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Impact of spraying incubated eggs submitted to high temperature with ascorbic acid on embryonic development, hatchability and some physiological responses of hatched chicks

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ABSTRACT

Three hundred and sixty Hubbard eggs were divided into two equal groups. In the 1st group, eggs were incubated at 37.5°C up to 19 day, while those in the 2nd group were incubated at the same degree except three days (6, 7 and 8d) and exposed to 40.0°C for 3hrs. Eggs were equally classified into three ascorbic acid/AA treatments. The first one was sprayed with distilled water (control), while the 2nd and 3rd treatments were sprayed with same solution but containing 20 and 30g AA/L, respectively. The highest embryo weight (EW/g) and lowest yolk sac weight (YSW/g) were obtained in the chronic group ($P \leq 0.05$). Embryonic mortalities were significantly ($P \leq 0.05$) increased, while the hatchability was significantly decreased in the chronic compared to control group. Chick weight (g), glucose and triiodothyronine (T3) levels were significantly lower ($P \leq 0.05$) in the chronic compared to control group. H/L ratio was significantly ($P \leq 0.01$) affected by incubation temperature. Body weight (g) and percentages of liver, spleen and bursa of fabricius were significantly ($P \leq 0.05$) lower of broilers in the chronic compared to control group. Referring to AA treatment, embryo weights (g) were not affected, whereas YSW/g, PCV/%, RBCs (10⁶) and H/L ratio and bursa percentage were significantly ($P \leq 0.05$) affected.

Key words: Incubation temperatures, Ascorbic acid, chick quality traits, hatchability, embryonic development

INTRODUCTION

Eggshell temperature during incubation period plays an important role in embryonic development, hatchability, survival and the performance of broilers (Willemsen *et al.* 2010; Molenaar *et al.* 2011a). Therefore, the embryonic growth rates during incubation period seem to be sensitive to temperature fluctuations (Molenaar *et al.* 2010). The results of some researchers as Lourens *et al.* (2007) and Nariņš *et al.* (2016) revealed that the optimum temperature until 19 days of incubation amounted 37.5°C for chickens to achieve the highest

hatchability with the best chick quality at hatch. In the first stage of incubation, live embryos in the eggs are absorbing heat from the surrounding air and leading to changes in the temperature of embryos (**Leksrisompong et al. 2007** and **Pulikanti et al. 2011**). During the second stage especially after the 9th day of incubation, the optimal temperature, it becomes difficult to achieve due to excessive heat production of the developing embryos (**Lourens et al. 2007**). Increasing incubation temperatures above 37°C was found to negatively affect the development of the broiler body weight as well as relative heart weight by about 17-31% (**Leksrisompong et al. 2007** and **Lourens et al. 2005**). The findings of **French (2000)** indicated that increasing the incubation temperature by 0.5 or 1°C (from 37.5 to 38.0 or 38.5°C) during the period from 3 to 25 days of incubation significantly affect the embryonic development of turkeys. Hence, controlling the embryo rather than the incubator temperature improves chick quality (**Hulet et al. 2007** and **Meijerhof, 2009**). The increase of eggshell temperature more than 39.5°C retarded the organ growth of embryos (**Leksrisompong et al. 2007**). The results of **Maatjens et al. (2014)** and **Badran et al. (2012)**, indicated that the development of crop, gizzard, proventriculus, liver, intestine development and heart was suppressed in the embryos exposed to high incubation temperatures. The temperature during incubation may influence thermoregulation of hatched chicks. Therefore, the peculiarity of epigenetic adaptation, which may occur during early pre- or postnatal ontogeny (**Nichelmann and Tzschentke 2002**), may contribute to alleviating problems associated with the thermoregulation during rearing. Therefore, it could be suggested that modifying the incubation temperatures may be a way to enhance post-hatching performance of poultry (**Yalcin and Siegel 2003**). Nowadays, ascorbic acid is used as anti-stress agent in raising poultry, as well as in incubating their eggs to reduce embryonic stress exposed to high temperatures. The ability of diluted ascorbic acid to interact with the eggshell cuticle was reported by (**Yassien et al. 2014**). In-ovo nutrition through egg injection or dipping with ascorbic acid has been applied during incubation to improve hatchability of chicken eggs (**Shafey, 2002**). Similarly, the injection of vitamins in-ovo has been applied to improve hatchability and hatchling body weight (**Robel, 1993**). In contrast, Ascorbic acid (Vitamin C) was found not to have positive effects on hatchability and hatchling body weight when eggs were incubated under intermittent heat stress (**Mohammed et al. 2011** and **Nowaczewski et al. 2012**). It is worth to mention that the injection into hatching eggs during the incubation period is considered as stressful manipulation for the growing of embryos, in addition to needed the skills and experience. Therefore, the current study was planned to investigate the impact of spraying hatching eggs exposure to high temperature with ascorbic-acid solutions

during early embryonic stage on embryonic vitality, hatchability, chick quality, haematological and some physiological body reactions.

MATERIALS AND METHODS

This study was performed at the Experimental Poultry Farm, Poultry Production Department, Faculty of Agriculture, Sohag University, Egypt.

Experimental eggs and management:

Eight hundred and seventy Hubbard broiler eggs were produced from hens at 42-weeks old and purchased from Elahiwa Station, Sohag governorate, Sohag, Egypt. From these incubator eggs, 360 eggs were taken and used in the present study. They were randomly distributed into two equal experimental groups (180 eggs/each). In the first, eggs were incubated at 37.5°C and 55-60 RH% from the first day to day 19 and considered as control group, while those in the 2nd group were incubated under the same degree except during the early embryonic stage (6-8days), since they were exposed to 40°C and to 55-60%RH for only 3 hours (12-15PM) daily. Eggs in each group were classified into three spraying treatments, in the 1st one; eggs were sprayed with only distilled water and considered as control, while those in the 2nd and 3rd treatments were sprayed with 20.0 and 30.0g AA/liter distilled water, respectively. The average egg weight amounted 63±2g. Temperature in the incubator was automatically adjusted and turned through 90° automatically every 2 h (Aygun *et al.* 2012).

Preparation of Ascorbic-acid solution and egg treatments: Ascorbic-acid solutions were freshly prepared by dissolving AA powder in distilled water and autoclaved in a dark environment due its high photosensitive to light. Distilled water and solutions were set in an incubator at 37.5°C for two hours before spraying.

Eggs treatments: After cleaning the egg surface with 100% ethanol, the eggshell was sprayed with ascorbic-acid with 50ml/ 1000 eggs after heat exposure. Eggs were placed horizontally, and the solution was applied in the egg surface of the opposite to the air chamber. On the 6, 7 and 8th day of incubation, eggs were transferred to the hatchery, and exposed to 40.0°C and 55-60% RH for three hours daily.

Studied traits:

Egg weight loss (water loss): Egg weight loss was calculated as the difference in egg weight before placement and on 18th day of incubation and expressed as a percentage of the initial egg weight. Eggs containing dead embryos and unfertile

eggs were excluded from the calculation for percentage egg weight loss (**Aygun and Sert, 2013**).

Embryonic parameters: At 18th day of incubation, 90 eggs (2 group's $\times$ 3 treatments $\times$ 5 eggs $\times$ 3 replicates) were randomly sampled to determine the embryonic development. Embryos in each treatment were killed by cervical dislocation. Therefore, both embryo and yolk sac weights were determined (**Willemsen et al. 2011**). Embryos were carefully separated from the yolk sac and embryonic membranes. After drying excessive embryonic fluids with absorbent papers, embryos and yolk sacs were weighed. Embryo length was measured from the tip of 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**).

Embryonic mortality, hatchability and duration of incubation: Early, intermittent and late-term embryonic mortality percentages were determined during 0-7, 8-14 and 15-21d, respectively. Late-term embryonic mortality was calculated as follows: (number of dead embryos at late term/all viable eggs at transfer) $\times$ 100, while chick culled rate was determined as the number of abnormal chicks /all viable chicks at hatch $\times$ 100. At hatch, the hatchability rates were expressed as percentages of fertile eggs (**Molenaar et al. 2011a**). The duration of incubation was determined as number of hours from placement to hatching.

Chick quality traits: Approximately after 12 hours from hatch, 90 dry chicks (2 group's $\times$ 3 treatments $\times$ 3 replicates $\times$ 5 chicks) were randomly taken to determine chick weight and length. Then, chicks were killed by cervical dislocation to determine the residual yolk sac weight and intestine length as well as the percentages of heart, intestine, bursa, gizzard and liver. Chick length (cm) was measured applying the same method used for determining of embryo length.

Physiological body reactions: The wing, head, shank, back and cloacal temperatures of chicks were determined and recoded using an infrared thermometer and the average **body surface temperature (BST/°C)** was calculated according the equation: $BST/^{\circ}C = (0.12 \times \text{wing } T) + (0.03 \times \text{head } T) + (0.15 \times \text{shank } T) + (0.70 \times \text{back } T)$, as described by **Richard (1971)**. The **cloacal temperatures (°C)** of the chicks were also measured by inserting a digital thermometer into the cloaca at deep 1cm with the nearest 0.1°C.

Productive traits: Ninety unsexed broiler chicks were randomly allocated into two groups (control and chronic high temperature and placed in 18 battery cages to

provide 5 chicks /replicate). Using a balance at ± 0.1 g precision, chicks were weighed on the first day of the growing period and fed on a standard pelleted broiler starter diet (22.52% CP and ME 12.78 MJ/kg of diet) from 1 to 14 days, a grower diet (22.12% CP and ME 14.14 MJ/kg of diet) from 15 to 28 days, and finally a finisher diet (21.0% CP and ME 13.56 MJ/kg of diet) from 29 to 42 days. Feed and water were offered *ad-libitum* during the experimental period. They were exposed to 23 hours of light and 1 hour of darkness (30 to 40 lux/m²) up to 42 day of age. Died chicks were daily recorded and the mortality rate were calculated.

Slaughter traits: At day 42 of the experiment, 72 broiler chicks (2 groups $\times$ 3 treatment $\times$ 3 replicates $\times$ 4 chicks) were randomly selected and slaughtered to measure body weight, liver, spleen, bursa of Fabricius and gizzard. The hearts were removed and dissected to obtain heart weights for calculating the RV: TV ratio (Huchzermeyer *et al.* 1988). The RV: TV ratio was calculated and the broilers with RV/TV ratios above 0.27 were classified as ascitic (Huchzermeyer *et al.* 1988 and Wideman, 2001).

Blood collection: At hatch, 90 samples were collected (2 group's $\times$ 3 treatment $\times$ 3 replicates $\times$ 5 chicks) were collected into heparinized tubules for haematological study. Blood samples were centrifuged to separate plasma out then decanted and deep frozen for analysis.

Estimation of haematological variables, glucose levels and T3 hormone concentration: Approximately 1 ml blood /chick was collected for hematological tests, including the packed cell volume (PCV), which was determined by micro-hematocrit method (Schalm *et al.* 1975). Hemoglobin (Hb) concentration was measured spectrophotometrically using cyanomet hemoglobin method (Schalm, 1975). Red blood cell (RBC/ 10^6) count was estimated using haemocytometer (Schalm *et al.* 1975). The granular and nongranular WBCs based on the procedures of Gross and Siegel (1983). Briefly, one drop of blood was being smeared on of the glass slide. The smears were stained using Wright's stain. One hundred leucocytes, including granular and nongranular, were counted on different microscopic fields representing 100 cells, and the heterophil to lymphocyte ratio was calculated. Plasma triiodothyronine (T3) was determined by the commercial kits. The glucose concentration was measured according to Trinder (1969) using commercial kits.

Statistical analysis: The obtained data were subjected to statistical analysis according to general linear model (GLM) of program (SAS, Institute, 2004)

according the following statically model: $Y_{ijk} = \mu + I_i + T_j + IT_{ij} + e_{ijk}$ where: Y_{ijk} = An observation, μ = Overall mean, I_i = Effect of incubation temperature ($i = 1, 2$), T_j = Effect of the ascorbic acid dose ($j = 1, 2, 3$), IT_{ij} = Effect of interaction between incubation temperature and ascorbic acid dose and e_{ijk} = Random error.

RESULTS AND DISCUSSION

Embryonic quality traits: The effects of incubation temperature and Ascorbic-Acid treatments on the embryonic parameters at the 18th day of incubation are presented in Table 1.

The results showed that the highest ($P \leq 0.05$) embryo weight and the embryo length with the lowest yolk sac weight were obtained from embryos exposed to chronic group. The increase in embryo weight under chronic temperature could be attributed to faster embryo development through the increased metabolic rate leading to higher gas exchange rate, which is essential to cover the requirements of the fast metabolism and guarantee the rapid development. These results are in agreement with those of **Walstra *et al.* (2010)** who reported that increasing incubation temperature to 40.7°C for 3 h/d, from days 15 to 17 of incubation, leading to decrease in yolk sac weight. Regarding the effect of ascorbic-acid treatments, the results showed no significant effects on egg weight, relative loss weight and embryo quality traits. There were no significant differences were found among treatments for embryo weight and length. The interaction had no significant effect on these parameters, except yolk sac percentage (Table1).

Embryonic mortality, hatchability and duration of incubation: Data presented in Table 2 showed significant effects ($P \leq 0.05$) for incubation temperatures on early and late embryonic mortalities as well as chick culled and hatchability percentages. Also, incubation period and time between external piping and hatching were positively influenced ($P > 0.05$) by the incubation temperature, being shorter when eggs were exposed to 40.0°C than at 37.5°C. The increased embryonic mortality rate in the high temperature group may be due to insufficient heat loss by the egg and consequently affects the homeostasis of embryos as well as may be due to excessive water loss from the eggs, causing high late mortality rates because of dehydration (**Ono *et al.* 1994**). Egg weight loss in broilers is 12-14% (**Rahn *et al.* 1981**), in quails, 10.33-10.78% (**Aygun and Sert, 2013; Aygun *et al.* 2012; Aygun and Sert, 2012**), in turkey, 9.6-11.5% (**Hulet *et al.* 1987**), in partridges, 9.84-26.53% (**Caglayan *et al.* 2009; Gomez-de-Travededo *et al.* 2014**).

The lowest hatchability percentage in chronic group could be attributed to the increased water loss and consequently to the insufficient egg contents needed to the embryonic development. This finding agree with those of **Morita *et al.* (2009)**, who recorded decreased of hatchability rates at temperatures higher than the usual for broiler chickens. Also, these findings showed that the duration of incubation was shortened when eggs were incubated at 40.0°C compared with 37.5°C. This could be attributed to accelerated in-ovo development, as confirmed by their higher conductive and evaporative heat losses and higher conductance, and consequently to the higher metabolic rates (**Sgavioli *et al.* 2014**). The early, intermediate and late embryonic mortalities in the treated eggs with AA decreased remarkably than those of control group. These results agree with those of **Yassein *et al.* (2014)**, who found that the early embryonic mortality (1-7days) and unhatched chicks (before and after piping) percentages were insignificantly affected. While, the author added that the embryonic mortality rates during both of the medium (8-14 days) and late (15-21 days) were significantly ($P \leq 0.01$) lower than those of the control group. The achieved results showed significant positive effects ($P \leq 0.05$) on the hatchability due to sprayed eggs as compared with control group. The increased hatchability in treated groups may be due to the beneficial effect of ascorbic-acid on egg shell conductance which is essential for the exchange of respiratory gases during incubation (**Tullett, 1978**) or due to the ability of AA as a weak acid to interact with the egg shell cuticle, which allows the embryos to break egg shell at hatch (**Shafey, 2002**). Contrarily, the embryonic mortality, dead after piping, hatch time and period between external piping and hatching were not affected by the treatment with Ascorbic-Acid or the interaction between incubation temperature and AA treatment.

Hatchling quality traits and physiological body reactions:

At 12 hour after hatch, the results presented in Tables 3&4 showed that chick weight in control was significantly higher ($P \leq 0.05$) than in chronic group. Also, the yolk sac weights and chick length were significantly higher ($P \leq 0.05$) in the chronic than in the control group. The increased chick weights in the control group could be attributed to the increased uptake of the yolk sac into the abdomen of the embryo, which provides the required nutrients for the chick during the first few days of life (**Meijerhof, 2009**). These results are in agreement with those of **Sozcu and Ipek (2015)**, who found that the higher chick and yolk sac weights were in the control, while the lower corresponding values were obtained in the chronic high group at 12

h after hatch. The body surface and cloacal temperatures of the chicks in the chronic group (31.64 and 39.08°C) increased significantly ($P \leq 0.05$) than those (31.32 and 38.65°C) of the control. This may be due to the effect of high incubation temperature, which led to the increased physiological body reactions of chicks. The heart, intestine, bursa Fabricius and Gizzard weights were significantly higher ($P \leq 0.01$) in the control than those of the chronic group. The decreased heart weight at hatch in the chronic group may be due to the increased susceptibility and to the incidence of metabolic disorders related with cardiovascular development later in life, such as ascites (**Leksrisompong *et al.* 2007**). Also, the decreased liver weight may be due to retarded liver development probably prejudiced the energetic metabolism and consequently the development and survival of the embryo, increasing the mortality rate mainly prior to the incubation period. These findings agree with those of **Sgavioli *et al.* (2014)**, who found that the absolute and relative weights of the yolk sac and the liver were higher and those of the heart and the gizzard were lower as the eggs were incubated at 39°C as compared with 37.5°C. The highest values of glucose (mg/dl) and T3 hormone (μ /L) for newly-chicks were found in the control, while the lowest one was recorded in chronic group ($P \leq 0.001$). The decreased glucose level in the chronic group may be attributed to faster absorption in the yolk sac. These results are in agreement with those of **Badran *et al.* (2012)**, who found that the levels of T3 were significantly ($P \leq 0.05$) lower in the chronic group as compared with the control during all embryonic stages. Similarly, **Yalçın *et al.* (2009)** found a reduction in plasma triiodothyronine (T3) level in heat acclimated than the control broilers. Regarding the effect of Ascorbic-acid treatments, chick weight was insignificantly increased in treated groups ($P > 0.05$) than that of the control. Also, chick and yolk sac weights as well as chick length were not affected by ascorbic acid treatments. These results agree with those of **Yassein *et al.* (2014)**, who indicated that body weight at hatch was insignificantly affected by spraying treatments, contained either 20 or 30g AA/liter. The relative weights of the heart, intestine, bursa, liver, and gizzard were insignificantly affected ($P \leq 0.05$) by AA treatment or interaction between incubation temperature and AA treatment. The weight of yolk sac and relative liver were higher for eggs incubated at 40.0°C, while those of the heart and the gizzard were significantly lower. The levels of glucose (mg/dl) and T3 hormone (μ /L) for newly-chicks were significantly influenced by AA treatments ($P \leq 0.001$). This could be attributed to the

positive effects of AA through improving the embryo weight and physiological status of treated chicks.

These findings are in agreement with those of **Sahin *et al.* (2002)** who found that the supplemental vitamin C increased T3 hormone concentration in Japanese quail.

Hematological parameters, leukocytes count and differential percentages in the newly hatched chicks:

From data Table 5, the PCV (%) and RBCs (10^6) were significantly influenced by incubation temperature ($P \leq 0.05$), while Hb (g/dl), Leukocyte counts (10^3), eosinophils, monocytes, basophils were not affected. After hatch, our findings showed that the heterophil percentages and the H/L ratio were higher, while the lymphocyte percentages were lower in chicks produced from eggs exposed to high temperature during incubation period. This could be related to the natural cellular immunity of the chicks during incubation period and immediately after the hatchability. Also, the higher lymphocyte percentage in relation to the other leukocytes showed difference between the neonatal chick and the adult bird. The heterophils has been considered the response to high and moderate stress in birds (**Maxwell *et al.* 1992**), while the H/L ratio is a known index to measure stress in chickens (**Gross and Siegel, 1983**).

Regarding the effect of AA treatments, the PCV (%), RBCs (10^6) and H/L ratio were significantly affected ($P \leq 0.05$), while Hb (g/dl), heterophils, Leukocyte counts (10^3), eosinophils, monocytes, basophils were not affected. These results disagree with those of **Yassein *et al.* (2014)**, who showed that the total count of white blood cells (WBC's) were significantly ($P \leq 0.01$) increased, while, RBC's count was not affected by spraying fertile eggs with either 20 or 30 g AA. The H/L ratio was significantly decreased ($P \leq 0.01$) in the treated groups, while the highest value (1.86) was obtained in the control. The percentages of heterophils, lymphocytes, basophils and H/L ratio were significantly ($P \leq 0.001$) affected by the interaction, while the PCV (%), HB(g/dl), RBCs (10^6), leukocytes (10^3), eosinophils and monocytes were not affected.

Productive traits of broiler chicks up to 42 day of age:

The initial body weight on day 1 was significantly ($P \leq 0.01$) higher in control than in the chronic group. There was a significant increase in the live weights allover experimental period in the control group than that of the chronic group, except at the 2nd week. At the end of experiment, the live weight of broilers in the control and

chronic groups were 2515.89 and 2442.22g, respectively ($P \leq 0.04$). The effects of incubation temperature fluctuations can impact the economic returns of the poultry industry by affecting post hatch broiler performance and processing yields (**Lourens and van Middelkoop, 2000**). During the growing period, the effect of incubation temperature on live weight was observed where broilers in the control were heavier body weight than those in the chronic group. This may be attributed to the variability in the chick initial body weight, which reflected the final body weight. These results are in agreement with those of **Hulet *et al.* (2007)**, who found that the changes in the eggshell temperature during the incubation period affected chick initial body weight. The cumulative feed consumption up to 42 days of age was significantly lower in the control than those of the chronic group ($P \leq 0.001$). During the 6-week growing period, the chronic (1.92) group had a significantly ($P \leq 0.01$) higher FCR relative to the control group (1.72). The improved feed consumption and feed conversion ratio for chicks produced from eggs under normal incubation temperature may be related to normal intestinal development at hatch. The obtained results are in agreement with those of **Palo *et al.* (1995)**, who reported that the development of the gastrointestinal tract has a major role in chick growth during the post hatch early growing period. These findings showed that the mortality rate due to ascites and total mortality were significantly ($P \leq 0.001$) higher in the chronic group, which clearly showed that the high temperatures increased the mortality rate during embryonic development due to ascites. The higher mortality rate due to ascites in the chronic group could be attributed to the adverse effects of high incubation temperature, resulting in a negative effect on the pulmonary vascular capacity, and increasing the oxygen metabolic demand, which leads to the development of ascites (**Lubritz and McPherson, 1994**). The achieved results of the current study indicated that the mortality rate due to the increased ascites in the chronic coinciding with the higher values of PCV, Hb, and RBC. Furthermore, a RV: TV ratio greater than 27% was considered as indicator for ascites (**Wideman, 2001**).

Regarding the effect of spraying hatching eggs with ascorbic-acid, it could be noted that body weight at (one day, 7 and 14 day of age) were significantly ($P \leq 0.05$) increased in AA treatments as compared with the control. However, body weight at 21, 28, 35 and 42 days of age were not affected by spraying treatments contained either 20 or 30 g AA. These results are in agreement with the findings of **Ghonim *et al.* (2009)**, who found that body weight was increased by 5.11, 6.55 and 0.74 % of

ascorbic-acid treatments by dipping, spraying and injection treatments as compared with control of ducklings during the first two weeks of age.

The results showed that the feed consumption during the growing period was insignificantly improved by ascorbic-acid spraying treatments (30 g AA/liter distilled water), while, feed conversion at different ages was not affected. This improve could be attributed to the action of AA in increasing metabolic rate. These findings agree with **Yassein *et al.* (2014)**, who found feed consumption at different ages was not affected by AA spraying treatments. Also, results in Table (6) showed that the mortality due to ascites and total mortality were significantly ($P \leq 0.001$) decreased in sprayed eggs than those of the control. The decreased mortality rate in chicks produced from sprayed eggs may be due to the role of AA in the regulation of body temperature, activation of the immune system and to its anti-stress properties **Kutlu (2001)**. Concerning the effect of interaction with AA dose, it could be observed that body weight and total weight gain, total feed consumption and feed conversion were insignificantly affected, while the mortality rate percentage was significantly affected (Table 6).

Slaughter weight, heart parameters and immune organs of broiler chick at (42d):

Data presented in Table 7, indicated that liver weight (g), liver (%), TV (g) and spleen (%) were significantly decreased in the chronic group than that of the control, while RV and RV: TV ratio were significantly increased ($P \leq 0.001$). The RV and RV: TV ratio were significantly ($P \leq 0.01$) higher in the chronic group (2.94 and 0.27) than in the control (2.61 and 0.21) group. The reduced heart weights at hatch due to higher eggshell temperatures may be due to the incidence of metabolic disorders related with cardiovascular development later in life, such as ascites (**Leksrisompong *et al.* 2007**). The RV: TV ratio was higher in the chronic than those in the control group. These results agree with those of **Molenaar *et al.* (2011a)**, who found that the ratio between the right and total ventricle mass of broilers was 1.1% higher in the high (38.9°C) as compared with control (37.5°C) at slaughter age. Also, the findings **Decuypere *et al.* (2000)** showed that the broilers susceptible to ascites present higher O₂ carrying capacity, blood viscosity, number of red blood cells, hematocrit value, and a lower relative heart weight than normal broilers. The slaughter weight (g), liver (%), RV (g), TV (g), RV: TV ratio and spleen (%) were not affected by AA treatments or by interaction, while the bursa of Fabricius (%) was significantly increased ($P \leq 0.05$). These results agree with the findings of **Selim *et al.* (2012)** who found that the relative

weights of thymus and bursa of Fabricius were comparatively higher in AA treatments as compared with the control group.

CONCLUSION

From the achieved results could be concluded that there was significant negative effects of high incubation temperature (40°C) during early embryonic stage on embryo traits, embryonic mortality, hatchability, immune organs, incubation duration and newly hatched chick weight and subsequently growth during growing period. Also, the findings revealed that spraying hatching eggs with 20 and 30g/liter ascorbic-acid after exposure to high incubation temperatures led to reduce early, intermittent and late embryonic mortalities by about 16.2, 24.6 and 11.3% as well as 16.2, 24.6 and 8.6%, respectively. Hatchability percentages and chick weights were significantly increased in the sprayed eggs as compared with the control. Thus, from these results could be recommended to use ascorbic-acid spraying at 20 or 30g /liter to minimize the adverse effect of high eggshell temperatures during early incubation period to improve hatchability, hatchling traits, chick parameters, hematological responses and physiological body reactions.

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For Review Only

Table 1. Effect of incubation temperature, ascorbic acid treatments on embryonic development parameters at 18th days of age

Traits → Treatment ↓	Initial egg weight (g)	Egg weight (g) 18 day	Relative water loss weight (%)	Embryo quality traits		
				Embryo weight 18d (g)	Yolk Sac (g)	Embryo Length (cm)
Effect of incubation temperature (IT)						
Control(37.5°C)	64.30	57.68	10.29	27.10	12.91	13.64
Chronic(40.0°C)	64.34	57.70	10.32	28.53	12.06	14.40
SEM	0.59	0.59	0.42	0.47	0.28	0.20
Effect of ascorbic acid (AA)						
Control	63.70	57.5	9.64	27.03	12.49	13.89
20g/Liter	65.03	57.7	11.27	28.30	11.94	14.05
30g/Liter	64.20	57.7	10.12	28.13	13.03	14.12
SEM	0.72	0.73	0.52	0.57	0.35	0.24
Probability						
IT	0.9685	0.9833	0.9142	0.0350	0.0382	0.0093
AA	0.4394	0.9614	0.1162	0.2455	0.0967	0.8035
IT×AA	0.8371	0.6539	0.4893	0.7594	0.0099	0.3474

Means with different superscripts in the same column are significantly different ($P \leq 0.05$).

Table 2. Effect of incubation temperature, ascorbic acid treatments on embryonic mortality and hatchability

Traits → Treatment ↓	Embryonic mortality			Dead after piping (%)	Hatchability (%)	Chick cull rate (%)	Incubation time (H)	TEPH (H)
	0-7	8-14	15-hatch					
Effect of incubation temperature (IT)								
Control (37.5°C)	2.44	2.32	4.61	3.11	85.55	1.78	513.11	13.83
Chronic (40.0°C)	3.94	2.49	6.28	3.28	81.14	2.71	497.78	10.61
SEM	0.237	0.23	0.538	0.37	0.66	0.17	4.028	0.40
Effect of ascorbic acid (AA)								
Control	3.58	2.88	5.83	3.58	81.05	2.57	502.50	11.83
20g/Liter	3.00	2.17	5.17	2.83	84.83	2.00	507.17	12.50
30g/Liter	3.00	2.17	5.33	3.17	84.17	2.17	506.67	12.33
SEM	0.29	0.28	0.659	0.45	0.81	0.208	4.93	0.49
Probability								
IT	0.0005	.6186	0.0460	0.7548	0.0003	0.0017	0.0196	0.0001
AA	0.2922	.1557	0.7621	0.5190	0.0117	0.1785	0.7681	0.6186
IT×AA	0.3110	.7974	0.4984	0.8135	0.3913	0.3583	0.5213	0.9624

Means with different superscripts in the same column are significantly different ($P \leq 0.05$). TEPH= Time between external piping and hatching

Table 3. Effect of incubation temperature, ascorbic acid treatments on hatchling quality traits

Traits → Treatment ↓	Initial egg weight (g)	Hatchling quality traits (After Hatching)			Physiological body reactions	
		Chick weight (g)	Yolk sac weight (g)	Chick length (cm)	Body surface temperature (°C)	Cloacal Temp. (°C)
Effect of incubation temperature (IT)						
Control (37.5°C)	64.30	42.16	5.23	19.50	31.32	38.65
Chronic (40.0°C)	64.34	40.70	5.90	20.01	31.64	39.08
SEM	0.59	0.45	0.16	0.21	0.09	0.096
Effect of ascorbic acid (AA)						
Control	63.70	40.42	5.64	19.53	31.893	39.54
20g/Liter	65.02	41.79	5.51	19.83	31.433	38.68
30g/Liter	64.24	42.07	5.54	19.93	31.123	38.37
SEM	0.72	0.55	0.20	0.26	0.11	0.118
Probability						
IT	0.9685	0.0260	0.0052	0.0966	0.0133	0.0024
AA	0.4394	0.0898	0.8990	0.4795	0.0001	0.0001
IT×AA	0.8374	0.8776	0.0009	0.7269	0.0735	0.3270

Means with different superscripts in the same column are significantly different ($P \leq 0.05$).

Table 4. Effect of incubation temperature, ascorbic acid treatments on hatchling organ percentages, glucose level and T3 hormone concentrations

Traits → Treatment ↓	Hatchling organ percentages						Glucose (mg/dl)	T3 (μL)
	Heart (%)	Intestine (%)	Bursa (%)	Gizzard (%)	Liver (%)	Intestine length (cm)		
Effect of incubation temperature (IT)								
Control (37.5°C)	1.00	4.69	0.15	4.24	2.30	34.93	163.45	109.49
Chronic (40.0°C)	0.83	4.45	0.10	3.79	2.83	38.28	150.27	100.98
SEM	0.04	0.14	0.003	0.04	0.05	0.54	0.35	0.70
Effect of ascorbic acid (AA)								
Control	0.92	4.53	0.13	3.97	2.52	35.73	146.14	95.00
20g/Liter	0.90	4.57	0.13	4.02	2.54	37.63	160.17	106.43
30g/Liter	0.93	4.77	0.12	4.04	2.65	36.46	164.27	114.27
SEM	0.05	0.17	0.004	0.05	0.057	0.67	0.429	0.86
Probability								
IT	0.0149	0.0990	0.0001	0.0001	0.0001	0.001	0.0001	0.0001
AA	0.9372	0.5966	0.9784	0.6629	0.2255	0.1370	0.0001	0.0001
IT×AA	0.1771	0.5966	0.6205	0.0186	0.3858	0.4850	0.0006	0.0629

Means with different superscripts in the same column are significantly different (P≤0.05).

Table 5. Effect of incubation temperature, ascorbic acid treatments on hematological parameters and leukocytes count and differential percentages in hatched chicks

Traits → Treatment ↓	Hematological parameters			Leukocytes count and differential percentages						H/L ratio
	PCV (%)	HB (g/dl)	RBCs (10 ⁶)	Count (10 ⁹)	Heterophils (%)	Eosinophils (%)	Lymphocyte s (%)	Monocytes (%)	Basophils (%)	
Effect of incubation temperature (IT)										
Control(37.5°C)	25.40	11.22	1.94	20.33	53.53	3.19	34.44	2.97	5.87	1.57
Chronic(40.0°C)	26.76	10.69	2.18	21.02	58.27	3.33	29.60	2.73	6.08	1.99
SEM	0.28	0.19	0.04	0.39	0.35	0.17	0.437	0.15	0.19	0.03
Effect of ascorbic acid (AA)										
Control	25.09	10.22	1.94	20.11	56.30	3.58	31.20	2.95	5.99	1.86
20g/Liter	27.02	11.24	2.10	20.81	55.80	3.00	32.60	2.80	5.80	1.72
30g/Liter	26.12	11.41	2.14	21.11	55.60	3.20	32.27	2.80	6.13	1.76
SEM	0.34	0.23	0.05	0.478	0.43	0.20	0.53	0.18	0.23	0.04
Probability										
IT	0.0010	0.2217	0.0004	0.2217	0.0001	0.5429	0.0001	0.2783	0.4489	0.0001
AA	0.0008	0.3284	0.0306	0.3284	0.4784	0.7462	0.1610	0.8040	0.6016	0.0028
IT×AA	0.2944	0.7630	0.7065	0.7630	0.0264	0.1293	0.0169	0.1759	0.0009	0.0537

Means with different superscripts in the same column are significantly different (P≤0.05).

Table 6. Effect of incubation temperature, ascorbic acid treatments on live weight (g), cumulative feed consumption (g) and feed conversion ratio (FCR) until 42 d of age

Traits → Treatment ↓	one day	7 days	14 days	21 days	28 days	35 days	42 days	TWG (g)	TFC (g) 1-42days	FCR (F/g) 1-42days	MR (%)	TMR (%)
Effect of incubation temperature (IT)												
Control (37.5°C)	42.29	145.24	412.88	759.22	1336.33	2056.11	2515.89	2473.60	4601.44	1.72	2.96	5.18
Chronic (40.0°C)	41.27	140.40	407.67	741.11	1274.78	1940.80	2442.22	2400.99	4236.11	1.92	5.18	11.11
SEM	0.348	1.318	2.58	5.57	13.74	19.38	25.36	25.62	11.52	0.019	0.34	1.05
Effect of ascorbic acid (AA)												
Control	40.87	137.70	405.33	740.00	1282.33	1980.87	2449.67	2408.79	4440.50	1.85	5.55	13.33
20g/Liter	42.31	142.43	409.33	750.67	1309.67	2016.67	2500.33	2458.02	4419.17	1.81	4.44	6.66
30g/Liter	42.09	148.33	416.17	759.83	1324.67	1997.83	2487.17	2445.07	4396.67	1.81	2.22	4.44
SEM	0.427	1.61	3.17	6.82	16.83	23.737	31.06	31.381	14.10	0.02	0.42	1.28
Probability												
IT	0.0337	0.0110	0.1575	0.0239	0.0021	0.0001	0.0430	0.0483	0.0001	0.0001	0.0004	0.0013
AA	0.0421	0.0001	0.0559	0.1266	0.2026	0.5681	0.4915	0.5188	0.0956	0.3236	0.0002	0.0006
IT×AA	0.7944	0.9488	0.7180	0.8510	0.6511	0.9601	0.9931	0.9929	0.9940	0.9924	0.0001	0.0001

Means with different superscripts in the same column are significantly different (P≤0.05). TWG (g) = Total weight gain (g), TFC (g) = Total feed consumption (g) and FCR (feed/gain) = Feed conversion ratio (F/g), MR (%) = Mortality Due to Ascites (%) and TMR = Total mortality rate

Table 7. Effect of incubation temperature, ascorbic acid treatments on slaughter weight, heart parameters and immune organs at 42 days of age

Traits → Treatment ↓	Slaughter weight (g)	Liver (%)	Heart parameters			Immune organs	
			RV (g)	TV (g)	RV/TV ratio	Spleen (%)	Bursa (%)
Effect of incubation temperature (IT)							
Control (37.5°C)	2539.33	2.42	2.61	12.12	0.21	0.113	0.138
Chronic (40.0°C)	2462.89	2.19	2.94	10.82	0.27	0.092	0.117
SEM	25.356	0.0488	0.058	0.22	0.003	0.004	0.005
Effect of ascorbic acid (AA)							
Control	2466.83	2.25	2.79	11.21	0.25	0.097	0.114
20g/Liter	2522.33	2.31	2.76	11.57	0.24	0.107	0.130
30g/Liter	2514.17	2.35	2.76	11.63	0.24	0.104	0.140
SEM	31.05	0.059	0.07	0.27	0.0037	0.005	0.006
Probability							
IT	0.0359	0.0010	0.0001	0.0001	0.0001	0.0006	0.0054
AA	0.3981	0.5226	0.9574	0.5184	0.1038	0.8976	0.0125
IT×AA	0.9888	0.6187	0.9770	0.9096	0.6360	0.3957	0.2194

Means with different superscripts in the same column are significantly different ($P \leq 0.05$). RV= Right Ventricle, TV= Total Ventricle and RV: TV ratio