Embryonic thermal manipulation and dietary fat source during acute heat stress: 2. Effect on broiler carcass characteristics and breast muscle myopathies

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Primary Audience: Nutritionist, Researchers, Production Managers, Meat Scientists

SUMMARY

The objective of this study was to evaluate the effect of embryonic thermal manipulation and dietary fat source during the finisher period on carcass characteristics and breast muscle myopathies following acute heat stress. Thermal manipulation (**TM**) of incubation temperatures has been shown to improve bird resistance to heat stress and enhance breast muscle growth. Fat supplementation is frequently used during heat stress and fat source may alter carcass composition, as fat sources rich in unsaturated fatty acids have been shown to reduce fat pad weight. Ross 708 eggs were incubated at 37.5˚C except during TM when temperature was increased to 39.5˚C for 12 h daily from embryo day 7 to 16. A total of 1,080 chicks were reared under standard conditions until acute heat stress at 43 d. Dietary treatments were applied during the finisher period beginning at 28 d, with diets including 4.5% of soya oil, poultry fat, or olive oil. At 49 d, 240 male birds were processed to assess carcass and portion weights, as well as breast quality. Carcass and portion weights were decreased by TM, however percent yield was similar to the controls. A decrease in breast muscle myopathies was noted but may have been due to the lower BW of the TM birds. Interactions between the treatments suggest that TM may alter lipid metabolism. Differences in dietary fat source did not affect carcass characteristics. The reduction in breast muscle myopathies may be negated by the negative impact of TM on carcass weights.

Key words: thermal manipulation, incubation, fat source, acute heat stress, carcass yield, meat quality, breast myopathy

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DESCRIPTION OF PROBLEM

Intensive genetic selection in poultry over the last half century has dramatically improved feed efficiency, growth rate, and carcass yield

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in commercial broiler strains (Havenstein et al., 2003a,b). However, these advances in both growth rate and body weight have also resulted in a reduced tolerance to heat stress. The increased metabolic heat production associated with fast growing broilers has led to a higher body temperature, while greater body mass relative to surface area has decreased the bird's capacity for sensible heat dissipation (Yalçin et al., 2001; Yahav et al., 2005). As broiler body weight increases approaching market age, so does susceptibility to heat stress and the potential for economic loss due to mortalities arising from heat stress (St-Pierre et al., 2003). Birds that survive heat stress can still incur production losses as broilers exposed to high temperatures may demonstrate decreased carcass yield, enhanced fat deposition, and reduced meat quality at processing (Baziz et al., 1996; Akşit et al., 2006; Song and King, 2015). As broiler market weights continue to increase annually (NCC, 2021), new strategies must be developed to improve broiler thermotolerance without sacrificing production or processing potential.

Production losses in broilers associated with high ambient temperatures may be reduced through prior acclimation to mild heat stress, but decreased feed intake and growth can still occur during this process (Abdelqader and Al-Fataftah, 2014). Recent work with thermal manipulation (**TM**) has demonstrated improved performance in broilers exposed to heat stress by acclimatizing birds during embryonic development (Moraes et al., 2003; Yahav et al., 2004; Piestun et al., 2008, 2011, 2013; Loyau et al., 2015). In chickens, thermoregulatory control systems begin to emerge halfway through incubation (Nichelmann and Tzschentke, 2002) and the neurological and endocrine pathways involved exhibit a high degree of plasticity during this period (Tzschentke and Basta, 2002). A major milestone in thermoregulatory development occurs during the second week of incubation, with the emergence of the hypothalamo-pituitary-thyroid and -adrenocortical axes (**HPT** and **HPA**, respectively) (Jenkins and Porter, 2004; McNabb, 2007). The HPT axis regulates thyroid hormones and by extension body temperature, while the HPA axis primarily controls corticosterone production but also contributes to thyroid regulation at the pituitary level (Debonne et al., 2008). Exposure to increased temperatures during the maturation of these axes may increase the neuronal hypothalamic temperature threshold for their response to high temperatures and improve bird heat tolerance post-hatch (Tzschentke and Basta, 2002). Furthermore, by acclimating the embryo to increased temperatures during incubation, it may be possible to improve heat tolerance in the bird without negatively impacting feed intake or growth.

In addition to influencing thermotolerance, TM may also alter carcass composition as increased breast portion yield at processing has been noted by some authors (Collin et al., 2007; Piestun et al., 2011, 2013; Loyau et al., 2013). The appearance of fetal myoblasts in the embryo coincides with the emergence of the HPT and HPA axes and may be positively influenced by TM exposure (Halvey, 2020). Satellite cell populations also develop during mid-incubation between the sarcolemma and the basal lamina of the muscle fibers and actively proliferate until hatch; these cells will contribute to the repair and regeneration of the skeletal muscles, as well as hypertrophic growth post-hatch (Stockdale, 1992). In meat birds selected for high yield, muscle myopathies such as wooden breast (**WB**) and white striping (**WS**) are becoming more prevalent as muscle growth surpasses the muscles' physiological capacity to support it (Velleman, 2015). These myopathies are detrimental to meat quality and reduce consumer acceptance of breast portions, resulting in economic loss for the industry (Petracci et al., 2019). Although the exact etiology of these myopathies is unclear, higher oxidation rates have been observed in breast muscle affected by these myopathies (Abasht et al., 2016; Soglia et al., 2016). Heat stress may further exacerbate the effects of WB and WS on meat quality as exposure to high temperatures is associated with increased oxidative stress in the skeletal muscle (Mujahid et al., 2007) and reduced muscle membrane integrity (Sandercock et al., 2001). Incubation profiles similar to TM have resulted in enhanced muscle morphology that may improve meat quality (Clark et al., 2017), but it

is unclear if these improvements can ameliorate the negative effects of heat stress or breast muscle myopathies. As TM application during embryonic development has the potential to improve breast yield and meat quality, further work is warranted to elucidate the practical applications of TM in broilers exposed to heat stress.

Dietary fat supplementation is commonly used in poultry diets to overcome reduced nutrient intake due to decreased feed consumption in heat stressed broilers. Supplemental dietary fat provides increased metabolizable energy at a reduced heat increment in comparison to carbohydrates and proteins, so that metabolic heat production is minimized in the bird (Dale and Fuller, 1979). In addition to improving dietary energy density, supplemental fat may also alter carcass composition depending upon the fatty acid profile of the fat source utilized. Diets higher in unsaturated fatty acids (**USFA**) have been shown to decrease abdominal fat deposition while diets higher in saturated fatty acids (**SFA**) resulted in greater fat deposition (Sanz et al., 2000; Crespo and Esteve-Garcia, 2002). During heat stress, fat deposition is increased (Baziz et al., 1996) despite reduced broiler feed intake. As poultry meat is perceived by the consumer to be a lean and healthy meat (Kennedy et al., 2004), excessive carcass fat can be viewed as an economic loss. Further investigation is needed to determine if the fatty acid composition of dietary fat sources can improve broiler carcass composition during heat stress.

The aim of this trial was to evaluate the effect of thermal manipulation during incubation and dietary fat source during the finisher period on carcass characteristics in birds exposed to acute heat stress during late rearing. Given the economic importance of breast meat as a premium portion, the occurrence of breast muscle myopathies under these conditions was also investigated.

MATERIALS AND METHODS

All animal procedures in this study were approved by the Institutional Animal Care and Use Committee at North Carolina State University. 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. Processing occurred at North Carolina State University Chicken Education Unit's broiler processing facility.

Incubation Treatments

Hatching eggs were collected from a 43-wk-old 708 Ross broiler breeder flock (Aviagen Inc., Huntsville, AL) and stored at 18˚C and 75% relative humidity (**RH**) for 2 to 5 d. Eggs were weighed during traying and sorted into two treatment groups with an average egg weight of 68.47 ± 1.04 g. Each incubation treatment consisted of 1,740 eggs across 12 replicate trays per treatment (3,480 eggs total). Pre-incubation occurred for 12 h in a forced ventilation cabinet at 26˚C before trays were placed into a Natureform incubator (I14, Natureform Inc, Jacksonville, FL). Trays were set in a generalized randomized complete block design with three trays from both treatments in each block (four blocks total) to reduce environmental effect of position within the incubator. Both treatments were initially set in the same machine (Control, **CN**) at a temperature of 37.5˚C and 56% RH for the first week of incubation.

On incubation day (**E**) 7, trays labelled as TM were moved to an adjacent incubator set at 39.5˚C and 65% RH for 12 h while the CN trays remained in the initial incubator at 37.5˚C and 56% RH. Relative humidity was increased in the TM machine to maintain similar moisture loss between treatments. Eggs in the TM treatment were moved at the same time daily between the TM and CN setters in 12 h cycles from E 7 to 16. A plastic tent heated to match CN setter temperature was placed over both incubators to prevent egg cooling during transfer between machines. After E 16, both treatments remained in the CN setter until transfer to a single hatcher on E 17.5 and hatching on E 21.

Bird Rearing and Dietary Treatments

Rearing occurred in a 60 pen environmentally controlled broiler house. Treatment pens were assigned in a randomized complete block

design with six treatments (two incubation profiles × three finisher diets) in 10 blocks. Each pen (1.2 × 1.5m) 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.

Standard commercial starter and grower diets were fed until 14 d and 28 d, respectively (Table 1). Dietary treatments began at 28 d, with birds being offered a finisher diet that differed in fat source. Treatment diets consisted of a saturated fatty acid source (poultry fat, **PF**), a monounsaturated fatty acid source (olive oil, **OO**) and a polyunsaturated fatty acid source (soya oil, **SO**). Each fat source was analyzed to determine the concentrations of free fatty acids (method Ca 5a-40, AOCS, 2017), SFA and USFA (method 996.06, AOAC, 2012), as shown in Table 2. Energy values were calculated for PF (35.45 MJ/kg), SO (37.63 MJ/kg), and OO (38.07 MJ/kg) using Wiseman's equation for birds older than 21 d (Wiseman and Salvador, 1989; Wiseman et al., 1998). Final dietary ME was determined to be 3,180 Kcal for the PF diet, 3,203 for the SO diet, and 3,208 for the OO diet (Table 1) based on a fat inclusion level of 4.5%. Diets were formulated to meet or exceed NRC (1994) requirements and were manufactured at the North Carolina State University Feed Mill.

Birds were reared under standard commercial lighting and temperature profiles (Aviagen, 2018) until the heat stress period at 43 d. At 43 d, an acute heat stress (**AHS**) challenge was imposed and temperatures within the house were increased from 21˚C to 32˚C for 4 h. Standard temperature profiles were implemented after the heat stress period until the end of the study at 49 d. Feed intake and body weights were measured weekly, but summarized for the entire trial as pre (0 to 42 d) and post (43 to 49 d) heat stress. Body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined per pen.

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

Ingredients (%)	Starter	Grower	Finisher
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	3,180
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]Finisher diets included either poultry fat, soybean oil, or olive oil at 4.5 %. The Wiseman equation (Wiseman and Salvador, 1989; Wiseman et al., 1998) 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).

[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.

Processing and Carcass Quality

At 49 d, all male birds were weighed individually and four birds from each pen were selected to be within one standard deviation of the average treatment BW. At 50 d, selected birds were held for 4 h without feed but ad libitum water access before being transported to the abattoir. Birds were processed within 2 h blocked by time for treatment effects.

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

Fatty acid (g/100g)[2]	Poultry fat	Soya oil	Olive oil
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[3]	5.46	0.55	0.77

[1]Each fat source included at 4.5 % to the base finisher diet.
[2]Method 996.06, AOAC (2012).
[3]Method Ca 5a-40, AOCS (2017).

Processing procedures were conducted in a small-scale abattoir under commercially viable conditions as described by Livingston et al. (2019). Live weight as well as hot and cold carcass weights were assessed along with cut yields including fat pad, *Pectoralis* major (**p. major**), *Pectoralis* minor (**p. minor**), wings, thighs, and frame. Meat quality of the boneless, skinless breast fillet (p. major) was evaluated for evidence of woody breast, white striping, and spaghetti breast on a 1 to 4 point ordinal scale. Breast myopathy scoring was performed by an experienced technician trained in using this system with no knowledge of the treatments.

Scoring for WB was assessed by palpation of the meat, with a score of 1 being considered normal with no signs of hardness. A score of 2 was assigned if increased firmness was noted in 50% or less of the breast. A score of 3 was awarded to portions where over 50% of the breast tissue was hardened, but some pliability remained. A WB score of 4 indicated an overall lack of pliability in the meat, with 90% or more of the breast being hard to the touch. Similarly, a score of 1 for WS evaluation indicated normal breast meat with no striations. As visible striations increased in severity from mild to moderate a WS score of 2 or 3 was assigned, respectively. A WS of 4 was assigned to breasts with severe striations across the ventral portion of the fillet. Breast portions were also scored for evidence of spaghetti breast, a myopathy where reduced structural integrity of the breast fillet is observed, to the extent that loose muscle fiber bundles can be manually separated (Petracci et al., 2019). Spaghetti breast (**SB**) scoring was assessed by palpation of the meat, with a SB score of 1 being considered normal with no signs of loose muscle structure of the cranial surface of the breast. A SB score of 2 was assigned to breasts that showed mild separation of muscle fibers. A SB score of 3 was assigned to breasts in which the muscle fibers could be separated and easily pulled apart. A SB score of 4 was assigned to breasts that showed severe muscle deterioration.

Color values (L* = lightness, a* = redness, b* = yellowness) for the boneless, skinless breast fillet portions were measured with a Minolta colorimeter (CR-300, Konica Minolta, Ramsey, NJ). The breast portion was wiped clean prior to measurements, which were taken with the colorimeter held perpendicular to the ventral portion of the breast. Three consecutive measurements were taken and their average recorded. Drip loss was assessed by weighing the breast portion and then suspending it in a whirl pack bag for 48 h. Portions were then reweighed and drip loss calculated as (weight initial − weight final) / weight initial. After the samples were weighed for drip loss, they were evaluated for cook loss. Breast samples were cooked until an internal temperature of 74˚C was reached, whereupon samples were removed from the oven, and cooled to room temperature. After cooling, samples were re-weighed and percentage cook loss was calculated as (raw weight − cooked weight) / raw weight.

Statistical Analysis

Data were analyzed as a 2 × 3 factorial design using the Mixed personality of JMP Pro 13 (SAS Institute, Cary, NC) with incubation and dietary treatments being the main effects. Rearing performance data (BWG, FI, and FCR)

were analyzed using pen as the experimental unit. Processing and meat quality data (individual BW, carcass and portion weights, percentage yields, myopathy scores, and color values) were analyzed using the individual broiler as the experimental unit (40 birds per treatment). Means were compared by Tukey's HSD test (Tukey, 1949) and statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Thermal Manipulation

Broiler performance prior to heat challenge was decreased for TM birds (Table 3), as demonstrated by a lower BWG ($P = 0.001$) and reduced FI ($P < 0.001$) from 0 to 42 d. Following AHS, BWG and FCR were noted to be similar between groups despite the continued decrease in FI for the TM birds ($P = 0.001$). Similar results were noted for TM birds on a biweekly basis from hatch as reported by Brannan et al. (2021), indicating that the reduction in BWG and FI was apparent at an early age. Portion yields are shown in Table 4, relative to the weight of cold carcass with fat pad which was also decreased in TM birds ($P = 0.019$). The percentage of portion yields remained similar between the treatments with the exception of abdominal fat pad (**AFP**) and p. minor, which were decreased ($P = 0.002$) and increased ($P = 0.008$) by TM respectively. Hot and cold carcass weights, as well as portion weights, for the main effects and interactions are provided as supplementary data in Table S1.

The effect of TM on broiler performance varies within the literature and has been demonstrated to be both enhanced (Piestun et al., 2009; Al-Zhgoul et al., 2013) and decreased (Loyau et al., 2013; Clark et al., 2017), likely due to variations in TM protocols and trial conditions. Improvements in breast weight and yield have been more consistently observed for TM birds (Halevy et al., 2006; Collin et al., 2007; Piestun et al., 2009, 2011, 2013;

Table 3. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) as influenced by incubation treatment and dietary fat source during the finisher period, as well as their interactions, pre- and post-heat challenge[1].

	0 to 42 d, pre-heat stress			43 to 49 d, post-heat stress		
	BWG, g/bird	FI, g/bird	FCR, g:g	BWG, g/bird	FI, g/bird	FCR, g:g
Incubation[2]						
CN	2795.2^{A}	4245.3^{A}	1.53	669.9	1405.5^{A}	2.08
TM	2692.5^{B}	4089.4^{B}	1.52	652.0	1329.9^{B}	2.04
SEM[3]	19.5	26.7	0.01	11.0	16.0	0.03
P-Value	0.001	<0.001	0.475	0.256	0.001	0.227
Diet[4]						
Olive	2758.9	4192.2	1.52	637.1	1357.9	2.11
Poultry	2737.1	4174.4	1.53	680.2	1381.6	2.04
Soya	2735.4	4135.6	1.52	665.5	1363.6	2.06
SEM	23.8	32.7	0.01	13.4	19.3	0.03
P-Value	0.741	0.463	0.965	0.081	0.670	0.196
Incubation x diet						
CN-Olive	2803.1	4248.0	1.52	627.6	1396.8	2.18^{A}
CN-Poultry	2793.8	4247.8	1.52	716.0	1411.1	1.97^{B}
CN-Soya	2788.7	4240.1	1.55	665.9	1408.5	2.12^{AB}
TM-Olive	2714.8	4136.4	1.53	646.6	1319.0	2.05^{AB}
TM-Poultry	2680.5	4100.9	1.53	644.4	1352.2	2.10^{AB}
TM-Soya	2682.2	4031.0	1.50	665.1	1318.6	1.99^{B}
SEM	33.7	46.3	0.02	18.8	27.3	0.04
P-Value	0.929	0.570	0.136	0.054	0.850	0.005

[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-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.

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

Table 4. Live weight at processing and carcass portions relative to the weight of cold carcass (including fat pad) as influenced by incubation treatment and dietary fat source, as well as their interactions, following heat stress[1] during the finisher period.

	Live Weight (50 d)	Thighs, %	Wings, %	*Pectoralis* major, %	*Pectoralis* minor, %	Fat pad, %	Frame, %	Cold carcass weight, g
Incubation[2]								
CN	3918.6	28.89	9.30	28.75	5.42^{B}	1.39^{A}	26.95	3190.1^{a}
TM	3813.2	28.72	9.27	28.67	5.61^{A}	1.20^{B}	26.76	3070.9^{b}
SEM[3]	43.0	0.16	0.07	0.20	0.05	0.04	0.54	35.7
P-Value	0.078	0.432	0.765	0.782	0.008	0.002	0.757	0.019
Diet[4]								
Olive	3878.9	28.69	9.28	28.62	5.47	1.29	27.26	3147.4
Poultry	3898.1	28.79	9.23	28.65	5.48	1.37	26.41	3178.9
Soya	3820.8	28.93	9.33	28.87	5.61	1.22	26.89	3065.1
SEM	51.4	0.18	0.08	0.24	0.06	0.05	0.44	43.7
P-Value	0.560	0.664	0.674	0.725	0.251	0.175	0.511	0.178
Incubation x diet								
CN-Olive	3892.9	28.75	9.28	28.40	5.31	1.43	28.20	3173.6
CN-Poultry	3772.7	28.74	9.17	29.13	5.41	1.36	26.04	3311.2
CN-Soya	3841.5	29.17	9.44	28.72	5.55	1.37	26.61	3085.4
TM-Olive	3866.0	28.62	9.28	28.83	5.64	1.16	26.32	3121.1
TM-Poultry	3773.7	28.83	9.30	28.16	5.54	1.37	26.78	3046.7
TM-Soya	3800.0	28.69	9.23	29.03	5.66	1.08	27.18	3044.9
SEM	72.4	0.26	0.11	0.34	0.08	0.08	0.76	61.5
P-Value	0.207	0.567	0.317	0.070	0.406	0.079	0.150	0.119

[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 12h 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.^{AB}Means in a column that possess different superscripts differ significantly ($P < 0.01$).^{ab}Means in a column that possess different superscripts differ significantly ($P < 0.05$).

Loyau et al., 2013), however such improvements were not apparent in the current results. Although an increase in yield for p. minor was noted for the TM birds (P = 0. 008), similar improvements were not evident for p. major. While the TM protocol applied in the current trial (39.5˚C for 12 h daily from E 7 to E 16) has previously been demonstrated to enhance breast portion (Piestun et al., 2009, 2011, 2013; Loyau et al., 2013), other factors may have contributed to the conflicting results presented here. Differences in embryonic heat production and temperature sensitivity have been noted between genetic strains, with the Ross 708 strain exhibiting less tolerance to temperature deviations when compared to Cobb (Hamidu et al., 2007, 2018; Nangsuay et al., 2015). In the present results, live performance and carcass weights were decreased in Ross 708 birds by a TM protocol that had previously been applied to Cobb birds with no negative effects (Piestun et al., 2009, 2011, 2013; Loyau et al., 2013). Conversely, improvements in breast development were noted for Ross birds (Collin et al., 2007) utilizing a TM protocol with a shorter duration (3 h daily) and exposure period (E 16 to E 18). This may suggest that the Ross strain embryos would benefit from milder TM exposure, possibly as a result of their increased temperature sensitivity (Hamidu et al., 2018). The development of strain specific TM protocols may reduce the negative effects on bird growth observed in the current trial, but further work is needed to identify the factors contributing to successful TM application.

Despite the decrease in carcass and portion weights, improvements in carcass quality were noted for TM birds. A reduction in the occurrence of WS (P = 0.002) and WB (P = 0.006) was observed in TM birds (Table 5), concurrent with the decrease in weight and percentage of AFP mentioned earlier (P = 0.002, Table 4). The TM period (E 7 to E 16) coincides with the establishment of fetal muscle myoblast and satellite cell proliferation, which may be

Table 5. The occurrence of breast muscle myopathies, meat color[1], and percentage loss as influenced by incubation temperature and dietary fat source, as well as their interactions, following heat stress[2] during the finisher period.

	White Stripe, ordinal scale	Wooden Breast, ordinal scale	Spaghetti breast, ordinal scale	L*	a*	b*	Drip loss, %	Cook loss, %
Incubation[3]								
CN	1.96^{A}	1.95^{A}	1.82	55.17	1.35	11.30^{B}	1.71	22.50
TM	1.71^{B}	1.70^{B}	1.83	55.75	1.26	12.02^{A}	1.66	23.33
SEM[4]	0.06	0.06	0.09	0.30	0.08	0.19	0.09	0.57
P-Value	0.002	0.006	0.961	0.173	0.430	0.004	0.751	0.290
Diet[5]								
Olive	1.90	1.93	1.77	55.75	1.35	12.36^{A}	1.76^{AB}	22.49
Poultry	1.86	1.81	1.91	55.29	1.36	11.62^{B}	1.92^{A}	23.61
Soya	1.74	1.73	1.80	55.34	1.21	10.99^{B}	1.37^{B}	22.64
SEM	0.07	0.08	0.10	0.37	0.23	0.23	0.12	0.67
P-Value	0.263	0.186	0.523	0.608	0.481	<0.001	0.005	0.415
Incubation x diet								
CN-Olive	2.04	2.14	1.79	55.06	1.52	11.96	1.74	22.33
CN-Poultry	1.95	1.95	2.00	55.66	1.24	11.37	2.06	23.41
CN-Soya	1.89	1.75	1.68	54.80	1.29	10.55	1.32	21.76
TM-Olive	1.77	1.72	1.74	56.45	1.19	12.75	1.78	22.65
TM-Poultry	1.78	1.68	1.83	54.92	1.48	11.88	1.79	23.82
TM-Soya	1.59	1.71	1.91	55.88	1.12	11.42	1.42	23.52
SEM	0.10	0.11	0.14	0.52	0.32	0.32	0.17	0.93
P-Value	0.785	0.249	0.353	0.073	0.074	0.814	0.499	0.719

[1]L* = lightness; a* = redness; b* = yellowness.

[2]Acute heat stress occurred at 32˚C for 4 h at 43 d.

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

[4]SEM: Standard error of mean.

[5]Poultry: poultry fat; Soya: soya oil; Olive: olive oil added at 4.5 % to finisher diet.

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

accelerated by increased incubation temperatures (Halevy et al., 2006; Piestun et al., 2009, 2011, 2015; Halevy, 2020). Following initial post-hatch muscle growth, the satellite cells within the muscle fiber become quiescent but may reenter the cell cycle in response to stress (Akasura et al., 2001; Hawke and Garry, 2001). The decreased vascularization and increased muscle fiber degeneration associated with intense breast muscle hypertrophy can activate satellite cell mediated muscle regeneration, potentially reducing the occurrence and severity of breast muscle myopathies (Velleman, 2015). However, as WS and WB are more prevalent in heavy broilers (Kuttappan et al., 2016) it is difficult to determine if the decreased occurrence here is associated with improvements in breast myogenesis or reduced hypertrophy. Clark et al. (2017) also reported a decrease in growth for TM birds, concurrent with a decrease in the severity of microscopic damage common in breast muscle myopathies. Although carcass weight was also reduced for TM birds, breast weights, yields, and average breast muscle fiber width remained similar to control birds (Clark et al., 2017). Considered with the present results, this may suggest improvements to breast muscle myogenesis arising from TM, independent of carcass or breast portion weight. Therefore, it may be that the increased incubation temperatures during TM resulted in increased satellite cell proliferation, which potentially contributed to improvements in breast muscle myopathy. However, a better understanding of the mechanisms involved in TM is needed to promote these improvements while limiting the negative impact on carcass growth observed here.

Similarly, the reduction in AFP may be due to improvements associate with TM but may also be related to the decreased growth demonstrated by the TM birds. Abdominal adipocyte size has been shown to be reduced in embryos exposed to increased temperatures (Almeida et al., 2016) and other studies involving TM in broilers have shown similar

decreases in AFP (Piestun et al. 2011, 2013; Loyau et al., 2013). However, it is unclear if the decrease in AFP associated with TM is due to a reduction in total carcass fat or rather a reallocation of adipose deposition. A surprising result in the current trial was the increase noted in breast yellowness (b*) for the TM birds (P = 0.004, Table 5), which may be an indication of increased fat deposition. Satellite cell reserves have been observed to be larger in small broilers in comparison to heavier birds as the cell population decreases with myocellular recruitment associated with hypertrophy (Daughtry et al., 2017). Satellite cells not recruited to myogenic hypertrophy or repair are likely to remain in a quiescent state until activated by stress (Hawke and Garry, 2001). However, despite their location in the myofiber, the multipotent nature of satellite cells may lead to differentiation into other cellular lineages (Akasura et al., 2001). Harding et al. (2015) observed that satellite cells isolated from skeletal muscle transdifferentiated to adipogenic lineage when exposed to high temperatures. Likewise, Piestun et al. (2017) noted increased fat deposition in the breast muscle satellite cells of chicks exposed to heat stress. It is conceivable that if the reduced growth of the TM birds led to larger satellite cell reserves, then AHS may have resulted in these cells being inducted into adipogenic differentiation and increased breast fat deposition. Further differences in carcass color, drip and cook loss were not observed between incubation treatments (Table 5). Overall, TM did not improve broiler performance or carcass and portion weights, despite a reduction in breast muscle myopathies and AFP. While the potential for improved breast meat quality associated with TM may exist, further research is needed to ensure the increased temperature exposure does not negatively impact carcass yield.

Dietary Fat Source

Dietary fat source as a main effect did not appear to influence BWG, FI, or FCR regardless of AHS (Table 3). Live weight and percent portion yields were also similar between dietary treatments (Table 4). In the current trial dietary fat source treatments were limited to the finisher period, as older broilers are more sensitive to heat stress (Yalçin et al., 2001) and economic loss is greatest prior to processing (St-Pierre et al., 2003). Although performance differences between fat sources are more apparent in younger birds (Noy and Sklan, 1995), other reports have noted differences in birds older than 21 d when offered diets varying in SFA and USFA concentrations (Pinchasov and Nir, 1992; Crespo and Esteve-Garcia, 2001, 2002; Newman et al., 2002). Abdominal fat pad weight has been shown to be particularly influenced by dietary fatty acid profile, with several studies indicating a reduction in AFP with increasing USFA concentration (Sanz, 1999; Sanz et al., 2000; Crespo and Esteve-Garcia, 2001, 2002; Ferrini et al., 2008). While there was a slight tendency towards AFP being decreased with increasing USFA in the present results ($P = 0.081$), overall bird performance and carcass weights remained similar between treatments. This may be due to the inclusion rate of the fat source, as the negative effects of SFA may be less apparent at lower inclusion levels (Wiseman and Salvador, 1991; De Witt et al., 2009). The current inclusion level (4.5%) was chosen for commercial relevance while an inclusion level of 6% or higher is more commonly seen in the literature (Pinchasov and Nir, 1992; Sanz, 1999; Sanz et al., 2000, ; Crespo and Esteve-Garcia, 2001, 2002; Ferrini et al., 2008). It may be that higher inclusion levels result in more pronounced differences between dietary fat sources and should be considered alongside fatty acid composition during diet formulation.

Dietary fat source was not shown to influence the occurrence of breast muscle myopathies but did affect other carcass quality measurements (Table 5). Differences were observed between the dietary treatments for meat yellowness ($P < 0.001$) and drip loss ($P = 0.005$). The increase in yellowness exhibited by the OO treatment is likely a result of the increased carotenoid content in olive oil when compared to the other fat sources (Minguez-Mosquera et al., 1990). The difference in drip loss may have occurred due to the higher levels of omega-3 and -6 fatty acids noted for SO and OO. Decreased drip loss has been associated with leaner breast meat (Le Bihan-Duval et al.,

1999; Berri et al., 2008) and intramuscular fat has been shown to decrease with diets rich in USFA (Wongsuthavas et al., 2008), particularly those high in omega-3 and 6 fatty acids (Qi et al., 2010). These results suggest that while dietary fat sources rich in USFA may offer some improvements in meat quality, carcass weights and yields do not appear to be significantly impacted.

Treatment Interactions

Significant interactions between the main treatments were not noted during the rearing phase prior to AHS (Table 3), nor in live weight and the percentage of carcass portions (Table 4) or carcass quality (Table 5). However, dietary fat source was observed to interact with the incubation treatments for post-heat stress FCR and p. major weights. The highest FCR was noted for the CN-OO birds while CN-PF and TM-SO resulted in the lowest FCR ($P = 0.005$) while the remaining interactions served as intermediates; a similar trend was noted for BWG ($P = 0.054$). For p. major weights, the PF diet within the CN and TM groups resulted in the heaviest (956.4 g) and lightest (858.5 g) portion weights, respectively ($P = 0.016$, Table S1).

Within the interactions, it would appear that the dietary fat sources were metabolized differently for the incubation treatments following AHS. Thermal manipulation in broilers is associated with a decreased body temperature, thought to arise from lower plasma thyroid hormone and decreased overall metabolism (Collin et al., 2005; Piestun et al., 2008, 2013, 2015; Loyau et al., 2013). Lipid metabolism may be particularly altered as decreased mRNA expression of lipase and fatty acid transporters have been noted in birds incubated under increased temperatures (Al-Zghoul et al., 2019). While these adaptations may reduce growth under standard rearing temperatures, the decreased metabolism associated with TM may allow the bird to better survive heat stress. Loyau et al. (2014) observed that the mRNA expression for enzymes involved in mitochondrial metabolism were less severely downregulated in birds exposed to TM compared to control birds during AHS challenge. Similarly, the expression of digestive enzymes and intestinal nutrient transporters has been shown to be upregulated upon initial exposure to heat stress in TM birds compared to controls (Al-Zghoul et al., 2019). Bird energy requirements are increased by heat stress (Hurwitz et al., 1980) and this increased demand along with differences in lipid metabolism between the incubation treatments may have exacerbated the differences between dietary fat sources. Specifically, the PF diet appeared to depress FCR, BWG, and p. major weights for the TM birds while improved values were noted for the CN-PF birds. Saturated fatty acids tend to be less readily absorbed and utilized by the bird, resulting in increased fat deposition (Sanz et al., 2000; Baião and Lara, 2005). It may be speculated that if the TM birds experienced a reduction in lipid metabolism due to their adaptation to heat stress, they may have been more negatively affected by the greater concentration of SFA in the PF diet. Along with the reduced BWG and carcass portions observed by the TM birds throughout the study, this may have led to the interactions noted between dietary fat sources within the incubation treatments.

The unexpected decrease in feed efficiency noted for CN-OO does not appear to agree with the above results and may be due to changes in mitochondrial function following heat stress. Avian uncoupling protein (**avUCP**) is a mitochondrial transport protein purported to be involved in the modulation of free radicals (Raimbault et al., 2001; Criscuolo et al., 2005). During heat stress, avUCP is downregulated which may contribute to the increased production of reactive oxygen species (Mujahid et al., 2007). The expression of avUCP mRNA appears to be sensitive to dietary fat source such that SFA results in downregulation while avUCP upregulation is associated with USFA (Seifi et al., 2018), concurrent with a decrease in mitochondrial superoxide production (Mujahid et al., 2009). However, as with TM, mitigating the damage of heat stress may come at the cost of production performance. Uncoupling proteins function as a bypass of ATPase in respiratory oxidation, negating ROS production but also limiting ATP production (Mailloux and Harper, 2011). If avUCP was upregulated in the current trial by OO, it may

have reduced feed efficiency in the birds due to increased mitochondrial uncoupling (Ojano-Dirain et al., 2007). The effect of avUCP may have been less apparent in the TM-OO birds due to the decreased thyroid hormone levels associated with TM, as avUCP is directly influenced by thyroid levels (Collin et al., 2003). Further work in our laboratory is currently underway to clarify if avUCP mRNA expression differed between treatments in the present results.

A limitation of the current trial is the absence of a control group that was not subjected to heat stress, due to facility constrains. The inclusion of such a control group may have provided further insight into how the incubation and dietary treatments, as well as their interactions, were altered independent of AHS.

CONCLUSIONS AND APPLICATIONS

1. Broiler performance and carcass yields were not enhanced by TM, however the occurrence of breast muscle myopathies was reduced in TM birds. Further work is needed to balance bird growth with improvements in muscle morphology with emphasis on strain specific profiles.
2. Dietary fat source did not influence live performance, carcass weights or yield in the current trial. At the inclusion levels used here (4.5%), the negative effects of SFA may not be apparent on bird performance and carcass composition.
3. Interactions between the incubation and dietary treatments were only apparent post-AHS and may indicate a change in lipid metabolism for the TM birds. Future investigations into how TM adaptations alter metabolism would be beneficial in understanding how to optimize nutrition and bird performance during heat stress.

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SUPPLEMENTARY MATERIALS

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