Embryonic thermal manipulation and dietary fat source during acute heat stress: 1. effect on hatchability and broiler performance

K. E. Brannan,[*,†,1,2] K. A. Livingston,[‡] and C. Jansen van Rensburg[†]

[*]*Piedmont Research Station, Research Stations Division, North Carolina Department of Agriculture and Consumer Services, Salisbury, NC 28147, USA;* [†]*Department of Animal Science, University of Pretoria, Pretoria 0002, South Africa; and* [‡]*Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA*

Primary Audience: Hatchery Managers, Nutritionist, Researchers, Production Managers

SUMMARY

Modern broilers have been selected for rapid growth but demonstrate reduced heat tolerance toward market age. As the poultry industry expands globally, strategies must be developed to support broiler performance in challenging climates. The objective of this study was to evaluate the effect of embryonic thermal manipulation (**TM**) and dietary fat source during the finisher period on broiler performance during acute heat stress (**AHS**) close to market age. The cyclic exposure to high temperatures during mid-incubation used in TM has been demonstrated to improve broiler tolerance to heat stress. However, high incubation temperatures can be detrimental to embryonic development and impair posthatch broiler performance. Embryos were exposed to 39.5°C for 12 h daily from incubation day 7 to 16 to assess the impact of TM on hatching and broiler performance. Dietary fat is commonly added to poultry diets during heat stress and it was theorized that differences in fat source may further impact bird performance. Finisher diets were supplemented with soya oil, poultry fat, or olive oil at 4.5% each. Broilers were exposed to a period of AHS at 43 d. Embryo mortality was increased, and hatchability was reduced by TM. Broiler performance was also decreased for the TM birds, but mortality during AHS was markedly reduced. Dietary fat source did not influence bird performance but was shown to interact with incubation treatment. Overall, the present data suggest optimal performance in modern broiler strains may be at odds with improved heat tolerance.

Key words: thermal manipulation, incubation, fat source, acute heat stress, broiler production

2021 J. Appl. Poult. Res. 30:100143
https://doi.org/10.1016/j.japr.2021.100143

DESCRIPTION OF PROBLEM

Broiler chickens have been genetically selected over the past several decades for rapid growth, improved feed efficiency, and increased body weight (**BW**); however, these advances have not been matched with similar developments in the birds' physiological support systems (Havenstein et al., 2003a,b). Increased BW has resulted in a greater body mass relative to body surface area, concurrent with a reduction in the capacity for sensible heat loss and a higher basal body temperature (Yalçin et al.,

[1]Present address: Faculty of Natural and Agricultural Sciences, Department of Animal Science, University of Pretoria, Private Bag X20, Hatfield, GP, 0028, South Africa.
[2]Corresponding author: kellyebrannan@gmail.com

2001; Yahav et al., 2005). Subsequently the risk for heat stress increases with bird BW, as does the potential for economic loss associated with heat stress mortality (St-Pierre et al., 2003). As poultry production continues to expand within the tropical regions of the globe, management programs must adapt to minimize the negative impact of heat stress on broiler performance (Daghir, 2008).

Controlled exposure to high temperatures during embryonic development has been demonstrated to decrease posthatch body temperature in broilers and improve bird thermotolerance to heat stress (Yahav et al., 2004; Collin et al., 2005; Piestun et al., 2008, 2011; Loyau et al., 2013; Al-Rukibat et al., 2017). Although the precise mechanisms behind this adaptation are unclear, it has been proposed that exposure to high temperatures during critical developmental periods may adjust the "set points" of the physiological systems involved in thermoregulation (Nichelmann and Tzschentke, 2002). The process has been termed thermal manipulation (**TM**) and relies on 3 main criteria for its success: embryonic developmental stage at application, severity, and duration (Yahav, 2009).

Although full homeothermy in chickens is only achieved 10 d after hatch, the ontogeny of the neural and endocrine pathways regulating body temperature begin during incubation (Nichelmann and Tzschentke, 2002). Around incubation day (**E**) 15, the hypothalamo-pituitary-thyroid and -adrenocortical axes (**HPT** and **HPA**, respectively) become functional and the embryo begins to respond to environmental temperatures through changes in metabolic rate induced by thyroid activity (Jenkins and Porter, 2004; McNabb, 2007). The HPT axis primarily regulates metabolism and body temperature through circulating thyroid hormones, whereas the HPA axis manages stress response; however, the 2 systems share a strong interrelationship that is already apparent during the embryonic stage. The HPA axis responds to stress with increased corticotrophin-releasing hormone secretion that can interact with both thyrotrophic and corticotropic cells at the pituitary level (Debonne et al., 2008). Consequently, a stress response can result in thyroid-stimulating hormone production, which then acts on the thyroid gland to increase thyroid hormone and influence body temperature (Geris et al., 1996). If the response threshold for both axes can be altered through TM, the potential exists to reduce bird body temperature and stress response so that the bird is more tolerant to high ambient temperatures. Therefore, the second week of incubation has been suggested as the optimal period for TM application to benefit from the plasticity of the HPT and HPA axes during embryonic development (Moraes et al., 2004).

Extreme deviations from the optimal incubation temperature of 37.8°C are known to result in decreased hatchability and chick quality (Wilson, 1991). However, experiments with TM utilizing increased temperatures have resulted in improve thermotolerance without losses in hatchability or chick quality when compared with standard incubation temperatures (Yahav et al., 2004; Collin et al., 2005). Although constant exposure to temperatures that exceed the ideal range for normal embryonic development can be detrimental to chick quality and broiler performance, TM exposure is cyclic which may reduce these negative effects (Piestun et al., 2008). Although variations in TM protocol are widespread throughout the literature, exposure to 39.5°C for 12 h daily from E 7 to E 16 appears to consistently yield the greatest improvements in posthatch thermotolerance without sacrificing hatchability, chick quality, or bird performance (Collin et al., 2005; Piestun et al., 2008, 2011). Yet even within the same TM protocol, variations in hatchability and chick quality occur (Yahav et al., 2004; Piestun et al., 2008, 2009; Zaboli et al., 2017) and further work is needed to determine how TM alters embryo development.

Birds with decreased thermotolerance reduce their feed intake during heat stress to limit dietary-induced thermogenesis. Although decreased feed consumption reduces the birds' metabolic heat production (Cooper and Washburn, 1998), inadequate energy intake coupled with elevated energy demands as the bird struggles to maintain body homeostasis depresses growth (Hurwitz et al., 1980). One strategy to maintain energy intake when feed consumption is depressed during heat stress is to supplement the diet with fat, which provides

increased energy density at a lower heat increment when compared with carbohydrates or proteins (Dale and Fuller, 1979; Tetter and Belay, 1996).

Concurrent with increased dietary energy density, differences in dietary fatty acid profile between fat sources may also contribute to improving broiler performance during high ambient temperatures. Under normal rearing temperatures, fatty acid profile has been shown to affect broiler BW and feed efficiency (Pinchasov and Nir, 1992; Zollitsch et al., 1997; Crespo and Esteve-Garcia, 2002). It has been suggested that unsaturated fatty acids (**USFA**) are more readily utilized by the bird when compared to saturated fatty acids (**SFA**) (Sanz et al., 2000a; Ferrini et al., 2010), leading to improvements in broiler performance. The high polarity of USFA compared to SFA allows for improved micelle formation and absorption (Garrett and Young, 1975), thereby increasing substrate availability for β-oxidation (Baião and Lara, 2005). Broilers fed a diet high in USFA have demonstrated an increase in β-oxidation (Sanz, 1999; Ferrini et al., 2010), which may significantly increase the energy availability during heat stress despite decreased feed intake. In birds approaching market age, heat stress susceptibility is increased along with the risk of economic loss due to mortality (Teeter and Belay, 1996; St-Pierre et al., 2003; Yahav et al., 2005). As energy requirements increase with bird age and BW (Sakomura et al., 2004) the benefits of dietary USFA may be greater for birds exposed to high environmental temperatures during the finishing stage of broiler rearing. Other researchers have demonstrated improvements in broiler performance associated with different fat sources during the finisher stage (Pinchasov and Nir, 1992; Sanz, 1999; Crespo and Esteve-Garcia, 2001), but further investigation is needed to determine if these benefits remain during heat challenge.

The objective of this trial was to evaluate the effect of TM during incubation on hatching and broiler performance during acute heat stress (**AHS**) close to market age. Dietary fat source and interaction with TM during the finisher period was also evaluated as a method of sustaining broiler performance under elevated environmental temperatures.

MATERIALS AND METHODS

Incubation and rearing for this experiment occurred at the North Carolina Department of Agriculture's Piedmont Research Station, in collaboration with North Carolina State University. All procedures carried out in this trial were reviewed and approved by the Institutional Animal Care and Use Committee at North Carolina State University.

Incubation Treatments

Hatching eggs were obtained from a 43-wk-old Ross 708 broiler breeder flock (Aviagen Inc., Huntsville, AL) with an average egg weight of 68.47 ± 1.04 g. Before incubation, eggs were stored at 18°C and 75% relative humidity (**RH**) for 2 to 5 d. Eggs were equally divided between the 2 incubation treatments, with a total of 1,740 eggs across 12 replicate trays per treatment (3,480 eggs total). Eggs were preincubated for 12 h in a forced ventilation cabinet at 26°C before being placed into a Natureform incubator (I14, Natureform Inc, Jacksonville, FL). Egg trays were arranged in a generalized randomized complete block design with 3 trays per treatment in each block (4 blocks total) to reduce any environmental effect of tray position within the incubator. All eggs were initially placed in the control (**CN**) machine set at 37.5°C and 56% RH until E 7 when the TM treatment began. Machine air temperatures and relative humidity were recorded 3 times daily.

On E 7, trays designated as TM were moved to a second machine set at 39.5°C and 65% RH for 12 h, whereas the CN trays remained in the initial incubator at 37.5°C and 56% RH. Relative humidity was increased in the TM setter to prevent excessive moisture loss from the eggs during exposure to high temperatures. The TM setter was adjacent to the CN setter to reduce transport stress when moving eggs. A plastic tent was erected over both machines and heated to match the set point of the CN machine during transfer to reduce heat loss from the eggs as they were moved between setters. After TM exposure, eggs were returned to the initial CN setter

Table 1. Composition of the starter, grower, and base finisher diet.

Item	Starter	Grower	Finisher
Ingredients (%)			
Corn	49.93	58.89	62.65
Soybean meal, 48%	34.65	26.50	21.57
Distillers dried grains with solubles	7.50	8.00	8.00
Fat[1]	3.50	3.43	4.50
Salt	0.29	0.26	0.24
Limestone	1.14	1.20	1.17
Dicalcium phosphate, 18.5%	1.54	0.85	0.46
DL-methionine, 99%	0.32	0.23	0.25
L-lysine-HCl, 78.8%	0.27	0.21	0.29
Choline chloride, 60%	0.18	0.18	0.18
Sodium bicarbonate	0.27	0.18	0.21
L-threonine, 98%	0.05	0.02	0.07
Coccidiostat[2]	0.05	0.05	0.05
Mineral premix[3]	0.23	0.20	0.20
Vitamin premix[4]	0.10	0.10	0.10
Phytase[5]	0.01	0.01	0.01
Calculated nutrient content			
Metabolizable energy, kcal/kg	2,950	3,050	*
Crude protein, %	22.00	18.95	17.11
Calcium, %	1.05	0.85	0.76
Available phosphorus, %	0.45	0.40	0.37
Digestible lysine, %	1.22	1.05	0.93
Sodium,%	0.24	0.23	0.19
Chloride, %	0.29	0.31	0.26

[1]Starter and grower diets included only poultry fat. Finisher diets included poultry fat (PF), soybean oil (SO), or olive oil (OO) at 4.5%.

[2]Coban 90 (Monesin) (Elanco Animal Health, Greenfield, IN) at 90 g/ton of feed.

[3]Trace minerals provided per kg of premix: manganese (MnO_2), 220 g; zinc (ZnO and $ZnSO_4$), 250 g; iron ($FeCO_3$), 75 g; copper (CuSO4 and $CuCl_2$), 10 g; iodine ($Ca(IO_3)_2$), 5 g; selenium (Na_2SeO_3), 1 g.

[4]Vitamins provided per kg of premix: vitamin A, 18,739,292 IU; vitamin D3, 6,613,868 IU; vitamin E, 66,139 IU; vitamin B12, 33 mg; riboflavin, 22,046 mg; niacin, 88,185 mg; d-pantothenic acid, 30,865 mg; menadione, 3,968 mg; folic acid, 2,646 mg; vitamin B6, 7,716 mg; thiamine, 5,512 mg; biotin, 176 mg.

[5]Quantum Blue 5G 5 at 0.20 lbs/ton (100 g/ton) to provide 500 FYT (AB Vista, Marlborough, UK) delivering 0.13% of available P, 0.06% of calcium and 0.03% of sodium.

*The Wiseman equation (Wiseman et al., 1998; Wiseman and Salvador, 1989) was used to calculate the energy value for each fat and final dietary ME was 3,180 kcal/kg (PF), 3,203 kcal/kg (SO), and 3,208 kcal/kg (OO).

for 12 h. Transfer between the machines occurred at the same time each day at 12 h intervals. Eggshell temperatures were measured from 5 eggs at different locations within each tray (for a total of 60 per treatment) twice a day, before and at the conclusion of TM exposure. Eggs were numbered so that the same eggs were monitored throughout incubation. Eggshell temperatures were measured at the equator of each egg using a Braun ThermoScan thermometer (IRT 4520, Thermoscan, Kronberg, Germany) that was allowed to equilibrate inside the CN setter for 10 min before measurements. The TM exposure continued daily until E16, at which point all eggs were incubated in the same machine at 37.5°C until transfer to the hatcher. At E 17.5, setter trays were transferred to their analogous hatching baskets, which were considered the replicate unit for hatching data. Egg trays were weighed before hatching and again at transfer to calculate moisture loss. Unhatched eggs were evaluated by experienced technicians to determine embryo stage at death. Age at embryo mortality was defined as early (E0–E7), middle (E8–E14), or late (E15–E21) and eggs that showed no indication of fertilization or development were categorized as infertile. Total hatchability and hatch of fertile

(**HOF**) eggs were calculated. Hatchability was defined as percentage of chicks per total eggs set, whereas HOF used the same formula but excluded eggs identified as infertile during breakout.

Good-quality chicks were sorted into groups by sex and average weight of incubation treatment before placement in rearing pens. For each treatment, 24 female and 24 male chicks were selected for sampling to evaluate the effect of incubation treatment on chick development. Body weight and yolk weight were measured from the sample chicks. Yolk-free BW and yolk weight relative to chick BW were calculated from these values.

Bird Rearing and Dietary Treatments

Chicks were placed in a 60 pen environmentally controlled broiler house, with pens assigned in a randomized complete block design with 6 treatments (2 incubation profiles × 3 finisher diets) in 10 blocks. Each pen (1.2 × 1.5 m) contained one tube feeder and 5 drinking nipples, with a supplemental feed pan and chick water font included during the first week of rearing. An equal number of chicks from both sexes were placed in each pen, for a total of 18 chicks per pen. Birds were reared under standard commercial lighting and temperature profiles (Aviagen, 2018) until the heat stress period at 43 d. Temperatures with in the house were gradually increased from 21°C to 32°C for 4 h at 43 d. Standard temperature profiles were implemented after the heat stress period until the end of the study at 49 d.

Birds were fed a standard commercial starter and grower diet until day 14 and 28, respectively. At 28 d, birds were presented one of 3 treatment finisher diets that differed in fat source. Treatment diets consisted of a monounsaturated fatty acid source (olive oil, **OO**), a polyunsaturated fatty acid source (soya oil, **SO**), and a saturated fatty acid source (poultry fat, **PF**). Fat sources were analyzed for free fatty acids (method Ca 5a-40, AOCS, 2017) as well as SFA and USFA concentrations (method 996.06, AOAC, 2012) and energy values were determined for each using the Wiseman equation for birds older than 21 d (Wiseman et al., 1998; Wiseman and Salvador, 1989). Diets were formulated based on the energy contribution of PF (35.45 MJ/kg), SO (37.63 MJ/kg), and OO (38.07 MJ/kg) at an inclusion level of 4.5%, with final dietary ME being 3,180 Kcal for the PF diet, 3,203 for the SO diet, and 3,208 for the OO diet (Table 1). All diets were formulated to meet or exceed NRC (1994) requirements. The fatty acid profiles and nutritional parameters of each fat source are shown in Table 2. All diets were manufactured at the North Carolina State University Feed Mill.

Feed intake (**FI**) and BW were recorded weekly for each pen, which served as the experimental unit for the rearing data. Mortalities were recorded daily and weighed on removal. Feed conversion ratio (**FCR**) adjusted for mortalities, was calculated at weekly intervals throughout rearing.

Statistical Analysis

Differences between eggshell temperatures and machine temperatures within incubation treatment were analyzed as nonparametric data using the Wilcoxon signed rank test, with egg tray serving as the experimental unit. Embryo, chick, and hatchability data were subjected to Shapiro-Wilk test to assess normality and Levene test to verify the equality of variances. Incubation data collected at hatch were analyzed by Welch's T-test using the Fit Y by X platform of JMP Pro 13 (SAS Institute, Cary, NC). Hatch basket treatment allocation was the same as egg tray and used as the experimental unit for all variables analyzed on day of hatch.

Rearing data (BW, FI, and FCR) before the addition of dietary treatment were analyzed as a one-way ANOVA using the fit model platform of JMP Pro 13 with incubation treatment (TM and CN) as the fixed effect. After the implementation of the dietary treatments (at 28 d), rearing data were analyzed as a 2 × 3 factorial (incubation × diet) using the mixed procedure of JMP. Incubation and dietary treatment were considered independent variables. Mortality data were not normally distributed and as such, transformed using Box-Cox transformation. Data were analyzed as means refit using best λ and untransformed means are presented. Differences between

Table 2. Fatty acid profile and nutritional parameters of the dietary fat sources[1].

Fatty acid profile	Poultry fat	Soya oil	Olive oil
Fatty acid (g/100g)			
C16:0	23.35	11.58	10.90
C16:1	6.17	0.18	0.17
C18:0	7.76	5.85	4.24
C18:1	40.80	22.78	29.07
C18:2	19.61	51.34	47.82
C18:3	0.81	7.11	7.03
Omega fatty acids			
Omega-3	1.16	7.11	7.03
Omega-6	19.61	51.34	47.82
Omega-9	41.08	23.00	29.22
Nutritional parameters (%)			
Total saturated fatty acids	31.86	18.37	15.67
Total unsaturated fatty acids	68.14	81.62	84.24
Unsaturated/saturated ratio	2.14	4.44	5.38
Total monounsaturated fatty acids	47.37	23.17	29.39
Total polyunsaturated fatty acids	20.77	58.45	54.85
Free fatty acids	5.46	0.55	0.77

[1]Each fat source included at 4.5% to the base finisher diet.

means were separated using Tukey's HSD test (Tukey, 1949) and significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Incubation and Hatch

Eggshell temperature (**EST**) and machine temperature from E7 to E16.5 for the TM and CN groups in the current trial are shown in Figures 1A and 1B, respectively. Initial EST was lower than the machine temperature for both groups and gradually increased with embryo age ($P < 0.05$). Although the difference between EST and machine temperature continued to increase while TM eggs were in the CN machine ($P < 0.05$), EST was largely similar to machine temperature in the TM setter from E11.5 until E16.5. Conversely, EST in the CN group continued to increase with embryonic age ($P < 0.01$). As the thermoregulatory capacity of the developing embryo is limited, embryo metabolism is contingent upon incubation temperature (Nichelmann and Tzschentke, 2002). Initial embryo metabolism and EST are strongly associated with machine air temperature (Lourens, 2005); however, with the emergence of the HPT and HPA axes mid-way through incubation (Jenkins and Porter, 2004; McNabb, 2007), increased heat production by the embryo contributes to the temperature experienced by the egg (Lourens et al., 2005). In the present results this can be observed in the eggshell temperatures for both incubation treatments, which began to exceed machine temperature set points at approximately E11. Eggshell temperature is used as a measurement of the temperature experienced by the embryo and is linearly related to embryonic metabolism during the latter half of incubation (Lourens et al., 2006). In the TM group, the EST reported during TM exposure remained equal to machine temperature past E11 but was shown to increase when eggs were returned to the CN machine. As embryonic metabolism is tied to heat production (Nichelmann and Tzschentke, 2002) and subsequent EST, the plateau observed for the TM group suggests that embryo growth was limited for the 12 h of TM exposure. The steady increase in EST for the TM group in the CN machine may indicate embryo recovery, but when compared with the progressive increase in EST shown by the CN group, it suggests that embryonic growth was dampened during TM exposure.

The increase in embryonic mortality and decreased hatchability noted for the TM group appear to support these findings. Embryo mortality, hatchability, and hatch of fertile eggs as influenced by incubation treatment can be seen

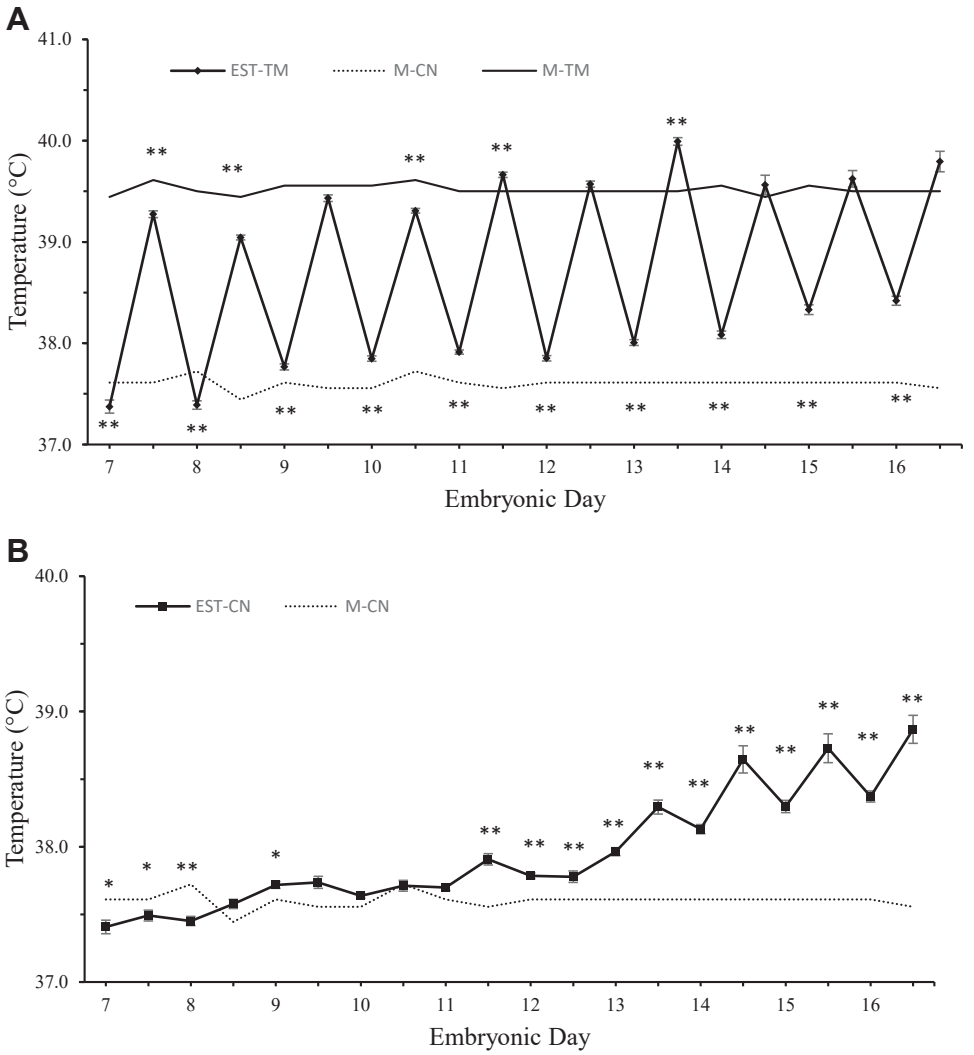

Figure 1. Eggshell temperature (EST) and machine air temperature (M) during incubation from embryonic day 7 to 16.5 in either (A) the thermal manipulation treatment (TM) that alternated between the control machine and a treatment machine set at 39.5°C and 65% RH in 12 h cycles or (B) the control group (CN) with a machine temperature of 37.5°C and 56% RH. Values are presented as means and standard error of means. * Means differ significantly ($P < 0.05$). ** Means differ significantly ($P < 0.01$).

in Table 3. Mid and late embryonic mortality were increased by TM ($P = 0.027$ and $P < 0.001$, respectively), developmental periods that coincide with the plateau in EST for the TM group during high temperature exposure. Consequently, hatchability and HOF were decreased in the TM group ($P < 0.001$). The effect of TM on hatchability in the literature has been shown to vary widely, with hatchability after TM exposure being increased (Yahav et al., 2004; Collin et al., 2005), decreased (Moraes et al., 2004; Yahav et al., 2004; Collin et al., 2007; Piestun et al., 2013), or similar (Yahav et al., 2004; Piestun et al., 2008) to standard incubation temperature profiles. Differences in genetic strain (Hamidu et al., 2018), flock age (Hamidu et al., 2007) and incubation design (French, 1997) can contribute to embryo heat production and EST, such that the same machine temperature profile may produce different results depending on the influence of these factors. These variations may explain the

Table 3. The effect of incubation treatment on embryo mortality, hatchability, and hatch of fertile eggs (%).

	Embryo mortality			Hatchability		
Incubation[1]	Early (E0–7)	Mid (E8–14)	Late (E15–E21)	Infertile	Hatch	Hatch of fertile
CN	4.3	0.1[b]	2.4[B]	7.9	89.5[A]	94.9[A]
TM	6.0	0.8[a]	17.3[A]	9.0	77.2[B]	82.2[B]
SEM	0.78	0.21	1.49	1.02	0.65	0.55
P-Value	0.148	0.027	<0.001	0.448	<0.001	<0.001

[A,B]Means in a column that possess different superscripts differ significantly ($P < 0.01$).

[a,b]Means in a column that possess different superscripts differ significantly ($P < 0.05$).

[1]TM = thermal manipulation at 39.5°C and 65% RH for 12 h from E7 to E16; CN = control remained at 37.5°C and 56% RH.

differences observed in the literature, with the effect of TM on hatchability fluctuating depending on how severely machine temperature impacted embryo development. Despite the increase in embryonic mortality and decrease in hatchability observed in the current results, chick BW and yolk utilization did not appear to be influenced by incubation treatment (Table 4).

Broiler Performance

The effects of TM on BWG, FCR, and FI during the starter (0–14 d) and grower (15–28 d) periods, before the addition of dietary treatments, are shown in Table 5. Feed intake was lower in the TM group during the starter period ($P < 0.001$), as was BWG ($P < 0.001$) and both remained decreased for the TM birds until AHS at 43 d ($P < 0.05$ for both). Although the TM birds did demonstrate improved feed efficiency during the first 2 wk of rearing ($P = 0.014$), no further differences in FCR were observed for the main effect of incubation. As incubation temperature and hatchability are strongly associated with posthatch performance (Joseph et al., 2006; Hulet et al., 2007; Yalçin et al., 2010), the influence of TM on broiler BW during rearing is also shown to vary widely within the literature. In market age TM birds, BW is typically equal (Collin et al., 2007; Piestun et al., 2008, 2013; Tona et al., 2008; Werner et al., 2010; Zaboli et al., 2017) or lower (Yalçin et al., 2008, 2010; Molenaar et al., 2011; Loyau et al., 2013; Piestun et al., 2013) in comparison with CN birds, although increased BW has been observed (Piestun et al., 2009; Al-Rukibat et al., 2017). Given the diversity of trial conditions, TM protocols, and responses presented in the literature, direct comparison of the current trial results is difficult. Despite the differences within the literature, the common objective throughout these trials is the use of TM to improve broiler tolerance to heat stress through exposure to increased incubation temperatures; however, this goal may not align with optimal broiler performance under standard rearing conditions. Ideal incubation temperature exists within the narrow range of 37°C to 38°C (Wilson, 1991) and extreme deviations outside this range are well established to be detrimental to embryonic development and subsequent broiler performance (Hulet et al., 2007; Meijerhof, 2009). The results presented here suggest that despite its cyclic exposure, the increased EST arising from TM exposure can be sufficient to negatively influence embryonic mortality, hatchability, and broiler performance up to market age.

The influence of dietary fat source in addition to incubation treatment, as well as their interactions, on BWG, FCR, and FI during the finisher period (29–42 d) is also shown in Table 5. Dietary treatments were introduced during the finisher period and continued until the end of the trial (49 d); however, these treatments were not shown to significantly influence BWG, FCR, or FI. While other trials have observed similar results (Andreotti et al., 2001; Potença et al., 2008), the findings presented here contrast with several other studies demonstrating improvements in broiler performance associated with increased dietary USFA supplementation (Pinchasov and Nir, 1992; Zollitsch et al., 1997; Sanz, 1999, 2000b; Crespo and Esteve-Garcia, 2002; Lopez-Ferrer et al., 2001; Newman et al., 2002). As the dietary treatments in the current trial focused only on the finisher period, it may be speculated that

Table 4. The effect of incubation treatment on chick body weight (BW), yolk weight, yolk-free BW, and yolk relative to BW.

Incubation[1]	Chick body weight, g	Yolk weight, g	Yolk-free body weight, g	Relative yolk weight, %
CN	46.8	4.1	42.8	8.7
TM	46.9	4.4	42.5	9.5
SEM	0.3	0.3	0.4	0.6
P-Value	0.922	0.376	0.543	0.382

[1]TM = thermal manipulation at 39.5°C and 65% RH for 12 h from E7 to E16; CN = control remained at 37.5°C and 56% RH.

broiler age offset differences in fat digestibility between the treatments. Differences in absorption and utilization between dietary fatty acid profiles are most evident in newly hatched chicks, in which overall lipid digestion is limited due to lower bile salt and lipase secretion (Noy and Sklan, 1995). However, while fat digestibility rapidly increases with bird age, differences in available metabolizable energy remain apparent between USFA and SFA in older birds (Wiseman and Salvador, 1991; Tancharoenrat et al., 2013).

The finisher period was chosen to evaluate fat sources in the present study due to the increased susceptibility to heat stress, mortality, and economic loss associated with older broilers exposed to AHS (Yalçin et al., 2001; St-Pierre et al., 2003). Other studies have demonstrated differences in broiler performance between dietary fat sources when treatments were applied to birds of 3 or more weeks of age (Pinchasov and Nir, 1992; Sanz, 1999; Sanz et al., 2000b; Crespo and Esteve-Garcia, 2002; Newman et al., 2002); however, these trials also utilized higher inclusion levels of the fat treatments in comparison with the present study. De Witt et al. (2009) reported a significant interaction between fat source and inclusion level, with performance differences between fatty acid composition being similar at lower levels (3%) but improvements being noted for USFA at higher levels (6%). While the inclusion level of the current trial (4.5%) was chosen to be commercially relevant, it may not have been sufficient to elicit differences in performance between the treatments.

Cumulative mortality (0–42 d) as affected by incubation treatment, dietary fat source, and their interactions was not shown to differ significantly prior to heat challenge (Table 5). Mortality within the treatment and interaction groups ranged from 1.9 to 5.0%, which was comparable with the current industry averages (NCC, 2019).

Acute Heat Stress Challenge

Despite the decrease in hatchability and broiler performance, mortality after AHS was dramatically reduced in TM birds (Table 6). Although these results may be due to the decreased BW exhibited by the TM birds, it may also suggest an improvement in heat tolerance. The improved livability during AHS shown here agrees with other works that observed decreased mortality during heat challenge for TM birds (Piestun et al., 2008; Zaboli et al., 2017) and may be related to improved thermal tolerance. Mortality associated with heat stress in market age broilers results in economic loss for the producer, as rearing costs expected to be recovered at processing are lost. As BWG was similar between incubation treatments after AHS (P = 0.256), the decrease in mortality demonstrated by the TM birds may be of greater benefit to producers and compensate for their reduced growth prior to heat stress. However, the decrease in hatchability observed with TM must also be considered. Increased placement of parent stock to account for hatchery losses may not be economically viable when compared with posthatch production losses. The present data suggest that while TM may decrease the cost of mortality at the producer level, hatcheries may experience economic loss due to decreased hatchability with TM programs. Additional financial analysis is needed to determine which is more costly, especially in settings where heat stress is unavoidable due to season or latitude.

Although interactions between the main effects of incubation and dietary treatments were not apparent before heat stress, BWG

Table 5. Average body weight gain (BWG), feed conversion ratio (FCR), feed intake (FI), and cumulative mortality[1] (0–42 d) as influenced by incubation treatment and dietary fat source during the finisher period, as well as their interactions.

	0–14 d			15–28 d			29–42 d			0–42 d
Treatment	BWG, g/bird	FCR, g:g	FI, g/bird	BWG, g/bird	FCR, g:g	FI, g/bird	BWG, g/bird	FCR, g:g	FI, g/bird	Mortality[5], %
Incubation[2]										
CN	513.2^{A}	1.21^{a}	619.1^{A}	1067.4^{a}	1.47	1567.7^{A}	1213.9^{A}	1.79	2058.5^{A}	3.5
TM	500.0^{B}	1.18^{b}	595.8^{B}	1037.1^{b}	1.47	1519.0^{B}	1154.5^{B}	1.71	1974.6^{B}	4.2
SEM[3]	2.4	0.01	2.5	8.5	0.01	10.4	12.2	0.01	18.0	1.0
P-Value	<0.001	0.014	<0.001	0.015	0.659	0.002	0.001	0.292	0.002	0.358
Diet[4]										
Olive							1186.4	1.71	2028.0	2.8
Poultry							1175.7	1.71	2016.3	3.6
Soya							1190.4	1.68	2005.3	5.0
SEM							15.0	0.01	22.0	1.2
P-Value							0.774	0.189	0.765	0.650
Incubation x diet										
CN-olive							1213.7	1.70	2057.0	1.9
CN-poultry							1204.6	1.70	2045.1	3.8
CN-soya							1223.4	1.69	2073.5	5.0
TM-olive							1159.1	1.72	1999.1	3.8
TM-poultry							1146.8	1.73	1987.4	3.8
TM-soya							1157.5	1.68	1937.2	5.0
SEM							21.2	0.02	31.1	1.8
P-Value							0.962	0.287	0.353	0.979

[A,B]Means in a column that possess different superscripts differ significantly ($P < 0.01$).

[a,b]Means in a column that possess different superscripts differ significantly ($P < 0.05$).

[1]Before acute heat stress.

[2]TM = thermal manipulation at 39.5°C and 65% RH for 12 h from E7 to E16; CN = control remained at 37.5°C and 56% RH.

[3]SEM = standard error of mean.

[4]Poultry = poultry fat; soya = soya oil; olive = olive oil added at 4.5% to finisher diet.

[5]Data were subjected to Box-Cox transformation ($\lambda = -0.440$). Values are presented as untransformed means and standard error of means.

($P = 0.054$) and FCR ($P = 0.005$) were shown to be affected by treatment interactions after AHS (Table 6). The most striking disparity occurred between fat sources within the CN group, which exhibited the best FCR and BWG (CN-PF birds) as well as the worst (CN-OO), while these values were similar between fat sources for the TM birds. The differences in FCR between incubation treatments on the same diets may suggest an altered nutrient metabolism arising from TM adaptation, particularly as this difference was only apparent after heat challenge. The hypothesis behind TM adaptation is that increased temperatures during incubation can lower the HPT and HPA set points of the embryo (Nichelmann and Tzschentke, 2002) in preparation for posthatch heat exposure. Birds exposed to TM during incubation consistently demonstrate a reduced body temperature, thought to arise from lower circulating triiodothyronine (Yahav et al., 2004; Collin et al., 2005; Piestun et al., 2008; Tona et al., 2008; Loyau et al., 2013; Al-Rukibat et al., 2017) and reduced obligatory heat production through decreased basal metabolic rate (Tona et al., 2008; Piestun et al., 2008). The decrease in basal metabolic rate supporting these adaptations appears to arise from a decrease in the expression of enzymes relevant to nutrient digestion (Al-Zghoul et al., 2019) and mitochondrial metabolism (Loyau et al., 2014, 2016) in TM birds under standard temperatures. It may be speculated that this reduction in nutrient digestion and metabolism could have resulted in the different fat sources being similar in terms of FCR and BWG within the TM group under heat stress but contributed to the poor performance noted for the TM birds before AHS. Further work is needed to understand the physiological modifications associated

Table 6. Average body weight gain (BWG), feed conversion ratio (FCR), feed intake (FI), and mortality as influenced by incubation treatment and dietary fat source, as well as their interactions following acute heat stress challenge occurred at 43 d[1].

	43–49 d			
Treatment	BWG, g/bird	FCR, g:g	FI, g/bird	Mortality[5], %
Incubation[2]				
CN	669.9	2.09	1405.5^{A}	18.5^{A}
TM	652.0	2.04	1329.9^{B}	9.3^{B}
SEM[3]	11.0	0.03	16.0	2.2
P-Value	0.256	0.227	0.001	0.006
Diet[4]				
Olive	637.1	2.11	1357.9	17.2
Poultry	680.2	2.04	1381.6	12.4
Soya	665.5	2.06	1363.6	12.1
SEM	13.4	0.03	19.3	2.7
P-Value	0.081	0.196	0.670	0.309
Incubation x diet				
CN-olive	627.6	2.18^{A}	1396.8	23.3
CN-poultry	716.0	1.97^{B}	1411.1	14.6
CN-soya	665.9	2.12^{AB}	1408.5	17.3
TM-olive	646.6	2.05^{AB}	1319.0	10.7
TM-poultry	644.4	2.10^{AB}	1352.2	10.3
TM-soya	665.1	1.99^{B}	1318.6	7.0
SEM	18.8	0.04	27.3	3.9
P-Value	0.054	0.005	0.850	0.518

^{A,B}Means in a column that possess different superscripts differ significantly ($P < 0.01$).

^{a,b}Means in a column that possess different superscripts differ significantly ($P < 0.05$).

[1]Acute heat stress occurred at 32°C for 4 h at 43 d.

[2]TM = thermal manipulation at 39.5°C and 65% RH for 12 h from E7 to E16; CN = control remained at 37.5°C and 56% RH.

[3]SEM = standard error of mean.

[4]Poultry = poultry fat; soya = soya oil; olive = olive oil added at 4.5% to finisher diet.

[5]Data were subjected to Box-Cox transformation ($\lambda = 0.412$). Values are presented as untransformed means and standard error of means.

with TM and how to best manage these birds during heat stress as well as under standard temperatures so that production losses are minimized.

The improvement in FCR for the CN-PF birds compared with the CN-OO group was unexpected, as performance between these interactions was similar before AHS. However, the difference in feed efficiency observed in the current results may not be due to the nutritional effects of the dietary fat treatments but their influence on mitochondrial metabolism. Avian uncoupling protein (**avUCP**) is a transporter protein found within the inner membrane of the mitochondria, so named as it uncouples cellular respiration from ATP synthesis during oxidative phosphorylation (Raimbault et al., 2001; Dridi et al., 2004). Although the function of avUCP remains unclear, it has been proposed to be involved in the modulation of reactive oxygen species (**ROS**). Increased ROS production is associated with AHS, possibly through the downregulation of avUCP (Mujahid et al., 2006). However, olive oil has been shown to increase avUCP mRNA expression during heat stress (Seifi et al., 2018) and may attenuate excessive ROS production (Mujahid et al., 2009). Conversely, SFA appear to downregulate avUCP mRNA expression (Seifi et al., 2018), possibly due to a decrease in triiodothyronine noted in birds fed diets rich in SFA (Ferrini et al., 2010) as avUCP shares a strong association with the thyroid hormone (Collin et al., 2003). Although avUCP may reduce ROS production, the uncoupling of oxidative phosphorylation results in reduced ATP production as ATP synthase is bypassed and energy production is decreased (Dridi et al., 2004). Therefore, it may be theorized that feed

efficiency was reduced in the CN-OO birds because of the upregulation of avUCP in response to heat stress ROS production. Current work within our laboratory analyzing avUCP mRNA expression may provide further elucidation for the results presented here.

A limitation of the present study is the lack of a heat stress control group, resulting from limitations in the trial facilities. Given the differences within the interactions before and after heat stress, further insight into how TM affects broiler performance may have been gained through comparison with a non–heat stressed group during the final week of rearing. However, given the similarities between treatment interactions for the 6 weeks before AHS, it may be hypothesized that their performance would have remained similar during the final week if maintained at standard rearing temperatures.

CONCLUSIONS AND APPLICATIONS

1. The higher incubation temperatures utilized in TM resulted in increased EST and embryonic mortality, as well as decreased hatchability and broiler performance. The mechanisms behind TM require further elucidation before a balance between heat acclimation and optimal broiler performance can be achieved.
2. Despite decreased broiler performance, livability was improved for the TM group during AHS. Future work examining the economics of live production is warranted to assess if the loss in hatchability is offset by decreased broiler mortality, particularly in operations where heat stress is expected due to season or region.
3. Broiler performance was shown to be similar between dietary fat sources at a 4.5% inclusion rate during the finisher period, before and after AHS.
4. Treatment interactions did not alter broiler performance until after heat stress. After AHS, TM birds performed similarly on the different diets while FCR and BWG were improved for PF in comparison with OO. Further research is needed to clarify how heat stress influences nutrient metabolism and what role embryonic acclimation may play.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the North Carolina Department of Agriculture and Consumer Services Piedmont Research Station, as well as the North Carolina State University Prestage Department of Poultry Science and Feed Mill. The authors would also like to thank B. J. Holtzhausen for his technical support in this experiment.

DISCLOSURES

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. The authors declare no conflicts of interest.

REFERENCES

Al-Rukibat, R. K., M. B. Al-Zghoul, W. M. Hananeh, M. Q. Al-Natour, and E. A. Abu-Basha. 2017. Thermal manipulation during late embryogenesis: effect on body weight and temperature, thyroid hormones, and differential white blood cell counts in broiler chickens. Poult. Sci. 96:234–240.

Al-Zghoul, M. B., H. Sukker, and M. M. Ababneh. 2019. Effect of thermal manipulation of broilers embryos on the response to heat-induced oxidative stress. Poult. Sci. 98:991–1001.

Andreotti, M. O., O. M. Junqueira, L. C. Cancherini, E. A. Rodrigues, and N. K. Sakomura. 2001. Valor nutricional de algumas fontes de gordura para frangos de corte. Proceedings of the 38th Annual Meeting of the Brazilian Society of Zootechnics Anais da 38° Reunião Anual da Sociedade Brasileira de Zootecnia, Piracicaba, SP. SBZ, Piracicaba.

AOAC. 2012. AOAC Official method 996.06: fats (total, saturated, and unsaturated in foods). Hydrolytic Extraction Gas Chromatographic method. in Official Methods of Analysis of the Association of Analytical Chemists. G.W. Latimer, ed. 19th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

AOCS. 2017. Official Method Ca 5a-40: free fatty acids in crude and refined fats and oils. in Official Methods and Recommended Practices of the AOCS. M.W. Collison, ed. 7th rev. ed. Amer. Oil Chem.' Soc., Champaign, IL.

Aviagen. 2018. Section 6 – Housing and environment. Pages 79–105 in Ross Broiler Management Handbook. Aviagen, Huntsville, AL.

Baião, N. C., and L. Lara. 2005. Oil and fat in broiler nutrition. Braz. J. Poult. Sci. 7:129–141.

Collin, A., C. Berri, S. Tesseraud, F. R. Rodon, S. Skiba-Cassy, S. Crochet, M. J. Duclos, N. Rideau, K. Tona, and J. Buyse. 2007. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poult. Sci. 86:795–800.

Collin, A., M. Picard, and S. Yahav. 2005. The effect of duration of thermal manipulation during broiler chick

embryogenesis on body weight and body temperature of post-hatched chicks. Anim. Res. 54:105–111.

Collin, A., M. Taouis, J. Buyse, N. B. Ifuta, V. M. Darras, P. Van As, R. D. Malheiros, V. M. Moraes, and E. Decuypere. 2003. Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chicken. Am. J. Physiol. Endocrinol. Metab. 284:E771–E777.

Cooper, M. A., and K. W. Washburn. 1998. The relationships of body temperature to weight gain, feed consumption, and feed utilization in broilers under heat stress. Poult. Sci. 77:237–242.

Crespo, N., and E. Esteve-Garcia. 2002. Dietary polyunsaturated fatty acids decrease fat deposition in separable fat depots but not in the remainder carcass. Poult. Sci. 81:512–518.

Daghir, N. J. 2008. Present status and future of the poultry industry in hot regions. Pages 1–12 in Poultry Production in Hot Climates. N. Daghir, ed. 2nd ed. CABI, Oxfordshire, UK.

Dale, N., and H. L. Fuller. 1979. Effects of diet composition on feed intake and growth of chicks under heat stress: I. Dietary fat levels. Poult. Sci. 58:1529–1534.

De Witt, F. H., S. P. Els, H. J. Van der Merwe, A. Hugo, and M. D. Fair. 2009. Effect of dietary lipid sources on production performance of broilers. S. Afr. J. Anim.Sci. 39:45–48.

Debonne, M., P. Baarendse, H. Van Den Brand, B. Kemp, V. Bruggeman, and E. Decuypere. 2008. Involvement of the hypothalamic-pituitary-thyroid axis and its interaction with the hypothalamic-pituitary-adrenal axis in the ontogeny of avian thermoregulation: a review. World Poult. Sci. J. 64:309–321.

Dridi, S., O. Onagbesan, Q. Swennen, J. Buyse, E. Decuypere, and M. Taouis. 2004. Gene expression, tissue distribution and potential physiological role of uncoupling protein in avian species. Comp. Biochem. Physiol. Part A. Mol. Integr. Physiol. 139:273–283.

Ferrini, G., E. G. Manzanilla, D. Menoyo, E. Esteve-Garcia, M. D. Baucells, and A. C. Barroeta. 2010. Effects of dietary n-3 fatty acids in fat metabolism and thyroid hormone levels when compared to dietary saturated fatty acids in chickens. Livestock Sci. 131:287–291.

French, N. A. 1997. Modeling incubation temperature: the effects of incubator design, embryonic development, and egg size. Poult. Sci. 76:124–133.

Garrett, R. L., and R. J. Young. 1975. Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. J. Nutr. 105:827–838.

Geris, K. L., S. P. Kotanen, L. R. Berghman, E. R. Kühn, and V. M. Darras. 1996. Evidence of a thyrotropin-releasing activity of ovine corticotropin-releasing factor in the domestic fowl (Gallus domesticus). Gen. Comp. Endocrinol. 104:139–146.

Hamidu, J. A., G. M. Fasenko, J. Feddes, E. E. O'dea, C. A. Ouellette, M. J. Wineland, and V. L. Christensen. 2007. The effect of broiler breeder genetic strain and parent flock age on eggshell conductance and embryonic metabolism. Poult. Sci. 86:2420–2432.

Hamidu, J. A., C. A. Torres, M. L. Johnson-Dahl, and D. R. Korver. 2018. Physiological response of broiler embryos to different incubator temperature profiles and maternal flock age during incubation. 1. Embryonic metabolism and day-old chick quality. Poult. Sci. 97:2934–2946.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003a. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500–1508.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003b. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509–1518.

Hulet, R., G. Gladys, D. Hill, R. Meijerhof, and T. El-Shiekh. 2007. Influence of egg shell embryonic incubation temperature and broiler breeder flock age on posthatch growth performance and carcass characteristics. Poult. Sci. 86:408–412.

Hurwitz, S., M. Weiselberg, U. Eisner, I. Bartov, G. Riesenfeld, M. Sharvit, A. Niv, and S. Bornstein. 1980. The energy requirements and performance of growing chickens and turkeys as affected by environmental temperature. Poult. Sci. 59:2290–2299.

Jenkins, S. A., and T. E. Porter. 2004. Ontogeny of the hypothalamo–pituitary–adrenocortical axis in the chicken embryo: a review. Domest. Anim. Endocrinol. 26:267–275.

Joseph, N. S., A. Lourens, and E.T. Moran Jr. 2006. The effects of suboptimal eggshell temperature during incubation on broiler chick quality, live performance, and further processing yield. Poult. Sci. 85:932–938.

Lopez-Ferrer, S., M. D. Baucells, A. C. Barroeta, and M. A. Grashorn. 2001. n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. Poult. Sci. 80:741–752.

Lourens, A., H. Van den Brand, M. J. Heetkamp, R. Meijerhof, and B. Kemp. 2006. Metabolic responses of chick embryos to short-term temperature fluctuations. Poult. Sci. 85:1081–1086.

Lourens, A., H. Van den Brand, R. Meijerhof, and B. Kemp. 2005. Effect of eggshell temperature during incubation on embryo development, hatchability, and posthatch development. Poult. Sci. 84:914–920.

Loyau, T., C. Berri, L. Bedrani, S. Métayer-Coustard, C. Praud, M. J. Duclos, S. Tesseraud, N. Rideau, N. Everaert, and S. Yahav. 2013. Thermal manipulation of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality. J. Anim. Sci. 91:3674–3685.

Loyau, T., S. Métayer-Coustard, C. Berri, S. Crochet, E. Cailleau-Audouin, M. Sannier, P. Chartrin, C. Praud, C. Hennequet-Antier, and N. Rideau. 2014. Thermal manipulation during embryogenesis has long-term effects on muscle and liver metabolism in fast-growing chickens. PLoS One 9:e105339.

Loyau, T., C. Hennequet-Antier, V. Coustham, C. Berri, M. Leduc, S. Crochet, M. Sannier, M. J. Duclos, S. Mignon-Grasteau, and S. Tesseraud. 2016. Thermal manipulation of the chicken embryo triggers differential gene expression in response to a later heat challenge. BMC Genom. 17:329.

McNabb, F. A. 2007. The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. Crit. Rev. Toxicol. 37:163–193.

Meijerhof, R. 2009. The influence of incubation on chick quality and broiler performance. Proc. Aust. Poult. Sci. Symp. 20:106 (Summary).

Molenaar, R., R. Hulet, R. Meijerhof, C. M. Maatjens, B. Kemp, and H. Van Den Brand. 2011. High eggshell temperatures during incubation decrease growth

performance and increase the incidence of ascites in broiler chickens. Poult. Sci. 90:624–632.

Moraes, V., R. D. Malheiros, V. Bruggeman, A. Collin, K. Tona, P. Van As, O. M. Onagbesan, J. Buyse, E. Decuypere, and M. Macari. 2004. The effect of timing of thermal conditioning during incubation on embryo physiological parameters and its relationship to thermotolerance in adult broiler chickens. J. Therm. Biol. 29:55–61.

Mujahid, A., K. Sato, Y. Akiba, and M. Toyomizu. 2006. Acute heat stress stimulates mitochondrial superoxide production in broiler skeletal muscle, possibly via down-regulation of uncoupling protein content. Poult. Sci. 85:1259–1265.

Mujahid, A., Y. Akiba, and M. Toyomizu. 2009. Olive oil-supplemented diet alleviates acute heat stress-induced mitochondrial ROS production in chicken skeletal muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 297:R690–R698.

Newman, R. E., W. L. Bryden, E. Fleck, J. R. Ashes, W. A. Buttemer, L. H. Storlien, and J. A. Downing. 2002. Dietary n-3 and n-6 fatty acids alter avian metabolism: metabolism and abdominal fat deposition. Br. J. Nutr. 88:11–18.

Nichelmann, M., and B. Tzschentke. 2002. Ontogeny of thermoregulation in precocial birds. Comp. Biochem. Physiol. Part A. Mol. Integr. Physiol. 131:751–763.

Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. Poult. Sci. 74:366–373.

NCC. 2019. U.S. Broiler Performance. National Chicken Council. Accessed Dec. 2020. https://www.nationalchickencouncil.org/statistic/us-broiler-performance/.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, J. Brake, and S. Yahav. 2008. Thermal manipulations during broiler embryogenesis: effect on the acquisition of thermotolerance. Poult. Sci. 87:1516–1525.

Piestun, Y., M. Harel, M. Barak, S. Yahav, and O. Halevy. 2009. Thermal manipulations in late-term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. J. Appl. Physiol. 106:233–240.

Piestun, Y., O. Halevy, D. Shinder, M. Ruzal, S. Druyan, and S. Yahav. 2011. Thermal manipulations during broiler embryogenesis improves post-hatch performance under hot conditions. J. Therm. Biol. 36:469–474.

Piestun, Y., S. Druyan, J. Brake, and S. Yahav. 2013. Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poult. Sci. 92:1155–1163.

Pinchasov, Y., and I. Nir. 1992. Effect of dietary polyunsaturated fatty acid concentration on performance, fat deposition, and carcass fatty acid composition in broiler chickens. Poult. Sci. 71:1504–1512.

Potença, A., A. E. Murakami, J. Fernandes, M. Matsushita, and E. L. Nakagawa. 2008. Performance, abdominal fat deposition and bone characteristics of broilers fed diets containing different lipid sources. Braz. J. Poult. Sci. 10:239–244.

Raimbault, S., S. Dridi, F. Denjean, J. Lachuer, E. Couplan, F. Bouillaud, A. Bordas, C. Duchamp, M. Taouis, and D. Ricquier. 2001. An uncoupling protein homologue putatively involved in facultative muscle thermogenesis in birds. Biochem. J. 353:441–444.

Sakomura, N. K. 2004. Modeling energy utilization in broiler breeders, laying hens and broilers. Braz. J. Poult. Sci. 6:1–11.

Sanz, M. 1999. Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. Br. Poult. Sci. 40:95–101.

Sanz, M., A. Flores, and C. J. Lopez-Bote. 2000a. The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. Br. Poult. Sci. 41:61–68.

Sanz, M., C. J. Lopez-Bote, D. Menoyo, and J. M. Bautista. 2000b. Abdominal fat deposition and fatty acid synthesis are lower and β-oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. J. Nutr. 130:3034–3037.

Seifi, K., M. Rezaei, A. T. Yansari, G. H. Riazi, M. J. Zamiri, and R. Heidari. 2018. Saturated fatty acids may ameliorate environmental heat stress in broiler birds by affecting mitochondrial energetics and related genes. J. Therm. Biol. 78:1–9.

St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86:E52–E77.

Tancharoenrat, P., V. Ravindran, F. Zaefarian, and G. Ravindran. 2013. Influence of age on the apparent metabolisable energy and total tract apparent fat digestibility of different fat sources for broiler chickens. Anim. Feed Sci. Technol. 186:186–192.

Teeter, R. G., and T. Belay. 1996. Broiler management during acute heat stress. Anim. Feed Sci. Technol. 58:127–142.

Tona, K., O. Onagbesan, V. Bruggeman, A. Collin, C. Berri, M. J. Duclos, S. Tesseraud, J. Buyse, E. Decuypere, and S. Yahav. 2008. Effects of heat conditioning at d 16 to 18 of incubation or during early broiler rearing on embryo physiology, posthatch growth performance and heat tolerance. Archiv Geflügelkd. 72:75–83.

Tukey, J. W. 1949. Comparing individual means in the analysis of variance. Biometrics 99–114.

Werner, C., C. Wecke, F. Liebert, and M. Wicke. 2010. Increasing the incubation temperature between embryonic day 7 and 10 has no influence on the growth and slaughter characteristics as well as meat quality of broilers. Animal 4:810.

Wilson, H. R. 1991. Interrelationships of egg size, chick size, posthatching growth and hatchability. Worlds Poult. Sci. J. 47:5–20.

Wiseman, J., and F. Salvador. 1989. The influence of age, chemical composition and rate of inclusion on the apparent metabolizable energy of fats fed to broiler chicks. Br. Poult. Sci. 30:653–662.

Wiseman, J., J. Powles, and F. Salvador. 1998. Comparison between pigs and poultry in the prediction in the prediction of the dietary energy value of fats. Anim. Feed Sci. Technol. 71:1–9.

Wiseman, J., and F. Salvador. 1991. The influence of free fatty acid content and degree of saturation on the apparent metabolizable energy value of fats fed to broilers. Poult. Sci. 70:573–582.

Yahav, S., R. S. Rath, and D. Shinder. 2004. The effect of thermal manipulations during embryogenesis of broiler chicks (Gallus domesticus) on hatchability, body weight and thermoregulation after hatch. J. Therm. Biol. 29:245–250.

Yahav, S., D. Shinder, J. Tanny, and S. Cohen. 2005. Sensible heat loss: the broiler's paradox. Worlds Poult. Sci. J. 61:419–434.

Yahav, S. 2009. Alleviating heat stress in domestic fowl: different strategies. Worlds Poult. Sci. J. 65:719–732.

Yalçin, S., S. Özkan, L. Türkmut, and P. B. Siegel. 2001. Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. Br. Poult. Sci. 42:149–152.

Yalçin, S., M. Çabuk, V. Bruggeman, E. Babacanoğlu, J. Buyse, E. Decuypere, and P. B. Siegel. 2008. Acclimation to heat during incubation. 1. Embryonic morphological traits, blood biochemistry, and hatching performance. Poult. Sci. 87:1219–1228.

Yalçin, S., E. Babacanoğlu, H. C. Güler, and M. Akşit. 2010. Effects of incubation temperature on hatching and carcass performance of broilers. Worlds Poult. Sci. J. 66:87–94.

Zaboli, G., S. Rahimi, F. Shariatmadari, M. A. K. Torshizi, A. Baghbanzadeh, and M. Mehri. 2017. Thermal manipulation during Pre and Post-Hatch on thermotolerance of male broiler chickens exposed to chronic heat stress. Poult. Sci. 96:478–485.

Zollitsch, W., W. Knaus, F. Aichinger, and F. Lettner. 1997. Effects of different dietary fat sources on performance and carcass characteristics of broilers. Anim. Feed Sci. Technol. 66:63–73.