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Effect of Strain, Thermal Manipulation during Incubation Phase on Hatching Quality Traits and Blood Parameters of Dokii-4 and Golden Montazah Chicks under Upper Egypt Conditions

Abuoghaba A. Abdel-Kareem and Fouad M. Abbas**Abstract**

This study investigated the impact of strain, thermal manipulations during incubation on the hatching quality traits and blood parameters of Dokii-4 and Golden Montazah chickens. A total of 2250 fertile eggs of both Golden Montazah and Dokii-4 egg produced from hens at 42 week old. The eggs of both strains were classified into 3 thermal treatments (2 strains \times 3 treatments \times 3 replicates \times 125 eggs). In the 1st group (control), eggs were incubated at 37.5°C and 55-60 % RH (one day-hatch), while those in the 2nd and 3rd groups were exposed daily to 35 and 40°C for 3 hours during successive days (5-7d) of incubation.

The obtained results summarized the follows:

1. The chick weight at hatch (CWAH) for eggs in Golden Montazah was significantly increased, while the percentages of spleen, intestines, stomach and liver were not affected compared with Dokii-4 chickens.
2. The cholesterol level in Golden Montazah was significantly decreased, while blood proteins, liver enzymes and blood minerals were not affected compared with Dokii-4 chickens.
3. The CWAH (g), spleen (%) and calcium level for chick exposed to high group (40°C) were significantly decreased while cholesterol level was significantly increased compared with those of low at (35°C) and the control at (37.5°C) groups.
4. It could be concluded that the hatching quality traits and blood parameters for Golden Montazah chickens produced from egg exposed to normal and low incubation temperature significantly improved as compared with those in high incubation temperature group.

Keywords: Strain, Thermal manipulations**Corresponding author:****M. A. Fouad**alwazery74@yahoo.com

INTRODUCTION

The incubation temperature is a critical factor in ensuring the normal development of chicken embryos (Yalçin et al., 2007). This temperature significantly influences hatchability, chick quality, and post-hatch growth performance (Lourens et al., 2005). Consequently, thermal manipulation, defined as exposing embryos to elevated or reduced temperatures during embryogenesis, is a strategy employed to enhance their capacity to adapt to hot or cold environments by modifying the thermotolerance of chickens post-hatch (Shinder et al., 2011). The optimal incubation temperature for chicken eggs was found to be between 37.0°C and 38.0°C (Wilson, 1991). The sources of temperature during the incubation phase include the incubator, heat exchange between the embryo and its environment, and the metabolic heat production of the embryo (French, 1997). During the incubation phase, eggshell temperature plays an important role in embryo development, hatchability, and the performance of chickens (Willemsen et al., 2011). The pattern of air flow around the egg is a significant factor affecting eggshell temperature, as the eggshell serves as a thermal barrier in determining the actual embryo temperature (Peebles, 2012). The findings of Elsayed et al. (2009) indicated that embryos treated with high temperatures exhibited higher hatching weights than the control group. Likewise, Ismail et al. (2016) demonstrated a notable increase in the weight and length of male Mamoura chicks produced from eggs exposed to 39°C for four hours during the incubation phase, compared to the control group that was subjected to 37.8°C. In contrast, Abuoghaba et al. (2018) observed no statistically significant differences in the weight of Sinai chicks at hatch between the thermal group (40°C) and the control group (37.5°C) during the early stages of embryogenesis (6-8 days of incubation). Similarly, Vesco et al. (2021) found that the hatchling weight at birth was not affected by maternal exposure to continuous heat stress (32°C and 60% RH) in comparison to females under thermoneutral conditions (23°C and 60% RH). Fouad et al. (2023) observed that the heart percentage of chicks produced from

eggs subjected to high thermal stress (40°C) was significantly higher than those from eggs incubated at normal (37.5°C) and low (35°C) temperatures. However, the gizzard percentage in the second group (40°C) was significantly lower, while the spleen and liver percentages remained unaffected. With regard to the effect of strain, the weight of the chick was found to be significantly greater in the Inshas strain than in the Dokii-4 strain (Ali et al., 2012). Similarly, significant differences were observed in the weight of male chicks at hatch between different strains. The Shaver C strain exhibited the highest weight, while the Mandarah strain exhibited the lowest weight (Taha et al., 2013). Therefore, this study aimed to evaluate the impact of thermal stress during early embryogenesis on hatching quality traits and blood parameters of native strains Dokii-4 and Golden Montazah chickens.

MATERIALS AND METHODS

This study was carried out at the research Poultry Farm, Poultry Production Department, Faculty of Agriculture Sohag University during the period from April 2020 to June 2021.

Experimental design

A total of 2,250 fertile eggs of both the Golden Montazah and Dokii-4 strains were obtained from hens at 42 weeks of age. The hens were purchased from the Animal Production Research Institute, Agricultural Research Center, Egypt. The eggs of both strains were classified into three equal thermal treatments (2 strains × 3 treatments × 3 replicates × 125 eggs). All eggs were incubated at 37.5°C and 55-60% RH from one day until hatch, and were considered the control group. The second and third groups were exposed daily to 35°C and 40°C for three hours (from 12PM to 3PM) for three successive days (five to seven days) of incubation. During the final three days of the incubation period, the eggs were subjected to a daily temperature of 37.5°C and a relative humidity of 60-65%.

Studied traits

Chick quality traits

Approximately 12 hours after hatching, 54 dry chicks (two strains \times three treatments \times three replicates \times three chicks) were randomly selected to determine their quality traits. The chick weights were determined by weighing to the nearest 0.1 g using an electronic balance. The chick length (cm) was measured from the tip of the beak to the tip of the middle toe by placing the chick face down on a flat surface and straightening the right leg (Hill, 2001). The chicks were slaughtered by cervical dislocation to determine the residual yolk sac weight, intestine percentage, as well as the percentages of heart, intestine, gizzard, and liver.

Blood parameters

After 12 hours from hatch, 54 blood samples (comprising two strains, three treatments, three replicates, and three chicks) were collected into heparinized and non-heparinized tubes for hematological and chemical analysis. The blood samples were then centrifuged to separate the plasma and serum, and subsequently decanted and deep frozen for analysis.

Hematological variables

The number of red blood cells (RBCs $\times 10^6$) was determined using a hemocytometer. A test tube was filled with 1.5 ml of salt solution and 0.5 microns of blood were added. The solution was then transferred into the hemocytometer, and the five squares of the slide were counted. The method used was that of West and Haines (2002). The bloodstream was employed to ascertain the WBC $\times 10^3$ count through the wedge method, which involved the preparation of a blood film by depositing a drop of blood on a slide. Subsequently, the Leishman stain, comprising a mixture of methylene blue and eosin in specific proportions, was utilized to pigment the slide after stabilization. The slide was positioned at approximately 45 degrees relative to the horizontal plane, and the subsequent staining process was conducted as follows: the eosinophils, heterophils, basophiles, lymphocytes, and monocytes were measured in

accordance with the methodology outlined by Lynch et al. (1969).

Blood biochemical determinations

Blood proteins (Total protein, albumin and globulin)

The estimation of serum total protein content was conducted in accordance with the methodology proposed by Gomal et al. (1949). The concentration of serum albumin was determined spectrophotometrically, following the approach outlined by Doumas et al. (1971), utilizing a spectrophotometer.

Liver enzymes

The AST and ALT enzymes in the serum were quantified using reagent kits purchased from Biodiagnostic Chemical Company (Egypt) and a spectrophotometer (50/20) according to the methodology described by Reitman and Frankel (1975).

Total lipids, cholesterol and triglycerides

The total lipids were determined in accordance with the methodology proposed by Zollner and Kirsch (1962), whereas the serum cholesterol was quantified in line with the approach outlined by Richmond et al. (1973). The serum triglycerides were measured using the spectrophotometer 50/20, as described by Fassati and Prencipe (1982).

Blood minerals

Serum calcium, phosphorus and potassium were determined by using the spectrophotometer 50/20.

Statistical analysis

The obtained data statistically analyzed by using GLM, produced by the statistical analysis systems (SAS, 2004). Duncan's new multiple ranges tests (Duncan, 1955) were used to determine significant differences between treatment means. The following linear model by applying: $Y_{ij} = \mu + S_i + TM_j + SiTM_j + e_{ij}$

Where, Y_{ij} = Observation measured, μ = Overall mean, S_i = Effect of strain ($i = 1, 2$), TM_j = Effect of thermal manipulation ($j = 1, 2$ and 3), $SiTM_j$ = Interaction between chicken strain and thermal

manipulation, E_{ij} = Random error component was normally distributed assumed.

RESULTS AND DISCUSSION

Effect of strain, thermal manipulations and their interaction on hatching quality traits:

With regard to the strain effect, the results presented in Table 1 revealed no statistically significant differences between the Golden Montazah and Dokii-4 chicken strains in terms of RCW and the percentages of spleen, heart, intestine, gizzard, and liver. However, the chick weight at hatch of the Golden Montazah was found to be significantly higher in comparison to that of the Dokii-4 chickens. The notable elevation in chick weight at hatch for the Golden Montazah strain may be attributed to a considerable augmentation in egg weight in comparison to that observed in the Dokii-4 strain. These findings align with those of Ali et al. (2012), who observed a notable increase in chick weight at hatch in Inshas when compared

to the Dokii-4 strain. Taha et al. (2013) also reported similar findings, noting that the hatch weight of males exhibited significant differences between the Shaver C, which demonstrated the highest significant values (45.16), and the Mandarah strain, which exhibited the lowest weight (34.97 g). The obtained results showed that there were no statistically significant differences in the percentages of the heart, intestine, gizzard, and liver. However, the percentage of spleen was significantly decreased in the group incubated at 40°C as compared with those incubated at 35°C and the control group incubated at 37.5°C. The significant decrease in the spleen percentage may be attributed to the observed reduction in immune stimulation in the chick. These findings align with those reported by Abuoghaba (2017), who documented a notable decline in spleen percentage in broiler chicks hatched from eggs subjected to chronic temperature exposure at 40°C, as compared to those produced under normal temperature conditions (37.5°C).

Table 1. Impact of strain, thermal manipulations and their interaction on hatching quality traits

Traits	CWH (g)	RCW (%)	Internal organs				
			Spleen (%)	Heart (%)	Intestine (%)	Gizzard (%)	Liver (%)
Effect of strain (s)							
Dokii-4	33.09 ^b	68.20	0.08	0.60	3.38	4.52	2.31
Golden Montazah	34.81 ^a	68.15	0.08	0.65	3.59	4.65	2.61
SEM	0.18	0.59	0.01	0.01	0.10	0.35	0.32
Effect of thermal manipulations (TM)							
1 st group (Control/37.5°C)	34.30 ^a	68.90	0.09 ^a	0.59	3.56	4.74	2.56
2 nd group (HIT/40°C)	33.07 ^b	66.49	0.06 ^b	0.69	3.12	4.11	2.11
3 rd group (LIT/35°C)	34.48 ^a	68.69	0.10 ^a	0.60	3.79	4.90	2.72
SEM	0.22	0.72	0.01	0.03	0.38	0.43	0.39
Probability							
S	0.001	0.953	0.001	0.953	0.860	0.240	0.641
TM	0.002	0.157	0.002	0.157	0.469	0.141	0.469
S×TM	0.273	0.835	0.273	0.835	0.906	0.744	0.977

^{A, b} Means with different superscripts in the same column are significantly different ($P \leq 0.05$). CWH (g) = Chick weight at hatch and RCW (%) = Relative chick weight. S= Strain, TM= Thermal manipulations, S×TM= interaction

The obtained findings indicated that the weight at hatch for chicks produced from eggs subjected to low temperature (35°C) exposure, as well as the control (37.5°C) group, exhibited a notable increase compared to the high

temperature (40°C) group. The notable increase in chick weight at hatch observed in the first and third groups may be attributed to the enhanced uptake of the embryo yolk sac, which provides essential nutrients during the initial stages of life

(Meijerhof, 2009). These findings align with those of Joseph et al. (2006), who discovered that the weight at hatch for chicks produced from eggs during the early incubation period (0 to 10 days) after exposure to a low incubation temperature (36.6°C) exhibited a notable increase due to the larger yolk sac weight in comparison to the control group. Additionally, the obtained results demonstrated that thermal manipulations did not exert a discernible influence on the relative chick weight percentage. No significant differences in all hatchling quality traits due to the interaction between strain and thermal manipulations.

Effect of strain, thermal manipulations and their interaction on blood parameters at hatch:

With regard to the strain effect, the results presented in Tables 2 and 3 demonstrated that there were no statistically significant differences between the two strains in terms of TP, Alb, Glb, Trigly, Ca, Ph, ALT, and AST values. However, the cholesterol values of the Dokii-4 strain were found to be significantly elevated in comparison to those of the Golden Montazah chickens. This significant increase may be attributed to the synthesis of egg cholesterol in the liver, its subsequent transport in the blood, and its incorporation into the egg yolk. These results align with those of Albokhadaim et al. (2012), who observed higher triglyceride levels (68.3 mg/dL) in SS males compared to adult Saudi chicken males. The results demonstrated that the impact of strain had no discernible influence on the concentrations of total protein (TP), albumin (Alb), globulin (Glb), triglycerides, phosphorus, alanine aminotransferase (ALT), and aspartate

aminotransferase (AST) in the chickens produced from eggs exposed to an incubation temperature of 40°C. Conversely, the cholesterol levels exhibited a notable increase in comparison to the low (35°C) and control (37.5°C) groups. The calcium level in the high-temperature group (40°C) exhibited a statistically significant decline in comparison to the low-temperature group (35°C) and the control group (37.5°C). The significant decrease in cholesterol concentration may be attributed to the release of glucocorticoids by chickens subjected to heat stress, which mobilizes lipids from adipose tissue to the liver in laying hens (Sahin & Küçük, 2001). This corroborates the function of HDL, which is to transport cholesterol from body tissues to the liver (Odihambo et al., 2006). The notable decline in calcium concentration may be attributed to the dietary sources of these minerals. The diminished concentrations of plasma minerals may be attributed to a reduction in feed intake or an impairment in the digestive and absorptive processes of nutrients. These findings align with those of Lee et al. (2020), who observed that heat stress rapidly elevated total cholesterol levels while reducing calcium concentrations significantly on day 1 post-heat treatment, compared to those of moderate and low-stressed laying hens. In contrast, Zaboli et al. (2016) observed no significant long-term impact on cholesterol, triglyceride, and HDL levels in male broilers exposed to a temperature range of 36 to 38°C for 24 hours on day 3 and 5 post-hatch, as well as in the control group. The interaction between strain and thermal manipulations did not result in any notable differences in blood parameters.

Table 2. Impact of strain, thermal manipulations and their interaction on blood proteins, cholesterol and triglycerides at hatch

Traits	Blood proteins			Blood lipids	
	TP (mg/dl)	Alb (mg/dl)	Glb (mg/dl)	Chol (mg/dl)	Trig (mg/dl)
Effect of strain					
Dokii-4	5.99	3.60	2.39	159.44 ^a	170.81
Golden Montazah	6.27	3.64	2.63	144.68 ^b	166.03
SEM	5.22	0.18	0.17	3.33	4.81
Effect of thermal manipulations (TM)					
1 st group (Control37.5°C)	6.20	3.66	2.54	146.67 ^b	163.85
2 nd group (HIT/40°C)	5.85	3.50	2.35	163.58 ^a	178.57
3 rd group (LIT/35°C)	6.34	3.70	2.64	145.94 ^b	162.86
SEM	0.32	0.22	0.20	4.08	5.89
Probability					
S	0.463	0.877	0.334	0.011	0.498
TM	0.557	0.800	0.623	0.019	0.158
interaction (S×TM)	0.999	0.999	0.993	0.782	0.938

^{A, b} Means with different superscripts in the same column are significantly different ($P \leq 0.05$). TP= total protein, Alb= Albumin, Glb= Globulin, Chol= Cholesterol, Trig= Triglycerides. S= Strain, TM= Thermal manipulations, S×TM= interaction

Table 3. Impact of strain, thermal manipulations and their interaction on liver enzymes and blood minerals of chicks at hatch

Traits	Liver enzymes		Blood minerals	
	ALT (mg/dl)	AST (mg/dl)	Calcium (ppm)	Phosphorus (ppm)
Effect of strain (S)				
Dokii-4	218.57	169.96	20.70	2.90
Golden Montazah	218.06	179.92	21.61	2.96
SEM	8.992	4.263	0.671	0.286
Effect of thermal manipulations (TM)				
1 st group (Control37.5°C)	211.39	173.43	22.33 ^a	3.00
2 nd group (HIT/40°C)	235.05	180.47	18.57 ^b	2.71
3 rd group (LIT/35°C)	208.51	170.91	22.57 ^a	3.07
SEM	11.01	5.22	0.82	0.35
Probability				
S	0.969	0.130	0.360	0.887
TM	0.223	0.437	0.010	0.744
Interaction (S×TM)	0.993	0.879	0.579	0.995

^{A, b} Means with different superscripts in the same column are significantly different ($P \leq 0.05$). S= Strain, TM= Thermal manipulations, S×TM= interaction.

CONCLUSION

From these findings could be concluded as follow:

The CWH (g) in Golden Montazah was significantly increased compared with Dokii-4 chickens. The cholesterol level was significantly increased in Dokii-4 as compared with Golden Montazah chickens. The chick weight at hatch for eggs exposed to low (35°C) and control (37.5°C) groups was significantly higher, while spleen percentage was significantly lower than that of the high (40°C) group. The cholesterol level was significantly increased in the high group (40°C) compared with those of low at (35°C) and the control at (37.5°C) groups, while the calcium level in the high group (40°C) was significantly decreased as compared with those of low (35°C) and control (37.5°C) groups. The interaction between strain and thermal manipulations did not result in a notable impact on the studied traits.

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