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ROLE OF BIOTIN IN IMPROVING HATCHABILITY AND PHYSIOLOGICAL STATUS OF TURKEY CHICKS AS AFFECTED BY AGE OF FLOCK

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ABSTRACT: An experiment was conducted to study the effects of flock age and biotin injection at different times of incubation on hatchability and physiological status of newly hatched chicks. One thousand and six hundred and twenty hatching turkey eggs representing two flock ages (32 and 48 wks). All eggs were randomly assigned to three injection time groups, (48 hours, 7 and 24 days of incubation), then each group was divided into six treatments according to the concentration of the vitamin. The first treatment as negative control, the second treatment as a sham-injected control (Dry punch) and the third treatment was injected with 1 ml saline in the air cell through the width end of the egg as sham control. The fourth, fifth and sixth groups were injected with 75, 100 and 125 µg biotin dissolved in 1 ml saline, respectively. Generally, the results indicated that egg fertility rate showed a positive relation with avidin expression in the young-age group than in old-age group. In addition, biotin injection at the late stages of embryogenesis positively affects hatchability, embryo survival percent and body weight of the day-old chicks compared to control and sham groups and influences insulin and thyroid hormones metabolism in post-hatched poults. Based on the findings of the present study injection of turkey eggs with biotin with doses 75 and 100 µg/egg on 24^{th} day of embryogenesis for younger and older hen's markedly improved hatchability and physiological responses for post-hatched chicks.

Key words: Turkey – biotin- injection eggs - avidin -hatchability.

INTRODUCTION

Avian embryogenesis is an external process, where embryonic growth and development is influenced by both endogenous and exogenous factors (Tong et al., 2013). However, turkey embryos are dependent upon the nutrient deposits in the egg, which have a direct effect on embryonic development, hatchability, hatchlings quality and subsequently on performance (Ondulla, 2005). Therefore embryo consumes the nutrients in eggs towards the end of the incubation and begins to use body reserves for emergence (Ferket, 2006), where embryo may lose weight in this period. Many researchers indicated that embryo weight may be increased by ovo nutrient injection during the period of incubation (Ferket, 2006). On the other hand, injection of fertilized eggs with nutrients plays an important role in replacing any deficiency in the synthesis of food materials involved in the composition of the egg which may occur as a result for the possible maternal malnutrition (Selim et al., 2012). In this context, biotin is important water-soluble vitamin and is an essential coenzyme for all known organisms. Its physiologically active form is linked to enzymes of metabolic importance like biotin carboxylase and biotin decarboxylase and seems to be a key-enzyme in important processes like gluconeogenesis and fatty acids and protein synthesis. Thus it is necessary to raise the level of dietary biotin for involvement of hatchability and chick's weight especially with the progression of maternal age, (Robel 2002). Furthermore, the decreases of fertility and hatchability observed with advance hen age may be due to a decline in the ability of older hens to retain spermatozoa in the utero vaginal sperm host glands (Fasenko et al., 1992). For this reason, this increased need of using biotin to increased hatchability with the advanced age of hen. In addition biotin has an extremely high binding affinity to avidin, the secretory glycoprotein which is found in the oviduct and deposited in the albumen fraction of eggs (Foye-Jackson et al., 2011). It is probable involvement of avidin in sustaining sperm viability in sperm storage tubules in turkeys and the high affinity of avidin to biotin suggest that supplementary biotin may increase oviductal avidin expression, thereby attenuating the adverse effect of aging on hen reproductive performance. Therefore, the main objective of the present study was to investigate the effects of turkey breeder flock age (32 and 48 wk) on the avidin content of egg and treated eggs with biotin solution during incubation period on hatchability and physiological status of newly hatched turkey.

MATERIALS AND METHODS

The present study was carried out at Mehallet Moussa Turkey Research Station, Animal Production Research Institute, belonging to Agriculture Research Center.

Turkey eggs and incubation

This experiment was carried out on a total number of 810 turkey eggs for each age weighed between 90 and 95 g obtained from local strain of Turkey Bronze which reared under good husbandry conditions. Birds were fed ad-libtuma. Breed diet formulated according to NRC, (1994), which are presented in table (1). Fresh water was available all time along the experimental period. Eggs were incubated at a temperature of 37.6 ± 0.3oC and a relative humidity of 60 – 65 % in a forced draught incubator. The experimental

design was 2x3x6 factorial arrangement, the main factors were the flock age (1 and 2), injection time (1, 2 and 3) and biotin concentrations (1 to 6 µg biotin solution).

Egg injection protocol

Eggs were collected at 32 and 48 wks of breeder age. Eggs were randomly assigned to three injection time groups (48 hours, 7 and 24 day of incubation), each group was divided into six treatments (biotin concentrations treatments) and each treatment consists of equal three replicates (45 eggs each) as follows:

The first treatment without injection (control), the second group was sham-injected control (dry punch), also the third treatment was injected in the air cell through the width end of the egg with 1ml saline as sham control. The fourth, fifth and sixth treatments were injected in the air cell through the width end of the egg with 75,100and 125µg biotin dissolved in 1 ml saline. Saline injection and dry punch were included as sham control, primarily to rule out a possible negative response caused by the stress of injection and handling. The treatment solutions or sham control were injected into the air cell by using graded insulin syringe (1 ml). Prior injection eggs, the injection site was disinfected with 70% ethanol and the solutions were warmed to 30°C. The pinhole site was sealed with sterile paraffin wax immediately after injection. The injected eggs were returned to the incubator after injection immediately.

Fertility and hatchability traits:

Fertility rate was determined as a percentage of fertile eggs to total eggs. Embryonic mortality during incubation for each group was calculated as a percentage of dead embryos in each term out of the total number of fertile eggs. Hatchability of fertile eggs for each treatment group was calculated as hatched turkey poults out of the total number of fertile eggs. Hatchability of total eggs for each treatment group was determined as hatched turkey poults out of the total number of eggs. Live body weight (LBW) of turkey poults was recorded at hatch.

Blood parameters:

Plasma biochemical analysis: During slaughter chicks after hatched individual blood samples were withdrawn from three chicks within each treatment in heparinized test tubes; then centrifuged at 3500 rpm for 15 minutes to get blood plasma. Plasma samples were stored at – 20 °C until analysis to determine total protein, albumin, globulin, however, globulin was obtained by subtraction of plasma albumin from total protein, total lipids, triglycerides and cholesterol concentrations were spectrophotometerically determined by using available commercial kits as described by the manufacturer companies (Spectrum, Diagnostics, Egypt. Co. for Biotechnology S.A.E). The radioimmunoassay (RIA) method was used for the determination of triiodothyronine (T_3) and insulin hormones using commercial RIA kits.

Electrophoresis method

Chemicals, equipment and reagents

All chemicals that used in the present study were purchased from Sigma Chemicals Co. Apparatus used in this study was from Cleaver Scientific Ltd, UK, Model V10-WCDC, size 20x10cm dual slab cell (SDS - PAGE). All reagents used in this study were prepared according to electrophoresis instruction manual of BioRad, Laemmli (1970). Sample preparation egg white (Albumin) samples were taken from the chosen breeds then the egg white was mixed with

distilled water at ratio of 1:10 v/v (1v egg white: 9v H2O) for each egg separately then the samples were homogenized using MSE sonicator at 28 db for 10 minutes. Subsequently the samples were aliquoted in tubes and stored at -20 °C until used. Running sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS- PAGE). SDS polyacrylamide gel electrophoresis was carried out according to Laemmli (1970) and Bio-Rad instruction manual. The detailed method was described by Shoukry et al., (2014).

Samples preparation for electrophoresis assay.

Statistical analysis:

Data were analyzed by the least squares analysis of variance using the General Linear Models procedure of the statistical analysis model (SAS, 2004). The statistical model was as follows:

Yijlk = μ + Ti +Aj +Bl+ (TA)ij+ (TB)il+(AB)jl+ (TAB)ijl+ eijlk where :

Yijlk = An observation;

μ = Overall mean;

T = Effect of flock age; i= (1 and 2) ;

A = Effect of injection time; j = (1, 2 and 3);

B = Effect of biotin level; l= (1,2,.. and 6);

TA= Effect of interaction between the flock age and injection time;

TB=Effect of interaction between the flock age and biotin level;

AB=Effect of interaction between injection time and biotin level;

TAB=Effect of interaction between the flock age and injection time and biotin level; and eijlk = Random error component assumed to be normally distributed. The significant differences among treatments were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Fertility rate and avidin expression:

In the finding of this study fertility rate was affected by flock age where it is decreased with advancing hens' age table (3). The observation is conceded with the previous studies reported by Brommer and Rattiste, (2008) who found that age had a significant effect on fertility of breeders. It is expected that the decrease in fertility with increase hen age may be due to a decline in the ability of older hens to retain spermatozoa in the uterovaginal sperm host glands (Fasenko et al., 1992), as well as the decrease in sperm storage in older hens than younger hen turkeys (Christensen, 1981). It is well known that fertility rate have directly correlated with the sperm storage function(s) of the sperm storage tubules (SST) and with the number of sperm-containing SST (Pierson et al., 1988). Clearly, from the former information, it a matter of fact, Avidin is characterized by a high affinity for biotin; where biotin is trace substances required by the developing embryo (White et al., 1976).

SDS polyacrylamide gel electrophoresis reflected a good separation of egg white proteins from hens at each age.The separation of egg white proteins for two breeds revealed breed differences at each age in protein band densitiesand percent figure (1) and table (2). The obtained results showed that in eggs produced from 32-wk-old hens have higher densities of 15.6 kDa band and frequency percentcompared to the same band from eggs produced from 48-wk-old hens. This band is coincident with molecular weights published by (Green, 1975), who reported that avidin is a tetrameric protein, composed of subunits of identical amino acid composition and sequence (15.6 kDa and 128 amino acids each) and

it is a trace component (0.05%) of egg white. The differences found in protein band densities and frequency percent of these breeds may be relevant to uterine fluid proteins proportions changed with the age of the birds and subsequently percent of protein that declined with age could be responsible for the deterioration of egg quality (Kaur et al. 2013). Results reported herein lend some support to the suggestion by previous authors (Daryabari et al., 2014) who reported that avidin mRNA expression might be associated with fertility rate, and that their expression is decreased in aging birds. Similar results were demonstrated by Foye-Jackson et al., (2011) who suggested that an antimicrobial property to avidin, which decreases the availability of biotin to microbial growth, thereby supporting sperm longevity and activation at the uterovaginal junction. It is probable involvement of avidin in sustaining sperm viability in sperm storage tubules in turkeys and the high affinity of avidin to biotin suggest that supplementary biotin may increase oviductal avidin expression, thereby attenuating the adverse effect of aging on hen reproductive performance.

Hatchability traits:

Effect of flock age.

The data presented in table (3) showed that eggs produced from flock at 32 weeks of age recorded significantly higher values of hatchability than eggs produced at 48 weeks of age. This may attributed to age of hens, may effect on eggs quality (Joseph and Moran, 2005). This finding was somewhat similar to the results of Elibol and Brake (2006), noted that hatchability of hatching eggs was significantly decreased by 24.41% by increasing flock age from 30 wks to 53 wks of age. However, hatchability percentage of fertile eggs significantly improved for hens aged 32-wks compared to those aged 48-wks. The results in the present study are in agreement with those obtained by Ulmer-Franco et al., (2010) who reported that flock age did significantly affected on hatchability of fertile eggs.

On the other hand, hatchability reduction of eggs resulted from older broiler breeders is a result of many contributing factors, including: larger egg size (Leeson and Summers, 2000), increased early and late embryo mortality (Elibol and Brake, 2003), poorer shell quality due to bigger surface area (Bennett, 1992) albumen quality deterioration (Tona et al., 2004) and increased the yolk cholesterol content (Dikmen and Sahan, 2007).

Effect of injection time.

As presented in table (3) the data indicated that injection time had highly significant effects on hatchability traits. However, in ovo administration of biotin on day 24 of incubation was more efficient than the other injection time It seems clear from this data that in ovo biotin especially at late embryonic life (24 of incubation) could show activity for processes of gluconeogenesis during embryonic life and consequently this vitamin contributes to such important processes as reproduction, bone development and growth (McMahon, 2002) therefore, it is probable that the most optimal time for the introduction of biotin into chicken eggs with the aim of improving hatchability during the period 21^{th} and 25^{th} d of incubation. This may indicate insufficient endogenous of biotin by embryos accompanied by a simultaneous, increased demand Biotin, especially towards the final period of incubation.

Effect of biotin dose.

Hatchability percentage of fertile eggs were significantly improved by injection biotin where the highest values observed for eggs injected with dose 100µg compared with the other treatments table (3). This finding agreed with Robel (2002) found that in ovo injection of turkey eggs with biotin improved hatchability of fertile eggs. Also, Robel and Christensen (1987) reported that the injection of turkey eggs with 87 µg /egg D-biotin at 25 day of incubation lead to improvement of hatchability compared with control.

It may be suggested by the fact that, the improvement in hatchability was due to a reduction in the numbers of early dead, late dead in shell embryos, where, eggs produced from breeders fed biotin deficient diets produced healthy embryos after the eggs were injected with biotin between 72 and 96 hours of incubation (Couch et. al., 1948). Biotin is important water-soluble vitamin, biotin is a cofactor in carboxylation and decarboxylation reactions. These reactions have important roles in anabolic processes and in protein metabolism (Calnek et al., 1997), and which reflect on embryo development and growth.

Results in table (4) showed that there were significant improvements in hatchability traits for eggs produced from hens aged 32-wks of age, which injected with a dose 75µg /egg of biotin followed by eggs produced from hens aged 48-wks old hens of age which received 100 µg biotin /egg of vitamin biotin with injection at day 24 of incubation as compared with other interaction.

Embryonic mortality percentage:

Effect of flock age.

Eggs produced from younger hens had lower embryonic mortality rate than those of the older ones table (3). This result was consistent with those reported by Elibol and Brake (2008) who found that embryonic mortality was much lower for broiler chickens, when age was 29-wks than those produced at 48-wks of age at all stages of embryo development. Also, the dead embryos increased during incubation period due to increase flock age (Zakaria et al. (2009). The high embryonic mortalities can be related to changes in egg weight and shell quality observed with increased hen age, which is presumed to ultimately influence the hatchability of eggs (Cloete et al., 2006).

Effect of injection time.

Injection time had highly significant effects on embryonic mortality rate. In ovo administration of biotin on day 24 of incubation was lower efficient on embryonic mortality than the other injection time treatments table (2). Generally, embryonic mortality occurs at the 10^{th} –13^{th} day of incubation. It is believed that the immediate cause of death of embryos is a disturbance of oxidation of fatty acids and the occurrence of hypoglycemia in this stage (White and lee, 1996).

The results of this experiment reveal that the injection of biotin at the beginning of embryogenesis may disturb the course of poult embryo development. It has been shown from previous studies on chicken embryo that only the lower dose of biotin (i.e. 60 µg) decreased hatchability which was evoked by a significant elevation in embryonic mortality following the manipulation. This explanation can be supported by fact that the sensitivity of the chicken embryo to in ovo manipulation is very high at early stages of embryogenesis and it decreases gradually during embryonic development. Several studies have demonstrated that

in-ovo injection of some nutrients may cause nutrient imbalance inside the eggs, and consequently may limit maximal growth and development of the embryo during early stages of incubation (Uni, 2014). It is thought that disturbance in embryo homeostasis caused by the applied in ovo manipulation is the main reason for its mortality (Bruggeman et al., 2003).

Effect of biotin dose.

Results in Table 3 showed that embryonic mortality of fertile eggs was significantly low for eggs injected with different levels of biotin as compared with the control groups. The increase in hatchability of turkey eggs, which was observed in this study following biotin injection may attributed to biotin is vitamin with important characteristics due to the presence of the inhibitors avidin in egg albumen, which affects biotin levels or biotin availability in the egg, and its availability to the embryo, and may affect egg hatchability (Robel, 1987). It is important an increased need of biotin for hatchability especially in aging hens is considered to coincide with the higher metabolic need of biotin with the advancing of age in the hen.

Results in table (4) showed that eggs produced at different ages which injected with 75 µg Biotin /egg at late stage of incubation had the lowest values for embryonic mortality compared with other interaction.

Chicks weight:

Effect of flock age.

Chicks weight (g) was significantly affected by flock age. Live body weight of chicks produced from 48-wks-old hen was increased as compared to those produced from 32-wk-old hens table (3). The same outcome was observed by Tona et al. (2004) who reported that chicks hatched from eggs produced from eder flock age had higher initial body weights than those produced from younger one . While, Applegate and Lilburn, (1999) found that hatching body weight (BW) in turkeys was greater in younger breeder hens than older breeder hens and that these differences are attributed to egg weights and also differences in villus height and width can affect BW and performance, and thus increased surface area available for nutrient absorption due to enhance growth and production. On the other side, in turkeys, because embryos from older parent flocks are normally larger than embryos from younger parent flocks (Lewis and Morris, 1998), it can be assumed that the embryos have more cells. Therefore, the increased metabolize in eggs from older parent flocks could be related to the higher number of embryonic cells present. This is clearly shown by the larger embryonic weight observed as the parent flock age increased.

Effect of Injection time.

Prolonged injection time at early days of incubation resulted in a decrease in chicks weight at hatch as compared with later days of incubation table (3). In the present study, results are consistent with the report of Amitav et al. (2007) on broiler body weight. Previous studies indicated that amino acid injected in to early stage (7 th d) of incubation might be detrimental on chick growth and could be avoided by injecting on 14 th d as demonstrated in our study, furthermore, that best time of injection to achieve highest chick weight at last week of incubation (Pulikanti et al., 2010) who reported that the main reason for this case is that accumulation of yolk-derived lipids increases in embryo tissues and absorption in this stage.

Effect of biotin dose.

Poult turkey weight (g) at hatch was significantly heavier for eggs produced from hens injected with 100 µg /egg biotin followed by 75 and 125µg /egg biotin than the control treatments. These results are in close agreement with those reported by Abdul -Lateif and Abdulateef (2012) who reported that in-ovo injection with biotin in chick egg improved chick weight at hatch. An adequate carry-over of biotin to the hatching chick is important for its subsequent growth and viability (Whitehead and Bannister 1980). The same authors stated that chicks deficient in biotin reserves show decreased growth potential and increased mortality even when fed diets adequate in biotin. It is well known that biotin is an essential coenzyme for all known organisms. Its physiologically active form is linked to enzymes of great metabolic importance like biotin carboxylase and biotin decarboxylase and a key-enzyme in important processes like gluconeogenesis and fatty acids and protein synthesis and consequently this vitamin contributes to such important processes as reproduction, bone development and growth (McMahon, 2002).

Results in Table 4 showed that the highest value of poult turkey weight obtained form eggs produced from 32-wks-old hens and which received 75 µg /egg of vitamin biotin followed by eggs produced from 48-wks-old hens which received 100 µg /egg of vitamin biotin with injection at late stage of incubation periods as compared with other interaction treatments.

Some blood parameters:

Total proteins:

Effect of flock age.

Blood serum proteins are a significant indicator of the health condition and production features of the organism because of their numerous roles in the physiology. Among numerous factors that influence the concentration of serum proteins, age, it plays an important role in the cell physiology. Plasma total proteins, albumin and globulin concentrations were significantly higher in turkey poults produced from 30-wks-old hens than those produced from 48-wks-old hens table (5). It is well known that yolk sac endoderm is the major, if not exclusive, source of lipid and protein constituents of embryonic serum and plays an important role in the regulation of vascular integrity in developing chick (Nakazawa et al., 2011). However, at hatch, the chicks of the older breeders had a higher yolk absorption but had lower relative yolk absorption compared with those of younger breeders. Also, rate of yolk absorption by embryos from 52 wks of age breeders decreased in comparison with those of 36 wks of age breeders (Şahan et al., 2014). This observation is consistent with results from previous studies, which demonstrated that the absorption and utilization of nutrients from the yolk sac by the embryo are affected by breeder age (Yadgary et al., 2010; Nangsuay et al., 2011). Furthermore, to evaluate metabolic status and health, apart from the concentration of protein fractions, it is important to know the increasing concentrations of the main total protein and albumin may be explained by the very quick somatic growth(mainly muscle tissue hypertrophic growth), as described in turkey (Vasicek et al., 1991). The strong increase in the serum globulin concentration may be associated with the improving immune status of the birds in

the post hatch period, Lisano and Kennamer (1977). This finding is in line with Flores et al., (2016) who found the highest immunoglobulin (IgM) titer was obtained in chicks from 36 wks old breeders, and the lowest, in chicks from 53 wks old breeders, while no differences in immunoglobulin (IgG, or IgA) titer were observed between breeder ages in Ross chicks.

Effect of Injection time.

Prolonged injection time at early days of incubation resulted in a decrease in plasma total proteins, albumin and globulin concentrations at hatch as compared with those injected at late days of incubation table (5). This result could be explained by albumen proteins are known to flow into the amniotic cavity, the yolk sac and finally the digestive tract of the embryo and are used as the main source of proteins for tissue synthesis at latest ages of incubation. (Willems et al 2014). Ratio of A/G was decreased at late days of incubation as compared with those injection data at early days of incubation which may indicates improved immunity. Immune system development begins early during embryogenesis until after post hating. Bursal precursor cells may be detected by 7 days of incubation, and cells expressing surface IgM, IgG, and IgA may be detected at 10 days of incubation (Bradley, 2012).

Effect of biotin dose.

Plasma total proteins (TP), albumin and globulin concentrations were increasing significant in group which injected with 75 µg biotin /egg compared with the other treatment groups table (5). The results of the current study in line with the findings of Abdul -Lateif and Abdulateef (2012) who found that plasma total proteins and proteins fractions were increasing significant in ovo injection biotin groups. From findings and observations in this work, it can suggest that elevated plasma TP levels in biotin treated groups may be due to physiological potential effect of this vitamin, where as, biotin might play an indirect role in protein synthesis in vivo through its involvement in the synthesis of oxalacetic acid for operation of the citric acid cycle as well as in ATP formation itself (Dakshinamurti and Litvak 1970), as well as, biotin is required for the synthesis of the inducible malic enzyme and ornithine trans carbamylase during it is involved indirectly in the biogenesis of a 4-carbon unit which is essential for synthesis of these enzymes. In addation, oxalacetic acid, the primary 4-carbon unit the synthesis of which is catalyzed by pyruvate carboxylase, is a source of aspartic acid.

Also, a significant increase was observed in the plasma globulin concentration in biotin groups compared to control groups and suggested that the increase of the plasma globulin may be due to the immunostimulant effect of biotin. This finding is in line with Yu et al., (2005) who reported that enhances development of broilers, elevates weight index, improves antibody titers to Newcastle disease, and significantly enhances blood B-lymphocyte and splenic T- and B-lymphocyte transformation ratios were resulted to add biotin to a wheat-casein diet in broilers.

In the present study the level of plasma A/G ratio showed significant decrease in biotin groups compared to the control groups. Ratio of A/G was decreased with the injection of biotin which may indicates improved immunity for the birds and this was implied by the increase in the globulin levels in this groups.

Results in table (6) showed that there were significant improvements in plasma total proteins of poults turkey obtained form eggs produced at different ages of turkey hens which injected with 100 µg /egg of vitamin biotin at late stage incubation periods as compared with other interaction treatments.

Plasma lipids profile:

Effect of flock age.

Plasma total lipids, triglycerides and cholesterol concentrations were significantly lower in turkey poults produced from 32-wks-old hens than those produced from 48-wks-old hens table (7). This finding was somewhat similar to the results of Latour et al. (1996) who noted that serum cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol concentrations were lower in those chicks from hens at 26 wk when compared with those at 36 wk of age. Breeder age can influence in metabolism of lipids in chicks and may be due to differences in the enzymatic activities of key lipolytic enzymes or the hormone levels in chicks. Normal yolk lipid transfer during the last week of incubation is accompanied by a large accumulation of lipid within the liver of embryos (Noble and Moore, 1964). However, biochemical and morphological evidence suggest that liver lipids in embryos from young hens are absorbed less efficiently than in embryos from mature hens (Noble and Cocchi, 1990). The observed changes suggest that many of the physiological and biochemical processes function differently in embryos from parents of different ages. On the other hand, this can be explained by a higher yolk absorption and yolk availability more greatly influencing the yolk-free BW from eggs of older hens compared with younger hens on d 18 and at hatch, that reflect changes in metabolism of lipids in chicks (Şahan et al., 2014).

Effect of Injection time.

Prolonged injection time at early days of incubation resulted in a increase in plasma total lipids and triglycerides (TG) concentrations at hatch as compared with those injected at late days of incubation table (7). Evidently, the embryo turkey orally consumes the amniotic fluid and yolk sac beginning at day 21- day 23 and continues slowly through incubation. It is therefore expected that improvement of plasma lipid metabolism these days. According to Speake et al. (1998) also they reported that during the last week of incubation, the chick's embryo mainly utilizes lipids from the yolk sac as an energy substrate for growth. The yolk residuals have been estimated to supply 90% of the total caloric needs of a chicken embryo (Noy and Sklan, 1999).

Effect of biotin dose.

Plasma total lipids, triglycerides and cholesterol concentrations were significantly decreased in biotin groups compared with the other treatment groups table (7). The results of the current study are in line with the findings of Abdul - Lateif and Abdulateef (2012), who shown improvement of plasma lipid metabolism in biotin groups compared to control group.

Biotin is a micronutrient required for promotion of growth and improvement of plasma lipid metabolism (Oloyo and Ogunmodede, 1989). For example, in process fatty acid synthesis, Wakil and Bressler, (1962) reported that acetyl CoA is carboxylated to malonyl CoA in the presence of a biotin-enzyme known as acetyl CoA carboxylase. It is therefore expected that biotin deficiency led to reduced acetyl CoA carboxylase activity

with consequent depression in lipogenesis. Also, the same trend, if malonyl CoA is an intermediate in cholesterol synthetic pathway as reported by Brodie et al. (1963).

Results in table 8 showed that there were significant improvements in plasma lipids profile of chicks turkey which gat us form eggs produced 32-wks-old hens which injected with 75 µg /egg of vitamin biotin followed by eggs produced from 48-wks-old hens which injected with 100 µg /egg of vitamin biotin at late stage of incubation periods as compared with other interaction treatments.

Insulin

Effect of flock age.

It is clear from the results of table (7) that insulin concentration was significantly changed with advancing flock age. The concentration of insulin was significantly increased in the blood plasma of turkey poults produced from 30-wks-old hens compared with those produced from 48-wks-old hens table (7).This can be explained by that quail liver and pancreas weights expressed as a percentage of whole body weight was greater in embryos from eggs produced by young hens compared with those from the oldest hens (Yildirim, 2005). Therefore, the possibility cannot be excluded increasing plasma insulin concentrations in poults from eggs produced by young hens compared with those from the old hens.

Effect of Injection time.

Prolonged injection time at early days of incubation resulted in a decrease in plasma insulin concentrations at hatch as compared with that injected at late days of incubation table (7).This finding may be attributed that insulin begins to be secreted from β-cells on day 4 of embryonic life, and the secretion rate could considerably increase on Days 15 and 22 of incubation to hatching day in chicks turkey (McMurtry et al, 1998).

Effect of biotin dose.

Plasma insulin concentrations were increased in biotin groups compared with the other treatment groups, table (7). The results of the current study are in agreement with the findings of Vega-Monroy et al., (2013) who found that serum insulin levels increased in biotin-supplemented mice. Moreover, this result reflected physiological response to biotin via increase in insulin of blood plasma. Where as, biotin supplementation augmented the proportion of beta cells by enlarging islet size and, unexpectedly, also increased the percentage of islets with alpha cells at the islet core (Bellairs and Osmond, 2005).

Results in table 8 showed that there were significant improvements in plasma insulin of poults turkey which gat us form eggs produced 32-wks-old hens which injected with125 µg /egg of vitamin biotin followed by eggs produced from 48-wks-old hens which injected with 125 µg /egg of vitamin biotin at late stage of incubation periods as compared with other interaction treatments.

Thyroid hormone

Effect of flock age.

There are related to changes in the embryonic metabolism associated with alterations in thyroid hormones concentration in blood circulation. It is well known that thyroid hormones play an important role in the development of many systems in all birds (Van der Geyten et al., 2002). The increase in tri iodothyronine (T_3) at the end of incubation is necessary for stimulation of growth and differentiation, and also for preparation of the chick for a life outside

the egg by regulating processes such as yolk sac retraction, the onset of pulmonary respiration, hatching, and the initiation of endothermic responses (Reyns et al., 2003).

In this concern, it appears that flock age has insignificant effects on plasma T_3 concentration. Statistical analysis of data reflect the slightly increase in plasma T_3 with age table, (7). Embryos from older parent flocks are normally larger than embryos from younger parent flocks (Lewis and Morris, 1998), it can be assumed that the embryos have more cells. Therefore, the increased heat production in eggs from older parent flocks could be related to the higher number of embryonic cells present. This is clearly shown by slightly higher plasma T_3 concentration observed as the parent flock age increased.

Effect of Injection time

Statistical analysis of data reflect the slightly increase in plasma T_3 for poults produced from egg injected at day 24 of incubation compared with that injected earlier table, (7). The increase in the thyroid hormone levels in the blood circulation at the last stage of embryogenesis is indispensable for the hatched chick (Deeming et al., 2004), and is correlated with the rate of the hatching processes (Sechman et al., 2006). It has already been established that the alterations in the T_4 and T_3 concentrations during the last phase of embryogenesis, and after the hatching process, resulted in the function of the hypothalamo-thyroid axis, which is responsible for thyroidal T_4 secretion, and the activity of deiodinase type I (D1) and type III (D3) in the liver and kidneys. According to Lu et al., (2007) observed constant levels of T_3 in chicken embryos during mid-incubation, but it peaked the day before hatch. A sharp rise in T_3 was associated to embryonic switching to lungrespiration.T_4 levels reached high levels and stayed high during amnion consumption (between 17 and 20 days of incubation in chickens and 22 and24 days in turkeys), and decreased after hatch. Elevated thyroxin levels are considered important for stimulating a variety of developmental and metabolic processes necessary for hatching. Therefore, it seems that any in ovo injection at early embryonic life can be harmful for the internal environment susceptibility and may have negative effects on hatching processes.

Effect of biotin dose.

Plasma T_3 concentrations were increased significant in biotin groups compared with the other treatment groups table (7). Nevertheless, in the available literature there are no data showing the influence of biotin on the function of the hypothalamic–pituitary–thyroid (HPT) axis during turkey embryogenesis. In the present experiment noted that the release of T_3 from the thyroid gland into the blood circulation after hatching in the group treated with biotin at a dose of 125 µg / egg was significantly higher than the other treatment groups. It can be concluded that the observed changes in T_3 levels are the result of changes in the metabolism of this hormone in peripheral tissues such as the liver under the influence of biotin.

Results in table (8) showed that there were significant improvements in plasma T_3 of chicks turkey obtained form eggs produced 32 wks old hens which injected with 75 µg /egg of vitamin biotin followed by eggs produced from 48 wks old hens which injected with 100 µg /egg of vitamin biotin at late stage of

incubation periods as compared with other interaction treatments.

CONCLUSIONS

It can be concluded from this study that the in ovo injection of biotin especially at the levels of 75 and 100 on 24 d of incubation would improve hatchability rate, survival embryo and certain blood serum characteristics. Negative effects of biotin on hatching success in the present experiments seem to be related to the early timing of its administration and advance the age. Beneficial effects of biotin on post hatch endocrine system may be expected in birds supplemented in ovo at the final stages of development. In order to understand the molecular mechanism of biotin action on expression of avidin in eggs, more research is needed in studies addressing age-related fertility.

Table (1): Composition and chemical analysis of the experimental rations used during the reproductive period.

Ingredients	Diets Quantity %
Yellow corn (8.5%)	70.01
Soybean meal (44 %)	19.80
Corn gluten meal (60%)	3.00
Limestone (CaCo3)	5.19
Calcium diphosphate (CaHPO4)	1.23
Sodium Chloride (Na Cl)	0.37
Pre-mix*	0.30
DL-Methionine	0.10
Total (kg)	100
Calculated analysis:	
ME (Kcal / kg)	2900
Crude protein %	16
Crude fat %	3.02
Crude fiber %	3.11

Table (2): Effect of flock age on protein fraction in egg albumin of turkey hens.

Lanes	*M.W.S	**M.w.	***Am. %	**M.w.	***Am. %
1	250	240	2.54	230	3.51
2		230	2	200	1.34
3	150				
4		130	5	128	5
5	100	110	10	110	10
6	75	77	15.55	77.5	15
7	50	46	7	45	6
8		45	11	44	11
9	37	34	6	35	5
10		32	16	32	15
11	25	28	11	28	12.57
12	20	24	10	24	7.53
13	15	15.5	0.05	15.5	0.03
14		14	2.46	14.2	1.41
15	10	12	1.4	12	6.61

* M.W.S = Molecular Weight Stander, , ** M.W = Molecular Weight, *** Am. % = Amount%

Table (3): Least-square means and standard errors for fertility, hatchability, embryonic mortality and hatch weight of different experimental groups as affected by studied factors.

Traits **Mean effects**	**Fertility rate%**	**Hatchability %** **(of total eggs)**	**Hatchability % (of fertile eggs)**	**Embryonic mortality %**	**Hatch weight (g)**
Flock age					
1(32 wks)	91.11±0.27 [a]	70.22±0.27[a]	82.77±0.28[a]	1.81±0.03 [b]	51.08±0.27[b]
2 (48 wks)	81.11±0.27 [b]	66.88±0.27[b]	77.60±0.28[b]	2.02±0.03 [a]	52.21±0.27 [a]
Time injection					
1 (48 h)		62.66±0.33 [c]	72.40±0.35 [c]	2.29±0.04 [a]	50.85±0.31[b]
2 (7 day)		66.00±0.33 [b]	80.04±0.35 [b]	2.07±0.04 [b]	51.65±0.35[a]
3 (24 day)		77.00±0.33 [a]	88.11±0.35 [a]	1.40±0.04 [c]	52.44±0.33 [a]
Levels of biotin					
Control		71.33±0.47 [b]	82.12±0.49[b]	1.79±0.05[c]	50.76±0.46[cd]
A shem		62.66±0.47[e]	72.79±0.49[c]	2.33±0.05 [a]	50.63±0.46[d]51
Sline		67.33±0.47[c]	74.19±0.49[c]	2.04±0.05 [b]	.23±0.48[bcd]
75 µg		68.33±0.47 [c]	89.47±0.49[a]	1.76±0.05 [c]	52.33±0.49[ab]
100 µg		77.66±0.47[a]	90.98±0.49[a]	1.62±0.05 [c]	52.74±0.48 [a]
125 µg		64.00±0.47[d]	71.54±0.49[d]	1.98±0.05 [b]	52.18±0.43[abc]
Overall mean	86.11	68.55	80.18	1.92	51.57

Mean having similar letter in each column are not significantly different ($P \leq 0.05$).

Table (4): Least-square means and standard errors for hatchability, embryonic mortality and hatch weight of different experimental groups as affected by interaction between studied factors.

Interaction			Hatchability % (of total eggs)	Hatchability % (of fertile eggs)	Embryonic mortality %	Hatch weight (g)
Flock age	Time injection	Levels of biotin				
1	1	1	76.00^{e}	80.95^{fg}	1.50^{gh}	48.88^{bcd}±1.14
		2	48.00^{L}	68.42^{j}	3.25^{a}	42.43^{e}±1.01
		3	72.00^{f}	65.22^{k}	1.75^{fg}	48.81^{cd}±1.05
		4	52.00^{k}	89.47^{cd}	3.00^{ab}	50.89^{abcd}±1.08
		5	76.00^{e}	93.33^{b}	1.50^{gh}	52.26^{abcd}±1.31
		6	56.00^{j}	59.09^{L}	2.50^{cd}	52.22^{abcd}±1.03
	2	1	72.00^{f}	80.00^{fg}	1.75^{fg}	48.73^{d}±1.18
		2	60.00^{i}	75.00^{h}	2.50^{cd}	52.43^{abcd}±1.22
		3	68.00^{g}	88.89^{d}	2.00^{ef}	51.59^{abcd}±1.31
		4	68.00^{g}	90.00^{cd}	2.00^{ef}	52.19^{abcd}±1.31
		5	76.00^{e}	89.47^{cd}	1.50^{gh}	52.16^{abcd}±1.22
		6	64.00^{h}	73.68^{hi}	1.91^{efg}	52.52^{abcd}±1.03
	3	1	72.00^{f}	80.00^{fg}	1.75^{fg}	50.02^{abcd}±1.11
		2	84.00^{c}	90.91^{cd}	1.00^{ij}	52.37^{abcd}±1.18
		3	68.00^{g}	86.71^{e}	2.00^{ef}	52.53^{abcd}±1.22
		4	97.00^{a}	97.31^{a}	0.08^{k}	54.12^{a}±1.26
		5	80.00^{d}	89.47^{cd}	1.25^{hi}	52.79^{abcd}±1.22
		6	75.00^{e}	92.00^{bc}	1.50^{gh}	52.47^{abcd}±1.11
2	1	1	68.00^{g}	81.82^{f}	2.00^{ef}	52.21^{abcd}±1.11
		2	56.00^{j}	60.87^{L}	3.00^{ab}	52.35^{abcd}±0.94
		3	68.00^{g}	52.00^{m}	2.00^{ef}	52.17^{abcd}±1.11
		4	60.00^{i}	79.17^{g}	2.50^{cd}	52.88^{abc}±1.03
		5	72.00^{f}	90.48^{cd}	1.75f^{g}	52.70^{abcd}±1.14
		6	52.00^{k}	48.00^{n}	2.75^{bc}	52.42^{abcd}±1.08
	2	1	72.00^{f}	85.00^{e}	1.75^{fg}	52.43^{abcd}±1.08
		2	56.00^{j}	69.57^{j}	2.75^{bc}	51.88^{abcd}±1.31
		3	64.00^{h}	67.33j^{k}	2.25^{de}	51.11^{abcd}±1.26
		4	64.00^{h}	90.00^{cd}	2.25^{de}	50.95^{abcd}±1.36
		5	68.00^{g}	86.36^{e}	2.00^{ef}	52.83^{abcd}±1.11
		6	60.00^{h}	65.22^{k}	2.25^{de}	51.02^{abcd}±1.08
	3	1	68.00^{g}	85.00^{e}	2.00^{ef}	52.32^{abcd}±1.11
		2	76.00^{e}	72.00^{i}	1.50^{gh}	52.32^{abcd}±1.14
		3	64.00^{h}	85.00^{e}	2.25^{de}	51.17^{abcd}±1.14
		4	69.00^{g}	90.91^{cd}	0.75^{j}	52.96^{abcd}±1.22
		5	94.00^{b}	96.82^{a}	1.75^{fg}	53.70^{a}±1.14
		6	77.00^{e}	91.30^{bcd}	1.00^{ij}	52.47^{abcd}±1.08
standard errors			±1.15	±1.22	±0.14	

a,b,c :means in the same column within each item with different superscript are significantly different P≤0.05

Table (5): Least-square means and standard errors for total protein, albumin, and globulin and A/G ratio of different experimental groups as affected by studied factors.

Mean effects	**Total protein (g/dL)**	**Albumin (g/dL)**	**Globulin (g/dL)**	**A/G ratio**
Flock age				
1(32 wks)	5.76±0.11^{a}	2.52±0.07	3.24±0.09^{a}	0.85±0.03^{b}
2 (48 wks)	5.54±0.11^{b}	2.64±0.07	2.89±0.09^{b}	0.96±0.03^{a}
Time injection				
1 (48 h)	5.42±0.13^{b}	2.65±0.09^{a}	2.77±0.11^{b}	1.01±0.04^{a}
2 (7 day)	5.50±0.13^{b}	2.52±0.09^{b}	2.98±0.11^{b}	0.90±0.04^{ab}
3 (24 day)	6.03±0.13^{a}	2.57±0.09^{b}	3.46±0.11^{a}	0.79±0.04^{b}
Levels of biotin				
Control	5.88±0.19^{ab}	2.81±0.12^{a}	3.07±0.15^{b}	0.94±0.06^{ab}
A shem	4.95±0.19^{c}	2.22±0.12^{b}	2.72±0.15^{b}	0.91±0.06^{ab}
Sline	5.69±0.19^{ab}	2.81±0.12^{a}	2.87±0.15^{b}	1.02±0.06^{a}
75 µg	5.99±0.19^{a}	2.79±0.12^{a}	3.20±0.15^{b}	0.90±0.06^{ab}
100 µg	6.06±0.19^{a}	2.39±0.12^{b}	3.67±0.15^{a}	0.76±0.06^{b}
125 µg	5.33±0.19^{bc}	2.45±0.12^{ab}	2.88±0.15^{b}	0.89±0.06^{ab}
Overall mean	5.65	2.58	3.07	0.91

Mean having similar letter in each column are not significantly different ($P \leq 0.05$).

Table (6): Least-square means and standard errors for total protein, albumin, globulin and A/G ratio of different experimental groups as affected by interaction between studied factors.

Interaction			Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio
Flock age	**Time injection**	**Levels of biotin**				
1	**1**	1	5.22^{efgh}	2.18^{defg}	3.04^{cdef}	0.76^{bcdef}
		2	5.21^{efgh}	3.02^{abcd}	2.19^{ef}	1.38^{a}
		3	5.83^{efgh}	2.83^{cdefg}	2.99^{cdef}	0.99^{abcde}
		4	5.98^{cdefgh}	2.83^{cdefg}	3.15^{cdef}	0.89^{abcdef}
		5	6.08^{bcdefg}	2.71^{defg}	3.37^{bcde}	1.05^{abcd}
		6	4.95^{efgh}	2.59^{defg}	2.36^{def}	1.09^{abcd}
	2	1	6.28^{abcdef}	3.01^{abcd}	3.26^{bcde}	0.93^{abcdef}
		2	5.02^{efgh}	1.99^{defg}	3.03^{cdef}	0.71^{bcdef}
		3	5.74^{efgh}	2.97^{bcde}	2.76^{cdef}	1.12^{abc}
		4	6.49^{abcde}	2.63^{defg}	3.86^{abc}	0.69^{bcdef}
		5	5.52^{efgh}	1.74^{g}	3.78^{abc}	0.55^{def}
		6	5.29^{efgh}	1.90^{efg}	3.38^{bcde}	0.66^{bcdef}
	3	1	6.33^{abcdef}	2.74^{defgh}	3.58^{abcd}	0.73^{bcdef}
		2	4.56^{gh}	1.91^{defg}	2.64^{cdef}	0.82^{bcdef}
		3	5.95^{efgh}	2.85^{bcdef}	3.09^{cdef}	0.93^{abcdef}
		4	7.10^{a}	2.92^{bcde}	4.18^{ab}	0.69^{bcdef}
		5	7.00^{ab}	2.29^{defg}	4.70^{a}	0.48^{ef}
		6	5.25^{efgh}	2.23^{defg}	3.02^{cdef}	0.76^{bcdef}
2	1	1	4.89^{fgh}	2.59^{defg}	2.30^{def}	1.11^{abc}
		2	4.29^{h}	2.34^{defg}	1.95^{f}	1.31^{ab}
		3	5.88ef^{gh}	2.66^{defg}	3.21^{cdef}	0.83^{bcdef}
		4	5.35^{efgh}	2.61^{defg}	2.74^{cdef}	0.95^{abcdef}
		5	6.03^{bcdefg}	2.96^{bcde}	3.07^{cdef}	0.96^{abcdef}
		6	5.30^{efgh}	2.45^{defg}	2.85^{cdef}	0.86^{abcdef}
	2	1	5.96^{defgh}	3.22^{a}	2.73^{cdef}	1.17^{abc}
		2	4.71^{fgh}	1.78^{fg}	2.92^{cdef}	0.62^{bcdef}
		3	6.18^{bcdefg}	3.24^{a}	2.94^{cdef}	1.11^{abc}
		4	4.92^{efgh}	2.66^{defg}	2.26^{def}	1.16^{abc}
		5	5.00^{efgh}	2.60^{defg}	2.40^{def}	1.08^{abcd}
		6	4.95^{efgh}	2.53^{defg}	2.41d^{ef}	1.04^{abcd}
	3	1	6.61^{abcd}	3.12^{ab}	3.49^{abcde}	0.89^{abcdef}
		2	5.90^{efgh}	2.31^{defg}	3.59^{abcd}	0.63^{bcdef}
		3	4.57^{gh}	2.33^{defg}	2.24^{def}	1.11^{abc}
		4	6.13^{bcdefg}	3.09^{abc}	3.03^{cdef}	1.01^{abcde}
		5	6.76^{abc}	2.04^{defg}	4.72^{a}	0.44^{f}
		6	6.26^{abcdef}	3.02^{abcd}	3.24^{cdef}	0.93^{abcdef}
standard errors			±0.48	±0.31	±0.38	±0.15

a,b,c :means in the same column within each item with different superscript are significantly different ($P \leq 0.05$).

Table (7): Least-square means and standard errors for total lipid, triglycerides, cholesterol, insulin and T_3 of different experimental groups as affected by studied factors.

Mean effects	**Total lipid (mg/dL)**	**Triglycerides (mg/dL)**	**cholesterol (mg/dL)**	**Insulin (ng/mL)**	**T_3 (ng/mL)**
Flock age					
1(32 wks)	1146.78±26.40 [b]	176.48±7.83[b]	346.93±3.35 [b]	0.48±0.018 [a]	2.92±0.07 [a]
2 (48 wks)	1194.76±26.40 [a]	230.15±7.83[a]	365.94±3.35[a]	0.39±0.018 [b]	2.50±0.07 [b]
Time					
injection	1285.28±32.34 [a]	253.46±9.59 [a]	367.93±4.10 [a]	0.23±0.02 [c]	2.54±0.08[b]
1 (48 h)	1156.33±32.34 [b]	210.53±9.59[b]	363.43±4.10 [a]	0.35±0.02 [b]	2.53±0.08 [b]
2 (7 day)	1070.69±32.34 [b]	145.96±9.59 [c]	337.94±4.10 [b]	0.72±0.02 [a]	3.05±0.08 [a]
3 (24 day)					
Levels of	1228.00±45.74 [b]	201.86±13.57[bc]	377.09±5.81 [a]	0.30±0.03 [c]	2.93±0.12[ab]
biotin	1498.33±45.74[a]	305.99±13.57 [a]	368.05±5.81 [a]	0.06±0.03 [d]	1.39±0.12[c]
Control	1190.00±45.74 [b]	237.12±13.57[b]	369.25±5.81 [a]	0.29±0.03 [c]	2.58±0.12 [b]
A shem	1051.11±45.74[c]	145.95±13.57 [d]	368.24±5.81 [a]	0.46±0.03 [b]	3.12±0.12 [a]
Sline	964.33±45.74 [c]	143.63±13.57 [d]	322.98±5.81 [b]	0.73±0.03 [a]	2.94±0.12[ab]
75 µg	1092.83±45.74[bc]	185.35±13.57 [c]	332.99±5.81 [b]	0.78±0.03 [a]	3.31±0.12 [a]
100 µg					
125 µg					
Overall mean	1170.76	203.31	356.43	0.44	2.71

Mean having similar letter in each column are not significantly different ($P \leq 0.05$).

Table (8): Least-square means and standard errors for total lipid, triglycerides, cholesterol, insulin and T_3 of different experimental groups as affected by interaction between studied factors.

Interaction			Total lipid (mg/dL)	Triglycerides (mg/dL)	cholesterol (mg/dL)	Insulin (ng/mL)	T_3 (ng/mL)
Flock age	Time injection	Levels of biotin					
1	1	1	1173.33^{cd}e	166.98^{cdef}	397.34^{abc}	0.35^{fghi}	2.63^{def}
		2	1783.33^{a}	355.41^{a}	335.22^{ghij}	0.08^{jk}	2.12^{efgh}
		3	1046.66^{cdefgh}	230.07^{bcde}	381.44^{abcdefg}	0.33^{ghij}	3.76^{b}
		4	1300.00^{bcd}	211.98^{bcde}	380.30^{abcdefg}	0.11^{ijk}	2.69^{cde}
		5	816.66^{efgh}	167.98^{cdef}	338.94^{defghi}	0.30^{ghijk}	1.64^{fghij}
		6	1026.66^{defgh}	316.71^{ab}	285.22^{k}	0.50^{efg}	3.70^{bc}
	2	1	1175.00^{cde}	168.31^{cdef}	397.34^{abc}	0.36^{fghi}	2.62^{def}
		2	1560.00^{ab}	155.20^{def}	336.89^{fghij}	0.09^{ijk}	0.91^{ij}
		3	1306.66^{bcd}	210.72^{bcde}	375.00^{bcdefg}	0.41^{fgh}	3.84^{b}
		4	1163.33^{cdef}	97.58^{f}	403.03^{abc}	0.40^{fgh}	2.42^{def}
		5	816.66^{efgh}	94.21^{f}	337.27^{efghij}	0.70^{de}	2.30^{defg}
		6	1028.66^{defgh}	154.78^{def}	322.34^{hijk}	0.50^{efg}	3.70^{bc}
	3	1	1175.33^{cde}	95.21^{f}	399.27^{abc}	0.36^{fghi}	2.62^{def}
		2	1686.66^{a}	271.71^{abc}	336.23^{fghij}	0.10^{ijk}	1.91^{fghi}
		3	1013.33d^{efgh}	257.41^{abcde}	312.12^{ijk}	0.35^{fghi}	3.86^{b}
		4	723.33^{h}	59.72^{f}	233.33^{L}	1.00^{c}	5.32^{a}
		5	817.66^{efgh}	96.55^{f}	336.27^{fghij}	1.33^{ab}	2.88^{bcde}
		6	1029.66^{defgh}	66.03^{f}	337.50^{efghij}	1.50^{a}	3.70^{bc}
2	1	1	1280.00^{bcd}	316.71^{ab}	355.68^{cdefghi}	0.24^{ghijk}	3.23^{bcd}
		2	1723.33^{a}	351.20^{a}	427.27^{a}	0.03^{k}	0.^{82j}
		3	1430.00^{abc}	305.36^{ab}	395.07^{abc}	0.22^{hijk}	1.34^{ghi}
		4	1576.66^{ab}	155.20^{def}	410.60^{ab}	0.07^{jk}	2.37^{def}
		5	1110.00^{cdefg}	211.98^{bcde}	322.91^{hijk}	0.20^{hijk}	3.30^{bcd}
		6	1156.66^{cdef}	251.94^{abcde}	385.22^{abcdef}	0.40^{fgh}	2.91^{bcde}
	2	1	1282.00^{bcd}	318.38^{ab}	355.68^{cdefghi}	0.24^{ghijk}	3.23^{bcd}
		2	1223.33^{bcd}	351.20^{a}	386.36^{abcde}	0.04^{k}	1.21^{hij}
		3	1266.66^{bcd}	262.95^{abcd}	364.77^{bcdefgh}	0.23^{ghijk}	1.34^{ghij}
		4	783.33^{fgh}	264.14^{abcd}	386.36^{abcde}	0.33^{ghij}	3.34^{bcd}
		5	1111.00^{cdefg}	211.98^{bcde}	323.05^{hijk}	0.60^{ef}	2.67^{cde}
		6	1159.66 ^{cdef}	236.80^{bcde}	373.10^{bcdefg}	0.40^{fgh}	2.91^{bcde}
	3	1	1282.33^{bcd}	145.53^{ef}	357.51^{cdefghi}	0.25^{ghijk}	3.26^{bcd}
		2	1013.33^{defgh}	351.20^{a}	386.36^{abcde}	0.05^{k}	1.35^{ghij}
		3	1076.66^{cdefgh}	156.20^{def}	387.12^{abcd}	0.23^{ghijk}	1.34^{ghij}
		4	760.00^{gh}	87.06^{f}	395.83^{abc}	0.90^{cd}	2.61^{def}
		5	1114.00^{cdefg}	79.07^{f}	279.44^{k}	1.25^{b}	4.82^{a}
		6	1156.66^{cdef}	85.80^{f}	294.58^{jk}	1.40^{ab}	2.91^{bcde}
standard errors			±111.90	±33.18	±14.37	±0.07	±0.30

a,b,c :means in the same column within each item with different superscript are significantly different ($P \leq 0.05$).

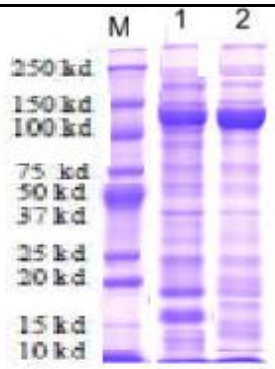

Figure (1): Electrophortic patterns of protein fraction in egg albumin
M (MolecularWeight Standards)- 1 = the eggs from 32-wk-old hens – 2=the eggs from 48-wk-old hens

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الملخص العربى

دور البيوتين في تحسين نسبة الفقس والخصائص الفسيولوجية لكتاكيت الرومي المتأثر بعمر القطيع

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أجريت هذه التجربة لتقييم تأثير عمر القطيع (32 أسبوع (صغير) و48 أسبوع (كبير)) وحقن بيض التفريخ بتركيزات مختلفة من البيوتين وفي أوقات مختلفة على نسبة الفقس وبعض صفات الدم لكتاكيت الرومي حديث الفقس. تم تقسيم ثمانمائة وعشرة بيضة من بيض الرومي عند كل عمر الى ثلاث مجموعات طبقا لوقت الحقن (48 ساعة و 7 و24يوم من عمر التحضين)، تم تقسيم كل مجموعة إلى ستة معاملات وفقا لتركيز الفيتامين. المعاملة الأولى كمجموعه مقارنه (بدون حقن)، المعاملة الثانية كمجموعه مقارنه يتم فيها ثقب القشرة وغلقها بدون حقن والمعاملة الثالثة تم حقنها ب1مل بمحلول ملحي فسيولوجي في الغرفة الهوائية من الجهة العرضية للبيضة. و تم حقن المجموعات الرابعة والخامسة والسادسة في نفس المكان بتركيزات 75و 100 و 125ميكروجرام / بيضة من البيوتين الذائبة في 1 مل من المحلول الملحي الفسيولوجى على التوالي.

وبشكل عام أشارت النتائج إلى أن معدل خصوبة البيض أظهر علاقة إيجابية مع تعبير بروتين الافيدين عند الأعمار الصغيرة عنها في الأعمار المتقدمة فى السن. وبالإضافة إلى ذلك حقن بالبيوتين في المراحل المتأخرة من التطور الجنيني يؤثر بشكل ايجابي على نسبة الفقس ونسبة الأجنة الحية ووزن الجسم من الكتاكيت مقارنة مع مجموعات الكنترول وكذلك التأثيرات على عمليات الايض للأنسولين وهرمون الغدة الدرقية للكتاكيت الرومى حديث الفقس.

ويمكن استنتاج أن حقن بيض الرومي بالبيوتين بجرعات 75 و 100 ميكروجرام / بيضة عند عمر 24 يوم من التطور الجنيني للدجاج الأصغر سنا والأكبر سنا على الترتيب .حيث تحسنت نسبة الفقس بشكل ملحوظ وكذلك الاستجابات الفسيولوجية لكتاكيت الرومى.