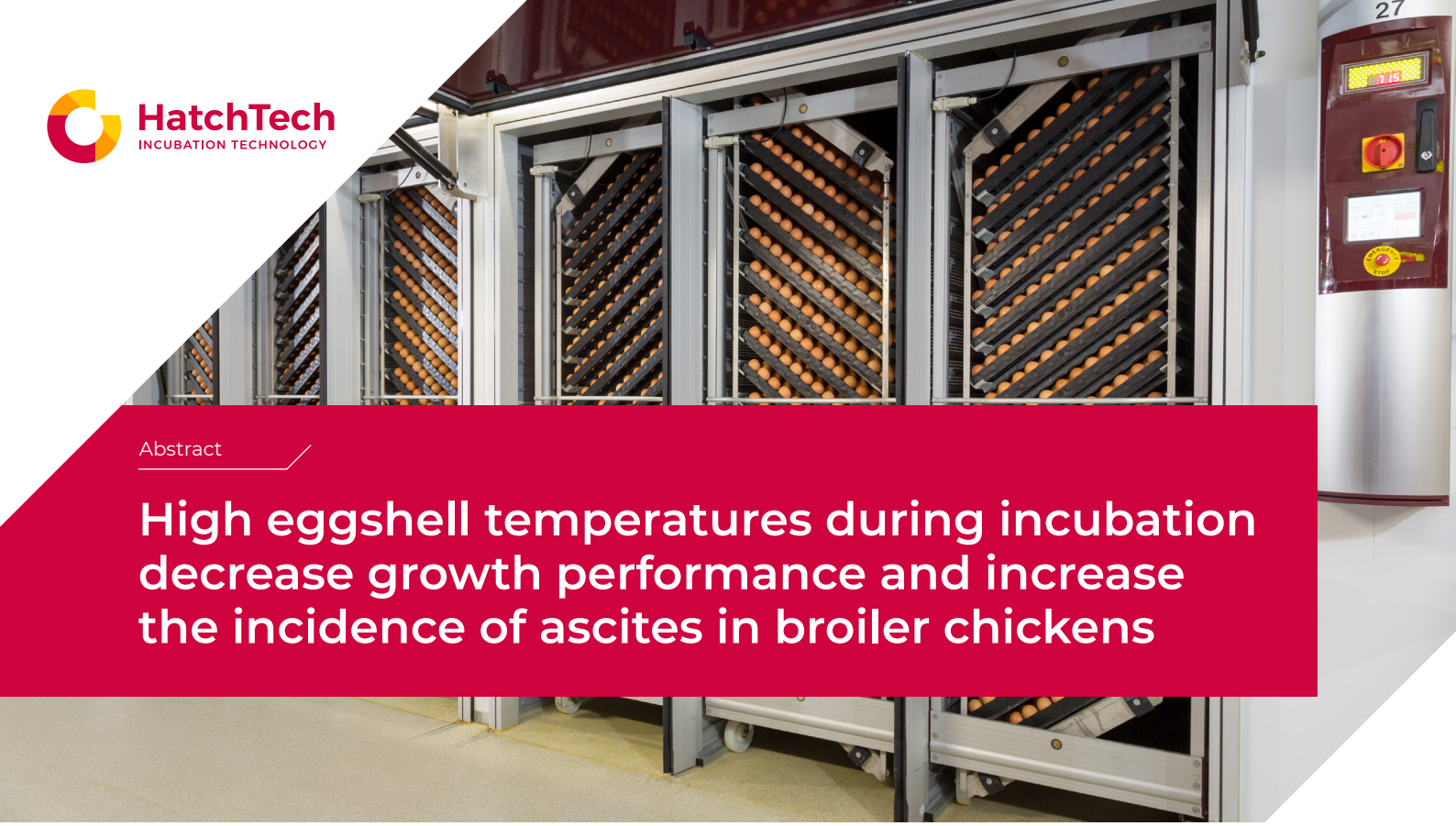
HatchTech
INCUBATION TECHNOLOGY



Abstract

High eggshell temperatures during incubation decrease growth performance and increase the incidence of ascites in broiler chickens

R. Molenaar*[1], R. Hulet[†],R. Meijerhof[‡], C. M. Maatjens*, B. Kemp[§],and H. van den Brand

High eggshell temperatures (EST;≥38.9°C) during the second half of incubation areknown to decrease the body and organ development of broiler hatchlings. In particular, relative heart weights are decreased by a high EST, and this may increase the incidence of metabolic disorders that are associated with cardiovascular development, such as ascites. The current study investigated the effects of a high EST on chick quality, subsequent performance, and the incidence of ascites later in life. Eggs were incubated at a normal (37.8°C) or high (38.9°C) EST from d 7 of incubation onward. After hatching, the chickens were housed per EST in pens, and a normal or cold temperature schedule was applied during the grow-out period.

Hatchability, hatchling quality, BW, feed conversion ratio, total mortality, mortality associated with ascites, slaughter characteristics, and ascites susceptibility at 6 wk of age were evaluated. Except for total ventricle weight, no interaction was found between EST and the grow-out temperature. Hatchability was comparable between the EST treatments, but the percentage of second-grade chickens was 0.7% higher at the high EST. Yolk-free body mass was 3.0 g lower, and heart weights were 26% lower at hatch in the high compared with the normal EST treatment. Body weight continued to be less during the grow-out period after the high EST incubation. However, breast meat yield was 1.0% higher in the high than in the normal EST.

Feed conversion ratio did not differ between EST treatments. Total mortality was 4.1% higher and mortality associated with ascites was 3.8% higher in the high compared with the normal EST treatment. The ratio between the right and total ventricle was 1.1% higher in the high compared with the normal EST treatment at slaughter age. In conclusion, a high EST from d 7 of incubation onward decreased hatchling quality and growth performance, but increased breast meat yield. Furthermore, high EST incubation increased the incidence of ascites, which may be related to the reduced heart development at hatch.

Introduction

Because of genetic selection, growth rates of broiler chickens have been increasing, and this has decreased the length of the production cycle by 60% within 40 yr (Wolanksi et al., 2004; Hulet, 2007; Baghbanzadeh and Decuypere, 2008). As a consequence, the incubation period has become a larger part of the total life span of a broiler chicken (Havenstein et al., 2003; Wolanski et al., 2004). Optimizing development and maturation during incubation is therefore important and will in turn optimize development during the grow-out period (Hulet, 2007). Several studies have shown that incubation conditions influence chick development (Freeman and Vince, 1974; Lourens et al., 2005, 2007) and that temperature is one of the most important environmental factors during incubation (Decuypere and Michels, 1992). Eggshell temperature (EST) often increases at the end of incubation because of the higher heat production of the embryos (Lourens et al., 2005) and problems with cooling and air velocity in the incubators (French, 1997; Elibol and Brake, 2008). High EST (≥38.9°C), as compared with normal EST (37.8°C), during the second half of incubation reduces hatchling quality, expressed by a lower yolk-free body mass (YFBM), a shorter chick length, and a poorer navel condition (Lourens et al., 2005, 2007; Hulet et al., 2007; Leksrisompong et al., 2007). In addition, several studies have shown that organ weights of hatchlings, and especially heart weights, are reduced because of a high EST (Wineland et al., 2000; Leksrisompong et al., 2007; Lourens et al., 2007).

The reduced heart weights at hatch that are due to a high EST may increase the susceptibility to and the incidence of metabolic disorders related to cardiovascular development later in life, such as ascites (Leksrisompong et al., 2007), but the relationship between EST and ascites was never investigated. Chickens that are incubated at a high EST may have an insufficient pulmonary vascular capacity, which may increase their metabolic demands for O_2 and result in the development of ascites (Lubritz and McPherson, 1994). As a result of the increased O_2 requirement, the O_2 carrying capacity is enhanced by an increase in the number of red blood cells (Decuypere et al., 2000). As a consequence, the viscosity of the blood increases; this can lead to increased cardiac output, pulmonary hypertension, and right ventricle hypertrophy, which are signs of the development of ascites (Scheele et al., 1991; Julian, 993; Lubritz and McPherson, 1994; Decuypere et al., 2000). An increase in the intravascular pressure results in fluid accumulation in the abdominal cavity and pericardium (Julian, 1993; Decuypere et al., 2000), and birds will eventually die from these lesions.

Ascites has become a major cause of mortality in modern broiler production, affecting approximately 5.0 to 8.0% of all broilers worldwide (Balog, 2003; Pavlidis et al., 2007). Modern broiler chickens are more sensitive to metabolic disorders such as ascites because of the genetic selection for rapid growth, low feed conversion ratio (FCR), and high meat yield (Scheele et al., 1991; Decuypere et al.,

2000; Balog, 2003; Arce-Menocal et al., 2009), which has resulted in decreased visceral organ development (Havenstein et al., 2003). Although genetic selection against ascites is effective and broiler breeder companies have implemented selection programs to reduce ascites (Wideman and French, 2000; Pakdel et al., 2005; Pavlidis et al., 2007; Arce-Menocal et al., 2009; Hassanzadeh et al., 2010), broilers susceptible to ascites are still present in commercial flocks and may account for almost 50% of the total mortality (Arce-Menocal et al., 2009). Therefore, the current study investigated the effect of high EST on chick quality, performance, and mortality, with particular attention given to the incidence of ascites. To induce ascites, a cold grow-out temperature was used (Balog, 2003).

Materials and methods

Experimental Design

The experiment was designed as a 2 × 2 factorial arrangement with 2 EST treatments (normal or high) during incubation and 2 temperature schedules (regular or cold) during the grow-out period. Chick quality was evaluated at hatch, and performance and ascites susceptibility and incidence were evaluated during the grow-out period and at slaughter age. The experimental protocol was approved by the Penn State University Animal Care and Use Committee.

Hatching Eggs

Twenty trays were used, each containing 132 firstgrade hatching eggs (n = 2,640) from commercial Ross × Cobb (500) breeders that were 33 wk of age. The average egg weight when the eggs were set was 60.1 g.

Storage and Incubation

Eggs were stored for 5 d at 18°C at the animal experiment facilities of Penn State University (University Park, PA). The eggs were then set in a Buckeye incubator (Chickmaster Inc., Medina, OH). Eggshell temperature was measured twice a day from 10 eggs with a Thermoscan instrument (Braun, Kronberg, Germany) and was maintained at 37.8°C. Eggs were turned hourly 90° between d 0 and 18 of incubation. On d 7 of incubation, eggs were randomly divided between 2 Buckeye incubators (Chickmaster Inc.). The empty trays in the incubator were filled with unfertilized eggs to ensure a uniform air speed around the eggs. The EST was again measured twice a day from 10 eggs with a Thermoscan instrument (Braun) and maintained at either the normal (37.8°C) or a high (38.9°C) EST. On d 18 of incubation, the eggs were transferred to hatching boxes in 2 separate Buckeye hatchers (Chickmaster Inc.), and the previous normal or high EST was maintained. From d 19 of incubation, the incubator temperature was fixed at the last EST set point, and the EST was allowed to increase during the hatching process. Relative humidity was set at 50% during the entire incubation process.

Hatch Until Slaughter Age

At hatch, all chickens were taken from both hatchers and vaccinated for Marek's disease. The hatchlings were classified as first- or second-grade chickens. A chick was classified as first grade when it was clean, dry, and without deformities or lesions (Tona et al., 2004). The other chickens were classified as second-grade chickens, and this also included the chickens that died in

the hatching basket after emerging from the eggshell. The percentage of first- and second-grade chickens was expressed as a percentage of fertile eggs. Fifty first-grade chickens per EST treatment were randomly selected and their BW were determined, along with the residual yolk, chick length, and navel condition. Chick length was measured from the top of the beak to the tip of the middle toe, excluding the nail (Hill, 2001). Navel ondition was scored as 1 (closed and clean navel area), 2 (black button up to 2 mm or black string), or 3 (black button exceeding 2 mm or open navel area). The hatchlings were decapitated and bled, and the residual yolk and heart were removed and weighed. The YFBM was calculated as BW minus residual yolk.

Nonhatched eggs were opened, and infertile eggs or embryo mortalities were classified as described by Lourens et al. (2006). The embryos in nonhatched eggs that were older than 18 d and not positioned with their head under the right wing were classified as malpositioned (Dove, 1935). The number of infertile eggs was expressed as a percentage of the total number of eggs. The embryonic mortalities per week and malpositioned embryos were expressed as a percentage of the fertile eggs.

Per EST treatment, 36 first-grade chickens were housed in a pen, with 24 replicates per EST treatment (n = 1,728). A cold or regular temperature schedule as applied to half of the pens per treatment during the grow-out period (Figure 1). Pens were prepared as described by Hulet et al. (2007). The chickens were fed a crumbled starter diet (3,086 kcal of ME/kg, 22.06% CP) until 14 d of age, a pelleted grower diet (3,152 kcal of ME/kg, 19.89% CP) until 28 d of age, and a pelleted finishing diet (3,219 kcal of ME/kg, 18.18% CP) until 42 d of age. Feed and water were provided ad libitum throughout the experiment by round pan hanging feeders and nipple drinkers, respectively. The light schedule was 20L:4D throughout the grow-out period. The chickens were weighed and their feed was weighed back per pen at 0, 7, 14, 21, 28, 35, and 42 d of age. Feed conversion ratio was calculated by dividing the total feed consumption per pen by the total growth per pen, with the growth of the birds that died being included as well. Dead chickens were removed and recorded daily per pen. Body weight, cause of death, and fluid in the pericardium and abdominal cavity were recorded, and the right ventricle (RV) and total ventricle (TV) were weighed. Pericardial and abdominal fluids were scored as either 0 if no fluid was present, or 1 if an accumulation of fluid was observed. Dead chickens were classified as having ascites when fluid was found in the pericardium and abdomen or when the calculated RV:TV ratio was greater than 27% (Wideman, 2001). At slaughter age on d 42 or 43, 20 males and 20 females per EST × grow-out treatment were processed; the BW, carcass weight, breast meat yield, and abdominal fat were weighed (n = 160). Furthermore, hematocrit, fluid in the pericardium and abdomen, and RV and TV weights were measured. Chickens were deprived of feed approximately 6 h before slaughter.

Statistical Analyses

Percentage of infertile eggs, first- and second-grade chickens, embryonic mortality per week, malpositioned embryos, and weight loss were analyzed using the GLM procedure of SAS (version 9.1; SAS Institute, 2004), with EST as the class variable and egg tray as the experimental unit. Body weight at hatch, YFBM, chick length, and heart and residual yolk weight were analyzed using the GLM procedure, with EST as the class variable and chicken as the experimental unit. Navel condition was analyzed using the LOGISTIC procedure, with EST as the class variable. Body weight and FCR during the grow-out period were

analyzed using the MIXED procedure for repeated measurements, with EST, grow-out temperature, and their interaction as class variables and the percentage of males per pen as a covariable. Pen was the repeated factor. The measurements taken at slaughter age (i.e., BW, carcass weight, breast meat yield, abdominal fat, and hematocrit) were analyzed using the GLM procedure, with EST, growout temperature, sex, and their interactions as the class variables and day of processing as a block. Breast meat yield was calculated as a percentage of carcass weight. Total mortality and mortality that was associated with ascites were calculated per pen and analyzed using the GLM procedure, with EST, grow-out temperature, sex, and their interactions as the class variables and the percentage of males per pen as a covariable. Distributions of the means and residuals were examined to check the model assumptions. In all analyses, nonsignificant interactions ($P > 0.05$) were excluded from the model. The least squares means were compared using Bonferroni adjustments for multiple comparisons. Data are presented as means ± SE. In all cases, a difference was considered significant at $P \leq 0.05$.

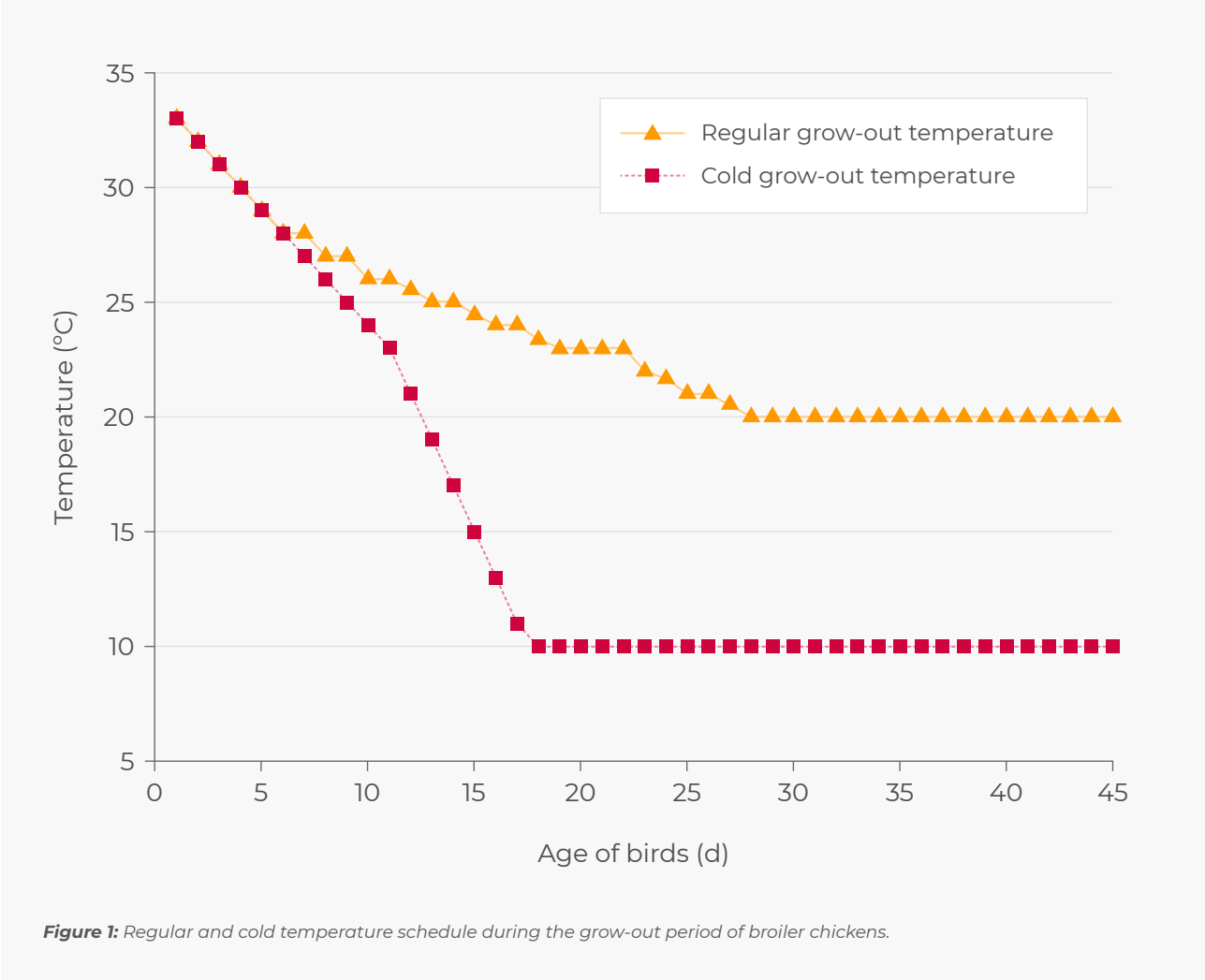


Figure 1: *Regular and cold temperature schedule during the grow-out period of broiler chickens.*

Item	n	Normal EST	High EST	P-value
Incubation[1]				
Infertile eggs[2] (%)	20	2.6 ± 0.45	3.4 ± 0.56	0.26
Hatchability of fertile eggs[3] (%)	20	94.5 ± 0.57	92.5 ± 1.04	0.12
Second-grade chickens[4] (%)	20	0.2 ± 0.11[b]	0.9 ± 0.30[a]	0.02
Embryo mortality[3] (%)				
wk 2	20	1.5 ± 0.27	1.1 ± 0.29	0.34
wk 3	20	1.9 ± 0.48	3.1 ± 0.68	0.17
Malpositioned embryos[3] (%)		1.4 ± 0.41	2.4 ± 0.67	0.24
Hatchling[5]				
BW (g)	100	40.6 ± 0.39[a]	37.2 ± 0.41[b]	<0.001
YFBM[6] (g)	100	36.9 ± 0.33[a]	33.9 ± 0.33[b]	<0.001
Chick length (cm)	100	19.5 ± 0.07	19.7 ± 0.07[b]	0.14
Residual yolk (g)	100	3.7 ± 0.15[a]	3.2 ± 0.18[b]	0.05
Navel condition	100			
1		50	18	
2		48	80	0.002
3		2	2	
Heart weight (g)	100	0.38 ± 0.00[a]	0.28 ± 0.00[b]	<0.001

[a,b] Means followed by different letters within a row are significantly different
[1] Tray was the experimental unit
[2] Expressed as a percentage of the total number of eggs
[3] Expressed as a percentage of fertile eggs
[4] Expressed as a percentage of hatched chickens
[5] Chick was the experimental unit
[6] Yolk-free body mass
[7] Percentage of chickens per treatment group that were scored with a navel condition of 1, 2, 3, where 1 = good, 2 = moderate, and 3 = poor

Table 1: *Percentages of infertile eggs, hatchability of fertile eggs, second-grade chickens, embryonic mortality, malpositioned embryos, and hatchling measurements for eggs incubated at a normal (37.8°C) or a high (38.9°C) eggshell temperature (EST) from 7 through 21 d of incubation*

Results

EST × Grow-Out Temperature

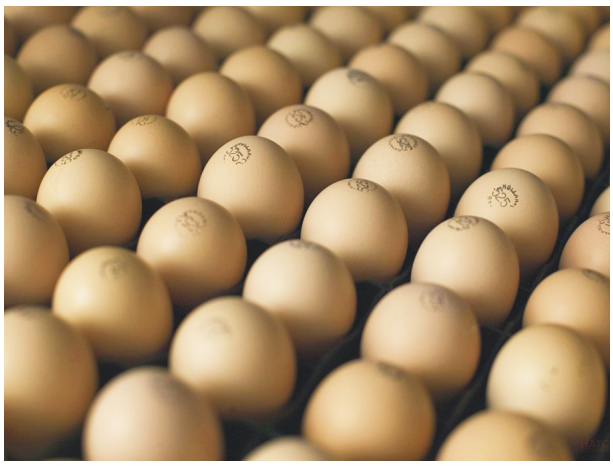
An interaction was found between EST and growout temperature for TV weight (P = 0.02). At the cold grow-out temperature, TV weight did not differ between EST and was on average 10.0 g. At the normal grow-out temperature, TV weight was less in the high EST treatment (8.4 g ± 0.21) than in the normal EST treatment (9.0 g ± 0.23).

EST

The percentage of infertile eggs was on average 3.0% and did not differ between EST treatments (P = 0.26; Table 1). Hatch of fertile eggs did not differ between EST treatments (P = 0.12), but percentage of secondgrade chickens was 0.7% higher (P = 0.02) in the high than in the normal EST treatment. Embryonic mortality in the second and third week of incubation and the percentage of malpositioned embryos did not differ between EST treatments (all P > 0.05). Body weight at hatch was 3.4 g less and YFBM was 3.0 g less in the high EST treatment when compared with the normal EST treatment (P < 0.001). Chick length did not differ between EST treatments (P = 0.14) and was on average 19.6 cm. Residual yolk weight was 0.5 g lower

in the high than in the normal EST treatment (P = 0.05). Navel condition was poorer in the high than in the normal EST treatment (P = 0.002). Heart weight at hatch was reduced by 26% in the high compared with the normal EST treatment (P < 0.001).

Body weight during the grow-out period was lower in the high than in the normal EST treatment (P < 0.001; Table 2). At 42 d of age, the BW was 41 g less in the high than in the normal EST treatment. The FCR between 0 and 42 d of age did not differ between the EST treatments and was on average 1.92 (P = 0.37; Table 3). Total mortality was 4.1% greater in the high than in the normal EST treatment (P = 0.008). Mortality associated with ascites was 3.8% greater in the high than in the normal EST treatment (P = 0.001). At slaughter age, BW, carcass weight, abdominal fat, hematocrit, pericardial and



abdominal fluid, RV and TV weights, and relative heart weight did not differ between the EST treatments (all P > 0.05; Tables 4 and 5). The percentage of breast meat yield was 1.0% and the RV:TV ratio was 1.1% greater in the high than in the normal EST treatment (P < 0.001 and P = 0.04, respectively).

Item	EST			Grow-out temperature		
	Normal	High	Change (normal-high)	Regular	Cold	Change (regular-cold)
Day						
0	41 ± 0.1[a]	38 ± 0.1[b]	3	40 ± 0.4[a]	40 ± 0.3[a]	0
7	165 ± 0.9[c]	164 ± 1.1[d]	2	163 ± 1.0[b]	167 ± 0.9[b]	-4
14	443 ± 2.3[e]	437 ± 2.4[f]	5	436 ± 1.8[c]	443 ± 2.7[c]	-7
21	925 ± 6.9[g]	891 ± 12.8[h]	34	930 ± 12.2[d]	886 ± 6.7[e]	44
28	1,520 ± 4.1[i]	1,470 ± 16.2[j]	50	1,545 ± 10.2[f]	1,445 ± 14.0[g]	100
35	2,208 ± 25.4[k]	2,161 ± 25.7[l]	47	2,272 ± 19.3[h]	2,097 ± 18.1[i]	175
42	2,895 ± 23.9[m]	2,854 ± 20.4[n]	41	2,948 ± 14.0[j]	2,800 ± 18.7[k]	148
Source of variation[1]	P-Value					
EST	< 0.001					
Grow-out temperature	< 0.001					
Day	< 0.001					
Grow-out temperature x day	< 0.001					

[a,b] Means followed by different letters within factor an row are significantly different (P ≤ 0.05)

[1] Interactions between EST x day, EST x grow-out temperature, and EST x grow-out temperature x day were not significant (P > 0.05)

Table 2: *Body weight (g) of birds incubated at a normal (37.8°C) or a high (38.9°C) eggshell temperature (EST) from 7 through 21d of incubation and grown at a regular or a cold temperature until 42 d of age*

Grow-Out Temperature

An interaction was found between grow-out temperature and age of the bird for BW (P < 0.001; Table 2). From 21 d of age onward, BW was lower in the cold compared with the regular grow-out temperature treatment. At 42 d of age, the BW difference was 148 g between the cold and regular grow-out temperature. The FCR between 0 and 42 d of age was poorer in the cold (1.98) than in the regular (1.86) grow-out temperature (P < 0.001; Table 3). Total mortality was 4.7% greater in the cold than in the regular grow-out temperature (P = 0.02). Mortality associated with ascites was 3.8% greater in the cold than in the regular grow-out temperature (P = 0.004).

Body weight at slaughter age and percentage of breast meat yield were not influenced by grow-out temperature (both P > 0.05; Table 4). Carcass weight was 92 g and abdominal fat was 6 g less in the cold than in the regular grow-out temperature (both P < 0.01). Hematocrit was 3% greater in the cold than in the regular grow-out temperature (P < 0.001). Pericardial and abdominal fluids were not affected by the grow-out temperature, and neither was RV weight (all P > 0.05; Table 5). The cold grow-out treatment had a lower RV:TV ratio and a higher relative heart weight than did the normal grow-out treatment (both P < 0.01).

At slaughter age, BW, carcass weight, RV weight, TV weight, and the RV:TV ratio were higher in males than in females (all P < 0.001; Tables 4 and 5). In addition, more males than females were scored with heart fluid at slaughter age (P = 0.004).

Discussion

EST × Grow-Out Temperature

Except for TV weight at slaughter age, no interaction was found between EST and grow-out temperature. A higher mortality associated with ascites could be expected in the high EST treatment and cold rearing treatment because both factors might be predisposing factors for ascites. However, TV weights were comparable between EST treatments at the cold grow-out temperature and were lower at the high than the normal EST at the normal grow-out temperature.

Treatment	n[1]	FCR, 0 through 42 d	Total mortality (%)	Mortality accosiated with ascites (%)
EST				
Normal	24	1.91 ± 0.02	8.4 ± 1.28[b]	2.8 ± 0.65[b]
High	24	1.93 ± 0.02	12.5 ± 1.16[a]	6.6 ± 1.02[a]
Grow-out temperature				
Regular	24	1.86 ± 0.01[b]	8.1 ± 1.00[b]	2.8 ± 0.69[b]
Cold	24	1.98 ± 0.02[a]	12.8 ± 1.37[a]	6.6 ± 1.00[a]
		P-Value		
Source of variation[2]				
EST		0.37	0.008	0.001
Grow-out temperature		<0.001	0.02	0.004

[a,b] Means followed by different letters within a column and factor are significantly different (P ≤ 0.05)
[1] Pen was the experimental unit
[2] Interaction between EST and grow-out temperature was not significant (P > 0.05)

Table 3: *Feed conversion ratio (FCR), total mortality, and mortality associated with ascites of birds incubated at a normal (37.8°C) or a high (38.9°C) eggshell temperature (EST) from 7 through 21 d of incubation and grown at a regular or a cold temperature until 42 d of age*

Cold growout temperatures increase the relative heart weights of broiler chickens, which may have partly counteracted the differences in relative heart weights resulting from the high EST treatment and depressed the mortality related to ascites.

EST

Eggshell temperature did not affect hatchability in the current study, but a high EST increased the percentage of second-grade chickens by 0.7%. A higher percentage of second-grade chickens is consistent with the results of other studies regarding high incubation temperatures; second-grade chickens are often characterized by a small, pale appearance and a poor navel quality (Romanoff, 1936; Byerly, 1938; Thompson et al., 1976; Leksrisompong et al., 2007; Piestun et al., 2009a). Second-grade chickens are often culled in practice; the current results therefore show that a high EST can decrease the number of salable hatchlings. Other studies have shown that a high EST in the second half of incubation can increase embryonic mortality in the last week of incubation (Byerly, 1938; French, 1994; Lourens et al., 2005). A higher third week embryonic mortality can be related to an increased number of malpositioned embryos (Byerly, 1938; French, 1994), but no indications of this problem were found in the current study, and this phenomenon may occur only at a higher EST. The hatchling quality of first-grade chickens was reduced in the high compared with the normal EST treatment, expressed by a lower BW and YFBM and a poorer navel quality, which is consistent with the results of other studies (Romanoff, 1936; Lourens et al., 2005, 2007; Joseph et al., 2006; Leksrisompong et al., 2007; Piestun et al., 2009a). Furthermore, high EST decreased subsequent performance, expressed by a lower average BW during the grow-out period. In the current study, measurements at slaughter age evaluated a smaller number of birds (n = 160), but they showed the same trend. Relative breast meat yield was 1.0% greater in the high than in the normal EST treatment, and this may be related to increased muscle cell proliferation and accelerated differentiation after high EST incubation (Piestun et al., 2009b). Different studies have shown that BW up to 21 d were lower in chickens incubated at a high EST (38.9 to 39.5°C) compared with a normal EST (approximately 37.8°C) at the end of incubation (Lourens et al., 2005; Joseph et al., 2006; Leksrisompong et al., 2009). This difference disappeared at slaughter age in the study by Joseph et al. (2006). Hulet et al. (2007) found a lower BW, of 48.1 g, at 44 d of ge in birds incubated from d 16 of incubation onward at an EST of 39.7°C compared with an EST of 37.5°C. However, the same study showed that an EST of 38.6°C from d 16 of incubation onward increased BW, with 49.5 g, compared with the normal EST of 37.5°C. The differences between studies on the effect of high EST on subsequent performance might be explained by compensatory growth or the temperatures that the birds experienced during their grow-out period. Piestun et al. (2008) and Yalçin et al. (2010) have shown that a continuous high temperature during incubation can improve the thermotolerance of broiler chickens. When birds incubated at a high temperature experience relatively high temperatures during the grow-out period, they may have an improved ability to cope with these temperatures and are better able to maintain growth compared with birds incubated at a normal incubation temperature (Yalçin et al., 2010).

Treatment	n[1]	BW (g)	Carcass weight (g)	Breast meat yield[2] (%)	Abdominal fat (g)	Hematocrit (%)
EST						
Normal	80	2,953 ± 0.0	2,188 ± 20.9	29.7 ± 0.2[b]	39 ± 1.6	34 ± 0.5
High	80	2,909 ± 0.0	2,166 ± 18.0	30.7 ± 0.2[a]	39 ± 1.3	34 ± 0.6
Grow-out temperature						
Regular	80	2,962 ± 0.0	2,223 ± 19.4[a]	30.0 ± 0.2	42 ± 1.3[a]	32 ± 0.6[b]
Cold	80	2,899 ± 0.0	2,131 ± 19.2[b]	30.4 ± 0.2	36 ± 1.4[b]	35 ± 0.5[a]
Sex						
Male	80	3,161 ± 0.0[a]	2,333 ± 16.8[a]	30.1 ± 0.2	38 ± 1.34	33 ± 0.6[b]
Female	80	2,700 ± 0.0[b]	2,022 ± 12.9[b]	30.4 ± 0.2	40 ± 1.47	35 ± 0.6[a]
		P-Value				
Source of variation[3]						
EST		0.27	0.46	<0.001	0.75	0.67
Grow-out temperature		0.11	0.003	0.16	0.002	<0.001
Sex		<0.001	<0.001	0.36	0.24	0.002

[a,b] Means followed by different letters within a column and factor are significantly different (P ≤ 0.05)
[1] Bird was the experimental unit
[2] As a percentage of carcass weight
[3] Interactions between EST, grow-out temperature, and sex were not significant (P > 0.05)

Table 4: *Slaughter and ascites characteristics of male and female birds incubated at a normal (37.8°C) or a high (38.9°C) eggshell temperature (EST) from 7 through 21 d of incubation and grown at a regular or a cold temperature treatment until 42 d of age*

Treatment	n	Paricardial fluid[1]		Amdominal fluid[1]		RV[2] (g)	TV[3] (g)	RV:TV[4] (g)	Heart (% of BW)
		0 (%)	1 (%)	0 (%)	1 (%)				
EST									
Normal	80	34	66	98	2	1.8 ± 0.05	9.5 ± 0.17	19.2 ± 0.40[b]	0.32 ± 0.00
High	80	32	68	98	2	1.9 ± 0.06	9.3 ± 0.18	20.3 ± 0.43[a]	0.32 ± 0.01
Grow-out temperature									
Regular	80	39	61	96	4	1.8 ± 0.06	8.7 ± 0.16	20.7 ± 0.47[a]	0.29 ± 0.00[b]
Cold	80	27	73	100	0	1.9 ± 0.05	10.0 ± 0.16	18.9 ± 0.34[b]	0.35 ± 0.00[a]
Sex									
Male	80	22	78	98	2	2.1 ± 0.05[a]	10.2 ± 0.15[a]	20.4 ± 0.41[a]	0.32 ± 0.00
Female	80	44	56	99	1	1.6 ± 0.05[b]	8.5 ± 0.15[b]	19.1 ± 0.42[b]	0.32 ± 0.01
		P-Value							
Source of variation[5]									
EST		0.68		0.56		0.21	0.31	0.04	0.75
Grow-out temperature		0.07		0.95		0.20	<0.001	0.002	<0.001
Sex		0.004		0.56		<0.001	<0.001	0.02	0.17
EST x grow-out temperature		NS		NS		NS	0.02	NS	NS

[a,b] Means followed by different letters within a column and factor are significantly different (P ≤ 0.05)
[1] Percentage of chickens per treatment group that were scored 0 or 1, where 0 = no fluid, and 1 = fluid accumulation
[2] Right ventricle
[3] Total ventricle
[4] Ratio between right and total ventricle
[5] Interactions between EST x sex, grow-out temperature x sex, and EST x grow-out temperature x sex were nog significant (P > 0.05)

Table 5: *Ascites characteristics of male and female birds at slaughter age that had been incubated at a normal (37.8°C) or a high (38.9°C) eggshell temperature (EST) from 7 through 21 d of incubation and grown at a regular or a cold temperature until 42 d of age*

Feed conversion ratio was not affected by EST treatment in the current study. However, BW was greater in the normal EST treatments at 42 d of age, and this increased the FCR. When the FCR was adjusted to 2 kg of BW (−0.01 FCR with every 25 g above 2 kg of BW), total FCR was 1.59 for the high EST treatment and 1.55 for the normal EST treatment. The higher FCR may be related to the lower intestinal development and maturation at hatch that is found with incubation temperatures above 37°C (Wineland et al., 2006), which may negatively affect nutrient utilization in later life. Different factors are known to be involved in the development of ascites; examples include genetics, diet composition, and environmental conditions during the grow-out period (Lubritz and McPherson, 1994; Acar et al., 2001; Aftab and Khan, 2005; Druyan et al., 2008; Baghbanzadeh and Decuypere, 2008; Izadinia et al., 2010). A relationship between high incubation temperatures and ascites was never investigated (Leksrisompong et al., 2007). The current study showed that high EST can also be a predisposing factor for ascites. The difference that was found in total mortality between EST treatments was almost completely explained by the difference in mortality associated with ascites. The higher ascites incidence in the high EST treatment may be related to the decreased heart development at hatch, which decreased the ability to supply the body with O_2 for its respiratory demands, the first step in the development of ascites.

Dewil et al. (1996) found a significantly lower relative heart weight in embryos of a fast-growing line that is susceptible to ascites when compared with embryos from a slow-growing line that is resistant to ascites. This reduction in heart weight may be related to a line difference, or it could be caused by a difference in EST that the eggs of the different lines experienced during incubation. Egg weight was higher for the ascitessusceptible line than for the ascites-resistant line, and both lines were incubated together in the same incubator. Lourens et al. (2006) showed that heat production of larger eggs is higher than that of smaller eggs in the second half of incubation. Therefore, the larger eggs of the ascites-susceptible line may have had higher EST despite identical incubation temperatures. As shown in the current study, the heart weights at hatch were lower at a high EST when compared with a normal EST and may be a predisposing factor for ascites. The decrease in heart weight in the high EST compared with the normal EST was 26% in the current study; this value was within the range found by other studies (17 to 31%; Wineland et al., 2000; Leksrisompong et al., 2007; Lourens et al., 2007; Molenaar et al., 2009). This reduction in heart weight is suggested to be caused by a decrease in cell division at high temperatures during the second half of incubation (Romanoff, 1960; Leksrisompong et al., 2007).

Furthermore, a higher susceptibility for ascites after high EST incubation may be indicated by the higher RV:TV ratio at slaughter age, although the average RV:TV ratio was still within the normal range (Wideman, 2001). Five birds from the high EST treatment and 2 birds from the normal EST treatment had an RV:TV ratio that was greater than 27%, which is an indicator for ascites (Wideman, 2001); these birds may have died from ascites at a later time. No further indications were found at slaughter age for ascites susceptibility, such as a higher hematocrit value or a lower relative heart weight.

Grow-Out Temperature

A low grow-out temperature combined with a fast growth rate is one of the main triggers that induces ascites because of the higher

metabolic rate and the increased O_2 demand (Scheele et al., 1991; Acar et al., 2001; Wideman, 2001). The current study also showed that chickens in the cold grow-out treatment had a more than 2-fold increase in mortality associated with ascites as compared with the normal grow-out temperature. The birds tried to adapt to the low grow-out temperature and higher metabolic rate by increasing the hematocrit value and relative heart weight, which is consistent with the results of other studies (Shlosberg et al., 1992; Lubritz and McPherson, 1994; Buys et al., 1999; Blahová et al., 2007). However, the RV:TV ratio was lower for the cold compared with the regular growout temperature in the current study, and this is in contrast with the results of studies by Shlosberg et al. (1992) and Lubritz and McPherson (1994). Higher hematocrit values increase cardiac output and can eventually result in a higher RV:TV ratio. The reason that no higher RV:TV ratio was found for the cold grow-out temperature might be that these chickens were not yet developing ascites.

Further consequences of the cold grow-out temperature were that the birds showed decreased BW and carcass weights, consistent with other studies (Lubritz and McPherson, 1994; Buys et al., 1999; Pakdel et al., 2005). To maintain body temperature at the cold grow-out temperatures, the birds consumed more feed; this resulted in a higher FCR, as shown by Buys et al. (1999) and Akşit et al. (2008). The abdominal fat content was lower in birds from the cold than the normal grow-out temperature, likely because more energy was used for heat production and the maintenance of body temperature instead of for fat deposition (Yunianto et al., 1997; Blahová et al., 2007).

Different studies have shown that male birds are more susceptible than female birds to ascites (Bendheim et al., 1992; Balog, 2003; Arce-Menocal et al., 2009) because of their high metabolic rate and growth. Although the current study found that male birds had a lower hematocrit value than female birds, the higher RV:TV ratio found in male birds indicated that male birds were more susceptible to ascites than female birds.

In conclusion, a high EST reduced body development both at hatch and in the grow-out period and increased the incidence of mortality associated with ascites, which may be related to the decreased cardiovascular development that was found at hatch. A cold grow-out temperature reduced subsequent performance and was, together with high EST incubation, a predisposing factor for ascites development in broiler chickens.

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