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EFFECT OF THERMAL MANIPULATION DURING INCUBATION AND SPRAYING JAPANESE QUAIL EGGS WITH ASCORBIC ACID ON EMBRYOGENESIS AND PHSIOLOGICAL RESPONSES OF HATCH CHICKS

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ABSTRACT: The objective of this study was to investigate the impact of spraying ascorbic acid on Japanese quail eggs submitted to high temperature during4th to 14th days of incubation period on embryogenesis, hatchability, embryonic mortality rate and chick quality.Six hundred Japanese quaileggs were divided into two equal groups of 300 eggs each according to incubation temperature. In the first group: eggs were incubated at 37.5°C (thermoneutral incubation temperature NIT 37.5°C)and 65% relative humidity (RH)from 1st to 14th days of incubation. The second group: (high incubation temperatureHIT 41°C): Eggs were incubated at 37.5°C during the first 3 days of incubation and from day 4th to 14th incubation temperature (IT) was raised to 41°C and RH65% for three hours per day. The eggs were evenly divided into three ascorbic acid (AA) treatments. The first one was sprayed with distilled water (control AA0), while, the second and third groups were sprayed with solutions containing 15 and 30gAA/L, respectively.The results concluded that HIT group had the highest embryo weight, embryonic mortalities and lower hatchability% compared to NIT group. The chicks weight (g) and triiodothyronine (T3) concentration were significantly lower (P≤0.05) in HIT group compared toNIT group. Glucose level and heterophil: lymphocyte (H: L) ratio wereincreased significantly (P≤0.01) as affected by thermal manipulations.The embryonic development, embryonic viability as well as hatchability improved by spraying ascorbic acid as compared to the untreated group.Values of glucose, T_3, RBCs, PCV% and HGB were significantly increased in the blood of hatching chicks by spraying AA solutionas compared to control while, residual yolk and H: L ratio was significantly(P≤0.05) decreased by spraying AA solution . The embryonic development, internal organs%, PCV% and HGB has been affected by interaction between incubation temperature and ascorbic acid.

It could be concluded that, spraying eggs of Japanese quail exposed to heat stress with AA solutions (15 or 30 g/L) daily during4th to 14th days of incubation period, may be an alternative method to minimize embryos heat stress and maximize the embryonic viability as well as hatchability, immunity of hatched chicks and chick quality.

Keywords:incubation temperature-ascorbic acid-chick quality-hatchability-embryogenesis.

INTRODUCTION

Poultry embryos are poikilothermic and have limited ability to thermo regulation their body temperature by control their heat production during incubation (Romjin and Lokhorst, 1955). In the early stage of embryonic development, the transfer of heat from the surrounding air in the incubator to egg embryos occurs, provided that the temperature of the embryos is less than the egg temperature (Pulikanti et al., 2011). Conversely, in the late stage of embryonic development, the embryos were increased metabolic rate and heat production thus, difficult to achieve the ranges of optimum eggshell temperature for normal embryos development (Molenaar et al., 2010). Thermal adaptation during the incubation period may contribute to reduces the problems associated with thermal regulation during the rearing periodand also, enhanced post-hatching performance of poultry (Yalčın and Siegel 2003). Several literatures have mentioned the importance of ascorbic acid (AA) it is necessary for numerous of biochemical processes such as biosynthesis of various as vital compounds such as collagen, adrenaline also, plays a critical role in vitamin D metabolism which is essential for the calcium regulation and calcification process (Nowaczewski et al., 2012). Furthermore, Ascorbic acid enhances the iron absorption, consequently increasing red blood cells and hemoglobin levels and improving the respiratory potential of the birds (Moura and Pedroso, 2003). In addition, ascorbic acid has an active role in regulation of body temperature, secretion of corticosterone and activation of the immune system (Kutlu, 2001). Ascorbic acid can used as an anti-stress agent to minimize embryonic heat stress effects and have positive effects on embryos development and hatchling body weight under thermoneutral or heat stress conditions. (Kutlu, 2001, and Nowaczewski et al., 2012). Ascorbic acid is a weak and the ability of diluted ascorbic acid to interact with the eggshell cuticle which, is considered part of the respiratory structure of the embryo that allow the movement of water vapor and gases exchange between the embryo and the external environment through eggshell microscopic pores as reported by (Shafey, 2002). Therefore, the objective of the present study was planned to investigate the beneficial effects of spraying ascorbic acid solutions on Japanese quail eggs subjected to thermoneutral (37.5°C) and chronic high temperature (41°C) during4th to 14th days of incubation on embryonic development, embryonic vitality, hatchability, chick quality, as well as chicks weights, hematological characteristics and some physiological body reactionsof hatched chicks.

MATERIALS AND METHODS

Treatment groups and incubation procedure:

Six hundred Japanese quail hatching eggs were individually weighed, numbered and randomly divided into two main groups of (300 eggs each) according to incubation temperature (IT). First group was served as a thermoneutral incubation temperature group (NIT), eggs were incubated at temperature of 37.5°C and relative humidity (RH) 65% for the first 14 days of incubation then, all eggs were transferred to hatchery during last three day of incubation at 37.0°C and 75% RH. The second group was incubated at the same temperature and relative humidity at the first 3 days of incubation after that, applied high chronic incubation temperature 41°C (HIT) for 3 h

(1200 to 1500) daily between the 4th to 14th days of incubation in quail embryos.

Eggs in each main groupwere divided into three sub-groups corresponded to spraying ascorbic acid (AA) treatments. In the first one, eggs were sprayed with distilled water (control, AA_0) while, those in the second and third treatments were sprayed with 15and 30 g/L (AA) solutions, respectively. The eggs were sprayed using a manual sprayer to cover the entire surface of the eggs.

Preparation of ascorbic acid solution and egg treatments:

Ascorbic acid solutions were freshly prepared by dissolving 15 and 30 g ascorbic acid powder in1000 ml distilled water and the mixture was saved in a dark bottledue to the sensitivity of ascorbic acid to light. The distilled water and ascorbic acid solution are placed in the incubator at 37.5 °C for 3 hours before used to spraying the eggs.

Eggs treatments:

To estimate the percentage of loss in egg weight, the eggs were weighed to the nearest 0.1 gm before placing the egg in the incubator and after 8 and 14 days of incubation period.Start time of incubation period was recorded after eggs were set and incubator was turned on to obtain the exact hatch time in hours and considered as zero time of incubation. The eggshell was cleaned with 100 % ethanol and then, the eggshell was sprayed with5 ml of AA solutions or distilled water per 100 eggs after heat exposure. From 4 to 14 days of incubation, eggs were transferred to the hatchery and exposed to 41.0 °C and 55 – 60% relative humidity for 3 h daily.

Studied traits

Embryonic development:

At 8th and 14th days of incubation, 30 eggs per period were selected randomly and weighed individually to determine embryonic development. The eggs were broken out gently and embryos were gently isolated from the yolk sac and embryonic membranes. The albumen, eggshell and yolk were weighed and estimated as ratio in relation to the egg weight after removing embryonic fluid and drying the embryo by filtration papers. Egg weight loss was calculated according to Aygun and Sert (2013). Egg weight loss% = initial egg weight ─ egg weight at day 8 or 14 of incubation × 100 / initial egg weight. Egg shell thickness (mm) and embryo shank length (cm) were measured. Embryo length (cm) was measured from the tip of beak to the end of the middle toe by extended the chick dorsal surface on a flat surface and straightening the right leg over a ruler (Hill, 2001).

Chick parameters:

All hatched chick body weight, length, shank length (cm) and incubation period (hrs) were determined for the all groups. The length of the chick (cm) was measured in the same method as determination of the length embryo.Random samples of 6 chicks were killed from each group for recording the internal organs measurements such as intestine length, weights(g) of breast, legs, liver, heart, gizzardand residual yolk sac were expressed as percentages of chick weights.

Embryonic mortality, hatchability, and duration of incubation

At the end of hatching time the un-hatched eggs were broken to determine the age of embryonic mortality (early 1-7 days, intermediate 8-14days and late 15-17 days). The remainder eggs were classified as infertile also, piped eggs was determined.

Embryonic mortality (%) = (dead embryos number) ×100 / (total incubated eggs). Hatchability percent was calculated basis of fertile eggs and the number of chicks

hatched for the all groups (Molenaar et al., 2011). The incubation period was determined as the number of hours from time of eggs setting in incubation to time of chicks hatching.

Physiological body reactions

The infrared thermometer device used to measured temperatures of chicks head, back, wing and shank. Temperature of cloacal, and the body surface (BST°C) was calculated according the equation:

BST°C = (0.03 × head Tem) + (0.70 × back Tem) + (0.12 × wing Tem) + (0.15 × shank Tem), as described by Richards (1971). The digital thermometer used to measure temperatures of chicks cloacal temperatures (°C) by inserting a digital thermometer 1 cm deep into the cloacal.

Blood parameters:

At hatch, blood was collected into heparin tubules for hematological tests from randomly six chicks per treatment. A portion of the fresh blood was used to investigate; red blood cell (RBCs10^6/mm^3) count using a hemocytometer (Schalm et al., 1975). Packed cells volume (PCV%) which was determined by the microhematocrit method (Schalm et al., 1975). Hemoglobin (HGB g/dl) concentration was spectrophotometrically measured using the cyanomet hemoglobin method (Schalm et al., 1975). White blood cells (WBCs 10^3/mm^3) differential counts and the heterophil to lymphocyte (H :L) ratio were estimated are based on the procedures of Gross and Siegel (1983). Plasma was obtained from the blood samples by centrifugation for 15 minutes at 3000 Rpm and stored at – 20 Cº until to be used in further analysis of blood constituents. Plasma triiodothyronine (T3ng/dl) hormone and glucose(mg/dl) concentration were determined by commercial kits according to Trinder (1969).

Statistical analysis

The data were statistically analyzed by using a two way analysis of variance (ANOVA) with incubation temperature (IT) and ascorbic acid treatments (AA) effects by applying the general linear model procedure (GLM) to analyze the differences between treatment groups using SAS software (SAS Institute, version 9.4, 2009). Duncan's multiple range test (Duncan, 1955) was used to detect the differences among least squares means of different groups. Prior to analysis, all values expressed in percentages were transformed to arcsine transformation to approximate normal distribution before analysis, according to (Steel and Torrie, 1980).

Statistical model:

$Y_{ijk} = \mu + T_i + A_j + TA_{ij} + E_{ijk}$, .Where: Y_{ijk} =an observation value, μ= the overall mean, T_i= the fixed effect due to incubation temperature (i = 1, 2), A_j= the fixed effect due to ascorbic acid level (j = 1, 2, 3), TA_{ij} = the fixed effect due to interaction between incubation temperature and ascorbic acid level, and E_{ij} = the random error.

RESULTS AND DISCUSSION

Embryonic quality traits

The effects of (IT) and (AA) at 8^{th} and 14^{th} days of incubation on the embryonic development are presented in Tables 1, 2, 3 and 4. The results showed embryonic development was faster for HIT group at 8^{th} and 14^{th} day of incubation compared to NIT group expressed by higher significant embryo length, embryo shank length, embryo weight (g), relative embryo weight percent and egg weight loss. The HIT group had lowest insignificant yolk sac weight; relative yolk sac weight, albumen; shell percentages and shell thickness were observed in embryos compared with NIT group. The increase in relative embryo weight percent from eggs submitted to high IT 41°C may be due to faster embryo growth through the increased metabolic rate and

heat production leading to increases embryos oxygen consumption and gases exchange rate, which is necessary to cover the requirements of the fast metabolism and guarantee the rapid development (Viviane et al., 2010). The egg weight losses percentage of the HIT group was higher compared with NIT group in both 8th and 14th day of incubation (Table 1, 3). This is a logical result due to exposure to thermal stress and thus, increase water evaporation rate from the eggshell also, HIT group had significant thinner eggshell thickness it increased the water loss rate (Tullett and Board, 1977). The increased weight loss may also have reduced yolk percent of embryos incubated at high temperature because increased the partial water vapor pressure between egg and surround air (Lourens et al., 2007). Data presented in Tables 1and 3 showed that eggshell percentage at 8th and 14th days of incubation and eggshell thickness at 8th were not influenced by IT. The eggs submitted to 41°C have thinner eggshell compared to those submitted to 37.5°C at 14th day of incubation. The thinner eggshell may due to the high temperatures accelerate embryos development which requires increased metabolism and thereby increased blood pH which related to increased phosphorus absorption from eggshells Sgavioli et al., (2013).

Referring to impacts of AA treatments, presented in Tables 1, 2, 3 and 4, the embryonic development expressed in embryo weight %, embryo length, shank length at the 8th and the 14th days of incubation tended to be higher (P>0.05) for eggs treated by spraying with AA solution than those of untreated eggs, especially eggs sprayed with AA 30g/L solution showed the highest records at the 8th and 14th days of incubation and at hatch. These results are in full agreement with those reported by Abuoghaba (2017). The same proportion of eggshell was used for embryonic development during incubation for AA treated groups and untreated one (Table 1, 3). Eggshell thickness of eggs treated by AA solution was similar to those untreated ones at the 8th day of incubation (Table 1). However, results showed a significant decline at the 14th day of incubation in eggs sprayed by AA solution compared to untreated this decline may be due to the interaction between the AA solution and eggshell layer that changes eggshell cuticle physical properties and porosity of shell causes a thinner eggshell cuticle resulting in promoted the eggshell conductance. This means that the use of AA solution increases embryos absorption the calcium and phosphorus of eggshell, movement of water vapour and gases exchange and thus improvement embryonic development (Shafey, 2002 and Sgavioli et al., 2013). This explained the increase in the egg weight loss% of the eggs treated with AA compared with untreated groups. The eggs treated with AA and exposed to high temperature had higher significant embryo weight, relative embryo weight %, embryo lengthand shank length compared to other groups at the 8th and the 14th days of incubation. Furthermore, there were no significant effects of interaction between IT and AA spraying in embryos consumption of albumen, shell %, egg water loss% and shell thickness at 8thday of incubation also, the yolk weight, yolk %, shell% egg water loss% and embryo shank at 14th day of incubation take the same trend. In addition, Sgavioli et al., (2013) concluded that spraying 15 or 30g/Lof AA solutions into eggs incubated under hot temperature decreased shell thickness at 14th day of incubation may due to increases the phosphorus absorption from eggshell. It seems that AA treatment could help the chick to overcome oxidative stress at hatch.

Embryonic mortality, hatchability, and duration of incubation

Temperature (41°C) increased embryonic mortalities ($P \leq 0.01$) during early and intermediate stages of incubation as well as decreased hatchability percentages for HIT group compared to NIT group (Table 5). The negative effects of HIT on embryonic mortality rate may be due to insufficient heat loss by the egg to keep the normothermia inside the eggs thus affecting the homeostasis of embryos. In addition, heat stress promote excessive water loss from the eggs, leads to the insufficient egg nutrients absorption, which negatively affected the embryonic development and causing high mortality rates due to of dehydration and consequently, reduction the hatchability (Viviane et al., 2010). Regarding hatch time, chicks were hatched earlier ($P>0.05$) for eggs exposed to 41°C (377.88 hrs) by about 17.38 hrs compared with 37.5°C (395.26 hrs) this may be due to higher metabolic rate accompanied with increased respiration rate, resulting in decreased O_2 supply and increased CO_2 concentrations inside the egg which, accelerate hatch time (Everaert et al., 2007). Sgavioli et al., (2014) reported that the elevated temperature generally accelerated hatching time but this was not in concert with the development of the organs. With respect to the effect of AA treatments, data indicate that the rate of embryonic mortality was decreased remarkably due to treatment with ascorbic acid and thus increased hatching rate compared with the untreated group (Table 5). The early stage and late stage of embryonic mortality and dead after piping were not affected by treatment with AA or the interaction between IT and AA treatment. While, intermediate stage had significant ($P<0.05$) lower embryonic mortality for AA groups than that of untreated fertile eggs (Table 5). Spraying quail eggs with either 15 or 30 g/L AA solutions led to an increase in hatchability of quail chick by 6.59 and 6.84 % of the control value, respectively at 4^{th} to 14^{th} days of incubation and it had no adverse effect on the chicks weight at hatch. Also,the results showed that chicks hatched from eggs AA treated, particularly AA 30 g/L solution have significantly ($P<0.05$) shorter hatching periods (382.26 hrs) than that of treated by AA 15 g/L solution and water (387.83, 389.63hrs) respectively, (Table 4). Interpretation plausible for the superiority of the AA treated groups in embryonic viability, hatchability and early chick hatching may be due to ascorbic acid may have a critical role in reducing stress by reduction of corticosterone which is associated with stress and has a negative impact in collagen synthesis and the metabolism of minerals and vitamin D thus the AA treated embryos reserved the viability and energy needed to emerge from the shell earlier than the control (Nowaczewski et al., 2012). In addition, the beneficial impacts of ascorbic acid on improved biological functions of the egg shell conductance as a result to the interact between AA solutions as a weak acid with eggshell cuticle layer that changes its properties which may have cause a thinner cuticle (Ghonim et al., 2009). This is allow adequate movement of water vapor and needful for the exchange of respiratory gases and helps embryos to break easily the eggshell at hatch (Shafey, 2002).The spraying AA on eggs submitted to high temperature significantly decreased incubation period and embryonic mortality as well as increased hatchability. These results correspond with the findings of Sgavioli et al., (2013) who indicated that pre-incubation injection of 4% vitamin C into eggs incubated under hot temperature decreases embryo mortality and improve hatchability. The advantageous of ascorbic

acid is attributed to its modifying influence on adrenal gland metabolism inhibiting the synthesis of 21-hydroxylase and 11-beta hydroxylase, i.e. enzymes which take active part in corticosterone production (Tullet, 1990). This hormone, in turn, is quite active in gluconeogenesis, supporting the production of energy during the period of adaptation to new conditions thermal stressespecially in later period of incubation (Nowaczewski et al., 2012).

Chick quality parameters and physiological body reactions

Chick quality parameters and physiological body reactions results are presented in Table 6. Our data demonstrated that embryos subjected to HIT (41^{o}C) from 4 until 14 days consumed less yolk and resulting in a significantly lower yolk free chick % at hatch and reduced hatched chick weights, chick length and chick shank length as compared to thermoneutral group. These results correspond to the results of Abuoghaba (2017). This effect may be explained by reduction in absorption of yolk% that reduced the available nutrients are necessary for meeting the nutritional requirements of embryo development as well as hatch weight and use an egg yolk to conserve energy for the hatching process (Willemsen et al., 2010). On the other hand, the increased chick weights in embryos exposed to NIT group could be attributed to the increased uptake of the yolk sac into the abdomen of the embryo, which use the yolk for development of muscle and organs and provides the nutrients needed for the chick during the first few days of life. It seems that relation between yolk uptake and body weight at time of hatch is associated with the establishment of a balance in energy expenditure for survival or the development of muscles and organs (Afsarian et al., 2016). The results of this study showed that the body surface and cloacal temperatures of the quail chicks in the HIT group were significantly increased in comparison to the NIT group. This increased in body surface and cloacal temperature indicated that embryo response to high temperature that leading to increase body physiological reactions of chicks. These results confirm those of Ipek et al., (2015) and Abuoghaba et al., (2018). The high temperature (41.0°C) has negatively effect on organs development as heart, gizzard and liver percentages that evidencing high sensibility to HIT. Thermal stress during incubation may affect the rate of cell division in these organs Leksrisomponget al., (2007). The decrease in chick heart weight at hatch in the HIT group may be due to the increased susceptibility and incidence of metabolic disorders related to cardiovascular development **(**Molenaar et al., 2011). The intestine length, legs, breast percentages were declined in the (HIT) group compared to (NIT) group. Referring to AA treatment, results showed the significant decreased in yolk sac weight and relative yolk sac weight in response to spraying AA compared to untreated one while, chick weight as well as chick length, chick shank length, body surface temperature and cloacal temperature were not influenced by AA treatments. These results agree with those of Zakaria and Al-anezi (1996) who found that eggs injected by a dose of 3 mg of AA at the 15th day of incubation increased body weight of chicks compared with the control. The intestine length was insignificantly affected by AA treatment while, the heart, gizzard, liver, legs and breast as relative weight were higher for chicks hatched from eggs treated with AA solution than those of untreated group. The yolk sac weighs, relative yolk percent and cloacal temperature were significantly affected by spraying fertile eggs subjected to HIT with AA solution. While, chick weight at hatch, chick length,

body surface temperature were not significant influenced by interaction between IT and spraying AA solution.

Glucose and T_3 hormone concentrations

Exposure eggs to 41°C resulted in a higher ($P \leq 0.001$) level of plasma glucose of newly hatched chicks compared to hatch chicks from eggs exposed to 37°C (Table, 8). The elevated blood glucose levels found in the HIT group could be explained by the concentrations of carbohydrate are low in the initial egg and the higher relative yolk weight in end stages of incubation this reflected the embryo could not use yolk lipids efficiently for energy. For those glucose precursors such as amino acids, glycerol, and lactate partly converted into glucose by hepatic gluconeogenesis to build up glycogen stores in heart, liver, intestines, leg and breast muscle, and yolk sac membrane (Garcia et al., 1986). This hypothesis was confirmed by Willemsen et al., (2010) who found the thermal stress during incubation increases glucose oxidation therefore, glycogen reserve are depleted more rapidly and increases blood glucose level which, are needed as energy sources during the last days of incubation to energy demanding hatching process. During the hatching process, glucose becomes an indispensable source for energy because muscle activity is high and O_2 availability is low (De Oliveira et al., 2008). During embryogenesis period the thermal stimulation an motivate the heat sensing neurons of hypothalamus increase secretion of embryo thyroid hormones T_3 and T_4 which, in turn regulates metabolic rate and affects growth of organs, such as the intestine and changes the sensitivity of these neurons to temperature fluctuation during the post-hatch period (Afsarian, et al., 2018). In present study, at hatch time it was shown a significant reduction in T_3 hormone concentration ($P<0.001$) for chicks hatched from eggs submitted to HIT compared to NIT group (Table 8). This reduction in T_3 may contribute to better thermoregulatory abilities of embryos exposed to heating at 41°C 3 hrs /d from 4^{th} to 18^{th} days of incubation. The superiority of T_3 levels in NIT group may be due to the less T_3 is necessary for oxidative metabolism, which leads to less T_3 uptake by hepatic cells and thus more T_3 remaining in the plasma (Reyns et al., 2002). These results are in agreement with those of Abuoghaba et al., (2018) found a reduction in (T_3) level at hatch in heat acclimated of embryos during incubation exposed to 41°C from 10 to 18 days of incubation period compared to the control broilers.

Referring to effects of AA treatment, the levels of glucose and T_3 hormone for one day old chicks were significantly ($P \leq 0.001$) increased in response to spraying with AA solution compared with control group. This may be due to the positive role played by ascorbic acid in improving the physiological status and embryos development of treated chicks. These results correspond with the findings of Sahin et al., (2002a, b), who found that the supplemental of ascorbic acid increased T_3 hormone level in plasma of Japanese quail.

The eggs subjected to HIT and spraying by AA showed higher significant ($P \leq 0.001$) level of blood glucose and decrease the concentration of T_3 compared to other groups. Generally, spraying eggs incubated under high temperature for 3 hrs by AA solution from 4^{th} to 14^{th} days resulted in consistent changes in post hatch plasma T_3 and glucose level explain some physiological imprinting mechanism of prenatal heat acclimation on these hormone axes and reduce thermal stress by ascorbic acid treatment.

Hematologic characteristics:

In relation to the erythrocyte parameters, it was observed that chicks of HIT group had a significant ($P \leq 0.05$) increase of RBCs, PCV% and HGB with potential improvement in the transport of gases (Table 8). Increased values of RBCs, PCV% and HGB after incubation at 41°C may be related to dehydration and consequently higher weight loss as well as lower hatched chicks weight and these results correspond with the findings of Sgavioli et al., (2013) and Ipek et al., (2015) who found that the higher RBCs, PCV% and HGB values were obtained in the HIT group as compared to the low incubation temperature and control groups. The leukocytes count, and differential percentages in the newly hatched chicks are presented in Table 9. At hatch, our findings showed that leukocyte counts, eosinophils, monocytes, and basophils percentages were not differed by incubation temperature. Lymphocyte and heterophils were the unique cell types significant ($P \leq 0.001$) influenced by incubation temperature. The heterophils values have been considered as a guide to response to light and moderate thermal stress in birds (Maxwell et al., 1992), while H: L ratio is a known index to measure stress in birds (Puvadolpirod and Thaxton, 2000 a, b). In present study, the heterophil value and H: L ratio were significantly($P \leq 0.001$) higher while, the lymphocyte value was lower in blood chicks produced from eggs exposed to HIT. These cells types and H: L ratio could be related with the natural cellular immunity of the birds, especially during the incubation period and consequently immediately at hatching. Kogutet al., (1998) described that during these cited phases the birds did not develop acquired immunity yet. Therefore, a higher values H: L ratio in the HIT (41°C) groups is a sign of embryos in thermal stress.

Referring to the effect of AA treatments, values of PCV, HGB and RBCs for chicks hatched from eggs sprayed with 15 and 30 g/L AA solutions were significantly higher than control group (Table 8). Considering that hematological respiratory variables is related to the gases transport, these data indicated that spraying AA solution increased respiratory potential (Moura and Pedroso, 2003 and Sgavioli et al., 2013). Results of counts of different white blood cells (%), eosinophils, monocytes and basophils did not differ significantly as a result of treatment by AA solution (Table 9). The heterophils and H: L ratio were significantly ($P \leq 0.01$) decreased, while lymphocytes% were increased in the AA treated groups compared to control group. These results may ensure that AA suppress corticostisterone synthesis and /or release from adrenal cortex, which acted important role as anti stressors(Ghonim et al., 2009). Chicks hatched from eggs spraying with AA solution had higher numbers of RBCs and greater HGB and PCV % when the incubation was performed at hot temperatures, which indicating an improvement in the gas exchange potential of the blood in those chicks and therefore an increase in hematopoietic process these results agree with Moura and Pedroso, (2003) and Sgavioli et al., (2013). The incubation temperature, spraying ascorbic acid and the interaction between both factors did not significantly influence the total leukocytes counts and the percentage of heterophils, lymphocytes, basophils, eosinophils, monocytes and the H/L ratio.

CONCLUSION

Spraying AA solutions (15 or 30 g/L) can be used as an effective anti-stress additive on Japanese quail eggs submitted tohigh temperature (41°C) and it may be alternative method to minimize the adverse effects of high incubation thus, maximize the

embryonic development, embryonic viability as well as hatchability, hematological variables responses in chicks and physiological body reactions.

Table (1): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on embryonic parameters at 8th day.

Embryonic parametersat 8th day						
Traits	**Egg Weight (g)**	**Yolk sac (%)**	**Albumen (%)**	**Shell (%)**	**Shell thickness (mm)**	**Egg water loss (%)**
Effect of incubation temperature (IT)						
NIT (37.5 C°)	11.52	37.57	38.05	8.26	20.40	6.00
HIT (41.0 C°)	11.72	36.6	36.82	7.97	20.28	6.46
SEM	0.06	0.73	0.97	0.21	0.26	0.40
Significance	0.21	0.38	0.38	0.35	0.76	0.40
Effect of ascorbic acid (AA)						
Control AA0	11.65	37.52	38.76	7.88	21.16[a]	5.87
AA 15 g L^{-1}	11.52	37.09	35.87	8.22	20.65[a]	6.99
AA 30 g L^{-1}	11.70	36.73	37.66	8.24	19.42[b]	5.798
SEM	0.07	0.90	1.19	0.26	0.048	0.55
Significance	0.2	0.82	0.24	0.56	0.21	0.18
Interaction (IT×AA)						
NITx AA0	11.74	35.83[b]	41.50	7.87	21.50	4.75
NIT x AA15	11.55	38.96[a]	35.33	7.87	20.50	7.02
NITx AA30	11.64	37.92[a]	37.31	8.17	19.80	6.20
HIT x AA0	12.03	39.20[a]	36.02	7.89	20.83	7.00
HIT x AA15	11.82	35.22[b]	36.42	8.59	20.80	6.98
HIT x AA30	11.62	35.53[b]	38.02	8.31	20.60	5.40
SEM	11.57	1.27	1.69	0.36	0.53	0.69
Significance	0.07	0.02	0.11	0.62	0.39	0.065

Means having different superscript letters within each column in each effect are significantly different ($P \leq 0.05$). IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA= interaction between incubation temperature and ascorbic acid.NIT=thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (2): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on embryonic development parameters at 8th day.

Incubation temperature-ascorbic acid-chick quality-hatchability-embryogenesis.

Embryonic development parameters at 8th day				
Traits	**Embryo weight (g)**	**Relative Embryo weight %**	**Embryo length (cm)**	**Embryo Shank length (cm)**
Effect of incubation temperature (IT)				
NIT (37.5 C°)	1.22[b]	10.41[b]	3.32[b]	0.43b
HIT (41.0 C°)	1.38a	11.76[a]	3.50[a]	0.49a
SEM	0.02	0.025	0.06	0.012
Significance	<.0001	0.0003	0.05	0.004
Effect of ascorbic acid (AA)				
Control AA0	1.20[b]	9.90[b]	3.21[b]	0.394[b]
AA 15 g L^{-1}	1.378[a]	11.80[a]	3.513[a]	0.499[a]
AA 30 g L^{-1}	1.33[a]	11.56[a]	3.515[a]	0.491[a]
SEM	0.02	0.24	0.015	0.24
Significance	<.0001	<.0001	0.017	<.0001
Interaction (IT×AA)				
NIT x AA0	1.23[b]	10.04[b]	3.36[a]	0.44[b]
NIT x AA15	1.246[b]	10.81[b]	3.27[b]	0.48[b]
NIT x AA30	1.200[b]	10.38[b]	3.07[b]	0.35[c]
HIT x AA0	1.169[b]	9.76[c]	3.41[a]	0.38[c]
HIT x AA15	1.51[a]	12.78[a]	3.76[a]	0.52[a]
41°C x AA30	1.47[a]	12.74[a]	3.62[a]	0.61[a]
SEM	0.11	0.23	0.11	0.02
Significance	<.0001	0.0048	0.013	<.0001

Means having different superscript letters within each column in each effect are significantly different ($P \leq 0.05$). IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA= interaction between incubation temperature and ascorbic acid. NIT = thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (3): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on embryonic parameters14th day

Embryonic parametersat14th day					
Traits	**Yolk Sac Weight (g)**	**Relative Yolk Sac Weight (%)**	**Shell %**	**Shell thickness (mm)**	**Egg water loss %**
Effect of incubation temperature (IT)					
NIT (37.5 C°)	2.92	24.58	8.24	20.40a	9.38b
HIT (41.0 C°)	2.87	24.54	7.98	19.80b	11.03a
SEM	0.13	0.74	0.27	0.26	0.3
Significance	0.82	0.93	0.51	0.011	0.05
Effect of ascorbic acid (AA)					
Control AA0	2.57^{b}	21.32^{b}	8.67	20.77_{a}	9.65^{b}
AA 15 g L^{-1}	2.93^{ab}	25.07^{ab}	7.98	20.55^{ab}	9.71^{b}
AA 30 g L^{-1}	3.19^{a}	27.30^{a}	7.68	19.70^{b}	12.50^{a}
SEM	0.169	0.912	0.31	0.06	0.58
Significance	0.05	0.015	0.12	0.05	0.006
Interaction (IT×AA)					
NIT x AA0	2.59	22.20	8.04	21.80^{a}	9.84^{c}
NIT x AA15	2.91	23.67	8.15	21.00^{a}	9.73^{c}
NIT x AA30	3.25	27.88	7.77	20.10^{a}	9.47^{c}
HIT x AA0	2.55	20.44	9.30	20.10^{a}	9.70^{c}
HIT x AA15	2.96	26.46	7.83	19.75^{b}	11.46^{b}
41°C x AA30	3.12	26.72	7.60	19.30^{b}	13.54^{a}
SEM	0.23	2.01	0.47	0.46	0.92
Significance	0.93	0.51	0.20	0.0051	0.050

Means having different superscript letters within each column in each effect are significantly different ($P \leq 0.05$). IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA = interaction between incubation temperature and ascorbic acid. NIT =thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (4): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on embryonic development parameters 14th day

Embryonic developmentparametersat14th day				
Traits	**Embryo weight (g)**	**Embryo weight %**	**Embryo length (cm)**	**Embryo Shank length (cm)**
Effect of incubation temperature (IT)				
NIT (37.5 C°)	5.91	49.81^{b}	7.65	1.19
HIT (41.0 C°)	6.15	52.23^{a}	8.03	1.21
SEM	0.106	0.41	0.17	0.03
Significance	0.124	0.025	0.1369	0.58
Effect of ascorbic acid (AA)				
Control AA0	5.69^{b}	48.59^{b}	7.20^{b}	1.12^{b}
AA 15 g L^{-1}	6.00^{b}	49.95^{b}	8.04^{a}	1.17^{b}
AA 30 g L^{-1}	6.39^{a}	54.52^{a}	8.28$_{a}$	1.31^{a}
SEM	0.13	0.50	0.17	0.21
Significance	0.0032	0.0002	0.004	0.006
Interaction (IT×AA)				
NIT x AA0	5.76	49.82	6.16^{c}	1.08
NIT x AA15	6.25	50.55	7.90^{b}	1.23
NIT x AA30	5.47	46.62	8.13^{a}	1.11
HIT x AA0	5.91	50.08	7.95^{b}	1.17
HIT x AA15	6.29	52.97	8.25^{a}	1.29
HIT x AA30	6.5	56.06	8.67^{a}	1.34
SEM	0.18	1.24	0.30	0.05
Significance	0.012	0.032	0.0002	0.19

Means having different superscript letters within each column in each effect are significantly different ($P \leq 0.05$). IT = effect of incubation temperature. AA = effect of ascorbic acid IT×AA= interaction between incubation temperature and ascorbic acid.NIT= thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (5): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on embryonic mortality, hatchability and incubation time.

Traits	Embryonic mortality (%)				Hatchability of fertile eggs (%)	Incubation time (hour)
Treatment	Early stage day 1–7	Intermediate stage 8–14d	Late stage 15 d to hatch	Dead after piping		
Effect of incubation temperature (IT)						
NIT (37.5 C°)	4.66b	2.44^{b}	2.60	0.86	86.56^{a}	395.26^{a}
HIT (41.0 C°)	8.49^{a}	4.98^{a}	2.38	1.61	78.98^{b}	377.88^{b}
SEM	0.78	0.36	1.07	0.38	1.49	0.68
Significance	0.0041	0.0040	0.52	0.16	0.0061	<.0001
Effect of ascorbic acid (AA)						
Control AA0	7.502	4.7^{3a}	3.14	1.64	80.50^{b}	389.63^{a}
AA 15 g L^{-1}	6.281	3.68^{ab}	2.22	0.50	85.81^{a}	387.83^{a}
AA 30 g L^{-1}	5.951	2.72^{b}	2.04	1.57	86.01^{a}	382.26^{b}
SEM	0.95	0.45	1.32	0.47	1.49	0.83
Significance	0.575	0.05	0.22	0.18	0.026	<.0001
Interaction (IT×AA)						
NIT x AA0	10.47^{a}	6.68^{a}	3.50^{a}	2.83^{a}	76.00^{b}	390.67ab
NIT x AA15	7.72^{b}	4.63^{b}	1.72^{c}	0.56^{c}	79.48^{b}	394.30a
NIT x AA30	7.29^{b}	3.64^{b}	2.58^{b}	1.47^{b}	81.48^{b}	400.83a
HIT x AA0	5.27^{c}	2.79^{c}	2.78^{b}	0.46^{c}	85.01^{a}	385.00b
HIT x AA15	4.54^{cd}	2.73^{c}	2.73^{b}	0.44^{c}	86.15^{a}	370.24^{c}
HIT x AA30	4.19^{d}	1.81^{d}	1.24^{c}	1.68^{b}	88.55^{a}	378.43^{c}
SEM	1.35	0.64	1.86	0.67	2.58	1.18
Significance	0.048	0.028	0.147	0.05	0.04	<.0001

Means having different superscript letters within each column in each effect are significantly different (P≤ 0.05). IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA= interaction between incubation temperature and ascorbic acid. NIT= thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (6): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on hatchling quality traits and Physiological body reactions.

Traits	**Chick quality parameters**				**Physiological body reactions**	
Treatment	**Chick 1day weight (g)**	**Chick length (cm)**	**Yolk Weight(g)**	**Relative Yolk (%)**	**Body surface temperature**	**Cloacal temperatures**
	Effect of incubation temperature (IT)					
NIT (37.5 C°)	9.19^{a}	11.39^{a}	0.89	7.58^{b}	31.35^{b}	38.35^{b}
HIT (41.0 C°)	8.86^{b}	11.01^{b}	0.93	8.48^{a}	31.7^{a}	38.79^{a}
SEM	0.099	0.079	0.04	0.45	0.1	0.06
Significance	0.027	0.0024	0.44	0.025	0.011	0.04
Effect of ascorbic acid (AA)						
Control AA0	8.62	11.497	0.87^{b}	9.37^{a}	31.29	38.84^{a}
AA 15 g L^{-1}	8.94	11.96	0.79^{a}	6.79^{b}	31.5	38.50^{b}
AA 30 g L^{-1}	9.00	11.13	0.78^{a}	7.15^{b}	31.4	38.22^{c}
SEM	0.12	0.09	0.05	0.55	0.13	0.07
Significance	0.27	0.22	0.0027	0.0054	0.07	<.0001
Interaction (IT×AA)						
NIT x AA0	9.29	11.62	0.78^{c}	6.25^{c}	31.73	38.81
NIT x AA15	8.64	11.38	0.76^{c}	6.59^{bc}	31.60	38.31
NIT x AA30	9.63	11.15	1.27^{a}	11.05^{a}	31.72	38.37
HIT x AA0	8.94	11.37	0.96^{b}	8.05^{b}	31.79	38.87
HIT x AA15	9.08	11.54	0.83^{bc}	7.00^{b}	31.90	38.70
HIT x AA30	8.55	11.11	0.88^{b}	7.68^{b}	31.82	38.08
SEM	0.17	0.13	0.075	0.78	0.09	0.11
Significance	0.062	0.06	0.0017	0.0049	0.082	0.0095

Means having different superscript letters within each column in each effect are significantly different ($P \leq 0.05$). IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA= interaction between incubation temperature and ascorbic acid.NIT= thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (7): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on hatchling organs percentages.

Traits	Intestine length (cm)	Breast (%)	Legs (%)	Heart (%)	Gizzard (%)	Liver (%)
Effect of incubation temperature (IT)						
NIT (37.5 C°)	16.70[a]	4.50[a]	9.52[a]	1.49[a]	5.20[a]	2.69
HIT (41.0 C°)	15.4[3b]	3.93[b]	8.72[b]	1.16[b]	4.41[b]	2.48
SEM	0.015	0.22	0.032	0.03	0.154	0.077
Significance	0.0144	0.032	0.02	0.002	0.002	0.06
Effect of ascorbic acid (AA)						
Control AA0	16.75	4.45[ab]	10.04[a]	0.94[b]	4.15[b]	2.38[b]
AA 15 g L^{-1}	15.64	3.67[b]	8.88[b]	1.061[b]	4.40[b]	2.94[a]
AA 30 g L^{-1}	15.81	4.53[a]	8.43[b]	1.99[a]	5.87[a]	2.43[b]
SEM	0.23	0.36	0.27	0.60	0.25	0.16
Significance	0.156	0.01	0.0012	0.088	<.0001	0.0007
Interaction (IT×AA)						
NIT x AA0	17.50	4.26[b]	10.73[a]	1.24	4.38[b]	2.47[a]
NIT x AA15	16.50	4.22[b]	9.29[ab]	1.19	4.83[b]	2.64[a]
NIT x AA30	16.12	5.02[a]	8.53[b]	1.05	4.02[b]	2.33[b]
HIT x AA0	16.00	4.63[b]	9.34[ab]	0.87	3.92[c]	2.30[b]
HIT x AA15	14.79	3.11[c]	8.48[b]	2.79	6.92[a]	2.25[b]
HIT x AA30	15.0	4.03[b]	8.33[b]	0.82	4.75[b]	2.53[a]
SEM	0.02	0.38	0.39	0.58	0.26	0.13
Significance	0.6331	0.03	0.036	0.11	0.0005	0.026

Means having different superscript letters within each column in each effect are significantly different ($P \leq 0.05$). IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA= interaction between incubation temperature and ascorbic acid. NIT=thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (8): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on hematological parameters, glucose level and T_3 hormone concentrations in hatched chicks.

Hematological parameters					
Traits	**Glucose (mg/dl)**	**T_3 (ng/dl)**	**RBCs (10^6/mm^3)**	**PCV (%)**	**HGB (g/dl)**
Effect of incubation temperature (IT)					
NIT (37.5 C°)	192.50^b	77.11^a	1.90^b	16.71^b	10.42^b
HIT (41.0 C°)	245.44^a	61.94^b	2.38^a	18.60^a	11.139^a
SEM	1.10	0.88	0.12	0.46	0.14
Significance	<.0001	<.0001	0.019	0.0086	0.0024
Effect of ascorbic acid (AA)					
Control AA0	191.83^c	60.16^c	1.73^b	15.00^c	9.76^b
AA 15 g L^{-1}	250.41^a	71.75b	2.41^a	17.25^b	11.29^a
AA 30 g L^{-1}	214.66^b	76.66a	2.29^a	20.71^a	11.27^a
SEM	1.35	1.08	0.152	1.11	1.44
Significance	<.0001	<.0001	0.0091	<.0001	<.0001
Interaction (IT×AA)					
NIT x AA0	181.50^e	76.16^b	1.44	12.80^d	9.95^b
NIT x AA15	220.66^c	61.00^c	2.01	17.20^b	11.33^a
NIT x AA30	175.33^f	94.16^a	1.96	16.00^c	10.37^a
HIT x AA0	202.16^d	67.33^c	2.31	18.50^b	9.58^b
HIT x AA15	280.16^a	59.33^d	2.51	18.83^b	11.24^a
41°C x AA30	254.00^b	59.1^{6d}	2.6	22.60^a	12.20^a
SEM	1.91	1.53	0.215	0.81	0.25
Significance	<.0001	<.0001	0.53	0.0004	0.0024

Means having different superscript letters within each column in each effect are significantly different (P≤ 0.05).IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA= interaction between incubation temperature and ascorbic acid.NIT= thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

Table (9): Effect of incubation temperature (IT) and spraying quail eggs by ascorbic acid (AA) on leukocytes count and differential percentages in hatched chicks.

Traits	WBCs (10^3/mm^3)	Heterophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)	H: L ratio
Effect of incubation temperature (IT)							
NIT (37.5 C°)	20.44	51.66[b]	37.44[a]	3.25	2.75	5.38	1.37[b]
HIT (41.0 C°)	20.05	56.27[a]	31.00[b]	3.26	2.82	6.63	1.83[a]
SEM	0.19	0.37	0.52	0.05	0.03	0.57	0.03
Significance	0.168	<.0001	<.0001	0.94	0.212	0.136	<.0001
Effect of ascorbic acid (AA)							
Control AA0	20.25	55.25[a]	32.58b	3.24	2.73	6.19	1.72[a]
AA 15 g L^{-1}	20.166	53.00[b]	35.25a	3.28	2.79	5.67	1.549[b]
AA 30 g L^{-1}	20.33	52.91[b]	34.83a	3.23	2.85	6.16	1.533[b]
SEM	0.23	0.45	0.63	0.06	0.04	0.70	0.038
Significance	0.8847	0.0015	0.0145	0.83	0.26	0.845	0.0031
Interaction (IT×AA)							
NIT x AA0	20.16	53.00	35.5	3.26	2.68	5.55	1.49
NIT x AA15	20.00	50.50	38.50	3.28	2.78	4.93	1.31
NIT x AA30	20.00	50.00	38.33	3.20	2.80	5.66	1.307
HIT x AA0	20.33	57.50	29.66	3.21	2.78	6.83	1.94
HIT x AA15	20.33	55.5	32.00	3.28	2.8	6.41	1.75
HIT x AA30	20.66	55.83	31.33	3.26	2.9	6.66	1.79
SEM	0.33	0.64	0.90	0.088	0.06	1.00	0.05
Significance	0.75	0.58	0.81	0.80	0.78	0.97	0.90

Means having different superscript letters within each column in each effect are significantly different ($P \leq 0.05$). IT = effect of incubation temperature. AA = effect of ascorbic acid. IT×AA= interaction between incubation temperature and ascorbic acid.NIT=thermoneutral incubation temperature 37.5°C. HIT= high incubation temperature 41°C. Control AA0= sprayed with distilled water. AA15 and 30 g AA / L = sprayed with solutions containing 15 and 30 g ascorbic acid / litter.

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الملخص العربى

تأثير المعاملة الحرارية و رش حامض الأسكوربيك علي بيض السمان الياباني أثناء التفريخ علي التطور الجنيني و الأستجابة الفسيولوجية للكتاكيت الفاقسة

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هدفت هذه الدراسة الى تقييم تأثير تعريض بيض السمان للمعاملة الحرارية أثناء التفريخ مع رش البيض بحامض الاسكوربيك من اليوم الرابع حتي الرابع عشرمن التفريخ علي معدل التطور الجنيني و النفوق الجنيني و نسبة الفقس وجودة الكتاكيت و بعض المعايير الفسيولوجية. أستخدم في هذه الدراسة عدد600 بيضة سمان ياباني قسمت لمجموعتين علي حسب المعاملة الحرارية . المجموعة الاولي (الكنترول): تم تعريض البيض لدرجة حرارة 37.5م° و رطوبة نسبية 65% من اليوم الاول و حتي الرابع عشر. المجموعة الثانية (المعاملة حرارياً): تم تعريض البيض بداية من اليوم الرابع و حتي الرابع عشر من التفريخ لدرجة حرارة 41 م° و رطوبة نسبية 65% لمدة 3 ساعات يومياً.و في نفس الوقت و قسم البيض لثلاث مجموعات علي حسب المعاملة بحامض الاسكوربيك.المجموعة الاولي (رش بالماء): تم رش البيض بالماء مرة كل يوم.المجموعة الثانية: تم رش البيض بمحلول من حامض الاسكوربيك بتركيز 15 جم/لتر ماء مرة كل يوم.المجموعة الثالثة: تم رش البيض بمحلول من حامض الاسكوربيك بتركيز 30 جم/لتر ماء مرة كل يوم. أشارت النتائج إلي أن تعريض البيض للحرارة 41م° 3 سا عات يومياً أدي إلي زيادة وزن الجنين و أرتفاع نسبة النفوق و إنخفاض نسبة الفقس مقارنة بالبيض المعرض لدرجة حرارة 37.5م.أنخفض وزن الكتاكيت الفاقسة عمر يوم و أرتفع مستوي الجلوكوز في الدمو أنخفض تركيز T_3 و معدل H: L نتيجة التعرض للحرارة التفريخ المرتفعة 41 م° مقارنة بمجموعة الكنترول. بالأشارة إلي تاثير معاملة البيض بالرش بحامض الاسكوربيك حدث تحسن واضح في كل من التطور الجنيني و حيوية الأجنة و أرتفاع نسبة الفقس في المجموعات المعاملة بحامض الاسكوربيك مقارنة بالمجموعات الغير معاملة. أدت المعاملة بحامض الاسكوربيك إلي زيادة مستوي الجلوكوز و كرات الدم الحمراء و الهيمتوكريت و الهيموجلوبين في دم الكتاكيت مقارنة بالمجموعات الغير معاملة. و أنخفض نسبة الصفار المتبقي و معدل H: L عندالمعاملة بحامض الاسكوربيك بالمقارنة بالمجموعات الغير معاملة.تأثر كلاًمن التطور الجنيني و نسب الاعضاء الداخلية للكتاكيت الفاقسة و نسبة الهيماتوكريت و الهيموجلوبين بالتفاعل بين المعاملة الحرارية و المعاملة بحامض الاسكوربيك لبيض السمان المخصب. يمكن أن نستخلص من هذه الدراسة أن رش البيض السمان و المعرض للاجهاد الحراري بمحلول حامض الاسكوربيك بمعدل (15 أو 30 جم /لتر) مرة يومياً أدي إلي تقليل الآثار السلبية لارتفاع درجة حرارة التفريخ و حسن من حيوية ومناعة الاجنة و حسن من نسبة الفقس و جودة الكتاكيت.