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SKELETAL EMBRYONIC DEVELOPMENT AND HATCHING TRAITS AS AFFECTED BY EGGSHELL OSTEOPONTIN PROTEIN DURING TWO STAGES OF EGG LAYING CYCLE

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ABSTRACT: The present experiment was carried out for determining the role of osteopontin protein (OPN) in eggshell on shell quality and consequent effect on skeletal embryos, hatching traits and some physiological parameters besides its relation with two stages of laying cycle of Mandarah chickens (32 to42 *vs* 52 to 62 wks. of age).

The obtained results are summarized as follows:-

1. Osteopontin concentrations (ng/µL) were decreased (P≤0.05) for eggshell of fresh eggs and hatched chicks for eggs produced from elder chickens compared with those from younger ones. The same trend of significant decrease of OPN was observed for tibia of hatched chicks and for chick's blood.
2. Osteopontin concentration decreased (P≤0.05) with the increase of embryonic age with respect to eggshell whereas, it increased for tibia.
3. Younger chickens represented significant increase of eggshell strength, eggshell index, eggshell and eggshell membranes thickness compared to those for elder ones.
4. Embryonic mortality %, hatch time (hr) and chick body weight at pull out (g) were significantly increased for eggs produced from elder chickens compared to those for younger ones while, hatchability of fertile eggs % was significantly decreased.
5. Calcium and phosphorus values were significantly decreased with the increase of flock age for eggshell of fresh eggs and eggshell through different times of incubation besides tibia of embryos at 15th day and for chicks at hatch.
6. Blood constituents of hatched chicks as calcium, phosphorus, and parathyroid hormone were significantly decreased with the increase of chicken age.
7. Relative asymmetry is higher (P≤0.05) for wing, tibia, tarsus and femur lengths of embryos and baby chicks for younger flock compared to elder one except that for tarsus of baby chicks.

The contributions of OPN as a major protein in shell structure and subsequent effects on hatching process are proven and this conclusion could set up alternative tool for eggshell quality evaluation and breeding selection programs.

Keywords: Osteopontin, eggshell quality, hatchability, calcium, parathyroid hormone

INTRODUCTION

The quality of the eggshell is of primary concern to the poultry industry (Hunton, 1995). The successful development of chicken embryos is dependent upon a robust eggshell for mechanical protection, prevention of water loss and as a primary source of calcium for the embryonic skeleton (Karlsson and Lilja, 2008). The eggshell is composed of 95% calcium carbonate in calcite form, it also contains a low percentage as 3.5% of organic matrix (Nys and Gautron, 2007). Matrix composition changes during the various phases of eggshell calcification are coincided with some alterations in eggshell mechanical properties that are observed at different physiological stages of the hen age (Gautron and Nys, 2006).

Osteopontin (OPN) is a phosphoglycoprotein which is rich in aspartic and glutamic acids and contains phosphoserine and phosphothreonine residues (Prince et al , 1987). In this context, OPN has been found in the eggshell and oviduct and suggested to be part of the array of macromolecules contributing to the regulation of eggshell mineralization and calcium metabolism of the eggshell gland (Lavelin et al., 1998 and 2000). Also, OPN was originally isolated from bones (Fernandez et al., 2003). Gene expression of OPN in different tissues suggests the involvement of this protein in a variety processes including development, wound healing, immunological response, bone resorption and calcification (Sodek et al., 2000) and regulation of its gene expression by calcitrophic substance such as parathyroid hormone (Noda and Rodan, 1989). Previous investigators reported that breeder age has important effects on eggshell characteristics (Tona et al., 2004 and Joseph and Moran, 2005). The deterioration in the eggshell thickness and embryonic mortalities increase are related to the age of the hens (Rizk et al., 2008). Alternatively, Lourens et al. (2006) showed that heavier eggs obtained usually from elder breeder chickens had a greater weight loss during incubation period. Furthermore, hatchability process decreases with increasing the age of breeder flock (Celen et al., 2009). Also, Hocking et al. (2001) found that a decline in calcium and phosphorus content in eggshell due to age of hen. In addition, breeder hen age affects plasma cholesterol, total lipids, globulin, and glucose levels for newly hatched chicks as mentioned by Latour et al. (1998) and Eshratkhah et al. (2011). Asymmetry refers to the random deviations from symmetry in the development of bilaterally symmetrical traits, fluctuating asymmetry, deviating in either direction with a normal distribution and a mean of zero (Moller, 1990).

This experiment is a part of series researches which open alternative criterion for determining eggshell quality through understanding the role of osteopontin protein in eggshell and the subsequent related functions besides its relation with flock age.

MATERIALS AND METHODS

The present study was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agriculture Research Center.

Experimental design:

One thousand and two hundreds hatching eggs produced from Mandarah hens through two stages of laying cycle (32 to 42 vs. 52 to 62 wks of age) were incubated representing three replicates for each flock age. All eggs were individually numbered and weighed prior to the beginning of the incubation and incubated in Egyptian made incubator at 99.5Fº and 55 %RH during setting phase of incubation. The time of setting eggs in the incubator was recorded for both ages to obtain the hatch time exactly in hours and considered as zero time of experiment. At 18 day (432 hrs) of

incubation, the eggs were weighed, candled and those with evidence of living embryos were transferred to the hatcher and incubated at 99ºF and 70 %RH. At 0,7, 14, 18 days of incubation, all eggs were individually weighed (gm), the percentage of egg weight loss for incubation intervals (0-7, 8-14, 15-18, 0-18) per each age were calculated. Beginning at 456 hrs of incubation and at 12 hrs intervals thereafter the hatcher was opened and chicks had fully emerged from eggs were removed and hatch window was calculated {The period of time elapsed from the hatching of the first chick to the hatching of the last one (Decupere et al., 2001)}. Body weights (g) for all chicks at the time of removal from the hatcher were recorded and termed as chick weight at pull out. Hatchability of fertile eggs percentage was determined. Eggs that failed to hatch at the end of incubation and having full opportunity for hatch were broken out and then examined with naked eye to estimate embryonic mortalities percentages during the intervals (early)1-7, (mid)8-14 and (late) 15-21 days of incubation.

Measurements:-

Before setting the eggs in the incubator, percentage of abnormal eggs was detected and discarded (i.e. rounded, oblonged, wrinkled, thinner eggshell and those with extra calcification, extremely large and small eggs).

Three fresh hatched eggs from each replicate for the two ages were taken to estimate the following parameters: eggs were individually weighed to nearest 0.1 g, egg shape index was determined as the percentage of the greatest width to the greatest length (Romanoff and Romanoff, 1949). Also the shells of broken eggs were weighed without the shell membranes to the nearest 0.1g then eggshell weight percentages were calculated. In addition to, shell thickness was measured with and without membranes by a micrometer to the nearest 0.01 mm. Eggshell strength (Newton) was estimated by single Radial Immune Diffusion Technique as described by Bennett et al. (1988) with Digital Force Gauge- FGC-50 at Agricultural Engineering Research Institute.

Proteins including osteopontin protein (OPN) were estimated at the Plant Pathology Research Institute, Central Lab of Biotechnology and analyzed by gel running in a dual Vertical Slab Unit (Hoper Scientific Instruments, san Francisco, CA, USA). The OPN concentration (ng/µL) was estimated in eggshell according to the method of Association of Official Analytical Chemists, AOAC (1998) and in bones according to the method of AOAC (1955) by Atomic Absorption Spectrometer.

The concentrations of osteopontin, calcium (Ca) and phosphorus (P) were measured in three eggshells from each replicate of the two flock ages representing fresh eggshell and eggshells at 9 and 15 days of incubation and at hatch. Also the same previous parameters were estimated in tibia bone at day 15 of incubation.

Morphometric traits were taken from embryos and baby chicks representing the experimented studied ages for measuring lengths(mm) of left and right face , wing , tarsus , tibia, and femur by using calipers as described by Yalcin and Siegel (2003) . Also, relatives of bilateral traits were detected as ({Left -right}/ [(Left+ right)/2]x100 (Yang et al., 1997) .

Blood samples were taken from slaughtered baby chicks and collected in heparinized tube immediately for measuring OPN protein in the blood besides plasma triiodothyonin, ng/ml (Beckman Coulter Company) and parathyroid hormones, ng ml (Sigma company). Also plasma globulin (mg/dl), Ca (mg/dl) P (mg/dl), glucose (mg/dl), total lipids (mg/dl) and cholesterol (mg/dl) were detected by available commercial Bio-diagnostics kits Egypt.

Osteopontin, Ca and P concentrations were measured in tibia bone for the same previous slaughtered baby chicks.

Statistical analyses:

Data obtained were statistically analyzed using general linear models (GLM) of SAS (2004). The significant differences among treatment means were tested according to Duncan (1955).

The following model was used

$Y_{ij} = \mu + L_i + e_{ij}$

μ = the overall mean,

L_i = flock age effect,

e_{ij} = random error

RESULTS AND DISCUSSION

Osteopontin protein:

Data of Table 1 represent osteopontin (OPN) concentration in eggshell, embryonic tibia through different days of incubation and in blood of baby chicks for eggs produced from two ages of Mandarah chickens. It can be observed from these data that OPN concentrations were significantly($P \leq 0.05$) decreased for shell of fresh eggs and at hatch for eggs produced from elder chickens (52-62 wks of age) compared with those from younger ones at (32-42 wks of age). The same trend of significant decrease with chicken age is observed for tibia of hatched chicks. Moreover, the observed decrease in OPN concentration for the elder chicken age compared to the younger one did not represent any statistical difference with respect to eggshell on days 9 and 15 of incubation and for embryonic tibia on day 15 of incubation. In addition to, OPN concentration in eggshell was decreased ($P \leq 0.05$) with the increase of embryonic age for both studied chicken's ages and realized the lowest value for eggshell of chicks at hatch. Opposite trend of OPN concentration is noticed in tibia as they increased ($P \leq 0.05$) for hatched chicks compared with the embryonic tibia at day 15 of incubation.

It could be concluded from the previous mentioned results that the decrease of OPN concentration decreases with the increase of chicken age and could be responsible or related to the deterioration of shell structure with the advanced chicken age.

The increase of OPN concentration in the tibia of hatched chicks compared with those for embryos at day 15 of incubation could be due to the depletion of OPN from eggshell to the embryos as they decreased from 15.44% for fresh eggs to 8.91 for eggshell of hatched chicks for eggs produced from younger chickens and from 11.34 % for fresh eggs to 5.77% for hatched chicks of elder ones (52-62 wks of age). Furthermore, significant increase is detected of OPN concentration was detected for blood of baby chick hatched from younger flock compared with elder one.

The results of OPN concentrations as shown in Table 1 for eggshell and tibia bone are confirmed by different research-workers. Nys et al. (2004) found that OPN is presented at high concentration in bone and in eggshell. In addition, OPN is suggested to be part of the array of macromolecules contributing to the regulation of eggshell mineralization (Fernandez et al., 2003).

The significant diminish of OPN concentration in eggshell with the increase of embryonic age and consequently the increase from tibia bone at day 15 of incubation to higher level for hatched chicks are in harmony with those reported by Hinck et al. (2012) who found that OPN incorporation into the eggshell may also serve some roles in the dissolution of the shell to provide calcium to the skeleton of the growing embryo. Kaweewong et al. (2013) referred to some differences in the types of proteins and their molecular functions were noted in eggshell at different incubation times. Also, the same authors reported that some proteins with different biological functions were presented at different times of embryo development which indicated activities involved during egg hatching. The results of significant decrease of OPN concentration with the increase of hen age as shown in first table is in harmony with that reported by Gautron and Nys (2006)

who found that a relationship between changes in eggshell mechanical properties induced by hen aging and level of matrix components.

Eggshell quality:

Effects of chicken age with reference to OPN on eggshell quality parameters are presented in Table 2. Younger chickens had significant ($P \leq 0.05$) increase compared with elder ones with respect to shell strength, eggshell index, eggshell and eggshell membranes thickness. Whereas, elder ones had significant increase of abnormal eggs%, egg weight , eggshell weight and eggshell weight percentage compared to those of the younger ones. From aforementioned results, it could be concluded that improvement of shell strength, eggshell index and eggshell and membrane thickness for the younger flock could partially due to the increase of OPN in eggshell.

The decreases of shell strength, shell thickness and shell membranes thickness are coincided with the increases of egg weight and eggshell weight for elder chickens compared with those for younger ones as documented in the previous literatures but looked at from another perspective. The egg weight increase with the hen age is due to the increase of yolk, albumen weight and yolk proportion (Suk and Park, 2001).Also, flock age had negative effects on shell quality, shell weight and shell strength (Rizk et al., 2008 and Tumova et al., 2014)

Shell strength is one of the most important external quality parameters of egg usually dependent on eggshell proportion and thickness. Ahmed et al. (2005) stated that material with smaller crystal size is solid and is therefore consistent with stronger shells. The correlation of egg size and thickness is = 0.85 and between shell thickness and strength is = 0.47 (Tumova and Ledvinka , 2009).

Eggshell matrix proteins are thought to influence the structure and mechanical properties of the eggshell (Nys et al., 1999).Fraser et al. (1998) reported that the changes are coincided with modifications with age in the matrix protein morphology and distribution throughout the palisade layer observed by transmission electron microscopy. Moreover, Panheleux et al. (2000) demonstrated that eggshell of similar shell weight and egg weight collected at the beginning and the end of the laying show lower eggshell breaking strength in aged birds and alteration in eggshell texture. Moreover the same authors observed that eggshell breaking strength decreased with increasing orientation of the calcite crystal and these modifications may be under the control of components of the organic matrix proteins. Chien et al. (2008) revealed that OPN was hypothesized to regulate eggshell formation by inhibiting calcite growth at specific crystallographic faces and compartmental boundaries. Also, Arazi et al. (2009) found that OPN expression was almost completely absent at the location corresponding to the cracks, whereas reduction in OPN synthesis is directly related to shell breakage. Also, Rodriguez-Navarro et al. (2002) found a significant correlation between crystallographic texture and eggshell breaking strength, whilst a random orientation of crystals was more consistent with stronger shell. This pattern however was only true for the eggs laid by young hens possibly because of on age related to change in the organic matrix or ultrastructure organization of the shells in the older group . Also, Dhawale (2008) showed that hen age may lead to formation of abnormal eggs and may be due to the organic matrix material of the shell has Ca binding properties and its organization during shell formation influences the shell abnormalities .

Hatching traits :

Effects of chicken age with reference to OPN on egg weight loss% , embryonic mortality , hatch time (hr) , hatch window (hr), hatchability of fertile eggs % and chick

weight at pull out are represented in Table 3.Egg weight loss was significantly(P≤ 0.05) increased among experimental incubation intervals (8-14, 15-18, 0-18) days for eggs produced from elder chickens compared with younger ones. Also the same trend of significant increase of egg weight loss for eggs of elder flock was reflected to the increase of embryonic mortality % among early, mid and late incubation intervals. Time of hatch was significantly (P≤ 0.05) delayed for eggs of elder chickens (507hr) compared with those for younger ones (495hr) and hatch window represented the same significant trend of increase. Both of hatchability % and chick body weight at pull out were significantly (P≤ 0.05) increased for younger chickens age compared with those for elder ones.

The significant increase of egg weight loss% for eggs produced from older hens compared with younger ones could be related to the egg weight increase. These results approach to those previously mentioned by Rizk et al.(2008) and Abudabos (2010). Moreover, the interpretation of the reported data of shell thickness as demonstrated in Table 2 could be the reason of increasing egg weight loss with the aging advance of chickens and this observation had been previously documented by Soliman et al. (1994) and Rizk et al. (2008). The results of increasing embryonic mortality for eggs produced from elder hens are in harmony with those mentioned previously by Rizk et al.(2008) and Abudabas (2010). The increase of late embryonic mortality with the increase of egg size for elder hens could be expected due to the greater difficulty for achieving adequate embryonic temperature and then losing embryonic metabolic heat during later incubation (Lourens et al ., 2006). Rizk et al.(2008) showed that rate of egg weight loss during incubation might be related to embryonic mortality. Besides parent flock age influences daily embryonic metabolism which coincides with the incidence of greater embryonic mortality during the late period of incubation (Hamidu et al., 2007). Supporting to our results regarding hatching time, Rizk et al. (2006) found that hatch time for chicks from elder flock age was significantly delayed compared to those from younger ones. Contradictory results were reported by Bruzual et al. (2000) who found that chick hatching time was not affected by hen age.

Shortest hatch window is preferable to obtain good results of hatch as staying the hatched chicks in the hatcher for a long period exposing the chicks to dehydration and losing a great amount of water and thereby decreasing the chick body weight at bull out.

The results of significant increase of hatchability for eggs of younger flock age compared to elder one are coincided with the results of Rizk et al. (2008) and could be related to the decrease of embryonic mortality. Different explanations of the decrease of hatchability for elder flocks could be due to decrease of shell egg thickness (Tsarenko, 1988), the change in the physical and functional qualities of eggs as the hens aged (Christensen et al., 1996), larger egg size (Leeson and Summers, 2000),and albumen quality deterioration (Tona et al., 2004).

The significant increase of chick weight for elder flock age compared to younger one could be related to the increase of egg weight as previously represented in Table 2. These results are documented by Tona et al. (2004); Rizk et al. (2006) and Abudabos (2010) who mentioned that hatched chicks from older breeder are larger.

The increase of OPN protein in eggshell for younger chickens could be considered as new commentary of increasing eggshell thickness and its relation to eggshell mineralization and crystallization and consequently affect egg weight loss , embryonic mortality and hatchability for younger chickens compared to elder ones.

Calcium and phosphorus concentrations:

Effects of chicken age with reference to OPN protein on Ca and P concentrations in eggshell and embryonic tibia are shown in Table 4. It is apparent from data of this table that ca and P were significantly($P \leq 0.05$) decreased with the increase of chicken age with respect to eggshell in fresh eggs and through different times of incubation besides tibia for embryos at 15th day and for chicks at hatch. Moreover, concentrations of Ca and P were significantly decreased with the embryonic development for both experimental chicken ages. The highest significant records for both Ca and P were observed for fresh eggshell eggs and the lowest ones were recorded for eggshell at hatch for both experimental ages. Opposite trend was observed for embryonic tibia as Ca and P significantly increased from day 15 of embryos to those for chicks at hatch for both chicken ages.

The trend results of Ca and P either in eggshell or embryonic tibia are related to embryonic age and flock age as observed in Table 4 and this observation is similar to the trend results of OPN in Table 1. This observation refers to the substantial relation and attitude between OPN with Ca and P and their behaviors in shell and bone texture. Different research works were published to support this idea as Fernandez et al. (2003) suggested that OPN could be part of the mechanism controlling the eggshell calcification arrest. Also, the same authors showed that involvement of OPN in variety processes including development, bone reabsorption and the bone calcification by OPN based on its tissue distribution, its affinity to calcium and its immunolocalization to electron- dense regions of mineralization. Also, Kaweewong et al. (2013) revealed that during chick embryo development, large amounts of calcium are mobilized from the eggshell to the developing embryo and OPN incorporation in the eggshell may be also serve some role in the dissolution of shell to provide calcium to the skeleton of the growing embryo. Furthermore, Ibelli et al. (2013) found that OPN involved with bone and mineralization. Mazzuco and Bertechini (2014) reported that OPN are known to be involved in calcium metabolism of eggshell.

The current results of significant decrease in Ca and P in eggshell and embryonic tibia with the increase of hen age are in harmony with that previously reported by Hocking et al. (2001) who found that a decline in Ca and P content in eggshell due to age as hen losses some of her ability to mobilize Ca from the bone and is less able to produce the needed calcium carbonate. Different authors have drawn the same our conclusion regarding the significant decrease of Ca and P of eggshell with advancing embryonic age and increasing in the tibia from day 9 as Pines (2007) found that eggshell is primary source of Ca for the embryonic skeleton of incubation to hatched chicks. Moreki (2005) showed that special relationship exists between Ca and P in bone formation as the two minerals occur in the body in combination with each other most of time. The significant increase of Ca as shown in Table 4 and OPN in Table1 for fresh eggshell is supposed to be the reason of eggshell quality improvement as appears in data of Table 2. These results are in accordance with that reported by Nowaczewski et al. (2016) who found that the main reason for shell quality decline over the laying period is due to a gradual loss in Ca deposition efficiency and withdrawal of the medullar bone. Gautron and Nys (2006) mentioned that shell matrix protein like OPN regulates shell mineralization and may influence its mechanical properties so there are relationship between eggshell matrix protein and egg quality.

Blood parameters :

Data of figure 1 illustrate that the parameters of plasma as globulin, glucose, phosphorus, calcium, T3 and PTH decreased ($P \leq 0.05$)with the increase of chicken age. Whereas, cholesterol and total

lipids increased (P≤ 0.05) with the increase of chickens age.

The plasma biochemical parameters of the baby chicks in this figure could be due to the maternal effect. Maternal effects are epigenetic modification of offspring phenotype provided by the mother during development (Dixon et al., 2016). Latour et al. (1996)mentioned that the observed changes suggest that many of the physiological and biochemical processes function differently in embryos from parents of different ages, a large proportion remains un used up to hatch and becomes sequestered into the body cavity and under appropriate conditions is utilized by 5days post hatch. The results of significant increase of plasma globulin for baby chicks produced from younger flock could be due to the increase of globulin concentration of parent flock at 32- 42wk of age as younger age as confirmed by Eshratkhah et al. (2011) who found significant increase in serum globulin at 32-34 weeks of old. From our point of view, we can refer that this increase of globulin concentration in blood of baby chicks for younger flock could be related to the increase of OPN either in the blood of mothers or from their eggshells as previously detected in Table 1. Furthermore, the results of cholesterol and total lipids decrease for baby chicks produced from younger flock are in accordance with that reported by Latour er al. (1998) who mentioned that serum lipids and cholesterol tended to be higher in chicks from elder hens than the younger one. This action may be due to lipid assimilation from the yolk continued while lipoprotein lipase activity was decreasing and the liver would not readily accept incoming lipid constituents and the relative concentrations of the lipids would be expected to increase in circulation. The significant increase of plasma glucose for baby chicks produced from younger flock is in harmony with that reported by Latour et al. (1996) who found that plasma glucose concentrations were greater in embryos and newly hatched chicks from the youngest aged hen and these values could be influenced by glycogenolysis . Moreover, data of the significant decrease in plasma T3 hormone for baby chicks related to elder hens are in accordance with those reported by McNabb and Wilson (1997). They referred that the younger hens may deposit more thyroid hormone into developing eggs or alternatively the deiodination of T4 may occur at different rates because of the maturity of ' monodeiodinase enzyme in the various tissues.

The significant increase of plasma Ca, Ph and PTH hormone for baby chicks of younger flock could be due to the increase of Ca , Ph and PTH levels for younger parents flock compared to that for elder one as pointed out by Preda et al. (2013) who found that significant increase in plasma Ca, P and PTH at 32 weeks of age (the peak of egg laying). The obtained results of increasing plasma PTH, Ca and P levels related to the increase of Ca and P levels in eggshell (Table 3) and eggshell quality (Table 2) and OPN concentration (Table 1) are keeping with those previously stated by Jiang et al. (2010) who mentioned that PTH controls levels of blood Ca and P and maintains the Ca homeostasis by regulating the Ca liberation from the bone and resorption from the kidney. Furthermore, Lavelin et al. (2000) found that the regulation of OPN gene expression by PTH hormone besides, Jiang et al. (2010) indicated that the higher serum calcium and PTH level may refer to better eggshell quality and the changes in bioactive PTH play an important role in eggshell calcification and consequently affect eggshell percentage and breaking strength.

Asymmetry:

Data of Table 5 represent the effect of Mandarah chicken age with reference to OPN on lengths of wing, tibia, tarsus and femur for embryos at day 15 and for baby chicks. Embryos at day 15 represent significant increase of tibia, tarsus and femur lengths for embryos produced from

elder flock compared to younger one, while wing length did not represent any statistical change. Moreover, baby chicks represented significant increase for elder flock with respect to wing, tibia and femur lengths except that for tarsus

Data of Table 6 show the effect of Mandarah chicken age with reference to OPN on relative asymmetry (RA) of wing, tibia, tarsus and femur lengths for of embryos at day 15 and for baby chicks. It can be observed that RA is higher ($P \leq 0.05$) for wing, tibia, tarsus and femur lengths of embryos and baby chicks of the younger flock compared to elder one expect that for tarsus of baby chicks. Also, data of the same table represent that baby chicks had significant ($P \leq 0.05$) decrease of RA for lengths of all studied bones compared with those for embryos. This observation of RA decrease for studied bones with the increase of embryonic development support the earlier study reported by Yalcin and Siegel (2003) who mentioned that the left and right sides asymmetries can be reduced with time episodes of compensational growth and asymmetries in skeletal traits that occurred early in incubation tended to decrease toward hatching.

The current results suggest that the length and developmental stability of studied bilateral traits generally increased with the increase of flock age. Besides, studied lengths of wing, tibia, tarsus and femur were increased for baby chicks compared with those for embryos at 15 days. Few earlier studies were reported regarding the effect of flock age on relative asymmetry. Alfonso-Torres et al. (2009) declared that different percentages of calcium in the eggshell might affect bone development in embryos. Also the same authors found that breeder age affect the absolute weight and the width of long bones of embryo. Also, Favero et al. (2013) found that midshaft width of tibia and femur was increased for embryos produced from younger flock compared to older one and bone calcification of embryos decreased with breeder age.

More researches are needed to study the effect of chicken age with reference to OPN on the developmental stability of bilateral traits and its influence on post hatch growth. The current knowledge of OPN and its incorporation into the eggshell may gain new insight regarding understanding the mechanical properties of eggshell and consequently embryonic development. Thus we hope to use this information in the application of breeding selection programs.

Table(1): Osteopontin protein concentration (ng/µ) in eggshell, embryonic tibia and blood of baby chicks representing two ages stages for Mandarah chickens

Chicken's age stage	Osteopontin concentration %						
	Eggshell				Tibia		
	Fresh eggs	During incubation		At hath	15th day of incubation	Hatched chicks	Baby chicks blood
		9th day	15th day				
32-42 Wks	15.44±0.50^{aA}	11.78±0.80^{B}	9.78±0.83^{B}	8.91±0.28^{aC}	9.78±0. 83^{B}	16.58±0.28^{aA}	12.78±0.08^{a}
52-62 Wks	11.34±0.54^{bA}	10.40±0.59^{AB}	8.72±0.86^{B}	5.77±0.75^{bC}	8.72±0. 86^{B}	11.07±0.54^{bA}	10.40±0.59^{b}

a and b means having different letters in the same column are significantly different (P≤0.05).
A, B and C means having different letters in the same row within the same trait are significantly different (P≤0.05).

Table (2): Effect of Mandarah chicken age with reference to osteopontin protein on eggshell quality parameters

Chicken's age stage	Eggshell quality parameters							
	Abnormal eggs %	Egg weight (g)	Eggshell weight (g)	Eggshell weight percentage	Shell strength (Newton)	Eggshell index	Eggshell thickness(mm)	Eggshell membranes thickness(mm)
32-42 Wks	9.28±0.44^{b}	52.50±0.02 ^{b}	5.33± 0.07^{b}	10.15±0.16^{b}	26.32±0.09^{a}	76.29±0.17^{a}	0.42± 0.01^{a}	0.05± 0.01^{a}
52-62 Wks	15.68±0.71^{a}	55.11±0.02^{a}	7.11±0.11^{a}	12.90±0.16^{a}	20.21±0.29^{b}	74.98±0.51^{b}	0.38±0.01^{b}	0.034±0.01^{b}

a and b means having different letters in the same column are significantly different (P≤0.05).

Table (3): Effect of Mandarah chicken age with reference to osteopontin protein on egg weight loss, embryonic mortality, hatch time, chick weight and hatchability

Chicken's age stage	Egg weight loss during incubation %				Embryonic mortality %			Hatch time (hr)	Hatch window (hr)	Chick weight at pull out(g)	Hatchability of fertile eggs %
	0-7day	8-14day	15-18day	0-18day	early (1-7days)	mid (8-14days)	late (15-20days)				
32-42 Wks	4.64±0.07	3.82± 0.08[b]	1.72± 0.07[b]	10.18± 0.10[b]	2.27± 0.17[b]	0.48± 0.09[b]	5.89 ±0.32[b]	495.33 ±2.22[b]	26.66 ±0.97[b]	35.55 ±0.25[b]	89.20 ±0.71[a]
52-62 Wks	4.73±0.10	5.20± 0.06[a]	2.07± 0.06[a]	12.00± 0.13[a]	3.63± 0.24[a]	1.28± 0.08[a]	9.43± 0.42[a]	501.00± 3.72[a]	32.00± 0.52[a]	37.00± 0.16[a]	84.43± 0.56[b]

a and b means having different letters in the same column are significantly different (P≤0.05).

Table (4): Effect of Mandarah chicken age with reference to osteopontin protein on calcium and phosphorus concentrations in eggshell and tibia

Chicken's age stage	Eggshell				Tibia	
	Fresh eggs	during incubation		At hath	embryos at 15th day	hatched chicks
		9th day	15th day			
	Calcium					
32-42 Wks	11.63±0.05^{aA}	10.29±0.01^{aB}	9.28±0.01^{aC}	8.85±0.04^{D}	11.01±0.01^{aB}	11.19±0. 28^{aA}
52-62 Wks	10.59±0.04^{bA}	9.43±0.02^{bB}	9.13±0.01^{bC}	8.58±0.07^{D}	9.64±0.01^{bB}	9.92±0. 02^{bA}
	Phosphorus					
32-42 Wks	5.89±0.08^{aA}	4.73±0.09^{aB}	4.28±0.05^{aC}	3.63±0.07^{D}	5.42±0.04^{aB}	5.7١±0.04^{aA}
52-62 Wks	4.76±0.04^{bA}	4.44±0.03^{bB}	4.16±0.02^{bC}	3.47±0.01 ^{D}	4.24±0.02^{bB}	4.58±0.02^{bA}

a and b means having different letters in the same column are significantly different (P≤0.05).

A,B ,C and D means having different letters in the same row within each trait are significantly different (P≤0.05).

Table(5): Effect of Mandarah chicken age stage with reference to osteopontin protein on lengths of wing, tibia , tarsus and femur of embryos at day 15 and baby chicks

Items	Mean length (mm)							
	Wing		Tibia		Tarsus		Femur	
	32-42 wks	52-62wks	32-42 wks	52-62wks	32-42 wks	52-62wks	32-42 wks	52-62wks
Embryos at day15	15.16±0.11	15.42±0.12	18.17±0.16^{b}	19.37±0.12^{a}	14.06±0.03^{b}	15.11±0.01^{a}	17.97±0.10 ^{b}	19.03±0 .10^{a}
Baby chicks	40.40±0.28^{b}	41.86±0.33^{a}	30.01±0.11^{b}	32.31±0.11^{a}	26.53±0.12	26.66±0.17	23.51±0.32 ^{b}	24.16±0.37 ^{a}

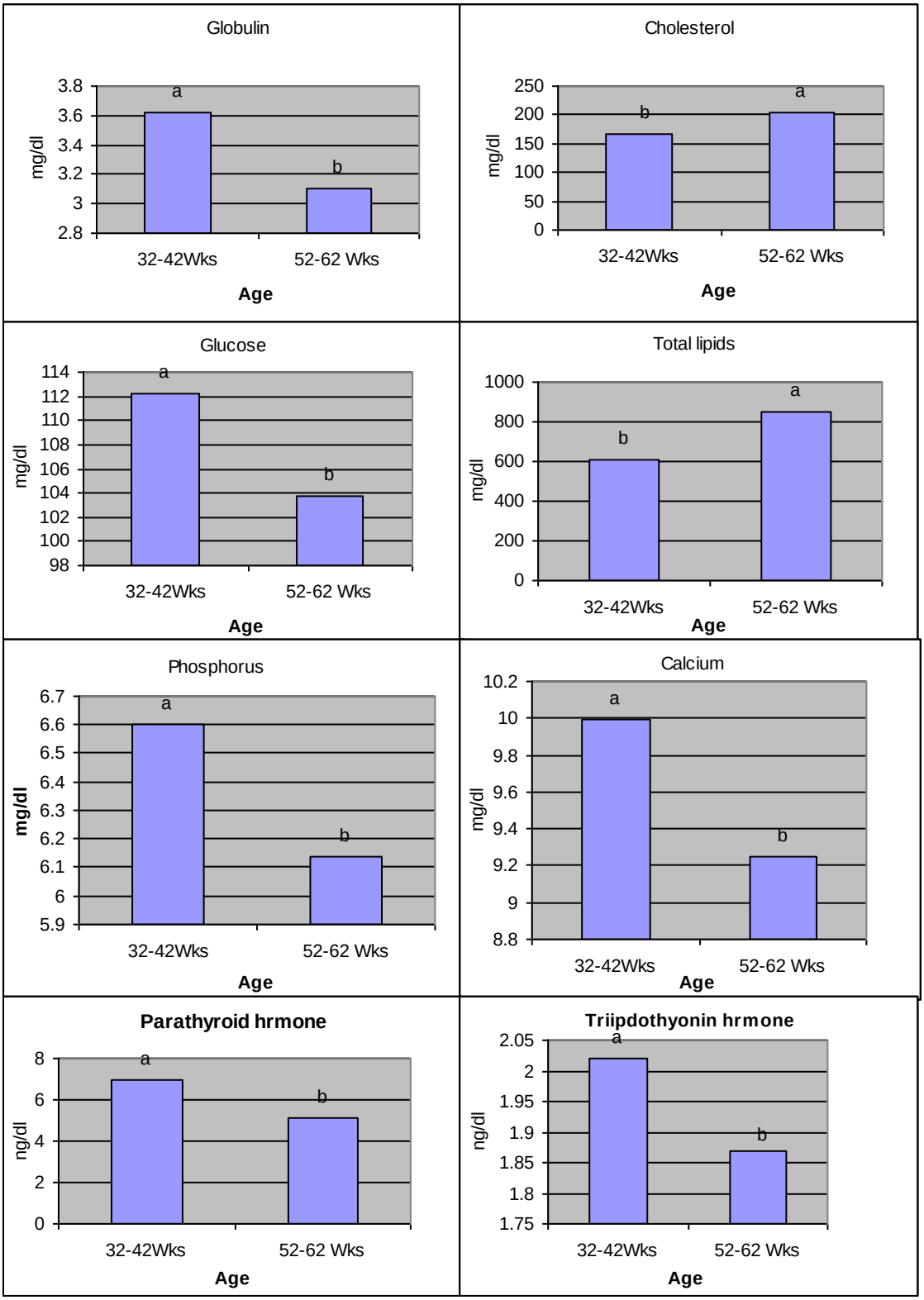
a and b means having different letters in the same row within each trait are significantly different (P≤0.05).

Table(6): Effect of Mandarah chicken age stage with reference to osteopontin protein on relative asymmetry of lengths for wing, tibia, tarsus and femur of embryos at day 15 and baby chicks

Items	Relative asymmetry (RA)							
	Wing length		Tibia length		Tarsus length		Femur length	
	32-42 wks	52-62wks	32-42 wks	52-62wks	32-42 wks	52-62wks	32-42 wks	52-62wks
Embryos at day 15	3.87±0.41^{bA}	5.06±0.40^{aA}	4.60±0.07^{bA}	5.94±0.15^{aA}	4.08±0.13^{bA}	5.36±0.19^{aA}	5.22±0.05^{aA}	5.88±0.04^{bA}
Baby chicks	2.58±0.28^{bB}	4.04±0.19^{aB}	3.07±0.13^{bB}	4.25±0.10^{aB}	3.04±0.09^{B}	3.25±0.13^{B}	3.87±0.14^{bB}	4.69±0.29^{a}

a and b means having different letters in the same row within each trait are significantly different (P≤0.05).
A and B means having different letters in the same column are significantly different (P≤0.05).

Fig1. Effect of parental Mandarah chicken age with reference to osteopontine protein on plasma biochemical parameters of hatched chicks



REFERENCES

Abudabos,A. 2010. The effect of broiler breeder strain and parent flock age on hatchability and fertile hatchability. Inter. J. of Poul. Sci., 9: 231-235.

Ahmed, A.M.; Rodriguez-Navarro, A.B.; Vidal, M.L.; Gautron, J.M.; Garcia-Ruiz, J.M., and Nys, Y. 2005. Changes in eggshell mechanical properties, crystallographic texture and in matrix proteins induced by molt in hens. Br. Poult. Sci., 46: 268-279.

Alfonso-Torres, K.A. ; Gargaglioni, L.H. ; Pizauro, J.M. ; Faria Filho, D.E. ; Furlan, R.L., and Macari, M. 2009. Breeder age and bone development in broiler chicken embryos. *Arq. Bras. Med. Vet. Zootec.,* 61: 219-226.

Arazi, H.; Yoselewitz, I.; Malka, Y.; Kelner, Y.; Genin,O., and Pines, M. 2009. Osteopontin and calbindin gene expression in the eggshell gland as related to eggshell abnormalities . Poul. Sci. 88:647–653

Association of Official Agricultural Chemists (AOAC). 1955. Official Methods of Analysis. 8th ed. Association of Official Agricul-tural Chemists, Washington, DC.

Association of Official Analytical Chemists (AOAC). 1998. Official Meth-ods of Analysis. 16th ed. AOAC International, Gaithers- burg, MD..

Bennett, J.K., R.K. Ringer, R.S. Bennett, B.A. Williams breaand P.E. Humphrey. 1988. Comarison of breaking strength and shell thickness as evaluators of eggshell quality. Environ. Toxicol. Chem., 7: 351-357.

Bruzual, J.J.; Peak, S.D.; Brake, J., and Peebles, E.D. 2000. Effects of relative humidity during the last five days of incubation and brooding temperature on performance of broiler chicks from young broiler breeders . Poult. Sci.,. 79:1385–1391.

Celen, M.F.; Yildirim, I.; Parlat, S., and Alkis, E., 2009. The effect of broiler breeder age and extra oxygen addition into incubator at high altitude (1700m) on hatching results and subsequent performance in broilers. J. Anim. Vet. Adv., 8:1438-1442.

Chien, Y.C.; Hincke, M.; Vali, H., and McKee, M., 2008. Ultrastructural matrix-mineral relationship in avian eggshell, and effects of osteopontin on calcite growth in vitro. J. of Structural Biology 163: 84-99.

Christensen ,V. L. ; Donaldson, W. E. , and Mc Murtry ,J. P. 1996. Physiological differences in late embryos from turkey breeders at different ages. Poult. Sci., 75: 172-178.

Decuypere, E.; Tona,K.; Bruggeman,V.,and Bamelis, F. 2001. The day-old chick : a crucial hinge between breeders and broiler . World's Poult. Sci. J., 57:127-138.

Dhawale, A. 2008. Abnormal eggs cause subnormal profits. World Poult., 24:20-23.

Dixon, L. M.; Sparks, N. H. C. , and Rutherford, K. M. D. 2016. Early experiences matter: a review of the effects of prenatal environment on offspring characteristics in poultry. Poult. Sci., 95: 489-499.

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

Durmus, I.; Yenica, E., and Demirtas, S.E., 2007. Egg abnormality. J. Poult. Res. 7: 66-71.

Eshratkhah, B.; Pourparsa, A.A.; Rostami,S., and Navid, M.R.A.2011. Relationship between the variations of blood thyroid hormones concentrations, their percentage and some biochemical parameters during different ages of layer breeder. Global Veterinaria, 6: 459-467.

Favero , A.; Vieira , S. L. ; Angel , C. R. ; Bos-Mikich , A.; Lothhammer , N., and Taschetto , D. ; Cruz , R. F. A. , and Ward T. L. 2013. Development of bone in chick embryos from Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from

inorganic and amino acid-complexed sources. Poult. Sci.,. 92 :402–411.

Fernandez, M.S.; Escobar, C.; Lavelin, I.; Pines,M., and Arias, J.L., 2003. Localization of osteopontin in oviduct tissue and eggshell during different stages of the avian egg laying cycle. J.Struct. Biol. 143:171-180.

Fraser, A.C.; Bain, M.M. and Solomon, S.E. 1998. Organic matrix morphology and distribution in the palisade layer of eggshells sampled at selected period during lay. Br. Poult. Sci. 39: 225-228.

Gautron, J. ,and Nys, Y. 2006. Eggshell matrix proteins and natural defenses of the egg. Symposium COA/NRA Scientific Cooperation in Agriculture, Taiwan, November 7-10.

Hamidu,J.A.; Fasenko, G.M.; Feddes, J.J.R.; O'Dea, E.E. ; Ouellette, C.A.; Wineland, M.J., and Christensen,V.L. 2007. The effect of broiler breeder genetic strain and parent flock age on eggshell conductance and embryonic metabolism. Poul. Sci., 86:2420–2432

Hincke, M.T.; Nys, Y.; Gautron, J.; Mann, K.; Rodriguez-Navarro, A.B., and McKee, M. 2012. The eggshell: structure, composition and mineralization. Frontiers in Bioscience, 17:1266-1280.

Hocking, P.M.; Maxwell, M.H.; Robertson, G.W., and Mitchell, M.A. 2001. Welfare assessment of modified rearing programmes for broiler breeders. Br. Poult. Sci., 42:424-432.

Hunton, P. 1995. Understanding the architecture of the eggshell. World's Poult. Sci. J. 51:140-147.

Ibelli, A.M.G. Fornari, M.B.; Neis, K.L.; Zanella, R.1,5; Tessmann, A.L.; Pandolfi, J.R.C.; Cantão, M.E.; Ledur, M.C. ,and Peixoto, J.O. 2013. Associations of the osteopontin gene with performance traits in a paternal broiler line. Resumos do 59 Congresso Brasileiro de Geneticia . 16 a 19 de setembro.

Jiang , R. S.; Xie ,Z.; Chen ,X.Y., and Geng, Z.Y. 2010. A single nucleotide polymorphism in the parathyroid hormone gene and effects on eggshell quality in chickens . Poul. Sci., 89 :2101–2105.

Joseph, NS. and Moran, Jr. E.T. 2005. Effect of flock age and postemergent holding in the hatcher on broiler live performance and further-processing yield. J. Appl. Poult. Res.14:512–520.

Kaweewong, K.; Garnjanagoonchorn, W.; Jirapakkul. W., and Roytrakul, S. 2013. Solubilization and identification of hen eggshell membrane proteins during different times of chicken embryo development using the proteomic approach. Protein J., 32:297-308.

Karlsson, O., and Lilja, C. 2008. Eggshell structure, mode of development and growth rate in birds, Zoology (jena)111: 494-502.

Latour, M.A.; Peebles, E.D.; Boyle, C.R.; Doyle, S. M.; Pansky, T., and Brake, **J.D**. **1996.** Effects of Breeder Hen Age and Dietary Fat on Embryonic and Neonatal Broiler Serum Lipids and Glucose.Poult. Sci., 75:695-701.

Latour, M.A,; Peebles, E. D. ;. Doyle, S. M .; Pansky T.; Smith, T. W. , and Boyle,C., 1998. Broiler breeder age and dietary fat influence the yolk fatty acid profiles of fresh eggs and newly hatched chicks. Poul. Sci., 77:47–53.

Lavelin, I.; Meiri, N., and Pines, M. 2000. New insight in eggshell formation. Poult. Sci., 79:1014-1017.

Lavelin, I., Yarden, N.; Ben-Bassat, S.; Bar, A. and Pines, M. 1998. Regulation of osteopontin gene expression during egg shell formation in the laying hen by mechanical strain. Matrix Biol. 17:615-623.

Leeson, S. and Summers, J.D. 2000. Broiler Breeder Production. University Books, Guelph, Ontario, Canada.

Lourens, A.; Molenaar, R.; Van den Brand, H; Heetkamp, M.J.W.;

Meijerhof, R., and kemp, B. 2006. Effect of egg size on heat production and the transition of energy from egg to hatchling. Poult. Sci., 85:770-776.

Mazzuco, H.,and Bertechini, A.G. 2014.Critical points on egg production: importance and incidence of eggshell breakage and defects. Cienc. Agrotec., Lavars, 38: 7-14.

McNabb, A. F. M., and Wilson C. M. 1997. Thyroid hormone deposition in avian eggs and effects on embryonic development. Amer. Zool. 37:553-560 .

Moller, A.P. 1990. Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. Anim. Behav., 40:1185-1187.

Moreki, J.C. 2005. The influence of calcium intake by broiler breeders on bone development and egg characteristics.Ph.D. Thesis,Fac.of Natural and Agricultural Sciences. University of the Free State.

Noda, M., and Rodan,G.A. 1989. Transcriptional regulation of osteopontin production in rat osteoblast-like cells by parathyroid hormone. J. Biol. Chem., 108: 713-718.

Nowaczewski ,S.; Babuszkiewicz, M., and Kaczmarek, S. 2016 Effect of broiler breeders' age on eggshell temperature, embryo viability and hatchability parameters Ann. Anim. Sci., 16 : 235–243.

Nys, Y., and Gautron, J., 2007. Structure and Formation of the Eggshell. In: Huopalahti, R., Lopez-Fandino, R., Anton, M., and Schade , R. eds., Bioactive Egg Compounds. Springer.

Nys, Y.; Gautron, J.; Garcia-Ruiz, J.M., and Hincke, M.T., 2004. Avian eggshell mineralization: biochemical and functional characterization of matrix proteins.CR Palevol., 3: 549-562.

Nys, Y.; Hincke, M.T.;Arias ,J.L.; Garcia-Ruiz, J.M., and Solomon, S.E. 1999. Avian eggshell mineralization. Poultry Avian Biol. Res., 10: 142-166.

Panheleux, M.; Nys, Y.;Williams, Y.; Gautron, J.; Boldicke,T., and Hincke, M.T. 2000. Extraction and quatification by ELIZA of eggshell organic matrix proteins (Ovocleidin-17, Ovoalbumin, Ovotransferrin) in shell from young and old hens. Poult. Sci., 79: 580-588.

Pines, M., 2007. The involvement of matrix protein in eggshell formation. Aust. Poult. Sci. Symp., 130-133.

Preda, C.; Balaceanu, R., and Dojana, N. 2013. Evolution of hormonal control of calcium and phosphorus metabolism in different hens breeds during the laying cycle.Veterinary Medicine 70: 286-292.

Prince, C.W.; Oosawa, T.; Butler, W. T.; Tomana, M.; Bhown, A.S.; Bhown, M., and Schrohenloher, R.E. 1987. Isolation, characterization and biosynthesis of a phosphorylated glycoprotein from rat bone. J. Biol. Chem., 262: 2900-2907.

Reis, L.H.; Gama,T., and Chaveiro,M.S., 1997. Effects of short storage conditions and broiler breeder age on hatchability, hatching time, and chick weights. Poult. Sci. 76:1459–1466.

Rizk, R.E.; Nadia A. EL-Sayed; Shahein E.H.A., and Hedaia M. Shalan, 2008. Relationship between eggshell membranes and embryonic development through different egg production periods in two developed chicken strains. Egypt. Poult. Sci. ,28: 535- 551.

Rizk, R.E.; Zeweil, H.S.; EL-Zayat, M.A., ana Salma A. H. Abouhafasa, 2006. Effect of flock age and dietary fat on production and reproduction performance in Japanese quail. The Scientific Committee of European Poultry Conference, Verona, Italy, 10-14 Sept.

Rodan, S.B.; Wesolowski, G.; Yoon, K., and Rodan, G. A., 1989. Opposing effects of fibroblast growth factor and pertussis toxin on alkaline phosphatase, osteopontin, osteocalcin and type 1

collagen mRNA levels in ROS 17\2.8 cells. J. Biol. Chem. 264: 19934- 19941.

Rodriguez-Navarro, A.B.; Kalin, O.; Nys, Y.;, and Garcia-Ruiz, J.M., 2002. Influence of microstructure on the shell strength of eggs laid by hens of different ages. Br. Poult. Sci., 43: 395-403.

Romanoff, A.L. and Romanoff A.J. 1949. The avian egg . John Wiely and Sons . Inc. New York

SAS., 2004. SAS\STAT Users Guide. Version 9.1 . SAS Inst. I.C., Carry,Nc,USA.

Effects of age of broiler breeders and egg storage on egg quality , hatchability, chicks quality, chick weight, and chick posthatch growth to forty –two days. Appl. Poult. Res., 13: 10 -18.

Tsarenko, P.P. 1988. Increasing the quality of poultry products : Table and

Tumova, E.; Gous,R.M., and Tyler, N., 2014. Effect of hen age, environmental temperature, and oviposition time on egg shell quality and egg shell and serum mineral contents in laying and broiler breeder hens. Czech J. Anim. Sci., 59:435– 443.

Yalcin, S., and Siegel, P. B. 2003. Exposure to cold or heat during

Sodek, J.;Ganss, B., and McKee, M.D. 2000. Osteopontin. Crit. Rev. Oral Biol. Med., 11: 279-303.

Soliman, F.N.K.; Rizk, R.E., and Brake, J. 1994. Relationship between shell porosity, shell thickness, egg weight loss and embryonic development in Japanese quaile eggs. Poult. Sci., 73:1607-1611.

Suk, Y.O., and Park, C. 2001. Effect of breed and age of hens on the yolk to albumen ratio in two different genetics stocks. Poult. Sci., 80:855-858.

Tona, K.; Onagbesan, O.; De Ketelaere, B.; Decuypere, E., and Bruggeman, V. 2004.

hatching eggs. Agropromizdat, Leningrad, Russia.

Tumova, E.; and Ledvinka, Z. 2009. The effect of time of ovipostion and age on egg weight, egg components weight and eggshell quality. Archiv Fur Gefl. 73:110-115.

incubation on developmental stability of broiler embryos.Poult. Sci., 82:1388-1392.

Yang, A, E.; Dunnington, A.; and Siegel, P.B.,1997. Developmental stability in stocks of white Leghorn chickens. Poult. Sci.,76:1632-1636

الملخص العربى

التطور العظمى و صفات الفقس و تأثرهم ببروتين الاستيوبونتين فى قشرة البيض خلال فترتين فى دورة أنتاج البيض

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اجريت هذه التجربة لتحديد دور بروتين الاستيوبونتين الموجود في قشرة البيض على جودة البيض و ما يترتب على ذلك من تأثيره على الهيكل العظمي للاجنة، وصفات الفقس وبعض القياسات الفسيولوجية، إضافة إلى علاقه ذلك بعمر دجاج سلالة المندرة خلال فترتين فى دورة أنتاج البيض(٣٢-٤٢ و ٥٢-٦٢ أسبوع) .

وتتلخص النتائج المتحصل عليها كما يلي: -

١. انخفض تركيز بروتين الاستيوبونتين (نانوجرام /ميكرو لتر) الموجود فى كل من قشرة البيض الطازج وقشر البيض عند الفقس وذلك للبيض الناتج من الدجاج الاكبر عمرا مقارنة بالبيض الناتج من العمر الأصغر . وقد لوحظ ايضا نفس الانخفاض المعنوى السابق فى تركيز بروتين الاستوبونتين فى كل من عظام الساق و دم الكتاكيت الفاقسة.

٢. حدث انخفاض معنوى فى تركيز بروتين الاستيوبونتين مع زيادة العمر الجنينى فى قشر البيض بينما زاد تركيزة فى عظمة الساق.

٣. سجل البيض الناتج من الدجاج الأصغر عمرا زيادة معنوية فى قوة القشرة ، دليل شكل البيضة و سمك القشرة واغشيتها مقارنة بالبيض الناتج من العمر الاكبر.

٤. سجل البض الناتج من الدجاج الاكبر عمرا زيادة معنوية فى نسب النفوق الجنينى و زمن الفقس (ساعة) و وزن الكتاكيت الفاقسةعند الخروج (جم) بتلك الناتجة من الدجاج الاصغر عمرا بينما زادت معنويا نسبة الفقس للبيض المخصب الناتج من الدجاج الاصغر عمرا مقارنة بالبيض الناتج من ا لدجاج الاكبر.

٥. حدث انخفاض معنوى فى تركيز كل من الكالسيوم والفوسفور مع زيادة عمر قطيع الامهات فى كل من قشرة البيض الطازج و قشرة البيض خلال اوقات مختلفة من التفريخ و ايضا فى عظام الساق لكل من الاجنة عند عمر ١٥ يوم و ايضا للكتاكيت الفاقسة .

٦. حدث انخفاض معنوى فى تركيز كل من الكالسيوم، والفوسفور، وهرمون الغدة الجاردرقية (الباراثيروويد) فى دم الكتاكيت الفاقسة الناتجة من الامهات الاكبر عمرا مقارنة بالأصغر.

٧. زاد معنويا التماثل العظمى لاطوال كل من عظام الجناح والساق والكاحل والفخذ للاجنة والكتاكيت االناتجة من الامهات الاصغر عمرا مقارنة بالناتجة من الاكبر عمرا.

اظهرت الدراسة مساهمة بروتين الاستيوبونتين كبروتين رئيسى فى تركيب قشرة البيض و أثره على عملية الفقس و قد يساهم ذلك فى أيجاد بدائل جديدة لتقييم جودة قشرة البيض و أستخدامها فى برامج الأنتخاب للدجاج