Periodical low eggshell temperatures during incubation and post hatch dietary arginine supplementation: Effects on performance and cold tolerance acquisition in broilers

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ABSTRACT An experiment was conducted to evaluate the effects of a periodically low eggshell temperature exposure during incubation and dietary supplementation of arginine on performance, ascites incidence, and cold tolerance acquisition in broilers. A total of 2,400 hatching eggs were randomly assigned to 2 treatment groups (16 replicates of 75 eggs per treatment). The eggs were incubated at a constant eggshell temperature (EST) of 37.8°C throughout the incubation period (CON) or were periodically exposed to 15°C for one hour on days 11, 13, 15, and 17 of incubation and the EST was measured (periodical low EST; PLE). After hatching, 240 one-day-old male broiler chicks from both treatment groups were reared for 42 d with or without dietary arginine supplementation in a completely randomized design with a 2 × 2 factorial arrangement. In order to induce ascites, all chicks were exposed to a 15°C room temperature from 14 d onwards. Results showed that second grade chicks and yolk sac weight were decreased, and final body weight was increased in the PLE group. Ascites mortality rate was decreased only in the PLE group and dietary arginine supplementation had no apparent effect. In the PLE group, the packed cell volume (PCV) percentage and red blood cell (RBC) count were decreased. In conclusion, the results showed that the PLE treatment during incubation was associated with improved hatchability, chick quality, and productive performance of broilers and decreased ascites incidence during post hatch cold exposure. Dietary arginine supplementation had no beneficial effects in cold exposed broilers.

Key words: arginine, ascites, hatchability, periodical low eggshell temperature, post-hatch performance

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INTRODUCTION

Due to genetic selection, the growth rate of broilers was increased considerably and the length of the growth period was decreased by 60% as compared with that of the past. Growth of body and muscles has not been always in balance with that of the bird visceral organs such as the cardiovascular and respiratory systems. Therefore, the bird capability to survive higher and lower than preferred ambient temperatures has been decreased, leading to an increase in metabolic disorders such as ascites syndrome (Janke et al., 2004).

One of the solutions to gain thermo-tolerance and to decrease metabolic diseases in broilers is epigenetic temperature adaptation. This adaptation is based on activity regulation and gene expression, and is not dependent on the gene sequence (Nichelmann and Tzschentke, 2002). One of approaches to induce epigenetic temperature adaptation is changing the incubation temperature (Tzschentke, 2007). However, it is important to know if temperature reduction relieves the embryos from heat stress, or induces a true cold stress (Lourens, 2008). Also, heat increments may bring cold-stressed embryos to optimum temperatures, or further into a more severe heat stress situation. It is, therefore, of utmost importance to measure eggshell temperature (**EST**) profiles during incubation and temperature treatments, to know the current thermal status of embryos and the temperature direction as a result of the treatment (Lourens et al., 2005).

An optimum EST of 37.8°C through d 19 of the incubation period resulted in the highest hatchability rate and chick quality with the highest yolk free body weight (**YFBW**) and longest chick length at hatching (Lourens et al., 2005, 2007). Literature has indicated that an EST of 39.5°C from d 14 of incubation retarded the growth of embryonic organs and increased the second grade chicks (Leksrisompong et al., 2007; Molenaar et al., 2011). Moreover, heart, liver, gizzard, proventriculus, and intestine development are suppressed in

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embryo exposed to high EST (Leksrisompong et al., 2007; Maatjens et al., 2014a). However, a few studies have addressed the effects of reduced EST on post-hatch thermal resistance. Maatjens et al. (2014a) applied an EST of 36.7, 37.8, and 38.9°C from d 19 of incubation through hatching. Results showed that lower EST (36.7°C) resulted in a higher yolk-free body weight, liver weight, and intestinal weight at hatching as compared to those recorded for an EST of 38.9°C.

Arginine is an essential amino acid for birds and besides its structural role in growth, it is the precursor of endothelial nitric oxide (**NO**; Dudzinski and Michel, 2007). It has been reported that the NRC's recommendations of arginine supports maximum growth and is not enough for the maximum production of NO (Dietert and Austic, 1994). NO is a very strong vasodilator (Dudzinski and Michel, 2007) anti-mitogenic (Tan et al., 2007) compound, contributing to relaxation of the smooth muscles of pulmonary vessels, thereby maintaining vascular tone (Govers and Rabelink, 2001). Thus, the decrease in NO production, because of the deficiency in arginine, may have an important contribution to the onset of ascites. There are also reports suggesting that arginine supplementation in the diet improved the cardiovascular function (Bautista-Ortega and Ruiz-Feria, 2010) and reduced ascites incidence (Tan et al., 2007) in chickens raised under low ambient temperatures.

There have been a number of studies dealing with the effects of short-term machine temperature reduction during incubation on hatchability and cold tolerance acquisition in broilers; however, these studies have not paid attention to controlling the EST. Also, the relation between periodically low EST treating during incubation in combination with the use of post-hatch vasodilator promoting feed additives, including arginine, on thermo-tolerance, ascites incidence, and broiler performance under low environmental temperatures are lacking. Therefore, the aim of the present experiment was to determine the effects of periodical low EST exposure during incubation and post-hatch arginine supplementation on ascites incidence, cold tolerance acquisition, and performance in broilers.

MATERIALS AND METHODS

Incubation, Experimental Treatments, and Sampling

All procedures in the current study were approved by the Animal Care and Welfare Committee of our institute. A total of 2,400 hatching eggs of approximately similar weights (65 ± 6.5 g) were obtained from a 36-wk-old Arian breeder flock (Babolkenar Arian Line Breeding Center, Babolkenar, Iran). The eggs were stored at 18°C and 65% relative humidity for 3 days, pre-incubated at 24°C for 8 h, and incubated in a single stage incubator (Capacity: 16,800; Petersime, Zulte, Belgium). The eggs were incubated at 37.8°C EST and at a relative humidity of 55 to 60% during the first 11 days of incubation. During incubation, the EST was recorded by an infrared digital thermometer (Braun, Kronberg, Germany) and machine temperature was adjusted in order to maintain the EST as close to 37.8°C as possible.

On d 11 of incubation, the eggs were candled, and those with viable embryos were randomly divided to 2 treatment groups (16 trays, 75 eggs per tray, and 1,200 eggs per treatment group). Each tray was considered as a replicate. The eggs were either incubated at a constant EST of 37.8°C throughout the incubation (**CON**) or periodically exposed to periodical low temperatures of 15°C machine temperature for 1 h on d 11, 13, 15, and 17 of incubation (periodical low EST; **PLE**). The EST during incubation in CON and PLE group was recorded using an infrared digital thermometer (Braun, Kronberg, Germany) by contact at the equator of the eggs.

At hatching, 2 chicks per tray (32 chicks per treatment group) were randomly selected and killed by cervical dislocation to determine chick weight, residual yolk sac weight (**RYSW**), and body weight without the yolk sac (yolk free body weight; **YFBW**). Relative yolk weight was calculated using the following formula:

$$\text{RYSW}(\%) = \frac{\text{yolk weight}}{\text{chick weight}} \times 100$$

All hatched chicks were classified as saleable or cull (Sozcu and Ipek, 2015). The rates of embryonic mortality, hatchability, and culled chicks were expressed as a percentage of fertile eggs (Sozcu and Ipek, 2015). Unhatched eggs were broken open to determine the stage of embryonic mortality as 0 to 3 d, 4 to 14 d, 15 to 18 d, or 19 to 21 d according to Hamburger and Hamilton (1951).

Broilers, Experimental Treatments, and Sampling

The broiler rearing experiment was conducted using 240 male chicks from CON and PLE groups. All chicks were fed a same standard diet until 14 d of age. From d 14 onwards, the chicks were fed on either the standard (**STD**) or arginine-supplemented (5 g/kg; **ARG**) diet. Both groups comprised 8 replicates of 15 chicks for each incubation treatment group (120 birds in total). This experiment was conducted as a 2 × 2 factorial arrangement with incubation treatment group and dietary treatment as factors. The birds were raised under standard conditions until 14 d of age (gradual reduction from 32 to 30°C) and lowered to a constant room temperature of 15°C from d 14 onwards (Akhlaghi et al., 2012). The birds were fed with conventional mash broiler diet (Table 1). Experimental diets met or exceeded the strain requirements as appropriate. All

Table 1. Composition of the experimental diets (%).

Item	Starter (0 to 13 d)	Grower (14 to 28 d)[1]	Finisher (29 to 42 d)[1]
Ingredient (%)			
Corn	55.72	58.33	64.47
Soybean meal	39.34	36.82	31.6
Oil	1.3	1.5	1.25
Sodium bicarbonate	0.1	0.1	0.1
Salt	0.38	0.35	0.35
Dicalcium- Phosphate	1.32	1.32	0.9
Limestone	1.4	1.2	1.1
DL- Methionine	0.27	0.27	0.13
L- Lysine HCL	0.1	0.1	0.03
Threonine	0.06	0.06	0.06
Phytase	0.005	0.005	0.005
Vitamin and mineral permix	0.5	0.5	0.5
Calculated nutrient content			
CP (%)	22	21.1	19.1
ME (kcol/kg)	2,850	2,896	2,958
Calcium (%)	0.96	0.87	0.73
Available phosphorus (%)	0.39	0.38	0.3
Methionine (%)	0.6	0.59	0.43
Lysine (%)	1.26	1.2	1.02
Arginine (%)	1.46	1.39	1.24

[1]Arginine was supplemented to standard diet at grower and finisher periods as 5 g/kg.

vitamin premix (kg): vitamin A, 14,000,000 IU; vitamin D_3, 5,000,000 IU; vitamin E, 60,000 IU; vitamin B_{12},24 mg; riboflavin, 12,000 mg; niacin, 80,000 mg;d-pantothenic acid, 20,500 mg;vitamin K, 2,700 mg; folic acid, 1,800 mg; vitamin B_6, 5,000 mg; thiamine, 4,000 mg; d-biotin, 150 mg; Ca, 1.20%; Mn, 30.0%; Zn, 21.0%; Cu, 8,500 mg; I, 2,100 mg; Se, 500 mg; Mo, 1,670 mg.

chicks had free access to feed and water throughout the study. Body weight (**BW**) and feed intake were determined at weekly intervals. Blood samples were taken from the brachial vein into EDTA-coated tubes. A portion of blood was stored at 4°C for determination of packed cell volume (**PCV**) and red blood cells (**RBC**) number after centrifugation (12 min at 1,800 × *g*). A small sample (0.5 mL) of blood was obtained from each bird, and a drop was used to prepare a blood smear slide. The slides were stained using a hematology staining kit (Polysciences Inc, Warrington, PA), air dried, and stored in a slide box. Heterophil/lymphocyte ratio was measured by taking the blood smear slides prepared earlier and observing them under 1,000 × magnifications using a microscope.

Another blood portion was centrifuged (12 min at 1,800 × *g*) and the plasma was stored at −20°C, pending for thyroid hormones, T3 and T4, using commercially available kits (Pars Azmon, Tehran, Iran) verified for avian quantifications (Akhlaghi et al., 2012). Mortality rate was recorded daily and evaluated for diagnosis of ascites, where birds with accumulation of abdominal fluid were considered ascitic (Luger et al., 2002). At 42 d, 2 birds per pen were killed by cervical dislocation to record heart, liver, spleen and bursa of Fabricius weights using the following formula:

$$\text{relative weight (\%)} = \frac{\text{organ weight (gr.)}}{\text{live body weight (gr.)}} \times 100$$

The overall ascites incidence was presented as the cumulative ascites mortality throughout the production period, plus ascites observed at necropsy on d 42.

Statistical Analysis

The data were subjected to analysis of variance (SAS Institute, 2002). An arc-sine transformation was used for percentage data to obtain normally distributed data. Significant differences among treatment means were determined by the Duncan's multiple range tests. Broiler data were analysed by the GLM procedure. Ascites mortality rate was analyzed using the logistic regression model by the GENMOD procedure of the same package. The treatment effect was expressed significant ($P \leq 0.05$) based on chi-square test.

RESULTS

Figure 1 summarizes the dynamics of the EST reduction during the egg exposure to 15°C machine temperature at d 11, 13, 15, and 17of incubation for 60 min. EST was most affected in eggs being incubated up to 11 d, and least influenced in older eggs. Lowering machine temperature at 17 d had only a small effect on EST, and EST was decreased by only 2.4°C after 60 minutes. The hatchability of fertile eggs did not affected by the thermal treatment ($P = 0.51$); however, the percentage of first grade chicks was higher ($P = 0.02$) in the PLE group than that of CON (Table 2). Embryonic mortality rate in the third week of incubation was significantly different between the EST treatments ($P = 0.01$). The BW and YFBM at hatching were not influenced in the PLE treatment. However, RYSW in the PLE treatment was 30% lower as compared to CON treatment ($P = 0.003$; Table 3). Final BW at the end of grow-out period in the PLE group was 69 g higher than that recorded for the CON treatment group ($P < 0.05$; Table 4). The FCR was decreased in the PLE treatment compared to the CON treatment (Table 4). Ascites mortality rate in the PLE treatment group was 6.9 percent as low as that found in the CON group ($P < 0.05$). The findings showed that dietary supplementation of arginine had no effect on growth performance, but it was associated reduced ascites mortality rate ($P < 0.05$; Table 4).

Relative spleen weight ($P < 0.05$) and the number of lymphocytes ($P < 0.05$) were greater in the PLE than in the CON treatment group (Table 5). Heterophil to lymphocyte ratio was lower in the PLE treatment ($P < 0.01$). Supplementary arginine in the diet was associated with an increased circulatory lymphocyte percentage ($P < 0.05$). Heart weight, RBC, PCV, and the plasma levels of T3 and T4 were lower in the PLE treatment than in the control group ($P < 0.05$; Table 6).

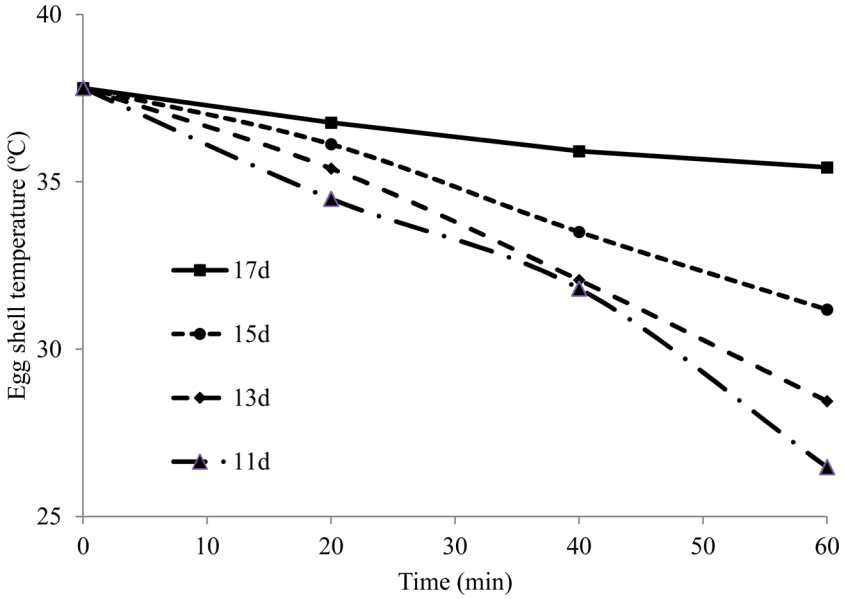

Figure 1. The eggshell temperature (EST) profiles in treatment groups during the periodically low EST treatment (PLE) on d11, 13, 15, and 17 of incubation.

Table 2. Effects of periodically low eggshell temperature (EST) during incubation on hatchability rate, chick quality and embryonic mortality.

Item	Hatchability (%)	First grade chicks (%)	Embryo mortality (as percent of fertile eggs)			
			0 to 3 d	4 to 14 d	15 to 18 d	19 to 21 d
EST,°C						
CON	84.03	78.42[b]	3.37	2.62	4.62[a]	0.87[b]
PLE	85.31	82.72[a]	3.31	2.56	2.93[b]	1.81[a]
SEM	1.48	1.46	0.52	0.48	0.41	0.22
P-value	0.51	0.04	0.93	0.93	0.01	0.01

[a,b]Within column, values with different superscripts differ significantly ($P \leq 0.05$).

CON = eggs incubated at 37.8° EST; PLE = Periodically Low EST exposure to 15°C for 1 h on d 11, 13, 15, and 17 of incubation.

Table 3. Effects of periodically low eggshell temperature (EST) during incubation on chick quality indices.

Item	BW (g)	RYSW (%)	YFBW (g)
EST,°C			
CON	49.62	8.84[a]	43.87
PLE	47.37	6.15[b]	43.37
SEM	1.30	0.38	1.32
P-value	0.24	0.003	0.79

BW = Body weight; RYSW = residual yolk sac weight; YFBW = yolk free body weight.

[a,b]Within column, values with different superscripts differ significantly ($P \leq 0.05$).

CON = eggs incubated at 37.8° EST; PLE = Periodically Low EST exposure to 15°C for 1 h on d 11, 13, 15, and 17 of incubation.

DISCUSSION

The aim of the present study was to determine the effects of periodical cold stress during incubation with or without arginine supplementation on hatchability rate, and cold tolerance acquisition in broilers. The chick quality has been demonstrated to be important for a good start for the chick and broiler performance (Meijerhof, 2009b). Absorption of the yolk sac into the abdominal cavity of the embryo during the late phase of the incubation period provides nutrients to the chicks during early post-hatch life. In this study, implementation of periodical cold stress during the incubation period considerably decreased the percentage of second grade chicks. This result agreed with other studies (Decuypere, 1984; Joseph et al., 2006). These authors reported that decreasing machine temperature to optimize EST in the setters, resulted in a decrease in the number second grade chicks.

As we know, the yolk sac is a vital impotence for embryonic development and the absorption of nutrients from the yolk sac is essential to initiate body growth (Meijerhof, 2009a) and for development of the small intestine (Noy and Skalan, 1999). If there was a large amount of residual yolk, then less development had occurred (Meijerhof, 2009b). It has clearly been shown that absorption of higher amount of yolk sac increased the development of the small intestine and lungs. In this study, PLE during incubation improved the yolk utilization and, hence, reduced the yolk sac weight. The results of this study agree with those of the previous

Table 4. Effects of periodical cold stress during incubation with or without arginine supplementation on growth performance and ascites mortality.

Item	Feed intake (g)	BW (g)	FCR	Ascites mortality (%)	Overall mortality (%)
EST,°C					
PLE	4,043	2,061[a]	1.963[b]	10.87[b]	21.39
CON	4,064	1,992[b]	2.040[a]	17.75[a]	23.90
Diet,					
ARG	4,019	2,028	1.981	14.37	21.87
STD	4,090	2,023	2.021	14.25	23.42
SEM	72.68	21.63	0.025	1.48	5.65
EST,°C × Diet					
PLE × ARG	4,091	2,082	1.965	11.25	23.75
PLE × STD	3,995	2,039	1.960	10.50	19.03
CON × ARG	3,946	1,974	1.998	17.50	20.00
CON × STD	4,183	2,008	2.082	18.00	27.81
SEM	102.79	30.58	0.035	4.11	7.99
Source of variation					
EST	0.84	0.04	0.05	0.01	0.76
Diet	0.51	0.88	0.28	0.98	0.85
EST × Diet	0.13	0.24	0.23	0.88	0.49

[a,b]Within columns, values with different superscripts differ significantly ($P \leq 0.05$).

CON = eggs incubated at 37.8° EST; PLE = Periodically Low EST exposure to 15°C for 1 h on d 11, 13, 15, and 17 of incubation; STD = standard diet; ARG = arginine-supplemented (5 g/kg).

Table 5. Effects of periodical cold stress during incubation with or without arginine supplementation on immune organs relative weight and immune cell counts.

Item	Spleen (%)	Bursa of Fabricius (%)	Leucocyte (× 10^3)	H (%)	L (%)	H/L
EST,°C						
PLE	0.161[a]	0.16	28.9	27.90	71.87[a]	0.39[b]
CON	0.108[b]	0.17	28.4	28.50	68.00[b]	0.42[a]
Diet,						
ARG	0.138	0.17	28.8	27.87	71.25[a]	0.39
STD	0.131	0.15	28.4	28.50	68.62[b]	0.41
SEM	0.015	0.02	0.86	0.80	0.55	0.01
EST,°C × Diet						
PLE×ARG	0.158	0.16	28.8	27.75	73.25	0.38
PLE ×STD	0.165	0.15	28.9	28.00	70.50	0.39
CON×ARG	0.118	0.17	28.8	28.00	69.25	0.40
CON ×STD	0.098	0.16	27.8	29.00	66.75	0.43
SEM	0.021	0.025	1.21	1.12	0.78	0.015
Source of variation						
EST	0.04	0.70	0.66	0.60	0.001	0.05
Diet	0.77	0.50	0.70	0.60	0.01	0.15
EST × Diet	0.53	1.00	0.66	0.75	0.87	0.74

[a,b]Within columns, values with different superscripts differ significantly ($P \leq 0.05$).

CON = eggs incubated at 37.8° EST; PLE = Periodically Low EST exposure to 15°C for 1 h on d 11, 13, 15, and 17 of incubation; STD = standard diet; ARG = arginine-supplemented (5 g/kg).

reports (Yalçin et al., 2012; Ipek et al., 2014; Maatjens et al., 2016). Improved yolk utilization is a reflection of improved embryonic development (Lourens et al., 2011) which might explain the increased thyroid hormones secretion from embryonic thyroid gland during PLE. Decuypere (1984) reported an inverse relationship between the incubation temperature and thyroid hormone secretion from the embryonic thyroid gland. In the PLE treatment, average BW was improved compared to the CON group. These findings were in accordance with that of Shinder et al. (2009) who reported that applying intermittent low temperatures during the later stages of incubation period improved the average BW of chickens reared in cold environments. Low ambient temperature increases the bird's requirements for energy; therefore, a larger part of the daily energy intake will be allocated to maintenance at the cost of performance (Shinder et al., 2002; Shinder et al., 2009). Broiler chicks belonging to the PLE group were more resistant to coldness and showed no increase in the levels of thyroid hormones. It can be suggested that the PLE broilers were therefore better able to allocate their energy efficiently in favor of growth instead of maintenance.

In the present experiment, the mortality caused by ascites in the PLE was lower than that of CON group. This finding was in agreement with the previous reports (Shinder et al., 2011; Shahir et al., 2012) where cold conditioning of embryos or post-hatch chicks was associated with a decreased ascites incidence. Shahir

Table 6. Effects of periodical cold stress during incubation with or without arginine supplementation on visceral organs, RBC, PCV and thyroid hormone status of broilers at 42 d of age.

Item	Heart (%)	Liver (%)	RBC ($\times 10^6$ /μL)	PCV (%)	T_3 (μg/dL)	T_4 (μg/dL)	T_3/T_4
EST,°C							
PLE	0.58[b]	2.42	2.37[b]	30.17[b]	2.87[b]	4.00[b]	0.73
CON	0.64[a]	2.41	2.55[a]	32.57[a]	3.70[a]	5.36[a]	0.69
Diet,							
ARG	0.61	2.49	2.41	30.75	3.01	4.42	0.70
STD	0.61	2.35	2.51	32.00	3.56	4.94	0.72
SEM	0.02	0.16	0.05	0.62	0.23	0.34	0.043
EST,°C × Diet							
PLE×ARG	0.60	2.57	2.34	30.40	2.70	3.74	0.74
PLE ×STD	0.55	2.27	2.40	29.95	3.03	4.26	0.71
CON×ARG	0.61	2.40	2.49	31.10	3.32	5.10	0.65
CON ×STD	0.66	2.42	2.62	34.05	4.08	5.63	0.73
SEM	0.03	0.22	0.07	0.87	0.33	0.48	0.06
Source of variation							
EST	0.04	0.98	0.03	0.02	0.03	0.02	0.54
Diet	0.96	0.54	0.22	0.18	0.12	0.30	0.66
EST × Diet	0.08	0.48	0.67	0.07	0.53	0.99	0.36

[a,b]Within columns, values with different superscripts differ significantly ($P \leq 0.05$).

CON = eggs incubated at 37.8° EST; PLE = Periodically Low EST exposure to 15°C for 1 h on d 11, 13, 15, and 17 of incubation; STD = standard diet; ARG = arginine-supplemented (5 g/kg).

et al. (2012) hypothesized that this effect might be attributed to thyroid hormone metabolism in cold conditioned birds. However, Shinder et al. (2011) related this finding to the change of post-hatch metabolic rate and body temperature of broilers. According to Tzschentke (2007), prenatal cold experience decrease neuronal hypothalamic cold sensitivity.

Dietary supplementation of arginine reduced the percentage of ascitic birds. This finding was in accordance with those of Tan et al. (2007), who reported a reduced ascites incidence for arginine supplemented diets, being mainly due to an enhanced synthesis of NO. Arginine is the precursor of NO, a very strong vasodilator causing relaxation and dilatation of pulmonary vessel's smooth muscle (Dudzinski and Michel, 2007).

In the present experiment, relative spleen weight and the number of lymphocytes in PLE broilers were increased. This finding contradicts to those of Santin et al. (2003), who suggested that changing the incubation temperature did not affect chicken immune responses. The difference found in these 2 experiments may be attributed to the duration and the level of induced cold stress. The mechanism by which the change in environmental temperature influences immune response has not completely been identified, but it seems that the level of circulating corticosteroids increases with a decrease in the ambient temperature. These hormones decrease thymus and spleen weights and reduce cell amplification factors and interleukin II (Siegel and Latimer, 1984). The ratio of heterophil to lymphocyte is a good indication of stress in broilers (Puvadolpirod and Thaxton, 2000). Therefore a decrease in the ratio of heterophil to lymphocyte in PLE chickens may indicate that these chickens accustomed to the temperature change in their growth environment.

In the present study, the average heart weight of PLE chickens was decreased. This finding is in accordance with Shinder et al. (2009, 2011), who related this changes to blood levels of thyroid hormones. Decreased incubation temperature is expected to increase the secretion of thyroid hormones from the embryonic thyroid gland, leading to a more efficient development in cardiovascular and pulmonary systems (Wittmann et al., 1983).

It was observed that under low post hatch environmental temperatures, the number of RBC and PCV of chickens in the PLE group was decreased in comparison with that of the control group and improved thermal resistance to cold, which is comparable to the findings of Shinder et al. (2009). Increased number of RBC and PCV as responses to decreased blood oxygen pressure (Julian et al., 1989; Schlosberg et al., 1998) is the bird's physiological adaptations when facing low environmental temperatures (Wideman et al., 1998a). An increase in PCV means a higher blood viscosity and more resistance toward the flow which leads to hypertension in pulmonary vessels and ends in ascites.

The results of the present experiment showed that levels of T3 and T4 in chickens of CON group were higher as compared to the PLE group. This finding was in partly accordance with those of Shahir et al. (2012) who reported that cold conditioning of broiler chicks decreased the circulatory T3 concentration. The PLE increases secretion of thyroid hormones from embryonic thyroid gland and this in turn affects the neurons responsible for temperature detection in hypothalamus and this changes neuron's sensitivity to temperature changes during post-natal growth (McNabb, 2006, 2007; Tzschentke, 2007). Decreasing the ambient temperature apparently did not over-stimulate thyroid secretion in PLE chickens in the current study.

It can be concluded that during incubation, it is of great importance to measure EST to determine the impact of treatments on embryonic development. Machine

temperature reduction during early stages in incubation has a far larger impact on the EST than late incubation when heat production is higher. Even though, the impact on EST was less during later stages of incubation, PLE during incubation increased the growth performance and decreased ascites incidence in low post-hatch temperature environments. Next to PLE, supplementation of arginine had no apparent effect on performance or ascites incidence when broilers were reared under low environmental temperatures. In conclusion, PLE treatment might be beneficial to embryonic development in broilers and might be used readily in the routine hatchery practice to improve hatching results and chick quality.

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