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Effects of injection of saline with and without vitamin C on heat tolerance of neonatal chicks

A.A.M. HAMDY[1,2], A.M. HENKEN[1], W. VAN DER HEL[1], A.K.I. ABD ELMOTY[2] & A.GH. GALAL[2]

[1]Department of Animal Husbandry, Wageningen Agricultural University, P.O. Box 338, NL 6700 AH Wageningen, Netherlands, [2]Department of Animal Production, Faculty of Agriculture, El-Minia University, Egypt

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Abstract

The effects of injection of saline (0.9 % NaCl) with and without vitamin C on heat tolerance of neonatal chicks were studied. Sham-treated chicks served as controls. The chicks hatched from eggs incubated at 45 % RH or 55 % RH. Between the first and the second treatment, a 48-hour exposure period to a constant environmental temperature of 39 °C took place. Consecutively, production parameters were studied during a 4-week growing period. During heat exposure, chicks hatched from eggs incubated at 45 % RH lost less body weight than those from eggs incubated at 55 % RH. At the end of exposure, body temperature was lower in chicks hatched from eggs incubated at 45 % RH compared to 55 % RH. Incubation RH did not affect growth rate, feed intake, feed conversion and mortality during the post-exposure growing period. Injection of saline with or without vitamin C before exposure resulted in a higher body weight after heat exposure compared to controls. Injection of saline enhanced body weight to a greater extent than saline with vitamin C. Injection of either solution before or after exposure did not affect production parameters in the 4-week period after exposure, except for mortality. Mortality of sham-sham treated chicks was higher than that of once or twice injected chicks. It is concluded that saline injection increased heat tolerance, but that addition of vitamin C did not have any contributing positive effect.

Keywords : Neonatal chicks, injection, saline, vitamin C, heat tolerance

Introduction

High temperatures occur frequently during transportation of neonatal chicks. Neonatal chicks cope with heat by evaporation of water (Henken et al., 1987; 1988). The water content of chicks may therefore be important with respect to heat tolerance. The water content can be influenced by varying incubation humidity and length of stay of chicks in the hatcher after hatching (Hamdy et al., 1991a). In contrast to these more 'natural' ways of influencing body water content and heat tolerance, also more artificial ways may be used, for instance injection

of saline (0.9 % NaCl) with or without vitamin C. Injection of saline may increase the amount of water available to evaporate and may therefore increase heat tolerance. Vitamin C has been claimed to improve resistance of chicks to heat (Schmeling & Nockels, 1978). Pardue & Thaxton (1982) noted that vitamin C appeared to reduce stress-related responses and to improve physiological adaptation. Subcutaneous injection of vitamin C will increase the vitamin C level in the blood (Satterfield et al., 1945).

The objectives of the present research were to determine the effects of injection of saline with and without vitamin C on heat tolerance of neonatal chicks hatched from eggs incubated at two different relative humidities.

Materials and methods

Eggs and incubation conditions

Hisex Brown layer eggs (Euribrid, Boxmeer, Netherlands), originating from inseminated layer hens of approximately the same age, were numbered (1 to 420) and weighed individually. The eggs were not older than 1 week (stored at 12 °C). The eggs were assigned to one of two incubators (Pass Reform, Zeddam, Netherlands). In one incubator, the relative humidity (RH) was kept at 55 % (normal RH) and in the other at 45 % (low RH). Temperature in both incubators was maintained at 37.7 °C. These conditions prevailed from day 0 to 19 of incubation.

Hatching conditions and procedures

At day 19, eggs from both incubators were reweighed and placed in a climate respiration chamber of 1.8 m^3 (Verstegen et al., 1987) which was used as a hatcher at a temperature of 37.7 °C and a RH of 55 %. From day 20 to 22, hatching occurred. Each chick was tagged and weighed immediately when emerging from the shell. Hatching time of each chick was recorded. To manipulate the chicks in the hatcher without disturbing the hatching process of the others, special measures were taken as described by Hamdy et al. (1991a). When about 90 % of all chicks had hatched, the hatcher was opened.

Experimental treatments and heat exposure

After collecting the chicks from the hatcher and individual weighing, 164 chicks from each RH class were assigned to one of three treatments (Figure 1):

1. Sham-treatment that served as control. These chicks were handled similarly as the chicks of Treatments 2 and 3, including inserting the needle subcutaneously (s.c.) in the neck. However, no solution was injected.
2. Treatment with saline (1 ml 0.9 % NaCl).
3. Treatment with saline and vitamin C (Merck, prod. nr. 500074) (1 ml 0.9 % NaCl + 1 % vitamin C (w/v)). A vitamin C concentration of 1 % is assumed

Figure 1. Schematic diagram describing the experimental design, and the numbers of chicks included.

Incubation day 0 to 19		Hatch day 19 to 22		Exposure[2] day 0 to 2 after hatch		Growing period[3] day 2 to week 4 after hatch			
						Sham	Saline	Saline + vitamin C	Sample
RH[1]	210	Climate chamber	164	Sham	68	20	19	19	10
				Saline	48	19	19	0	10
				Saline + vitamin C	48	19	0	19	10
				Total	164	Total			164
		Control + Sample	10	+ 10 +					
		Other	26						

[1] RH is 45% for one chamber, 55% for the other.
[2] Treatment on day 0 after hatch.
[3] Treatment on day 2 after hatch.

to be comparable to a dietary level of 1000 ppm (Stilborn et al., 1988) assuming a feed intake of maximally 20 g per bird in two days and a vitamin C digestibility of minimally 50 %.

Average age, i.e. time period between hatching and opening the hatcher, of each treatment group was similar. Both sexes were represented equally in each treatment. After treatment, the chicks were assigned to one of two identical climate respiration chambers of 1.8 m^3 each (Verstegen et al., 1987). Chicks hatched from eggs incubated at 55 % RH were placed in one chamber and those from 45 % RH in the other. These 164 chicks were kept in one large group within a circular confinement of about 0.6 m^2. Temperature in both chambers was maintained at 39 °C constantly and RH at 60 %. The exposure period in the chambers lasted for 48 h. No feed or water was provided in this period. Light was on continuously with a light intensity of about 65 lux at chick level.

Experimental procedure and measurements

Thirty minutes after placing the chicks in the chambers, rectal temperature was measured (Dual Digital Thermometer) of 10 chicks from each treatment group within each chamber. These measurements were repeated just before opening the chambers after exposure. Chicks were weighed individually a third time directly after exposure. Initially, before placing 164 chicks in each chamber, each RH class consisted of at least 184 chicks. Ten untreated chicks were taken randomly and

sacrificed before exposure to determine initial water content of the whole body, of the yolk sac and of the remainder of the body. After exposure, 10 chicks from each treatment group were randomly chosen and sacrificed to determine body composition at the end of exposure (Figure 1).

The growing period and its procedure

Before exposure, 10 untreated chicks from each RH class were placed in a grower cage with feed (commercial starter, CP, 20.8 %; ME, 2800 Kcal per kg; calcium, 0.99 %; and available phosphorus, 0.45 %) and water available ad libitum. These chicks served as untreated, non-exposed controls. After heat exposure, the chicks from each treatment group were assigned to post-exposure treatments as shown in Figure 1. Thus, 7 treatment groups in each RH class can be specified with respect to the growing period.

1. A sham-sham group
2. A sham-saline group
3. A sham-saline + vitamin C group
4. A saline-sham group
5. A saline-saline group
6. A saline + vitamin C-sham group
7. A saline + vitamin C-saline + vitamin C group

The chicks of each of the seven treatment combinations were assigned to one of two grower cages. These cages and those with the untreated non-exposed controls belonged to one battery line in the University poultry house. Environmental temperature was maintained at about 31 °C during the first week and was decreased thereafter in a stepwise-fashion by 2 °C per week until about 25 °C in the 4th week. Light was on continuously. The vaccination schedule adopted was: Marek's disease (day 1 of growing period, intramuscularly), Infectious Bronchitis (day 2 of growing period, intra-ocularly (i.o)), Infectious Bursal disease (day 12 of growing period, i.o) and Newcastle disease (day 18 of growing period, i.o). The experimental growing period lasted 4 weeks.

The body weight of each chick (n = 288) was determined at the start of the growing period (day 2 of age) and each week thereafter until week 4. Also, feed intake per cage, feed conversion per cage (feed : gain), and body temperature (5 chicks from each cage) were determined weekly. Mortality was recorded daily per cage.

Calculations and statistics

Since body composition is related to body weight (Henken et al., 1987), regression equations can be calculated relating composition data of specific parts to body weight on basis of sampled chicks before and after exposure separately ($y = a + b \times x$, with y = water content of the specific part, a = the intercept, b = the regression coefficient and x = body weight). Thus, water content of the whole

body, of the yolk sac and of the remainder of each individual chick at start and end of exposure can be estimated. The average sample composition for a specific part was used in case the regression was not significant ($P \geq 0.10$).

Estimates of initial and final composition of each chick were obtained by substituting weight before treatment and after exposure, respectively, for x in the equations. Subsequently, water loss of the whole body, of the yolk sac and of the remainder during exposure were calculated and expressed as loss percentages of the respective initial values.

The data on egg weight at day 0 and 19 of incubation, on body weight at hatch, before treatment and at end of exposure, on body weight loss and water loss of specific body parts and on initial and final body temperature were analysed with the General Linear Model procedure of the SAS Institute (SAS, 1985). Main factors included in the model were incubation RH (low or normal) and injection (sham, saline and saline + vitamin C). Two and three-way interactions were dropped from the initial model when not significant ($P \geq 0.10$). As the initial status of a parameter is important with respect to heat tolerance, the initial status was added to the model as a covariable (e.g. initial body weight as covariable when analysing for body weight loss during exposure) (Hamdy et al., 1991a).

The data on daily gain, feed intake, feed conversion and body temperature during the 4-week growing period were analysed with exposure history (yes or no), incubation RH (low or normal) and treatments included as main factors in the model (SAS, 1985). Interactions were analysed initially, but deleted from the model if not significant ($P \geq 0.10$).

The daily recordings of mortality were used to calculate the number of chick days per cage within a week when determining feed intake per chick and feed conversion.

The data on mortality were analysed by Fisher's exact test (Dean et al., 1990).

Results

Weights of eggs and chicks

The data on egg weight at day 0 and 19 of incubation, on body weight at hatch, at start and at end of exposure are shown in Table 1. Egg weight at day 0 of the two RH classes was similar ($P \geq 0.10$). At day 19, eggs incubated at normal RH were heavier ($P \leq 0.001$) than those incubated at low RH. Chicks hatched from eggs incubated at normal RH were heavier ($P \leq 0.05$) at hatch than those from eggs incubated at 45 % RH. When egg weight at day 19 was 1.0 g higher, hatch weight increased by 0.8 g, approximately.

Body weight before treatment was not affected by incubation RH, and was similar for each group assigned to one of the treatments ($P \geq 0.10$).

Body weight at end of exposure was similar for the two RH classes ($P \geq 0.10$). However, injection of saline with or without vitamin C at the start of exposure increased ($P \leq 0.001$) body weight at the end of exposure by about 0.55 g compared to sham-treatment. Both experimental treatments had similar effects.

Table 1. Least squares means, regression coefficient (b), residual SD and coefficient of determination (*R*-square) of the parameters studied before and during the exposure period.

Parameter[1]	Relative humidity		Treatments			b[3]	Residual[4] SD	*R*-square
	low	normal	sham	saline	saline + vitamin C			
Egg weight at day 0 (g)	62.5	62.8	62.6	62.6	62.5	•	1.45	0.03
Egg weight at day 19 (g)	54.2^{α}	55.3^{β}	54.7	54.9	54.8	0.84***	1.67	0.43
Body weight at hatch (g)	46.2^{a}	46.9^{b}	46.6	46.6	46.5	0.78***	1.24	0.67
Body weight at start (g)	41.4	41.6	41.6	41.5	41.4	0.73***	1.43	0.52
Body weight at end (g)	33.2	33.1	32.8^{α}	33.4^{β}	33.3^{β}	0.84***	1.03	0.74
Body weight loss (%)	19.8	20.4	21.2^{β}	19.5^{α}	19.6^{α}	–0.16	2.46	0.08
Body water loss (%)	21.4	21.1	21.9^{β}	20.6^{α}	$21.3^{\alpha\beta}$	–0.34***	2.84	0.08
Yolk sac water loss (%)	68.7^{α}	73.4^{β}	73.2^{τ}	68.4^{α}	71.6^{β}	–0.02	6.72	0.88
Remainder water loss (%)	17.9^{β}	16.7^{α}	17.8^{b}	16.9^{a}	17.4^{ab}	–0.81***	3.18	0.13
Body temperature at start (°C)[2]	40.7	40.8	40.5^{A}	40.8^{B}	40.8^{B}	–0.01	0.28	0.25
Body temperature at end (°C)[2]	40.9^{α}	41.2^{β}	41.1	41.0	41.0	–0.01	0.36	0.19

^{a,b} Means for each factor within a row with different superscripts differ significantly ($P < 0.05$).
^{A,B} Means for each factor within a row with different superscripts differ significantly ($P < 0.01$).
$^{\alpha,\beta,\tau}$ Means for each factor within a row with different superscripts differ significantly ($P < 0.001$).
***$P \leq 0.001$.
• *no covariable used.*
[1] The statistical analysis was based on $n = 328$.
[2] The statistical analysis of body temperature at start and end was based on n= 60.
[3] Covariables used were: egg weight at day 0 for egg weight at day 19, egg weight at day 19 for hatch weight, hatch weight for weight at start and weight at start for weight at end, the respective initial value for loss of each specific part and initial body weight for body temperature.
[4] Root mean squares error of the statistical model used.

Body weight loss and water loss during heat exposure

The data on percentages of body weight loss, body water loss, yolk sac water loss and remainder water loss are shown in Table 1. A slight difference ($0.5 < P < 0.10$) in percentage body weight loss between chicks hatched from the two RH classes existed, chicks hatched from eggs incubated at 45 % RH were loosing less body weight than those hatched from eggs incubated at 55 %.

When using body weight before treatment as a reference, differences ($P \leq 0.001$) in body weight loss between saline with or without vitamin C injected birds and sham-treated ones were –1.6 and –1.7 %, respectively. Body weight loss of saline and saline + vitamin C injected chicks was similar ($P \geq 0.10$). Incubation RH did not affect ($P \geq 0.10$) percentage body water loss. Treatment significantly affected body water loss, yolk sac water loss and remainder water loss. Sham-treated chicks lost more in each case than chicks injected with saline or saline + vitamin C, if compared with body weight before treatment. Differences between the latter two treatments were minor except for yolk sac water loss. In all cases, loss percentages of chicks injected with saline + vitamin C were intermediate between those of sham- and saline-treated chicks.

Body temperature

The data on body temperature are shown in Table 1. Incubation RH did not affect body temperature at the start of exposure. After exposure, chicks hatched from eggs incubated at 45 % RH had a lower ($P \leq 0.001$) body temperature (–0.3 °C) than chicks hatched from eggs incubated at 55 % RH. Differences between treatments in body temperature at start of exposure were significant ($P \leq 0.01$). Injection with saline with or without vitamin C increased body temperature at the start of exposure by 0.3 °C compared to sham-treatment. After exposure all treatment groups had similar body temperature.

Growth performance

Data on daily gain, feed intake, feed conversion and body temperature during the

Table 2. Least squares means, residual SD and coefficient of determination (*R*-square) of the parameters studied during the growing period.

Parameter	Week	Exposure		Relative humidity		Average of treatments[4]	Residual SD[5]	*R*-square
		yes	no	low	normal			
Daily gain (g)[1]								
	1	4.3	4.4	4.3	4.4	4.4	1.14	0.03
	2	7.6	7.7	7.4	7.8	7.6	1.51	0.04
	3	11.8	11.8	11.9	11.7	11.9	2.16	0.03
	4	15.1	15.0	15.2	14.9	15.2	3.24	0.03
Daily feed intake (g)[2]								
	1	9.3	9.9	9.7	9.4	9.6	0.81	0.27
	2	15.9	15.9	16.0	15.8	15.6	1.58	0.31
	3	25.6	25.6	25.6	25.6	25.6	1.12	0.18
	4	35.5	35.6	35.7	35.4	35.6	1.81	0.15
Feed conversion[2]								
	1	2.7	2.6	2.7	2.7	2.7	2.70	0.37
	2	2.1	2.1	2.2	2.0	2.1	0.39	0.23
	3	2.2	2.2	2.2	2.2	2.2	0.13	0.17
	4	2.4	2.4	2.4	2.4	2.3	0.20	0.16
Body temperature (°C)[3]								
	1	40.9[a]	41.0[b]	41.0[b]	40.9[a]	41.0	0.33	0.10
	2	41.3[a]	41.4[b]	41.4[b]	41.3[a]	41.3	0.25	0.07
	3	41.5	41.4	41.5	41.4	41.4	0.27	0.07
	4	41.2	41.3	41.3	41.2	41.3	0.24	0.05

[a,b] Means for each factor within a row with different superscripts differ significantly ($P < 0.05$).
[1] In each experiment the statistical analysis was based on $n = 288$ with substraction of the number of dead chicks weekly.
[2] The statistical analysis was based on n = 30.
[3] The statistical analysis was based on n = 150.
[4] No significant differences between treatments were found during the growing period.
[5] Root mean squares error of the statistical model used.

growing period are presented in Table 2. Chicks exposed to the experimental temperature regimens in the chambers had similar daily gain, feed intake and feed conversion as non-treated and non-exposed controls during the 4-week growing period. The same is true with respect to the chicks of the two incubation RH classes and the experimental treatments. Exposure and incubation RH, but not treatment, affected ($P \leq 0.05$) body temperature in the 1st and 2nd week, in which exposed chicks and chicks from the 55 % RH group had a 0.1 °C lower body temperature than controls and chicks from the 45 % RH group, respectively.

Mortality

No mortality occurred during the 2-day exposure period in the chambers. Mortality only occurred during the first two weeks of the 4-week growing period after exposure (Table 3). Exposure did not affect mortality significantly (2/20 for control and 16/268 for exposed chicks). Also incubation relative humidity did not affect mortality.

Compared with the pooled total of the other 6 treatments, a significantly ($P \leq 0.001$) higher proportion of sham-sham treated chicks died during the growing period (7/228 vs. 9/40, respectively).

Discussion

Eggs incubated at 45 % RH lost more weight from day 0 to 19 of incubation than eggs incubated at 55 % RH. The differences in egg weight at day 19 and in chick weight at hatch between the two RH classes were significant. This result must be due to increased water loss at the lower water vapor pressure in the incubator (45 % RH). Similar results were reported by Peebles et al. (1987) and Hamdy et

Table 3. Mortality in numbers of chicks during the growing period.

Week[1]	Exposed chicks Relative humidity		Control chicks[2] Relative humidity		Treatment[3]						
	low RH	normal RH	low RH	normal RH	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	5	6	–	1	7***	–	–	3	–	1	–
2	3	2	1	–	2	–	2	–	–	–	1
Total	8	8	1	1	9***	–	2	3	–	1	1

***$P \leq 0.001$ between treatment (1) and the other treatments (2) to (7) together.

[1] No mortality occurred in the 3rd and 4th week.

[2] Control groups were not exposed to the experimental temperatures, but placed in grower cages at normal thermal conditions with feed and water available directly after hatch.

[3] Treatment: (1) sham-sham, (2) sham-saline, (3) sham-saline + vitamin C, (4) saline-sham, (5) saline-saline, (6) saline + vitamin C-sham, (7) saline + vitamin C-saline + vitamin C.

al. (1991a). During the period between hatch and start of exposure, this difference in hatch weight between RH groups disappeared. Staying time in the hatcher as such is also an important factor for heat tolerance (Hamdy et al., 1991a). During the exposure period, chicks hatched from eggs incubated at 45 % RH lost less weight than those hatched from eggs incubated at 55 % RH, which agrees with earlier results of Hamdy et al. (1991a). During the growing period at normal conditions, chicks hatched from eggs incubated at low RH initially had a higher body temperature than those hatched from eggs incubated at 55 % RH. A reason for this increased warmth may be that heat production is different between the chicks of the two RH groups. Heat production is related to feed intake. Although differences were not significant, chicks hatched from eggs incubated at low RH consistently ate more than those from eggs incubated at 55 % RH. It can be concluded that in the present experiment a low incubation RH did not affect heat tolerance of chicks negatively. On the contrary, short-term heat tolerance tended to be positively affected. However, on the longer term, feed conversion and mortality were not affected.

In earlier experiments, Hamdy et al. (1991b) found that mortality after exposure was significantly reduced in chicks hatched from eggs incubated at low compared to normal RH. In those experiments, however, mortality was at a higher level than in the present experiment. The age of the laying hens was the same. Environmental conditions were also similar. There may be a difference in level of heat tolerance between batches of chicks. A reason might be the difference in body weight at the start of exposure. In the present experiment, average body weight at the start was about 41.5 g, while in the experiment of Hamdy et al. (1991b) it was 38 g. Apparently, this difference is an advantage for the chicks with higher body weight, resulting in an increased heat tolerance.

It should be realized that the obtained results concerning body weight loss during the heat exposure period heavily depended on the choice of the reference value. Body weight of injected chicks before and after treatment differed by about 1 g (i.e. the approximate weight of 1 cc of fluid), representing ca 2.3 % of the body weight of the chick.

Using the weight after injection as a reference, injected chicks lost both relatively and absolutely more weight during heat exposure, although their final body weight is higher. Apparently, these chicks used part of the injected volume for evaporation.

Another aspect associated with the injection of fluid is its temperature, which was about 25 °C at the time of injection. This implicates that 2.3 % of the total body mass had to be warmed up to approximately 40.5 °C. Perhaps the higher body temperature for the injected chicks at the start of exposure can partially be explained by the volume and/or the temperature aspect associated with the injection of fluid, resulting in an overshoot with respect to body temperature.

As injection of saline with or without vitamin C resulted in more body water at the end of exposure, this may indicate an increased heat tolerance during exposure.

Adding vitamin C did not have any beneficial effect. On the contrary, vitamin C seemed to counteract the saline effect, because the saline + vitamin C group

was mostly intermediate between the sham- and the saline-treated group.

Injection after heat exposure to help the chicks to recover from the heat stress did not have any effect on the parameters measured during the growing period. The absence of such effects of saline and vitamin C in the growing period is also reported by Maurice & Deodato (1982) and Stilborn et al. (1988). However, injection as such did reduce mortality compared to sham-sham treatment.

In conclusion, it can be stated that injection of saline reduced body weight loss during heat exposure compared to non-treated controls, by reducing water loss when body weight before treatment is used as a reference. Vitamin C seemed to have no additional positive effect on heat tolerance when given in combination with saline. Treatment effects on parameters after heat exposure were not present with one exception. Mortality of sham-sham treated chicks was higher than that of injected chicks.

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