CHANGES OF SOME BLOOD COMPONENTS ASSOCIATED WITH OSTRICH EMBRYONIC DEVELOPMENT DURING INCUBATION

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Abstract: *A total number of 60 ostrich eggs weighing between 1300 to 1500 g were obtained from commercial farm located at Cairo. Eggs were incubated at multi stage incubator. At 20, 30, 34, 36, 38, 39, 40, 41, 42 and 43 days of incubation, 4 embryos were removed from the fertile eggs and examined for some hematological parameters as well as some blood chemistry. Furthermore, calcium and phosphorus content in plasma, leg bones and eggshell of the growing embryos and hatching chicks were determined.*

The results showed that: As the embryos developed, erythrocyte and leukocyte counts, hemoglobin concentration, hematocrite value (PCV), plasma glucose, total protein, albumen, globulin and albumen to globulin ratio (A/G), urea, uric acid, and alkaline and acid phosphates concentration increased gradually with increasing embryo age, all these parameters reached their highest values approximately at 41 or at 42-d of age. However, plasma concentrations of (T3) and (T4) increased with increasing the embryos in age and reached its maximum value at 41 d of incubation, meanwhile, both T3 and T4 concentration in the plasma of the ostrich embryos decreased at pipping and before hatching. Moreover, plasma total lipids, cholesterol and triglyceride concentration increased gradually as the embryo advanced in age and reached their peak at 34, 36 and 38-d-old respectively. On the other hand, eggshell content of (Ca) and (P) decreased gradually as the embryo advanced in age. On the contrary, (Ca) and (P) concentration in the embryos plasma and bones increased with increasing the embryos age. These results establish base line information on ostriches hematological and biochemical parameters. Moreover, any reduction in these base line parameters during the last stage of the ostrich embryonic development may cause high mortality rate.

Keywords: Ostrich Embryo; Hematological Parameters; Biochemical Parameters; Plasma, Bone, Egg Shell Ca and P.

INTRODUCTION

Recently, ostrich farming are considered as an untraditional way for solving the gap in meat production. Ostriches produce high protein red meat with a very low cholesterol levels, and high concentration of iron, phosphorus, potassium, copper and manganese (Sales and Horbanczuk 1998). The leather is proven to be very hard in texture, while the feathers are sold to the fashion industry (Kreibich and Sommer 1995).The hatchability of ostrich eggs is low and varied, ranging from 33% to 80% (Mushi et al 2008; Dzoma and Motshegwa 2009). Embryonic mortality reaches its maximum during the last few days of incubation (Deeming 1995; Brown et al1996).

Moreover, 28 – 50% of the fertile egg fail to hatch (Badley 1997 and Nashat 2005).

The poor understanding of the pattern of embryonic development especially during hatching and the lack of information dealing with the hematological parameters and plasma chemistry values in ostrich embryos acts as limiting factor in the expansion of the ostrich industry worldwide (Deeming and Ayres 1995).

This study was carried out to study some internal physiological parameters that may affect ostrich embryonic development during artificial incubation. In this respect, the hematological and biochemical parameters in the plasma of ostrich embryo's during incubation as well as Ca and P concentrations in Plasma, bones and egg shell were studied to establish reference hematological and plasma chemistry values. Measurement of these physiological parameters on the embryos provides valuable information for evaluating ostrich embryo's health status which reflects on the capability of the embryos to liberate themselves from the eggs and therefore the hatchability may increase.

MATERIALS AND METHODS

The present study was carried out at the Poultry Production Unit, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt. Data were collected and analyzed during the period from August 2006 till October 2009.

Egg Collection and Preparation:

A total number of 60 ostrich eggs weighed between 1300 to 1500 g were collected daily as soon as possible after laying and cleaned immediately with a dry clean tissue paper, disinfectant solution sprayed on the surface of each egg and the shell was wiped dry with a clean toilet paper (Deeming 1997). Eggs were stored for up to 7 days in a clean storage room at 18 °C and 69 % relative humidity as recommended by Gonzalez et al (1999). Eggs collected per week were left over night at room temperature before introducing to the incubator.

Egg Incubation:

Eggs were incubated in multi stage incubator. Each egg was numbered using permanent marker and weighed using digital electronic balance (accuracy two decimal points). During incubation, eggs were set in metal – framed egg trays in a vertical position at 36.5 °C and 25 % relative humidity (RH) and were turned every 1 hour through 45° up to 39 days. During incubation period, eggs were candled at weekly intervals. After two weeks incubation (second candling), infertile eggs were excluded. On day 39 after incubation, the fertile eggs were transferred to the Hatcher where the temperature and the humidity were adjusted to36 °C and 40 % RH up to hatching time.

Egg and Embryo Sampling:

Four fertile eggs were taken randomly after 20 days of incubation for embryo's blood sampling and eggshell study. At 30, 34, 36, 38, 39, 40, 41and 42 days of incubation, four fertile eggs were taken randomly for blood, bone and eggshell sampling respectively. Sacrificed embryos were separated carefully from all surrounding egg contents as described by Peebles et al (1998) and stored frozen at -20^0 C.

Blood and plasma parameters:

1- Blood sampling technique:

Till 40 days of incubation blood samples were collected from the umbilical vein of the embryo using clean dry needle 21 G. After 41 days of incubation, blood samples were collected by slaughtering embryos or hatched chicks. Blood samples were collected into heparinzed test tubes and each blood sample was divided into two test tubes: one to determine the blood hematology and the other was centrifuged immediately at the speed of 4000 r.p.m. for

5 minutes; plasma samples were kept in plastic tubes and stored at -20^0 C until biochemical analysis.

2- Hematological parameters:

Red blood cells (RBCs) and white blood cells (WBCs) counts were determined according to Natt and Herrick (1952). Hemoglobin concentration (Hb) % and packed cell volume (PCV) were determined according to Dacie and Lewis (1991).

3-Plasma biochemical parameters:

Plasma Total protein was measured calorimetrically according to Henry (1964). Plasma albumin was determined calorimetrically by the method described by Doumas et al (1971). The concentration of globulins in each sample was obtained by subtracting the albumin values from the total protein concentration from which the albumin / globulin ratio (A/G) was obtained. Plasma urea was determined according to Patton and Crouch (1977). Plasma Uric Acid was determined according to Caraway (1955). Determination of Plasma cholesterol was carried out according to the method of Watson (1960). Plasma total lipids were determined according to Lxingth et al (1972). Plasma triglycerides were determined using chemical commercial kits as described by the manufacture companies (spectrum, Diagnostics, Egypt, Co. for Biotechnology, S.A.E.). Alkaline phosphates were determined calorimetrically according to Belfield and Goldberg (1971). Acid Phosphates was measured calorimetrically according to Kind and King (1954). Total thyroxin (T4) and triiodothyronine (T3) were measured in plasma by radioimmunoassay Kits (Coat-A-Count. PC Diagnostic Products Corporation Los Angles, CA90045). Plasma inorganic phosphorus and calcium were determined calorimetrically according to El-Merzabani et al (1977) and Gindler and King (1972), respectively.

Bone sampling and Ca and P measurements:

Femur, Tibiotarsus and Tarsometatarsus bones of developing embryos were separated fromflesh, cleaned from all adhering tissues, rinsed with distilled water and dried over night at 100 ^{0}C. A constant portion from each bone was grinded using steel mixture then 1 g was subjected to wet ashing according to a method described by Charles et al (1984). A calorimetric technique of El-Merzabani et al (1977) and Gindler and King (1972) were used to determine Ca and P content in the ash respectively.

Eggshell parameters:

The eggshells of each egg were collected immediately after removing the embryo , cleaned of adhering shell membrane and washed with distilled water to remove all albumen and dried overnight at 60 ° C then kept dry in dissector.

A portion of each eggshell was grinded using steel mixture then 1 g was subjected to wet ashing according to method described by Charles et al (1984). Eggshell phosphorus and calcium were determined calorimetrically in the wet ash according to the methods of El-Merzabani et al (1977) and Gindler and King (1972), respectively.

Statistical analysis: .

Data were analyzed using statistical package system software (SPSS, Version 10). Duncan (1955), multiple range and multiple F tests was used to test the effect of age on different parameters.

RESULTS AND DISCUSSION

Embryonic Hematology:

The effect of ostrich embryo's age on hematological parameters is presented in Table 1. Erythrocyte numbers increased gradually with age. The erythrocyte count reaches its maximum value (3.15 million

per mm^3) at the 42- d-old embryos. This value is significantly higher ($P \leq 0.05$) than that at 20 days (1.37 million per mm^3). WBCs counts increased gradually from week to week during embryonic development but the increment was not significant. Starting from the 40^{th} - d till the 42^{nd} day of incubation the increment in WBCs counts were significantly higher ($P \leq 0.05$) than that at any embryonic age. Hemoglobin content in the embryo's blood increased significantly from day 20 of incubation (3.86 g/dl) to day 30 of incubation (6.0 g/dl) and from day 30 to day 34 of incubation (6.9 g/dl), hemoglobin concentration at 36, 38 and 39 days of incubation increased slightly with no significant differences between these days, starting from the 40^{th} day until the 42^{nd} day of incubation there were a significant differences ($P \leq 0.05$) between these days in the Hb concentrations, the Hb concentration reached it's maximum level (9.86 g/dl) in the embryos blood at the 42 day of incubation. Meanwhile, as the Hematocrite value (PCV) reflects the status of RBCs counts and Hb content of the blood, it logically increased with advanced in age to reach its highest level (51.00 %) also in the 42- d-old embryos.

The significant increase in erythrocyte count, Leukocytes count, Hb concentration and PCV value of ostrich embryo with age advance is in agreement with Romanoff (1960) who reported that chicken embryonic RBCs, Hb and PCV increased several folds during the incubation period and the greatest increase takes place during the latter stage of incubation to reach values comparable to adults.

The primary physiological function of the red blood cells (RBCs) is to carry hemoglobin which sequentially carries oxygen to tissues. The increase in RBCs and Hb in the blood during embryonic growth might have been initiated by the increase of erythropoietin from kidney and this resulted in increased rate of erythropoiesis Baumann and Dragon (2005).

Khorrami et al (2008) reported that O_2 transport to the embryo's metabolically active tissues depends upon the number of red blood cells and the concentration and character of the avian embryonic hemoglobin. Dragon and Baumann, 2003; Maina, 2004 reported that changes in hematocrite and hemoglobin concentration and thus changes in blood O_2 transporting capacity are inducible well before hatching. Chan and Burggren, 2005 reported that erythropoiesis assists the chicken embryo in maintaining normal levels of blood O_2 transport. Avian embryos exhibit relatively complex chemoreceptor reflexes by at least the last third of embryonic development period (Khandoker et al 2003), and arterial hypoxia may additionally stimulate cardiac output, enhanced erythropoiesis raising hematocrite and redistribution of blood flow within the tissues. Hematocrit has the potential to affect developing embryos. Regulation of hematocrit is an important component in maintaining blood oxygen homeostasis. (Khorrami 2004) reported that hematocrite was significantly affected by developmental stage of chicken embryos and increased significantly from day 15 to day 17. hematocrite value increases progressively with development in chicken embryos (Khorrami 2004). At the beginning of development, the single cell consumes oxygen to generate the energy required to divide, reorganize, and differentiate. As the embryo continues to grow and mature, its metabolic rate increases to support a larger mass, continued growth, functioning organ systems, and movements (Black 2003). As the embryo approaches' hatching its oxygen requirement exceeds the rate of oxygen diffusion across the shell, (Reeves 1984). During the first 80% of incubation the physiology of the cardiovascular and respiratory systems is sufficient to support the metabolic requirements of the growing

embryo (Tazawa et al 1988). There is little change in the oxygen consumption until internal pipping and the beginning of pulmonary respiration at 90% of incubation, when convective respiration and direct access to air increase the amount of oxygen available to the embryo (Rahn et al 1974). Following external pipping, chorioallantois membrane (CAM) circulation is reduced and the circulatory pathways to the lungs open to prepare the embryo for hatching (Tazawa and Takenaka, 1985).

Blood Chemistry of ostrich embryos during incubation:

1 -Plasma Glucose Concentration:

Plasma glucose concentrations of ostrich embryos during incubation are illustrated in Table (2). Examination of the data clearly showed that plasma glucose level increased significantly between 20, 30, 34 and 36 days of incubation followed by insignificant differences in plasma glucose levels between 36, 38and 39 days of incubation. No significant differences in plasma glucose levels were observed between days 40 and 41. However, at 42 days of the embryonic development plasma glucose increased significantly ($P \leq 0.05$) than that in days 40 and 41 respectively. The data clearly showed that plasma glucose levels increased significantly during early and late embryonic development. Meanwhile, it can be observed that plasma glucose level at hatch - 42-d old - (15.2 mmol/l) reach approximately 7 folds than that at 20-d old (2.15 mmol/l).

The observed patterns of plasma glucose during ostrich embryonic development is in agreement with Suvarna et al (2004), who reported that plasma glucose levels increased significantly with increasing turkey embryos age. In this respect, Lu et al (2007) reported a significant increase in plasma glucose levels with increasing age of Cobb 500 chick embryos from 10 d to hatch. However, Lu et al (2004) reported a significant increase in plasma glucose from 13 d to 18 d suggesting that glucose may act as an important regulator to protein anabolism in the chick embryos via suppressing amino acid oxidation.

Bhattacharyya et al (2009) reported that maintenance of glucose homeostasis during few days pre and post hatch is a great challenge in a chick life. Moreover, Suvarna et al (2004) demonstrated that the increased plasma glucose during late embryonic development may result from the increased power of glucagon, causing glycogenolysis.

2-Plasma total lipids, cholesterol and triglyceride concentration:

Plasma total lipid, triglyceride and cholesterol concentration in ostrich embryos during incubation are presented in Table (2). Statistical analysis indicated that the mean plasma total lipids concentration increased gradually as the embryo advanced in age, and reached a peak at 34, 36-d-old (1255, 1143.9 mg/dl) respectively. Also, Plasma cholesterol levels continued to increase significantly with age and reached a peak at 36, 38- d-old (309.7, 323.7 mg/dl) respectively. Plasma triglyceride concentration followed the same pattern, continued to increase significantly with age and reached a peak at 38-d-old (340 mg/dl).

Roux et al (2000) reported that cholesterol is considered an essential agent for embryonic development. Deficiency in cholesterol during embryogenesis and organogenesis cause severe abnormalities. Cholesterol not only plays a role in the physical structure of the membrane (eg, its viscosity and interference with phospholipids), but also in the synthesis of a number of steroid hormones.

The significantly higher plasma total lipids, triglyceride and cholesterol concentration of ostrich embryo during incubation with progress of age are possibly

du to the fact that ostriches use nutritional reserves from the yolk sac, which is rich in this metabolites Miranda et al (2008). Bouda et al (2004) attributed the significant increase of plasma total lipids, triglyceride and cholesterol fivefold higher in 4-day-old ostriches than in older birds to the high reserves contained in the yolk sac. Stokes et al (1952) showed that the conversion rate of acetate to cholesterol is greatest towards the end of the 10^{th} day of incubation; it is observed that the percentage of activity in the embryo increases with age. The highest concentration of total lipid occurred in the plasma of chick embryo from the 10^{th} to the 18^{th} day of incubation. Also, Wood (I972) showed an increase of embryonic and mature brain, heart and in total lipids from the 10^{th} day to hatching (21^{st} day). While triglyceride levels increased dramatically after the 16^{th} day. Peres et al (1999) reported that plasma triacylglyceride concentrations and liver glycogen content may be related to the mechanism of glucose regulation.

3-Plasma alkaline phosphates and acid phosphates Concentration:

Statistical analysis of the data in Table (2) indicated that the mean plasma alkaline phosphates concentration increased gradually as the embryo advanced in age, the greatest value (140.9IU/L) was recorded at hatch (42- d-old). Plasma acid phosphates concentration reached a peak at 36- d-old embryos (13.36U/L) and sharply declined thereafter to reach its minimum value (5.9 U/L) at hatch. Alkaline (ALP) and acid phosphates are enzymes widely distributed in various organs (liver and bone) of the developing chick embryo, they are associated with osteoblastic processes (Costa et al 1993).In addition; alkaline phosphates activity is also an indicator of the metabolic activity of the minerals associated with skeletal ossification (Sigler, 1995; Holle and Benson, 2001). Increased alkaline phosphates activity is an indication of bone repair or remodeling (Hopkins, 1995). Bonucci et al. (2001) proposed a functional correlation between the acid phosphates activity of osteocytes and the calcium levels in the body fluid. Nakano et al (2004) reported that enzymatic activity of acid phosphates has been regarded as one of the reliable markers for osteoclasts and their precursors. The significant increase in plasma alkaline phosphates and acid phosphates concentration in ostrich embryo plasma with age advance agrees with Kubota et al (1981) who reported a significant increase in plasma alkaline phosphates and acid phosphates activities of broiler tibiae during embryonic development which represent osteoblastic activity. Moreover, plasma alkaline phosphates and acid phosphates activities began to increase at days 10-12, reached maximal values at day 19 and sharply declined thereafter. Both bone enzyme activities were highly correlated with Ca2+-binding activity in the chorioallantoic membrane. The reason that embryonic bone formation has to be completed by day 19, therefore, appears to be closely related to chorioallantoic function. Also the sharp decrease after day 19 of the bone alkaline phosphates activities, which reflect osteoblastic activity, indicates the completion of the embryonic bone formation by day 19. Bone resorption also does not seem to occur, since bone acid phosphates activity is very low throughout embryonic life. With regard to ALP activity values determined in the present research work, it can be concluded that this activity corresponds to the intensity of the ossification process and skeletal development during the embryonic development. Miranda et al (2008) attributed the higher concentrations of alkaline phosphatase to the release of large amounts of bone isoenzymes during the intense process of bone formation (osteogenesis) and skeletal development of the birds in the embryonic period.

4- Plasma total protein, Albumin, Globulin and A/g ratio:

Plasma total protein, albumin, globulin and albumin to globulin ratio (A/G) of ostrich embryos during incubation are presented in Table (2). The data showed that the mean plasma total protein, albumen, globulin and albumin to globulin ratio (A/G) increased gradually as the embryos advanced in age; the highest values were recorded at hatching time (42-d-old embryos).

The significant increase in plasma globulin of ostrich embryos especially during the last days before hatching seems to be associated with antibody formation. Kwak et al (1999) indicated that plasma globulin portion mainly involved in antibody formation as well as cell membrane activities and cell division and cell proliferation. However, Soliman (2000) showed that antibodies are globulin in nature and are involved in immune reactivity (immunoglobulins, Igs).

Serum albumin is the most plentiful and the most familiar plasma protein, albumin has been assigned numerous physiological roles (Peters 1975). It is the principle agent responsible for the osmotic pressure of the blood, for transport of fatty acids, for sequestration and transport of bilirubin and conveyance of tryptophan, cystine and various hormones including thyroxin and steroids. Serum albumin also has a nutritive role as a reservoir of amino acids for peripheral tissues. Serum albumins are also reflected by total proteins levels. Albumin in fact represents a large part of total proteins and its trend follows that of total proteins.

It can be concluded that the significant increase in plasma concentrations of total protein, albumin and globulins of ostrich embryo with increasing the embryonic age is associated with the rapid increase in the embryos weight and this increment in plasma total proteins, albumin and globulin may be due to increased metabolic rates to meet rapid tissue formation, rapid growth, the increase in muscle mass, and as a consequence of high protein demands (Palomeque et al 1991). Moreover, Quintavalla et al (2001) reported that total proteins play an important role in transport of vitamins, hormones, enzymes and electrolytes.

5- Urea and Uric acid Concentration:

Uric acid is one of the most important end products of nitrogen metabolism in birds (Lumeij 1997). Blood nitrogen (urea), has been shown to be an indicator of protein metabolism and kidney function (Ubaldi et al 1982).

Plasma urea and uric acid concentration of ostrich embryos during incubation are presented in Table (3). Data indicated that the mean of plasma urea and uric acid concentration increased gradually as the embryos advanced in age, plasma urea and uric acid concentrations reached its maximum level at 42- d-of incubation.

The significant increase in Plasma urea and uric acid concentration of ostrich's embryo during incubation may be due to high protein metabolism. Miranda et al (2008) attributed the higher values of plasma urea to the large protein reserves contained in the yolk sac. The higher uric acid values indicate high purine metabolism and high recycling of nucleic acids, since birds excrete uric acid via urine. The concentration of uric acid in blood varies directly according to the demand of amino acids for protein synthesis (Costa et al 1993). Fahmy (2008) observed dilated urethra field with urates in the dead ostrich embryos.

6- Thyroxin (T4) and Tri-iodothyronine (T3) Concentration:

Thyroid hormones triiodothyronine (T3) and thyroxin (T4) are involved in numerous physiological processes in mammals and birds (McNabb 2000). In

addition, thyroid hormones regulate heat production during the incubation of chick eggs (McNabb 2000). McNabb et al 1993 reported that the thyroid determines the characteristic stage of development and it's hormones play major roles in tissue differentiation and in the final maturation of many tissues just prior to hatching. Dawson et al (1994) showed a significant correlation between thyroxin and body weight. Moreover, King and May (1984) reported that thyroid hormones are involved in ossification. Gado (1973) indicated that thyroid hormones are essential for normal growth in fowl and the overall body growth, maturation and growth of specific tissue being affected. Also, thyroid gland plays a major role in the regulation of growth in birds and thyroid hormones play a major role in regulating the oxidative metabolism in birds (Peebles and Marks 1991).

Plasma concentrations of triiodothyronine (T3) and tetraiodothyroxine (T4) of ostrich embryo during incubation are presented in Table (2).

Statistical analysis indicated that the mean plasma concentrations of triiodothyronine (T3) significantly increased with age and reached a peak at 40 and 41d-of embryo age (450, 650 pg/mL) respectively, then declined to 333 pg/mL at pre pipping day(42). Meanwhile, plasma concentrations of thyroxin (T4) increased significantly with increasing embryo ages and reached a peak at the 40d of the embryo age (5.43ng / ml). Moreover, both T3 and T4 concentration in the plasma ostrich embryo decreased at pipping and before hatching Table (2). The previous results are in contrast with Christensen et al (2002) who found that the T3 concentration were elevated at external pipping, and in agreement with Suvarna et al (2004) who reported a significant increase in plasma concentrations of T3 and T4 as the turkey poults embryos aged. Moreover he observed that T3 and T4 increased dramatically at the 27 days of embryo development then declined at pre pipping and before hatching. Christensen and Davis (2004) reported that the embryo survival rates improved during the latter times of embryo development when embryonic thyroid hormone concentrations increased. Lu et al (2007) reported a significant increase in plasma triiodothyronine and thyroxin levels during late chick embryo development. Plasma triiodothyronine increased 4-fold from 18d to 20d, and thyroxin increased 3-fold from 16d to 19d. Also, Lu et al (2004) showed a significant increase in plasma T3 and T4 levels during the third week of incubation and reached a peak at 19-20 d. Also our results are consistent with McNabb (2000) and Reyns et al (2003), they observed a sharp rise in T3 activities at the developmental stage when the embryo switches to lung respiration, this period is a critical time for the chick embryos to survive because they need more oxygen and energy for hatching.

The ability to use glycogen reserves respectively in liver and pectoral muscle during the last 3 days of incubation has been positively correlated to thyroid hormone levels (De Oliveira 2007). Moreover, Christensen et al (2003) observed that embryos and hatchlings with low levels of T3 were not able to utilize their energy reserves even when liver and muscle glycogen was plentiful. Bellabarba et al (1988) reported that the increase in plasma T4 may be related to elevation in thyroid hormone receptors in the liver and brain during mid-stage chick embryogenesis. The significant elevation in plasma T4 levels before hatch at this study is in agreement with previous results reported by McNabb (2000) and Reyns et al (2003), moreover they concluded that the significant increment in thyroxin levels during this stage is very important for stimulating a variety of developmental, physiological and metabolic processes necessary for successful hatching. Thyroid

hormones, both T4 and T3, showed significant positive correlations with chick embryonic BW, suggesting that thyroid hormones appear to be critically important in maintaining normal growth and development during chick embryogenesis.

Depending on the previous results, it can be concluded that the observed significant increase in the thyroid hormones during embryonic development in this study is logic since they are necessary for most body functions. They directly affect a number of physiological processes and are required for the permissive actions of other hormones; moreover, thyroid hormones are very important for embryo growth and stimulating a variety of developmental, physiological and metabolic processes necessary for successful hatching.

7- Calcium (Ca) and phosphorus (P) content in ostriches egg shell, embryos plasma and femur, tibiotarsus and tarsometatarsus bones:

7-1- Eggshell (Ca) and (P) concentration.

Eggshell calcium (Ca) and phosphorus (P) content of ostrich eggs during incubation are shown in Table (3). The eggshell content of calcium (Ca) and (P) decreased gradually as the embryo advanced in age. It reached its minimum (15.42 mg/dl) and (0.48 mg/dl), respectively at hatching day (42d).

The egg-shell consists of several mutual layers of calcium carbonate (CaCO3). The major elements in the eggshell are calcium, magnesium, sodium, and carbon. The elemental composition of eggshell has been reported to be about 98.2% calcium, 0.9% magnesium and 0.9% phosphorus (Powrie 1972; Kermanshah and Hadavi 2006; Vetter and Grady 2005). Approximately 80% of the calcium requirements of the chick by the time of hatch are derived from the eggshell (Simkiss 1961; Richards and Packard 1996; Packard and Packard 1991). Most of the calcium in the chicken egg (1526 mg in this example egg of 58 g) is in the shell, where is available to the embryo after it is mobilized (Richards and Packard 1996). Abdel-Salam et al (2006) reported that calcium is the most important constituent of the eggshell. Phosphorus (P) stores are more problematic given that the shell contains very minimal amounts of P, and thus the only amounts available for all functions, including phospholipid synthesis, bone growth and mineralization, are found primarily in the yolk (Richards and Packard 1996).

The observed lower Ca content in the eggshell of hatched eggs in this study is in agreement with many literatures which are dealing with this research point, they attributed the reduction in the Ca content of the shell to Ca withdraw from the eggshell for embryo bone formation during incubation. In this respect, Saleh (1988) attributed the thin shell of hatching eggs to the more consumption of Ca by the live embryos than the dead in shell ones. Also, Davis and Christensen (1995) showed that pores became wider during ostrich egg incubation which may be due to a reduction in the shell thickness. Furthermore, Tuan and Zrike (1978) reported that after the early period of embryonic growth, the primary source of Ca (over 80%) was mobilized via shell absorption (dissolution of the shell) by the chorioallantoic circulation by mechanisms similar to bone resorption and the shell was the only sources of Ca in late incubation. Akins and Tuan (1993); Elaroussi et al (1994); and Narbaitz et al 1981 reported that chicken eggshell supplies approximately 80% of the calcium found in the hatchling chick. This process requires the active transport of calcium across the ectodermal cell layer of the extra embryonic chorioallantoic membrane (Terepka et al 1969).

7-2- Plasma (Ca) and (P) concentration of ostrich embryos during incubation:

Plasma (Ca) and (P) concentration of ostrich embryos during incubation are presented in Table (3). Embryos plasma (Ca) and (P) concentration increased gradually as the embryo advanced in age, the increase reached its maximum (6.75 and 5.02 mg/dl) at hatching day (42d). This finding is in agreement with Elaroussi and Elbarkouky (2003) who reported that plasma (Ca) and (P) concentration of Japanese quail embryos increased gradually as the embryo advanced in age, with the greatest increase at hatching day. Simpraga et al (2004) attributed the high phosphorus content in the embryos serum to the fact that during embryonic development, the energetic metabolism is very active, and phosphorus, as a component of energy-rich compounds (creatine phosphate and adenosin triphosphate) plays a very important role.

Christensen and Biellier (1982) stated that increased plasma Ca in embryos at the time of pipping and hatching increased physiological muscular mechanism and muscular activity of the embryo to break and free itself from the shell. Grabowski (1966) and Christensen and Eden (1985) found that increasing plasma Ca in turkey eggs at 25 days of incubation improved hatchability. Christensen and Biellier (1982) reported that increased plasma Ca may play a role in increasing muscular activity and ionic Ca stimulates muscular contraction.

7-3- Calcium (Ca) and phosphorus (P) concentration of Femur, Tibiotarsus and Tarsometatarsus bones of Ostrich embryos

Calcium and phosphorous are essential macro minerals, they play an important role in the normal bone system development of embryos (Julian 2005; Almeida et al 2006). Phosphorus may affect more biological systems than any other element. It is an important element in many body functions including bone formation, phospholipid synthesis, acid-base balance and metabolism of fat, proteins, carbohydrates and lipids (Celebi et al 2005).

The bone (Ca) and (P) concentration of ostrich embryo during incubation are presented in Table (3) .Calcium and phosphorus content increased significantly ($P<0.05$) as the embryo grows up, a much higher (Ca) and (P) content at hatching day (42d) were observed . The previous results, is in agreement with Elaroussi and Elbarkouky (2003) who found that bone (Ca) and (P) concentration of Japanese quail increased gradually as the embryo advanced in age, with a maximal concentrations at hatching day. The significant observed increase in bone (Ca) and (P) of the ostriches embryo in this study is mainly due to the rapid calcification of the skeleton that take place during the last week of incubation.

During embryonic skeletal calcification in the chick embryo, the chorioallantoic membrane (through its circulatory system) is responsible for mobilizing over 100 mg of calcium from the egg shell into the embryonic circulation (Romanoff 1967). The chorioallantoic membrane lines the interior of the egg shell and beings to transport calcium at days 10-12 of embryonic life until hatching at day 21 (Tuan and Scott 1977). Crooks et al (1976) demonstrate that the Ca2+-transport function of the chorioallantoic membrane is highly developmentally regulated, after 12 to14 days of incubation, and continuing to increase steadily until hatching. The ossification of the skeleton of chicken embryos occurs mainly in the later half of incubation and is well advanced at the time of hatching (Simkiss 1962). Kubota et al (1981) on chickens, reported that the fresh weight and calcium content of embryonic tibiae during incubation began to increase at day 12 and attained maximal values at day 19 (2 days before hatching) when the

bone calcium content almost reached a maximum. The maximal value of Ca^{++} binding activity of the chorioallantoic membrane was about 10 times higher than the basal value at day 12.

The above mentioned results of the hematological and biochemical parameters of the ostriches embryos in this study, establish base line information on the normal hematological and biochemical values which are important for nutritional studies, metabolic disorders, monitoring health status and for appropriate early diagnosis and treatment of diseases. Moreover, it was reported that most of the embryonic mortality of the ostrich embryos occurred in the final stage of embryogenesis (Deeming 1995; Brown et al1996 and Wiercińska and Szczerbińska 2005). This final stage of the embryonic development is associated with the increase in most of the biochemical and hematological parameters in the embryos blood. So, any reduction in these base line parameters during the final stage of incubation will reflect on the embryos health and thereafter on there capability to hatch.

Table (1): Effect of ostrich embryos age on blood Hematology (Mean ± S.E).

Parameter	Embryo Age(day)								
	20	30	34	36	38	39	40	41	42
RBCs×10^6	1.37± 0.05 ^{e}	2.40± 0.03 ^{d}	2.43± 0.04 ^{d}	2.52± 0.03 ^{cd}	2.52± 0.04 ^{cd}	2.69± 0.07 ^{bc}	2.74± 0.07 ^{b}	3.10± 0.05 ^{a}	3.15± 0.13 ^{a}
WBCs×1000	4.33± 0.67 ^{d}	4.66± 0.33 ^{cd}	4.66± 0.33 ^{cd}	5.00± 0.58 ^{cd}	5.33± 0.67 ^{cd}	5.33± 0.88 ^{cd}	6.66± 0.33 ^{bc}	8.00± 0.58 ^{ab}	9.33± 0.88 ^{a}
Hb (g/dl)	3.86± 0.09 ^{g}	6.00± 0.15 ^{f}	6.93± 0.18 ^{e}	7.70± 0.25 ^{d}	8.00± 0.21 ^{d}	8.13± 0.15 ^{d}	8.76± 0.15 ^{c}	9.30± 0.10 ^{b}	9.86± 0.09 ^{a}
PCV%	25.00± 1.15 ^{g}	34.00± 0.58 ^{f}	38.66± 0.67 ^{e}	41.30± 0.67 ^{d}	43.00± 0.58 ^{cd}	44.00± 1.15 ^{c}	45.00± 0.00 ^{c}	47.33± 0.33 ^{b}	51.00± 0.58 ^{a}

Means in the same row with different superscripts are significantly different (P<0.05).

Table (2): Effect of ostrich embryos age on blood chemistry (Mean ±S.E).

Parameter	Embryo Age(day)								
	20	30	34	36	38	39	40	41	42
Glucose (mmol/l)	2.15± 0.13^{f}	6.58± 0.16^{e}	7.91± 0.54^{d}	10.7± 0.44^{c}	10.7± 0.15^{c}	10.9± 0.11^{c}	13.3± 0.19^{b}	14± 0.48^{b}	15.2± 0.32^{a}
T. Lipids (mg/dl)	415.7± 13.94^{f}	644.9± 18.18^{cd}	1255± 16.75^{a}	1143.9± 71.38^{ab}	1101.5± 53.12^{b}	760.9± 44.95^{c}	584.3± 20.91^{de}	572.3± 50.12^{de}	500.5± 37.65^{ef}
Triglycerides (mg/dl)	100± 6.83^{f}	133± 2.00^{e}	163± 1.92^{d}	238.3± 13.59^{b}	340± 20.81^{a}	195.5± 2.44^{c}	178.3± 4.17^{d}	75.3± 3.53^{f}	43± 3.51^{g}
Cholesterol (mg/dl)	162.8± 13.2^{e}	232.9± 8.54^{d}	295.9± 9.9^{ab}	309.7± 15.39^{a}	323.7± 7.05^{a}	290.3± 3.18^{abc}	258.4± 1.11^{cd}	240.2± 20.95^{d}	233± 8.5^{d}
Alk.Phospha. (IU/L)	31.53± 3.34^{f}	55.7± 2.54^{e}	72.53± 2.91^{d}	74.2± 3.12^{d}	88.7± 4.26^{c}	99.5± 1.06^{bc}	104.76± 3.35^{b}	107.9± 6.94^{b}	140.9± 7.78^{a}
Acid phosphatase (U/L) Total activity	5.98± 0.15^{d}	8.83± 0.32^{bc}	10.1± 0.34^{b}	13.36± 0.69^{a}	12.8± 1.18^{a}	7.76± 0.15^{c}	5.67± 0.23^{d}	6± 0.17^{d}	5.9± 0.35^{d}
T. Protein (g/dl)	0.79± 0.08^{f}	1.01± 0.01^{e}	1.04± 0.1^{de}	1.17± 0.01^{cde}	1.21± 0.01^{cd}	1.26± 0.02^{c}	1.5± 0.08^{b}	1.59± 0.03^{b}	1.9± 0.06^{a}
Albumin (g/dl)	0.29± 0.03^{e}	0.41± 0.03^{d}	0.5± 0.04^{cd}	0.51± 0.02^{c}	0.51± 0.01^{c}	0.56± 0.02^{c}	0.66± 0.05^{b}	0.7± 0.03^{b}	0.83± 0.01^{a}
Globulin (g/dl)	0.5± 0.06^{e}	0.6± 0.02^{cde}	0.54± 0.08^{de}	0.66± 0.03^{cd}	0.7± 0.01^{c}	0.7± 0.01^{c}	0.84± 0.04^{b}	0.9± 0.01^{b}	1.05± 0.07^{a}
A/G ratio	0.6± 0.1^{b}	0.67± 0.07^{b}	0.95± 0.15^{a}	0.77± 0.06^{ab}	0.72± 0.01^{ab}	0.8± 0.03^{ab}	0.78± 0.02^{ab}	0.78± 0.03^{ab}	0.8± 0.05^{ab}
Urea (mg/dl)	4.62± 0.07^{e}	6.00± 0.37^{d}	9.04± 0.54^{c}	9.78± 0.50^{bc}	10.9± 0.05^{b}	10.9± 0.05^{b}	10.91± 0.05^{b}	13.01± 0.41^{a}	13.86± 0.78^{a}
Uric (mg/dl)	4.11± 0.15^{e}	4.8± 0.19^{de}	5.57± 0.07^{d}	5.57± 0.07^{d}	7.45± 0.38^{c}	8.23± 0.15^{bc}	8.59± 0.29^{b}	9.9± 0.29^{a}	10.6± 0.22^{a}
T3 (pg/ml)	138.3± 1.68^{g}	186.7± 3.35^{f}	205.7± .66^{f}	213.3± 1.68^{ef}	255± 2.89^{de}	273.3± 1.68^{d}	450± 28.9^{b}	650± 28.9^{a}	333.3± 16.7^{c}
T4 (ng/ml)	0.35± 0.03^{h}	1.733± 0.14^{g}	2.43± 0.07^{f}	2.77± 0.07^{e}	5.07± 0.07^{b}	5.13± 0.07^{b}	5.43± 0.07^{a}	4.07± 0.07^{c}	3.2± 0.17^{d}

Means in the same row with different superscripts are significantly different(P<0.05).

Table (3): Effect of ostrich embryos age on calcium and phosphorus concentration (mg/dl) in plasma, bone and eggshell (Mean ± S.E).

	Criteria	Embryo Age(day)									
		0	20	30	34	36	38	39	40	41	42
Plasma	Calcium (mg/dl)		1.69± 0.06^{h}	2.2± 0.10^{gh}	2.51± 0.05^{fg}	3.06± 0.17^{ef}	3.57± 0.08^{de}	4.12± 0.06^{cd}	4.66± 0.09^{bc}	5.11± 0.14^{b}	6.75± 0.54^{a}
	Phosphorus (mg/dl)		1.44± 0.04^{f}	1.8± 0.10^{e}	1.87± 0.04^{e}	2.5± 0.13^{d}	3.09± 0.09^{c}	3.23± 0.06^{c}	3.42± 0.06^{c}	4.01± 0.05^{b}	5.02± 0.28^{a}
Bone	Bone Calcium (mg/dl)			4.93± 0.12^{g}	6.0± 0.06^{f}	6.93± 0.06^{e}	7.64± 0.31^{d}	9.1± 0.05^{c}	9.4± 0.03^{bc}	9.85± 0.16^{b}	10.72± 0.29^{a}
	Bone Phosphorus (mg/dl)			3.94± 0.03^{g}	4.75± 0.09^{f}	5.043± 0.04^{e}	5.343± 0.08^{d}	5.56± 0.02^{c}	5.72± 0.07^{c}	6.1 ± 0.03^{b}	6.57± 0.14^{a}
Eggshell	Calcium Eggshell (mg/dl)	16.95± 0.22^{a}	16.46± 0.03^{b}	16.28± 0.02^{bc}	16.22± 0.02^{c}	15.97± 0.01^{d}	15.92± 0.02^{d}	15.83± 0.00^{d}	15.76± 0.03^{de}	15.6± 0.02^{ef}	15.42± 0.05^{f}
	Phosphorus Eggshell (mg/dl)	0.69± 0.01^{a}	0.64± 0.00^{b}	0.63± 0.00^{c}	0.62± 0.00^{c}	0.59± 0.00^{d}	0.58± 0.00^{de}	0.58± 0.00^{e}	0.57± 0.00^{e}	0.56± 0.00^{f}	0.48± 0.01^{g}

Means in the same row with different superscripts are significantly different (P<0.05).

REFERENCES

Abdel-Salam, Z.A., Abdou, A.M., and Harith, M.A. (2006). *Elemental and Ultrastructural Analysis of the Eggshell: Ca, Mg and Na Distribution during Embryonic Development via LIBS and SEM Techniques. International Journal of Poultry Science 5 (1): 35-42.*

Akins, R. E., and Tuan, R.S. (1993). *Transepithelial calcium transport in the chick chorioallantoic membrane I. Isolation and characterization of chorionic ectoderm cells. Journal of Cell Sci. 105:369-379*

Almeida Paz ICL, Mendes AA, Quinterio RR, Vulcano LC, Takahashi SE, Garcia RG, Komiyama CM, Balog A, Pelícia K, Wescheler FS, Scudeller PSO, Piccinin A. (2006). *Bone Mineral Density of Tibae and Femura of Broiler Breeders: Growth, Development and Production. Brazilian Journal of Poultry Science Revista Brasileira de Ciência Avícola. 8(2):75 – 82.*

Badley, A.R. (1997) *Fertility, hatchability and incubation of ostrich (Struthio camelus) eggs. Poult. and Avian Biology Reviews, 8(2) 53-76.*

Baumann, R. and Dragon, S. (2005). *Erythropoiesis and red cell function in vertebrate embryos. Eur. J. Clin. Invest. 35 Suppl. 3,2 -12.*

Belfield, A and Goldberg, D.M. (1971). *Colourimetric determination of alkaline phosphatase activity Enzyme. 12,561-568.*

Bellabarba, D., S. Belisle, N. Gallo-Payet, and J.-G. Lehoux. (1988). *Mechanism of action of thyroid hormones during chick embryogenesis. Am. Zool. 28:389–399.*

Bhattacharyya,A., Majumdar, S., Bhanja, S.K., Mandal, A.B., Dash, B.B., and Agarwal, S.K. (2009). *Effect of in ovo injection of glucose on growth, immunocompetence and development of digestive organs in turkey poults. 16th European Symposium on Poultry Nutrition.*

Black, Juli (2003). *Developmental patterns of metabolism and hematology in the late stage chicken embryo (Gallus domesticus) at two incubation temperatures. Master of Science (Biology), University Of North Texas. May 2003, 84 pp.*

Bonucci E, Mocetti P, Silvestrini G, Ballanti P, Zalzal S, Fortin M, Nanci A (2001) *The osteoblastic phenotype in calcium-depleted and calcium-repleted rats: a structural and histomorphometric study. J Electron Microsc 50:333–347.*

Bouda J., Quiroz-Rocha G.F., Ramírez E.S., Peña J.E. & Flores J.L.D. (2004). *Valores bioquímicos selectos em plasma sanguíneo de avestruces de diferentes edades y sexo. Vet. Méx. 35(1):1-10.*

Brown, C.R., Peinke, D., And Loveridge, A. (1996). *Mortality in near term ostrich embryos during artificial incubation. Brit. Poult. Sci., 37. 73-85.*

Caraway, W. (1955). *Determination of uric acid in serum and urine. Am. J. Clin. Path. 25, 840.*

Çelebi, S., Bölükbasi, S.C., and Utlu, N. (2005). *The Influence of Dietary Phosphorus Level on Plasma Calcium and Phosphorus, Eggshell Calcium and Phosphorus. International Journal of Poultry Science 4 (7): 497-499.*

Chan, T. and Burggren, W.W. (2005). *Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (Gallus gallus). Respir. Physiol. Neurobiol. 145,251 - 263.*

Charles, C., Mc.Cormic. and Cunningham, D. (1984) *Forced resting by high dietary, zinc: tissue zinc accumulation and reproductive organ weigh changes. Poult. Sci. 63: 1207-1212.*

Christensen, V.L. and Edens, F.W. (1985). *Magnesium calcium, and phosphorus content of shells from hatching and non hatching turkey eggs. Poult. Sci., 64: 1020 1027.*

Christensen, V.L. and Davis, G.S.(2004). *Maternal Dietary Iodide Influences Turkey Embryo Thyroid Function. International Journal of Poultry Science 3 (9): 550-557.*

Christensen, V.L., and Biellier, H.V. (1982) *Physiology of turkey embryos during pipping and hatching. IV. Thyroid function in embryos from selected hens. Poult Sci 61:2482-2488.*

Christensen,- V.L., Davis, G.S. and Nestor, K.E. (2002). *Environmental incubation factors influence embryonic thyroid hormones. Poult. Sci. 81:442–446.*

Christensen, V.L., Ort, D.T., and Grimes, J.L. (2003). *Physiological factors associated with weak neonatal poults (Meleagris gallopavo). Int J Poult. Sci. 2:7-14.*

Costa N.D., McDonald D.E. and Swan R.A. (1993). *Age-related changes in plasma biochemical values of farmed emus (Dromaius novae-hollandiae). Aust. Vet. J. 70(9):341-344.*

Crooks, R.J., Kyriakides, C.P.M., and Simkiss, K. (1976). *Routes of calcium movement across the chick chorioallantois. Q. J. Exp. Physiol. 61, 265-274.*

Dacie, J., and Lewis, S. (1991) *Practical Hematology. Seventh Edition; Churchill Livingstone, pp. 37, 38, 39, 48.*

Davis, G.S., and Christensen, V.L. (1995). *Eggshell conductance and other functional qualities of ostrich eggs. The Ostrich News, 8 (80), 60.*

Dawson, A., McNaughton, F. J., Goldsmith, A. R., and Degen, A. A. (1994). *Ratite-like neoteny induced by neonatal thyroidectomy of European starlings, Sturnus vulgaris). J. Zool. London 232, 633–639.*

De Oliveira, J.E. (2007). *Effects of In ovo Feeding on Turkey Embryos Development, Energy Status, Intestinal Maturation, Gene Expression and Post-hatch Development. A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy Nutrition Raleigh, North Carolina*

Deeming, D.C. (1995). *Possible effect of microbial infection on yolk utilization in ostrich chicks. Vet. Rec. 136(11):270 -271.*

Deeming, D.C. (1997). *Ratite egg incubation. A practical Guide. Ratite Conference, Buckingham shire, UK.*

Deeming, D.C., and Ayres, L. (1995) *Factors affecting the growth rate of ostrich (Struthio camelus) chicks in captivity. The Veterinary Record, in press.*

Doumas, B.T.; Waston, W.D. and Biggs, H.G. (1971). *Albumin standard and the measurements of serum albumin with bromocresol green. Clin. Chem. Acta.31, 87.*

Dragon, S. and Baumann, R. (2003). *Hypoxia, hormones, and red blood cell function in chick embryos. News Physiol. Sci. 18,77 -82.*

Duncan, D. B. (1955). *Multiple range and multiple F tests, Biometrics, 11, 1-42.*

Durgun, Z., Keskin, E. Col, R. and Atalay, B. (2005). *Selected haematological and biochemical values in ostrich chicks and growers. Archiv Fur Geflugelkunde 69(2): 62-66.*

Dzoma, B.M. and Motshegwa, K. (2009). *A retrospective Study of Egg Production, Fertility and Hatchability of Farmed Ostriches in Botswana International Journal of Poultry Science 8 (7): 660-664.*

Elaroussi, M. A., Smitha, A.U., Hellwiga,W., and DeLuca, H. F.(1994). *The role of vitamin D in chorioallantoic membrane calcium transport. Biochimica et Biophysica Acta (BBA) – Biomembranes. 1192(1): 1-6.*

Elaroussi, M.A., and Elbarkouky, E. (2003). *Development changes associated with Japanese quail embryogenesis during the latter part of incubation. Journal Egypt German Society of Zoolgy, Vertebrate Anatomy& Embryology, 40B: 99-111.*

El-Merzabani, M.M., El-Aaser, A.A. and Zakhary, N.I. (1977). *Determination of inorganic phosphorus in serum. J. Clin. Chem. Clin. Biochem. 15 :715 – 718.*

Fahmy, M.O. (2008). *Performance of ostriches (Struthio Camelus) fed on different levels of protein and energy under hot climate. Egypt. Poult.Sci. Vol (28) (IV) (977-1002).*

Gado, M.S. (1973). *Effect of thyroid gland hormone on egg production moulting and egg quality in both egg and meet type of fowl. Ph.D. Thesis Ret: Academy Moscow. USSR.*

Gindler M. King J.D. (1972) *Am. J. Clin. Path. 58,376.*

Gonzales, A., Satterlee, D. G., Moharer, F., and Cadd, G.G. (1999). *Factors affecting ostrich egg hatchability. Poult. Sci., 78: 1257-1262.*

Grabowski, C. (1966). *Teratogenic effects of calcium salts on chick embryos. J. Embryol. Exp. Morphol. 15: 113 - 118.*

Henry, R.J. (1964) *Clinical chemistry, Harper and Row publishers,New York, pp. 181.*

Hoiby,M., Aulit, A. and Bjonees, P.D.(1987). *Anaerobic metabolism in fowl embryos during normal incubation. Comparative Biochemistry Physiology. 86:91-94.*

Holle, D., F. Benson (2001): *Understanding Production. Ostrich Nutrition. 2001 Blue Mountain Feeds, Inc. South Africa. pp. 8-13.*

Hopkins, B. A. (1995). *Blood count and serum chemistry analysis. In: Ratite Encyclopedia, Ostrich - Emu - Rhea, 1st ed., (Drenowatz, C., Ed). Charley Elrod & Helen Wilborn. pp. 409-412.*

Julian RJ (2005). *Patologias ósseas em aves. In: Conferência Apinco de Ciência e Tecnologia Avícolas; (2005); Santos, SP. Brasil. p. 107-122.*

Kermanshahi, H., and Hadavi, A. (2006). *Effect of Added Extra Calcium Carbonate into the Diets, One Hour Before Starting Dark Period on Performance and Egg Quality of Laying Hens. International Journal of Poult. Sci. 5 (10): 946-948.*

Khandoker, A. H., Dizalowski, E. M., Burggren, W. W. and Tazawa, H. (2003). *Cardiac rhythms of late pre-pipped and pipped chick embryos exposed to altered oxygen*

environments. Comp. Biochem. Physiol. 136A,289 -299.

Khorrami, S. (2004). *Hematocrit, hematocrit regulation and its effect on oxygen consumption in the late stage chicken embryo (Gallus domesticus). Master of Science (Biology), University Of North Texas , 34 pp.1.*

Khorrami,S., Tazawa, H., and Burggren, W. (2008). *Blood-doping' effects on hematocrit regulation and oxygen consumption in late-stage chicken embryos (Gallus gallus) .Journal of Experimental Biology 211, 883-889.*

Kind, P.R, and King, E.J.(1954). *Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol. 7(4):322–326*

King, D.B., and May, J.D. (1984). *Thyroidal influence on body growth. J. Exp. Zool.*

Kreibich, A., and Sommer, M. (1995) *Miscellaneous. In: kreibich, A., and Sommer, M. (eds.). Ostrich farm management, (Landwirtschafts verg, Gmbh, Munster- Hiltrup, Germany), 166-169.*

Kubota, M., Abe, E., Shinki, T. and Suda, T. (1981) . *Vitamin D metabolism and its possible role in the developing chick embryo. J. Biochem194, 103-109.*

Kwak, H.. Austic, R. E.. and Dietert, R.R. (1999). *Influence of dietary arginine concentration on lymphoid organ growth in chickens. Poult. Sci., 78: 1536-1541.*

Lu, J., McMurtry, J.P., Coon, C.N. (2004). *Developmental changes of plasma insulin, glucagons, IGF-I, IGF-II, thyroid hormones, and glucose concentration in chick embryo. Poultry Science. 83 (Suppl.1):192.*

Lu, J.W., McMurtry, J.P., and Coon, C.N. (2007). *Developmental Changes of Plasma Insulin, Glucagon, Insulin-like Growth Factors, Thyroid Hormones, and Glucose Concentrations in Chick Embryos and Hatched Chicks. Poultry Science 86:673–683.*

Lumeij JT. Avian clinical biochemistry. In: Kaneko JJ, Harvey JW, Bruss ML, editors. (1997). *Clinical biochemistry of domestic animals. 5th ed. San Diego (Ca): Academic Press: 857-883.*

Lxingth, J.A., Andrson, S., and Rowle, J.K. (1972) *Chemical bases of the sulfa phoshovanilin reaction for estimating total lipids. Clinical Chem. (18c3).*

Maina, J. N. (2004). *Systematic analysis of hematopoietic, vasculogenetic, and angiogenetic phases in the developing embryonic avian lung, Gallus gallus variant domesticus.Tissue Cell 36,307 -322.*

McNabb, F. M. A. (2000). *Thyroids. Pages 461–471 in Sturkie's Avian Physiology. G. Whittow, ed. Academic Press, New York, NY.*

McNabb, F.M.A., Dunnington, E.A., Siegel, P.B., and Suvarna, S. (1993). *Perinatal thyroid hormones and hepatic 5' deiodinase in relation to hatching time in weight-selected lines of chickens. Poult. Sci., 72: 1764-1771.*

Miranda, R.L., Mundim, A.V., Costa, A. S., Guimarães, R. V., and Silva, F. O.C. (2008). *Serum biochemistry of 4-day-old ostriches (Struthiocamelus). Pesq. Vet. Bras. 28(9):423-426.*

Mushi, E.Z., M.J. Binta, R.G. Chabo and O. Galetshipe, (2008). *Problems associated with artificial incubation and hatching of ostrich (Struthio*

camelus) eggs in Botswana. Res. J. Poult. Sci., 2: 21-26.

Nakano, Y., Toyosawa,S., and Takano,Y. (2004). *Eccentric Localization of Osteocytes Expressing Enzymatic Activities, Protein, and mRNA Signals for Type 5 Tartrate-resistant Acid Phosphatase (TRAP). Journal of Histochemistry and Cytochemistry 52 (11): 1475-1482.*

Narbaitz, R., Stumpf, W.E., and Madhabananda, S. (1981). *The role of autoradiographic and immunocytochemical techniques in the clarification of sites of metabolism and action of vitamin D. J Histochem. Cytochem. 29: 91-100.*

Nashat, S.I. (2005). *Studies On Some Factors Affecting Ostrich Production In Egypt. Thesis, M.Sc., Al-Azher University*

Natt, M.P. and Herrick, C.A. (1952) *A new blood count diluents for counting the erythrocytes and leucocytes of chicken. Poultry Science, 31: 735-738.*

Packard, M.J., and G.C. Packard. (1991). *Patterns of mobilization of calcium, magnesium, and phosphorus by embryonic yellow-headed blackbirds (Xanthocephalus xanthocephalus). J. Comp. Physiol. B 160:649-654.*

Packard, M.J., Clark, N.B. (1996). *Aspects of calcium regulation in embryonic lepidosaurians and chelonians and a review of calcium regulation in embryonic archosaurians. Physiol Zool 69:435–66.*

Palomeque, J., Pinto, D. and Viscor, G.(1991). *Hematologic and Blood Chemistry Values Of the Masai Ostrich (Struthio Camelus). Journal of Wildlife Diseases, 27(1), pp. 34-40*

Patton, C.J., and Crouch, S.R, (1977) *Spectrophotmetric and kinetics investigations of the Berthelot reaction for the determination of ammonia. Analytical Chemistry, 49, 464-469.*

Peebles, E.D., and Marks, H.L. (1991). *Effects of selection on plasma thyroxin concentration in Japanese quail under thioracil and protein stress. Poul. Sci. 63:207-213.*

Peebles, E.D., T. Pansky, S.M. Doyle, C.R. Boyle, T.W. Smith, M.A. Latour, and P.D. Gerard. (1998). *Effects of dietary fat and eggshell cuticle removal on egg water loss and embryo growth in broiler hatching eggs. Poultry Sci. 77:1522-1530.*

Peres, H., Goncalves, P., and Oliva-Tales, A. (1999). *glucose tolerance in gilthead seabream Sparus aurata and European seabass (Dicentrachus Labrax). Aquaculture, 179:415-423.*

Peters T. (1975). *The Plasma Proteins (edited by Putnam F. W.), 1: 133-181. Academic Press, New York.*

Powrie, W.D. (1972). *Chemistry of eggs and eggs products. In: egg science and technology, Stadelman, W.J, and Cotterill, O.J.(Eds), pp (65-91), the AVI publishing company, INC. Westport, Connecticut.*

Quintavalla F., Bigliardi E. and Bertoni P. (2001). *Blood biochemical baseline values in the ostrich (Struthio camelus). Annali Fac. Med. Vet. 21:61-71.*

Rahn, H., Paganelli, C.V., Ar, A. (1974). *The avian egg: air-cell gas tensions, metabolism, and incubation time. Respir. Physiol. 22, 297-309.*

Raukar, J. and M. Simpraga (2005). *Haematological parameters in the blood of one day old ostriches. Israel Journal of Veterinary Medicine 60(4): 112-116.*

Reeves, R.B. (1984). *Blood oxygen affinity in relation to yolk-sac and chorioallantoic gas exchange in the developing chick embryo. In: Seymour, R. S. (Ed.), Respiration and metabolism of embryonic vertebrates. Dr. W. Junk Publishers, Dordrecht.*

Reyns, G. E., K. Venken, G. M. de Escobar, E. R. Ku" hn, and V. M. Darras. (2003). *Dynamics and regulation of intracellular thyroid hormone concentrations in embryonic chicken liver, kidney, brain, and blood. Gen. Comp. Endocrinol. 134:80–87.*

Richards, M. P., and M. J. Packard. (1996). *Mineral metabolism in avian embryos. Poult. Av. Biol. Rev. 7:143-161.*

Romanoff, A. L. 1967. *Chemical composition of the whole embryo, Table 1 and 11. Pages 38-43 in Biochemistry of The Avian Embryo. John Wylie and Sons, New York.*

Romanoff, A.L. (1960) *The avian embryo; structural and functional development. Macmillian, New York, NY.*

Roux, C., Wolf, C., Mulliez, N., Gaoua, W., Cormier, V., Chevy, F., and Citadelle, D.(2000). *Role of cholesterol in embryonic development1. American Journal of Clinical Nutrition, 71(5): 1270S-1279s.*

Saleh, K. (1988) *Porosity and thickness of eggshell and their relation to hatchability of fertile eggs in three species of poultry. Egypt Poult. Sci. , 8:410-419.*

Sales J. and Horbanczuk J. (1998). *Ratite meat. World's Poultry Science Journal, 54, 1, 59-67.*

Sigler, D. H. (1995): *Basic nutrition for ratites. In: Ratite Encyclopedia, Ostrich – Emu - Rhea, 1st ed., (Drenowatz, C., Ed.), Charley Elrod & Helen Wilborn, Ratite Records, San Antonio, TX, USA. pp. 287-294.*

Simkiss, K. (1961). *Calcium metabolism and avian reproduction. Biological Reviews of the Cambridge Philosophical Society, 36: 321-367.*

Simkiss, K.(1962). *The sources of calcium for the ossification of the embryos of the giant leathery turtle. Comparative Biochemistry and Physiology, 7 (1-2): 71-74*

Šimpraga, M., Raukar, J., Novak I.L. (2004). *Calcium, phosphorus and magnesium levels and alkaline phosphatase activity in the blood of one-day-old ostriches. Vet. Arch. 74(3):177-188.*

Soliman, R. (2000) *The immune response. In: Notes on immunology. Chapter 6, Printed by: Printing and Publishing Committee, Faculty of Veterinary Medicine, Cairo University. PP. 106 – 142.*

Stokes, W.M. Fish, W.A. and Frederick C. Hickey, 0. P. (1952). *Metabolism Of Cholesterol In The Chick Embryo I. The Synthesis Of Cholesterol (From the Research Laboratory of Providence College and St. Joseph's Hospital, Providence, Rhode Island)*

Suvarna, S., Christensen, V.L. , Ort, D.T. and Croom, W.J.(2004). *Ontogeny of Intestinal Glucose Transport in Heavy and Light Body Weight Turkey Poults. International Journal of Poultry Science 3 (12): 783-790.*

Tazawa, H., Nakazawa, S., Okuda, A. and Whittow, G. C. (1988). *Short-term effects of altered shell conductance on oxygen uptake and hematological variables of late chicken embryos. Respir. Physiol. 74,199 -209.*

Tazawa, H., Takenaka, H. (1985). *Cardiovascular shunt and model analysis in the chick embryo. Cardiovascular Shunts: Alfred Benzon*

Symposium 21. K. Johansen and W. W. Burggren. Copenhagen, Munksgaard: 179-198.

Terepka, A.R., Stewart, M.E. and Merkel, N. (1969). *Transport functions of the chick chorio-allantoic membrane. II. Active calcium transport, in vitro. Exp. Cell Res. 58: 107-117.*

Tuan, R., and Zrike, (1978) *Functional involvement of carbonic anhydrase in calcium transport of the chick chorioallantoic membrane. Biochem. J. 176:67-74.*

Tuan, S., and Scott, W.A. (1977). *Calcium-binding protein of chorioallantoic membrane: Identification and developmental expression. Proc. Natl. Acad. Sci. USA 74: 1946-1949.*

Ubaldi A., Corbella E., Montanari P. (1982): *Diagnostica chimico-clinica veterinaria. Casa Editrice Ambrosiana – Milano*

Vetter, A.E., and Grady, M.O (2005). *Sodium and anion transport across the avian uterine (shell gland) epithelium. The Journal of Experimental Biology 208, 479-486.*

Watson, D. (1960). *A simple methods for the determination of serum cholesterol. Clin. Chem. ACTa. 5. 637.*

Wiercińska, M. and Szczerbińska D. (2005). *The Ostrich And Emu Egg Hatchability With Reference To Dead Embryo Analysis, Electronic Journal of Polish Agricultural Universities (EJPAU) 8(4), 41. Available Online: http://www.ejpau.media.pl/volume8/issue4/art-41.html*

Wood, R. (1972). *Relationship between embryonic and tumor lipids: I. Changes in the neutral lipids of the developing chick brain, heart and liver. Journal Lipids 7:(9)596-603.*

الملخص العربي

بعض التغيرات في مكونات الدم المصاحبة لتطور نمو أجنة النعام أثناء التفريخ الآصطناعى

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** قسم التطبيقات البيولوجية- شعبة تطبيقات النظائر المشعة- مركز البحوث النووية- هيئة الطاقة الذرية

أجريت هذه الدراسة على أجنة النعام لمتابعة التغيرات فى بعض المقاييس الفسيولوجية الحيوية خلال التفريخ وتقديم مرجع للقيم البيوكيميائية فى الدم ومعلومات عن بعض التغيرات المصاحبة بنمو وتطور أجنة النعام الطبيعي خلال مدة التفريخ حيث لا توجد دراسة مشابهة ومعلومات كاملة متعلقة بهذا الموضوع من البحث. تم تحديد صورة دم كاملة تشمل عد كرات الدم الحمراء والبيضاء وتركيز الهيموجلوبين والهيموتكريت فى عينات دم الأجنة على أعمار ٢٠ ، ٣٠ ، ٣٤ ، ٣٦ ، ٣٨ ، ٣٩ ، ٤٠ ، ٤١ ، ٤٢ ، و ٤٣ يوم من التفريخ . كذلك تم فصل بلازما الدم لتقييم تركيزات البلازما من البروتينات الكلية-الألبيومين- الجلوبيولين- الجلوكوز- الآلكالين فوسفاتيز – أسيد فوسفاتيز – اليوريا – اليوريك أسيد – الكولسترول – الليبيدات الكلية – الجلسريدات الثلاثية- هرمونات الغدة الدرقية – كذلك تم قياس تركيز ومحتوى الكالسيوم والفسفور فى البلازما وقشرة البيض وعظام أرجل الأجنة. أظهرت النتائج الآتي :- يزداد عدد كرات الدم الحمراء والبيضاء وتركيز الهيموجلوبين والهيموتكريت تدريجيا بتطور الجنين ويصل لأعلى قيمة عند عمر ٤٢ بشكل معنوي. يزداد مستوى الجلوكوز معنويا مبكرا عند عمر ٢٠ وحتى عمر٣٦ يوم وكذلك متأخرا عند عمر ٤٠ وحتى ٤٢ من التطور الجنينى. يزداد تركيزات هرمونات الغدة الدرقية **T4, T3** معنويا بتقدم العمر ويصل لأعلى قيمة عند عمر جنينى ٤١ و ٤٠على الترتيب ويقل عند النقر وقبل الفقس . يزداد معنويا بتقدم العمر الجنينى تركيزات كل من البروتينات الكلية-الألبيومين-الجلوبيولين- نسبة الجلوبيولين الى الألبيومين - الآلكالين فوسفاتيز – أسيد فوسفاتيز – اليوريا – اليوريك أسيد وأعلى زيادة كانت عند عمر الفقس ٤٢ يوم . أيضا يزداد تركيز البلازما من الكولسترول – الليبيدات الكلية – الجلسريدات الثلاثية تدريجيا بتقدم العمر الجنيني ويصل لأعلى قيمة عند عمر ٣٤ و ٣٦ و ٣٨ يوم على الترتيب. يقل محتوى القشرة من الكالسيوم والفوسفور بالتقدم فى العمر بينما يزداد تركيز البلازما وعظام أرجل الأجنة من الكالسيوم والفوسفور معنويا بالتقدم فى العمر وتكون أعلى زيادة عند عمر الفقس ٤٢ يوم. وتؤسس هذه النتائج المتحصل عليها من هذه الدراسة قاعدة بيانات للتركيب الكيميائي والهيماتولوجي لدم أجنة النعام خلال النمو الجنيني. علاوة علي ذلك اي نقص عن المقاييس المذكورة سابقا في قاعدة البيانات للتركيب الكيميائي والهيماتولوجي لدم اجنة النعام خلال المرحلة الجنينية المتأخرة قد تفسر ظاهرة ارتفاع نسبة نفوق الأجنة.