achieved in Hisex white compared to Sudanese Baladi. In spite of the higher mean values of the egg characteristic of birds kept under semi closed system compared to those kept under traditional system the egg weight, egg volume and shell thickness no significant effect were observed of management. The IgY concentration was affected by breed and management system. The result obtained variation of IgY concentration between breed. The results showed that the IgY had an antibacterial effect against E. coli, Staphylococcus and Pseudomonas. In spite of the significant difference in IgY concentration between the two breed there was no significant difference due to breed in the inhibition zone.

Fed egg yolk powder at starter period was improved the performance, and the birds fed higher level showed, best feed conversion rate and higher body weight and weight gain, The best feed conversion ratios determined in the whole production period were obtained in the broilers fed 0.4% diet.

6.2. Recommendations:

The findings of this study recommended that the local Sudanese Baladi can be product good egg quality and more IgY concentration with option breed and management. IgY is good alternative of antibiotic for inhibit the growth bacteria. Inclusion high of egg yolk powder in broiler diet may be give good result in broiler performance. Although the existing results seemed encouraging, much more research needed on using egg yolk powder for growth promotion in poultry.

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Appendixes

2.1 Uses of IgY for passive immunization in chickens

Pathogen/antigen	Target species	Effects of IgY	Reference
Escherichia coli	chickens	Improved intestinal health and immune responses in broilers challenged with O78:K80	Mahdavi, *etal*. 2010
Salmonella spp.	chickens	Reduced rate of Salmonella contaminated eggs in Salmonella Enteritidis (SE)-infected chickens	Gurtler,*etal*. 2004
	chickens	Reduced fecal shedding and cecal colonization in SE-infected broilers	Rahimi,*etal*. 2007
Infectious bursal disease Virus.	chickens	Protected chicks from IBDV infection	Yousif *et al*. 2006
Eimeriaspp.	chickens	Protected chicks against avian coccidosis	Lee *et al*. 2009a,b

Table (4.8) Effect of different level of egg yolk powder inclusion on broiler diet week one

Egg yolk powder %	Initial body weight (g)	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
0%	95.83 ± 1.1	228.33 ± 8.2	191.66 ± 6.9	95.83 ± 7.3	2.39 ± 0.3
0.2%	95.00± 1.1	201.38 ± 1.2	180.83 ± 12.7	85.83 ± 11.8	2.37 ± 0.2
0.4%	95.027± 1.1	190.83 ± 1.6	192.22 ± 9.5	96.94 ± 10.0	1.97 ± 0.2
Sign,	NS	*	NS	NS	**

N= 30 bird per replicate

NS= Not significant

Table (4.9) Effect of different level of egg yolk powder inclusion on broiler performance at week two

Egg yolk powder %	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
0%	314.16 ± 17.0	393.61 ± 21.5	201.94 ± 15.1	1.55 ± 0.1
0.2%	304.47 ± 10.0	366.38 ± 12.3	181.11 ± 11.5	1.69 ± 0.2
04%	333.88 ± 17.5	413.88 ± 28.2	214.44 ± 20.4	1.57 ± 0.1
Sig.	*	*	*	NS

N= 30 bird per replicate

NS= Not significant

Table (4.10) Effect of different level of egg yolk powder inclusion on broiler performance at week three

Egg yolk powder %	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
0%	432.77 ± 15.0	690.55 ± 20.5	266.11 ± 15.3	1.62 ± 0.2
0.2%	419.44 ± 36.3	593.88 ± 55.4	219.44 ± 34.0	1.91 ± 0.2
0.4%	435.55 ± 37.6	654.16 ±22.6	221.94 ± 15.9	1.96 ± 0.1
Sig.	NS	*	*	**

N= 30 bird per replicate

NS= Not significant

Table (4.11) Effect of different level of egg yolk powder inclusion on broiler performance at finisher period week four

Egg yolk powder %	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
0%	598.05 ± 56.5	1016.10 ±44.3	291.66 ± 11.7	2.05 ± 0.3
0.2%	560.00 ± 15.4	833.88 ± 29.9	300.55 ±27.7	1.88 ± 0.2
0.4%	588.05 ± 42.7	904.44 ± 32.3	250.27 ±22.3	2.36 ± 0.5
Sig.	NS	**	*	*

N= 30 bird per replicate: NS= Not significant: *significant at (P< 0.05): **significant at (P< 0.001)

Table (4.12) Effect of different level of egg yolk powder inclusion in diet on broiler at week five

Egg yolk powder %	Feed intake wk.5(g)	Body weight wk.5 (g)	Weight gain wk.5 (g)	FCR wk5
0%	1013.31 ±63.1	1632.19 ± 118.37	456.69 ±41.2	2.22 ± 0.2
0.2%	990.69 ±43.8	1638.31 ±50.5	544.53 ±23.1	1.81 ± 0.2
0.4%	806.56 ±65.2	1435.89±118.8	564.22 ± 84.8	1.47 ± 0.3
Sig.	**	*	*	**

N= 30 bird per replicate NS= Not significant: * significant difference at (P< 0.05): **significant at (P< 0.001)

Table (4.3): IgY concentration reported by some authors

Author	IgY concentration	Units	Remarks
Shimizu *et al*., (1988)	1.0 – 3.2	mg/ml	Varied in tow groups.
Hansen, *et al*., (1998)	0.6	mg/ml	Ammonium sulphate (60% v/v)
Carlander, *et al*., (2001)	3 – 7	mg/ml	IgY concentration varies significant among individual
Bizanov and Jonauskiene (2003).	1-3.8 1.6-2.0 1.6-1.8 0.4 – 0.9	mg/ml mg/ml mg/ml mg/ml	varied in tow groups Ammonium Sulphate method. Ammonium Sulphate method. Unimmunized birds.
Hamal, *et al*., 2006)	1.5 2.6	mg/ml mg/ml	Lower concentrate in line chicken Lower concentrate in other line chicken
Carlander, *et al*., (2010)	2.21 1.95 1.62 0.33,0.35 and 0.40	mg/ ml mg/ml mg/ml mg/ml	Compare between three breeds. Three individual of Rode Island Red have very low IgY concentration
Gadde, *et al*., (2015)	8 – 25	mg /ml	PEG
Agrawal, *et al.*, (2016) Nafisa (2017)	2.4 2.3 2.6 2.5 16.7- 30.9	mg/ml mg/ml mg/ml mg/ml mg/ml	Concentration of IgY between four breeds Varied between different lines of chicken.
Cutting and Roth (1973)	1 -25	mg/g -yolk	Range variation of (IgY) concentration.
Carlander, *et al*., (2001)	42 – 105	mg/g- yolk	
Hamal, *et al*., (2006)	22.5 – 43.9	mg/g- yolk	
Kitaquchi, *et al*., (2008).	12.2 6.2 5.7	mg/g-yolk mg/ g- yolk mg/g- yolk	PNP/DO strain DeKalb strain Nagoya strain
Ulmer- Franco, *et al*.,(2012)	9.3 – 11.3	mg/g- yolk	Higher level among different strain
Nafisa (2017)	4.03 – 6.8 206.01 -392.03	mg/g-egg mg/ egg	Varied between different lines of chicken.
Rose *et al*., (1974)	100 – 150	mg/egg	zinc sulphate. ammonium sulphate.