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Effects of thermal manipulation during late incubation period on post-hatch thermotolerance in ostrich

M.A. Elsayed

Biological Application Department, Nuclear Research Centre, Atomic Energy Authority, Cairo, Egypt

ABSTRACT: The effects of thermal manipulation (TM) during late ostrich embryonic development on hatchability, body weight, biochemical and hormonal changes, and the ability of hatching chicks to cope with thermal challenge in days 6–8 of age were examined. At 35 days of incubation, two hundred fertile eggs were weighed and divided into two equal groups with five replicates. The first group was exposed to 36.5°C and 25% relative humidity (RH) (control), while the second group was exposed to 38.5°C and 45% RH for 3 h daily in days 35–37 of incubation (thermal manipulation). At days 6–8 of age, the hatched chicks from each treatment were randomly divided into two groups: control group (exposed to 32 ± 1°C), and thermal challenge group (exposed to 40 ± 1°C for 3 h daily). Hatchability rate was significantly lower with high incubation temperature as compared to the normal incubation temperatures. Embryonic TM and thermal challenge in days 6–8 of age reduced significantly total protein, albumin, and triiodothyronin concentrations and elevated uric acid, creatinine, triglycerides, and glucose concentrations as compared with the control. The level of corticosterone was significantly higher in the thermal challenge group as compared to the control. In conclusion, exposing the ostrich embryos to TM (38.5°C) during late embryonic development induced physiological changes that may represent epigenetic adaptation to TM. The same mechanisms are employed for increasing the ability to improve thermotolerance post-hatch.

Keywords: ostrich embryo; heat stress; hatchability; blood biochemicals; epigenetic

INTRODUCTION

Ostrich chicks are susceptible to disease and infection, various disorders, and stress during the first few weeks of their life (Barri et al. 2005). These problems often result in high mortality, which is one of the major welfare problems in the ostrich industry. A special attention is required during this period to ensure that the chick welfare is not compromised. Once the chicks reach three months of age, they are relatively hardy and only need protection from bad weather (Gladys 2000). It is important to keep young ostriches warm, raise them in stress free environment, and give them sufficient space for exercise. Temperature recommended for ostrich chicks in a brooder box varies because the rearing environmental conditions differ. In Egypt, ostrich chicks are exposed to high environmental temperature especially during summer (up to 48°C). When young ostrich chicks are heat stressed for long periods, normal development of the immune system is affected (as observed in other avian species) resulting in poor appetite, increased risk of dehydration, and bacterial infections (Gladys 2000). Therefore, summer heat with the progressing global warming is one of major concerns in the current poultry industry because chickens generally are low tolerant to heat. Various countermeasures were reported to reduce the negative effect of heat stress on chickens, but the problem has not yet been resolved. Under high environmental temperatures, chickens are known to acclimatize to heat by modulating their behavioural and/or physiological responses for thermoregulation (Yahav 2000). Due to immature thermoregulation in young chicks, thermal condi-

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tioning is a process in which chicks are exposed to high environmental temperature for 24 h during the first week of life (Yahav 2000). The treatment results in significantly increased feed intake and body weight gain and decreased mortality with heat tolerance (Yahav and Plavnik 1999). However, such treatments are difficult to use in practice, and impose a heavy stress load on chicks. To reduce the load in thermal conditioning, it is necessary to consider the quality and quantity of the load, such as duration and temperature. Experiments covering various chicken breeds and lines would lead to more detailed insight on the treatment, too.

Low hatchability of ostriches is well known and many chicks that do hatch subsequently die. The factors that affect hatchability include egg hygiene, egg storage conditions and period, incubation temperature, humidity, egg orientation, egg turning, ventilation, and sanitation. Incubation temperature for ratites ranges from 35.9 to 36.5°C (Wilson 1997). Estimated relative humidity (RH) requirements are 15–20% during incubation and 40% during hatching. Egg weight loss of 12–17% during 38 days of incubation is recommended for ostrich eggs. Major factors contributing to egg weight loss are shell porosity and RH. Other factors include egg size and incubation temperature (Wilson 1997). Increasing the incubation temperature from 36.0 to 37.2°C reduces hatchability from 73 to 44% (Stewart 1996). Similar results were found by Hassan et al. (2004), who reported that mortality was increased at 37.5°C if compared to 36.5°C. The incubation period was reduced by about 2 days when the incubation temperature increased from 36.5 to 37.5°C (Hassan et al. 2004). However, Stewart (1996) noticed that increasing the incubation temperature from 35.0 to 36.7°C decreased the incubation period by 3 days. Deeming et al. (1993) suggested that the incubation period decreased by 2 days for every degree increase in incubation temperature.

During the critical periods of embryonic or early post hatch development changes in climatic conditions (e.g. ambient temperature) may cause long-lasting changes in the epigenetic programming of respective body functions ("imprinting" of physiological control systems), or induce epigenetic temperature adaptation, which results in a long-lasting cold or warm adaptation in poultry (Tzschentke 2008). Thermal conditioning during the first days post-hatch improved thermotolerance in later life. But thermal manipulation (TM) during incubation seems to be more easily applicable. The application of TM in the last days of incubation seems to be beneficial. Thus, the application of TM during incubation would likely lead to effective alterations in the thermoregulatory response thresholds of the embryo and the hatched chick, depending on the timing and severity of the manipulation. The timing of TM has to be linked to the development of the hypothalamus–hypophysis–thyroid axis to change the heat production threshold response (Yahav et al. 2004b), and to the development of the hypothalamus–hypophysis–adrenal axis to avoid the increase in stress response (Tzschentke 2008).

Therefore, we hypothesized that TM with high temperatures during certain periods in late incubation in ostrich could induce epigenetic (temperature) adaptation and affect the adaptation capacity and performance of the chicks post-hatch.

MATERIAL AND METHODS

The present work was carried out at the ostrich farm located at the Nuclear Research Center, Atomic Energy Authority, Inshas, Egypt.

Eggs handling. Two hundred and fifty eggs (1300–1400 g) from ostrich hens were collected from breeding yards at 15:00 h each day, washed with disinfectant (TH_4^+), then allowed to dry for 30 min. Eggs were stored, thereafter, at 17°C and 75% RH for 7 days in a vertical position until incubation. Eggs were setting (setter model: NMC-1000; NatureForm Hatchery Technologies, Jacksonville, USA) at 36.5°C and 25% RH for 39 days. At day 14 of incubation, eggs underwent lighting examination to remove infertile eggs.

Thermal manipulation (TM). At day 35 of embryonic development, two hundred fertile eggs were divided into two equal groups with five replicates for each group (20 fertile eggs for each replicate). The first group was exposed to 36.5°C and 25% RH and served as a control, while the second group was exposed to 38.5°C and 45% RH for 3 h daily during the last period of incubation (days 35–37) and served as TM-treated (TM). The treated eggs from the second group were transferred into another incubator of the same model for 3 h daily from 12:00 to 15:00 and then were put back to the incubator with the regular conditions of 36.5°C and 25% RH. The eggs in all incubators were turned every 2 h at a 45° angle. On day 39 of incubation,

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all eggs were weighed to calculate percentage of water loss, then transferred to a hatcher (model PTO; PTO Company, Alexandria, Egypt; 35.5°C and 40–45% RH) for 3 days until hatching (days 41–42).

Percentage of water loss was calculated using the following equation:

Water loss (%) = [(EW before incubation – – EW at transfer)/EW before incubation] × 100

where:
EW = egg weight

At hatch, hatching percentage was calculated as follows:

Hatching percentage = (number of hatched eggs/number of fertile eggs) × 100

Total embryonic mortality rate (after removing infertile eggs) was calculated using the equation:

Embryonic mortality (%) = [(number of incubated fertile eggs – number of hatched eggs)/number of incubated fertile eggs] × 100

Fifteen birds from each group (control and TM; 3 birds for each replicate) were weighed and rectal temperatures (RT) measured, then slaughtered. Blood samples were taken immediately into non-heparinized tubes, then centrifuged to separate serum. Liver, heart, gizzard, and proventiculus weights were obtained and calculated as a percentage of body weight.

Thermal challenge treatment. Residual hatched chicks (60 ostrich chicks for the TM and 60 chicks for the control group) were randomly divided into two groups per 30 birds, control group and a heat stressed group (thermal challenge), with five replicates for each group (6 birds for each replicate). All chicks were placed in a controlled room under regular rearing conditions (32 ± 1°C) and exposed to 24 h light all day long. The birds were fed starter ration *ad libitum* (22% protein and 2680 kcal metabolizable energy (ME)/kg). The components and chemical analysis of the diet were as shown in Table 1. In days 6–8 of age, the challenge groups were exposed to 40 ± 1°C for 3 h daily, while the control chicks continued under the regular conditions. Feed consumption in days 6–8 of age was determined. After the heat exposure period (at 8 days of age), RT of fifteen birds from each group (3 birds for each replicate) was measured, birds were slaughtered, and the same organs like previously were collected, weighed, and calculated as a percentage of body weight. Blood samples were taken for biochemical and hormonal analyses.

Table 1. Ingredients and chemical analysis of the experimental commercial diet

Ingredients (kg/t feed)	
Yellow corn	430
Soybean meal	335
Lucerne	150
Dicalcium phosphate	37
Limestone	16
Salt	3
Synthetic lysine	1
Synthetic methionine	1
Antitoxic	2
Mineral[1] & vitamin premix[2]	12
Oil	12
Zinc pastracine	1
Total (kg)	1 000
Chemical analysis (per kg feed)	
Metabolizable energy (kcal/kg)	2 680
Protein (%)	22
Crude fibre (g/kg)	6
Calcium (g/kg)	1.5
Phosphorus (g/kg)	0.75

[1]every 4 kg of mineral premix contain the following elements: choline chloride 1800 g, manganese 225 g, zinc 150 g, iron 60 g, copper 13 g, iodine 1.5 g, selenium 0.60 g, cobalt 0.90 g, magnesium 250 g, $CaCO_3$ added to complete total weight of 4 kg (inclusion rate 4 kg/t)

[2]every 2 kg of vitamin premix contain the following vitamins: vitamin A 37 500 000 MIU, vitamin D3 11 250 000 MIU, vitamin E 300 g, vitamin B1 3300 mg, vitamin B2 3300 mg, vitamin B6 4400 mg, vitamin B12 100 mg, pantothenic acid 20 000 mg, nicotinic acid 60 000 mg, folic acid 2000 mg, biotin 200 mg, $CaCO_3$ added to complete total weight of 2 kg (inclusion rate 4 kg/t)

Serum total protein (TP), albumin (Alb), uric acid (UA), creatinine (Creat), triglycerides (Trig), and glucose (Gluc) were determined colorimetrically using colorimetric kits (Stanbio Laboratory LP, Boerne, USA) and measured using scanning spectrophotometer Spectronic 1201 (Milton Roy, Ivyland, USA). Serum globulin (Glob) values were calculated by subtracting Alb values from their

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corresponding TP values of the same sample. Serum corticosterone (Cort) was determined using ELISA kit (EIAab, Wuhan, China), while triiodothyronine (T_3) concentration was determined using radioimmunoassay kit (Institute of Isotopes Co., Ltd., Budapest, Hungary) and samples were counted on a Packard Cobra Gamma Counter, Model 5002 (PerkinElmer, Waltham, USA).

Statistical methods

Thermal manipulation treatment. To determine the effect of TM during late ostrich embryonic development on parameters, data were statistically analyzed by one way analysis of variance using the General Linear Models procedure of the SAS software (Statistical Analysis System, Version 5.0, 1998). Significance level was set at $P < 0.05$. Mean values were compared using Duncan's Multiple Range Test (Duncan 1955) in the case of significant differences.

The model used was:

$$X_i = \mu + T_i + e_i$$

where:

X_i = any value from the overall population

μ = overall mean

T_i = effect of the i^{th} treatment (i = 1, control group and thermal manipulation group)

e_i = random error associated with the i^{th} individual

Thermal challenge treatment. Two way (with interaction) analysis of variance was used to evaluate the effect of thermal challenge from 6 to 8 days of age on the studied parameters. Significance level was set at $P < 0.05$. Mean values were compared using Duncan's Multiple Range Test (Duncan 1955) in the case of significant differences.

The model used was:

$$X_{ij} = \mu + T_i + S_j + ST_{ij} + e_{ij}$$

where:

X_{ij} = any value from the overall population

μ = overall mean

T_i = effect of the i^{th} treatment (i = 1, control 1 and thermal manipulation treatment (experiment in incubation period))

S_j = effect of the j^{th} heat exposure (j = 1, control 2 and thermal challenge treatment (heat stress experiment after hatch))

ST_{ij} = effect of interaction between the i^{th} treatment and the j^{th} heat exposure

e_{ij} = random error associated with the ij^{th} individual

Table 2. Effects of thermal manipulation (TM) during late ostrich embryonic development on eggs water loss

Treatments	Initial egg weight (g)	Egg weight on day 39 (g)	Egg water loss (%)
TM	1355	1170	13.6
Control	1370	1178	14.0
SEM	11.4	11.2	3.2
Probability	0.55	0.23	0.45

SEM = standard error of the means

RESULTS

Effect of TM on eggs weight loss. The effect of TM during late ostrich embryonic development on percentage of egg water loss at 39 days of embryonic development is presented in Table 2. It could be stated that TM had no effect on the percentage of egg water loss.

Effect of TM on hatchability. The effects of TM during late ostrich embryonic development on hatchability, embryonic mortality rate, and RT are summarized in Table 3. TM significantly decreased hatchability by about 4.3% and increased mortality by about 4.3% as compared to the control. Results show that at hatch, RT of the TM ostrich chicks was significantly lower than of the control.

Body and internal organs weight percentage at hatch. The effects of TM during late ostrich embryonic development on body and some organs weight percentage at hatch are shown in Table 4. Body weight (BW) was significantly ($P \leq 0.05$) reduced by the TM treatment as compared to the normal incubation temperature (control) treatment. The data from Table 4 also indicate that liver and heart percentages were significantly

Table 3. Effects of thermal manipulation (TM) during late ostrich embryonic development on some hatched traits

Treatments	Hatchability (%)	Mortality rate (%)	Rectal temperature at hatch (°C)
TM	76.5[b]	23.5[a]	37.9[b]
Control	80.8[a]	19.2[b]	38.2[a]
SEM	3.2	3.2	0.051
Probability	< 0.001	< 0.001	< 0.001

SEM = standard error of the means

[a,b]means for each parameter in the same column with different superscript are significantly different due to treatment ($P < 0.05$)

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Table 4. Effects of thermal manipulation (TM) during late ostrich embryonic development on hatch body weight (BW) and percentage of some internal organs weights[1] (n = 15 per each group)

Treatments	BW[1] (g)	Liver (%)	Heart (%)	Gizzard (%)	Proventiculus (%)
TM	850[b*]	2.26[b]	1.20[b]	3.024	1.340
Control	885[a]	2.55[a]	1.57[a]	2.994	1.366
Standard error of the means	1.78	0.014	0.014	0.017	0.014
Probability	< 0.001	< 0.001	< 0.001	0.245	0.228

[a,b]means for each parameter in the same column with different superscript are significantly different due to treatment (P < 0.05)
[1]organs weights calculated as a percentage of body weight
*values are means

lower ($P \leq 0.05$) in TM chicks as compared with the control. There were no significant differences between the TM and the control group in gizzard or proventiculus weight percentage.

Blood biochemical and hormonal levels at hatch. Data in Table 5 indicate that at hatch, the TP, Alb, and Glob concentrations were significantly ($P \leq 0.05$) lower in the TM group during late embryonic development as compared to the control. UA and Creat concentrations were significantly ($P \leq 0.05$) higher in the TM group as compared to the control.

In addition, Table 6 indicates that Trig, Gluc, and Cort concentrations, unlike T_3 level, were significantly ($P \leq 0.05$) higher in the TM group during late embryonic development as compared to the control.

Effect of TM on post hatched chicks. Data concerning the effect of thermal challenge for ostrich chicks at 8 days of age on RT, mortality rate, and feed consumption are presented in Table 7. On day 8 of age, RT of TM ostrich chicks was significantly lower than of those of the control for TM treatment group (C1), while it was significantly higher in the thermal challenge treated group (TCH) than in the TCH control group (C2). Additionally, data in Table 7 indicate that on day 8 of age, mortality rate was significantly increased in

Table 5. Effects of thermal manipulation (TM) during late ostrich embryonic development on the levels of some blood biochemical parameters at hatch (n = 15 per each group)

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
TM	3.49[b*]	2.05[b]	1.44[b]	6.87[a]	0.52[a]
Control	3.99[a]	2.25[a]	1.74[a]	4.55[b]	0.39[b]
Standard error of the means	0.008	0.008	0.01	0.007	0.009
Probability	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.001

[a,b]means for each parameter in the same column with different superscript are significantly different (P < 0.05)
*values are means

Table 6. Effects of thermal manipulation (TM) during late ostrich embryonic development on the levels of some blood biochemical parameters at hatch (n = 15 per each group)

Treatments	Triglyceride (mg/dl)	Glucose (mg/dl)	Triiodothyronine (ng/dl)	Corticosterone (ng/ml)
TM	145.5[a*]	199.5[a]	74.8[b]	28.23[a]
Control	132.0[b]	179.7[b]	115.1[a]	27.72[b]
Standard error of the means	0.087	0.095	0.077	0.11
Probability	< 0.0001	< 0.0001	<0.0001	0.004

[a,b]means for each parameter in the same column with different superscript are significantly different (P < 0.05)
*values are means

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Table 7. Effects of thermal challenge at 8-day-old ostrich chicks on rectal temperature, mortality rate, and feed consumption (n = 15 for rectal temperature measurement)

Treatments	Rectal temperature (°C)	Mortality rate (%)	Feed consumption (g/day)
Thermal manipulation during hatch (TMDH)			
C1	38.90[a]	2.63[a]	92.2[b]
TM	38.45[b]	2.35[b]	103.3[a]
Thermal challenge after hatch (TCHAH)			
C2	38.50[b]	2.365[b]	106.8[a]
TCH	38.95[a]	2.615[a]	88.7[b]
SEM	0.045	0.009	0.2
Interactions			
C1 × C2	38.7[b]	2.44[b]	105.0[a]
C1 × TCH	39.1[a]	2.82[a]	78.5[c]
TM × C2	38.5[b]	2.29[b]	107.7[a]
TM × TCH	38.4[b]	2.41[b]	98.8[b]
SEM	0.063	0.002	0.2
Probability			
TMDH	< 0.0001	< 0.0001	< 0.0001
TCHAH	< 0.0001	< 0.0001	< 0.0001
TMDH × TCHAH	< 0.0001	< 0.0001	< 0.0001

SEM = standard error of the means, TM = thermal manipulation, TCH = thermal challenge, TMDH = thermal manipulation during hatchability, TCHAH = thermal challenge after hatch, C1 = control for TM group, C2 = control for TCH group

[a–c]means with different superscripts within main effects or the interaction are significantly different ($P < 0.05$)

C1 than in TM treatment and generally, rates of mortality were higher in the TCH ostrich chicks than in C2. Finally, on day 8 of age, feed consumption was generally decreased in C1 as compared with the TM treatment, while TCH ostrich chicks exhibited lower feed consumption than those in C2.

The data presented in Table 7 showed that there was an interaction between the treatments during hatch and the treatment post-hatch on RT, mortality rate, and feed consumption.

Body and internal organs weight percentage on day 8 post-hatch. Table 8 indicates that BW, liver, and heart weight percentages on day 8 post-hatch were significantly lower in the TM group than in C1 and in the TCH treatment than in C2. Also, data in Table 8 reveal that on day 8 of age there was a numerical effect by TM or TCH treatment on gizzard percentage weight as compared to control groups. Whereas there was no significant effect on proventiculus weight percentage. Data in Table 8 show that there was an interaction between treatments during hatch and treatment post-hatch on BW, liver, heart, and gizzard weight percentage.

Blood biochemical and hormonal levels on day 8 of age. On day 8 of age, TP and Alb concentrations were significantly lower in the TM group than in C1, Glob concentration was higher in the TM than in C1 (Table 9). TP, Alb, and Glob concentrations were significantly ($P \leq 0.05$) lower in the TCH group than in C2. In addition, data in Table 9 indicate that UA and Creat concentrations were significantly ($P \leq 0.05$) higher in the TM group than in C1, and significantly ($P \leq 0.05$) higher in the TCH group as compared to C2. Data in Table 9 show that there was an interaction between treatments during hatch and treatment post-hatch on TP, Alb, Glob, UA, and Creat.

Data given in Table 10 indicate that both serum Trig and Gluc concentrations, unlike T_3 level, were significantly higher in the TM group than in C1, and in the TCH group than in C2. Finally, however, Cort concentration was lower in the TM group than in C1, Cort concentration was significantly higher in the TCH group than in C2. Data in Table 10 show that there was an interaction between treatments during hatch and treatment post-hatch on serum Trig, Gluc, T_3, and Cort concentrations.

DISCUSSION

Thermal manipulation effects. The rate of water loss during incubation was directly related to the rate of embryonic development, piping oxygen consumption rate, metabolic rate, and egg shell gas conductance (Tan et al. 2010). Water loss in our study was not significantly different between TM and normal incubation temperature. This may be due to the higher RH (45%) during the heat treatment period to avoid an increase in water evaporation from the eggs. This finding is in agreement with the findings of El-Sheikh (1997) concerning a broiler breeder hatching egg subjected to temperatures of 37.7, 37.2, and 37.8°C at RH of 59%, and he found that any of these temperatures had no effect on egg weight loss from the first day to the time of external piping.

In the current study, the lower hatchability and higher mortality rate as a result of the TM as compared to the control are in agreement with Yalcin

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Table 8. Effects of thermal challenge at 8-day-old ostrich chicks on body weights and some organs weight percentage[1]

Treatments	BW (g)	Liver (%)	Heart (%)	Gizzard (%)	Proventiculus (%)
Thermal manipulation during hatch (TMDH)					
C1	947[a]	3.017[a]	1.351[a]	3.714[b]	1.473
TM	905[b]	2.538[b]	1.215[b]	3.985[a]	1.479
Heat stress after hatch (TCHAH)					
C2	957.5[a]	2.82[a]	1.424[a]	3.818[b]	1.480
TCH	895.0[b]	2.73[b]	1.142[b]	3.907[a]	1.471
SEM	1.45	0.009	0.01	0.012	0.009
Interactions					
C1 × C2	975[a]	2.586[c]	1.510[a]	3.712[d]	1.486
C1 × TCH	920[c]	3.056[a]	1.338[b]	3.924[b]	1.476
TM × C2	940[b]	2.490[d]	1.192[c]	3.768[c]	1.476
TM × TCH	870[d]	2.978[b]	1.092[d]	4.046[a]	1.482
SEM	2.05	0.013	0.014	0.017	0.012
Probability					
TMDH	0.0001	0.0001	0.0001	0.0001	0.42
TCHAH	0.0001	0 0001	0.0001	0.0001	0.63
TMDH × TCHAH	0.002	0.0001	0.02	0.08	0.21

SEM = standard error of the means, TM = thermal manipulation, TCH = thermal challenge, TMDH = thermal manipulation during hatchability, TCHAH = thermal challenge after hatch, C1 = control for TM group, C2 = control for TCH group, BW = body weight

[a–d]means with different superscripts within main effects or the interaction are significantly different ($P < 0.05$)

[1]organs weights calculated as a percentage of body weight

Table 9. Effects of heat challenge at 8-day-old ostrich chicks on the levels of some blood biochemical parameters

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Thermal manipulation during hatch (TMDH)					
C1	4.52[a]	2.595[a]	1.930[b]	6.65[b]	0.395[b]
TM	4.41[b]	2.340[b]	2.065[a]	6.82[a]	0.480[a]
Heat stress after hatch (TCHAH)					
C2	4.57[a]	2.55[a]	2.015[a]	6.42[b]	0.395[b]
TCH	4.31[b]	2.38[b]	1.930[b]	7.06[a]	0.480[a]
SEM	0.006	0.006	0.006	0.007	0.006
Interactions					
C1 × C2	4.72[a]	2.77[a]	1.95[c]	6.27[d]	0.42[b]
C1 × TCH	4.42[b]	2.34[b]	2.08[a]	6.56[c]	0.37[b]
TM × C2	4.22[d]	2.42[b]	1.80[d]	7.37[a]	0.54[a]
TM × TCH	4.39[c]	2.34[b]	2.05[b]	6.74[b]	0.42[b]
SEM	0.008	0.009	0.009	0.01	0.009
Probability					
TMDH	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TCHAH	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TMDH × TCHAH	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0005

SEM = standard error of the means, TM = thermal manipulation, TCH = thermal challenge, TMDH = thermal manipulation during hatchability, TCHAH = thermal challenge after hatch, C1 = control for TM group, C2 = control for TCH group

[a–d]means with different superscripts within main effects or the interaction are significantly different ($P < 0.05$)

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Table 10. Effects of heat challenge at 8-day-old ostrich chicks on the levels of some blood biochemical parameters

Treatments	Triglyceride (mg/dl)	Glucose (mg/dl)	Triiodothyronine (ng/dl)	Corticosterone (ng/ml)
Thermal manipulation during hatch (TMDH)				
C1	152.75^{b}	179.4^{b}	120.4^{a}	30.51^{a}
TM	158.20^{a}	181.4^{a}	115.5^{b}	29.07^{b}
Heat stress after hatch (TCHAH)				
C2	144.95^{b}	170.2^{b}	123.25^{a}	29.05^{b}
TCH	166.00^{a}	190.6^{a}	112.60^{b}	31.83^{a}
SEM	0.059	0.36	0.1	0.054
Interactions				
C1 × C2	149.9^{c}	171.0^{c}	125.3^{a}	28.19^{c}
C1 × TCH	140.0^{d}	169.4^{d}	121.2^{b}	29.91^{b}
TM × C2	166.5^{a}	187.8^{b}	115.5^{d}	32.84^{a}
TM × TCH	165.5^{b}	193.4^{a}	119.5^{c}	28.22^{b}
SEM	0.084	0.51	0.1	0.076
Probability				
TMDH	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TCHAH	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TMDH × TCHAH	< 0.0001	< 0.0001	< 0.0001	< 0.0001

SEM = standard error of the means, TM = thermal manipulation, TCH = thermal challenge, TMDH = thermal manipulation during hatchability, TCHAH = thermal challenge after hatch, C1 = control for TM group, C2 = control for TCH group
$^{a–d}$means with different superscripts within main effects or the interaction are significantly different ($P < 0.05$)

et al. (2009) who reported that high incubation temperatures (37.5–39.0°C) during the last three days of incubation led to accelerated embryonic development and hatching time but decreased maximum hatchability and increased mortality rates.

Furthermore, reduction in hatch BW in the TM treatment as compared to the control in the present study is in agreement with Yahav et al. (2004a) who reported that BW was significantly reduced at high incubation temperature as compared with normal incubation temperature. Chick embryos have previously been reported to respond to elevated incubation temperature with accelerated growth and development but the accelerated development has been reported to negatively affect hatchling BW. This effect may also extend to a reduction in yolk sac absorption that reduces the nutrients available for embryo development and hatch weight (Yahav et al. 2004a).

The data from the present study are in line with Elsayed et al. (2009) reporting that with increasing incubation temperature there was a significant decrease in liver weight percentage associated with a similar decrease in the liver glycogen content. They stated that it may be due to the activation of hepatic enzyme systems that are involved in glycogen degradation (glucogenesis) to Gluc. Also, this decrease may be due to the stimulation of fat absorption from yolk sac and activation of fat metabolism (lipolysis) to produce energy. In addition, Leksrisompong et al. (2007) arrived to similar results in their study. They reported that high setter and hatcher temperatures result in smaller heart. Many common poultry problems such as sudden death syndrome and ascites are related to problems with cardiovascular system development and function.

In addition, our results are in agreement with Piestun et al. (2008) who concluded that the exposure to TM during a late period of embryogenesis caused lower TP, Alb, and Glob concentrations in serum possibly due to changes in body temperature leading to a shift in tissue fluids resulting in the change in the serum proteins concentration (Yalcin et al. 2009). In the same trend to our results, Harr (2002) reported that exposing embryos to high temperatures during incubation raised UA and Creat concentrations. The author referred this increase in the TM group at hatch to the increase in protein catabolism as a result of the increase in the

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glucocorticoid hormones as well as to the decrease in protein anabolism as a result of the decrease in T_3 level at heat stress conditions (Harr 2002). In addition, the results of Elsayed et al. (2009) that blood Gluc concentration was significantly higher when embryos were exposed to high incubation temperature (40.7°C for 3 h/day in days 15–17 of incubation) correspond with the present results. Yalcin et al. (2009) studied the effects of heat acclimation of embryos on blood metabolites and hormones of broilers. Eggs obtained from breeders were divided into 2 groups. One group of eggs was incubated at control incubation temperature (ITCONT), whereas the second group was heat-acclimated at 38.5°C for 6 h/day in days 10–18 of incubation (ITHA). There was a reduction in plasma triiodothyronine (T_3) level in ITHA than in ITCONT treated broilers. Finally, Yahav et al. (2004a) found that TM significantly reduced chick's body temperature and plasma thyroid hormones concentration, but had no effect on plasma Cort concentration.

Thermal challenge effects. Body temperature is a good indicator of the level of metabolic rate and is directly linked to acclimation. Our results are in agreement with Yahav et al. (2004a, b) and Tan et al. (2010) who found that thermal conditioning at an early age of chickens results in improved heat tolerance and reduces mortality when they are re-exposed to heat later in life. Also, they found that the initiation times for behavioural responses (panting and wing-droop posture) in experienced chicks were later than in those of the control. At the end of heat exposure treatment, the RT in experienced chicks was lower than that in the control. This finding suggests that heat tolerance may act on the thermoregulatory mechanism in chicks to increase the sensible heat loss with the progress of time (Vishwajit et al. 2012).

High environmental temperature results in a significant economic loss as a result of increasing body temperature and mortality rate and reducing performance (May and Lott 2000). Mortality rate was increased in ostrich chicks exposed to heat stress in days 6–8 after hatch as compared to the control group or birds hatched from eggs previously exposed to TM during incubation. Our results are in line with Barri et al. (2005) who reported that ostrich chicks are susceptible to stress during the first few weeks of their life. These problems often result in high mortality, which is one of the major welfare problems in the ostrich industry. Therefore, eggs exposure to high incubation temperature may help avoid the negative effect of high environmental temperature post hatch.

In general, high ambient temperature reduced feed consumption, body and organs weight, and growth rate of hens (Yalcin et al. 2009). This is in agreement with our study, proving that exposure to high temperature lowered feed consumption and weight of body and some organs of ostrich chicks. Absorption of the yolk sac provides nutrients for ostrich chicks for the first few days, but differences of opinion still remain on when feed should be provided to them. Generally, ostrich chicks lose weight in the first few days of life. For example, weight loss of ostrich chicks occurs in the first 7 days due to utilization of egg yolk and then the weight increases by about 1.3 kg/week up to 12 weeks (Mushi et al. 1998). The authors recommended that ostrich chicks should have access to feed and water from day 1 to promote the development of the digestive tract. They reported that feed consumption soon after hatch increases the rate of yolk utilization. Therefore, the decrease in feed consumption, body and some organs weight post hatch may refer to other factors than heat stress.

Our results are in agreement with Kalamah (2001) who reported that heat stress caused a significant reduction in plasma TP, Alb, and Glob in chicks. Recent evidence suggested that plasma TP and the increased synthesis of a group of proteins known as the heat shock proteins (HSPs) might point to the effect of heat stress in birds (Etches et al. 1995). However, Vishwajit et al. (2012) found that no significant changes were detected in plasma TP and UA investigated in 7-, 14-, and 21-day-old heat-exposed chicks as compared to the control groups.

In addition, our results are in line with Habeeb et al. (1993) who found that at heat stress conditions, UA and Creat concentrations were significantly elevated than in chickens from thermoneutral group. This increase may refer to the increase in protein catabolism as a result of the increase in the glucocorticoid hormones as well as to the decrease in protein anabolism as a result of the decrease in T_3 level at heat stress conditions (Habeeb et al. 1993).

Our results are also in agreement with Yalcin et al. (2008) who reported that serum Trig were significantly higher in the heat acclimated than the control chicks. While, Moraes et al. (2003) speculated that the decrease in circulating blood

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Trig levels may be attributed to food intake reduction associated with heat stress because of sensitivities of Trig concentrations to changes in energy intake.

In addition, our results are in agreement with Vishwajit et al. (2012) who found that plasma Gluc concentration gradually increased in 14– 21-day-old heat-exposed chicks. This increase was associated with the activation of hepatic enzyme system which is involved in Gluc production (Yalcin et al. 2008).

The importance of the thyroid gland in the adaptation to heat stress is related to the control role that thyroid hormones play in the regulation of metabolic rate of birds. Epigenetic heat adaptation involves changes in blood contents, hormonal and metabolic regulations that enhance heat endurance. The T_3 values measured in the present study agree with those given by Yahav et al. (2004a) and Yalcin et al. (2008, 2009) who reported lower T_3 levels in broilers incubated at high fluctuating temperatures. This indicates a carry-over effect of pre-hatch temperature treatment on post-hatch life. In addition, thermal challenge in our study induced an intensive development of hyperthermia in the control chicks, which was accompanied by a diminished capacity to reduce serum T_3 concentration, compared with the control on day 8 of age.

Finally, Cort is the most common glucocorticoid in birds and its role, under heat stress conditions, is well known (Halevy et al. 2001). Glucocorticoids released during stress mobilize lipids from adipose tissue, which supports gluconeogenesis. Stored Trig are broken down into non-esterified fatty acids, which then can be processed by the liver and other tissues for energy supply through synthesis of adenosine triphosphate. Also, glucocorticoids inhibit the synthesis of Trig from non-esterified fatty acids (Halevy et al. 2001). In the present study, the thermal treatment during late embryonic development had a numerical effect on the serum level of Cort at hatch, while the level of Cort was significantly higher in the TCH group as compared to the control. Our results are in agreement with Zulkifli et al. (2009) and Vishwajit et al. (2012) who reported that heat challenge may elevate Cort in broiler chickens. These results suggest that the application of TM at 38.5°C in late embryonic development succeeded in reducing the negative effect of heat stress in those chicks during early exposure to stressful environmental conditions.

CONCLUSION

In conclusion, the present study indicates that exposing the ostrich embryos to TM (38.5°C) during late embryonic development induced physiological changes that may represent epigenetic adaptation to TM. These same mechanisms are employed for raising the ability to cope with post-hatch heat stress.

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Corresponding Author

Dr. Mohamed Abd Elmohsen Elsayed, Atomic Energy Authority, Nuclear Research Centre, Biological Application Department, Abu Zaabal, P.O. 13759, Cairo, Egypt
Phone: + 201 141 190 052, e-mail: Mohamedmabrouk341@yahoo.com