Physiological responses on aflatoxin-induced broiler eggs to *Urginea epigea* capped with Ag-ZnO nanoparticles

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DECLARATION

I, Dina Oluwole Opeyemi affirm that.

i. This is my original research.

ii. The use of information and materials from any other sources has been fully acknowledged.

iii. This thesis is not submitted for any degree or examination at any university other than Northwest University.

iv. The reported results were generated by me and not by any other scholar or organization.

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Signed; Date ; 15-11-2023

Supervisor…….

Signed……. Date…….

ABSTRACT

The presence of Aflatoxins B_1 (AFB_1) and Aflatoxin B_2 (AFB_2) in a contaminated feed ingredient produced by moulds (fungi) has proven to be harmful to bird growth performance and reduced egg production. Furthermore, Aflatoxins, when combined, have a detrimental effect on fertilized eggs. This has a great impact on egg hatchability and embryo viability, resulting in a great economic loss. This study was carried out to investigate the physiological responses of aflatoxin-induced broiler eggs to *Urginea epigea* capped with Ag-ZnO nanoparticles. 1500 fertile eggs of broiler chickens were sanitized, weighed, and randomly allocated to twelve experimental treatments involving three (3) levels of dosage of AFB1 [Control = no AFB1, Positive Control =Phosphate Buffered Saline (Pbs), and 45 ng AFB1/egg], 2 levels of AFB2 (0 ng AFB2/egg and 15 ng AFB2/egg), 3 levels of Ag-ZnO [Control = Minus Ag-ZnO (without injection), Plus Ag-ZnO without extract (injected with conventional Nano- Ag-ZnO), plus Ag-ZnO with extract (injected with *U. epigea* extract-fabricated Nano- Ag-ZnO)] in a 3 x 2 x 3 x 2 factorial arrangement in a completely randomized design. Each treatment consisted of 150 eggs with 10 replicates of 15 each. Administration of both the aflatoxins and nanoparticles was done using a syringe and needles with sterile tips at a level of 2.5 ppb, 5 ppb and 10 ppb respectively, followed by incubation in an incubator for 7, 14 and 19 days. The egg's weight, width, length, egg shape index, shell weight, albumen weight, albumen length, albumen height, yolk height, yolk weight, yolk diameter, embryo weight, embryo length, tibia length, albumen index, yolk index, and yolk index were all measured on days 7, 14, and 19 of incubation. The phyto-synthesized nano Ag-ZnO significantly reduced AF-induced toxicity in broiler embryos. However, 10 ppb of AFB1+AgZnOGreen syntheses improved the embryonic length at 19 days. Moreover, the combined effect of AFB1+AFB2+ AgZnO Green syntheses and AFB2+AgZnO chemical syntheses show a strong correlation in terms

of egg length and egg shape index by serving as a good indicator of egg weight. The combination of AFB1+AFB2+ Ag-ZnO Green syntheses at 2.5 ppb improved the shell weight at a duration of 7 days compare to oter treatment. The albumen weight, albumen length and yolk weight were improved in phyto-synthesized extract-treated egg embryos compared to those exposed to AF alone ($P > 0.05$). The result from this study shows that nano material also exhibited an antioxidant potential by reducing the damaging effects of free radicals in the embryo eggs.

Keywords: Aflatoxins, *Urginea epigea,* silver zinc oxide,

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Lastly, all praises should go up to the loving GOD who makes everything possible. Thank you, Father I want to thank God for all the blessings he has bestowed on me. I am who I am because I know the Lord "Know the Lord, He is God, it is He who has made us, and not we ourselves; we are His people and the sheep of His pasture- Psalm 100: 3

DEDICATION

I dedicate this thesis to my beloved late Father Thomas Sunday Dina.

May his soul rest in eternal peace.

"When they ask you how you did it, tell them God is good all the time hallelujahs."

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

AFB1:	Aflatoxin B1
AFB2:	Aflatoxin B2
AFM1:	Aflatoxin M1
AgNps:	Silver Nanoparticles
AgZnO:	Silver Zinc Oxide
AuNP:	Gold Nanoparticles
BBB:	Blood–Brain Barrier
CNS:	Central Nervous System
DNA:	Deoxyribonucleic Acid
ELISA:	Enzyme-Linked Immunosorbent Assay
FAO:	Food and Agricultural Organization
FLD:	Fluorescence Detection
GST:	Glutathione S-transferase
GSH:	Glutathione
HPLC:	High-Performance Liquid Chromatography
LDL:	Low-Density Lipoprotein

LOD:	Logarithm of the odds
NaOH:	Sodium Hydroxide
NPS:	Nanoparticles
ZnO:	Zinc Oxide
PC:	Positive Control
NC:	Negative Control

1. INTRODUCTION

Chicken meat and eggs are the most valuable sources of protein for human nutrition globally. They are sources of high-quality protein, vitamins, minerals (Korish & Attia, 2020), and essential fatty acids (Zaheer, 2015, Ahmad *et al.*, 2018). According to FAO chicken meat and eggs play a vital role in fighting hunger and malnutrition in poor countries (Oznurlu *et al.*, 2012). Eggs provide mostly pure protein of high quality; they also provide adequate vitamins and choline for young children. During human pregnancy, the essential fatty acids present in eggs are very important. Eggs are one of the most popular foods in many countries and it is easily accessible and can be produced at affordable prices. Chicken meat as a source of protein has led to increases in chicken consumption worldwide (Quinn, 2017).

However, there is a serious concern about mycotoxins that affect poultry production. Mycotoxins are secondary compounds produced by fungi on feed substrate (Bhat *et al.*, 2010). When consumed, these metabolites can cause major health issues ranging from acute to chronic poisoning to hepatocarcinoma, hepatitis, cirrhosis and DNA damage (Coulombe, 1993). There are various types of mycotoxins, but those that present a concern to human health and livestock include aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone, and nivalenol/deoxynivalenol (Kumar *et al.*, 2008).

As a secondary metabolite, mycotoxin can cause severe health problems and even death in humans and other animals. Mycotoxins are produced by the *Aspergillus* fungi; aflatoxins are one class of mycotoxins that cause serious effects on poultry production. They are carcinogenic secondary metabolites formed primarily by *A. flavus* and *A. parasiticus* in agricultural foodstuffs such as peanuts, maize grains, cereals and animal feeds (Sarma *et al.*, 2017). In poultry, aflatoxins cause several adverse effects including decreased growth rate, reduced feed conversion efficiency, decreased egg production and hatchability leading to increased

susceptibility to disease (Rawal *et al.*, 2010). Aflatoxins pose a major threat to the poultry industry and cause economic losses to producers because of their often sub-lethal but toxic effects. Dietary aflatoxin reduces weight gain and feed intake and worsens feed efficiency (Yang *et al.*, 2016). In addition, aflatoxin intake in birds affects the liver, kidneys and the immune system thereby causing deleterious biochemical, haematological, reproductive, and pathological changes (Ortatatli & Oğuz, 2001).

Moreover, aflatoxins have a negative impact on egg and shell formation, leading to poor egg and shell quality (pale eggs/small, fragile shell/bloodspots/ meat spots) due to their biological effects and widespread toxicity (Fayjaloun *et al.*, 2019). Aflatoxin B1 is the most important foodborne mycotoxins (Kihara *et al.*, 2000). In fertilized eggs, aflatoxin contamination and residues constitute a serious problem in the poultry industry (Oznurlu *et al.*, 2012). Mostly, fertile egg cause economic losses due to left-over aflatoxins in the feed by reducing embryo viability and hatchability and leading to several organ malformations (Qureshi *et al.*, 1998a).

Maternal transfer of aflatoxins to embryonated eggs retards the development of chicken embryos and inhibits the growth of bone tissue, especially tibia in chickens (Celik & Hotchkiss, 2000, Yin *et al.*, 2017). Maternal exposure to aflatoxin B1, in particular, has been shown to cause functional defects in the immune system of the developing embryo (Celik & Hotchkiss, 2000). During the growth and maturation of impaired chicks, the immune system's dysfunction increases sensitivity to pathogens. Such animals acquire a weak immune system and a lack of tolerance to infectious conditions (Qureshi *et al.*, 1998b). However, no studies have hitherto investigated the effects of *in vivo* exposure to combined aflatoxins on egg quality, immunity and embryo development. Embryonic development is one of the major targets of aflatoxin and it increases the mortality of embryos, increases the number of infertile eggs and impairs hatchability (Surai *et al.*, 2002).

One possible approach to alleviating aflatoxin-induced toxicity in eggs is by administration of plant-derived compounds (phytochemicals) that inhibit the physiological changes caused by aflatoxins and help in the biotransformation of aflatoxins and their epoxides. In this regard, Glutathione S-transferase (GST) is one of the critical candidate enzymes because of its involvement in GSH-mediated aflatoxin detoxification and degradation (Guengerich *et al.*, 1998). In general, pre-treatment with GST isoform inducers and antioxidants capable of elevating cellular levels of GSH protects against AFB_1-induced DNA damage and carcinogenesis (Buetler *et al.*, 1995). Evidence has accumulated that *in vivo* administration of phytochemicals ameliorates the effects of AFB1 by increasing GSH availability (El-Bahr, 2015).

Several studies has indicated that number of phytochemicals (sesquiterpenoids) found in *Urginea epigea*, as well as bufadienolides, may act as an anti-amoxin mechanism by upregulating GST expression and other antioxidant mechanisms, but the idea is that administration in ovo will prevent aflatoxin toxicity (Koorbanally *et al.*, 2004). *Urginea epigea* is an indigenous bulbous plant inhabiting semi-arid and rocky grasslands in many southern African countries, particularly along the eastern seaboard, and commonly used by Zulu, Swati, and Xhosa traditional healers as an oral tonic for soothing body pains, healing bone fractures in human beings and farm animals also used as an aphrodisiac. *U. epigea* is known to possess cardiotonic activity and its bulb sap usually induces skin irritation upon handling, due to the presence mainly of bufadienolides (Koorbanally *et al.*, 2004). Bufadienolides are a group of 24-carbon cholesterol-related polyhydroxy steroids and their glycosides (Kolodziejczyk-Czepas & Stochmal, 2017). Their most investigated pharmacological activities are cardiotonic and anti-cancer properties (Deng *et al.*, 2014).

However, phytochemicals are chemically unstable and easily degradable upon exposure to environmental conditions such as high temperature, oxygen, pH and light before being absorbed (Assadpour & Jafari, 2019). Therefore, it is necessary to protect these bioactive compounds to ensure their controlled release at the right place and time. Nanoencapsulation is one of the most effective technologies for entrapping, protecting and delivering bioactive compounds into biological systems (Koshani & Jafari, 2019, Dima *et al.*, 2020). This technology has versatile benefits for targeted site-specific delivery and efficient absorption through cells. Due to their induction of skin irritation upon handling (Koorbanally *et al.*, 2004), hydrophobic nature, and weak antioxidant potential (Kolodziejczyk-Czepas & Stochmal, 2017), which might limit their direct addition into animal feed formulations, bufadienolides are ideal phytochemical candidates for nanoencapsulation.

1.2 Problem statement

Maternal exposure to dietary aflatoxins and their metabolites in chicken parent stocks and hatcheries leads to toxin transfer to eggs, resulting in embryotoxicity, heart abnormalities and teratogenic effects (Van Rensburg, 2007). The occurrence of aflatoxins in poultry diets reduces the hatchability, hatchling weight, growth rate, meat and egg production, meat and egg quality and vaccination efficiency, as well as impairing the feed conversion ratio and increasing the exposure of birds to disease and mortality (Sur & Celik 2003). Dietary aflatoxins cause antimicrobial resistance on livestock health and pose a considerable risk to human economic losses. In a lot of developing countries, people are in danger of dietary aflatoxin contamination (Ortatatli & Oğuz, 2001). Lack of responsiveness to aflatoxin contamination increases the threat of damage to livestock. Accumulation of dietary aflatoxin in livestock causes liver cancer and results in liver damage (Negash, 2018).

1.3. Justification

Food and nutrition security in Africa is at risk from aflatoxins, not only because they harm animal and human health but also because they pose a serious threat to food and nutrition security. They are of particular concern and present a challenge that may compromise food sustainability in this era of the rapidly rising human population with ever-growing demands for food (Unnevehr & Grace, 2013). The occurrence of these diseases is more likely and their consequences are more severe in rural communities where most indigenous chickens are raised extensively for meat and eggs due to poor feed handling and storage methods (Mtileni *et al.*, 2012). The existence of aflatoxin species, as is co-occurrence with other mycotoxins, is a natural phenomenon almost everywhere these toxins occur, resulting in synergistic actions and increased risk of toxicity (Omotayo *et al.*, 2019). Yet very few studies have focused on this issue of aflatoxin (and mycotoxin) co-occurrence.

1.4 Aim and Objectives

This study aims to investigate the ameliorative effects of *in vivo* administration of *Urginea epigea* extract phyto-synthesized silver-zinc oxide bimetallic nanoparticles on the quality and physio-pathological characteristics of chicken eggs subjected to aflatoxicosis.

The objectives of this study are mainly:

a. To determine changes *in vivo* administration of AFB1 and AFB2, singly and combined, with or without *Urginea epigea* bulb extract-fabricated silver-zinc oxide nanoparticles on shell quality, egg weight, egg colour, and morphological characteristics.

b. To determine the effects of *in vivo* administration of *Urginea epigea* bulb extract-fabricated silver-zinc oxide bimetallic nanoparticles on shell quality and physio-

pathological characteristics of chicken eggs and embryos subjected to AFB1 and AFB2, singly and combined.

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2. LITERATURE REVIEW

2.1 Background

Mycotoxin research began in 1962 in response to an unusual veterinary crisis near London, England, in which approximately 100,000 turkey chicks died (Lim *et al.*, 2017). Mycotoxicosis may lead to chronic diseases that affect a large portion of people in developing countries and their prevalence continues to increase due to high intake levels of agricultural products (Gbashi *et al.*, 2018). Various types of moulds (fungi) naturally produce mycotoxins. These moulds are commonly found on or in cereals, dried fruits, nuts and spices, among other foods. Mould can grow before or after harvest, during storage, on and inside the food itself under warm, humid, and damp conditions, both before and after harvest (Misra *et al.*, 2019). Poultry health is adversely affected by mycotoxins in several ways, such as reduced poultry productivity and immune suppression, increased disease and susceptibility to parasites/diseases and death (Abo-Al-Ela *et al.*, 2021). According to previous reports, low fungal toxin levels are a major contributor to the decline in domestic bird performance (Magnoli *et al.*, 2017). The method of modifying these toxins is influenced by several factors, including the type of poultry, treatment time and organisms. Despite being extremely rare, mycotoxins have not been thoroughly studied in chicks hatched under aflatoxins during incubation (García-Béjar *et al.*, 2020). One of the most critical health hazards of naturally occurring mycotoxins is aflatoxins (AFs), which are primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Valencia-Quintana *et al.*, 2020). In poultry aflatoxicosis, AFB1 is considered the most toxic and carcinogenic aflatoxin among the four major classes, namely B1, B2, G1, and G2. AFB1 can cause immunosuppression and hepatotoxic effects (Rushing & Selim, 2019). Chicken diets contain aflatoxins ranging from 10 to 20 grams per kg, which may be carried over to egg production in the ratio of 1/2000 to 1/2500 (Elwan *et al.*, 2022). In chickens, aflatoxicosis is a major concern

because it leads to significant economic losses by reducing feed usage, egg production, body weight gain and mortality rates. AFB1 residues may also be passed from the laying hens to fertilized eggs during egg formation, decreasing the viability and hatchability of embryos as well as causing numerous organ malformations (Yin *et al.*, 2017). As well, chicken embryo development was retarded by AFB1 transfer from layer feed to embryonic eggs, which inhibited the growth of bone tissue, especially the tibia (Fouad *et al.*, 2019). Toxin-induced teratogenesis was shown in an *in vitro* experiment on chick embryos due to a decrease in cell proliferation during early morphogenesis (Qureshi *et al.*, 2014).

2.2 Taxonomy of *Urginea*

Hundreds of species of *Urginea* are found throughout India, Africa and the Mediterranean region. *Urginea* belongs to the division Liliophyta, class Liliopsida, order Liliales and tribe Scilleae *(Dobhal, 2016). Urginea indica* is a perennial herb and is found in deserts, shrubs, grasslands, dunes, and forests. It is a perennial geophyte with dark green leaves and whitish flowers that are bisexual, hypogynous, campanulate, bracteate and dropping. The bracts are solitary with long or short pedicels (Lakshman & Paramasivam, 2012). The roots are six to ten inches in length, protruding from the base of a large, globular, tunicate bulb. The outer scales of the bulb are papery and red, orange, or brown. *U. indica* has two distinct varieties. The first category is unique with underground bulbs producing inflorescence without vegetative leaves, immediately after the first shower followed by severe summer. Plants in the second category produce their vegetative leaves along with the inflorescence axis shortly after the first monsoon shower (Lakshman & Paramasivam, 2012).

2.1.1 Therapeutic benefits of *Urginea*

Urginea is reputed for several therapeutic benefits. Bufadienolides are used as cardiotonic agents in the bulb of the plant. A long history of medicinal use of the plant can be found in both pharmaceutical and biocidal applications (Sultana *et al.*, 2010). According to a literature survey, the extracts of *Urginea indica* bulbs have been reported for pharmacological and biological activities which are antifungal antioxidant, gastrointestinal stimulant, anti-inflammatory, anti-carcinogenic, cardio-tonic, antimicrobial, anti-insecticidal, anti-arthritic and antidiabetic (Shenoy *et al.*, 2006, Dobhal, 2016). The plant extracts have also been used for dermatological, diuretic properties, abortifacient effects, effects on the menstrual cycle, a cough expectorant, cure for renal failure, chronic rhinitis, and chronic pulmonary disorder (Koh *et al.*, 2009).

2.3 Utilizing *in ovo* injection to provide supplemental nutrients to broiler embryo.

Recent research has been focused on exploring the use of *in-ovo* injection as a way of providing supplemental nutrients to broiler embryos. Such supplemental nutrients include carbohydrates (Hou & Tako, 2018). Commercial strain broiler embryo development could be compromised by a lack of essential nutrients in their eggs due to intense selection for rapid growth (Ismail, 2018). Several factors affect the successful use of *in-ovo*-injected nutrients, including the site and timing of injection (Kermanshahi *et al.*, 2017). Additionally, the type of nutrient, composition and volume of carrier solution injected (McGruder *et al.*, 2011) all influence how well he nutrients are taken up. As described by Freeman and Vince (1974), on the seventh day of incubation, fertile broiler breeder eggs have four compartments: the extra-embryonic coelom, the allantoic sac, the amnion cavity, and the yolk sac (Kermanshahi *et al.*, 2017).

According to (Ohta *et al.*, 2001) they injected amino acid solutions into the yolk sac and extraembryonic coelom of fertile eggs to determine the best site of injection for amino acid

injection in broiler breeder eggs. They found that when injected into these compartments, the subsequent chick's body weight was higher than when injected into non-fertile eggs. Although the moisture and fat levels of eggs are sufficient or higher than what is needed for embryonic development, the levels of amino acids and proteins available are lower than those that are required for optimal growth and development (De Oliveira *et al.*, 2008). Studies have shown that *in-ovo* injections of amino acids lead to increased chick weight at hatch (Ohta *et al.*, 2001).

2.4 Effects of aflatoxicosis on morphological aspects of eggs

The internal and external qualities of eggs play vital roles in embryo development and they may directly or indirectly influence hatchability results (Demirel & Kırıkçı, 2009, Boleli *et al.*, 2016). During egg incubation and hatching, eggshells serve primarily two purposes: to protect and provide calcium to the embryos as well as to exchange gases and water between the embryos and the external environment (Portugal *et al.*, 2014). In the period of incubation, the eggshell strength is significant for preventing water loss, microbial penetration and untimely gas diffusion before incubation (Cook *et al.*, 2005).

During storage, the eggshell affects the development of the embryo in the egg's physical aspect and may decrease resistance to gas diffusion, producing necrosis and regressive changes in the blastoderm, which cause embryo development failure (Egbeyale *et al.*, 2013). The size and weight of eggs are considered to influence the analysis of internal and external qualities of eggs. Egg size varies with egg shell characteristics as well as the external quality of eggs. Egg size also affects incubation success, embryonic death, and hatchability (Shafey, 2002). Egg quality focuses on eggshell cleanliness, texture and shape, while internal quality refers to abdomen, transparency and viscosity, air cell size, yolk shape and yolk membrane strength (Stadelman *et al.*, 2017). Additionally, the egg size can be used to evaluate the quality of eggs in various aspects, including the nutritional content, the egg's integrity for commercialization, storage, and

incubation, as well as its preservation during storage (Hegab & Hanafy, 2019). In the period of incubation, the eggshell strength is significant for preventing water loss, microbial penetration, and untimely gas diffusion before incubation.

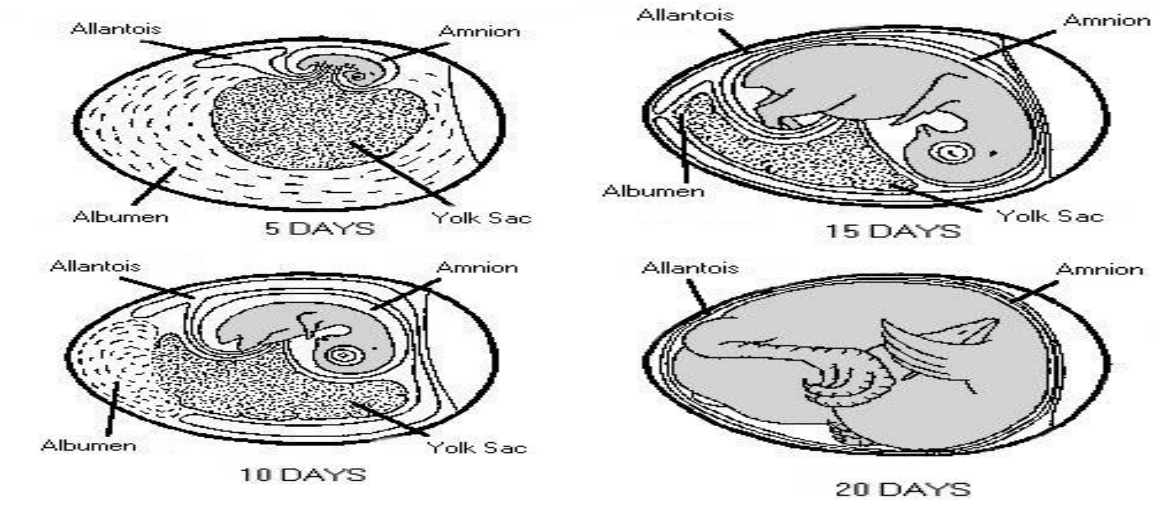

Figure 2.1: Successive changes in the position of the chicken embryo and its embryonic membrane (Rilden & Johnson, 1964).

Homeostasis is one of the major physiological processes arising during the pre-hatch period and it is used in the maintenance of glucose levels. Vieira and Moran Jr (1998) reported that inadequate glycogen makes the embryo mobilize more muscle protein for gluconeogenesis, thus reducing early growth and development. In the incubator, the liver and muscle are not affected by a metabolic pathway on the last day of incubation. More so physiological changes also take place in the yolk during the last week of incubation due to the high energy requirement of the hatching process and the relatively low availability of oxygen and the fatty acids cannot supply all the necessary energy (Moran Jr, 2007). The yolk comprises about 50% water, 15% protein, 33% fat, and less than 1% carbohydrates; however, this composition depends significantly upon egg weight, genetic strain, and hen age (van der Wagt *et al.*, 2020). The main source of energy is the York during embryonic development, and the only source of lipids for embryo tissue growth over the surface of the yolk sac (Uni et al. 2012).

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2.4.1 Symptoms of aflatoxicosis

Aflatoxins are secondary mould metabolites produced by some strains of *Aspergillus flavus*, among others (Klich, 2007). Ruminants metabolize aflatoxins in the liver and excrete them in bile. Aflatoxin B1 is the most potent mycotoxin produced by moulds in livestock (Wogan, 1966). AFB1 is more prevalent in monogastric animals than in ruminants, due to the ability of bacteria from the rumen to metabolize mycotoxins. Depending on the type of aflatoxin and the level of consumption, swine exposure to aflatoxins can cause a variety of chronic or acute syndromes; in swine, aflatoxins can increase the susceptibility to infectious diseases and the risk of death, cause weight loss, poor performance, reduce reproductive capability, change clinical biochemical patterns, and suppress the immune system (Bou Dergue, *et al*. 2009).

In chicken, aflatoxin has several detrimental effects on their health and production such as reduced growth and weight gain, decreased feed efficiency, immune suppression, liver damage, and reduced egg production quality, all this leads to economic losses to poultry producers (Oznurlu *et al.*, 2012).

2.4.2 Bioavailability and toxicity of aflatoxin

Various methods, such as physical separation, thermal inactivation, irradiation, microbial degradation, and treatment with a variety of chemicals have been used for the detoxification or inactivation of AF-contaminated feedstuff (Zhu *et al.*, 2016). The most promising approach is the use of sorbents in diets that adsorb AF in the gastrointestinal tract of animals and reduce bioavailability and toxicity (Shi *et al.*, 2006). Zeolite is one of these sorbent agents. The basic mechanism of action of zeolites is binding to AF in food irreversibly; thus, limiting AF absorption in the digestive tract (Shabani *et al.*, 2016).

2.4.3 Effects of aflatoxin B-1 *in-ovo* on the proximal tibial growth plate of broiler chickens: Histological, histometric, and immunohistochemical findings

Poultry producers encounter aflatoxins regularly in feedstuffs, resulting in severe economic losses and health problems. (Akande *et al.*, 2006). Even though the maximum tolerable levels in Turkey in terms of total AFs are 20 mg/kg for layer feed and 50 mg/kg for feedstuffs (Kocasari *et al.*, 2013). There have been some reports of higher levels being found in poultry food and foodstuffs, ranging from 5 to 100 parts/109 (Oguz, 2011).

Due to this instability, the compound reacts with nuclear DNA and forms an adduct at the N (7)-position of guanine. This adduct could cause mutations that inhibit the production of RNA and, hence, enzymes and other proteins (Sur & Celik, 2003). Oznurlu *et al.* (2012) reported that the formation of DNA-AF adducts results in malignant transformations along with deletions and sister chromatid exchanges. The adverse effects of AFs on poultry particularly anorexia, poor feed utilization, decreased body weight gain, decreased egg production, and susceptibility to unseen and unknown diseases, remain a major economic concern despite numerous studies investigating their toxic effects (Sur & Celik, 2003, Dhama *et al.*, 2007). There is less evidence about the toxicity and detoxification processes in the early embryonic cells; however, the chick embryo starts to gain its detoxifying ability between day 5 and day 6 of incubation, shortly after which the liver and kidneys are fully functional (Sur & Celik, 2003). Embryonic deaths happened at the earlier stages of incubation in AFB1-treated eggs. The higher mortality of fertilized eggs has been reported to coincide with increasing toxin doses in a positive dose-response manner (Sur & Celik, 2003).

There is evidence that AF inhibits the growth and development of bone tissue in broiler chickens, which results in retarded development of the skeletal system. The effects are more pronounced in the tibia (Elwan *et al.*, 2021). Researchers also found that breaking strength and

tibial diameter decreased significantly in the birds exposed to high AF levels (5.0 and 10 mg/g) and ochratoxin levels (4.0 and 8.0 mg/g) (Oznurlu *et al.*, 2012). Studies have shown that the length of the femur and tibia, and the weight of the femur, tibia, radius, and ulna in birds fed 5 mg/g AF in the feed for 6 weeks were significantly lower than in birds that did not receive AF (Cui *et al.*, 2021). Study shows that retardation of skeletal development that AFB 1-treated groups showed were lower mean tibia lengths and weights when evaluated with the control group (PB 0.05) (Oznurlu *et al.*, 2012). In developing embryos, the yolk is the main source of energy and supplies more than 90% of the total energy required by the embryo through the oxidation of yolk lipids (Speake *et al.*, 1998). In embryos, the fatty acid composition of the yolk has a significant effect on the weight and percentage of dry matter accumulation, Therefore, the yolk and its fatty acid content meet the nutritional demands of developing embryos (Yalcin *et al.*, 2008). Data has also demonstrated that the embryo weights significantly declined in the AFB1-treated groups. Among the mycotoxin-induced bone abnormalities, bone fractures are another economic concern for the animal industry (Bryden, 2012). As a key factor in skeletal development, the epiphyseal growth plate plays a key role in bone metabolism, which can lead to skeletal abnormalities (Leach & Gay, 1987). As bones develop and grow rapidly, including the tibia, growth plate cartilage determines how fast the bones grow as well as how long they will be in the long run (Farquharson & Jefferies, 2000).

2.5 Nanotechnology; Solutions in the food and pharmaceutical industries

In the food and pharmaceutical industries, currently, nanotechnology offers a range of promising solutions (Ahmadi & Kurdestany, 2010). Silver nanoparticles have been used as antimicrobial agents in medicine (Rai *et al.*, 2009). Incorporating nanoparticles into other substrates results in novel systems with new applications, called nanocomposites (Evanoff & Chuanov, 2005).

The properties of nanocomposites depend on their constituent properties, particle size, shape, and interaction with the surface (Urbańska *et al.*, 2015).

2.5.1 Nanomaterials for Green Synthesis

Nanomaterials are substances with at least one external dimension in the size range of approximately 1–100 nanometers due to their unique property like high mobility, small size and chemical reactivity, with potential for wide-ranging therapeutic applications (Shahverdi *et al.*, 2007). The application of nanotechnology has enabled the chemical, physical, and optical properties of metals to be altered significantly (Rai *et al.*, 2009). There is a growing concern for the nutritional supplementation of poultry diets with nanoparticles due to today's consumption trends and society's expectations. Nanoparticles are being used to supply various elements in poultry nutrition today. Silver nanoparticles in animal production have good potential for animal nutrition (Grodzik & Sawosz, 2006, Sawosz *et al.*, 2009). Nanoparticles made of metal can bind to more biomolecules, such as antibodies, aptamers, enzymes, and so on. This increases the number of binding sites for biomolecules on the surface (Jans & Huo, 2012). In livestock production, silver nanoparticles may affect intestinal microbial populaces and improve the health and immunological status of the birds and provide the birds with a chance to expend less metabolic effort for immunological control purposes, with nutrients for other physiological and productive purposes (Ferket, 2011). Burrell *et al.* (1999) stated that numerous studies have recognized the antimicrobial activity of silver nanoparticles *in vitro* have effective biocidal activity against a broad spectrum. Silver nanoparticles have some physical and chemical characteristics associated with their larger equivalents because of a very high surface-to-volume ratio, physical activity and chemical stability (Singh *et al.*, 2009).

Zinc is an important micronutrient for metabolic activities and is found in all enzyme groups, (Broadley *et al.*, 2012). ZnO plays an important role in physiological developments and has a

lot of potential in bionanotechnology. In addition, oxygen is one of the commonest elements on the earth, making up about 21% of the atmosphere (Cloud, 1965). Also, zinc oxide has an advantage over other metal oxides due to its biodegradability, biocompatibility, ease of synthesis and low cost in terms of structural aspect ratio (Zhou *et al.*, 2017). Study has shown a simple, low-cost technique for producing ZnO, Ag, and Ag-ZnO nanoparticles using simply available plant sources that do not require the use of hazardous chemicals as reducing agents (Nasrollahzadeh *et al.*, 2019). In natural medicine, plants comprise biomolecules (such as carbohydrates, proteins, and coenzymes) with a high tendency to convert metal salts to nanoparticles plant extracts have been explored as reducing agents and capping molecules for the green synthesis of nanoparticles to enhance their biocompatibility and biological application (El-Seedi *et al.*, 2019).

Several techniques are under investigation to study the physiological changes of eggs during the incubation period using a technique that requires the injection of a nutrient solution a few days to provide an external source of energy to the embryo and the hatchlings (De Oliveira *et al.*, 2008). In previous research, Arafa *et al.* (1981) reviewed that broiler chickens are detected to be more resistant to aflatoxin toxicity than other poultry species. In poultry terminology, AFBI is called the silent murderer because it affects the birds immediately after consumption. Celik *et al.* (2000) reported that the sensitivity of the chicken embryo to aflatoxin can be determined by using fertilized eggs with *in-ovo* injections.

2.5.2 Recent development in aflatoxin detection using nanoparticles.

Aflatoxin is a class of heterocyclic aromatic hydrocarbons produced by *Aspergillus flavus* and *Aspergillus parasiticus* under humid conditions (Garden & Strachan, 2001, Khlangwiset *et al.*, 2011). In terms of median lethal dose (LD50) rats or mice, AFB1 is poisonous at the dose of 0.36mg per kilogram of body weight (Squire, 1981). Also, broiler chickens have been identified

to be more resistant to aflatoxin toxicity than other poultry species (Arafa *et al.*, 1981). Various strategies have been established to help manage the risk of aflatoxin within the feed. Despite these efforts, the occurrence of aflatoxin in animal feeds cannot yet be eradicated (Pinotti *et al.*, 2016). Therefore, nanoparticles have broad application prospects in the chemical industry, life sciences, environmental protection and other fields (Gao *et al.*, 2019). To further advance the sensitivity and ease of aflatoxin analysis and detection, more types of nanomaterials are being used, such as metals nanomaterials, metal oxides, hydroxide and carbon nanomaterials.

2.5.3 Metal Nanomaterials for Mycotoxin Detection

Metal nanomaterials which are easy to combine with biomolecules in food safety detection also have decent biocompatibility. The increase of available sites for binding biomolecules on nanoparticle surfaces can also be seen as a major benefit given that they can bind to more biomolecules, such as antibodies, aptamers, enzymes and so forth (Jans & Huo, 2012). Metal nanomaterials have intense adsorption features and can be combined with different biological macromolecules. They can be used to determine a variety of enzyme activities and they do not affect regular biological activities. More so, the unique fluorescence and electrical properties of metal nanomaterials are the main sources for food analysis.

In addition, Sun *et al.* (2014). reported that different emission or absorption wavelengths of the corresponding metal nanomaterials and aflatoxin can be quantitatively and qualitatively analyzed. The small particle size of these materials will have a quantum size effect and demonstrate optical, electrical, chemical and catalytic properties. Several researchers have employed different types of metal nanoparticles in determining aflatoxin. Yan *et al.* (2020b) built an electrochemical immunoassay method for AFB1 by using gold nanoparticle (AuNP)-modified PEDOT-GO. In this process, graphene oxide was not only used as a fixed platform but also facilitated the rapid transmission of electrons and was also confirmed to apply to corn

AFB1 assay, with a LOD of 0.09 ng/Ml. Li *et al.* (2020) reported that AFB1 was detected by using a gold nanodot-modified rGO nanomaterial on conductive glass and a composite electrode immobilized with the antibody. The detection limit was 6.9 pg/mL.

2.5.4 Metal Oxides and Hydroxides Nanomaterials for Mycotoxin Detection

Since metal oxides and hydroxides are stable and cost-effective, they are also widely used in aflatoxin detection (Gao *et al.*, 2019). Recently, the identified metal oxides and hydroxide nanomaterials are proving to be promising candidates for detecting various target analytics through various EC technologies due to their greater physicochemical properties such as high mechanical strength, large surface area, high catalytic performance, and abundant electronic properties. Moreover, Singh *et al.* (2013) considered an electrochemical sensor by immobilizing Sm_2O_3 nanorods and nickel nanoparticles on the surface of the electrode. A common metal oxide, Fe_3O_4 has been generally considered for its low cost, nontoxicity, ease of production and storage. The anti-AFB1 antibody was used as the gratitude element and can detect 10–700 pg/mL AFB1 in food. In addition, a reusable electrochemical quartz crystal microbalance (QCM) immunosensor for the finding of AFB1 in food was established. In the test, AFB1 from a corn sample was captured on the electrode surface by the capture antibody while the free antigen was washed away, and the finding antibody retained the ability to bind AFB1 in the sample, thereby forming a sandwich complex on the electrode surface (Chauhan *et al.*, 2016).

2.5.5 Carbon Nanomaterials for Mycotoxin Detection

The use of carbon nanomaterials in electrochemical testing is often not limited to only role and function but most of the time contains the actual superposition of multiple functions to complete the improvement of aflatoxin analysis performance (Yan *et al.*, 2020a). Additionally,

carbon nanomaterials possess excellent electrical properties (such as wider potential windows, good chemical stability, good shape controllability and biocompatibility), easy functionalization, and other characteristics that make them well-suited to analysis and detection (Wang *et al.*, 2020).

Subsequently, the AFM1 electrochemical analysis method in which GQDs, α-cyclodextrin, and silver nanoparticles (AgNPs) were sequentially put on GCE to prepare a ternary composite film, and the AFM1 detection technique was established by linear sweep voltammetry (LSV) (Shadjou *et al.*, 2018).

Because of the above, various nanomaterials (AgNPs AuNPs, NiNPs, Fe_3O_4NPs, Sm_2O_3 nanorods) would be synthesized from the extract of maize feed purchased from the South African market. The fabricated nanoparticles obtained from maize feed extract would be characterized mainly using X-ray diffractive analysis (XRD), scanning electron microscopy (SEM), Fourier Transform Infra-Red (FT-IR) Spectroscopy, and UV–V analysis. Subsequent determination of aflatoxin in the fabricated nanoparticles will be measured spectroscopically.

Previous studies have applied several methods for the detection of aflatoxin such as thin-layer chromatography (Castro *et al.*, 2001), HPLC (Dobolyi *et al.*, 2013), mass spectroscopy (Lattanzio *et al.*, 2007), enzyme-linked immune-sorbent assay (ELISA) (Mushtaq *et al.*, 2020), electrochemical immunosensor (Zhang *et al.*, 2016) and ICP-OSE (Karasakal, 2020) The more accurate, confidential and the advance procedure is high-performance liquid chromatography (HPLC) (Manetta & Control, 2011). High-performance liquid chromatography (HPLC) is the most commonly and frequently used method for the determination of aflatoxins in the laboratory to date (Yibadatihan *et al.*, 2014). HPLC has numerous advantages in contrast to other analytical methods such as good repeatability, low detection limit and high sensitivity and it is comparatively the key value in a technique where a mixture of compounds is present.

HPLC method consists of two-phase, the normal phase and the reversed-phase which have been established by using both ultraviolet (UV) and fluorescence detection (FLD). It gives fast and accurate findings and final results while the normal phase systems uses ultraviolet (UV) monitoring at wavelengths of 254 and 365 nm (Wacoo *et al.*, 2014). The complex operational system, high instrument cost, complexity in the cost of extraction, and longer time are the major disadvantages over other methods (Mushtaq *et al.*, 2020).

2.6 Phytochemical Activity

Botanicals are considered environmentally friendly and safer alternative sources of bioagents for the control of fungi and mycotoxins in food and feed. They contain various phytochemicals with pharmacological properties against numerous diseases. Botanicals are applied as bio-fungicides and nutraceuticals to amend the spread of toxigenic fungi and mycotoxin contamination in poultry (Dikhoba *et al.*, 2019, Kavitha *et al.*, 2020). The use of phytochemicals has gained significant consideration due to increasing worry over the protection of synthetic chemicals and the emergence of antibiotic-resistant strains of microorganisms (Salamci *et al.*, 2007).

Plants as drugs have several advantages due to their abundance in nature and wide geographical distribution. Drugs have been synthesized from plant extracts based on their uses in traditional medicine (Dias *et al.*, 2012). Different schemes have been established to inhibit aflatoxin production, such as gene silencing and transgenic plants mostly from green synthesis from African plant species, perennial herbs and South African marine plants (Labib *et al.*, 2020). Plant extracts have shown the potential influence of inhibiting aflatoxins because of their phytochemical composition. The genus *Urginea epigea*, which is in the family of Hyacinthaceae, is primarily found in Africa with some species found in the Mediterranean. It produces flowers that range from white to pale yellow or pink (Mulholland *et al.*, 2013). The

bulb has been widely used in South Africa for medicinal purposes and it possesses antimutagens, antimicrobials and anticarcinogens capable of compromising the toxic and genotoxic effects of mycotoxins (Kamanja, 2014). *Urginea epigea* is capable of inhibiting enzymes in cell wall components (Fusi *et al.*, 2008). The plant has been used as a bioactive additives compound to prevent fungal growth (Loi *et al.*, 2020) and aflatoxin contamination in poultry while it also reduces the risks of mutagenicity and carcinogenicity of aflatoxins (Munteanu & Vasile, 2020).

Subsequently Pineda *et al.* (2012b) stated that the metabolism of layer embryos can be modified with the *in-ovo* injection of silver nanoparticles (Ag Nano) on day 1 of incubation, increasing the metabolic rate in layer embryos. Moreover, Ferket (2011) reported that the *in-ovo* method of introducing silver nanoparticles could be a valuable practice for the earlier establishment of immunity and intestinal integrity of the birds, which are important to achieve maximum potential for growth and feed efficiency.

However, physiological changes of eggs as well as the physiopathology during incubation must be observed to attain good and quality birds after hatchling. A lot of factors affect the development of the embryo during incubation which is mostly caused by eggshell, weight, and size of the eggs and has a high influence on the growth and performance of birds after hatchery. Therefore, it is very important to develop systems that can stop or inhibit the effect of aflatoxins by using synthesized silver nanoparticles from *Urginea epigea.*

Urginea epigea has phytochemicals including bufadienolide that has the potential of ameliorating the aflatoxins, but it is unstable. Therefore, it is necessary to encapsulate its chemical composition with nanoparticles and prevent the ease of degradation under environmental conditions which will control the aflatoxin-induced effects via upregulation of antioxidant enzyme gene expression and increase the viability of GSH.

2.7 Conventional application and limitations of nanotechnology

Traditional anti-cancer drugs are associated with many complications including insolubility in water, rapid clearance from the bloodstream and insufficiency of drug delivery to cancer cells. While conventional drugs are relatively safe, they can cause non-specific toxicity and insufficiency of drug delivery to cancer cells (Kumari *et al.*, 2016). Several biological pathways are used by nanoparticles (NPs) to deliver payloads to cellular and intracellular targets, including crossing the blood-brain barrier (BBB). The ability for nanoparticles to cross the BBB appears to be enabled by receptor-mediated endocytosis through brain endothelial cells.

Considering employing nanoparticles to deliver proteins or other macromolecules across the BBB, it seems that this technology holds great promise for the non-invasive treatment of CNS diseases, including neoplasms (Wohlfart *et al.*, 2012). Since NPs have an affinity to acidic environments, as typified by tumour tissue, selective targeting strategies with NPs can facilitate more effective cancer detection and treatment with minimal side effects on normal cells (Kreuter, 2014). There have been extensive research projects on non-cytotoxic doses of silver nanoparticles (AgNPs) because of their potential to induce gene expression for impaired cell cycle progression, DNA damage and apoptosis in human cells. According to previous research, silver nanoparticles caused DNA damage leading to cell cycle arrest in the G2/M phase and enhanced apoptosis in tumour cells, including those that harbour glioblastoma multiforme (GBM) (Urbańska *et al.*, 2015).

2.7.1 Nano-Nutrition of Chicken Embryos

Several amino acids in eggs are insufficient to fully support embryonic development and *in-ovo* nutrient administration may be used to supply extra nutrients and energy to the embryo (Grodzik *et al.*, 2013). Modern broiler types are intensively selected for growth rate and muscle size (including pectoral muscles). As a result, chicken embryos have an increased requirement

for energy and protein, and consequently, the imbalance between the need for and reserves of nutrients may limit the maximum size and growth of chicken embryos (according to genotype) (Griffin & Goddard, 1994). Some authors have revealed that concentrations of certain amino acids in the egg are not sufficient to fully support embryonic development (Beck *et al.*, 2015). Further, since eggs can store limited amounts of carbohydrates, amino acids act as substrates for glycogen synthesis, thereby limiting their availability for protein synthesis (Fujimoto *et al.*, 2013). *In ovo* nutrition has been demonstrated to be one possibility for supplying embryos with nutrients and energy. A recent study demonstrated that l-glutamine *in ovo* administration to chicken embryos increased VEGF-A mRNA and protein levels which is responsible for endothelial cell proliferation, stimulates vasculogenesis and angiogenesis and affects pectoral muscle morphology (Grodzik *et al.*, 2013).

2.7.2 Nano-Nutrition of chicken embryos; its effect on gene expression

In modern broilers, the quantity and quality of nutrients stored in eggs might not be optimal for fat rate embryo development; hence, *in ovo* injection of nutrients might be beneficial (Pineda *et al.*, 2012a). Recent research showed that *in ovo* feeding reduces post-hatch mortality and skeletal disorders, it increases muscle growth and breast meat yield (Sawosz *et al.*, 2013).

2.7.3 Photocatalytic performance of Ag-ZnO nanocomposites

A growing number of different applications for nano-sized semiconductor materials have emerged recently, including photoelectric energy conversion materials as well as water and air purification catalysts (Murata *et al.*, 2000, Evgenidou *et al.*, 2005). As a result of its high photocatalysis and relatively low cost, ZnO has attracted much attention for the degradation of various pollutants. Several methods have been described to prepare AG-ZnO nanocomposites, including photoreduction (Behnajady *et al.*, 2006, Hong *et al.*, 2006). There has been recent interest in using nanocomposites as photocatalysts to degrade environmental pollutants.

Among various semiconductor photocatalysts, ZnO nanomaterials have garnered considerable attention due to their thermal and chemical stability, non-toxicity, low cost, and high catalytic efficiency (Lai *et al.*, 2010).

2.8 Summary of the literature review

Literature has fully emphasized that there is a growing use of plant extracts as food additives because of their bioactive compounds, such as polyphenols and carotenoids, which exhibit antimicrobial and antioxidant properties, especially against LDL cholesterol and DNA. Results from different scholars have shown that plants are abundant in nature and have a wide geographic distribution. They are excellent sources for drugs, as well as traditionally used plants have been used for drugs synthesized from their extracts. No research has been done using *Urginea epigea* extract bulbs; therefore, the synthesis of *Urginea epigea* has the potential to stop or inhibit the effect of aflatoxins, as it contains phytochemicals, such as bufadienolide, that can ameliorate the effects of aflatoxins.

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3. Investigating the Ameliorative Effects of *in-ovo* Administration of *Urginea epigea* Extract Phyto-synthesized Silver-Zinc Oxide Bimetallic Nanoparticles on Quality and Physio-pathological Characteristics of Chicken Eggs Subjected to Aflatoxicosis

3.1 INTRODUCTION

The poultry industry is concerned about the contamination of poultry feed with aflatoxins, since aflatoxicosis causes poor feed utilization, reduced body weight gain, lowered egg production, and decreased survival rates (Mgbeahuruike *et al.*, 2018). It is well known that dietary AFs and their metabolites are carcinogenic, teratogenic, mutagenic and growth inhibitory, and they also penetrate and accumulate in poultry soft tissues and fat depots (Pal *et al.*, 2019). It is important to recognize that aflatoxin contamination of agricultural commodities can have significant economic consequences since it can cause severe yield losses as well as acute and chronic toxicity in both humans and animals (Cinar & Onbaşı, 2019). As a result of climate change, several reports indicate its incidence is rising. Therefore, different strategies such as biological control, development of resistant cultivar, fungicides and maintenance of near-optimum water relations in the crop through irrigation; control of pod and seed-feeding insects; optimal timing of digging and harvest; avoidance of mechanical damage during cultivation, harvest and post-harvest handling; rapid post-harvest drying; and maintenance of low temperature and relative humidity in storage have have been extensively studied for the past many years has be implemented before and after harvest to reduce aflatoxin impact on food and feed chains.

Further, because of its biological effects and widespread toxicity, aflatoxin B1 is considered to be the most important foodborne mycotoxin due to its impact on egg and shell formation (Kihara *et al.*, 2000). There is a serious problem of aflatoxin contamination and residues in fertilized eggs in the poultry industry (Oznurlu *et al.*, 2012). Most of the economic losses are

attributed to the fertilized egg due to left-over aflatoxins in the feed affecting embryo viability and hatchability, as well as causing organ malformations (Qureshi *et al.*, 1998a). In the early stage of development, there are a lot of changes that occur such as cell proliferation, migration and apoptosis in the developing embryo (Fayjaloun *et al.*, 2019).

AF-induced embryotoxicity in chickens can be prevented with cost-effective and practical methods, but currently limited. It is currently necessary to include AF-binding adsorbents in feeds to prevent laying hens from being exposed to AF-contaminated diets, thus reducing the carry-over of AF residues into fertilized eggs. It is therefore important to develop an effective strategy to control AF-induced embryotoxicity in chickens.

A variety of chemical and synthetic compounds have been used to inhibit toxic fungi for many years. But as green chemistry and sustainability become increasingly important, scientists are becoming more interested in using alternative pest and disease control methods including natural antifungal agents that do not harm the environment and humans.

The phytochemical composition of some plant extracts has shown that they can inhibit aflatoxin production. The genus *Urginea epigea*, which belongs to the family Hyacinthaceae, is mainly found in Africa, but some species grow in the Mediterranean. It produces white to pale yellow or pink flowers (Mulholland *et al.*, 2013). Several antimutagens, antimicrobials, and anticarcinogens have been found in the bulb, which can impede the toxic and genotoxic effects of mycotoxins. (Kamanja, 2014). *Urginea epigea* is capable of inhibiting enzymes in cell wall components (Fusi *et al.*, 2008) and the plant has been used as a bioactive additives compound to prevent fungal growth (Loi *et al.*, 2020) and aflatoxin contamination in poultry. Thus it reduces the risks of mutagenicity and carcinogenicity of aflatoxins (Munteanu & Vasile, 2020).

A successive study by (Pineda et al., 2012) found that silver nanoparticles (Ag Nano) can be injected *in ovo* into layer embryos on the first day of incubation to increase their metabolism.

Moreover, (Ferket, 2011) reported that *in ovo* method of introducing silver nanoparticles could be a valuable practice for the earlier establishment of immunity and intestinal integrity of the birds, which are important to achieve maximum potential for growth and feed efficiency.

However, it is crucial to observe the physiological changes in eggs as well as the pathophysiology during incubation.to attain good and quality birds after hatchling. A lot of factors affect the development of the embryo during incubation. This is primarily caused by the eggshell, weight, and size of the eggs and plays an influential role in the growth and performance of birds after hatching. Therefore, it is very important to develop systems that can stop or inhibit the effect of aflatoxins by using synthesized silver nanoparticles from *Urginea epigea.*

There are phytochemicals in Urginea epigea, including bufadienolide, that may ameliorate aflatoxins, but they are unstable, so nanoparticles must be used to encapsulate their chemical composition and prevent it from degrading when exposed to the environment..

3.2 Materials and Methods

3.2.1 Study site

The study was conducted at Molelwane Farm situated 6 km west of the Northwest University, Mafikeng Campus, South Africa. The geographical coordinates of Molelwane are 25º48′00″S and 25º38′21″E. The area has summer months from August to March with temperatures ranging from 22 °C to 35°C and a long-term average annual rainfall of 450 mm. The province has winter months from May to July, with sunny dry days and chilly nights with average minimum and maximum temperatures of 2°C and 20°C respectively.

3.2.2 Source and preparation of materials

Fertile eggs of broiler chickens were obtained and used in this study. *Urginea epigea* plants were purchased from Muthi Shops in Ermelo (Mpumalanga) and Durban (KwaZulu-Natal). The bulbs were air-dried at room temperature and ground using a laboratory mill. Zinc acetate, silver nitrate and ethanol reagents for nanoparticle synthesis were purchased from Merck, South Africa. The green synthesis of silver zinc oxide fabricated with *U. epigea* extract and standard aflatoxin was used in this experiment.

3.2.3 Experimental design, treatments, sampling, and measurements

The fertile eggs (1500) of broiler chickens were sanitized by fumigation, weighed and randomly allocated to different experimental treatments involving 3 levels of AFB1 [Control = no AFB1, Positive Control = Phosphate-buffered saline and 45ng AFB1/egg], 2 levels of AFB2 (5ng AFB2/egg and 15ng AFB2/egg), 3 levels of Ag-ZnO [Control = minus Ag-ZnO (without injection), Plus Ag-ZnO without extract (injected with conventional Nano-Ag-ZnO), and Plus Ag-ZnO with extract (injected with *U. epigea* extract-fabricated Nano- Ag-ZnO)] in a 3 x 2 x 3 x 2 factorial arrangement in a completely randomized design. Each treatment had 150 eggs

with 10 replicates of 15 each. The administration of both the aflatoxins and nanoparticles was done on the same day (Day 1) immediately before placing the eggs in the incubator. On this day, the eggshells were drilled using micro dremel at the blunt end and injected with 20 uL of respective aflatoxin solution into the air space (Parlat *et al.*, 1999). A syringe and needles with sterile tips were used for administering reactant materials. Subsequently, both conventionally synthesized and *U. epigea* extract-fabricated Ag-ZnO solutions (50 mg/L) were administered into the air space at a dose of 0.3 mL/egg. The holes were sealed immediately with sterile tape and the eggs were placed in an incubator at 37 °C at 65% relative humidity and turned through 90° every 2 hours for 21 days. All eggs were weighed on day 1 of incubation to determine the egg weight at setting and shell quality was determined by the colour of the shell (brown) from day 1 of incubation to day 21.

3.2.4 Measurement of external and internal egg quality traits

3.2.4.1 External

The eggs were weighed from each treatment including the control on days 7, 14 and 19 using a sensitive weighing balance and were recorded.

3.2.4.2 Egg shape index

The egg shape index was calculated by dividing the short diameter by the length of each egg and multiplying by 100. The egg length and width were measured with the aid of the digital calliper; the shape indexes of the eggs were calculated using the following formula given by (Khurshid *et al.*, 2004).

$$\boldsymbol{Egg\ shape\ index} = \frac{\textbf{egg width}}{\textbf{egg lenght}}\ \boldsymbol{x}\ \mathbf{100}$$

3.2.4.3 Albumen and yolk heights

Day 7 of the experiment albumen and yolk heights were measured at their highest point using a spherometer. After removal of the egg contents, the shells of the broken eggs were washed, air-dried, and then weighed to measure shell weight in grams to calculate the shell weight percentages of egg (g) using an electronic balance.

3.2.4.4 Shell thickness

Shell thickness of each treatment within each egg weight category was measured, without the shell membrane, using a digital calliper (ORKA Technology LLC, Bountiful, UT) with a sensitivity of 0.001 mm at the broader end, the equator, and the pointed end of the fertilized egg and then average thickness was calculated.

3.2.4.5 Albumen weight

Albumen weight (g) was calculated as egg weight - (yolk weight + eggshell weight). Haugh units (HU) were calculated as HU = 100 × log (albumen height - 1.7 × egg weightx0.37+7.6), according to Fernandez *et al.* (2011).

An observational study of the eggs was carried out from day 7, day 14, and day 19 of incubation. The eggs were broken to determine the pathophysiology changes of the embryo. The embryo of each treatment including the control will be measured with a measuring ruler.

3.2.4.6 Embryo colour

The colour of the embryo was observed by taking the picture and comparing it with the normal colour of healthy embryo which looks like a tiny white donut or bullseye. From day 7, day 14 and day19 an egg was broken and examined to see the changes in the embryogenesis stages of the chicken until the end of the study. Breaking the eggs was done carefully, each egg was

broken at one side and then the eggshell was taken off to prevent any damage to the contents of the egg, then the content of the egg was put gently onto a plate and several photos were taken using neue digital camera.

3.2.4.7 Embryo Weight

The weight of the embryo was recorded using the standard described by Hamburger and Hamilton (1951) with some modification i.e., Embryo weight/Egg weight x 100.

3.2.4.8 The Yolk Diameter

The yolk diameter was measured in mm using a Vernier calliper from each treatment and the weight was determined using an electronic balance (0.01-g accuracy).

3.2.4.9 The Yolk Sac

The yolk sac was measured with a measuring ruler and the allantois and amnion of each treatment with the control from day 7, day 14 and day19 of incubation were measured with a measuring ruler.

3.2.4.10 The internal quality

The internal quality and embryonic development of each treatment was observe by checking the growth rate of all the treatment including the control were recorded. The mean relative embryo weight and relative yolk sac weight of each group were calculated using the equations as provided below: Relative embryo weight = [(embryo weight on day 7, day 14 and on day19 /egg weight on day 7, day 14 and on day 19) × 100%)]; Relative yolk sac weight = [(yolk sac weight on day 18/egg weight on day 18) × 100%]. The development status of chicken embryos was compared with the standard described by Hamburger and Hamilton (1951) according to

the Hamburger-Hamilton stages with some modifications (Hamburger & Hamilton, 1951). Also candling of the eggs was carried out.

3.2.5 Statistical Analysis

Data were statistically analyzed by analysis of variance (ANOVA) using the IBM Spss software package and were presented as Least Square Means (LSM) with respective pooled SEMs. Where significant differences ($P<0.05$) between treatments were observed, LMS was compared using Turkey's test.

4. Results and Discussion

4.1 Results

Aflatoxin B1 on the physiological responses of induced broiler eggs to *Urginea epigea*, revealed that there was no significant ($p>0.05$) difference in all the parameters measured as presented in Table 1. However, in control groups, embryo weight was significantly higher in the positive control compared to the negative control. Embryo weight in eggs injected with 10ppb AFB1 was significantly greater compared to other treatment groups, while a lower value was observed in 5ppb. There was a significant difference between the sampling days at 7 days in embryo weight, although no significant difference was observed in the embryo weight between eggs treated and sampling days of 14 and 19 days respectively.

Table 1a: Effect of Aflatoxin B1 on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	62.92±4.55	59.10±4.67	64.97±2.40	**0.267**
	14	57.00±4.06	60.10±1.96	55.96±7.89	62.38±2.58	62.76±3.19	**0.266**
	19	62.46±2.23	63.23±2.15	59.79±3.93	63.94±0.66	63.86±3.20	**0.223**
Width	7	4.53±0.25	4.27±0.25	4.37±0.21	4.23±0.15	4.40±0.36	**0.714**
	14	4.27±0.12	4.50±0.10	4.27±0.15	4.43±0.23	4.47±0.21	**0.535**
	19	4.50±0.10	4.33±0.12	4.37±0.15	4.43±0.23	4.53±0.25	**0.656**
Length	7	5.37±0.15	5.60±0.20	5.70±0.17	5.50±0.17	5.73±0.12	**0.222**
	14	5.77±0.21	5.83±0.23	5.57±0.31	5.47±0.12	5.67±0.35	**0.692**
	19	5.67±0.40	5.63±0.15	5.70±0.20	5.67±0.25	5.87±0.21	**0.531**
Egg shape index	7	84.47±4.22	76.14±1.84	77.27±2.76	76.97±1.81	76.82±7.51	**0.993**
	14	74.00±0.88	77.22±3.47	76.70±1.89	81.05±2.48	75.64±8.46	**0.451**
	19	79.71±6.58	76.93±1.44	76.62±2.96	78.32±4.92	77.23±1.65	**0.817**
Shell weight	7	5.07±0.73	5.66±0.22	5.32±0.99	5.65±0.33	5.61±0.34	**0.792**
	14	5.63±0.25	5.57±0.48	5.02±0.41	5.31±0.15	5.40±0.52	**0.51**
	19	5.40±0.21	5.26±0.13	5.53±0.60	5.44±0.07	5.37±0.53	**0.916**

[abc]Means in the same columns with different superscripts differ significantly (P<0.05)

Table 1b: Effect of Aflatoxin B1 on internal quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	4.00±1.00	3.33±0.58	4.00±1.00	**0.593**
	14	1.67±0.58	1.67±0.57	2.00±1.00	1.67±0.58	1.67±0.58	**0.824**
	19	1.00±0.00	1.33±0.57	1.33±0.58	1.33±0.58	1.33±0.58	**1**
Albumen length	7	47.67±4,51	50.67±2.52	53.00±6.24	47.67±2.52	50.67±2.08	**0.341**
	14	28.00±2.00	27.00±2.00	24.67±2.08	26.33±2.08	27.00±1.00	**0.414**
	19	20.33±2.52	21.00±2.00	19.33±1.15	20.33±2.31	19.67±1.53	**0.78**
Albumen height	7	23.60±1.57	24.04±1.53	22.82±1.48	23.61±1.34	24.34±1.07	**0.42**
	14	6.27±1.05	5.67±0.58	5.14±0.29	5.35±0.14	5.94±0.63	**0.121**
	19	3.94±0.58	3.93±0.67	3.88±0.47	4.34±0.52	3.73±0.52	**0.362**
Yolk height	7	5.00±1.00	5.33±0.58	6.00±1.00	5.00±1.00	4.67±0.58	**0.593**
	14	2.33±0.58	2.33±0.58	2.33±0.58	2.67±0.58	2.33±0.58	**0.729**
	19	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.33±0.58	**0.422**
Yolk weight	7	27.41±1.24	24.51±2.05	25.95±0.48	25.91±2.60	24.71±2.25	**0.236**
	14	8.92±2.13	8.43±0.82	8.91±0.48	7.25±1.78	7.08±2.09	**0.367**
	19	5.27±0.88	4.78±0.41	5.18±0.51	5.10±0.70	5.40±0.38	**0.791**
Yolk diameter	7	56.00±2.00	61.33±2.52	59.00±4.00	57.67±3.51	56.67±4.93	**0.702**
	14	24.33±1.53	28.00±6.56	29.00±1.00	31.00±2.65	27.33±2.08	**0.166**
	19	20.67±2.08	20.00±2.00	20.00±2.00	24.33±2.52	19.33±1.53	**0.48**
Embryo weight	7	$1.31±0.12^{ab}$	$1.25±0.17^{b}$	$1.28±0.02^{b}$	$1.14±0.06^{c}$	$1.33±0.08^{a}$	**0.019**
	14	11.26±1.06	12.30±0.90	11.16±0.60	12.13±0.57	11.75±1.23	**0.427**
	19	30.63±1.23	29.29±1.15	30.15±0.66	28.93±0.47	30.28±1.02	**0.13**
Embryo length	7	25.67±4.04	27.33±3.06	26.00±3.00	25.67±1.53	30.00±1.00	**0.071**
	14	62.67±2.52	56.00±3.00	60.67±3.06	62.00±5.67	59.00±4.00	**0.711**
	19	85.00±2.00	82.00±2.00	85.33±3.21	62.33±29.73	82.67±4.16	**0.284**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	11.67±2.52	12.00±1.00	14.67±2.08	**0.205**
	19	27.33±1.53	23.67±3.51	26.67±2.52	28.33±1.53	24.00±2.45	**0.065**
Albumen index	7	8.21±1.18	7.88±1.82	7.50±1.39	8.38±1.93	6.96±1.10	**0.544**
	14	5.88±1.79	6.10±1.84	7.93±3.40	6.43±2.52	6.10±1.83	**0.685**
	19	4.97±0.61	6.54±3.45	6.85±2.74	6.81±3.27	6.67±2.89	**0.997**
Yolk index	7	11.48±2.09	11.55±0.80	9.89±1.08	11.79±1.95	12.19±0.51	**0.155**
	14	10.72±1.80	12.39±4.02	12.83±2.45	13.61±1.99	12.11±2.61	**0.751**
	19	10.33±1.56	10.00±1.00	10.00±1.00	12.17±1.26	8.61±2.11	**0.075**

The relative egg weight, width, length, egg shape index, shell weight, albumen weight, albumen length, albumen height, yolk height, yolk weight, yolk diameter, embryo weight, embryo length, tibia length, albumen index and yolk index on sampling days of 7, 14, and 19 of incubation as depicted in Table 2. Results revealed that administration of eggs with 20% PBS (negative control) did not significantly affect the shell weight and embryo weight when compared with the positive control. Eggs injected with 2.5ppb demonstrated significantly higher shell weight and embryo weight as compared to the other treatment at a sampling day of 19. Although no significant difference in the shell weight and embryo weight between the sampling days of 7 and 14 respectively were observed among the treatment.

The yolk weight of egg injected with 10ppb was found to be significantly lower as compared to the negative control and positive control. In addition, 5ppb of AFB2 significantly increased the yolk weight treated egg at 14 days duration of sampling. However, no significant effect was observed at 7 and 19 days of sampling respectively.

Table 2a: Effect of Aflatoxin B2 on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	60.17±2.40	61.27±2.80	62.43±4.70	**0.737**
	14	57.00±4.06	60.10±1.96	61.29±3.05	63.12±6.35	63.36±2.19	**0.814**
	19	62.46±2.23	63.23±2.15	64.06±4.74	64.48±7.11	65.97±3.06	**0.898**
Width	7	4.53±0.25	4.27±0.25	4.43±0.21	4.37±0.31	4.27±0.31	**0.768**
	14	4.27±0.12	4.50±0.10	4.37±0.31	4.47±0.06	4.37±0.12	**0.77**
	19	4.50±0.10	4.33±0.12	4.33±0.15	4.33±0.25	4.37±0.15	**0.97**
Length	7	5.37±0.15	5.60±0.20	5.50±0.20	5.57±0.21	5.43±0.15	**0.702**
	14	5.77±0.21	5.83±0.23	5.63±0.25	5.77±0.06	5.77±0.21	**0.638**
	19	5.67±0.40	5.63±0.15	5.80±0.17	5.53±0.15	5.83±0.25	**0.205**
Egg shape index	7	84.47±4.22	76.14±1.84	80.76±6.62	76.43±1.56	77.62±2.98	**0.485**
	14	74.00±0.88	77.22±3.47	78.00±4.21	77.47±1.56	75.74±0.93	**0.579**
	19	79.71±6.58	76.93±1.44	74.72±1.71	78.59±6.26	74.88±3.80	**0.422**
Shell weight	7	5.07±0.73	5.66±0.22	5.35±0.98	5.49±0.32	5.33±0.15	**0.938**
	14	5.63±0.25	5.57±0.48	5.27±0.79	5.21±0.15	5.48±0.30	**0.281**
	19	5.40±0.21	5.26±0.13	5.89±0.19^{a}	5.34±0.14^{bc}	5.40±0.21^{b}	**0.018**

^{abc}Means in the same rows with different superscripts differ significantly (P<0.05)

Table 2b: Effect of Aflatoxin B2 on internal quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	3.67±0.58	3.67±1.15	3.67±0.58	**1**
	14	1.67±0.58	1.67±0.57	2.33±0.58	1.67±0.58	2.00±1.00	**0.579**
	19	1.00±0.00	1.33±0.57	1.33±0.58	1.33±0.58	1.33±0.58	**1**
Albumen length	7	47.67±4.51	50.67±2.52	52.00±4.00	51.33±3.21	58.00±6.56	**0.252**
	14	28.00±2.00	27.00±2.00	26.33±2.51	27.67±1.53	26.67±3.51	**0.819**
	19	20.33±2.52	21.00±2.00	18.67±1.53	22.33±2.08	19.00±1.00	**0.058**
Albumen height	7	23.60±1.57	24.04±1.53	23.25±0.89	21.91±1.58	22.10±0.64	**0.346**
	14	6.27±1.05	5.67±0.58	5.31±0.06	4.59±0.47	5.08±1.10	**0.478**
	19	3.94±0.58	3.93±0.67	3.76±0.56	4.13±0.79	4.02±0.30	**0.738**
Yolk height	7	5.00±1.00	5.33±0.58	5.33±1.15	5.00±1.00	5.67±1.15	**0.77**
	14	2.33±0.58	2.33±0.58	3.33±0.58	2.67±1.15	3.00±1.00	**0.702**
	19	2.00±0.00	2.00±0.00	2.33±0.58	2.00±0.00	2.00±0.00	**0.422**
Yolk weight	7	27.41±1.24	24.51±2.05	25.63±1.56	25.42±1.08	24.74±1.68	**0.751**
	14	8.92±2.13^{a}	8.43±0.82^{ab}	7.68±0.60^{b}	8.73±0.63^{ab}	5.09±0.61^{c}	**0.001**
	19	5.27±0.88	4.78±0.41	5.85±±0.33	5.68±1.35	5.21±0.61	**0.667**
Yolk diameter	7	56.00±2.00	61.33±2.52	63.33±2.08	60.33±3.06	59.67±1.53	**0.198**
	14	24.33±1.53	28.00±6.56	26.67±2.52	28.33±2.08	30.00±3.61	**0.405**
	19	20.67±2.08	20.00±2.00	20.00±3.00	20.33±3.21	18.67±1.53	**0.736**
Embryo weight	7	1.31±0.12	1.25±0.17	1.24±0.07	1.33±0.09	1.33±0.09	**0.452**
	14	11.26±1.06	12.30±0.90	11.85±0.62	12.68±1.53	13.02±0.34	**0.379**
	19	30.63±1.23	29.29±1.15	30.77±0.56^{a}	28.34±0.97^{b}	30.52±0.83^{a}	**0.019**
Embryo length	7	25.67±4.04	27.33±3.06	27.67±2.52	25.33±2.08	26.67±4.16	**0.663**
	14	62.67±2.52	56.00±3.00	62.67±5.51	62.67±3.06	62.33±7.02	**0.996**
	19	85.00±2.00	82.00±2.00	86.00±3.61	86.00±1.00	85.33±3.21	**0.947**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	14.00±1.00	13.00±1.00	13.67±1.53	**0.609**
	19	27.33±1.53	23.67±3.51	27.67±1.53	26.67±2.52	28.67±1.87	**0.485**
Albumen index	7	8.21±1.18	7.88±1.82	7.08±1.29	7.22±2.60	6.31±0.64	**0.79**
	14	5.88±1.79	6.10±1.84	8.92±2.37	6.05±2.18	8.67±4.45	**0.512**
	19	4.97±0.61	6.54±3.45	7.34±3.83	5.95±2.41	6.94±2.66	**0.85**
Yolk index	7	11.48±2.09	11.55±0.80	12.36±3.38	12.34±2.06	10.77±1.80	**0.69**
	14	10.72±1.80	12.39±4.02	8.22±1.95	10.33±4.01	11.23±2.53	**0.485**
	19	10.33±1.56	10.00±1.00	8.72±1.19	8.78±2.34	9.33±0.76	**0.874**

Result in Table 3 revealed that the shell weight of silver zinc oxide injected egg decreased in a dose-dependent manner in comparison to controls (positive and negative control). However, shell weight treated with 2.5ppb of silver zinc oxide green concentration showed a significant higher value ($p<0.05$) during the 7 days incubation compared to other treatment. Between the days of sampling, no significant effect was observed in 14 and 19 durations respectively. The albumen weight obtained was significantly greater in the positive and negative control compared to the 10ppb treated egg. Moreover, 2.5ppb concentration experiences a higher value when compared to other treatment groups at 7-day sampling period. The albumen length of the 10ppb treated group of silver zinc oxide green was higher ($p<0.05$) on incubation days of 7 compared to the positive control. The albumen index of the positive control and 2.5ppb experience a significantly higher value than those of the other treatment group. A non-significant effect of all parameters recorded in the incubation period of 14 and 19 days of the different concentration were observed.

Table 3a: Effect of silver zinc oxide green synthesis on external quality and physiological of induced broilers eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	62.39±5.18	57.76±3.53	64.65±1.80	**0.153**
	14	57.00±4.06	60.10±1.96	64.89±2.55	67.07±3.62	64.73±1.31	**0.524**
	19	62.46±2.23	63.23±2.15	60.58±4.20	62.42±3.79	62.35±3.91	**0.819**
Width	7	4.53±0.25	4.27±0.25	4.53±0.21	4.33±0.06	4.37±0.31	**0.517**
	14	4.27±0.12	4.50±0.10	4.43±0.25	4.27±0.15	4.43±0.31	**0.65**
	19	4.50±0.10	4.33±0.12	4.47±0.29	4.33±0.25	4.37±0.15	**0.783**
Length	7	5.37±0.15	5.60±0.20	5.57±0.15	5.47±0.06	5.47±0.06	**0.422**
	14	5.77±0.21	5.83±0.23	5.73±0.23	5.53±0.32	5.63±0.31	**0.712**
	19	5.67±0.40	5.63±0.15	5.90±0.20	5.60±0.10	5.80±0.30	**0.296**
Egg shape index	7	84.47±4.22	76.14±1.84	81.41±2.24	78.79±1.78	79.38±5.27	**0.644**
	14	74.00±0.88	77.22±3.47	77.42±5.69	77.18±2.53	78.65±1.18	**0.873**
	19	79.71±6.58	76.93±1.44	75.67±2.91	77.34±3.12	75.33±1.37	**0.619**
Shell weight	7	$5.07±0.73^{c}$	$5.66±0.22^{b}$	$5.96±0.13^{a}$	$5.24±0.09^{b}$	$5.22±0.40^{bc}$	**0.016**
	14	5.63±0.25	5.57±0.48	5.67±0.18	5.54±0.19	5.40±0.22	**0.327**
	19	5.40±0.21	5.26±0.13	5.42±0.53	5.67±0.13	5.28±0.08	**0.362**

Table 3b: Effect of silver zinc oxide green synthesis on internal quality and physiological of induced broilers eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00[a]	4.00±1.00[a]	4.67±0.58[a]	3.00±0.00[bc]	3.33±0.58[b]	**0.011**
	14	1.67±0.58	1.67±0.57	1.33±0.58	2.33±0.58	2.00±0.00	**0.098**
	19	1.00±0.00	1.33±0.57	1.33±0.58	1.00±0.00	1.33±0.58	**0.63**
Albumen length	7	47.67±4,51[b]	50.67±2.52[ab]	57.67±3.21[ab]	47.00±5.29[b]	60.33±5.69[a]	**0.033**
	14	28.00±2.00	27.00±20	27.00±1.00	25.67±1.53	25.00±1.73	**0.302**
	19	20.33±2.52	21.00±2.00	20.00±1.00	21.33±2.52	20.67±2.08	**0.723**
Albumen height	7	23.60±1.57	24.04±1.53	22.40±2.21	22.36±0.88	22.09±1.71	**0.97**
	14	6.27±1.05	5.67±0.58	5.93±0.17	5.63±0.21	5.81±0.57	**0.626**
	19	3.94±0.58	3.93±0.67	4.03±0.90	3.83±0.53	4.32±0.26	**0.644**
Yolk height	7	5.00±1.00	5.33±0.58	5.00±1.00	4.67±1.15	6.00±1.00	**0.34**
	14	2.33±0.58	2.33±0.58	2.33±0.58	2.67±0.58	2.67±1.15	**0.85**
	19	2.00±0.00	2.00±0.00	2.33±0.58	2.00±0.00	2.00±0.00	**0.422**
Yolk weight	7	27.41±1.24	24.51±2.05	25.86±0.88	24.82±0.52	25.85±0.66	**0.197**
	14	8.92±2.13	8.43±0.82	9.61±1.59	9.21±0.28	8.44±0.84	**0.436**
	19	5.27±0.88	4.78±0.41	5.61±0.33	5.78±1.20	4.99±0.58	**0.485**
Yolk diameter	7	56.00±2.00	61.33±2.52	66.00±2.65	58.33±5.86	61.67±4.16	**0.185**
	14	24.33±1.53	28.00±6.56	26.67±3.06	31.00±2.65	27.33±2.89	**0.218**
	19	20.67±2.08	20.00±2.00	19.67±2.08	19.33±2.52	20.33±1.53	**0.84**
Embryo weight	7	1.31±0.12	1.25±0.17	1.90±0.11	1.24±0.16	1.22±0.04	**0.869**
	14	11.26±1.06	12.30±0.90	12.50±0.73	12.18±0.62	11.93±0.70	**0.614**
	19	30.63±1.23	29.29±1.15	29.57±0.79	29.99±1.43	30.39±1.05	**0.683**
Embryo length	7	25.67±4.04	27.33±3.06	27.33±2.52	24.00±2.65	26.67±2.52	**0.312**
	14	62.67±2.52	56.00±3.00	62.00±3.61	63.67±4.04	67.00±6.08	**0.462**
	19	85.00±2.00	82.00±2.00	64.00±31.43	86.67±3.79	84.33±4.51	**0.324**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	13.00±1.00	14.00±1.00	14.00±2.00	**0.63**
	19	27.33±1.53	23.67±3.51	27.33±1.53	26.33±1.53	28.00±3.61	**0.713**
Albumen index	7	8.21±1.18[a]	7.88±1.82[ab]	8.07±0.58[a]	6.38±0.67[b]	5.54±0.92[c]	**0.015**
	14	5.88±1.79	6.10±1.84	4.85±1.99	8.95±1.93	9.01±1.76	**0.058**
	19	4.97±0.61	6.54±3.45	6.77±2.82	4.81±0.57	6.54±3.03	**0.582**
Yolk index	7	11.48±2.09	11.55±0.80	13.63±3.26	12.78±1.70	10.39±1.06	**0.255**
	14	10.72±1.80	12.39±4.02	11.89±3.17	11.94±2.31	13.83±0.76	**0.537**
	19	10.33±1.56	10.00±1.00	8.78±2.34	9.11±2.46	10.17±0.76	**0.692**

No significant effect was obtained between the incubation sampling of 7, 14 and 19 days respectively of the treated group and controls (positive and negative control) in silver zinc oxide chemical induced broiler eggs as presented in Table 4. However, significant variation ($p<0.05$) in albumen index of broiler eggs was observed at the 7 days incubation of 10ppb and positive control where the overall value was observed. Furthermore, no significant effect was shown in 14 and 19 incubation period of the experiment.

Table 4a: Effect of silver zinc oxide chemical on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	60.45±4.26	64.97±2.40	62.38±4.70	**0.42**
	14	57.00±4.06	60.10±1.96	62.04±1.51	59.26±3.15	64.23±1.55	**0.085**
	19	62.46±2.23	63.23±2.15	62.61±1.84	62.68±1.82	61.13±2.17	**0.575**
Width	7	4.53±0.25	4.27±0.25	4.57±0.21	4.40±0.36	4.07±0.12	**0.118**
	14	4.27±0.12	4.50±0.10	4.67±0.15	4.47±0.12	4.50±0.20	**0.329**
	19	4.50±0.10	4.33±0.12	4.60±2.00	4.50±1.00	4.50±0.26	**0.787**
Length	7	5.37±0.15	5.60±0.20	5.53±0.21	5.73±0.12	5.60±0.20	**0.433**
	14	5.77±0.21	5.83±0.23	5.63±0.25	5.80±0.17	5.57±0.15	**0.388**
	19	5.67±0.40	5.63±0.15	5.80±0.10	5.93±0.15	5.70±0.20	**0.263**
Egg shape index	7	84.47±4.22	76.14±1.84	82.52±1.98	76.82±7.51	72.67±3.26	**0.119**
	14	74.00±0.88	77.22±3.47	82.90±3.05	77.07±3.69	80.83±2.70	**0.154**
	19	79.71±6.58	76.93±1.44	79.34±4.34	75.86±2.10	78.92±3.16	**0.429**
Shell weight	7	5.07±0.73	5.66±0.22	5.61±0.36	5.61±0.34	5.55±0.09	**0.959**
	14	5.63±0.25	5.57±0.48	5.55±0.21	5.77±0.38	5.55±0.18	**0.556**
	19	5.40±0.21	5.26±0.13	5.57±0.08	5.48±0.13	5.35±0.32	**0.454**

[abc]Means in the same rows with different superscripts differ significantly (P<0.05)

Table 4b: Effect of silver zinc oxide chemical on internal quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	3.33±0.58	4.00±1.00	4.33±0.58	**0.317**
	14	1.67±0.58	1.67±0.57	1.33±0.58	1.33±0.58	1.67±0.58	**0.729**
	19	1.00±0.00	1.33±0.57	1.00±0.00	1.00±0.00	1.67±0.58	**0.079**
Albumen length	7	47.67±4,51	50.67±2.52	53.33±8.02	50.67±2.08	49.33±1.52	**0.35**
	14	28.00±2.00	27.00±2.00	27.67±2.52	27.33±2.08	27.33±0.58	**0.815**
	19	20.33±2.52	21.00±2.00	21.33±2.52	22.33±2.08	21.00±2.00	**0.754**
Albumen height	7	23.60±1.57	24.04±1.53	22.27±1.93	24.34±1.07	25.02±1.13	**0.125**
	14	6.27±1.05	5.67±0.58	5.94±1.10	5.97±0.44	5.88±0.41	**0.989**
	19	3.94±0.58	3.93±0.67	3.58±0.44	3.47±0.47	3.98±0.13	**0.288**
Yolk height	7	5.00±1.00	5.33±0.58	5.00±1.00	4.67±0.58	5.33±1.15	**0.702**
	14	2.33±0.58	2.33±0.58	2.33±0.58	2.33±0.58	2.33±0.58	**0.1**
	19	2.00±0.00	2.00±0.00	3.47±0.47	2.00±0.00	2.00±0.00	
Yolk weight	7	27.41±1.24	24.51±2.05	25.32±1.13	24.71±2.25	27.58±1.16	**0.147**
	14	8.92±2.13	8.43±0.82	10.42±1.03	9.12±1.93	7.92±0.37	**0.135**
	19	5.27±0.88	4.78±0.41	4.72±0.39	5.06±0.95	5.30±0.59	**0.607**
Yolk diameter	7	56.00±2.00	61.33±2.52	62.00±3.00	56.67±4.93	61.00±3.00	**0.259**
	14	24.33±1.53	28.00±6.56	25.00±3.00	29.00±5.00	31.00±2.65	**0.21**
	19	20.67±2.08	20.00±2.00	22.67±2.52	21.00±1.73	24.33±2.52	**0.278**
Embryo weight	7	1.31±0.12	1.25±0.17	1.31±0.07	1.33±0.08	1.20±0.09	**0.166**
	14	11.26±1.06	12.30±0.90	12.49±0.97	11.99±0.47	12.07±0.51	**0.652**
	19	30.63±1.23	29.29±1.15	30.09±1.49	29.93±1.44	29.03±0.50	**0.555**
Embryo length	7	25.67±4.04	27.33±3.06	25.67±2.52	30.00±1.00	28.33±2.52	**0.116**
	14	62.67±2.52	56.00±3.00	63.00±3.00	59.00±6.00	66.33±8.62	**0.417**
	19	85.00±2.00	82.00±2.00	84.33±5.51	84.00±4.36	82.67±2.52	**0.884**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	15.00±2.00	13.00±2.00	13.00±1.00	**0.332**
	19	27.33±1.53	23.67±3.51	27.00±1.00	27.67±2.08	28.33±1.15	**0.579**
Albumen index	7	8.21±1.18^{a}	7.88±1.82^{ab}	6.03±0.58^{bc}	6.10±0.12^{b}	8.83±1.02^{a}	**0.004**
	14	5.88±1.79	6.10±1.84	4.74±1.67	4.91±1.72	6.22±2.30	**0.611**
	19	4.97±0.61	6.54±3.45	4.73±0.55	4.58±0.55	5.24±1.20	**0.611**
Yolk index	7	11.48±2.09	11.55±0.80	12.78±2.84	11.96±0.72	10.77±1.17	**0.449**
	14	10.72±1.80	12.39±4.02	10.94±1.59	12.89±3.75	13.44±1.90	**0.504**
	19	10.33±1.56	10.00±1.00	11.33±1.26	10.43±0.67	11.54±1.75	**0.574**

The data obtained in AFB1 + silver zinc oxide green on the physiological responses of induced broiler eggs to *Urginea epigea* as showed in Table 5 revealed the combination of aflatoxin b1 + silver oxide green on egg shape index at a duration of 7 days incubation in comparison to respective treated group of incubation interval. Higher values were observed in the positive control and treatment group of 5ppb compared to the negative control and 10ppb treatment group respectively. Significant difference ($p<0.05$) was seen among treatment group of albumen length, albumen height and yolk weight at 10ppb concentration in 19 days of incubation, while a lower value was obtained in negative control, 2.5ppb, and 5ppb respectively. Moreover, the embryo length was significantly higher in positive control and 5ppb of administered nanoparticle compared to 10ppb were a reduce value was recorded.

Table 5a: Effect of aflatoxins B1+ silver zinc oxide green on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	59.57±5.85	63.15±4.86	62.34±2.71	**0.636**
	14	57.00±4.06	60.10±1.96	61.02±5.21	62.31±2.53	61.68±3.07	**0.918**
	19	62.46±2.23	63.23±2.15	63.61±0.94	63.76±1.23	60.98±2.05	**0.106**
Width	7	4.53±0.25	4.27±0.25	4.30±0.26	4.53±0.25	4.37±0.31	**0.592**
	14	4.27±0.12	4.50±0.10	4.40±0.10	4.57±0.15	4.40±0.30	**0.544**
	19	4.50±0.10	4.33±0.12	4.47±0.15	4.50±0.10	4.50±0.10	**0.918**
Length	7	5.37±0.15	5.60±0.20	5.43±0.15	5.37±0.15	5.57±0.21	**0.41**
	14	5.77±0.21	5.83±0.23	5.63±0.21	5.67±0.06	5.60±0.30	**0.93**
	19	5.67±0.40	5.63±0.15	5.60±0.20	5.93±0.15	5.80±0.26	**0.229**
Egg shape index	7	84.47±4.22^{a}	76.14±1.84^{b}	77.62±2.99^{ab}	84.47±4.22^{a}	76.43±1.56^{b}	**0.039**
	14	74.00±0.88	77.22±3.47	78.66±2.56	80.60±3.37	78.31±4.80	**0.765**
	19	79.71±6.58	76.93±1.44	79.76±0.53	75.86±2.10	77.22±3.47	**0.202**
Shell weight	7	5.07±0.73	5.66±0.22	4.86±0.49	5.32±0.22	5.63±0.35	**0.107**
	14	5.63±0.25	5.57±0.48	5.43±0.22	5.55±0.38	5.37±0.24	**0.736**
	19	5.40±0.21	5.26±0.13	5.28±0.32	5.51±0.31	5.71±0.37	**0.349**

^{abc}Means in the same rows with different superscripts differ significantly (P<0.05)

Table 5b: Effect of aflatoxins B1+ silver zinc oxide green on internal quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	4.00±1.00	3.33±0.58	3.33±0.58	**0.492**
	14	1.67±0.58	1.67±0.57	2.00±1.00	1.33±0.58	2.00±1.00	**0.593**
	19	1.00±0.00	1.33±0.57	1.33±0.58	1.00±0.00	1.67±0.58	**0.296**
Albumen length	7	47.67±4,51	50.67±2.52	50.33±1.53	56.67±5.69	53.00±3.61	**0.228**
	14	28.00±2.00	27.00±2.00	27.00±1.00	26.67±1.53	25.33±1.15	**0.296**
	19	20.33±2.52^{bc}	21.00±2.00^{b}	19.33±2.52^{c}	22.33±2.08^{ab}	27.00±2.00^{a}	**0.015**
Albumen height	7	23.60±1.57	24.04±1.53	23.67±1.17	22.54±0.10	22.61±1.28	**0.368**
	14	6.27±1.05	5.67±0.58	4.74±0.44	5.53±0.92	5.69±0.29	**0.21**
	19	3.94±0.58^{bc}	3.93±0.67^{bc}	4.37±0.49^{b}	3.72±0.37^{bc}	5.73±0.56^{a}	**0.006**
Yolk height	7	5.00±1.00	5.33±0.58	4.33±0.58	6.00±1.00	5.00±1.00	**0.145**
	14	2.33±0.58	2.33±0.58	3.00±1.00	2.33±0.58	2.33±0.58	**0.492**
	19	2.00±0.00	2.00±0.00	2.67±0.58	2.00±0.00	2.33±0.58	**0.296**
Yolk weight	7	27.41±1.24	24.51±2.05	27.55±1.26	24.79±0.61	25.75±1.39	**0.063**
	14	8.92±2.13	8.43±0.82	8.60±0.85	8.00±0.36	8.90±0.67	**0.304**
	19	5.27±0.88^{b}	4.78±0.41^{c}	5.04±0.72^{bc}	5.01±0.98^{c}	8.51±1.29^{a}	**0.009**
Yolk diameter	7	56.00±2.00	61.33±2.52	57.00±3.00	59.67±1.53	60.33±3.06	**0.327**
	14	24.33±1.53	28.00±6.56	28.33±2.08	31.00±2.65	29.67±0.58	**0.323**
	19	20.67±2.08	20.00±2.00	20.33±2.52	21.00±1.73	28.00±6.56	**0.119**
Embryo weight	7	1.31±0.12	1.25±0.17	1.30±0.13	1.33±0.94	1.35±0.12	**0.896**
	14	11.26±1.06	12.30±0.90	13.38±1.20	12.59±0.85	11.85±0.56	**0.2**
	19	30.63±1.23	29.29±1.15	30.54±1.46	29.71±1.31	28.81±0.40	**0.265**
Embryo length	7	25.67±4.04	27.33±3.06	26.33±4.16	27.67±3.21	25.00±2.00	**0.627**
	14	62.67±2.52	56.00±3.00	65.33±4.16	58.00±2.00	60.33±2.52	**0.062**
	19	85.00±2.00^{a}	82.00±2.00^{ab}	82.67±1.53^{ab}	83.33±4.04^{a}	56.00±3.00^{b}	**0**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	13.33±1.53	12.33±1.15	13.00±1.00	**0.63**
	19	27.33±1.53	23.67±3.51	27.33±2.08	26.00±1.00	23.67±1.53	**0.077**
Albumen index	7	8.21±1.18	7.88±1.82	8.21±1.18	6.30±0.64	7.23±2.59	**0.433**
	14	5.88±1.79	6.10±1.84	7.60±3.98	4.82±1.81	7.67±3.81	**0.53**
	19	4.97±0.61	6.54±3.45	7.19±3.98	4.57±0.55	6.28±1.53	**0.47**
Yolk index	7	11.48±2.09	11.55±0.80	11.48±2.09	10.77±1.80	12.33±2.06	**0.649**
	14	10.72±1.80	12.39±4.02	8.72±1.93	13.89±3.79	13.17±2.75	**0.143**
	19	10.33±1.56	10.00±1.00	7.89±2.00	10.40±0.69	12.39±4.02	**0.19**

No significant difference was observed ($p>0.05$) in all parameters measured in broiler eggs expose with AFB1 + silver zinc oxide chemical as revealed in Table 6. However, there was an increasing dose-dependent value in yolk height in incubation period of day 14, with a greater value observed in dose treatment of 10ppb. The incubation days of 7, 14 and 19 days of the experiment did not significantly improve the external and internal quality of the developing embryo, but there was an increasing numerical value across the group with dose and duration in tibia length.

Table 6a: Effect of aflatoxins B1 + silver zinc oxide chemical on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	60.78±3.96	60.05±6.28	65.68±0.69	**0.293**
	14	57.00±4.06	60.10±1.96	63.04±3.38	62.73±2.44	60.59±1.52	**0.487**
	19	62.46±2.23	63.23±2.15	64.83±0.98	63.58±3.85	63.81±0.34	**0.786**
Width	7	4.53±0.25	4.27±0.25	4.37±0.31	4.10±0.10	4.57±0.06	**0.061**
	14	4.27±0.12	4.50±0.10	4.33±0.15	4.23±0.12	4.50±0.95	**0.091**
	19	4.50±0.10	4.33±0.12	4.37±0.15	4.40±0.20	4.50±0.20	**0.675**
Length	7	5.37±0.15	5.60±0.20	5.47±0.06	5.57±0.25	5.73±0.06	**0.178**
	14	5.77±0.21	5.83±0.23	5.57±0.21	5.60±0.35	5.60±0.58	**0.942**
	19	5.67±0.40	5.63±0.15	5.77±0.23	5.60±0.10	5.63±0.10	**0.382**
Egg shape index	7	84.47±4.22	76.14±1.84	79.38±5.27	73.77±4.33	78.90±1.61	**0.248**
	14	74.00±0.88	77.22±3.47	77.97±5.46	75.86±6.51	79.41±1.93	**0.7**
	19	79.71±6.58	76.93±1.44	75.73±1.01	78.54±2.17	80.35±3.11	**0.115**
Shell weight	7	5.07±0.73	5.66±0.22	5.22±0.40	5.57±0.35	5.60±0.34	**0.42**
	14	5.63±0.25	5.57±0.48	5.42±0.36	5.52±0.41	5.71±0.38	**0.667**
	19	5.40±0.21	5.26±0.13	5.54±0.37	5.29±0.41	5.40±0.20	**0.692**

Table 6b: Effect of aflatoxins B1 + silver zinc oxide chemical on internal quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	3.33±0.58	4.00±1.00	3.33±2.08	**0.797**
	14	1.67±0.58	1.67±0.57	1.67±0.58	1.67±0.58	2.00±1.00	**0.824**
	19	1.00±0.00	1.33±0.57	1.33±0.58	1.33±0.58	1.33±0.58	**1**
Albumen length	7	47.67±4,51	50.67±2.52	60.33±5.69	48.00±2.65	42.33±17.62	**0.194**
	14	28.00±2.00	27.00±2.00	27.00±3.00	25.33±1.53	37.00±17.44	**0.382**
	19	20.33±2.52	21.00±2.00	21.67±1.53	19.33±0.58	20.67±2.08	**0.25**
Albumen height	7	23.60±1.57	24.04±1.53	22.09±1.71	23.90±0.97	18.45±11.93	**0.644**
	14	6.27±1.05	5.67±0.58	5.80±0.53	5.65±0.55	5.72±0.52	**0.94**
	19	3.94±0.58	3.93±0.67	4.09±0.61	3.68±0.61	4.27±0.36	**0.315**
Yolk height	7	5.00±1.00	5.33±0.58	6.00±1.00	5.00±1.00	3.67±1.53	**0.135**
	14	2.13±0.58	2.23±0.58	2.33±0.58	2.67±0.58	2.69±0.58	**0.729**
	19	2.00±0.00	2.00±0.00	1.67±0.58	1.67±0.58	1.67±1.15	**1**
Yolk weight	7	27.41±1.24	24.51±2.05	25.34±1.12	26.88±2.14	17.18±9.50	**0.158**
	14	8.92±2.13	8.43±0.82	9.74±1.44	6.74±1.67	8.10±0.43	**0.078**
	19	5.27±0.88	4.78±0.41	5.79±0.39	4.89±0.52	5.13±0.16	**0.619**
Yolk diameter	7	56.00±2.00	61.33±2.52	61.67±4.16	57.33±3.06	47.00±19.08	**0.338**
	14	24.33±1.53	28.00±6.56	31.00±2.64	30.33±3.21	31.00±2.65	**0.947**
	19	20.67±2.08	20.00±2.00	20.33±1.53	21.67±0.58	24.00±3.60	**0.219**
Embryo weight	7	1.31±0.12	1.25±0.17	1.19±0.09	1.16±0.08	11.76±17.91	**0.407**
	14	11.26±1.06	12.30±0.90	12.89±1.27	11.64±0.85	11.68±0.41	**0.242**
	19	30.63±1.23	29.29±1.15	30.31±0.97	29.98±1.14	29.73±0.60	**0.752**
Embryo length	7	25.67±4.04	27.33±3.06	26.67±2.52	27.00±3.61	48.33±32.62	**0.344**
	14	62.67±2.52	56.00±3.00	64.00±7.81	61.33±6.51	57.67±4.16	**0.51**
	19	85.00±2.00	82.00±2.00	83.00±5.20	84.33±0.58	84.67±6.43	**0.905**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	15.00±1.00	12.33±1.53	12.33±1.15	**0.062**
	19	27.33±1.53	23.67±3.51	23.67±3.51	27.33±1.53	27.00±2.00	**0.218**
Albumen index	7	8.21±1.18	7.88±1.82	5.54±0.92	9.02±0.93	7.79±2.81	**0.131**
	14	5.88±1.79	6.10±1.84	6.07±1.70	6.58±2.28	5.42±1.45	**0.752**
	19	4.97±0.61	6.54±3.45	6.07±1.70	6.58±2.28	5.42±1.45	**0.752**
Yolk index	7	11.48±2.09	11.55±0.80	10.39±106	11.72±1.94	12.12±0.35	**0.303**
	14	10.72±1.80	12.39±4.02	12.22±2.68	11.72±2.61	13.22±3.67	**0.831**
	19	10.33±1.56	10.00±1.00	10.17±0.76	10.67±0.58	10.61±2.64	**0.919**

Table 7 indicates that all parameters measured had no significant improvement (p>0.05) on broiler eggs administered aflatoxin b2 + silver zinc oxide in terms of dose and duration of incubation.

Table 7a: Effect of aflatoxins B2 + silver zinc oxide green on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	60.19±6.90	59.29±2.14	60.44±5.62	**0.962**
	14	57.00±4.06	60.10±1.96	61.76±3.51	64.36±2.69	64.26±1.55	**0.459**
	19	62.46±2.23	63.23±2.15	62.51±2.23	65.80±5.53	64.20±4.89	**0.681**
Width	7	4.53±0.25	4.27±0.25	4.50±0.20	4.27±0.38	4.47±0.29	**0.61**
	14	4.27±0.12	4.50±0.10	4.50±0.10	4.43±0.12	4.33±0.15	**0.326**
	19	4.50±0.10	4.33±0.12	4.47±0.21	4.30±0.20	4.33±0.12	**0.523**
Length	7	5.37±0.15	5.60±0.20	5.50±0.10	5.50±0.26	5.47±0.06	**0.961**
	14	5.77±0.21	5.83±0.23	5.53±0.15	5.80±0.20	5.67±0.15	**0.237**
	19	5.67±0.40	5.63±0.15	5.70±0.20	5.70±0.20	5.70±0.20	**0.1**
Egg shape index	7	84.47±4.22	76.14±1.84	81.87±5.13	77.38±3.19	81.21±5.44	**0.49**
	14	74.00±0.88	77.22±3.47	81.35±2.35	76.45±1.32	76.51±1.32	**0.06**
	19	79.71±6.58	76.93±1.44	77.87±1.57	75.41±0.87	76.04±1.34	**0.13**
Shell weight	7	5.07±0.73	5.66±0.22	5.58±0.39	5.36±0.09	5.23±0.09	**0.279**
	14	5.63±0.25	5.57±0.48	5.44±0.27	5.39±0.40	5.44±0.21	**0.971**
	19	5.40±0.21	5.26±0.13	5.39±0.34	5.42±0.27	5.64±0.70	**0.793**

[abc]Means in the same rows with different superscripts differ significantly (P<0.05)

Table 7b: Effect of aflatoxins B2 + silver zinc oxide green on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	4.00±1.00	4.00±1.00	3.33±0.58	**0.593**
	14	1.67±0.58	1.67±0.57	1.67±0.58	2.00±1.00	2.67±0.58	**0.317**
	19	1.00±0.00	1.33±0.57	1.00±0.00	1.33±0.58	1.33±0.58	**0.63**
Albumen length	7	47.67±4.51	50.67±2.52	55.00±7.81	52.00±4.36	45.33±2.52	**0.158**
	14	28.00±2.00	27.00±2.00	25.00±3.00	27.67±2.08	26.00±1.00	**0.381**
	19	20.33±2.52	21.00±2.00	19.67±2.08	22.33±2.08	21.67±2.89	**0.416**
Albumen height	7	23.60±1.57	24.04±1.53	22.47±2.29	21.55±1.03	22.97±2.53	**0.708**
	14	6.27±1.05	5.67±0.58	5.59±0.47	5.11±1.10	5.67±0.47	**0.64**
	19	3.94±0.58	3.93±0.67	4.06±0.22	4.40±0.56	4.53±1.56	**0.829**
Yolk height	7	5.00±1.00	5.33±0.58	5.33±1.53	5.00±1.00	4.33±0.58	**0.562**
	14	2.33±0.58	2.33±0.58	2.67±0.58	2.67±1.15	3.33±1.15	**0.661**
	19	2.00±0.00	2.00±0.00	2.00±0.00	2.33±0.58	2.00±0.00	**0.422**
Yolk weight	7	27.41±1.24	24.51±2.05	25.68±2.39	24.68±1.72	26.54±1.91	**0.563**
	14	8.92±2.13	8.43±0.82	9.99±1.48	8.38±0.74	9.79±1.38	**0.297**
	19	5.27±0.88	4.78±0.41	5.64±0.55	5.88±1.32	6.45±1.72	**0.742**
Yolk diameter	7	56.00±2.00	61.33±2.52	59.67±4.04	58.67±2.08	56.00±2.00	**0.337**
	14	24.33±1.53	28.00±6.56	26.67±3.06	30.00±3.61	29.67±5.51	**0.59**
	19	20.67±2.08	20.00±2.00	20.67±3.06	20.00±2.65	25.33±7.57	**0.412**
Embryo weight	7	1.31±0.12	1.25±0.17	1.22±0.19	1.26±0.06	1.24±0.16	**0.954**
	14	11.26±1.06	12.30±0.90	11.72±0.55	12.60±0.55	12.99±1.48	**0.411**
	19	30.63±1.23	29.29±1.15	30.10±0.56	29.31±0.89	24.13±10.39	**0.467**
Embryo length	7	25.67±4.04	27.33±3.06	26.33±2.52	24.00±2.65	25.00±4.36	**0.698**
	14	62.67±2.52	56.00±3.00	64.00±4.00	63.33±7.37	62.67±6.11	**0.964**
	19	85.00±2.00	82.00±2.00	85.67±4.04	85.67±1.15	76.67±15.70	**0.448**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	13.33±1.53	14.33±0.58	13.67±1.53	**0.648**
	19	27.33±1.53	23.67±3.51	27.33±2.52	25.67±1.53	22.33±8.62	**0.532**
Albumen index	7	8.21±1.18	7.88±1.82	7.21±0.98	7.74±2.19	7.27±0.94	**0.896**
	14	5.88±1.79	6.10±1.84	6.74±2.57	7.77±1.95	6.23±1.80	**0.684**
	19	4.97±0.61	6.54±3.45	5.12±0.51	5.95±2.41	7.00±3.00	**0.614**
Yolk index	7	11.48±2.09	11.55±0.80	11.65±2.43	12.00±2.07	13.03±1.27	**0.69**
	14	10.72±1.80	12.39±4.02	10.33±2.52	12.17±3.21	9.89±2.01	**0.562**
	19	10.33±1.56	10.00±1.00	10.33±1.53	7.66±1.34	13.00±3.61	**0.088**

Table 8 revealed the administration of aflatoxin B2 + silver zinc oxide chemical on the physiological responses of induced broiler eggs. There was no significant difference in all the treated group in terms of dose and duration. However, length had no significant effect in the control groups (positive and negative control), except in the treated group of 2.5ppb which increased significantly compared to other dose treated groups. Furthermore, no significant effect was observed among the sampling days of 7 and 14 duration period and between the controls and sampling day of 7 and 14 respectively.

Table 8a: Effect of aflatoxins B2 + silver zinc oxide chemical on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	62.97±4.78	63.07±3.10	64.98±1.99	**0.773**
	14	57.00±4.06	60.10±1.96	63.52±1.01	62.46±1.07	63.85±1.38	**0.373**
	19	62.46±2.23	63.23±2.15	63.60±1.83	63.75±1.25	64.32±1.03	**0.81**
Width	7	4.53±0.25	4.27±0.25	4.23±0.25	4.37±0.35	4.43±0.15	**0.661**
	14	4.27±0.12	4.50±0.10	4.23±0.12	4.34±0.21	4.47±0.15	**0.291**
	19	4.50±0.10	4.33±0.12	4.50±0.10	4.37±0.15	4.47±0.15	**0.506**
Length	7	5.37±0.15	5.60±0.20	5.73±0.12	5.53±0.25	5.73±0.12	**0.332**
	14	5.77±0.21	5.83±0.23	5.60±0.35	5.73±0.23	5.53±0.23	**0.68**
	19	5.67±0.40	5.63±0.15	5.93±0.15^{a}	5.53±0.06^{b}	5.60±0.20^{ab}	**0.035**
Egg shape index	7	84.47±4.22	76.14±1.84	73.84±4.43	78.98±2.70	77.34±2.80	**0.247**
	14	74.00±0.88	77.22±3.47	75.86±6.52	75.29±6.23	80.88±5.92	**0.519**
	19	79.71±6.58	76.93±1.44	75.86±2.10	78.91±2.74	79.76±0.533	**0.119**
Shell weight	7	5.07±0.73	5.66±0.22	5.54±0.21	5.66±0.19	5.59±0.59	**0.923**
	14	5.63±0.25	5.57±0.48	5.61±0.13	5.71±0.35	5.59±0.23	**0.828**
	19	5.40±0.21	5.26±0.13	5.62±0.13	5.59±0.13	5.62±0.21	**0.972**

^{abc}Means in the same rows with different superscripts differ significantly (P<0.05)

Table 8b: Effect of aflatoxins B2 + silver zinc oxide chemical on internal quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	4.00±1.00	4.00±1.00	3.33±0.58	**0.593**
	14	1.67±0.58	1.67±0.57	1.67±0.58	1.67±1.15	1.67±0.58	**0.1**
	19	1.00±0.00	1.33±0.57	1.00±0.00	1.33±0.58	1.33±0.58	**0.63**
Albumen length	7	47.67±4,51	50.67±2.52	51.00±2.00	53.00±4.00	50.67±6.43	**0.798**
	14	28.00±2.00	27.00±2.00	25.33±1.53	35.67±18.47	26.67±4.16	**0.497**
	19	20.33±2.52	21.00±2.00	22.00±2.65	21.67±2.31	19.33±2.52	**0.416**
Albumen height	7	23.60±1.57	24.04±1.53	25.13±1.52	22.51±0.38	23.82±1.30	**0.089**
	14	6.27±1.05	5.67±0.58	5.79±0.55	6.14±0.77	5.74±0.70	**0.742**
	19	3.94±0.58	3.93±0.67	3.74±0.38	4.18±0.57	4.08±0.79	**0.662**
Yolk height	7	5.00±1.00	5.33±0.58	5.00±1.00	5.67±0.58	5.67±1.15	**0.63**
	14	2.33±0.58	2.33±0.58	2.67±0.58	2.33±0.58	2.33±0.58	**0.729**
	19	2.00±0.00	2.00±0.00	2.00±0.00	2.00±1.00	2.66±0.58	**0.422**
Yolk weight	7	27.41±1.24	24.51±2.05	26.25±2.36	25.14±0.75	25.56±0.87	**0.679**
	14	8.92±2.13	8.43±0.82	6.78±1.31	7.64±1.01	8.39±1.82	**0.435**
	19	5.27±0.88	4.78±0.41	5.06±0.95	4.80±0.48	5.11±0.87	**0.879**
Yolk diameter	7	56.00±2.00	61.33±2.52	59.33±1.53	61.33±3.51	59.67±4.16	**0.736**
	14	24.33±1.53	28.00±6.56	30.33±3.21	30.67±3.06	28.33±2.89	**0.623**
	19	20.67±2.08	20.00±2.00	21.00±1.73	22.67±2.08	20.33±2.52	**0.438**
Embryo weight	7	1.31±0.12	1.25±0.17	1.38±0.04	1.32±0.04	1.29±0.04	**0.085**
	14	11.26±1.06	12.30±0.90	11.49±0.90	11.59±1.32	12.57±0.90	**0.459**
	19	30.63±1.23	29.29±1.15	30.35±1.29	29.37±1.03	29.99±1.20	**0.667**
Embryo length	7	25.67±4.04	27.33±3.06	28.33±2.08	26.33±2.08	27.00±2.65	**0.581**
	14	62.67±2.52	56.00±3.00	61.33±6.51	58.33±3.06	65.33±3.51	**0.254**
	19	85.00±2.00	82.00±2.00	83.33±4.04	82.00±2.00	82.67±1.53	**0.842**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	13.33±1.53	13.00±1.00	15.00±1.00	**0.173**
	19	27.33±1.53	23.67±3.51	27.33±1.53	25.00±1.00	27.67±2.52	**0.221**
Albumen index	7	8.21±1.18	7.88±1.82	8.09±1.95	7.62±2.33	6.56±0.32	**0.579**
	14	5.88±1.79	6.10±1.84	6.58±2.28	4.45±0.79	6.44±2.77	**0.44**
	19	4.97±0.61	6.54±3.45	4.59±0.59	6.40±3.57	7.19±3.98	**0.602**
Yolk index	7	11.48±2.09	11.55±0.80	11.00±1.21	10.86±0.72	10.68±1.70	**0.955**
	14	10.72±1.80	12.39±4.02	11.72±2.61	13.11±2.61	12.78±3.85	**0.88**
	19	10.33±1.56	10.00±1.00	10.50±0.87	9.78±1.35	7.89±2.01	**0.164**

The combined effect of aflatoxins B1 + aflatoxins B2 chemical on egg weight, width, length, egg shape index, shell weight, albumen weight, albumen length, albumen height, yolk height, yolk weight, yolk diameter, embryo weight, embryo length, tibia length, albumen index and yolk index as presented in Table 9 shows that data obtained were not significantly affected ($p>0.05$) among the treated egg in dose and duration of incubation.

Table 9a: Effect of aflatoxins B_1 + aflatoxins B_2 chemical on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	62.12±3.74	63.17±3.78	61.06±2.91	**0.771**
	14	57.00±4.06	60.10±1.96	64.62±3.65	64.65±2.09	63.84±2.92	**0.932**
	19	62.46±2.23	63.23±2.15	65.53±5.73	64.05±2.13	67.07±3.62	**0.684**
Width	7	4.53±0.25	4.27±0.25	4.30±0.17	4.43±0.15	4.40±0.36	**0.797**
	14	4.27±0.12	4.50±0.10	4.47±0.15	4.40±0.10	4.50±0.20	**0.739**
	19	4.50±0.10	4.33±0.12	4.43±0.29	4.47±0.12	4.33±0.25	**0.772**
Length	7	5.37±0.15	5.60±0.20	5.60±0.17	5.57±0.21	5.60±0.26	**0.977**
	14	5.77±0.21	5.83±0.23	5.63±0.21	5.50±0.17	5.60±0.26	**0.75**
	19	5.67±0.40	5.63±0.15	5.57±0.31	5.70±0.20	5.60±0.10	**0.75**
Egg shape index	7	84.47±4.22	76.14±1.84	76.80±2.76	79.77±5.53	78.63±3.59	**0.656**
	14	74.00±0.88	77.22±3.47	79.42±5.54	80.05±3.09	80.39±2.65	**0.955**
	19	79.71±6.58	76.93±1.44	79.76±6.32	78.42±3.36	77.34±3.12	**0.81**
Shell weight	7	5.07±0.73	5.66±0.22	5.18±0.21	5.68±0.36	5.31±0.12	**0.113**
	14	5.63±0.25	5.57±0.48	5.67±0.39	5.79±0.34	5.43±0.17	**0.406**
	19	5.40±0.21	5.26±0.13	5.48±0.24	5.37±0.66	5.67±0.13	**0.682**

[abc]Means in the same rows with different superscripts differ significantly (P<0.05)

Table 9b: Effect of aflatoxins B_1 + aflatoxins B_2 chemical on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	3.00±1.00	2.00±1.00	3.67±1.53	**0.304**
	14	1.67±0.58	1.67±0.57	1.67±0.58	1.67±0.58	2.00±0.00	**0.63**
	19	1.00±0.00	1.33±0.57	1.00±0.00	1.00±0.00	1.00±0.00	
Albumen length	7	47.67±4,51	50.67±2.52	43.00±15.39	34.33±16.44	44.00±16.09	**0.73**
	14	28.00±2.00	27.00±2.00	25.33±3.06	27.33±2.52	26.67±2.08	**0.648**
	19	20.33±2.52	21.00±2.00	22.00±3.47	20.33±1.53	21.33±2.52	**0.747**
Albumen height	7	23.60±1.57	24.04±1.53	16.70±9.22	10.75±9.29	16.80±9.18	**0.673**
	14	6.27±1.05	5.67±0.58	5.66±0.48	6.01±0.46	5.64±0.58	**0.627**
	19	3.94±0.58	3.93±0.67	4.09±0.24	4.18±0.69	3.83±0.53	**0.711**
Yolk height	7	5.00±1.00	5.33±0.58	4.67±2.52	3.67±2.01	4.33±2.01	**0.859**
	14	2.33±0.58	2.33±0.58	2.33±0.58	2.33±0.58	3.00±1.00	**0.492**
	19	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	
Yolk weight	7	27.41±1.24	24.51±2.05	18.06±9.46	13.02±9.80	18.24±9.57	**0.761**
	14	8.92±2.13	8.43±0.82	7.33±0.94	9.44±1.67	9.86±1.68	**0.157**
	19	5.27±0.88	4.78±0.41	5.71±1.29	5.13±0.96	5.78±1.20	**0.765**
Yolk diameter	7	56.00±2.00	61.33±2.52	50.67±19.86	40.00±21.79	49.00±18.25	**0.789**
	14	24.33±1.53	28.00±6.56	27.67±2.52	32.67±2.31	27.67±3.21	**0.103**
	19	20.67±2.08	20.00±2.00	19.33±0.58	22.33±3.06	19.33±2.52	**0.262**
Embryo weight	7	1.31±0.12	1.25±0.17	1.27±0.10	1.26±0.43	1.32±0.09	**0.692**
	14	11.26±1.06	12.30±0.90	12.30±0.97	11.92±0.38	12.61±0.79	**0.563**
	19	30.63±1.23	29.29±1.15	28.87±0.58	30.03±2.16	29.99±1.43	**0.61**
Embryo length	7	25.67±4.04	27.33±3.06	40.33±29.26	51.33±21.13	41.00±28.69	**0.855**
	14	62.67±2.52	56.00±3.00	67.00±6.25	65.33±5.51	62.00±7.00	**0.634**
	19	85.00±2.00	82.00±2.00	85.33±0.58	87.67±2.08	86.67±3.79	**0.556**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	13.67±1.53	13.33±2.52	13.33±1.15	**0.967**
	19	27.33±1.53	23.67±3.51	27.67±2.08	27.00±2.00	27.00±1.00	**0.87**
Albumen index	7	8.21±1.18	7.88±1.82	7.07±0.66	6.11±2.88	8.30±1.68	**0.426**
	14	5.88±1.79	6.10±1.84	6.78±2.87	6.02±1.79	6.38±2.10	**0.922**
	19	4.97±0.61	6.54±3.45	4.63±0.80	4.93±0.36	4.81±0.57	**0.836**
Yolk index	7	11.48±2.09	11.55±0.80	11.81±2.98	11.39±3.37	11.92±1.94	**0.971**
	14	10.72±1.80	12.39±4.02	12.44±3.60	14.44±2.88	10.55±4.07	**0.455**
	19	10.33±1.56	10.00±1.00	8.64±2.03	11.17±1.53	9.11±2.46	**0.34**

Table 10 shows the combined effects of AFB1 + AFB2 green from all treated groups. However, the value obtained in embryo length of the negative control was higher in comparison with the treatment groups at a sampling day of 14. But 2.5ppb concentration of the injected eggs had a higher value compared to the treated groups. Moreover, significantly higher value was observed in shell weight and yolk weight at dose treatment of 5ppb and 2.5ppb respectively compared to the other treated groups at a duration of 14 days incubation.

Table 10a: Effect of aflatoxins B_1 + aflatoxins B_2 green on external quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Egg Weight	7	59.51±6.94	61.37±4.48	60.66±2.31	61.69±4.93	63.01±1.41	**0.69**
	14	57.00±4.06	60.10±1.96	60.52±0.93	63.76±1.75	63.21±1.43	**0.064**
	19	62.46±2.23	63.23±2.15	61.56±0.99	63.68±1.53	64.81±1.83	**0.095**
Width	7	4.53±0.25	4.27±0.25	4.43±0.21	4.50±0.26	4.43±0.15	**0.908**
	14	4.27±0.12	4.50±0.10	4.47±0.15	4.43±0.25	4.33±0.15	**0.691**
	19	4.50±0.10	4.33±0.12	4.57±0.15	4.50±0.26	4.53±0.06	**0.903**
Length	7	5.37±0.15	5.60±0.20	5.63±0.12	5.57±0.15	5.53±0.15	**0.694**
	14	5.77±0.21^{ab}	5.83±0.23^{a}	5.70±1.00^{ab}	5.30±1.00^{b}	5.53±0.12^{b}	**0.01**
	19	5.67±0.40	5.63±0.15	5.60±0.10	5.53±0.32	5.63±0.19	**0.847**
Egg shape index	7	84.47±4.22	76.14±1.84	78.73±4.41	80.80±3.08	80.11±0.55	**0.724**
	14	74.00±0.88	77.22±3.47	78.40±4.03	83.72±6.33	78.32±2.90	**0.333**
	19	79.71±6.58	76.93±1.44	81.59±4.16	80.92±9.06	80.49±1.26	**0.973**
Shell weight	7	5.07±0.73	5.66±0.22	5.65±0.62	5.77±0.36	5.56±0.36	**0.858**
	14	5.63±0.25^{ab}	5.57±0.48^{b}	5.84±0.56^{ab}	6.46±0.32^{a}	5.43±0.27^{b}	**0.054**
	19	5.40±0.21	5.26±0.13	5.31±0.16	5.50±0.37	5.14±0.28	**0.369**

^{abc}Means in the same rows with different superscripts differ significantly (P<0.05)

Table 10b: Effect of aflatoxins B_1 + aflatoxins B_2 green on internal quality and physiological responses of induced broiler eggs to *Urginea epigea*

		Dosage of Administration					
Parameters	**Day**	**PC**	**NC**	**2.5ppb**	**5ppb**	**10ppb**	**P-value**
Albumen weight	7	4.00±1.00	4.00±1.00	4.00±1.00	4.67±0.58	3.67±0.58	**0.317**
	14	1.67±0.58	1.67±0.57	1.67±0.58	1.67±0.58	1.67±0.58	**1**
	19	1.00±0.00	1.33±0.57	1.00±0.00	1.33±0.58	1.33±0.58	**0.63**
Albumen length	7	47.67±4.51^{c}	50.67±2.52^{ab}	49.00±2.65^{b}	56.67±3.06^{ab}	58.00±4.58^{a}	**0.041**
	14	28.00±2.00	27.00±2.00	25.67±1.53	29.00±1.00	25.67±2.08	**0.068**
	19	20.33±2.52	21.00±2.00	21.33±2.31	19.67±2.08	19.67±3.06	**0.664**
Albumen height	7	23.60±1.57	24.04±1.53	22.33±1.69	22.77±2.29	22.36±0.88	**0.94**
	14	6.27±1.05	5.67±0.58	6.14±0.66	5.67±0.59	5.93±1.12	**0.796**
	19	3.94±0.58	3.93±0.67	3.91±0.61	3.89±0.70	4.66±0.45	**0.278**
Yolk height	7	5.00±1.00	5.33±0.58	5.67±0.58	5.67±1.53	6.00±1.00	**0.914**
	14	2.33±0.58	2.33±0.58	2.67±0.58	2.33±0.58	2.33±0.58	**0.729**
	19	2.00±0.00	2.00±0.00	2.33±0.58	2.33±0.58	2.67±0.58	**0.729**
Yolk weight	7	27.41±1.24	24.51±2.05	23.99±1.53	25.38±0.89	24.76±0.55	**0.346**
	14	8.92±2.13^{b}	8.43±0.82^{b}	10.41±0.65^{a}	6.78±1.31^{c}	9.08±1.21^{ab}	**0.018**
	19	5.27±0.88	4.78±0.41	5.81±0.54	5.16±0.79	4.99±0.84	**0.412**
Yolk diameter	7	56.00±2.00	61.33±2.52	64.66±2.52	63.33±4.50	64.67±2.52	**0.854**
	14	24.33±1.53	28.00±6.56	26.67±6.66	26.33±2.52	27.67±1.53	**0.923**
	19	20.67±2.08	20.00±2.00	21.00±2.65	22.67±1.53	21.00±3.61	**0.703**
Embryo weight	7	1.31±0.12	1.25±0.17	1.19±0.05	1.17±0.13	1.33±0.08	**0.159**
	14	11.26±1.06	12.30±0.90	12.19±0.64	12.49±1.30	11.52±0.43	**0.432**
	19	30.63±1.23	29.29±1.15	31.45±0.63	29.75±0.77	30.27±1.12	**0.121**
Embryo length	7	25.67±4.04	27.33±3.06	26.33±4.73	26.00±1.00	28.67±1.53	**0.516**
	14	62.67±2.52	56.00±3.00	67.33±3.79	64.67±3.51	60.67±2.08	**0.11**
	19	85.00±2.00	82.00±2.00	86.00±3.61	80.67±2.08	83.67±2.52	**0.144**
Tibia length	7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	14	12.67±2.52	12.00±1.00	15.00±2.00	13.67±1.53	12.67±1.15	**0.276**
	19	27.33±1.53	23.67±3.51	25.33±3.21	27.67±2.52	26.33±2.52	**0.611**
Albumen index	7	8.21±1.18	7.88±1.82	8.11±1.63	7.62±0.63	6.86±1.45	**0.536**
	14	5.88±1.79	6.10±1.84	6.52±2.37	5.71±1.85	6.43±2.02	**0.874**
	19	4.97±0.61	6.54±3.45	4.72±0.48	9.14±3.99	4.71±0.48	**0.095**
Yolk index	7	11.48±2.09	11.55±0.80	11.50±1.37	11.97±4.48	11.03±2.28	**0.929**
	14	10.72±1.80	12.39±4.02	10.78±5.43	11.83±3.40	12.39±3.24	**0.892**
	19	10.33±1.56	10.00±1.00	9.44±2.88	10.05±2.20	8.11±2.01	**0.62**

Discussions

Aflatoxins are mycotoxins responsible for the contamination of poultry feed ingredients through the production of secondary metabolites, leading to aflatoxicosis in bird, resulting in poor growth performance and infectious disease (Yin *et al*., 2017). Moreover, the transfer of aflatoxins from hen to eggs raises public health awareness, and the residual effect of the toxin adversely reduce growth and feed efficiency, decreased egg production and distort the development of emerging embryo viability and hatchability, and a resultant effect to disease and organ malfunction (Oznurlu *et al.*, 2012). Therefore, a protective strategy is needed to shield the fertilized egg and developing embryo from aflatoxicosis which is require for the sustainability of the poultry industry (Yin *et al*., 2017).

The results obtained from Tables 3 and 4 revealed that the dose treatment of 10ppb and 2.5ppb exposed to AFB_1 and AFB_2 within an incubation period of 7 and 19 days of embryo weight were ($P<0.05$) significantly affected. This result support the report of Celik *et al.* (2000) and Oznurlu *et al.* (2012) who observed that the development of chicken embryos was adversely affected in the presence of 10 and 100 ng of AFB_1/egg. The deleterious effects of AFB_1 and AFB_2 as documented by (Bahey *et al.*, 2015; Moudgil *et al.*, 2013) confirmed the metabolism of the toxins' transformation into metabolites in the liver. Aflatoxins are not toxic per se but require metabolic conversion by hepatic enzymes (cytochrome P450 family) to the metabolically active metabolite exo AFB1 8, 9 epoxides to exert toxicity. This particular cellular compound binds with proteins, DNA and RNA to influence normal cellular activities, and is considered the active form responsible for carcinogenicity and mutagenicity effects (Gallagher *et al.*, 1994; Oznurlu *et al.*, 2012). Yin., *et al.* (2017) observed increase in embryo weight by 3.3% carvacrol and 17% trans-cinnamaldehyde when compared with egg injected

with 75 ng Aflatoxin with 0.1% CR and TC. This difference could be because of the bioactive compound potential to decrease AF-induced toxicity in chicken embryos. Phytochemicals improvement on the embryo weight inoculated with AFB_1 is suggestive of the protective effect of carvacrol and trans-cinnamaldehyde to AFB_1 by increasing the survivability of the embryos by 55%. However, there is limited information about the detoxification mechanisms of early embryonic cells, Studies have shown that chicken embryos possess the ability of detoxify shortly after the development of the liver by day five and six of incubation (Oznurlu *et al.*, 2012; Stefaniak *et al.*, 2019). The embryo length increased significantly in AFB1+AgZnO-Green synthesised injected eggs at an incubation interval of 19 days, showing the protective capacity of the phytochemicals to mitigate the toxic effect in the liver without an appreciable improvement in embryo growth.

In comparison with the positive and negative control, the significant interacting effect between aflatoxins B1 and aflatoxins B2 on embryo weight show that the exposure of the toxins causes' embryo organ to develop various gross, skeletal, and visceral anomalies with added doses of the toxin during organogenesis. Vuguin, (2007) observed that AFB1 (300 to 400 ug/rat) administered in pregnant rats caused increased prenatal deaths and haemorrhages at the utero placental junction, and fetal growth was retarded. (Woo *et al.*, 2013) reported that aflatoxins (75% AFBI and 25% AFB2) the doses of 7, 3.5, 1.4 and 0.7 mg/kg body weight, injected subcutaneously in rats on 8 to 16 days of pregnancy, did not reveal malformations in the foetus in any treated group, except wrinkled skin, enlarged head and stunted growth observed at higher dose levels. In experimentally induced chronic aflatoxicosis in non-pregnant rabbits, (Wangikar *et al*. 2005) observed anorexia, icterus, lethargy, emaciation, dehydration and death in the rabbits given 0.05 and 0.0625 mg/kg body weight for 14 days and reported the acute oral LD_{50} of AFB1 for rabbits as 0.3 mg/kg body weight. Whereas treatment of pregnant rabbits with 1 and 3 ppm of AFB1 from zero days of pregnancy to one-week post-partum was found

to cause maternal mortality and reabsorptions into the system (Pare *et al*., 2001). Evidence of outbreaks resulting from aflatoxin poisoning in Eastern and Central Provinces of Kenya, from January to July 2004 revealed high levels of aflatoxin (20 to 8,000 ppb) in maize samples collected from patient households (Centers for Disease Control and Prevention, 2004). Moreover, similar levels of aflatoxins were also detected in the animal feedstuffs and cereals tested in the laboratory, thus these levels refer to a comparable dose range in humans and even in poultry birds (Wangikar *et al.,* 2005).

There is a significant interaction effect exists in AFB1+AgZnOGreen systheside nanoparticle compared to AFB1+AgZnO chemical and was observed in the embryo length of broiler eggs. This could be explained based on the effect on the developing embryo exhibited by broiler chicken in response to the administration of AFB1+AgNanoparticles Zinc oxide at the end of embryogenesis which did not affect embryo weight (Pineda *et al.,* 2017). (Łukasiewicz *et al.* 2020) reported an increase in the heart weight and a decreased in the liver weight of embryos at sampling of 19 day.

There is a strong correlation between egg length, egg shape index and egg weight. This implies, the longer the length of the egg, the higher the weight (Abebe, 2015). Egg length has been significantly reported to affect egg weight (Alkan *et al.*, 2015; Shi *et al.*, 2009). The egg shape index is indicative of increased embryo weight and hatched weight (Khalil *et al.*, 2016). (Taskin *et al.,* 2015) reported that egg length and egg shape index are good indicators of egg weight. The injection of phyto-synthesized AFB1+AgZnO green synthesis, AFB1+AFB2+green synthesis and AFB2+AgZnO chemical synthesis induced physiological changes in aflatoxins inoculated broiler eggs on length and egg shape index because of the bioactive element in the phyto-synthesized green and zinc acetate, indicative of improved egg quality (Lidia and Ewa, 2009). However, the administration of the extract at varying level had a significant effect of

aflatoxins administration in the current study such as egg length, egg width and egg shape index as the administration of aflatoxins at all levels altered the egg shape index compared with eggs produced by other treatment of broiler eggs. In contrast the mean value obtained for egg width and egg shape index in the current study differed significantly from the finding of (Attia, 2018) who reported that the incorporation of 1.5 and 3% turmeric powder in the layer diet did not show significant improvement on egg shape index among the groups. (Buğdaycı *et al*. 2018) reported that the addition of essential oil and ginger had no significant impact on the egg shape index.

The shell weight was affected by Aflatoxin B2 at an incubation duration of 19 days when the egg was exposed to 2.5 parts per billion of the toxins. This might be due to the tendency of aflatoxins, affecting egg formation through an impairment of the shell gland to mobilize the shell to the uterus. The result of the current study agrees with the report of (Devegowda & Ravikiran, 2008) who found a linear correlation between reduction in egg quality and aflatoxin level. Whereas, (Zaghini *et al.*, 2005) observed reduced eggshell weight when birds were fed AFB1. The significant improvement in the shell weight of the Silver Zinc Oxide green and the combination of AFB1+AFB2+Green synthesis depict the potential of the phytochemical compound present to mobilize and improve small environment in the uterus (site of calcium deposition) and consequently increase shell weight more than the other treatments and controls. The correlation between Aflatoxin B2, silver zinc oxide green and combination of AFB1+AFB2+green is suggestive of the inherent potential of the bioactive compound to up-regulate calcium absorption in the uterus. The significant effect between the extract and the parameters on the shell weight was not in complete agreement with another previous study (Washburn *et al*., 1985). In conformity with the previous experiment the dietary aflatoxin at 5mg/kg did not have a detrimental effect on shell weight as assessed by deformation, breaking strength, specific gravity, or thickness. (Washburn *et al*., 1985) reported that groups receiving

aflatoxin laid eggs with stronger shells than the controls, which was highly significant for deformation, breaking strength, and thickness. (Manafi, 2011) observed the decreased shell weight due to feeding of AF and ochratoxin or their combinations might be due to the poor calcium and phosphorus absorption and interference in vitamin D_3 metabolism resulting in poor shell quality at higher level of toxins. Likewise, (Denli *et al*., 2009) observed that aflatoxicosis causes poor availability of calcium and phosphorus.

The albumen weight of an egg plays an important role during embryonic development. Apart from the formation of sub-embryonic fluid, albumen proteins are known to flow into the amniotic cavity, the yolk sac and finally the digestive tract of the embryo and are used as a main source of proteins for tissue synthesis (Willems *et al.*, 2014). In the present study, we found a significant increase in albumen weight in eggs injected with 10 parts per billion of silver zinc oxide green synthesis compared with the control (positive and negative). The increase in albumen weight revealed that the phytochemical compounds in the silver zinc oxide green synthesis can stimulate the growth of the epithelial cells and tubular gland cells in the magnum of the reproductive tract to synthesize and secrete substantial amount of albumen compared to the rest of the groups (Saraswati *et al.*, 2013). The albumen (length and height) was affected significantly by silver zinc oxide green synthesis, aflatoxins b1+Silver zinc oxide green synthesis (albumen length and albumen height) and the combination of AFB1+AFB2+Green synthesis. The albumen contained a biologically active substance called lysozyme. The lysozyme is a natural barrier that inhibits the growth and invasion of bacteria and aflatoxins into the yolk (Surai, 2002)). A high concentration of lysozyme in the broiler egg protects the embryo from infection prior to the production of its own immunoglobulins (Lewko & Gornowicz, 2009). Apart from the protective function, it is responsible for the gelatinous structure of albumen and egg freshness (Lewko & Gornowicz, 2009; Sah & Mishra, 2018). However, the significant effect observed in the treatment is suggestive of the protective effects

of phyto-synthesized nanoparticles green to activate and enhanced the activities of lysozyme in the albumen while maintaining the gelatinous structure and freshness. Furthermore, more in-depth investigation should be carried out to explain the protective mechanisms of the Nano particle green synthesis on albumen. The injection of aflatoxins B1 and aflatoxins B2 into eggs did not significantly affect the albumen length and albumen height contradicting the report of (Sawhney *et al.*, 1973) that albumen height was adversely affected when Japanese quails were exposed to AFB1.

Additionally, egg yolk is the main source of energy for the developing embryo, which supplies more than 90% of the total energy requirements of the embryo by oxidation of yolk lipids (Wong & Uni, 2021; Yin *et al.*, 2017). The yolk sac weight is an external embryonic tissue that surrounds the yolk, and absorbs, digests and transports nutrients during incubation of the chicken egg (van der Wagt *et al.*, 2020). Moreover, yolk weight and yolk content are important for supporting the development of the embryo during the entire phase of developmental and formative stage of the embryo (Onbaşılar *et al.*, 2017; Yalcin *et al.*, 2008). In the experiment, we discover an increase of yolk weight in eggs exposed with 10ppbs aflatoxins b1+Silver zinc oxide green synthesis (incubation period of 19 days) and the combination of AFB1+AFB2+Green synthesis at 2.5ppb, which indicate phyto-synthesized green particle treated eggs did not facilitate a better development of the embryo. This significant difference observed in AFB2 and AFB1 indicates a reduced embryos weight development in the aflatoxins treated eggs. From this study, the embryo weight obtained revealed a significant reduction in AFB1 exposed eggs at an incubation of 7 days, thereby supporting the report of Yin *et al.* (2017) who observed a direct relationship between yolk weight and embryo weight where the phytochemicals employed reduced aflatoxins induced toxicity in chicken embryo.

Metal nano particles are believed to be safe, stable and they possess salient properties for applications (Pandurangan and Kim, 2015). In addition, it appears that nano materials hold lot of potential to pass some of the barriers by efficiently targeting cells and molecules in many diseases (Atef *et al.*, 2016). The albumen index measured the quality and freshness of the egg. The significant difference observed in AgZnO Green synthesis and AgZnO chemical synthesis at the first half of incubation period (7 days) indicates antioxidant potential of the nano material to prevent the inoculated embryo eggs from deterioration, by detoxifying free radicals. These factors protect cells from reactive oxygen species damaging effects in aflatoxicosis while retaining freshness (Bhatti *et al.*, 2022).

5 Conclusion

This study can therefore be concluded to evaluate the potential protective *in ovo* effects of phyto-synthesized nano AgZnO on the physiological changes and embryo development in aflatoxins inoculated broiler eggs. The nano material also exhibited an antioxidant potential by reducing the damaging effects of free radicals in the embryo eggs. Moreover, future studies using an *in vivo* chicken model are required to establish the results of the present study by investigating the efficacy of in-feed supplementation of phyto-synthesized Nano AgZnO for reducing the adverse effect of aflatoxins in laying birds.

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