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Heat stress prevention and performance: Evaluation of embryonic thermal manipulation and dietary fat source in market age broilers

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Heat stress continues to be a major economic issue for the poultry industry, particularly for modern commercial strains. Genetic selection for high-yield broilers has led to a disproportionate increase in body mass relative to body surface area, which has resulted in reduced heat dissipation and higher basal body temperatures (Yalçin *et al*., 2001; Yahav *et al*., 2005).

This imbalance between heat production and loss is particularly apparent in birds approaching market age, with higher rates of heat stress mortality being associated with heavier bodyweights (St-Pierre *et al*., 2003). Birds that survive high temperatures may still incur production losses due to decreased carcass quality, as hyperthermia is associated with increased fat deposition (Baziz *et al*., 1996) and undesirable meat characteristics (Sandercock *et al*., 2001).

Although several strategies have been proposed to support broiler performance during high ambient temperatures, no intervention has been shown to completely ameliorate the negative effects of heat stress. Variations in environmental conditions and individual bird characteristics make it difficult to adequately predict and prevent the negative effects of heat stress (Lara and Rostagno, 2013).

Combining heat stress strategies may better address performance problems associated with hyperthermia, but research evaluating interactions between interventions is needed to determine their impact on broiler production during heat stress.

Background

Supplementation of dietary fat is a common practice during heat stress, as fat provides increased energy at a lower heat increment when compared to carbohydrates or protein (Dale and Fuller, 1979). Conventionally, dietary fat is chosen based on cost of inclusion or availability; however, the fatty acid profile of the fat source may offer improvements in performance.

Unsaturated fatty acids (USFA) are more readily encapsulated into micelles when compared to saturated fatty acids (SFA), resulting in improved gut absorption and increased substrate availability for β-oxidation (Garrett and Young, 1975; Sanz *et al*., 2000). Under standard rearing temperatures, diets rich in USFA have been reported to improve bodyweight gain and feed efficiency in broilers, concurrent with a reduction in abdominal fat deposition (Zollitsch *et al*., 1997; Crespo and Esteve-Garcia, 2002); however, it is unclear if these benefits are sustained during heat stress.

During hyperthermia, energy requirements are increased as the bird struggles to maintain thermal homeostasis (Hurwitz *et al*., 1980) and despite reduced feed intake, fat deposition may be increased as protein retention is decreased (Gonzalez-Esquerra and Leeson, 2005). These negative effects may be amplified in older birds as energy requirements and susceptibility to heat stress are increased with bird age and bodyweight (Sakomura *et al*., 2004).

Given the improvements in broiler performance associated with USFA under standard temperatures, evaluation of fat sources differing in fatty acid composition is needed to determine if similar benefits can be obtained during heat stress; particularly during the finisher stage where risk for economic loss is greatest (St-Pierre *et al*., 2003).

Acclimation to mild increases in temperature is another heat stress management strategy; however, production losses may still occur due to the narrow thermal tolerance range of modern broiler strains (Yalçin *et al*., 2001; Abdelqader and Al-Fataftah, 2014). Embryonic acclimation or thermal manipulation (TM) during incubation may provide a viable alternative by exposing the embryo to cyclic high temperatures during key developmental periods (Moraes *et al*., 2003).

Approximately mid-way through incubation, the hypothalamo-pituitary-thyroid and -adrenocortical axes (HPT and HPA, respectively) coalesce and the embryo begins to respond to environmental temperature through metabolic changes (Jenkins and Porter, 2004; McNabb, 2007). As full homeothermy is only achieved post-hatch, there exists a high degree of plasticity in these emerging thermoregulatory systems and increased temperature exposure during this period has been suggested to alter the 'set points' of these axes (Nichelmann and Tzschentke, 2002).

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

Ingredients (%)	Starter	Grower	Finisher
Maize	49,93	58,89	62,65
Soya bean 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			
Metabolisable 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, soya bean 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 3,180kcal/kg (PF), 3,203kcal/kg (SO) and 3,208kcal/kg (OO). [2] Coban® 90 (Monensin) (Elanco Animal Health, Greenfield, IN) at 90g/ton of feed. [3] Trace minerals provided per kg of premix: manganese (MnO_2), 220g; zinc (ZnO and $ZnSO_4$), 250g; iron ($FeCO_3$), 75g; copper (CuSO4 and $CuCl_2$), 10g; iodine ($Ca(IO_3)_2$), 5g; selenium (Na_2SeO_3), 1g. [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, 33mg; riboflavin, 22,046mg; niacin, 88,185mg; d-pantothenic acid, 30,865mg; menadione, 3,968mg; folic acid, 2,646mg; vitamin B6, 7,716mg; thiamine, 5,512mg; biotin, 176mg. [5] Quantum Blue 5G® 5 at 0,20lbs/ton (100g/ton) to provide 500 FYT (AB Vista, Marlborough, UK) delivering 0,13% of available P, 0,06% of calcium and 0,03% of sodium.

Table 2: Fatty acid profile and nutritional parameters of the dietary fat sources1.

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.

Broilers exposed to TM during incubation have been reported to exhibit lower body temperature, decreased circulating thyroid hormones and improved tolerance to heat stress, suggestive of a reduced response from the HPT and HPA axes (Yahav *et al*., 2004; Piestun *et al*., 2011; Loyau *et al*., 2013; Al-Rukibat *et al*., 2017). Other work has suggested carcass composition may also be improved by TM, as increased breast yield has been reported in TM birds (Collin *et al*., 2007; Piestun *et al*., 2011; Loyau *et al*., 2013).

Satellite cell populations associated with myoblasts also begin to proliferate midway through incubation and may be stimulated by the increased temperatures implemented during TM (Halevy, 2020). In addition to muscle hypertrophy, increased satellite cells may potentially contribute to the repair and regeneration of breast muscle myopathies such as wooden breast and white striping (Stockdale, 1992; Velleman, 2015).

However, the potential benefits of TM remain controversial. While exposure to high temperatures during TM is cyclic, it is well established that deviations from the accepted range of 37,8°C can be determinantal to embryonic development (Wilson, 1991). Further work is needed to elucidate the risks and potential benefits of TM before it can be commercially applicable.

The objective of this trial was to determine the influence of TM and dietary fat source on broiler performance in birds exposed to heat stress during the finisher phase. Carcass yield was also evaluated along with the occurrence of breast muscle myopathies due to the economic importance of the breast as a premium portion.

Materials and methods

The trial was conducted at the North Carolina Department of Agriculture's Piedmont Research Station with

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

	28 to 42 days, pre-heat stress				43 to 49 days, post-heat stress			
	BWG, g/bird	FI, g/bird	FCR, g:g	Mortality, %	BWG, g/bird	FI, g/bird	FCR, g:g	Mortality, %
Incubation[2]								
CN	1 213,9^{A}	2 058,5^{A}	1,79	1,1	669,9	1 405,5^{A}	2,08	18,5^{A}
TM	1 154,5^{B}	1 974,6^{B}	1,71	0,9	652,0	1 329,9^{B}	2,04	9,3^{B}
SEM[3]	12,2	18,0	0,01	0,5	11,0	16,0	0,03	2,2
P-Value	0,001	0,002	0,292	0,725	0,256	0,001	0,227	0,006
Diet[4]								
Olive	1 186,4	2 028,0	1,71	1,0	637,1	1 357,9	2,11	17,2
Poultry	1 175,7	2 016,3	1,71	0,9	680,2	1 381,6	2,04	12,4
Soya	1 190,4	2 005,3	1,68	1,0	665,5	1 363,6	2,06	12,1
SEM	15,0	22,0	0,01	0,6	13,4	19,3	0,03	2,7
P-Value	0,774	0,765	0,189	0,997	0,081	0,670	0,196	0,309

[1] Acute heat stress occurred at 32°C for 4 hours at 43 days. [2] TM = thermal manipulation at 39,5°C and 65% RH for twelve hours 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. ^{A,B} Means in a column that possess different superscripts differ significantly ($P < 0,01$).

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

	Cold carcass weight, g	Fat pad, %	Thighs, %	Wings, %	Breast, %	White stripe, ordinal scale	Wooden breast, ordinal scale
Incubation[2]							
CN	3 190,1^{a}	1,39^{A}	28,89	9,30	34,06	1,96^{A}	1,95^{A}
TM	3 070,9^{b}	1,20^{B}	28,72	9,27	34,29	1,71^{B}	1,70^{B}
SEM[3]	35,7	0,04	0,16	0,07	0,20	0,06	0,06
P-Value	0,019	0,002	0,432	0,765	0,429	0,002	0,006
Diet[4]							
Olive	3 147,4	1,29	28,69	9,28	34,09	1,90	1,93
Poultry	3 178,9	1,37	28,79	9,23	34,12	1,86	1,81
Soya	3 065,1	1,22	28,93	9,33	34,31	1,74	1,73
SEM	43,7	0,05	0,18	0,08	0,25	0,07	0,08
P-Value	0,178	0,175	0,664	0,674	0,802	0,263	0,186

[1] Acute heat stress occurred at 32°C for four hours at 43 days. [2] TM = thermal manipulation at 39,5°C and 65% RH for twelve hours 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. ^{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 ($P < 0,05$).

approval from the Research Ethics committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria (NAS200/2020). A total of 3 480 eggs (average egg weight 68,5 ± 1g) were selected from a 43-week-old Ross 708 flock and equally allocated between the control (CN) or thermal manipulation (TM) groups.

All eggs were initially incubated within the same setter at 37,5°C and 56% relative humidity (RH) until incubation day seven, when half of the trays were moved to an adjacent incubator set at 39,5°C and 65% RH for TM application. Following the twelve-hour exposure, TM trays were returned to the CN setter for twelve hours and this cycle continued from incubation day seven to 16 (Brannan *et al*., 2021a).

Hatching occurred in a single machine, after which first grade chicks from both groups were sexed and placed equally in a 60-pen environmentally controlled broiler house, with 18 chicks per pen. A randomised complete block design with the six treatments (two incubation profiles × three finisher diets) was used for pen allocation, with each treatment combination being replicated ten times within the house. Mortalities were recorded daily while bodyweight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined per pen on a weekly basis.

Standard commercial starter and grower diets were fed until 14 days and 28 days, respectively (*Table 1*). Fat source treatments were applied during the finisher period beginning at 28 days and 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).

Analysis was conducted for free fatty acids (method Ca 5a-40, AOCS, 2017) as well as SFA and USFA concentrations (method 996,06, AOAC, 2012) as shown in *Table 2*, and energy values were determined for each fat source using the Wiseman equation for birds older than 21 days (Wiseman and Salvador, 1991; Wiseman *et al*., 1998). Diets were formulated based on the energy contribution of each fat source at an inclusion level of 4,5%, with final dietary ME being 3,180 kcal for the PF diet, 3,203 kcal for the SO diet and 3,208 kcal for the OO diet. All diets were formulated to meet or exceed NRC (1994) requirements.

Lighting and temperature profiles were applied as per standard commercial practice (Aviagen, 2018) until the acute heat challenge at 43 days, when temperatures within the house were gradually increased from 21°C to 32°C for four hours. Following heat stress, standard temperature profiles were implemented until the end of the study at 49 days.

Male birds were selected for sampling to represent average treatment BW and processing occurred in a small-scale abattoir as described by Livingston *et al*. (2019). Cold carcass weight was assessed along with portion yields of abdominal fat pad (AFP), thighs, wings and breast (*Pectoralis* major and minor). The occurrence of wooden breast (WB) and white stripping (WS) were determined using a 1-to-4-point ordinal scale as outlined in Brannan *et al*. (2021b), with 1 indicating normal tissue and 4 representing severe WB or WS.

Statistical analysis

Data were analysed as a one-way ANOVA with incubation treatment as the main effect until 28 days, after which fat source treatments were introduced and data were analysed as a two-way ANOVA. Mortality data were subjected to Box-Cox transformation ($\lambda = -0,440$) to improve normality prior to analysis, though untransformed means are presented here.

Carcass data were also analysed as a two-way ANOVA using the mixed procedure of JMP. Pen served as the experimental unit for rearing data, while the individual bird was the experimental unit for carcass data. Differences between means were separated using Tukey's HSD test (Tukey, 1949) and significance was determined at $P < 0,05$.

Results and discussion

Bodyweight gain and feed intake were significantly decreased by TM during the starter and grower period (Brannan *et al*., 2021a), as well as during the finisher period prior to heat stress ($P < 0,01$; *Table 3*). Despite the cyclic exposure to high temperatures, hatchability was decreased by approximately 12% in the TM group (Brannan *et al*., 2021a).

Increased incubation temperatures have been shown to negatively impact post-hatch broiler performance and although TM exposure was cyclic, the stress was sufficient to impair embryo development.

While TM birds did demonstrate improved liveability following AHS and a decreased occurrence of breast muscle myopathies, these improvements may also be attributed to the reduced growth reported for this group. Ultimately, TM may not ameliorate the negative effects of hyperthermia but may instead shift the economic burden of heat stress.

While the reduced mortality and improved carcass quality following heat stress may be advantageous, it remains to be seen if these benefits are sufficient to offset the reduced hatchability and growth associate with TM. As such, until pre-heat stress performance is improved, TM is unlikely to be a commercially accepted heat stress intervention.

Differences in dietary fat source were not apparent during the finisher period nor at processing (*Table 4*). While younger birds tend to be more sensitive to increased SFA levels due to decreased lipase secretion (Noy and Sklan, 1995), performance in older birds has been shown to be altered by dietary fatty acid composition as well (Pinchasov and Nir, 1992; Crespo and Esteve-Garcia, 2002; Newman *et al*., 2002).

However, the inclusion level in these trials was also 6%, while the present treatments were only supplemented at 4,5% to be commercially relevant. Other authors have suggested that in older birds, differences in dietary fatty acid profiles may only be apparent at higher inclusion rates (Wiseman and Salvador, 1991; De Witt *et al*., 2009) and the results here appear to support this proposal.

Future work exploring the relationship between inclusion level and bird age would help identify optimal fat source combinations and improve broiler performance.

Conclusion

Ultimately, these data suggest that heat stress remains a problem for which there are no easy answers. While TM did significantly reduce heat stress mortality, further research is needed to ensure these improvements do not occur at the cost of hatchery and rearing performance.

Dietary fat source does not appear to offer any advantages during AHS, but results may vary with bird age and inclusion level. Given the wide variety of factors that determine the severity of heat stress, successful interventions may also need to be developed based on individual circumstances if both broiler performance and survival are to be optimised.

The decrease in BWG for TM birds was also apparent in the lower cold carcass weight at slaughter (*Table 4*); however, the relative portion yields were similar to CN birds. ❖

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