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Effects of cold stress during transportation on hatchability and chick quality of broiler breeder eggs

Ahmad SALAHI[1,*], Mozhdeh MOOSANEZHAD KHABISI[2], Abbas PAKDEL[3], Ali BAGHBANZADEH[4]

[1]Department of Animal Science, Varamin–pishva Branch, Islamic Azad University, Varamin, Tehran - IRAN

[2]Department of Animal Science, Kahnooj Branch, Islamic Azad University, Kahnooj, Kerman - IRAN

[3]Department of Animal Science, Faculty of Agricultural Sciences and Engineering, University of Tehran, Karaj - IRAN

[4]Section of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran - IRAN

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Abstract: In this experiment 72,000 broiler breeder eggs (Ross 308 strain) collected from 36-week-old flocks were subjected to cold stress during transportation. Eggs were allocated to 4 temperature groups (treatments): 1.2 °C, 1-2 °C, 2.5-3.9 °C, 4-6 °C, and a control group, 21-22 °C. Each treatment had 14,400 eggs, and data were analyzed in a completely randomized design. The results of this study showed that cold stress had a significant effect on percentage of egg weight loss ($P < 0.001$), and minimal egg weight loss occurred in the control group. The percentage of exploders and early hatched chicks and chick weight were higher in the below zero temperature treatment than the other groups ($P < 0.01$). Cold stress had a significant effect on chick length, hatchability, and the hatching of fertile eggs ($P < 0.001$). The effects of cold stress on chick yield and body weight uniformity were significant ($P < 0.01$). The effect of cold stress on hatchery byproduct efficiency was significant ($P < 0.001$), but did not affect fertility. Cold stress also had significant effects on early (1-8 days), middle (9-17 days), and late mortality (20-21 days); total embryo mortality; and exposed brain. Ectopic viscera was significant ($P < 0.001$), and most mortality was observed in below 4 °C treatments. Total percentages of malpositions and deformity ($P < 0.001$) and egg contamination at 1-9 days (first stage) and 10-21 days (second phase) were affected by cold stress ($P < 0.001$). Cold stress also had a significant impact on the number of cull chicks; percent of string navel, button navel, total string, and button; omphalitis; full body cavity; red hocks; dehydration; dirty chickens; and stubby down. Cold stress affects performance during incubation and overall chick quality.

Key words: Cold stress, egg transportation, hatchability, chick quality, malposition and deformity, culls, egg weight loss, embryo mortality and contamination

Introduction

Incubation provides proper conditions for fertile eggs to produce high quality day old chicks. This occurs when the fertile eggs are delivered under standard conditions from breeder farm to hatchery (1,2). It is necessary to protect hatching eggs against any heat or cold stress during collection in the barn; storage at the farm; and transportation to the hatchery, egg grading room, incubator, and hatcher. For example, Cobb-Vantress, Inc—one of the primary breeders—

* E-mail: ahmad.salahi2010@gmail.com

recommends that the temperature of trucks delivering hatching eggs from farm to hatchery be between 20 and 23 °C (3).

Temperature is one of the important factors affecting the growth and development of the embryo at all stages of the incubation period. The optimum temperature for fertile eggs during the storage period depends on the age of eggs, the age of broiler breeder flock, and the genetic strain of the birds (1-4). It must be noted that, as storage time increases, hatching eggs should be stored at lower temperatures. Guidelines are provided by primary breeder companies. For instance, Aviagen recommends storing eggs 1-3 days or older (e.g., 4 days) at 19 °C and 16-18 °C, respectively (5).

It has been shown that storing eggs at −18.33 °C for 10 h reduced the internal temperature of the eggs to −1 °C. However, this did not have any impact on hatchability rate (6). Storing turkey hatching eggs at −18.33 °C for 1, 2, 3, and 4 h led to a higher number of female embryos compared to eggs stored from 10 to 12.7 °C; when the temperature was reduced to −16 °C, hatchability decreased (7). If the temperature of the egg center over 120 min reaches −7 to −10 °C, embryos will develop normally until they reach 8 days of age (6). Keeping eggs at −3 °C for 4 days resulted in the development of the embryos to the blood ring stage—with a wide and enlarged blastoderm—after which only the ectoderm and endoderm had the opportunity to grow (6). Wilson (8) showed that cold stress (−2 °C) before incubation increased embryo mortality, and the peak of mortality occurred after 16 days. Lundy (9) showed that eggs maintained at −2 to −3 °C caused ice crystal formation, which led to irreversible damage to embryo tissues.

Deeming (10) reported that cold stress reduced yolk consumption by the embryo. It also prevented growth of the embryo and reduced the water loss or vapor exchange of the egg surface. A reduction of ambient temperature in broiler breeder hens increased food consumption and serum corticosterone. It reduced egg production; feed efficiency; and concentrations of some vitamins, minerals, insulin, ascorbic acid, alpha tocopherol, and retinol in plasma. However, the level of malondialdehyde; the production rate of free radicals; and requirements for vitamins A, C, and E increased (11-14).

Cold stress caused weakness in lymphoid organs and decreased immune function in hens. The effects of cold stress in chickens can be produced by different mechanisms. The cold changes the endocrine system; the hypothalamus-pituitary-adrenal axis, sympathetic-adrenal-medullary axis, and the thypothalamus-pituitary-thyroid axis become active, and, as a result, hormone responses to stress will be evident. Cold stress caused a significant drop in corticosterone levels and affected cellular immunity, but had no effect on T4 (15).

Several climate changes may take place during egg transportation. From an economic perspective it is a very important issue for day old chick producers. Therefore, in the current study the effects of cold stress and freezing conditions on broiler breeder eggs during transportation, and subsequent chick quality and incubation characteristics were investigated.

Material and methods

The altitude of Tehran Province, where the experiment was carried out, is 972 m. The experiment started in January, when the ambient temperature during delivery of the hatching eggs to the hatchery was about −15 °C. Due to road closures as a result of cold weather conditions, all eggs were kept inside the truck for 40 h. Internal temperature of the eggs was measured by thermometer (in 144 eggs, by breaking the eggs and measuring the temperature at the large end). The thermometer (Testo, Co.) was capable of measuring temperatures from −40 to 230 °C, with accuracy to 0.1 °C. Temperature recording for each egg took about 90 s. We did our best to minimize the effect of ambient temperature on the thermometer. A total of 72,000 fertile and settable eggs were used in this study. Of this total number, 12% (equal to 48 cartons) were selected; 3 eggs from each carton. In total, the internal temperatures of 144 eggs (48 × 3) were measured.

There were a total of 4 treatments (temperature group) −1.2 °C, 1 to 2 °C, 2.5 to 3.9 °C, 4 to 6 °C, and a control group, 21-22 °C. Each treatment had 3 replicates, and each replicate contained 4800 eggs (equivalent to a Petersime trolley). Each treatment had 14,400 eggs in total. In a completely randomized design, eggs were evaluated after 11 h prewarming

and then set in the incubator. From each treatment 720 eggs (3600 eggs total) were individually weighed by digital scale (Berlini, model KV 2001).

Egg prewarming or preheating was completed in 11 h which is 2 h more than the usual time allotted. In order to synchronize and prevent delay in the removal of chicks from the hatcher, the egg setting was changed to 1 h earlier than the usual time. Every effort was made to prevent eggs from sweating while they were still in the preheating room.

Eggs were individually weighed while in the setter and at the time of transfer from setter trays to the hatcher, at day 19 of incubation (19 days plus 6 h). In the incubator, eggs usually lose part of their weight as water vapor (egg weight loss) (4). To calculate the percentage of egg weight loss, the following formula was used: $\frac{Egg\ wrt\ pre - Egg\ wk\ trans}{\text{Egg wt pre}} \times 100$

wt = weight; pre = at beginning of prewarming; trans = transfer day.

It must be noted that we calculated prewarming weight and not setting weight, as there might have been some weight loss during the 8 to 11 h of prewarming. In order to determine fertility, we candled the eggs at day 18 of incubation. Cobb-Vanress, Inc. recommends candling between days 10 and 12 of incubation (3); however, the Aviagen management guide indicates that the right time for candling is day 18 of incubation (5).

Measuring chick length is a fast method for evaluating chick quality (16). We measured from the beginning of the beak to the end of the middle toe by ruler. Chicks were stretched along a ruler by HatchTech method (16). According to Molenaar (16), only 25 chicks are needed to measure length; however, we evaluated 150 chicks. We calculated the chick yield (%) or chick weight/initial egg weight ratio from this formula (4):

$$\frac{Chick\ weight(g)}{\text{Initial egg weight}(2)} \times 100$$

We also performed an egg breakout on unhatched eggs (equivalent 20.83% of total), and results were recorded and evaluated. After the hatch, all residue, dead embryos, debris, culls, and shells were collected in order to prepare them for the production of hatchery waste. During this process, the above-mentioned materials are heated to 200 °C for 5 to 6 h in the processing tank, and the end product is milled into hatchery waste (hatchery by product). All data were analyzed with SAS software version 9.1. Statistical models used for data analysis were as follows (17):

$Yij = \mu + Ti + Eij$ Yij = observation *ij*

μ = mean of observations (overall mean)

Ti = effect of i treatment [i = 1, −1.2 °C, i = 2, 1 to 2 °C, i= 3, 2.5 to 3.9 °C, i= 4, 4 to 6 °C, and i = 5, control (21 to 22 °C)] Eij = experimental error

Results

Our data showed that the rate of egg weight loss during transportation from farm to hatchery was 1.49%. It must be mentioned that the ambient temperature during transport from broiler breeder farm to the hatchery reached below zero. This did not cause freezing in the egg yolks or albumen; however, wrinkling of the vitelline membrane and color spots in egg yolks (manifested as darker or lighter areas) were observed. In this study egg weight loss was affected by temperature ($P < 0.001$), and maximum weight loss (14.20%) was found in treatment 2. A number of contaminated eggs exploded during the transfer stage or incubation. In this study, percentage of exploded eggs was influenced by temperature ($P < 0.01$); most exploders (0.05%) were in treatment 1. The number of early hatched chicks in the incubator was significantly higher in treatment 1 ($P < 0.001$) than in other treatments. Some chicks pipped the outer shell of the egg while still in the incubator. Under normal conditions we observed 1%-1.5% of eggs in this situation. As indicated in Table 1, cold stress did not have a significant effect on eggs pipped. The effect of cold stress on chick weight was significant ($P < 0.01$), and mean weight of chicks in treatment 1 (−1.2 °C) was higher than in other treatments. Moreover, chick yield in treatment 1 was higher than in other treatments ($P < 0.01$). Chick length was affected by cold stress ($P < 0.001$); minimum chick length (17.90 cm) was observed in treatment 1, and the greatest chick length was found in the control group. Chick length decreased with reduction in temperature during transport.

Table 1. Effect of cold stress on performance and chick quality.

Treatment	Initial egg weight (g)	Egg weight loss (%)	Exploder (%)	Early hatched (%)	Egg pipped (%)	Chick weight (g)	Chick length (cm)	Chick yield (%)
1	61.76	13.66^{a}	0.056^{a}	0.016^{a}	0.66^{ab}	42.83^{a}	17.90^{c}	69.35^{a}
2	61.80	14.20^{a}	0.015^{b}	0.0066^{b}	1.206^{a}	41.67^{b}	17.98^{c}	67.43^{bc}
3	61.77	13.52^{a}	0.015^{b}	0.00^{c}	1.14^{a}	41.53^{b}	18.07^{c}	67.23^{bc}
4	61.83	14.06^{a}	0.023^{b}	0.0046^{bc}	1.19^{a}	41.34^{b}	18.30^{b}	66.86^{c}
Control	61.71	12.16^{b}	0.020^{b}	0.00^{c}	0.02^{b}	41.93^{b}	18.61^{a}	67.95^{b}
SEM	0.187	0.222	0.0053	0.002	0.292	0.210	0.079	0.291
P value	0.992	0.0005	0.0012	0.0007	0.0674	0.0042	0.0002	0.0011

$^{a-c}$: Means within a column without a common superscript differ significantly ($P \leq 0.05$).
Treatments: 1 = (−1.2 °C), 2 = (1 to 2 °C), 3 = (2.5 to 3.9 °C), 4 = (4 to 6 °C), control =) 21 to 22 °C).

As shown in Table 2, hatchability and hatch of fertile eggs decreased as the severity of cold stress increased. Cold stress had a significant effect on percent hatchability and hatch of fertile eggs ($P < 0.001$). Cull percentage and hatchery byproduct efficiency increased when temperature was reduced ($P < 0.001$); cold stress also significantly reduced the percentage of chick weight uniformity ($P < 0.01$).

As presented in Table 3, increase in cold stress before incubation resulted in a significant increase in the rate of contamination ($P < 0.001$). Cold stress also affected total embryo mortality ($P < 0.001$), and, with decrease in temperature below 4 °C, total mortality increased. Reducing the temperature did not affect the infertility rate of eggs. Ectopic viscera (ECV) is a condition in which the intestines appear outside the abdominal cavity when the chicken is fully developed (4). In this study, cold stress significantly affected the incidence of ECV ($P < 0.001$); with a decrease in temperature, the rate of ECV increased.

As shown in Table 4, most malpositions (1.10%) were observed in treatment 1. It seemed that temperatures below 2 °C increased the incidence of malpositions. Total malpositions and also head between thighs, beak above right wing, and head under left wing increased. Cold stress caused a

Table 2. Effect of cold stress on hatchability, culls, uniformity, fertility, hatchery by product efficiency.

Treatment	Hatchability (%)	Hatch of fertile (%)	Culls (%)	Uniformity of chick weight (%)	Fertility (%)	Hatchery by product efficiency (%)
1	85.40^{e}	87.31^{d}	1.84^{a}	73.63^{d}	97.81	51.35^{a}
2	86.39^{d}	88.28^{c}	1.49^{b}	77.57^{cd}	97.85	51.19^{ab}
3	86.85^{c}	88.72^{c}	1.21^{c}	80.36^{bc}	97.89	51.02^{bc}
4	87.84^{b}	89.86^{b}	1.09^{d}	82.50^{ab}	97.75	50.96^{c}
Control	90.36^{a}	92.58^{a}	0.84^{e}	85.33^{a}	97.60	50.30^{c}
SEM	0.095	0.219	0.029	1.400	0.250	0.070
P value	<0.0001	<0.0001	<0.0001	0.0014	0.9262	<0.0001

$^{a-c}$ Means within a column without a common superscript differ significantly ($P \leq 0.05$).
(Treatments: 1 = (−1.2 °C), 2 = (1 to 2 °C), 3 = (2.5 to 3.9 °C), 4 = (4 to 6 °C), control =) 21 to 22 °C)

Table 3. Effect of cold stress on infertility, embryo mortality, and contamination.

Treatment	Infertility	1-8 days (early mortality)	9-17 days (middle mortality)	18-19 days (turned mortality)	20-21 days (late mortality)	Total mortality	Contamination		Ectopic viscera
							1-9 days (early)	10-21 days (late)	
1	2.19	6.15[a]	2.20[a]	1.53[b]	0.88[a]	10.77[a]	1.21[a]	1.21[a]	0.28[a]
2	2.14	5.24[b]	1.78[c]	2.02[ab]	0.85[b]	9.90[b]	1.10[b]	0.73[b]	0.17[b]
3	2.10	5.47[b]	2.01[b]	2.60[a]	0.84[b]	10.94[a]	1.00[c]	0.66[c]	0.13[c]
4	2.25	4.39[c]	1.08[d]	2.59[a]	0.81[c]	8.87[c]	0.58[d]	0.67[c]	0.09[d]
Control	2.40	3.48[d]	0.87[e]	1.86[b]	0.78[d]	7.00[d]	0.24[e]	0.12[d]	0.04[e]
SEM	0.250	0.149	0.043	0.179	0.005	0.130	0.019	0.006	0
P value	0.9262	<0.0001	<0.0001	0.0065	<0.0001	<0.0001	<0.0001	<0.0001	< 0.0001

[a–c] Means within a column without a common superscript differ significantly (P ≤ 0.05).
(Treatments: 1 = (–1.2 °C), 2 = (1 to 2 °C), 3 = (2.5 to 3.9 °C), 4 = (4 to 6 °C), control =) 21 to 22 °C)

Table 4. Effect of cold stress on embryo malposition.

Treatment	Malposition					
	Head between thighs	Head in the small end of egg	Head under left wing	Head not directed toward air cell	Beak above right wing	TOTAL
1	0.39[a]	0.15[c]	0.06[b]	0[b]	0.50[a]	1.10[a]
2	0.22[b]	0.48[b]	0.00[d]	0[b]	0.37[b]	1.07[a]
3	0.22[b]	0.48[b]	0.06[b]	0[b]	0.13[c]	0.89[b]
4	0.17[c]	0.58[a]	0.12[a]	0.02[a]	0.00[d]	0.89[b]
Control	0.06[d]	0.16[c]	0.04[c]	0[b]	0.00[d]	0.26[c]
SEM	0	0.015	0	0	0	0.015
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a–c] Means within a column without a common superscript differ significantly (P ≤ 0.05).
(Treatments: 1 = (–1.2 °C), 2 = (1 to 2 °C), 3 = (2.5 to 3.9 °C), 4 = (4 to 6 °C), control =) 21 to 22 °C)

significant increase in the rate of malpositions in treatments compared to the control group (P < 0.001).

As indicated in Table 5, cold stress caused significant increases in the incidence of chicken deformities in treatments compared to the control group.

As shown in Table 6, cold stress significantly increased the number of chicks culled due to conditions such as red hocks, button navel, summation of button, and string navel.

Discussion

The vitelline membrane contains many proteins, carbohydrates, and lipids. Eggs stored for 6 months at temperatures ±1 °C experience weight loss, loss of nitrogen, and changes in the chemical composition of vitelline (18). The vitelline membrane loses its natural state (selective nature) between 20 and 30 days of storage (19). After 6 weeks eggs stored at 4 °C showed a decrease in the elastic properties of the vitelline membrane; this resulted in problems that

Table 5. Effect of cold stress on embryo deformity and disorders.

Treatment	Deformity				TOTAL
	Exposed brain	Without eye(s)	4 legs	Deformed beak	
1	0.22[a]	0[c]	0.13[a]	0.90[c]	1.25[c]
2	0.11[c]	0.40[b]	0.05[ab]	2.48[a]	3.04[b]
3	0.09[d]	0[c]	o[b]	0[d]	0.06[d]
4	0.13[b]	1.33[a]	o[b]	2.36[b]	3.82[a]
Control	0.02[e]	0[c]	o[b]	0[d]	0.02[d]
SEM	0	0.007	0.03	0	0.033
P value	<0.0001	<0.0001	0.0396	<0.0001	<0.0001

[a–c] Means within a column without a common superscript differ significantly (P ≤ 0.05).
(Treatments: 1 = (–1.2 °C), 2 = (1 to 2 °C), 3 = (2.5 to 3.9 °C), 4 = (4 to 6 °C), control =) 21 to 22 °C)

Table 6. Effect of cold stress on culled chicks.

Treatment	Culls and deaths							
	String navel	Button navel	String + button	Body cavity full	Red hock	Dehydrated	Stubby down	Dirty chicks
1	1.77[e]	20.79[a]	22.56[a]	58.09[d]	4.29[a]	7.82[b]	4.08[d]	3.15[a]
2	6.27[c]	12.79[b]	19.07[b]	56.29[e]	4.18[a]	4.99[d]	12.98[c]	2.49[b]
3	12.53[b]	6[c]	18.53[b]	76.36[a]	0[d]	5.10[c]	0[e]	0[c]
4	14.47[a]	0[e]	14.47[c]	62.63[b]	1[c]	4.53[e]	17.37[a]	0[c]
Control	5[d]	2[d]	7[d]	61.56[c]	1.43[b]	11[a]	16[b]	0[c]
SEM	0.172	0.264	0.292	0.323	0.133	0	0.082	0.038
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a–c] Means within a column without a common superscript differ significantly (P ≤ 0.05).
(Treatments: 1 = (–1.2 °C), 2 = (1 to 2 °C), 3 = (2.5 to 3.9 °C), 4 = (4 to 6 °C), control =) 21 to 22 °C)

facilitated the rupture of the vitelline membrane (20). Storage time and temperature are 2 factors that affect the vitelline membrane. The strength of the vitelline membrane decreases with increasing storage time. This may allow nutrients in the yolk to become available to any microorganism present in the albumen (21). Wrinkling of the vitelline membrane in sub-zero temperatures, under cold stress, is in line with this finding.

Egg water loss during storage has a positive effect on hatchability (22). The percentage of egg weight loss between 1 and 19 days in Cobb 500 and Ross 308 strains is 10.9% and 12.9%, respectively. Age of the flock (before 44 weeks) does not affect egg weight loss, but the effect of strain is significant (23). Egg weight loss reaches 12% up to 18 days, and as the altitude increases, the drop in egg weight increases (3). According to Aviagen, egg weight loss should be 12% from the time eggs set in the incubator up to transfer; under these conditions hatchability and chick quality are ideal. If eggs are stored for more than 6 days, egg weight loss will reach 11.5% (4). In the current study, egg weight loss in control eggs was 12.16%, which conforms to the observations of

Aviagen and Cobb-Vantress. When compared with the control group, cold stress caused an increase in the egg weight loss. However, there was not a significant difference between the treatments.

An increase in exploder eggs can be caused by a dirty nest, egg laying on the floor, egg washing, using dirty egg cleaner or sandpaper, high levels of dust in the nest, transportation and cold conditions, sweating eggs, spraying water on eggs, broken eggs, hand contamination of egg handlers, contamination of the setter floor, air filters, and the moisture supply system (24). The current study demonstrated that cold stress is another factor affecting the percentage of exploder eggs.

Early hatch in the incubator can be attributed to small eggs, differences between breeds, as well as high temperatures and low humidity in the incubator (24). In the present study, cold stress—especially at temperatures below zero—increased the rate of early hatched chicks.

Chick weight is affected by egg weight (25,26). A decrease in temperature (from 37.8 to 36.6 °C) during incubation resulted in an increase in chick body weight, from 39 to 40 g, and yolk sac weight, from 3 to 4.3 g (27). These findings are consistent with the findings of others; there were overweight chicks in treatment 1 and a lack of yolk uptake and inappropriate yolk usage.

Chick yield or chick weight-to-egg ratios are normally between 66% and 68% (3). This ratio in fresh eggs (stored for a short period of time) was between 67% and 67.5% (4). Other research reported chick yield at 36 weeks at 73.1% (23). In the current study, the chick weight-to-egg ratio was higher in treatment 1. Improper use of nutrients inside the egg and yolk sac prevents chicks from growing enough. A temperature decrease from 37.8 to 36.6 °C during incubation reduced chick length from 16.8 to 16.3 cm (27). Chick length in this experiment was affected by cold stress; reducing the temperature during transport also reduced chick size.

Percentage hatchability and hatch of fertile eggs (Ross 308, 36 weeks of age) were 80.8% and 85.9%, respectively (23). A temperature decrease from 37.8 to 36.6 °C in incubation caused a reduction in hatchability (salable chicks) from 87% to 81% (27). In the current study, reducing the temperature level reduced the hatch of fertile eggs and hatchability. The rate of cull chicks was 0.97%. Although it was 0.68% at 38 weeks and 0.42%-0.67% at under 43 weeks of age, the highest rate was 1.49% at 63 weeks (Cobb 500) (28). In the current study, the percentage of cull chicks and hatchery byproduct (hatchery waste) increased as the temperature was reduced.

Uniformity of flock is the percentage of chicks within ±10% (29) or ±15% (3) of the average body weight at a certain age. The goal is 80% uniformity in a flock. Uniformity is affected by many factors including egg size, shell quality, genetic variation of parents, flock density, quantity or quality of feed consumed, parasites, environmental conditions (nest temperature), photoperiod programs, feed restriction programs, broiler breeder body weight (30), incubator type or model (single stage or multistage), incubation conditions, duration of egg storage, initial egg weight, chick room conditions, condition of trucks and transportation system, variation between eggs (non-uniform chicks produced from small or large eggs), mixing eggs from young and old flocks, mixing eggs from different strains, storage periods, different patterns of egg storage, ventilation, non-uniform setter and hatcher conditions, diseases, and stress (24). Hatchability in flocks with 55%-59% and 75%-80% uniformity was 69.19 ± 1.93% and 83.93 ± 1.65%, respectively (30). In the current study, cold stress severely reduced uniformity of body weight.

Egg contamination rates from 0 to 21 days (in a flock 31-45 weeks of age) were nearly 0.5% (4). Placing cold stress on eggs before incubation significantly increased the number of contaminated eggs and chicks with full body cavity.

Cold stress had a significant effect on total embryonic mortality, and with reduction in temperature (below 4 °C) the rate of mortality increased. Reducing the temperature did not affect infertility. Egg shell contamination, nest contamination, ventilation status, and hatchery disinfection can cause increases in embryo mortality (31). An increase in the duration of egg storage, fumigation within 12 to 16 h of incubation, high or low temperatures, egg damage during transportation (jarring), illness, an old or aging flock, contamination, drugs, and pesticides are factors

that can increase embryonic mortality (24). Early embryonic mortality (36 weeks, Ross 308) was 2.5% (23). A decrease in incubator temperature from 37.8 to 36.6 °C increased early embryonic mortality from 2.7% to 2.8% (27). Mortality at 12-17 days is caused by inappropriate incubator temperature, moisture, nutritional deficiency (vitamins, phosphorus, and linoleic acid), and lethal genes (24). Reduction in incubator temperature from 37.8 to 36.6 °C increased embryonic mortality at the middle stage from 0.2% to 0.5% (27). Embryo mortality in the middle stage (36 weeks, Ross 308) was 3% (23). Wilson (25) showed that cold stress (−2 °C) before the incubation period increased embryo mortality and most mortality reported after the 16th day. In the current study, we found that early stage embryo mortality in treatment 1 was higher than in other treatments.

Eggshell pipping can result from upside-down eggs, setting fumigation at a high concentration, high temperature, high humidity, low turning, old eggs, large-sized eggs, and ventilation problems (24). Embryo mortality at the end of incubation (late stage) in a 36 week Ross 308 was 1.1% (23).

A reduction in incubator temperature from 37.8 to 36.6 °C increased embryonic mortality at the pipping stage (beak entering air cell) from 0.8% to 1.4% and at external pipping (tip of beak puncturing the eggshell) from 0.9% to 2.4% (27). The incidence of ECV is increased by high incubator temperatures, inheritance, and lethal genes (24). In the current study, cold stress significantly increased ECV rates. The quality of chickens during incubation depends on various factors such as maternal age, flock status, length of storage and storage conditions, and incubation conditions (32). Inside the egg a normal embryo must position itself with the head (beak) under the right wing and placed directly into the air cell. Usually 1%-2% of chickens have malpositions and deformity, and these disorders are observed in the last week of incubation. In this study, malpositions occurred in 1.2%-1.8% of chicks (average 1.5%). Most embryos have a type of malposition that prevents the use of oxygen and they die in the shell (dead in shell); only a few of these embryos are able to leave the shell. Type 6 malpositions account for 48% of malpositions (beak above right wing); type 5 (feet on the head), 20%; type 1 (head between the thighs), 12.5%; type 2 (head towards the end thin egg), 7.5%; type 3 (head under left wing), 7.5%; and type 4 (head not directed toward air cell), 4.5% (33).

Cold stress significantly affected total malpositions. Most malpositions (1.10%) occurred in treatment 1. A decrease in temperature towards −2 °C increased the incidence of malpositions. One of the important factors affecting the occurrence of malpositions is insufficient egg weight loss in the incubator (33). In the present study, the rate of egg weight loss in all treatments was higher than in the control group and the total rate of malposition was associated with it.

Deformity or abnormalities prevent embryos from leaving the eggs. The rate of deformity was 0.22%-0.3%. Most abnormalities occur between 15 and 21 days of incubation and include brain hernia (29%), beak deformity (27%), failure to develop eyes (25%), 4 legs (10%), lack of upper beak (8%), and twisted legs (1%). High incubation temperatures cause abnormalities in the brain and eyes, while low temperatures affect chicken growth. At normal hatchability status (85%) the rate of deformity does not exceed more than 0.3% (33).

In our experiment, cold stress caused a significant increase in the rate of deformity in treatment groups compared to the control. Inheritance and viral infection cause cross beaked chickens and chickens without eye(s); these and other malformations are also caused by high temperatures, problems in egg handling, or delay in hatch time due to low temperatures during incubation (3). We observed that cold stress increased dirty chicks, red hock, button navel, and total string and button navel.

In conclusion, cold stress during the delivery of eggs from farm to hatchery affects the performance of the developing embryo during incubation; cold stress also impacts chick quality at the time of hatch and at later stages of life.

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