

Broiler performance and avian uncoupling protein mRNA expression during acute heat stress as influenced by thermal manipulation and dietary fat source.

by

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SUMMARY

Acute heat stress (**AHS**) in broilers results in reduced performance, increased mortality, and economic loss for producers. The effects of AHS are intensified as birds approach market age, as susceptibility to hyperthermia increases with age and body weight. Embryonic acclimation to high temperatures through thermal manipulation (**TM**) has been proposed to improve bird thermal tolerance post-hatch and may enhance breast muscle yield. However, high incubation temperatures can be detrimental to embryonic development and subsequent post-hatch growth and it is unclear if the cyclic application of TM is sufficient to reduce these negative effects. Dietary supplementation with unsaturated fatty acids (**USFA**) has also been demonstrated to improve bird performance and reduced fat deposition under standard rearing conditions, although it is unclear if these benefits remain during AHS. Both TM and USFA may also contributed to a reduction in oxidative stress, possibly through the upregulation avian uncoupling protein (**avUCP**).

Ross 708 hatching eggs were exposed to a TM protocol of 39.5°C for 12 h daily from incubation days 7 to 16. Standard commercial diets were offered during until 28 d, and evaluation of dietary fat occurred during the finisher period. Dietary treatments consisted of fat sources varying in fatty acid composition and were formulated to include either soya oil, poultry fat, or olive oil at 4.5 % each. At 42 d, AHS was imposed, and a sample of male birds were selected for processing at 49 d.

Increased eggshell temperatures were observed in the TM group, resulting in an increase in embryo mortality and decrease in hatchability. Although chick weight and yolk-free body mass were similar at hatch, TM birds exhibited decreased feed intake and body weight for most of the rearing period. Livability was approximately double for TM birds following AHS,

although it is unclear if this was due to TM adaptation or smaller BW. Carcass and portion weights were decreased for TM birds, as was the occurrence of breast muscle myopathies; however, as this advantage may be related to the reduced growth demonstrated by the TM birds. Dietary fat source was not shown to significantly alter broiler performance or carcass characteristics. An interaction between the treatments was noted following AHS and may suggest modifications to lipid metabolism arising from TM.

Neither TM nor dietary fat source influenced avUCP mRNA expression or oxidative stress as measured by concentration of thiobarbituric acid reactive substances (**TBARS**). Exposure to AHS resulted in the downregulation of avUCP concurrent with increased TBARS, which may support the role of avUCP in free radical regulation. Males demonstrated a higher level of avUCP expression as well as increased TBARS, which may be associated with their increased growth rate and susceptibility to oxidative stress as compared to females. This is the first report of sex differences in avUCP mRNA expression in broilers and future work into heat interventions may benefit from increased focus on male birds.

DECLARATION

I, **Kelly Elizabeth Brannan**, declare that the thesis, which I hereby submit for the degree PhD – Animal Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institute.

Signature:

Date:

DEDICATION

This work is dedicated to Dr. Carmen “Carm” Parkhurst (1942 – 2019) who sparked my interest in poultry science and Dr. John T. Brake (1952 – 2018) who fostered my enthusiasm for research.

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To my parents, thanks for your support, love, and free dinners for when I forget to eat properly. To my sister, thank you for continuing to force me to set the bar higher. To my husband, thank you for encouraging me each step of the way and reminding me to have fun along the way.

PREFACE

This thesis is based on work conducted by me at the North Carolina Department of Agriculture's Piedmont Research Station, under the supervision of Dr. Christine Jansen van Rensburg through the Department of Animal Science, University of Pretoria. The study has been divided into chapters, which are written as separate manuscripts for publication in peer reviewed journals.

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LIST OF ABBREVIATIONS

a*	Meat color score for redness
AFP	Abdominal fat pad
AHS	Acute heat stress
ANOVA	Analysis of variance
avUCP	Avian uncoupling protein
b*	Meat color score for yellowness
BW	Body weight
BWG	Body weight gain
C	Celsius
CN	Control
Cort	Corticosterone
d	Day of age, post-hatch
E	Day of embryo age during incubation
EST	Eggshell temperature
FCR	Feed conversion ratio
FI	Feed intake
g	Gram
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HOF	Hatch of fertile
HPA	Hypothalamo-pituitary-adrenocortical axis
HPT	Hypothalamo-pituitary-thyroid axis
L*	Meat color score for lightness
MDA	Malondialdehyde
NST	Non-shivering thermogenesis
OO	Olive oil treatment
p. major	Pectoralis major
p. minor	Pectoralis minor
PF	Poultry fat treatment
RH	Relative humidity
ROS	Reactive oxygen species
RT-qPCR	Real-time quantitative polymerase chain reaction
SB	Spaghetti breast
SFA	Saturated fatty acid
SO	Soy oil treatment
T3	Triiodothyronine
Tb	Body temperature
TBARS	Thiobarbituric acid reactivate substance
TH	Thyroid hormone
TM	Thermal Manipulation
UCP	Uncoupling protein
USFA	Unsaturated fatty acid
WB	Wooden breast
wk	Week
WS	White striping

INTRODUCTION

Global population growth is projected to reach 9 billion over the next three decades, concomitant with a rise in food demand and decreased agricultural land due to increasing urbanization (FAO, 2017). Poultry production is currently the leading animal protein source globally and is projected to increase in importance due to its affordability, convenience, and consumer demand (FAO, 2017; Mottet and Tempio, 2017). Intensive genetic selection in broilers over the past several decades has resulted in a highly efficient bird that exhibits a 400% higher growth rate concurrent with a 50% lower feed conversion ratio as compared to its 1950s predecessor (Zuidhof, et al., 2014). However the push for higher growth rate and carcass yield has not been matched by similar developments in the birds physiological supports systems (Havenstein, et al., 2003a, 2003b), resulting in an increased susceptibility to stress. At the pedigree level, such physiological imbalances may not be apparent as breeding stock are typically managed in environmentally controlled facilities; however their progeny may be exposed to a wide variety of rearing conditions and lack the physiological plasticity to adapt (Daghir, 2008). Modern broilers appear to exhibit heightened sensitivity to even minor deviations in rearing conditions and may be more prone to stress as excessive selection for production traits has reduced the metabolic capacity for stress response (Rauw, et al., 1998; Cahaner and Deeb, 2000).

Heat stress in particular is a major issue within poultry production, as the increased growth rate of commercial breeds results in higher body temperatures yet the increased body mass relative to surface area has resulted in decreased capacity for sensible heat loss (Yalcin, et al., 2001; Yahav, et al., 2005). In the United States alone, average annual losses associated with heat stress in poultry have been estimated to be approximately \$150 million (St-Pierre, et al., 2003); however given the increases in broiler growth rate over the past two decades, the current cost is likely higher (NCC,

2021). Although environmentally controlled housing is common in the United States, in developing countries with warmer climates such facilities are less common due to cost and availability of resources (Glatz and Pym, 2013). Where climate control houses are available, heat stress can still be an issue as stocking densities may be increased by producers to recoup investment costs (Samarakoon and Samarasinghe, 2012). Poultry production is currently the leading agricultural sub-sector, particularly in developing countries as the poultry industry leads the global meat market in providing affordable protein (Mottet, et al., 2017; OECD/FAO, 2020). Therefore, the development of practical heat stress interventions is needed if production is to be maximized or losses associated with heat loss at least minimized.

Although several strategies for managing broilers during heat stress have been proposed, no solution has been demonstrated to be completely effective; particularly in cases of acute heat stress, when ambient temperature rises rapidly over a short period of time and the bird may be unable to adequately adjust (Teeter and Belay, 1996). Variations in environmental factors (such as humidity, ventilation, etc...) and individual bird characteristics (such as age, body weight, etc...) also make it difficult to predict the severity with which heat stress may impact bird performance and which intervention may be most appropriate (Lara and Rostagno, 2013). Additionally, heat stress management strategies may also reduce bird performance, although to a lesser extent than heat stress itself. Feed restriction, acclimation to moderate heat stress, and inclusion of slower growing genetic stock may reduce the negative effects of AHS, but also diminish broiler performance under normal rearing conditions (Teeter, et al., 1996; Lin, et al., 2006b; Saeed, et al., 2019). Therefore, there exists a need to develop heat stress interventions that promote optimal broiler performance under standard conditions and ameliorate (or at least minimize) losses during heat challenges.

The modification of existing methods such as acclimation and dietary fat supplementation may provide such an opportunity. Acclimation to heat stress during the perinatal stage, when birds are more tolerant of high temperatures, may allow increased thermotolerance without the decrease in performance noted in older birds (Arjona, et al., 1988; Iqbal, et al., 1990; Abdelqader and Al-Fataftah, 2014). Furthermore such early adaptation to high temperatures may have positive effects on broiler performance and carcass composition (Piestun, et al., 2013; Al-Rukibat, et al., 2017). Similarly the addition of dietary fat during heat stress is a common heat stress management tool (Dale and Fuller, 1979) that may potentially improve bird performance under standard rearing temperatures with the inclusion of fat sources rich in unsaturated fatty acids (Pinchasov and Nir, 1992). Further synergy may exist in combining these strategies to develop a multiprong approach, such as addressing the physiological challenges of heat stress via acclimation concurrent with the nutritional challenges of reduced feed intake and increased energy demand with fat supplementation. However further research is needed to determine the efficiency of these strategies before they can be considered commercially viable.

Additionally, successful heat stress interventions must consider the negative impact of oxidative stress (Lin, et al., 2006a). Management strategies during heat stress primarily focus on production variables such as body weight gain, feed intake, and mortality, as these are easily quantified and economically significant. However oxidative stress may represent a less obvious but no less detrimental cost. Birds surviving heat challenge may suffer from oxidative damage arising from mitochondrial dysfunction and increased free radical production (Akbarian, et al., 2016). While reactive oxygen species (**ROS**) occur during normal metabolic activity, excessive levels may result in lipid and protein peroxidation, as well as cell death (Cross, et al., 1987). These effects may not be visible to producers, but can decrease carcass quality and result in losses at

processing (Song and King, 2015; Zaboli, et al., 2019). Avian uncoupling protein (**avUCP**) is a recently discovered protein that may contribute to the regulation of ROS through increased mitochondrial uncoupling and proton conductance (Raimbault, et al., 2001); however, acute heat stress results in the downregulation of avUCP, which may contribute to the high levels of ROS observed during heat stress (Mujahid, et al., 2006). Recent work has suggested that existing heat stress interventions may contribute to the upregulation of avUCP and support a reduction in oxidative stress as well as upholding production performance. Both prenatal acclimation (Al-Zghoul, et al., 2019) and dietary supplementation with unsaturated fatty acids (Mujahid, et al., 2009) have been associated with higher avUCP mRNA expression during heat challenge. While these results are promising, further research is needed to evaluate if avUCP expression is increased sufficiently to limit oxidative damage as well as to determine if broiler performance is enhanced.

Overall, further work is needed to evaluate the effectiveness of heat stress interventions in preparing birds for high temperatures without sacrificing performance. The deleterious effects of oxidative stress must also be considered for successful heat stress management, as broiler performance extends to carcass quality as well. Modifications of existing strategies such as acclimation and fat supplementation have presented promising results, but further research is needed to establish the impact of these interventions on bird growth, carcass composition, and oxidative stress. Additionally, the potential for synergy between heat stress solutions may offer more comprehensive protection from the negative effects of AHS and requires further investigation.

In this thesis, the effects of combining heat stress strategies during acute heat stress on broiler performance is evaluated. The negative effects of heat stress are discussed in Chapter 1, along with an overview of embryonic thermal manipulations and dietary fat supplementation as

heat stress interventions. The role of avian uncoupling protein in oxidative stress is also considered along with the potential for its upregulation by heat stress interventions. In Chapter 2, the effects of thermal manipulation and dietary fat source on hatchability, embryonic development, and live performance are presented with a discussion on the commercial applicability of these strategies. Chapter 3 evaluates carcass characteristics as influenced by thermal manipulation and dietary fat, with emphasis on the occurrence of breast muscle myopathies. The influence of the previously discussed heat stress interventions as well as bird sex and heat stress itself on avian uncoupling protein and oxidative damage are considered in Chapter 4. This chapter is the first to report sexual dimorphism in avian uncoupling protein mRNA expression and may explain previous discrepancies in the literature as to the role of this protein during heat stress. Finally, in Chapter 5 an overview of thermal manipulation and dietary fat source as viable heat stress interventions is given. Future research topics concerning avian uncoupling protein and oxidative stress in relation to heat stress are discussed as well as reinterpretations of existing literature based on bird sex. Conclusions of the present findings along with potential applications of this research are summarized and a final overview of the work is presented.

CHAPTER 1

Literature Review

1.1 Heat stress in the broiler chickens

Intensive genetic selection in broilers over the past several decades has resulted in birds with increased growth rate, improved feed efficiency, and heavier body weights (Havenstein, et al., 2003a). However, these advances have not been matched with similar developments in the physiological support systems of the bird (Havenstein, et al., 2003b). Consequently, producers must carefully balance maximizing the genetic potential of modern broilers while minimizing physiological stress that can limit bird growth. Heat stress has become a growing concern for managing modern broilers as selection for rapid growth rate and increased body weight (**BW**) has inadvertently resulted in reduced heat tolerance (Cahaner, et al., 2000). Broilers selected for rapid growth rate demonstrate greater metabolic activity and increased heat production, resulting in a higher basal body temperature (Yalcin, et al., 2001). Likewise, the larger body mass relative to surface area exhibited by commercial broiler strains selected for high carcass yield has resulted in a decreased capacity for sensible heat loss (Yahav, et al., 2005). Despite the increased risk of hyperthermia associated with rapid growing broilers, future genetic selection will likely continue to focus on maximizing bird growth and feed efficiency (Tavárez and Solis de los Santos, 2016). As such, the development of strategies to successfully manage these birds under heat stress conditions must also be a priority if broiler genetic potential is to be achieved.

Heat stress is characterized by an imbalance between heat production and heat loss resulting in increased body temperature. Ambient temperature is generally the primary consideration for inducing heat stress, however individual bird characteristics such as age, BW, and growth rate also contribute to how the bird responds to high temperatures and the severity of

the imbalance (Lara, et al., 2013). The duration of high temperature exposure also plays a role in heat stress response: chronic heat stress (**CHS**) is generally defined as exposure to high ambient temperatures for an extended period and allows birds to acclimate, while acute heat stress (**AHS**) is characterized by a sudden, rapid increase in temperature over a short period of time, with no opportunity for acclimation. In broilers approaching market weight, AHS can be particularly detrimental as susceptibility to heat stress is increased with bird age and BW (St-Pierre, et al., 2003). Mortality rate associated with AHS has been observed to be over 20 times higher as compared to CHS, resulting in significant economic loss due to the failure to recoup rearing cost at processing (Rodrigues, et al., 2019). Surviving birds may represent further economic loss as reductions in meat quality and increased fat deposition have been associated with AHS (Sandercock, et al., 2001; Gonzalez-Esquerria and Leeson, 2005; Wang, et al., 2009). Prior acclimation to AHS with milder heat exposure has been suggested to reduce the negative impacts of severe heat challenges, but may also result in economic loss due to reduced bird performance during the acclimation period (Teeter, et al., 1996; Abdelqader, et al., 2014). Therefore, heat stress interventions must maintain a balance between sustaining bird performance with minimizing economic loss in order to be successful.

1.2 Thermal manipulation

While acclimation to heat stress in post-hatch broilers can result in reduced performance, acclimation during the perinatal period when chicks are more tolerant of high temperatures may be beneficial. Chicks exposed to AHS at 5 d of age demonstrated a significant reduction in mortality when exposed to similar temperatures at market age, with no negative effects on BW or feed efficiency (Arjona, et al., 1988). However, the increased heating cost associated with early age acclimation and logistics of ensuring uniform temperature exposure may not be practical in

commercial facilities. Acclimation during incubation has been demonstrated to similarly improve bird thermotolerance post-hatch and may be a more practical solution. Several authors have reported embryos exposed to high incubation temperatures to be less negatively impacted by heat stress during rearing (Iqbal, et al., 1990; Moraes, et al., 2003; Yahav, et al., 2004b; Collin, et al., 2005; Piestun, et al., 2008a; Loyau, et al., 2015). Although the precise mechanisms behind this adaptation, termed thermal manipulation (**TM**), are not fully understood, three main factors have been identified to be influential: embryo age at application, temperature severity and exposure duration (Yahav, 2009).

In chickens, full homeothermy is only achieved 10 d post-hatch; however the neural and endocrine pathways necessary for thermoregulation begin to coalesce mid-way through embryonic development (Nichelmann and Tzschenke, 2002). The emergence of the hypothalamo-pituitary-thyroid and -adrenocortical axes (**HPT** and **HPA**, respectively) at incubation day (**E**) 15 are the first indications of thermoregulatory capacity and allow the embryo to respond to environmental temperature change through alterations in metabolic rate (Jenkins and Porter, 2004; McNabb, 2007). Post-hatch, both axes contribute to managing the bird's response to heat stress: the HPT axis through altering metabolism and body temperature via thyroid activity and the HPA axis via corticosterone secretion in response to stress (Debonne, et al., 2008). Theoretically, exposure to increased temperatures during the developmental stage of these axes may potentially alter the regulatory set points and reduce their response to similar temperatures post-hatch (Nichelmann, et al., 2002). Application of TM during the second week of incubation has been associated with decreased thyroid and corticosterone response during heat stress post-hatch, suggesting successful alteration of the HPT and HPA axes (Yahav, et al., 2004a; Piestun, et al., 2008b; Loyau, et al., 2013). Therefore, the second week of incubation has

been suggested as the ideal embryonic age for TM exposure as it coincides with HPT and HPA development.

The severity of high temperature exposure is also a critical factor in TM application, as it must be sufficient to induce adaptation but not be detrimental to the growing embryo. Optimal incubation temperature has been established to be 37.8°C and deviations from this value can negatively affect embryo development, resulting in a reduction in hatchability, chick quality, and subsequent broiler performance (Wilson, 1991; Hulet, et al., 2007; Leksrisompong, et al., 2007; Molenaar, et al., 2011). These negative effects are primarily associated with constant high incubation temperatures, while TM exposure is cyclic and may be less harmful (Piestun, et al., 2008a). Although improvements in hatchability, as well as thermotolerance, have been reported with TM (Yahav, et al., 2004b; Collin, et al., 2005), discrepancies exist within the literature as other studies have noted hatchability to be unaltered (Yahav, et al., 2004b; Piestun, et al., 2008a) or even decreased by TM (Moraes, et al., 2004; Collin, et al., 2007; Piestun, et al., 2013). Variations in TM protocol account for some of the inconsistencies, but even under the same protocol differences in hatchery performance have been demonstrated (Yahav, et al., 2004b; Piestun, et al., 2008a; Piestun, et al., 2009; Zaboli, et al., 2017). The most consistent improvements in thermotolerance occur with TM exposure of 39.5°C for 12 h daily from E 7 to E 16 (Collin, et al., 2005; Piestun, et al., 2008a; Piestun, et al., 2011; Zaboli, et al., 2017), however further research is needed to determine how this protocol impacts embryonic growth, hatchability, and post-hatch broiler performance.

Concurrent with improved heat tolerance, TM may also improve carcass composition as breast portion yield has been reported to be increased in TM birds (Collin, et al., 2007; Piestun, et al., 2011; Loyau, et al., 2013; Piestun, et al., 2013). Proliferation of fetal myoblasts and

satellite cells occurs during the second week of incubation, when TM application is optimal, and may be enhanced by increased incubation temperatures (Halevy, et al., 2006; Piestun, et al., 2015). Larger populations of myogenic cells appear to contribute to greater myofiber hypertrophy (Piestun, et al., 2009) while increased satellite cell populations associated with muscle fibers may also contribute to post-hatch muscle tissue repair and hypertrophic growth (Stockdale, 1992), both functions that may improve carcass yield and quality at processing. The breast portion is considered a premium cut, resulting in increased genetic selection for higher breast yields in modern broiler strains (Zuidhof, et al., 2014). However, muscle myopathies such as wooden breast (**WB**) and white striping (**WS**) have become more prevalent in high yield broilers during recent years as breast muscle growth exceeds the muscles' physiological capacity to support it. While the etiology of these disorders remains to be established, intensive genetic selection for increased muscle fiber diameter in the breast may contribute to their development. Larger muscle fibers decrease the available space for connective tissue and vascularization within the muscle, leading to muscle fiber degeneration and necrosis (Velleman, 2015). Satellite cells are activated by stress arising from muscle damage and can contribute to the repair and regeneration of the tissue, potentially reducing the damage associated with WB and WS (Asakura, et al., 2001; Hawke and Garry, 2001). The potential for TM to improve heat tolerance and breast muscle development in broilers would provide significant economic benefit to producers, however trial results have been somewhat inconsistent (see Table 1). From the literature, 39.5°C for 12 h daily from E 7 to E 16 (Collin, et al., 2005; Piestun, et al., 2008a; Piestun, et al., 2011; Zaboli, et al., 2017) has been observed to improve heat resistance without sacrificing hatchability or growth and is current the best candidate for further TM research.

Table 1.1. Summary of literature on embryonic thermal manipulation (TM) protocols, effect on performance¹, and response to heat stress².

Citation	Breed	TM temperature (°C)	Embryonic age (E) at TM	Duration of TM (h)	Hatch	Final BW	Condition of heat challenge (age/temperature/duration)	Response to heat stress
Yahav et al., 2004a	Ross PM3, 44wk	39.5	E8 to E10	3	=	=	3d/41°C /6h	- Tb
		41	E8 to E10	3	=	=		- Tb
		39.5	E16 to E18	3	+	=		- Tb,- Cort
		41	E16 to E18	3	+	=		- Tb
Collin et al., 2005	Ross PM3, 40wk	39.5	E16 to E18	3	=	=	3d/41°C /6h	- Tb
		39.5	E16 to E18	6	=	+		- Tb
		39.5	E16 to E18	12	=	+		- Tb
		39.5	E16 to E18	24	=	=		- Tb
Collin et al., 2007	Ross PM3, 43wk	39.5	E8 to E 10	3	+	=	42d/35°C/6h	+ mortality
		39.5	E16 to E18	3	+	=		+ mortality
		39.5	E8 to E1 and E16 to E18	3	-	=		+ mortality
Piestun et al., 2008a	Cobb, 39wk	39.5	E7 to E16	12	=	=	35d/35°C/5	- mortality, - TH, - Cort
		39.5	E7 to E16	24	-	-		- mortality, - TH, - Cort
Piestun et al., 2008b	Cobb, 37wk	39.5	E7 to E16	12	=	=	3d/41°C/3h or 10d/38°C /6h	- Tb, - TH, - Cort
		39.5	E7 to E16	24	-	-		
Piestun et al., 2011	Cobb, 31wk	39.5	E7 to E16	12	=	=	21 to 35d/32°C /12h daily	+ %breast, - %AFP, - Tb
Loyau et al., 2013	Cobb, 38 to 45wk	39.5	E7 to E16	12	=	-	34d/32C/5h	+ %breast, - %AFP
Zaboli et al., 2017	Ross 308, 36wk	39.5	E7 to E16	12	=	=	28 to 42d/26 to 34°C /14d	- mortality, - FCR, + BWG, - %AFP

¹ ND: no data; ‘-’ : TM exhibited significantly lower value as compared to control; ‘+’ : TM exhibited significantly higher value as compared to control; ‘=’ : TM values not statistically different as compared to control.

² TH: thyroid hormones; Cort: corticosterone; Tb: bird body temperature; %Breast: breast portion relative to carcass weight; %AFP: abdominal fat pad weight relative to carcass weight; FCR: feed conversion ratio; BWG: body weight gain.

1.3 Dietary fat source

At the onset of heat stress, feed consumption is decreased to limit metabolic heat production and restore body temperature balance (Cooper and Washburn, 1998), however energy demands are simultaneously increased as the bird attempts to maintain homeostasis (Hurwitz, et al., 1980). The subsequent metabolic imbalance results in reductions in body weight gain and feed efficiency along with economic loss due to delayed processing or increased feed costs (Liu, et al., 2020). Increased supplementation of dietary fat is a common heat stress intervention, as fat provides a greater energy density and lower heat increment in comparison to carbohydrates or proteins (Dale, et al., 1979; Teeter, et al., 1996). Conventionally, fat supplementation strategies during heat stress have focused on inclusion level, but fatty acid composition of the fat source may also offer opportunities for improved broiler performance. Fat sources rich in unsaturated fatty acids (**USFA**) are thought to be more readily utilized by the bird as compared to saturated fatty acids (**SFA**) due to their increased polarity, which allows for improved micelle formation and absorption (Garrett and Young, 1975; Baião and Lara, 2005). Concurrent with increased uptake, higher rates of β -oxidation have also been noted in birds fed USFA rich diets (Sanz, et al., 2000a), which may increase energy availability during heat stress despite reduced feed intake.

Differences in digestibility between USFA and SFA are most notable in young birds, which exhibit lower lipase activity and bile salt secretions that limit SFA utilization (Wiseman and Salvador, 1991; Noy and Sklan, 1995). Although capacity for fat digestion increases rapidly with age, AME for SFA remains lower in older birds when compared to USFA (Tancharoenrat, et al., 2013). The increased energy availability arising from USFA may be most beneficial in broilers approaching market age as energy requirements are increased with bird age and BW

(Sakomura, 2004). Susceptibility to hyperthermia and risk of economic loss is also highest prior to processing (Teeter, et al., 1996; St-Pierre, et al., 2003) and broilers exposed to heat stress during the finisher period may also benefit from increased dietary USFA supplementation. Improvements in performance under standard rearing temperatures have been reported for broilers fed higher concentrations of USFA during the finisher stage (Pinchasov, et al., 1992; Zollitsch, et al., 1997; Sanz, 1999; Crespo and Esteve-Garcia, 2001; Newman, et al., 2002), however it is unclear if these benefits are maintained during heat stress.

Similar to TM, increased dietary USFA inclusion may also have a positive effect on carcass composition. Decreased abdominal fat deposition has been reported in broilers fed diets with greater USFA levels when compared to diets richer in SFA, which were shown to increase fat deposition (Pinchasov, et al., 1992; Sanz, 1999; Sanz, et al., 2000b; Crespo and Esteve-Garcia, 2002). While decreased fat deposition associated with USFA has primarily been reported for birds under standard temperatures, it may be advantageous to heat stressed birds as well. Despite the reduction in feed intake during hyperthermia, fat deposition is increased during heat stress (Baziz, et al., 1996), with the effect being more notable in older birds (Gonzalez-Esquerro, et al., 2005). Aside from the reduction in feed efficiency associated with increased fat deposition, further losses can occur at processing due to carcass composition. Poultry meat is viewed by consumers to be a lean, healthy meat and excess carcass fat can reduce sales and consumption (Kennedy, et al., 2004; Tůmová and Teimouri, 2010). The potential for dietary fat source to increase energy availability as well as improve carcass composition would be of economic relevance to producers, particularly during periods of heat stress. However, reports of these improvements have currently been limited to standard rearing temperatures and further research is needed to evaluate the effect of USFA in birds exposed to heat stress.

1.4 Avian uncoupling protein

Oxidative stress is another consequence of hyperthermia in broilers that can contribute to poor performance and reduced carcass quality (Sandercock, et al., 2001; Lin, et al., 2006a; Song, et al., 2015). At the onset of heat stress, energy demand and mitochondrial activity are initially increased as the bird tries to maintain homeostasis (Hurwitz, et al., 1980). During oxidative phosphorylation, electron carriers from metabolic cycles occurring within the mitochondria are oxidized by a series of enzymes within the inner mitochondrial matrix. At each step, electrons are shuttled between complexes as protons are simultaneously pumped outside the matrix and an electrochemical potential is established across the membrane. The reentry of these protons into the inner mitochondrial matrix drives ATP synthase and results in the formation of water from molecular oxygen, which serves as the final electron acceptor. The reduction of oxygen during this process results in reactive oxygen species (**ROS**) intermediates, which during normal metabolic activity serve as signaling molecules or are fully reduced to water (Turrens, 2003; D'Autréaux and Toledano, 2007). However during periods of stress such as hyperthermia, increased metabolic activity concurrent with a decrease in antioxidant capacity can result in excessive free radical formation (Akbarian, et al., 2016). Excessive ROS accumulation can result in lipid and protein oxidation and eventual cell death (Cross, et al., 1987), negatively impacting broiler performance and carcass quality (Sandercock, et al., 2001; Lin, et al., 2006a; Wang, et al., 2009).

Uncoupling proteins (**UCP**) are mitochondrial carrier proteins located within the inner mitochondria matrix that may assist in the reduction of ROS by uncoupling oxidative phosphorylation via increased proton conductance (Ricquier and Bouillaud, 2000a). While bypassing ATP synthase may decrease ATP production, the reentry of protons into the matrix

decreases further free radical formation and limits oxidative stress (Brookes, 2005). The first UCP (thermogenin) was discovered in the brown adipose tissue (**BAT**) of mammals, where the energy resulting from the disruption of the electrochemical proton gradient contributes to non-shivering thermogenesis (**NST**) (Ricquier and Kader, 1976). Later discovery of additional UCP homologues in mammals prompted thermogenin to be renamed UCP1 and expanded the tissue distribution and functions of these proteins. While UCP1 is exclusive to BAT (Desautels, et al., 1978), UCP2 is ubiquitously expressed in mammals (Fleury, et al., 1997) and UCP3 is primarily expressed in both skeletal muscle and BAT (Boss, et al., 1997). Neither UCP2 nor UCP3 appear to contribute to thermogenesis, as knockout mice for these genes do not demonstrate cold sensitivity or a decreased capacity for non-shivering thermogenesis (Arsenijevic, et al., 2000; Vidal-Puig, et al., 2000). Instead, these UCP have been proposed to mediate ROS production, insulin regulation, and energy metabolism (Brand and Esteves, 2005). As UCP continue to be identified in a diverse range of species, their roles in mitochondrial regulation continue to expand (Ricquier and Bouillaud, 2000b).

Currently only one UCP has been characterized in avian species (**avUCP**) and has been suggested to contribute to thermogenesis as it shares approximately 55% homology with UCP1 (Raimbault, et al., 2001). Primarily expressed in skeletal muscle tissue (Evock-Clover, et al., 2002), avUCP expression has been reported to be upregulated during torpor in humming birds (Vianna, et al., 2001), cold exposed penguins (Talbot, et al., 2004), and cold acclimated ducklings (Teulier, et al., 2010). As birds lack BAT, skeletal muscle has been proposed as the primary tissue involved in avian NST (Duchamp and Barre, 1993) and upregulation of avUCP in muscle tissue has been noted in cold exposed birds concurrent with increased NST and heat production (Collin, et al., 2003a; Teulier, et al., 2010). Several studies have demonstrated

increased avUCP mRNA expression and protein levels with cold exposure (Ueda, et al., 2005; Dridi, et al., 2008; Teulier, et al., 2010) and vice versa, with avUCP downregulation occurring during acute heat stress (Taouis, et al., 2002; Mujahid, et al., 2006; Kikusato and Toyomizu, 2013). However discrepancies exist within the literature, with reports of avUCP expression being unchanged or decreased during cold exposure (Hirabayashi, et al., 2005; Mujahid, 2010) as well as upregulation being demonstrated during chronic heat stress (Dridi, et al., 2008; Azad, et al., 2010). Additionally, avUCP has been reported to be upregulated in response to other stimuli, such as fasting (Abe, et al., 2006), hypoxia (Rey, et al., 2010b), and transportation stress (Zhang, et al., 2010a), when increased thermogenesis would not be not advantageous. Consequently, other functions besides NST have been proposed for avUCP.

As avUCP shares a greater similarity in tissue expression and amino acid sequence with UCP2 and UCP3 (approximately 70%), it has been suggested that they may also share similar physiological roles (Fleury, et al., 1997; Vidal-Puig, et al., 1997; Raimbault, et al., 2001). Both UCP2 and UCP3 have been associated with uncoupling activity in response to excessive superoxide production and a subsequent reduction in ROS generation, a function apparently supported by increased ROS production exhibited in knockout mice for both genes (Arsenijevic, et al., 2000; Brand, et al., 2002; Echtay, et al., 2002). Similar findings have been reported for avUCP, as upregulation has been noted in response to increased superoxide production concurrent with an increase in proton conductance and suggestive of uncoupling activity (Talbot, et al., 2003; Criscuolo, et al., 2005; Rey, et al., 2010a). A role in free radical regulation would explain the variety of stimuli reported to upregulate avUCP mRNA expression as oxidative stress has been demonstrated to be increased during fasting (Abe, et al., 2006), hypoxia (Rey, et al., 2010b), and transportation (Zhang, et al., 2010b). However, stressors such as AHS result in

excessive ROS production concurrent with (and possibly resulting from) a downregulation in avUCP (Mujahid, et al., 2006; Kikusato, et al., 2013) and may suggest that factors beyond the potential function of avUCP can alter its expression.

Aside from ROS, free fatty acids and thyroid hormone have been suggested as two regulators of avUCP and may offer further insight into the protein's function. Upregulation of avUCP during cold was initially associated with glucagon, the primary hormone in NST, and contributed to its putative thermogenic function (Duchamp, et al., 1993; Raimbault, et al., 2001). Glucagon is a highly lipolytic hormone and circulating concentrations are increased with energy demands, such as during fasting (Christensen, et al., 2013) and transportation (Mitchell and Kettlewell, 1998), which may contribute to the increased avUCP expression noted during these stressors. Concurrent with increased free radical production, stress also results in the mobilization of fatty acids and the upregulation of avUCP may serve to curb oxidative damage as well as limit lipid peroxidation arising from excessive ROS (Mujahid, et al., 2007). However, the increase in avUCP expression noted for birds in response to high fat diets under standard conditions suggests that plasma lipids may be a greater factor in avUCP regulation than stress alone and may indicate a role in lipid metabolism (Collin, et al., 2003b; Collin, et al., 2009).

Fatty acid saturation may also be a factor affecting avUCP expression, as USFA appear to upregulate avUCP while SFA result in decreased expression (Seifi, et al., 2018). This relationship may offer a solution to the increased ROS production observed during AHS with the downregulation of avUCP. Mujahid et al. (2009) reported increased avUCP expression concurrent with decreased free radical production in birds fed a diet higher in USFA during AHS; however, differences between fat source inclusion levels within the study make interpretation difficult as a USFA fat source was included in addition to the basal diet fat

(Mujahid, et al., 2009). Similar results were observed in birds exposed to CHS while fed isocaloric diets differing in fatty acid composition and may indicate avUCP upregulation associated with USFA (Seifi, et al., 2020). However given the differences in metabolic response between CHS and AHS, further work is needed to verify if avUCP is upregulated by USFA during AHS (Akbarian, et al., 2016). As dietary fat supplementation during heat stress is already an established intervention, investigation into whether differences in fat source may offer further benefits may help improve broiler performance during AHS.

Similar to free fatty acids, the influence of thyroid hormone on avUCP expression contributed to it being considered a thermogenic protein. Given the importance of triiodothyronine (**T3**) in regulating body temperature, the positive relationship between avUCP, plasma T3 levels, and heat production in cold acclimated birds appeared to support a role for avUCP in thermogenesis (Collin, et al., 2003c; McNabb, 2007). Yet work in cold acclimated and T3 treated birds also demonstrated a decrease in ROS production parallel with increased avUCP expression for these birds (Rey, et al., 2010a), making it difficult to construe the true function of avUCP. While the ultimate function and regulation of avUCP remains to be elucidated, the strong influence of T3 over avUCP expression may offer insight into how the bird responds to heat stress. Exposure to TM has been proposed to alter the HPT and HPA axes, which may inadvertently alter thyroid function and avUCP expression as the reduction in body temperature observed in TM birds is consistently accompanied by lower plasma T3 levels (Yahav, et al., 2004b; Collin, et al., 2005; Piestun, et al., 2008b; Loyau, et al., 2013). Decreased T3 levels in TM birds would presumably result in reduced avUCP expression, however the improved heat tolerance imparted by TM adaptation may also reduce the need for avUCP regulation of ROS. Evaluation of avUCP levels in TM birds remains inconclusive, with reports of avUCP mRNA

expression being either unaltered (Al-Zghoul, et al., 2015) or increased (Loyau, et al., 2014; Al-Zghoul, et al., 2019) following TM adaptation. Given the potential of avUCP to limit oxidative damage, further investigation is warranted to determine the effects of TM on avUCP expression and oxidative damage arising from AHS.

1.5 Conclusions

Genetic selection for improved growth rate and increased breast portion yield in commercial broilers has resulted in a reduced tolerance to heat stress. As birds approach market weight, susceptibility to hyperthermia is greatly increased and economic loss due to heat stress mortality becomes a major issue. Birds that survive heat challenge may still suffer poor performance and decreased carcass quality arising from oxidative stress. Heat interventions such as TM and dietary fat may assist in ameliorating the negative effects of heat stress on broiler performance and potentially improve carcass composition. Higher incubation temperatures during TM may alter thermoregulatory systems in the developing embryo and improve bird heat tolerance to similar temperatures post-hatch. Myoblast and satellite cell proliferation during incubation may also benefit from TM, potentially enhancing breast yield and meat quality. Increased dietary fat is commonly used during heat stress to improve diet density without increasing metabolic heat production. Fat sources rich in USFA may provide further improvements through increased digestibility and decreased fat deposition. Both heat stress interventions may impact the expression of avUCP, which has been suggested to reduce ROS formation and subsequent oxidative damage during stress. Although avUCP has been demonstrated to be downregulated during AHS, TM has been shown to alter thyroid hormone (a proposed regulator of avUCP) and may modify avUCP mRNA expression. Dietary fatty acid composition has also been suggested to influence avUCP expression, with increased USFA

concentrations resulting in avUCP upregulation concurrent with decreased oxidative damage. However, the literature concerning the effects of TM and dietary fat source on broiler performance and avUCP expression is lacking. Further work is needed to evaluate effective strategies that can sustain broiler production and carcass quality during AHS, as well as to determine if any synergies between these interventions may exist.

1.6 Objective

The objective in the current study was to evaluate the effects of both TM and dietary fat source on broiler performance during acute heat stress at market age. Along with live weight and feed efficiency, carcass yield and quality were also evaluated. Given the economic significance of the breast portion as a premium cut, the occurrence of breast muscle myopathies was also investigated. Finally, the effect of TM and dietary fat source on avUCP mRNA expression and MDA concentration were determined to assess if these strategies could reduce oxidative damage in heat stressed broilers.

1.7 References

- Abdelqader, A., and A.-R. Al-Fataftah. 2014. Thermal acclimation of broiler birds by intermittent heat exposure. *J. Therm. Biol.* 39:1-5.
- Abe, T., A. Mujahid, K. Sato, Y. Akiba, and M. Toyomizu. 2006. Possible role of avian uncoupling protein in down-regulating mitochondrial superoxide production in skeletal muscle of fasted chickens. *FEBS Letters*. 580:4815-4822.
- Akbarian, A., J. Michiels, J. Degroote, M. Majdeddin, A. Golian, and S. De Smet. 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *J. Anim. Sci. Biotechnol.* 7:1-14.
- 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., S. M. El-Bahr, R. K. Al-Rukibat, S. D. Abd Elhafeed, T. A. Althnaian, and S. Y. Al-Ramadan. 2015. Biochemical and molecular investigation of thermal manipulation protocols during broiler embryogenesis and subsequent thermal challenge. *BMC Vet. Res.* 11:292.
- 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.
- Arjona, A., D. Denbow, and W. Weaver Jr. 1988. Effect of heat stress early in life on mortality of broilers exposed to high environmental temperatures just prior to marketing. *Poult. Sci.* 67:226-231.
- Arsenijevic, D., H. Onuma, C. Pecqueur, S. Raimbault, B. S. Manning, B. Miroux, E. Couplan, M.-C. Alves-Guerra, M. Gubern, and R. Surwit. 2000. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat. Genet.* 26:435-439.

- Asakura, A., M. A. Rudnicki, and M. Komaki. 2001. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Differentiation*. 68:245-253.
- Azad, M. A. K., M. Kikusato, T. Maekawa, H. Shirakawa, and M. Toyomizu. 2010. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. *Comp. Biochem. Phys. A*. 155:401-406.
- Baião, N. C., and L. J. C. Lara. 2005. Oil and fat in broiler nutrition. *Braz. J. Poult. Sci.* 7:129-141.
- Baziz, H. A., P. A. Geraert, J. C. F. Padilha, and S. Guillaumin. 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. *Poult. Sci.* 75:505-513.
- Boss, O., S. Samec, A. Paoloni-Giacobino, C. Rossier, A. Dulloo, J. Seydoux, P. Muzzin, and J.-P. Giacobino. 1997. Uncoupling protein- 3: a new member of the mitochondrial carrier family with tissue- specific expression. *FEBS Letters* 408:39-42.
- Brand, M. D., and T. C. Esteves. 2005. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab.* 2:85-93.
- Brand, M. D., R. Pamplona, M. Portero-Otín, J. R. Requena, S. J. Roebuck, J. A. Buckingham, J. C. Clapham, and S. Cadenas. 2002. Oxidative damage and phospholipid fatty acyl composition in skeletal muscle mitochondria from mice underexpressing or overexpressing uncoupling protein 3. *Biochem. J.* 368:597-603.
- Brookes, P. S. 2005. Mitochondrial H leak and ROS generation: an odd couple. *Free Rad. Biol. Med.* 38:12-23.
- Cahaner, A., and N. Deeb. 2000. Reduced heat tolerance in fast-growing broilers. *BSAP Occas. Publ.* 27:85-86.
- Christensen, K., J. McMurtry, Y. Thaxton, J. Thaxton, A. Corzo, C. McDaniel, and C. Scanes. 2013. Metabolic and hormonal responses of growing modern meat-type chickens to fasting. *Br. Poult. Sci.* 54:199-205.

- Collin, A., C. Berri, S. Tesserand, F. E. 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., J. Buyse, P. Van As, V. M. Darras, R. D. Malheiros, V. M. Moraes, G. E. Reyns, M. Taouis, and E. Decuypere. 2003a. Cold-induced enhancement of avian uncoupling protein expression, heat production, and triiodothyronine concentrations in broiler chicks. *Gen. Comp. Endocrinol.* 130:70-77.
- Collin, A., R. D. Malheiros, V. M. B. Moraes, P. Van As, V. M. Darras, M. Taouis, E. Decuypere, and J. Buyse. 2003b. Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. *Br. J. Nutr.* 90:261-269.
- 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., Q. Swennen, S. Skiba-Cassy, J. Buyse, P. Chartrin, E. Le Bihan-Duval, S. Crochet, M. J. Duclos, R. Joubert, and E. Decuypere. 2009. Regulation of fatty acid oxidation in chicken (*Gallus gallus*): interactions between genotype and diet composition. *Comp. Biochem. Physiol. B.* 153:171-177.
- Collin, A., M. Taouis, J. Buyse, N. B. Ifuta, V. M. Darras, P. Van As, R. D. Malheiros, V. M. B. Moraes, and E. Decuypere. 2003c. 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. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poult. Sci.* 80:71-78.
- 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.

- Criscuolo, F., M. d. M. Gonzalez-Barroso, Y. L. Maho, D. Ricquier, and F. Bouillaud. 2005. Avian uncoupling protein expressed in yeast mitochondria prevents endogenous free radical damage. *Proc. R. Soc. B.* 272:803-810.
- Cross, C. E., B. Halliwell, E. T. Borish, W. A. Pryor, B. N. Ames, R. L. Saul, J. M. McCord, and D. Harman. 1987. Oxygen radicals and human disease. *Ann. Intern. Med.* 107:526-545.
- D'Autréaux, B., and M. B. Toledano. 2007. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol.* 8:813-824.
- Daghir, N. J. 2008. Broiler feeding and management in hot climates. Pages 227-260 in *Poultry Production in Hot Climates*. 2nd ed. N. J. Daghir, ed., Oxford, 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.
- Debonne, M., P. J. J. 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. *Worlds Poult. Sci. J.* 64:309-321.
- Desautels, M., G. Zaror-Behrens, and J. Himms-Hagen. 1978. Increased purine nucleotide binding, altered polypeptide composition, and thermogenesis in brown adipose tissue mitochondria of cold-acclimated rats. *Can. J. Biochem.* 56:378-383.
- Dridi, S., S. Temim, M. Derouet, S. Tesseraud, and M. Taouis. 2008. Acute cold- and chronic heat- exposure upregulate hepatic leptin and muscle uncoupling protein (UCP) gene expression in broiler chickens. *J. Exp. Zool. A Ecol. Genet. Physiol.* 309:381-388.
- Duchamp, C., and H. Barre. 1993. Skeletal muscle as the major site of nonshivering thermogenesis in cold-acclimated ducklings. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 265:R1076-R1083.
- Echtay, K. S., D. Roussel, J. St-Pierre, M. B. Jekabsons, S. Cadenas, J. A. Stuart, J. A. Harper, S. J. Roebuck, A. Morrison, and S. Pickering. 2002. Superoxide activates mitochondrial uncoupling proteins. *Nature.* 415:96-99.

- Evock-Clover, C. M., S. M. Poch, M. P. Richards, C. M. Ashwell, and J. P. McMurtry. 2002. Expression of an uncoupling protein gene homolog in chickens. *Comp. Biochem. Physiol. A.* 133:345-358.
- FAO, F. 2017. The future of food and agriculture—Trends and challenges. Annual Report.
- Fleury, C., M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi-Meyrueis, F. Bouillaud, M. F. Seldin, R. S. Surwit, and D. Ricquier. 1997. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* 15:269-272.
- 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.
- Glatz, P., and R. Pym. 2013. Poultry housing and management in developing countries. Pages 24-28 in *Poultry Development Review*, FAO, Rome, IT.
- Gonzalez-Esquerria, R., and S. Leeson. 2005. Effects of acute versus chronic heat stress on broiler response to dietary protein. *Poult. Sci.* 84:1562-1569.
- Halevy, O., S. Yahav, and I. Rozenboim. 2006. Enhancement of meat production by environmental manipulations in embryo and young broilers. *Worlds Poult. Sci. J.* 62:485-497.
- 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.
- Hawke, T. J., and D. J. Garry. 2001. Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol.* 91:534-551.
- Hirabayashi, M., D. Ijiri, Y. Kamei, A. Tajima, and Y. Kanai. 2005. Transformation of skeletal muscle from fast-to slow-twitch during acquisition of cold tolerance in the chick. *Endocrinology.* 146:399-405.

- 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.
- Iqbal, A., E. Decuypere, A. Abd El Azim, and E. R. Kühn. 1990. Pre-and post-hatch high temperature exposure affects the thyroid hormones and corticosterone response to acute heat stress in growing chicken (*Gallus domesticus*). *J. Therm. Biol.* 15:149-153.
- Jenkins, S. A., and T. E. Porter. 2004. Ontogeny of the hypothalamo–pituitary–adrenocortical axis in the chicken embryo: a review. *Dom. Anim. Endocrinol.* 26:267-275.
- Kennedy, O. B., B. J. Stewart- Knox, P. C. Mitchell, and D. I. Thurnham. 2004. Consumer perceptions of poultry meat: a qualitative analysis. *Nutr. Food Sci.* 32::122-129.
- Kikusato, M., and M. Toyomizu. 2013. Crucial role of membrane potential in heat stress-induced overproduction of reactive oxygen species in avian skeletal muscle mitochondria. *PLoS One.* 8:e64412.
- Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. *Animals.* 3:356-369.
- Leksrisompong, N., H. Romero-Sanchez, P. W. Plumstead, K. E. Brannan, and J. Brake. 2007. Broiler incubation. 1. Effect of elevated temperature during late incubation on body weight and organs of chicks. *Poult Sci* 86:2685-2691.
- Lin, H., E. Decuypere, and J. Buyse. 2006a. Acute heat stress induces oxidative stress in broiler chickens. *Comp. Biochem. Physiol. A.* 144:11-17.
- Lin, H., H. Jiao, J. Buyse, and E. Decuypere. 2006b. Strategies for preventing heat stress in poultry. *World's Poultry Science Journal* 62:71-86.
- Liu, L., M. Ren, K. Ren, Y. Jin, and M. Yan. 2020. Heat stress impacts on broiler performance: a systematic review and meta-analysis. *Poult. Sci.* 99:6205-6211.

- Loyau, T., L. Bedrani, C. Berri, S. Métayer-Coustard, C. Praud, V. Coustham, S. Mignon-Grasteau, M. J. Duclos, S. Tesseraud, and N. Rideau. 2015. Cyclic variations in incubation conditions induce adaptive responses to later heat exposure in chickens: a review. *Animal*. 9:76-85.
- 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.
- McNabb, F. M. A. 2007. The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. *Crit. Rev. Toxicol.* 37:163-193.
- Mitchell, M., and P. Kettlewell. 1998. Physiological stress and welfare of broiler chickens in transit: solutions not problems! *Poult. Sci.* 77:1803-1814.
- 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. M. B., R. D. Malheiros, V. Bruggeman, A. Collin, K. Tona, P. Van As, O. M. Onagbesan, J. Buyse, E. Decuypere, and M. Macari. 2003. Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. *J. Therm. Biol.* 28:133-140.
- Moraes, V. M. B., 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.

- Mottet, A., and G. Tempio. 2017. Global poultry production: current state and future outlook and challenges. *Worlds Poult. Sci. J.* 73:245-256.
- Mujahid, A. 2010. Acute cold-induced thermogenesis in neonatal chicks (*Gallus gallus*). *Comp. Biochem. Physiol. A.* 156:34-41.
- 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.
- Mujahid, A., Y. Akiba, C. H. Warden, and M. Toyomizu. 2007. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. *FEBS Letters* 581:3461-3467.
- Mujahid, A., K. Sato, Y. Akiba, and M. Toyomizu. 2006. Acute heat stress stimulates mitochondrial superoxide production in broiler skeletal muscle, possibly via downregulation of uncoupling protein content. *Poult. Sci.* 85:1259-1265.
- 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. A.* 131:751-763.
- Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. *Poult. Sci.* 74:366-373.
- OECD/FAO. 2020. OECD-FAO Agricultural Outlook 2020-2029. Rome, IT.
- 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.
- 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., 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., D. Shinder, M. Ruzal, O. Halevy, J. Brake, and S. Yahav. 2008a. Thermal manipulations during broiler embryogenesis: effect on the acquisition of thermotolerance. *Poult. Sci.* 87:1516-1525.
- Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, and S. Yahav. 2008b. The effect of thermal manipulations during the development of the thyroid and adrenal axes on in-hatch and post-hatch thermoregulation. *J. Therm. Biol.* 33:413-418.
- Piestun, Y., S. Yahav, and O. Halevy. 2015. Thermal manipulation during embryogenesis affects myoblast proliferation and skeletal muscle growth in meat-type chickens. *Poult. Sci.* 94:2528-2536.
- 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.
- 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.
- Rauw, W., E. Kanis, E. Noordhuizen-Stassen, and F. Grommers. 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livest. Prod. Sci.* 56:15-33.
- Rey, B., D. Roussel, C. Romestaing, M. Belouze, J.-L. Rouanet, D. Desplanches, B. Sibille, S. Servais, and C. Duchamp. 2010a. Up-regulation of avian uncoupling protein in cold-acclimated and hyperthyroid ducklings prevents reactive oxygen species production by skeletal muscle mitochondria. *BMC Physiol.* 10:5.
- Rey, B., M. Spée, M. Belouze, A. Girard, J. Prost, D. Roussel, and C. Duchamp. 2010b. Oxygen recovery up-regulates avian UCP and ANT in newly hatched ducklings. *J. Comp. Physiol. B.* 180:239-246.

- Ricquier, D., and F. Bouillaud. 2000a. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *J. Physiol.* 529:3-10.
- Ricquier, D., and F. Bouillaud. 2000b. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem. J.* 345:161-179.
- Ricquier, D., and J.-C. Kader. 1976. Mitochondrial protein alteration in active brown fat: a sodium dodecyl sulfate-polyacrylamide gel electrophoretic study. *Biochem. Biophys. Res. Commun.* 73:577-583.
- Rodrigues, M., M. Garcia Neto, S. Perri, D. Sandre, M. Faria Jr, P. Oliveira, M. Pinto, and R. Cassiano. 2019. Techniques to Minimize the Effects of Acute Heat Stress or Chronic in Broilers. *Braz. J. Poult. Sci.* 21:1-6.
- Saeed, M., G. Abbas, M. Alagawany, A. A. Kamboh, M. E. Abd El-Hack, A. F. Khafaga, and S. Chao. 2019. Heat stress management in poultry farms: A comprehensive overview. *J. Therm. Biol.* 84:414-425.
- Sakomura, N. K. 2004. Modeling energy utilization in broiler breeders, laying hens and broilers. *Braz. J. Poult. Sci.* 6:1-11.
- Samarakoon, S. M. R., and K. Samarasinghe. 2012. Strategies to improve the cost effectiveness of broiler production. *Trop. Agric. Res.* 23:338-346.
- Sandercock, D. A., R. R. Hunter, G. R. Nute, M. A. Mitchell, and P. M. Hocking. 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. *Poult. Sci.* 80:418-425.
- 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.
- Seifi, K., M. Rezaei, A. T. Yansari, M. J. Zamiri, G. H. Riazi, and R. Heidari. 2020. Short chain fatty acids may improve hepatic mitochondrial energy efficiency in heat stressed-broilers. *J. Therm. Biol.* 89:102520.
- Song, D. J., and A. J. King. 2015. Effects of heat stress on broiler meat quality. *Worlds Poult. Sci.* 71:701-709.
- 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.
- Stockdale, F. E. 1992. Myogenic cell lineages. *Dev. Biol.* 154:284-298.
- Talbot, D. A., C. Duchamp, B. Rey, N. Hanuise, J. L. Rouanet, B. Sibille, and M. D. Brand. 2004. Uncoupling protein and ATP/ADP carrier increase mitochondrial proton conductance after cold adaptation of king penguins. *J. Physiol.* 558:123-135.
- Talbot, D. A., N. Hanuise, B. Rey, J.-L. Rouanet, C. Duchamp, and M. D. Brand. 2003. Superoxide activates a GDP-sensitive proton conductance in skeletal muscle mitochondria from king penguin (*Aptenodytes patagonicus*). *Biochem. Biophys. Res. Commun.* 312:983-988.
- 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.
- Taouis, M., V. De Basilio, S. Mignon-Grasteau, S. Crochet, C. Bouchot, K. Bigot, A. Collin, and M. Picard. 2002. Early-age thermal conditioning reduces uncoupling protein messenger RNA expression in pectoral muscle of broiler chicks at seven days of age. *Poult. Sci.* 81:1640-1643.
- Tavárez, M. A., and F. Solis de los Santos. 2016. Impact of genetics and breeding on broiler production performance: a look into the past, present, and future of the industry. *Anim. Front.* 6:37-41.

- Teeter, R. G., and T. Belay. 1996. Broiler management during acute heat stress. *Anim. Feed Sci. Technol.* 58:127-142.
- Teulier, L., J.-L. Rouanet, D. Letexier, C. Romestaing, M. Belouze, B. Rey, C. Duchamp, and D. Roussel. 2010. Cold-acclimation-induced non-shivering thermogenesis in birds is associated with upregulation of avian UCP but not with innate uncoupling or altered ATP efficiency. *J. Exp. Biol.* 213:2476-2482.
- Tůmová, E., and A. Teimouri. 2010. Fat deposition in the broiler chicken: a review. *Sci. Agric. Bohem.* 41:121-128.
- Turrens, J. F. 2003. Mitochondrial formation of reactive oxygen species. *J. Physiol.* 552:335-344.
- Ueda, M., K. Watanabe, K. Sato, Y. Akiba, and M. Toyomizu. 2005. Possible role for avPGC-1 α in the control of expression of fiber type, along with avUCP and avANT mRNAs in the skeletal muscles of cold-exposed chickens. *FEBS Letters* 579:11-17.
- Velleman, S. G. 2015. Relationship of skeletal muscle development and growth to breast muscle myopathies: a review. *Avian Dis.* 59:525-531.
- Vianna, C. R., T. Hagen, C.-Y. Zhang, E. Bachman, O. Boss, B. Gereben, A. S. Moriscot, B. B. Lowell, J. E. P. W. Bicudo, and A. C. Bianco. 2001. Cloning and functional characterization of an uncoupling protein homolog in hummingbirds. *Physiol. Genomics.* 5:137-145.
- Vidal-Puig, A., G. Solanes, D. Grujic, J. S. Flier, and B. B. Lowell. 1997. UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem. Biophys. Res. Commun.* 235:79-82.
- Vidal-Puig, A. J., D. Grujic, C.-Y. Zhang, T. Hagen, O. Boss, Y. Ido, A. Szczepanik, J. Wade, V. Mootha, and R. Cortright. 2000. Energy metabolism in uncoupling protein 3 gene knockout mice. *J. Biol. Chem.* 275:16258-16266.
- Wang, R. R., X. J. Pan, and Z. Q. Peng. 2009. Effects of heat exposure on muscle oxidation and protein functionalities of pectoralis majors in broilers. *Poult. Sci.* 88:1078-1084.

- 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. 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. 2009. Alleviating heat stress in domestic fowl: different strategies. *Worlds Poult. Sci. J.* 65:719-732.
- Yahav, S., A. Collin, D. Shinder, and M. Picard. 2004a. Thermal manipulations during broiler chick embryogenesis: effects of timing and temperature. *Poult. Sci.* 83:1959-1963.
- Yahav, S., R. S. Rath, and D. Shinder. 2004b. 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.
- Yalcin, S., S. Özkan, L. Türkmüt, and P. B. Siegel. 2001. Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. *Br. Poult. Sci.* 42:149-152.
- Zaboli, G.-R., 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.
- Zaboli, G., X. Huang, X. Feng, and D. U. Ahn. 2019. How can heat stress affect chicken meat quality?—a review. *Poult. Sci.* 98:1551-1556.
- Zhang, L., H. Yue, S. Wu, L. Xu, H. Zhang, H. Yan, Y. Cao, Y. Gong, and G. Qi. 2010a. Transport stress in broilers. II. Superoxide production, adenosine phosphate concentrations, and mRNA levels of avian uncoupling protein, avian adenine nucleotide translocator, and avian peroxisome proliferator-activated receptor- γ coactivator-1 α in skeletal muscles. *Poult. Sci.* 89:393-400.

Zhang, L., H. Y. Yue, S. G. Wu, L. Xu, H. J. Zhang, H. J. Yan, Y. L. Cao, Y. S. Gong, and G. H. Qi. 2010b. Transport stress in broilers. II. Superoxide production, adenosine phosphate concentrations, and mRNA levels of avian uncoupling protein, avian adenine nucleotide translocator, and avian peroxisome proliferator-activated receptor- γ coactivator-1 α in skeletal muscles. *Poult. Sci.* 89:393-400.

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.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poult. Sci.* 93:2970-2982.

CHAPTER 2

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

2.1 Summary

Modern broilers have been selected for rapid growth but demonstrate reduced heat tolerance towards 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 close to market age. The cyclic exposure to high temperatures during mid-incubation used in TM have been demonstrated to improve broiler tolerance to heat stress. However high incubation temperatures can be detrimental to embryonic development and impair post-hatch broiler performance. Embryos were exposed to 39.5°C for 12 h daily from incubation days 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 either soya oil, poultry fat, or olive oil at 4.5 % each. Broilers were exposed to a period of acute heat stress (**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

2.2 Description of problem

Broiler chickens have been genetically selected over the past several decades for rapid growth, improved feed efficiency, and increased body weight; however these advances have not been matched with similar developments in the birds' physiological support systems (Havenstein et al., 2003a, b). Increased body weight 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 (Yalçın et al., 2001; Yahav et al., 2005). Subsequently the risk for heat stress increases with bird body weight, 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 post-hatch 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 upon three 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 post-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 while the HPA axis manages stress response, however the two 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 corticotrophic cells at the pituitary level (Debonne et al., 2008). Consequently, a stress response can result in thyroid stimulating hormone production, which then acts upon 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 in order 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 to 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). While 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 post-hatch 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 to 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 body weight 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 body weight (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 (Sanz, 1999; Pinchasov and Nir, 1992; 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 thermal manipulation during incubation on hatching and broiler performance during acute heat stress 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.

2.3 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, USA) with an average egg weight of 68.47 ± 1.04 g. Prior to incubation, eggs were stored at 18°C and 75% relative humidity (**RH**) for 2 to 5 d. Eggs were equally

divided between the two incubation treatments, with a total of 1,740 eggs across 12 replicate trays per treatment (3,480 eggs total). Eggs were pre-incubated for 12 h in a forced ventilation cabinet at 26°C before being placed into a Natureform incubator (I14, Natureform Inc, Jacksonville, FL, USA). Egg trays were arranged in a generalized randomized complete block design with three trays per treatment in each block (four 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 three 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 while 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 for 12 h. Transfer between the machines occurred at the same time each day at 12 h intervals. Eggshell temperatures were measured from five 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 minutes prior to 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 prior to 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 to E7), Middle (E8 to E14), or Late (E15 to 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 while 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 prior to 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 (**BW**) and yolk weight were measured from the sample chicks. Yolk free body weight (**YFBW**) as well as 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 six treatments (two incubation profiles × three finisher diets) in 10 blocks. Each pen (1.2 x 1.5 m) contained one tube feeder and five 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 within 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 days 14 d and 28 d, respectively. At 28 d, birds were presented one of three 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 and Salvador, 1989; Wiseman et al., 1998). 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 2.1). 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 2.2. 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 upon removal. Feed conversion ratio (**FCR**) adjusted for mortalities, was calculated at weekly intervals throughout rearing.

Table 2.1. 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	*
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 either 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 (MnO₂), 220 g; zinc (ZnO and ZnSO₄), 250 g; iron (FeCO₃), 75 g; copper (CuSO₄ and CuCl₂), 10 g; iodine (Ca(IO₃)₂), 5 g; selenium (Na₂SeO₃), 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 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).

Table 2.2. Fatty acid profile and nutritional parameters of the dietary fat sources¹.

Fatty acid (g/100g)	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	5.46	0.55	0.77

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

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) prior to 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 x 3 factorial (incubation x 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 means were separated using Tukey's HSD test (Tukey, 1949) and significance was determined at $P < 0.05$.

2.4 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 Figure 2.1a and 2.1b, 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

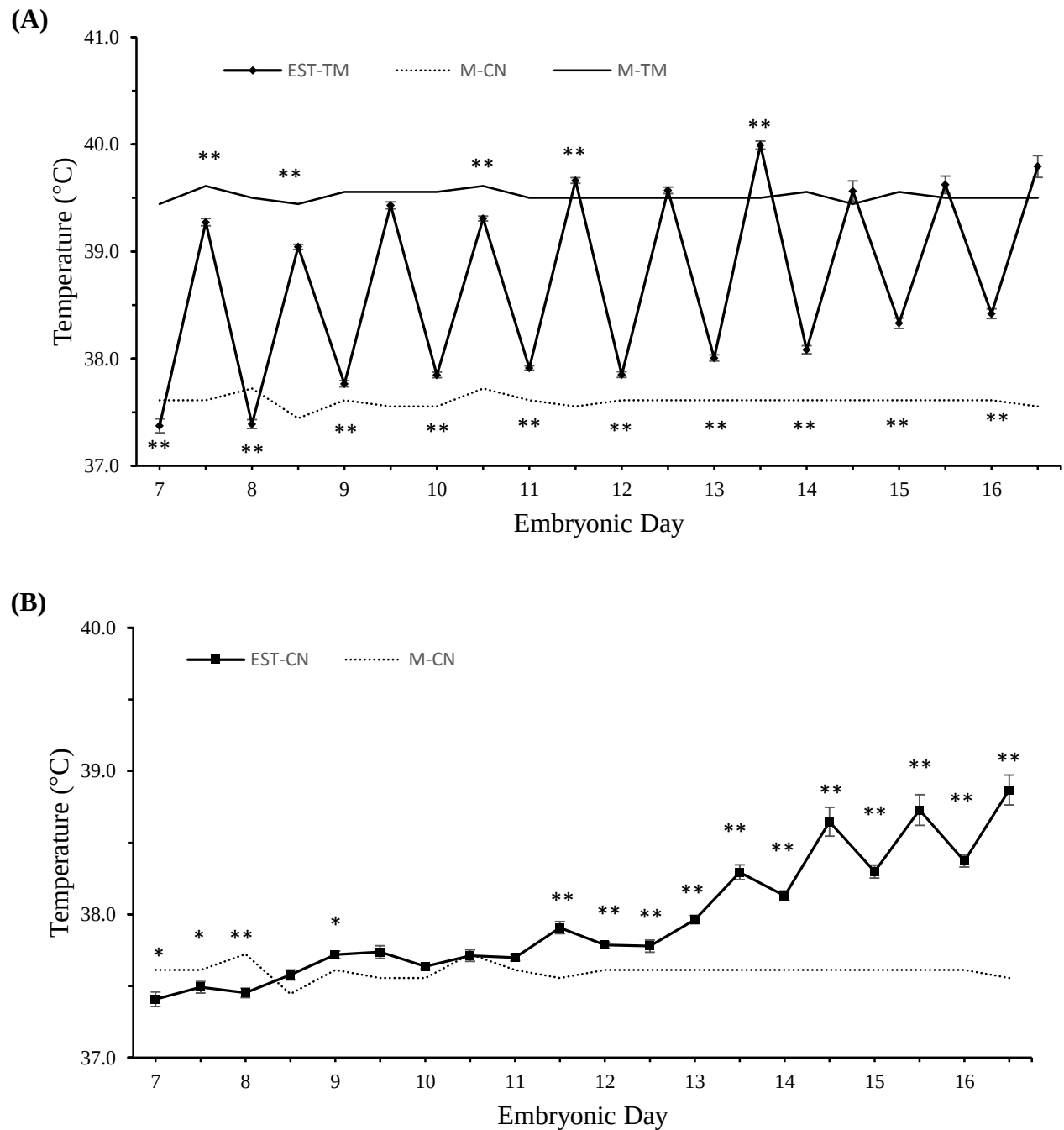


Fig. 2.1. Eggshell temperature (EST) and machine air temperature (M) during incubation from embryonic day 7 to 16.5 in either (A) the thermal manipulation treatment (TM) that alternated between the control machine and a treatment machine set at 39.5°C and 65 % RH in 12 h cycles or (B) the control group (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 ($P < 0.01$).

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 to 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 in Table 2.3. 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 following TM exposure being increased (Yahav et al., 2004; Collin et al., 2005), decreased (Moraes et al.,

Table 2.3. The effect of incubation treatment on embryo mortality, hatchability, and hatch of fertile eggs (%).

Incubation ¹	Embryo mortality			Hatchability		
	Early (E0 to 7)	Mid (E8 to 14)	Late (E15 to 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 ²	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

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.

² SEM = Standard error of mean.

^{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).

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 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 2.4).

Table 2.4. 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 ²	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.

² SEM = Standard error of mean.

Broiler Performance

The effects of TM on BWG, FCR, and FI during the starter (0 to 14 d) and grower (15 to 28 d) periods, prior to 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 two weeks 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 post-hatch 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 to 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 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 to 42 d) is also shown in Table 2.5. 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 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 (Yalçın 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

Table 2.5. Average body weight gain (BWG), feed conversion ratio (FCR), feed intake (FI), and cumulative mortality¹ (0 to 42 d) as influenced by incubation treatment and dietary fat source during the finisher period, as well as their interactions.

Treatment	0 to 14 d			15 to 28 d			29 to 42 d			0 to 42 d
	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 , %
Incubation²										
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
Diet⁴										
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

¹ Prior to 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.

^{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 ($P < 0.05$).

birds of three 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 to the current 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 to 42 d) as affected by incubation treatment, dietary fat source, and their interactions was not shown to differ significantly prior to heat challenge (Table 2.5). Mortality within the treatment and interaction groups ranged from 1.9% to 5.0%, which was comparable to the current industry averages (NCC, 2019).

Acute Heat Stress Challenge

Despite the decrease in hatchability and broiler performance, mortality following AHS was dramatically reduced in TM birds (Table 2.6). While 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 post 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

Table 2.6. 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 d¹.

Treatment	43 to 49 d			
	BWG, g/bird	FCR, g:g	FI, g/bird	Mortality ⁵ , %
Incubation²				
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
Diet⁴				
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

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.

^{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 ($P < 0.05$).

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 to post-hatch 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 prior to heat stress, BWG ($P = 0.054$) and FCR ($P = 0.005$) were shown to be affected by treatment interactions following 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 post 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 post-hatch 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 prior to AHS. Further work is needed to understand the physiological modifications associated 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 to the CN-OO group was unexpected, as performance between these interactions were similar prior to 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). While 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 due to the upregulation of avUCP in response to heat stress ROS production. Current work within our lab analyzing avUCP mRNA expression may provide further elucidation for the results presented here.

A limitation of the current study is the lack of a heat stress control group, resulting from limitations in the trial facilities. Given the differences within the interactions prior to and following 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 six weeks prior to AHS, it may be hypothesized that their performance would have remained similar during the final week if maintained at standard rearing temperatures.

2.5 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, prior to and following AHS.
4. Treatment interactions did not alter broiler performance until after heat stress. Following AHS, TM birds performed similarly on the different diets while FCR and BWG were improved for PF in comparison to OO. Further research is needed to clarify how heat stress influences nutrient metabolism and what role embryonic acclimation may play.

2.6 Acknowledgements

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2.7 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(1):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(2):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. *Anais da 38ª 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*. 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*. 7th rev. ed. Amer. Oil Chem.' Soc., Champaign, Ill.
- Baião, N. C., and L. Lara. 2005. Oil and fat in broiler nutrition. *Braz. J. Poult. Sci.* 7:129-141.
- 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., 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. 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-Endoc M.* 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*. 2nd ed. N. Daghir, 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, Van der Merwe, H J, 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 Poultry 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 Science.* 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(9).
- 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(1-2):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 downregulation 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(3-4):186-192.
- Teeter, R. G., and T. Belay. 1996. Broiler management during acute heat stress. *Anim. Feed Sci. Technol.* 58(1-2):127-142.
- Tona, K., O. Onagbesan, V. Bruggeman, A. Collin, C. Berri, M. J. Duclos, S. Tesseraud, J. Buyse, E. Decuyper, 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 für Geflügelkunde.* 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., 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.
- Wiseman, J., Powles, J., 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.
- 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(4-5):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.
- Yalçın, S., S. Özkan, L. Türkmüt, and P. B. Siegel. 2001. Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. *Br. Poult. Sci.* 42:149-152.
- Yalçın, 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.
- Yalçın, 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(1-4):63-73.

CHAPTER 3

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

3.1 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

3.2 Description of problem

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

3.3 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 x 1.5m) contained one tube feeder and five 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 3.1). 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 3.2. 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 3.1) 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.

Table 3.1. 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 (MnO₂), 220 g; zinc (ZnO and ZnSO₄), 250 g; iron (FeCO₃), 75 g; copper (CuSO₄ and CuCl₂), 10 g; iodine (Ca(IO₃)₂), 5 g; selenium (Na₂SeO₃), 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.

Table 3.2. 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.

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.

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. 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 $(\text{weight initial} - \text{weight final}) / \text{weight initial}$. 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 $(\text{raw weight} - \text{cooked weight}) / \text{raw weight}$.

Statistical Analysis

Data were analyzed as a 2 x 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 $P < 0.05$.

3.4 Results and discussion

Thermal Manipulation

Broiler performance prior to heat challenge was decreased for TM birds (Table 3.3), 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 3.4, 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 S3.1.

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; Piastun et al., 2009, 2011, 2013; 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

Table 3.3. 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 challenge¹.

	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
Incubation²						
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
Diet⁴						
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.

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

Table 3.4. 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 stress¹ during the finisher period.

	Live Weight (50 d)	Thighs, %	Wings, %	<i>Pectoralis</i> major, %	<i>Pectoralis</i> minor, %	Fat pad, %	Frame, %	Cold carcass weight, g
Incubation²								
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
Diet⁴								
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.

^{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 ($P < 0.05$).

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 3.5), concurrent with the decrease in weight and percentage of AFP mentioned earlier ($P = 0.002$, Table 3.4). The TM period (E 7 to E 16) coincides with the establishment of fetal muscle myoblast and satellite cell proliferation, which may be 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.

Table 3.5. The occurrence of breast muscle myopathies, meat color¹, and percentage loss as influenced by incubation temperature and dietary fat source, as well as their interactions, following heat stress² during the finisher period.

	White Stripe, ordinal scale	Wooden Breast, ordinal scale	Spaghetti breast, ordinal scale	L*	a*	b*	Drip loss, %	Cook loss, %
Incubation³								
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
Diet⁵								
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.

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

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 et al., 1999, 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 et al., 1999, 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; Crisuolo 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 constraints. 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.

3.5 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.

3.6 Acknowledgement

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3.7 References

- Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. *PloS One*. 11:e0153750.
- Abdelqader, A., and A. Al-Fataftah. 2014. Thermal acclimation of broiler birds by intermittent heat exposure. *J. Therm. Biol.* 39:1-5.
- Akşit, M., S. Yalcin, S. Özkan, K. Metin, and D. Özdemir. 2006. Effects of temperature during rearing and crating on stress parameters and meat quality of broilers. *Poult. Sci.* 85:1867-1874.
- Al-Zghoul, M. B., A. R. S. Alliftawi, K. M. M. Saleh, and Z. W. Jaradat. 2019. Expression of digestive enzyme and intestinal transporter genes during chronic heat stress in the thermally manipulated broiler chicken. *Poult. Sci.* 98:4113-4122.
- Al-Zhgoul, M., S. D. Abd Elhafeed, M. M. Ababneh, K. I. Jawasreh, K. A. Al Busadah, and Z. B. Ismail. 2013. Thermal manipulation during chicken embryogenesis results in enhanced Hsp70 gene expression and the acquisition of thermotolerance. *Res. Vet. Sci.* 95:502-507.
- Almeida, V. R., V. S. Morita, S. Sgavioli, T. I. Vicentini, D. Castiblanco, and I. C. Boleli. 2016. Incubation temperature manipulation during fetal development reduces adiposity of broiler hatchlings. *Poult. Sci.* 95:316-324.
- 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*. 19th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.
- AOCS. 2017. in *Official Methods and Recommended Practices of the AOCS*. 7th rev. ed. American Oil Chemists' Society, Champaign, Ill.

- Asakura, A., M. A. Rudnicki, and M. Komaki. 2001. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Differentiation*. 68:245-253.
- Aviagen, 2018. Section 6 – Housing and environment, in: *Ross Broiler Management Handbook*. Aviagen, Huntsville, AL, pp. 79-105.
- Baião, N. C., and L. Lara. 2005. Oil and fat in broiler nutrition. *Braz. J. Poult. Sci.* 7:129-141.
- Baziz, H. A., P. A. Geraert, J. Padilha, and S. Guillaumin. 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. *Poult. Sci.* 75:505-513.
- Berri, C., J. Besnard, and C. Relandeau. 2008. Increasing dietary lysine increases final pH and decreases drip loss of broiler breast meat. *Poult. Sci.* 87:480-484.
- Brannan, K.E., K. A. Livingston, and C. J. van Rensburg. 2021. Embryonic thermal manipulation and dietary fat source during acute heat stress. 1. Effect on hatchability and broiler performance. *J. Appl. Poult. Res.*, 100143.
- Clark, D. L., K. G. Walter, and S. G. Velleman. 2017. Incubation temperature and time of hatch impact broiler muscle growth and morphology. *Poult. Sci.* 96:4085-4095.
- 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.

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.

Crespo, N., and E. Esteve-Garcia. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. Poult. Sci. 80:71-78.

Criscuolo, F., M. d. M. Gonzalez-Barroso, Y. L. Maho, D. Ricquier, and F. Bouillaud. 2005. Avian uncoupling protein expressed in yeast mitochondria prevents endogenous free radical damage. Proceedings of the Royal Society B: Biological Sciences. 272(1565):803-810.

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.

Daughtry, M. R., E. Berio, Z. Shen, E. Suess, N. Shah, A. E. Geiger, E. R. Berguson, R. A. Dalloul, M. E. Persia, and H. Shi. 2017. Satellite cell-mediated breast muscle regeneration decreases with broiler size. Poult. Sci. 96:3457-3464.

De Witt, F. H., S. P. Els, Van der Merwe, H J, 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. Decuyper. 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. *Worlds Poult. Sci. J.* 64:309-321.
- Ferrini, G., M. D. Baucells, E. Esteve-Garcia, and A. C. Barroeta. 2008. Dietary polyunsaturated fat reduces skin fat as well as abdominal fat in broiler chickens. *Poult. Sci.* 87:528-535.
- Halevy, O. 2020. Timing Is Everything—The High Sensitivity of Avian Satellite Cells to Thermal Conditions During Embryonic and Posthatch Periods. *Front. Physiol.* 11:235.
- Halevy, O., M. Lavi, and S. Yahav. 2006. Enhancement of meat production by thermal manipulations during embryogenesis of broilers. *New Insights into Fundamental Physiology and Perinatal Adaptation of Domestic Fowl*, Nottingham University Press, UK:77-88.
- 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.
- Harding, R. L., D. L. Clark, O. Halevy, C. S. Coy, S. Yahav, and S. G. Velleman. 2015. The effect of temperature on apoptosis and adipogenesis on skeletal muscle satellite cells derived from different muscle types. *Physiol. Rep.* 3:e12539.

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

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

Hawke, T. J., and D. J. Garry. 2001. Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol.* 91:534-551.

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.

Kennedy, O. B., B. J. Stewart- Knox, P. C. Mitchell, and D. I. Thurnham. 2004. Consumer perceptions of poultry meat: a qualitative analysis. *Nutr. Food Sci.* 34:122-129.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. *Poult. Sci.* 95:2724-2733.

Le Bihan-Duval, E., N. Millet, and H. Remignon. 1999. Broiler meat quality: effect of selection for increased carcass quality and estimates of genetic parameters. *Poult. Sci.* 78:822-826.

- Livingston, M. L., C. Landon, H. J. Barnes, and J. Brake. 2019. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. *Poult. Sci.* 98:217-226.
- Loyau, T., L. Bedrani, C. Berri, S. Métayer-Coustard, C. Praud, V. Coustham, S. Mignon-Grasteau, M. J. Duclos, S. Tesseraud, and N. Rideau. 2015. Cyclic variations in incubation conditions induce adaptive responses to later heat exposure in chickens: a review. *Animal.* 9:76-85.
- 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.
- Mailloux, R. J., and M. Harper. 2011. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic. Biol. Med.* 51:1106-1115.
- McNabb, F. A. 2007. The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. *Crit. Rev. Toxicol.* 37(1-2):163-193.
- Minguez-Mosquera, M. I., B. Gandul-Rojas, J. Garrido-Fernandez, and L. Gallardo-Guerrero. 1990. Pigments present in virgin olive oil. *J. Am. Oil Chem. Soc.* 67:192-196.

Moraes, V., R. D. Malheiros, V. Bruggeman, A. Collin, K. Tona, P. Van As, O. M. Onagbesan, J. Buyse, E. Decuypere, and M. Macari. 2003. Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. *J. Therm. Biol.* 28:133-140.

Mujahid, A., Y. Akiba, and M. Toyomizu. 2007. Acute heat stress induces oxidative stress and decreases adaptation in young white leghorn cockerels by downregulation of avian uncoupling protein. *Poult. Sci.* 86:364-371.

Mujahid, A., N. R. Pumford, W. Bottje, K. Nakagawa, T. Miyazawa, Y. Akiba, and M. Toyomizu. 2007. Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. *J. of Poult. Sci.* 44:439-445.

Nangsuay, A., R. Meijerhof, I. Van Den Anker, M. Heetkamp, B. Kemp, and H. van den Brand. 2015. Development and nutrient metabolism of embryos from two modern broiler strains. *Poult. Sci.* 94:2546-2554.

NCC. 2021. U.S. Broiler Performance. National Chicken Council. Accessed March 2021.
<https://www.nationalchickencouncil.org/statistic/us-broiler-performance/>.

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.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Ojano-Dirain, C. P., N. R. Pumford, M. Toyomizu, and W. G. Bottje. 2007. Association of mitochondrial function and feed efficiency. *J. Poult. Sci.* 44:221-237.
- Petracci, M., F. Soglia, M. Madruga, L. Carvalho, E. Ida, and M. Estévez. 2019. Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. *Comp. Rev. Food Sci. Food Saf.* 18:565-583.
- 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.
- 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., 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., T. Patael, S. Yahav, S. G. Velleman, and O. Halevy. 2017. Early posthatch thermal stress affects breast muscle development and satellite cell growth and characteristics in broilers. *Poult. Sci.* 96:2877-2888.

Piestun, Y., S. Yahav, and O. Halevy. 2015. Thermal manipulation during embryogenesis affects myoblast proliferation and skeletal muscle growth in meat-type chickens. *Poult. Sci.* 94:2528-2536.

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.

Qi, K. K., J. L. Chen, G. P. Zhao, M. Q. Zheng, and J. Wen. 2010. Effect of dietary $\omega 6/\omega 3$ on growth performance, carcass traits, meat quality and fatty acid profiles of Beijing-you chicken. *J. Anim. Physiol. Anim. Nutr.* 94:474-485.

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.

Sandercock, D. A., R. R. Hunter, G. R. Nute, M. A. Mitchell, and P. M. Hocking. 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. *Poult. Sci.* 80:418-425.

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., C. J. Lopez-Bote, D. Menoyo, and J. M. Bautista. 2000. 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.
- Soglia, F., L. Laghi, L. Canonico, C. Cavani, and M. Petracci. 2016. Functional property issues in broiler breast meat related to emerging muscle abnormalities. *Food Res. Int.* 89:1071-1076.
- Song, D. J., and A. J. King. 2015. Effects of heat stress on broiler meat quality. *Worlds Poult. Sci. J.* 71:701-709.
- 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.
- Stockdale, F. E. 1992. Myogenic cell lineages. *Dev. Biol.* 154:284-298.
- Tukey, J. W. 1949. Comparing individual means in the analysis of variance. *Biometrics.* 1:99-114.
- Tzschentke, B., and D. Basta. 2002. Early development of neuronal hypothalamic thermosensitivity in birds: influence of epigenetic temperature adaptation. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 131:825-832.
- Velleman, S. G. 2015. Relationship of skeletal muscle development and growth to breast muscle myopathies: a review. *Avian Dis.* 59:525-531.

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., 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.

Wiseman, J., Powles, J. 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.

Wongsuthavas, S., S. Terapuntuwat, W. Wongsrikeaw, S. Katawatin, C. Yuangklang, and A. C. Beynen. 2008. Influence of amount and type of dietary fat on deposition, adipocyte count and iodine number of abdominal fat in broiler chickens. *J. Anim. Physiol. Anim. Nutr.* 92:92-98.

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(4-5):245-250.

Yahav, S., D. Shinder, J. Tanny, and S. Cohen. 2005. Sensible heat loss: the broiler's paradox. *Worlds Poult. Sci. J.* 61(3):419-434.

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

3.8 Supplementary data

Table S3.1. Carcass and portion weights (g) of 50 d male broilers as influenced by incubation treatment and dietary fat source, as well as their interactions, following heat stress¹ during the finisher period.

	Hot carcass weight	Cold carcass weight without fat pad	Thighs	Wings	<i>Pectoralis</i> major	<i>Pectoralis</i> minor	Fat pad	Frame
Incubation²								
CN	3224.3 ^a	3145.5 ^a	915.6	293.9 ^a	913.9	172.3	44.6 ^A	858.5 ^a
TM	3093.3 ^b	3034.0 ^b	886.4	284.9 ^b	881.7	172.4	36.9 ^B	820.6 ^b
SEM ³	35.9	35.1	11.0	3.0	11.7	2.7	1.5	13.4
P-Value	0.011	0.026	0.062	0.033	0.053	0.978	0.001	0.047
Diet⁴								
Olive	3170.7	3106.5	904.0	291.5	898.0	171.7	40.9	852.2
Poultry	3202.0	3135.3	914.8	292.0	907.5	173.9	43.6	846.5
Soya	3103.7	3027.4	884.1	284.7	888.1	171.4	37.7	820.0
SEM	43.9	42.9	13.5	3.6	14.5	2.9	1.8	16.3
P-Value	0.285	0.199	0.273	0.310	0.634	0.793	0.081	0.363
Incubation x diet								
CN-Olive	3197.2	3128.0	909.3	292.6	899.6 ^{ab}	168.4	45.6	880.3
CN-Poultry	3342.8	3265.6	941.4	300.3	956.4 ^a	177.6	45.6	875.4
CN-Soya	3132.9	3042.9	896.0	288.8	885.8 ^{ab}	170.9	42.5	819.7
TM-Olive	3144.2	3084.9	898.7	290.4	896.3 ^{ab}	175.0	36.2	824.1
TM-Poultry	3061.1	3005.1	888.2	283.6	858.5 ^b	170.3	41.6	817.5
TM-Soya	3074.5	3012.0	872.2	280.6	890.4 ^{ab}	171.9	32.9	820.3
SEM	62.0	60.5	19.0	5.1	20.2	4.1	2.6	23.0
P-Value	0.099	0.099	0.492	0.340	0.016	0.220	0.451	0.381

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.

^{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).

CHAPTER 4

Influence of incubation, diet, and sex on avian uncoupling protein expression and oxidative stress in market age broilers following exposure to acute heat stress

4.1 Abstract

Genetic selection for rapid growth in broilers has inadvertently resulted in increased susceptibility to heat stress, particularly in male birds. Increased oxidative stress associated with hyperthermia may be reduced by avian uncoupling protein (avUCP), which has been proposed to modulate free radical production. However, the relationship between avUCP expression and current heat stress management strategies is unclear. Embryonic acclimation or thermal manipulation (TM) and dietary fat source are two heat stress interventions that may alter avUCP expression, but the literature is inconclusive. The objective of this trial was to investigate the effect of TM and dietary fat source on avUCP expression in market age broilers following acute heat challenge. The influence of bird sex and heat challenge were also evaluated. Concentration of thiobarbituric acid reactive substances (TBARS) was measured as a marker of oxidative stress. Embryonic TM occurred from incubation day 7 to 16 for 12 h daily at 39.5°C. Dietary treatments were applied from 28 to 43 days of age using either poultry fat, soya oil, or olive oil supplemented at 4.5% in the diet. Acute heat stress (AHS) occurred on day 43 at 32°C for 4 h. Neither avUCP nor TBARS concentrations were significantly influenced by TM or dietary fat source. Downregulation of avUCP was observed following AHS, concurrent with an increase in TBARS concentration. Male birds exhibited higher levels of both avUCP expression and TBARS compared to females prior to AHS. A significant interaction was noted for heat stress by sex, with avUCP expression being greatest in males prior to AHS. The increase in avUCP expression and TBARS concentrations in male birds may be associated with an increased susceptibility to stress arising from the increased growth rate noted for male broilers.

Key words: thermal manipulation, dietary fat source, acute heat stress, avian uncoupling protein, oxidative stress

4.2 Introduction

Heat stress remains an issue for the poultry industry as genetic selection for rapid growth and heavy body weight have resulted in an increased susceptibility to hyperthermia (Cahaner and Deeb, 2000; Havenstein et al., 2003). Increased metabolic activity coupled with a larger body mass relative to surface area has led to a higher body temperature and decreased capacity for sensible heat loss in modern broilers (Yalçın et al., 2001; Yahav et al., 2005). During exposure to high ambient temperatures, bird feed intake is lowered to minimize metabolic heat production, but subsequent body weight gain and feed efficiency are also reduced (Teeter and Belay, 1996; Deeb and Cahaner, 2002).

Bird performance during heat stress may be further impaired due to increased production of reactive oxygen species (**ROS**) that can result in oxidative damage (Altan et al., 2003; Lin et al., 2006). Although ROS generation occurs during normal aerobic respiration (Hensley et al., 2000), excessive ROS levels such as that arising from heat stress can result in lipid peroxidation, protein oxidation, DNA damage, and cell death (Cross, 1987). Mitochondria are the primary site of ROS generation as the reduction of oxygen during cellular oxidation results in free radical intermediates (Pryor, 1986; Mailloux and Harper, 2011). However, ROS production may be curbed by increased proton leak into the matrix, as mitochondrial proton conductance shares a negative feedback loop with ROS generation (Skulachev, 1996; De Grey, 2002). Uncoupling proteins (**UCP**) are mitochondrial carrier proteins that may contribute to this process by increasing proton conductance through the uncoupling of oxidative phosphorylation (Ricquier and Bouillaud, 2000). Although energetically inefficient, the uncoupling associated with UCP

homologues serve various physiological functions. In mammals, UCP1 contributes to non-shivering thermogenesis (Enerbäck et al., 1997) while UCP2 and UCP3 have been proposed to modulate ROS production (Vidal-Puig et al., 2000; MacLellan et al., 2005).

Currently, avian uncoupling protein (**avUCP**) is the only UCP identified in birds (Raimbault et al., 2001; Vianna et al., 2001). Although its precise function remains unclear, several studies have presented evidence supportive of avUCP playing a role in the regulation of ROS production (Talbot et al., 2004; Abe et al., 2006; Mujahid et al., 2006; Rey et al., 2010; Zhang et al., 2010), similar to mammalian UCP2 and UCP3 which both share approximately 70% homology with avUCP (Raimbault et al., 2001). In broilers, heat stress is strongly associated with increased ROS production and oxidative damage (Mujahid et al., 2005; Azad et al., 2010), however the effect of heat stress on avUCP mRNA expression is less apparent. Although work has suggested increased ROS levels during heat stress may be exacerbated by the down regulation of avUCP (Mujahid et al., 2006; Kikusato and Toyomizu, 2013), others have reported avUCP expression to be unaltered by hyperthermia (Al-Zghoul et al., 2015). Several reports have substantiated the upregulation of avUCP in response to ROS formation associated with stressors such as hypoxia (Rey et al., 2010), transport (Zhang et al., 2010), and fasting (Abe et al., 2006). Upregulation of avUCP during heat stress may similarly reduce free radical damage, but the effect of high ambient temperatures on avUCP mRNA expression remains to be clarified. Heat stress interventions which can promote the upregulation of avUCP may potentially represent an opportunity to reduce ROS levels in broilers and limit oxidative damage during high temperature exposure.

One strategy for managing heat stress is embryonic acclimation to post-hatch temperature challenges through periods of controlled exposure to similar conditions, or thermal manipulation

(**TM**) (Moraes et al., 2003). At approximately incubation day (**E**) 15, the hypothalamo-pituitary-thyroid and -adrenocortical axes (**HPT** and **HPA**, respectively) coalesce and the embryo begins to respond to environmental temperatures through changes in metabolic rate induced by thyroid activity (Jenkins and Porter, 2004; McNabb, 2007). Exposing the embryo to increased temperatures during this developmental period may influence the “set points” of these thermoregulatory systems and improve heat stress tolerance post-hatch (Nichelmann and Tzschentke, 2002). Several studies have reported a decrease in post-hatch body temperature and plasma thyroid hormones concurrent with improved resistance to heat stress in birds exposed to TM (Yahav et al., 2004; Collin et al., 2005; Piestun et al., 2008; Loyau et al., 2013), which suggests modification of the HPT and HPA axes. However, the reduction in sensitivity for these axes may indirectly result in lower avUCP levels as triiodothyronine (Collin et al., 2003a) and the β -adrenergic system (Bugajski et al., 1991; Joubert et al., 2011) have both been proposed as regulators of avUCP mRNA expression. The current literature concerning the influence of TM on avUCP has been inconclusive, with reports of TM having no effect (Al-Zghoul et al., 2015) or increasing avUCP mRNA expression in TM birds (Loyau et al., 2014; Al-Zghoul et al., 2019). While there is evidence of improved heat tolerance in TM birds (Collin et al., 2005; Piestun et al., 2008; Loyau et al., 2013), it is unclear if the benefits of TM include the upregulation of avUCP. However, given the potential of avUCP to reduce oxidative damage in broilers during heat stress, further investigation is warranted to determine if this protein may contribute to TM adaptation.

Increased dietary fat is a frequent intervention employed during heat stress to sustain bird performance. Supplemental fat provides increased energy density at a lower heat increment when compared to carbohydrates or proteins so that bird performance can be maintained during heat

stress despite reduced feed intake (Dale and Fuller, 1979; Tetter and Belay, 1996). High levels of dietary fat have also been demonstrated to upregulate avUCP and may suggest the involvement of lipids in the regulation of avUCP mRNA expression (Collin et al., 2003b; Joubert et al., 2011). The fatty acid composition of the diet may further influence avUCP expression, as upregulation was noted in broilers fed diets rich in unsaturated fatty acids (**USFA**) while diets greater in saturated fatty acids (**SFA**) resulted in downregulation (Seifi et al., 2018). Increased avUCP expression associated with USFA may decrease oxidative damage during heat stress, as olive oil was observed to upregulate avUCP and reduce ROS production in broilers exposed to high temperatures (Mujahid et al., 2009). Although fat supplementation is a well-established heat management strategy, choice of dietary fat source tends to be based on least-cost formulation and olive oil is not a cost-efficient ingredient for broiler diets. Soya oil is a more conventional USFA fat source that may potentially promote avUCP expression similar to olive oil without dramatically increasing feed cost. Heat stress interventions must be practical as well as effective to be commercially relevant; therefore, evaluation of different fat sources on avUCP expression is needed to establish if USFA can enhance broiler performance during heat stress.

Aside from management strategies, the influence of bird sex during hyperthermia must also be considered as genetic selection for rapid growth rate and muscle yield has amplified sexual dimorphism in modern broilers (Zuidhof et al., 2014). Improved growth is already apparent in males during embryonic development (Henry and Burke, 1998) and the difference in growth rate and body weight between the sexes continues to increase with age post-hatch (Nogueira et al., 2019). Such differences likely contribute to performance in males being more severely impacted during heat stress when compared to females (Osman et al., 1989), particularly in strains selected for rapid growth (Cahaner and Leenstra, 1992; Settar et al., 1999).

As ROS are a byproduct of normal metabolic activity (Hensley et al., 2000), genetic selection for increased growth rate and body weight in modern male broilers may have inadvertently resulted in greater ROS production due to increased metabolic rate. Brothers et al. (2019) reported that males exhibited an upregulation of genes involved in oxidative stress response when compared to females in birds reared under normal conditions, however avUCP was not listed among the genes investigated. Given that avUCP has been proposed to regulate ROS production, differences in avUCP expression between the sexes may potentially contribute to the increased susceptibility to oxidative stress exhibited by male broilers. As commercial breeding programs continue to select for enhanced growth rates, it is likely that male broiler performance will become increasingly sensitive to high temperatures (Settar et al., 1999; Daghir, 2008). The influence of bird sex on avUCP expression has not previously been confirmed and may contribute to a better understanding of the protein, as well as the effects of sexual dimorphism in broilers.

Therefore, the objective of this trial was to determine the influence of TM and dietary fat source on avUCP mRNA expression and oxidative damage in male and female broilers exposed to heat stress. Broiler performance was also evaluated to determine if these factors may have commercial significance. As the risk of heat stress and economic loss are greatest in birds closer to market age (St-Pierre et al., 2003), broiler response to high temperatures was assessed during the finisher stage of production.

4.3 Materials and methods

Incubation and rearing occurred at the North Carolina Department of Agriculture's Piedmont Research Station, in collaboration with North Carolina State University. All procedures used within this study were reviewed and approved by the Institutional Animal Care

and Use Committee at North Carolina State University as well as the Research Ethics committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria (NAS200/2020).

Incubation and Rearing

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 and stored at 18°C and 75% relative humidity (**RH**) for 2 to 5 d prior to incubation. Eggs were distributed between the two incubation treatment groups, with 1,740 eggs set into 12 replicate trays per treatment (3,480 eggs total). Pre-incubation occurred in a forced ventilation cabinet for 12 h at 26°C, after which all trays were placed into a single incubator (Natureform Inc., Jacksonville, FL) set at 37.5°C and 56% RH. Tray placement within the incubator was arranged in a generalized randomized complete block design, with three trays per treatment in each block (four blocks in total) to reduce position effect within the machine. Turning occurred through 90° hourly.

At incubation day (**E**) 7, trays in the TM treatment were transferred to a second incubator set at 39.5°C and 65% RH. Relative humidity in the TM incubator was increased to prevent excessive moisture loss from the eggs during exposure to increased temperatures. After 12 h of high temperature exposure, the TM trays were returned to the initial incubator for 12 h and this cycle was repeated at the same time daily from E7 to E16. Trays in the control group (**CN**) remained in the original setter at a constant temperature throughout incubation. The TM incubator was adjacent to the CN incubator to reduce transport stress. A plastic tent was erected over both machines and heated to 37.5°C to minimize heat loss by the eggs during transfer between machines. Trays in the CN group were also handled twice daily during TM transfer so that any handling stress would be similar between groups. The TM exposure continued until E16

and at E17.5 eggs were transferred to hatching baskets and both groups were placed in a single hatcher until completion of incubation.

At hatch, good quality chicks were sexed and placed in a 60 pen environmentally controlled broiler house. Treatment pens were assigned in a randomized complete block design with six treatments (two incubation profiles $\times$ three finisher diets) in 10 blocks. Each block contained six pens, representing each treatment combination for a total of 10 replicates per treatment. An equal number of chicks for both sexes were placed in each pen for a total of 18 chicks per pen. All pens (1.2 x 1.5 m) used wood shavings for bedding and contained one tube feeder as well as five drinking nipples. A supplemental feed pan and chick water font was included in each pen for the first week of rearing and removed at 7 d. Standard commercial lighting and temperature profiles (Aviagen, 2018) were implemented until the acute heat challenge at 43 d, when temperatures within the house were gradually increased from 21°C to 32°C for 4 h. Following heat stress, standard temperature profiles were implemented until the end of the study at 49 d.

Birds were fed a standard commercial starter and grower diet until 14 d and 28 d, respectively, after which dietary treatments were applied during the finisher period from 28 to 49 d. Treatment diets differed in fat source, which 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 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 (Table 4.1). 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 4.2. All diets were manufactured at the North Carolina State University Feed Mill.

Table 4.1. 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	*
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 either 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 (MnO₂), 220 g; zinc (ZnO and ZnSO₄), 250 g; iron (FeCO₃), 75 g; copper (CuSO₄ and CuCl₂), 10 g; iodine (Ca(IO₃)₂), 5 g; selenium (Na₂SeO₃), 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 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).

Table 4.2. Fatty acid profile and nutritional parameters of the dietary fat sources¹.

Fatty acid (g/100g)	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	5.46	0.55	0.77

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

Body weight (**BW**) and feed intake (**FI**) were recorded by pen on a weekly basis. Feed conversion ratio (**FCR**) was calculated as was production efficiency factor (**PEF**) using the following formulae.

$$FCR = \frac{\text{Feed intake}}{\text{Body weight gain}}$$

$$PEF = \frac{\text{Live weight (kg)} \times \text{Livability (\%)}}{\text{Age (d)} \times FCR} \times 100$$

Breast muscle tissue samples were taken at 42 d (pre-AHS) and 43 d (post-AHS) to evaluate lipid peroxidation through malondialdehyde (**MDA**) levels and avUCP mRNA expression. Tissue samples were taken from one male and one female bird selected from each pen by BW to be close to the average BW of the treatment pen for each sex. Sample birds were euthanized via cervical dislocation by trained technicians and tissue was collected from the center of the left breast muscle (*Pectoralis major*), approximately 2.5 cm from the keel bone.

Tissue samples were flash frozen in liquid nitrogen immediately upon collection and stored at -80°C until analysis.

Thiobarbituric acid reactive substance assay

Lipid peroxidation, as determined through measuring the amount of MDA that reacts with 2-thiobarbituric acid, was used to estimate oxidative stress (Armstrong and Browne, 1994; Yagi, 1998). A thiobarbituric acid reactivates substance (**TBARS**) assay kit was used to evaluate MDA levels in the breast tissue samples taken pre- and post-heat stress (42 and 43 d, respectively). Samples were analyzed within 3 wk of collection to prevent any changes in MDA levels due to long-term storage. Approximately 50 mg (± 5 mg) of tissue from each sample was mixed with 500 μ L of homogenization buffer, which contained 50 mM TrisHCl pH 8, 150 mM NaCl, 1% NP-40, 1 mM Na₃VO₄, 0.1% SDS, 2 uM leupeptin, 2 uM pepstatin, 10 KIU/mL aprotinin, and 400 uM PMSF, and then homogenized with a Tissue Tearor (Model 985370, Biospec Products Inc., Bartlesville, OK) before being centrifuged at 1,600 x g for 10 minutes at 4°C. The resultant supernatant was subjected to bicinchoninic acid assay to determine protein concentration, and samples were normalized to the lowest concentration, with each sample having a final total volume of 100 μ L. The colorimetric version of the TBARS assay was performed per kit instructions (item no. 700870, Cayman Chemical, Ann Arbor, MI) and absorbance was read at 532 nm (Bio-Rad xMark, Bio-Rad Laboratories Inc., Hercules, CA). Results are presented here as MDA relative to the amount of sample protein (nmol/mg).

Real-Time Quantitative PCR

The expression of avUCP mRNA in the breast tissue was determined using RT-qPCR. Methodology was performed as described by Saini et al. (2019) and followed the Minimum Information for Publication of Quantitative Real-Time Experiments (MIQE) guidelines (Bustin

et al., 2009). Trizol reagent (item no. 15596026, Invitrogen, Carlsbad, CA) was used for total RNA isolation according to the manufacturer's instructions. Sample RNA purity was confirmed by spectrophotometry (NanoDrop 200, Thermo Scientific, Waltham, MA) and RNA integrity was evaluated by gel electrophoresis. Isolated RNA was stored at -80°C. Synthesis of cDNA was performed using 1 µg of RNA with random hexamer primers and ImProm-II™ Reverse Transcriptase (item no. A3803, Promega, Madison, WI) diluted to a concentration of 1 µg/uL, prepared and synthesized as per manufacturer's instructions. Following synthesis, cDNA was stored at -20°C.

In preparation for RT-qPCR analysis, cDNA was added to a PCR reaction mix that included 2 uM of both forward and reverse primers (IDT, Coralville, IA), as well as 2x SYBR® Green Supermix (item no. 1725271, Bio-Rad, Madison, WI). The forward and reverse primers used for avUCP (GenBank® accession no. AF433170.2) were GTCACTTCGTGGCTGCCTTC and CTCGGCACATTGCGGTACTG, respectively. Glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**, GenBank® accession no. NM_204305.1) served as the reference gene and the GAPDH primer sequences were forward ATGACCACTGTCCATGCCATCA and reverse AGGGATGACTTTCCCTACAGCCTT. Dilution of cDNA occurred at a 1:10 concentration for avUCP and 1:1000 for GAPDH so that Ct values between the target and reference genes would be no more than 5 Ct values apart. Amplification efficiency for all primers was between 90 to 110%. RT-qPCR was conducted using a PCR Thermal Cycler (Bio-Rad CFX384 Real-Time System, Bio-Rad Laboratories Inc., Hercules, CA) and cycles consisted of 95°C for 2 min followed by 45 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 30 s. Incorporation of the fluorescent dye was measured at the end of each amplification cycle and an analysis of the melting curve occurred upon the completion of the final cycle to confirm amplification

specificity. Samples were run in triplicate and target gene expression was normalized to GAPDH. Mean relative expression change was determined using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001) for statistical analysis. Abundance of GAPDH was not affected by bird sex, heat exposure, incubation, or dietary treatment.

Statistical analysis

All data were subjected to the Shapiro-Wilk test to assess normality and Levene's test was used to verify the equality of variances. Production data (BWG, FI, and FCR) were analyzed as a two-way ANOVA (incubation x diet) using the fit-model platform of JMP Pro 13 (SAS Institute, Cary, NC) with rearing pen as the experimental unit. A forward selection stepwise regression with Bayesian information criterion (BIC) was conducted to determine the optimal model for evaluating the effect of incubation, diet, sex, and heat stress on avUCP gene expression and TBARS levels. Heat stress and sex were selected to be significant variables, while incubation and dietary treatments were removed from the model. Subsequently, gene expression and TBARS data were analyzed as a two-way ANOVA with sex and heat stress as the main effects. Concentrations of TBARS were not normally distributed and were therefore log-transformed for analysis while the untransformed values are presented in the results section. Differences between means were separated using Tukey's HSD test (Tukey, 1949) and significance was determined at $P < 0.05$.

4.4 Results

Rearing performance

The effect of incubation and dietary treatments, as well as their interactions, on broiler performance during the finisher phase is shown in Table 4.3. Decreased BWG and FI were noted for the TM birds prior to the heat challenge ($P < 0.001$ and $P < 0.002$, respectively). Although

BWG was shown to be similar following AHS, FI remained lower for the TM group ($P < 0.001$). Differences in FCR and PEF scores between the incubation treatments were not observed, regardless of heat challenge. Dietary fat source was not shown to influence broiler performance. Bird mortality has been previously reported (Brannan et al., 2021) and was approximately halved in the TM group (9.3%) in comparison to the CN birds (18.5%) following heat stress ($P = 0.006$). Mortalities prior to heat stress were not noted to significantly differ between treatments nor interactions.

A significant interaction was observed for FCR and PEF during the post-heat stress period. Birds in the CN-PF and TM-SO exhibited improved feed efficiency while the CN-OO group demonstrated the poorest ($P = 0.005$). Likewise, CN-PF and TM-SO resulted in the highest PEF scores while CN-OO had the lowest ($P = 0.049$). A similar trend ($P = 0.054$) was noted for BWG, with CN-PF exhibiting the highest BWG and CN-OO the lowest.

Table 4.3. Weekly average body weight gain (BWG), feed conversion ratio (FCR), and feed intake (FI) as influenced by incubation treatment and dietary fat source during the finisher period, as well as their interactions pre- and post-heat challenge¹.

Treatment	29 to 42 d, pre-heat stress				43 to 49 d, post-heat stress			
	BWG, g/bird	FI, g/bird	FCR, g:g	PEF	BWG, g/bird	FI, g/bird	FCR, g:g	PEF
Incubation²								
CN	1213.9 ^A	2058.5 ^A	1.79	456.5	669.9	1405.5 ^A	2.09	189.3
TM	1154.5 ^B	1974.6 ^B	1.71	439.8	652.0	1329.9 ^B	2.04	201.4
SEM ³	12.2	18.0	0.01	6.5	11.0	16.0	0.03	5.5
P-Value	0.001	0.002	0.292	0.072	0.256	0.001	0.227	0.124
Diet⁴								
Olive	1186.4	2028.0	1.71	456.8	637.1	1357.9	2.11	187.4
Poultry	1175.7	2016.3	1.71	445.4	680.2	1381.6	2.04	200.4
Soya	1190.4	2005.3	1.68	442.3	665.5	1363.6	2.06	198.1
SEM	15.0	22.0	0.01	7.9	13.4	19.3	0.03	6.7
P-Value	0.774	0.765	0.189	0.403	0.081	0.670	0.196	0.352
Incubation x diet								
CN-Olive	1213.7	2057.0	1.70	466.5	627.6	1396.8	2.18 ^A	174.2 ^b
CN-Poultry	1204.6	2045.1	1.70	456.7	716.0	1411.1	1.97 ^B	208.3 ^a
CN-Soya	1223.4	2073.5	1.69	446.4	665.9	1408.5	2.12 ^{AB}	185.5 ^{ab}
TM-Olive	1159.1	1999.1	1.72	447.1	646.6	1319.0	2.05 ^{AB}	200.7 ^{ab}
TM-Poultry	1146.8	1987.4	1.73	434.1	644.4	1352.2	2.10 ^{AB}	192.6 ^{ab}
TM-Soya	1157.5	1937.2	1.68	438.2	665.1	1318.6	1.99 ^B	210.7 ^a
SEM	21.2	31.1	0.02	11.2	18.8	27.3	0.04	9.7
P-Value	0.962	0.353	0.287	0.795	0.054	0.850	0.005	0.049

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.

^{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).

TBARS

Stepwise regression analysis revealed no influence of dietary or incubation treatment on TBARS levels (Supplementary Table 4.1). Concentration of TBARS as influenced by bird sex and acute heat stress are shown in Figure 4.1. Male broilers exhibited higher levels of TBARS in comparison to females ($P = 0.0001$) and heat stress was shown to increase TBARS for all birds ($P < 0.0001$). Significant differences were not apparent for interactions between bird sex and heat challenge ($P = 0.3540$).

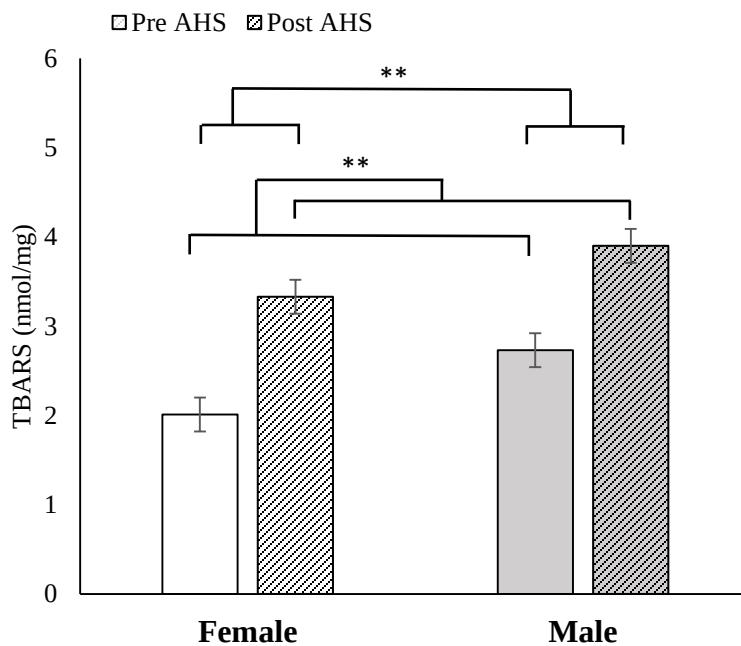


Fig. 4.1. Concentration of thiobarbituric acid reactive substances (TBARS) in pectoralis major of broiler chickens as influenced by broiler sex ($P=0.0001$) and exposure to acute heat stress (AHS) ($P<0.0001$). Heat challenge occurred at 43 d for 4 h at 32°C. Data were subjected to log transformation for statistical analysis. Values are presented as untransformed means and standard error of means. $N = 60$ individuals per treatment interaction.

** Means differ significantly ($P \leq 0.0001$).

avUCP mRNA expression

Gene expression of avUCP was not shown to be significantly affected by dietary or incubation treatments (Supplementary Table 4.1), as determined by stepwise regression analysis. The effect of bird sex, heat challenge, and their interaction on avUCP expression is displayed in Figure 4.2. Increased avUCP expression was noted for males ($P < 0.0001$) while acute heat stress resulted in a significant down regulation of avUCP ($P = 0.0003$). A significant interaction was noted for sex and AHS, with males prior to heat exposure exhibiting the greatest abundance of avUCP mRNA ($P = 0.0001$).

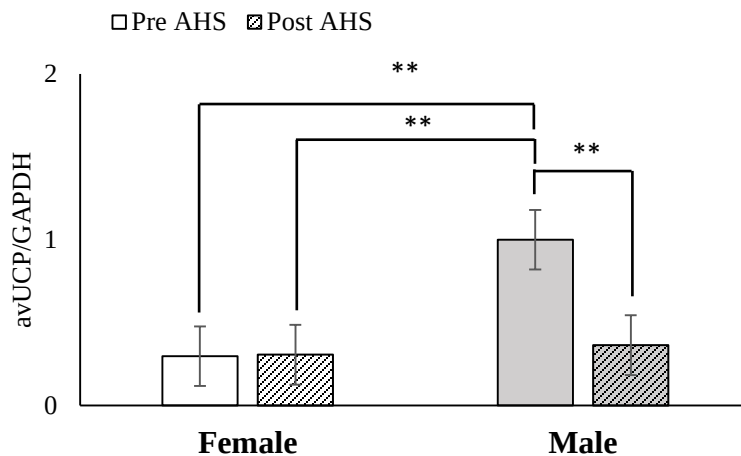


Fig. 4.2. Avian uncoupling protein (avUCP) expression in pectoralis major of broiler chickens as influenced by the interaction between broiler sex and exposure to acute heat stress (AHS) ($P=0.0001$). Pre-AHS samples were taken on 42 d and post AHS samples were taken immediately following a heat challenge at 32°C for 4 h on 43 d. Values are presented as means and standard error of means. $N = 60$ birds per treatment interaction group.

** Means differ significantly ($P < 0.0001$).

4.5 Discussion

The expression of avUCP mRNA in breast muscle was not influenced by TM, in agreement with previous studies (Loyau et al., 2014; Al-Zghoul et al., 2015). Although upregulation of avUCP has been observed in the liver of TM birds (Al-Zghoul et al., 2019), avUCP expression in skeletal muscle, as evaluated in the current trial, was not altered by incubation treatment. This disparity likely represents differences in tissue regulation as other work has demonstrated variations in avUCP expression between skeletal muscle and liver tissue (Vianna et al., 2001; Del Vesco et al., 2013). The breast portion was chosen for sampling in the current trial as skeletal muscle has been shown to exhibit the greatest abundance of avUCP mRNA in broilers, while liver and adipose tissues displayed the lowest (Evock-Clover et al., 2002; Dridi et al., 2004). Additionally, breast meat is considered a premium portion and ROS accumulation resulting from high ambient temperatures has been linked to undesirable meat characteristics that can result in economic loss at processing (Sandercock et al., 2001; Wang et al., 2009). A reduction in breast muscle oxidative damage, through either TM adaptation or avUCP expression, would presumably result in a corresponding reduction in losses associated with poor meat quality in heat stressed broilers. However, similar to avUCP expression, significant differences in TBARS concentrations in breast meat were not apparent between the incubation treatments. The decrease in BWG and FI noted for the TM birds presented here further suggests that TM may have been detrimental to bird performance, although a decrease in mortality following AHS was noted by our group (Brannan et al., 2021) and other authors (Piestun et al., 2008; Zaboli et al., 2017). However, the improved livability noted for the TM birds did not compensate for their reduced growth, as demonstrated by the similar PEF between the groups in the current results. While the balance between bird performance and heat stress

mortality resulting from TM remains to be elucidated, the present results do not support a role for avUCP expression or reduced oxidative damage in TM adaptation.

Dietary fat source was not shown to significantly alter avUCP expression or TBARS concentration in the present trial. The difference between the current results and previous work demonstrating significant avUCP upregulation associated with USFA (Mujahid et al., 2009; Seifi et al., 2018) are likely due to differences in trial design. The heat challenge implemented by Seifi et al. (2018) occurred for a period of 10 d and chronic heat stress has been reported by other authors to upregulate avUCP, independent of diet (Dridi et al., 2008; Azad et al., 2010). Similarly, the method of olive oil supplementation utilized by Mujahid et al. (2009) may have resulted in a greater difference in diet energy between treatment diets when compared to the present trial diets. Higher levels of dietary fat have been shown to upregulate avUCP expression (Collin et al., 2003b), while the current trial diets were determined to be similar in energy content and representative of a commercially relevant diet. Levels of TBARS were also unaltered by dietary fat source which may be related to the similarities between diets in avUCP expression, based on the proposed role of avUCP as a modulator of oxidative stress. Overall, the current results suggest that dietary fat source does not play a role in avUCP regulation nor the reduction of oxidative damage during AHS.

Although dietary fat source alone was not shown to affect production performance, a significant interaction was noted for fat source and incubation treatment for FCR and PEF following heat stress, concurrent with a similar trend in BWG. The differences in FCR and PEF between treatment interactions were only apparent following AHS and may be linked instead to the adverse effects of hyperthermia on broiler performance (Teeter and Belay, 1996). The calculations for FCR and PEF are subject to variations in BWG, FI, and livability which

fluctuated widely within the interactions following the heat challenge. Previous work in our group noted a numerically larger mortality rate for the CN-OO birds (Brannan et al., 2021), which likely contributed to the poorer PEF in the present results. Heavier birds are more susceptible to heat stress in terms of reduced growth and increased mortality (Cahaner and Leenstra, 1992) and it may be speculated that the impact of AHS on these top performing birds resulted in increased variation for FCR and PEF. However, the similarities between the interaction groups for avUCP expression and TBARS levels do not indicate a role for these variables in influencing bird performance.

In the current trial, a significant increase in TBARS was observed concurrent with the downregulation of avUCP expression following AHS exposure. As heat stress is well-established to induce free radical production, lipid peroxidation, and oxidative damage in broilers (Altan et al., 2003; Mujahid et al., 2005; Lin et al., 2006; Azad et al., 2010), the rise in TBARS post-AHS was to be expected. In agreement with other authors (Mujahid et al., 2006; Kikusato and Toyomizu, 2013) avUCP expression was decreased following AHS, with males being notably affected as demonstrated in the heat stress by sex interaction results. To the authors knowledge, this is the first report of differences in broilers between the sexes in avUCP mRNA expression and may explain the disparities of heat stress on avUCP expression in the literature. Previous work demonstrating avUCP downregulation in broilers exposed to heat stress exclusively sampled male birds (Mujahid et al., 2006, 2009; Kikusato and Toyomizu, 2013) while more ambiguous results occurred in mixed sex trials (Al-Zghoul et al., 2015). Given the present results, it may be speculated that in mixed sex studies the ratio of male to female birds may have greater effect on avUCP expression than heat stress and should be considered as an influencing factor in future trials.

The higher expression of avUCP noted for males prior to AHS may in part be driven by the increased TBARS concentrations observed for these birds in the current results. Males have been shown to exhibit a greater susceptibility to oxidative stress, which has been proposed to arise from their rapid growth rate when compared to female broilers (Brothers et al., 2019). As free radical production is a normal product of increased mitochondrial activity (Hensley et al., 2000), male broilers may potentially generate higher levels of ROS than their female counterparts under standard conditions due to their increased metabolic rate (Meltzer, 1983). The increase in avUCP expression may be in response to the increased free radical generation but may not be sufficient to curb oxidative damage. Similar to the occurrence of ascites in broilers (Julian, 1998), it may be speculated that intensive selection for production traits such as rapid growth rate was not concurrent with selection for the physiological systems to support these traits, resulting in metabolic imbalance. Under normal conditions, this asymmetry may not be notably detrimental to male broiler performance but likely contributes to the higher levels of stress noted for males as well as their increased susceptibility to heat stress (Cahaner and Leenstra, 1992; Brothers et al., 2019). Therefore, research into heat interventions that account for bird sex may prove more effective in limiting the negative effects of heat stress on broiler performance.

4.6 Conclusion

Neither TM nor dietary fat source were shown to contribute to avUCP expression, decreased TBARS levels, or improvements in bird performance in the present trial. However, the current results highlight the importance of bird sex in developing heat stress management strategies, as males demonstrated the highest levels of TBARS and avUCP expression. As male broilers demonstrate a higher growth rate, heavier BW and greater carcass yield, interventions that successfully attenuate the effects of heat stress for males will likely have a greater success in

maintaining whole flock performance. To the authors' knowledge, this is the first report of sexual dimorphism in avUCP mRNA expression in broilers. Future work into broiler management during high temperatures, oxidative stress, and avUCP expression would benefit from including sex as a variable.

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4.8 References

- Abe, T., Mujahid, A., Sato, K., Akiba, Y., Toyomizu, M., 2006. Possible role of avian uncoupling protein in down-regulating mitochondrial superoxide production in skeletal muscle of fasted chickens. *FEBS Lett.* 580, 4815-4822.
- Altan, Ö, Pabuçcuoğlu, A., Altan, A., Konyalioğlu, S., Bayraktar, H., 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Br. Poult. Sci.* 44, 545-550.
- Al-Zghoul, M., El-Bahr, S.M., Al-Rukibat, R.K., Abd Elhafeed, S.D., Althnaian, T.A., Al-Ramadan, S.Y., 2015. Biochemical and molecular investigation of thermal manipulation protocols during broiler embryogenesis and subsequent thermal challenge. *BMC Veterinary Research* 11, 292.
- Al-Zghoul, M.B., Sukker, H., Ababneh, M.M., 2019. Effect of thermal manipulation of broilers embryos on the response to heat-induced oxidative stress. *Poult. Sci.* 98, 991-1001.
- 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*. 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. 7th rev. ed. Amer. Oil Chem.' Soc., Champaign, Ill.
- Armstrong, D., Browne, R., 1994. The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory, in: Free Radicals in Diagnostic Medicine. Springer, Boston, MA, pp. 43-58.
- Aviagen, 2018. Section 6 – Housing and environment, in: Ross Broiler Management Handbook. Aviagen, Huntsville, AL, pp. 79-105.
- Azad, M., Kikusato, M., Maekawa, T., Shirakawa, H., Toyomizu, M., 2010. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.* 155, 401-406.
- Brannan, K.E., Livingston, K.A., van Rensburg, C.J., 2021. Embryonic thermal manipulation and dietary fat source during acute heat stress. 1. Effect on hatchability and broiler performance. *J. Appl. Poult. Res.*, 100143.
- Brothers, B.K., Zhuo, Z., Papah, M., Abasht, B., 2019. RNA-seq analysis reveals spatial and sex differences in pectoralis major muscle of broiler chickens contributing to difference in susceptibility to wooden breast disease. *Front. Physiol.* 10, 764.
- Bugajski, J., Turoń, M., Gadek-Michalska, A., Borycz, J.A., 1991. Catecholaminergic regulation of the hypothalamic-pituitary-adrenocortical activity. *J. Physiol. Pharmacol.* 42, 93-103.
- Bustin, S.A., Benes, V., Garson, J.A., Helleman, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clin. Chem.* 55, 611-622.
- Cahaner, A., Deeb, N., 2000. Reduced heat tolerance in fast-growing broilers. *BSAP Occas. Publ.* 27, 85-86.
- Cahaner, A., Leenstra, F., 1992. Effects of high temperature on growth and efficiency of male and female broilers from lines selected for high weight gain, favorable feed conversion, and high or low fat content. *Poult. Sci.* 71, 1237-1250.

- Collin, A., Picard, M., Yahav, S., 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., Taouis, M., Buyse, J., Ifuta, N.B., Darras, V.M., Van As, P., Malheiros, R.D., Moraes, V.M., Decuypere, E., 2003a. Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chicken. *Am. J. Physiol. Endocrinol. Metab.* 284, E771-E777.
- Collin, A., R. D. Malheiros, V. M. Moraes, P. Van As, V. M. Darras, M. Taouis, E. Decuypere, and J. Buyse. 2003b. Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. *Br. J. Nutr.* 90, 261-269.
- Cross, C.E., Halliwell, B., Borish, E.T., Pryor, W.A., Ames, B.N., Saul, R.L., McCord, J.M., Harman, D., 1987. Oxygen radicals and human disease. *Ann. Intern. Med.* 107, 526-545.
- Daghir, N. J., 2008. Broiler feeding and management in hot climates in: *Poultry Production in Hot Climates*, 2nd ed. CABI, Cambridge, MA, pp 227-260.
- Dale, N., Fuller, H.L., 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 Grey, A.D., 2002. HO₂•: The forgotten radical. *DNA Cell Biol.* 21, 251-257.
- Deeb, N., Cahaner, A., 2002. Genotype-by-environment interaction with broiler genotypes differing in growth rate. 3. Growth rate and water consumption of broiler progeny from weight-selected versus nonselected parents under normal and high ambient temperatures. *Poult. Sci.* 81, 293-301.
- Del Vesco, A.P., Gasparino, E., Neto, A.R.O., Rossi, R.M., Soares, M.A.M., da Silva, S.C.C., 2013. Effect of methionine supplementation on mitochondrial genes expression in the breast muscle and liver of broilers. *Livest. Sci.* 151, 284-291.
- Dridi, S., Onagbesan, O., Swennen, Q., Buyse, J., Decuypere, E., Taouis, M., 2004. Gene expression, tissue distribution and potential physiological role of uncoupling protein in avian species. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 139, 273-283.

- Dridi, S., Temim, S., Derouet, M., Tesseraud, S., Taouis, M., 2008. Acute cold- and chronic heat- exposure upregulate hepatic leptin and muscle uncoupling protein (UCP) gene expression in broiler chickens. *J. Exp. Zool. A: Ecol. Genet. Physiol.* 309, 381-388.
- Enerbäck, S., Jacobsson, A., Simpson, E.M., Guerra, C., Yamashita, H., Harper, M., Kozak, L.P., 1997. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387, 90-94.
- Evock-Clover, C.M., Poch, S.M., Richards, M.P., Ashwell, C.M., McMurtry, J.P., 2002. Expression of an uncoupling protein gene homolog in chickens. *Comp. Biochem. Physiol. A: Molecul. Integr. Physiol.* 133, 345-358.
- Havenstein, G.B., Ferket, P.R., Qureshi, M.A., 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poult. Sci.* 82, 1500-1508.
- Henry, M.H., Burke, W.H., 1998. Sexual dimorphism in broiler chick embryos and embryonic muscle development in late incubation. *Poult. Sci.* 77, 728-736.
- Hensley, K., Robinson, K.A., Gabbita, S.P., Salsman, S., Floyd, R.A., 2000. Reactive oxygen species, cell signaling, and cell injury. *Free Radic. Biol. Med.* 28, 1456-1462.
- Jenkins, S.A., Porter, T.E., 2004. Ontogeny of the hypothalamo–pituitary–adrenocortical axis in the chicken embryo: a review. *Domest. Anim. Endocrinol.* 26, 267-275.
- Joubert, R., Métayer-Coustard, S., Crochet, S., Cailleau-Audouin, E., Dupont, J., Duclos, M.J., Tesseraud, S., Collin, A., 2011. Regulation of the expression of the avian uncoupling protein 3 by isoproterenol and fatty acids in chick myoblasts: possible involvement of AMPK and PPAR α ? *Am. J. of Physiol. Regul. Integr. Comp. Physiol.* 301, R201-R208.
- Julian, R.J., 1998. Rapid growth problems: ascites and skeletal deformities in broilers. *Poult. Sci.* 77, 1773-1780.
- Kikusato, M., Toyomizu, M., 2013. Crucial role of membrane potential in heat stress-induced overproduction of reactive oxygen species in avian skeletal muscle mitochondria. *PLOS One* 8, e64412.

- Lin, H., Decuypere, E., Buyse, J., 2006. Acute heat stress induces oxidative stress in broiler chickens. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 144, 11-17.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta CT$ method. *Methods* 25, 402-408.
- Loyau, T., Berri, C., Bedrani, L., Métayer-Coustard, S., Praud, C., Duclos, M.J., Tesseraud, S., Rideau, N., Everaert, N., Yahav, S., 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., Métayer-Coustard, S., Berri, C., Crochet, S., Cailleau-Audouin, E., Sannier, M., Chartrin, P., Praud, C., Hennequet-Antier, C., Rideau, N., 2014. Thermal manipulation during embryogenesis has long-term effects on muscle and liver metabolism in fast-growing chickens. *PLOS One* 9, e105339.
- MacLellan, J. D., Gerrits, M. F., Gowing, A., Smith, P. J., Wheeler, M. B., Harper, M. E., 2005. Physiological increases in uncoupling protein 3 augment fatty acid oxidation and decrease reactive oxygen species production without uncoupling respiration in muscle cells. *Diabetes*, 54, 2343-2350.
- Mailloux, R.J., Harper, M., 2011. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic. Biol. and Med.* 51, 1106-1115.
- 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.
- Meltzer, A., 1983. Thermoneutral zone and resting metabolic rate of broilers. *Br. Poult. Sci.* 24, 471-476.
- Moraes, V., Malheiros, R.D., Bruggeman, V., Collin, A., Tona, K., Van As, P., Onagbesan, O.M., Buyse, J., Decuypere, E., Macari, M., 2003. Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. *J. Therm. Biol.* 28, 133-140.

- Mujahid, A., Sato, K., Akiba, Y., Toyomizu, M., 2006. Acute heat stress stimulates mitochondrial superoxide production in broiler skeletal muscle, possibly via downregulation of uncoupling protein content. *Poult. Sci.* 85, 1259-1265.
- Mujahid, A., Yoshiki, Y., Akiba, Y., Toyomizu, M., 2005. Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poult. Sci.* 84, 307-314.
- Mujahid, A., Akiba, Y., Toyomizu, M., 2009. Olive oil-supplemented diet alleviates acute heat stress-induced mitochondrial ROS production in chicken skeletal muscle. *m. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R690-R698.
- Nichelmann, M., Tzschentke, B., 2002. Ontogeny of thermoregulation in precocial birds. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 131, 751-763.
- Nogueira, B., Reis, M.P., Carvalho, A.C., Mendoza, E., Oliveira, B.L., Silva, V.A., Bertechini, A.G., 2019. Performance, growth curves and carcass yield of four strains of broiler chicken. *Braz. J. Poult. Sci.* 21, 4.
- NRC, 1994. Nutrient requirements of poultry, ninth revised ed. National Academy Press, Washington, DC. pp. 19-34.
- Osman, A. M. A., Tawfik, E. S., Ristic, M., Hebel, W., Klein, F. W., 1989. Effect of environmental temperature on growth, carcass traits and meat quality of broilers of both sexes and different ages, 3: Tissue composition of breast and thighs. *Arch. Für Geflügelkunde*, 53, 244-250.
- Piestun, Y., Shinder, D., Ruzal, M., Halevy, O., Brake, J., Yahav, S., 2008. Thermal manipulations during broiler embryogenesis: effect on the acquisition of thermotolerance. *Poult. Sci.* 87, 1516-1525.
- Pryor, W.A., 1986. Oxy-radicals and related species: their formation, lifetimes, and reactions. *Annu. Rev. Physiol.* 48, 657-667.
- Raimbault, S., Dridi, S., Denjean, F., Lachuer, J., Couplan, E., Bouillaud, F., Bordas, A., Duchamp, C., Taouis, M., Ricquier, D., 2001. An uncoupling protein homologue putatively involved in facultative muscle thermogenesis in birds. *Biochem. J.* 353, 441-444.

Rey, B., Spée, M., Belouze, M., Girard, A., Prost, J., Roussel, D., Duchamp, C., 2010. Oxygen recovery up-regulates avian UCP and ANT in newly hatched ducklings. *Journal of Comparative Physiology B* 180, 239-246.

Ricquier, D., Bouillaud, F., 2000. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *J. Physiol.* 529, 3-10.

Saini, N., Helfrich, K.K., Kwan, S.T.C., Huebner, S.M., Abazi, J., Flentke, G.R., Blohowiak, S.E., Kling, P.J., Smith, S.M., 2019. Alcohol's dysregulation of maternal–fetal IL- 6 and p-STAT3 is a function of maternal iron status. *Alcohol. Clin. Exp. Res* 43, 2332-2343.

Sandercock, D.A., Hunter, R.R., Nute, G.R., Mitchell, M.A., Hocking, P.M., 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. *Poult. Sci.* 80, 418-425.

Seifi, K., Rezaei, M., Yansari, A.T., Riazi, G.H., Zamiri, M.J., Heidari, R., 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.

Settar, P., Yalçın, S., Turkmut, L., Ozkan, S., Cahanar, A., 1999. Season by genotype interaction related to broiler growth rate and heat tolerance. *Poult. Sci.* 78, 1353-1358.

Skulachev, V.P., 1996. Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants. *Q. Rev. Biophys.* 29, 169-202.

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

Talbot, D.A., Duchamp, C., Rey, B., Hanuise, N., Rouanet, J.L., Sibille, B., Brand, M.D., 2004. Uncoupling protein and ATP/ADP carrier increase mitochondrial proton conductance after cold adaptation of king penguins. *J. Physiol.* 558, 123-135.

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

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

- Vianna, C.R., Hagen, T., Zhang, C., Bachman, E., Boss, O., Gereben, B., Moriscot, A.S., Lowell, B.B., Bicudo, J.E.P., Bianco, A.C., 2001. Cloning and functional characterization of an uncoupling protein homolog in hummingbirds. *Physiol. Genomics* 5, 137-145.
- Vidal-Puig, A.J., Grujic, D., Zhang, C., Hagen, T., Boss, O., Ido, Y., Szczepanik, A., Wade, J., Mootha, V., Cortright, R., 2000. Energy metabolism in uncoupling protein 3 gene knockout mice. *J. Biol. Chem.* 275, 16258-16266.
- Wang, R.R., Pan, X.J., Peng, Z.Q., 2009. Effects of heat exposure on muscle oxidation and protein functionalities of pectoralis majors in broilers. *Poult. Sci.* 88, 1078-1084.
- Wiseman, J., Salvador, F., 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.
- Wiseman, J., Powles, J., Salvador, F., 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.
- Yagi, K., 1998. Simple assay for the level of total lipid peroxides in serum or plasma. *Methods Mol. Biol.* 108, 101-106.
- Yahav, S., Rath, R.S., Shinder, D., 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., Shinder, D., Tanny, J., Cohen, S., 2005. Sensible heat loss: the broiler's paradox. *Worlds Poult. Sci. J.* 61, 419-434.
- Yalçın, S., Özkan, S., Türkmüt, L., Siegel, P.B., 2001. Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. *Br. Poult. Sci.* 42, 149-152.
- Zaboli, G., Rahimi, S., Shariatmadari, F., Torshizi, M. A. K. , Baghbanzadeh, A., Mehri, M., 2017. Thermal manipulation during pre and post-hatch on thermotolerance of male broiler chickens exposed to chronic heat stress. *Poult. Sci.* 96, 478-485.
- Zhang, L., Yue, H.Y., Wu, S.G., Xu, L., Zhang, H.J., Yan, H.J., Cao, Y.L., Gong, Y.S., Qi, G.H., 2010. Transport stress in broilers. II. Superoxide production, adenosine phosphate concentrations, and mRNA levels of avian uncoupling protein, avian adenine nucleotide

translocator, and avian peroxisome proliferator-activated receptor- γ coactivator-1 α in skeletal muscles. Poult. Sci. 89, 393-400.

Zuidhof, M.J., Schneider, B.L., Carney, V.L., Korver, D.R., Robinson, F.E., 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult. Sci. 93, 2970-2982.

4.9 Supplementary data

Table S4.1. Concentrations of thiobarbituric acid reactive substances (TBARS¹) and avian uncoupling protein (avUCP) mRNA expression in pectoralis major of broiler chickens as influenced by the main effects of incubation treatment² and dietary fat source³, as well as their interactions.

Treatment	Pre-AHS		Post-AHS	
	TBARS, nmol/mg	avUCP, avUCP/GAPDH	TBARS, nmol/mg	avUCP, avUCP/GAPDH
Incubation				
CN	2.17	0.09	3.67	0.029
TM	2.57	-0.08	3.56	-0.028
SEM ⁴	0.14	0.25	0.26	0.130
P-Value	0.134	0.486	0.835	0.660
Diet				
Olive	2.29	0.17	3.64	0.010
Poultry	2.22	-0.01	3.40	0.024
Soya	2.58	-0.14	3.81	-0.030
SEM	0.18	0.31	0.32	0.161
P-Value	0.527	0.5993	0.770	0.941
Incubation x diet				
CN-Olive	2.16	0.67	3.53	0.011
CN-Poultry	2.04	-0.10	3.26	-0.012
CN-Soya	2.31	-0.14	4.23	0.091
TM-Olive	2.43	-0.18	3.75	0.020
TM-Poultry	2.42	0.09	3.53	0.061
TM-Soya	2.86	-0.14	3.39	-0.138
SEM	0.25	0.44	0.45	0.227
P-Value	0.833	0.290	0.377	0.611

¹ TBARS data were subjected to log transformation for statistical analysis. Values are presented as untransformed means and standard error of means.

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 Poultry = poultry fat; Soya = soya oil; Olive = olive oil added at 4.5 % to finisher diet.

⁴ SEM = Standard error of mean.

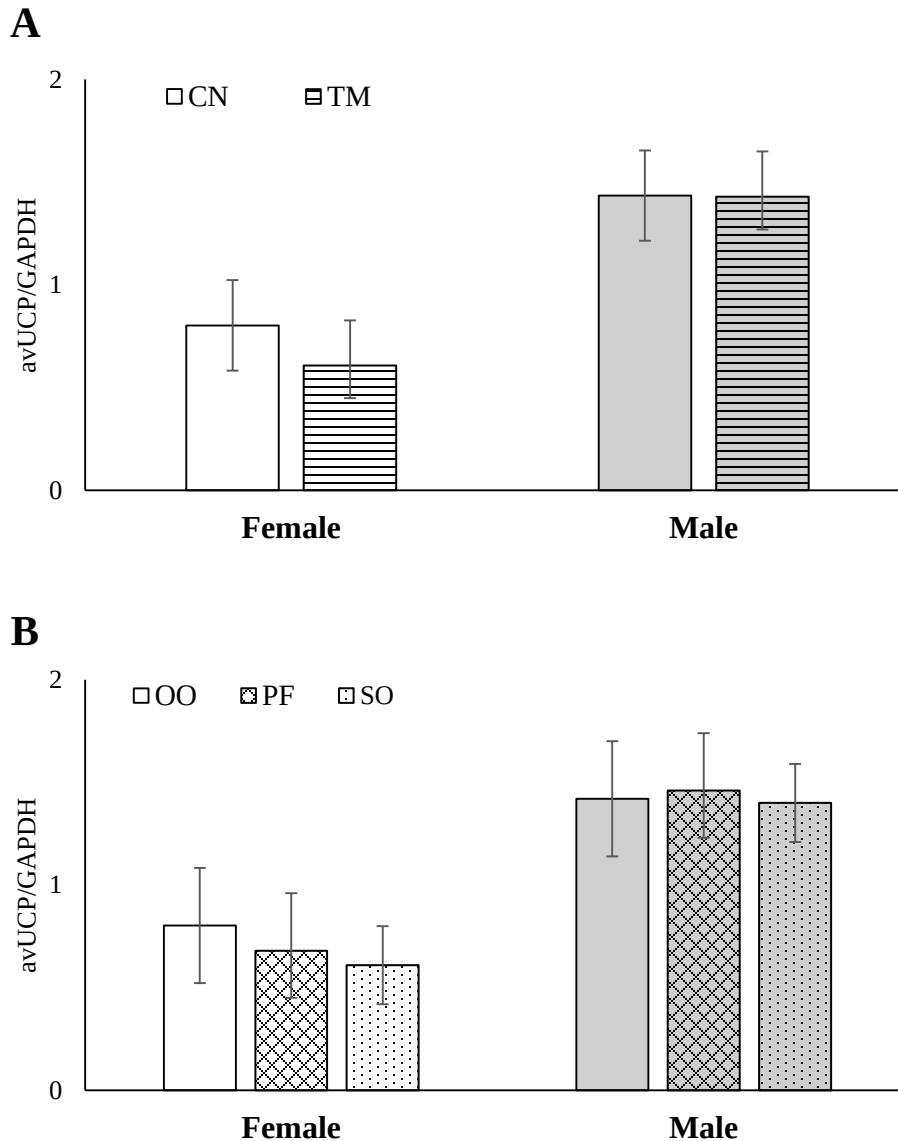


Fig. S1. Avian uncoupling protein expression in pectoralis major of broiler chickens as influenced by the interaction between broiler sex and **(A)** incubation treatment ($P = 0.3040$) and **(B)** dietary fat source ($P = 0.6893$). Incubation treatments consisted of either thermal manipulation (TM) that alternated between the control machine and a TM machine set at 39.5°C and 65 % RH in 12 h cycles or the control group (CN) with a machine temperature of 37.5°C and 56 % RH. Fat sources included olive oil (OO), poultry fat (PF), and soy oil (SO) at 4.5% each in the finisher diet. Values are presented as means and standard error of means. $N = 60$ birds per incubation interaction group and $N = 40$ birds per diet fat interaction.

CHAPTER 5

Conclusions, applications, and future research prospects

5.1 Thermal manipulation

Although TM did improve bird livability during AHS, hatchability and broiler performance were greatly depressed. The increase in embryo mortality and subsequent decrease in hatchability observed for the TM group was likely due to the increased EST observed during TM exposure. Eggshell temperatures are generally accepted to be a more accurate representation of the temperature experienced by the embryo, as opposed to machine air temperature (Meijerhof and Van Beek, 1993). Embryo temperature is a balance between metabolic heat production by the embryo, evaporative heat loss, and incubator temperature (French, 1997). In the current study, relative humidity was adjusted to result in similar moisture loss between treatments so that differences in machine temperature were emphasized as part of the TM treatment. While both groups demonstrated an increase in EST beyond machine set point from approximately E10, the difference was gradually reduced for the TM group with embryonic age and suggested a reduction in embryonic metabolism. Concurrent with the increase in mid to late embryonic mortality, it is clear the high incubation temperatures applied during TM were harmful to embryo growth. The present study is one of the few to report embryonic mortality and EST in conjunction with TM application and demonstrates the importance of including these parameters in incubation research. Differences in genetic strain (Hamidu, et al., 2018), flock age (Hamidu, et al., 2007), and incubator design (French, 1997) alter how the embryo responds to its environment and contribute to variations in development when incubation is based on machine temperature alone. Future work into TM protocols that utilize temperature profiles based on EST can reduce these variations and will likely produce more consistent results between trials.

The increased EST experienced by the TM group likely contributed to their poor post-hatch performance, as several reports have noted a relationship between high EST and decreased broiler performance (Lourens, et al., 2005; Joseph, et al., 2006; Hulet, et al., 2007; Leksrisonpong, et al., 2009; Molenaar, et al., 2011). Although TM did support reduced mortality during AHS, the improvements in livability may have been associated with the smaller BW of the TM birds rather than heat stress adaptations. Heavier birds are known to be more susceptible to heat stress (Cahaner and Leenstra, 1992) due to their decreased capacity for heat loss (Yahav, et al., 2005), while the lower BW and FI of the TM birds may have resulted in improved heat loss as well as decreased metabolic heat production. Similarly, the decreased carcass weights demonstrated by TM birds likely contributed to their reduction in WB and WS, as BMM tend to be more prevalent in high yield broilers (Velleman, 2015). Although the results here support improved livability during AHS for TM birds, the negative effects on performance noted at the hatchery, rearing, and processing levels do not make TM a viable heat stress intervention. The costs incurred by extending the rearing period to reach BW targets would likely negate any economic benefits of reduced AHS mortality and additional losses at the hatchery and processing levels would result in further economic cost for integrated companies. Future research concerning TM profiles based on EST may offer improvements in thermotolerance without the negative effects observed here; however, such work must also consider commercially relevant parameters in order to be applicable.

5.2 Dietary fat source

Dietary fat source during the finisher period was not shown to alter bird performance or response to AHS. Despite improvements in meat color and drip loss with increased USFA levels in the current results, more economically significant traits such as carcass composition or yield

remained similar between diets. In the present trial, dietary fat sources were applied during the finisher stage as older birds are more susceptible to heat stress and it was hypothesized that the benefits of USFA would be more apparent. However, it may be that at commercially relevant inclusion levels, fatty acid profile is less influential on bird performance (De Witt, et al., 2009). As feed costs constitute nearly 75% of production costs, these results suggest that nutritionist may have flexibility when creating least-cost formulations. The similarities in performance between dietary fat sources presented here indicate that in markets where prices for USFA ingredients are high, SFA fats such as poultry by-product may be an economical alternative for finisher diets. However, consideration should be given to the inclusion levels of SFA during heat stress as higher levels may result in performance losses greater than the reduced formulation costs.

Despite performance remaining similar between dietary fat source treatments prior to AHS, a significant interaction was noted between incubation treatment and dietary fat source following the heat challenge. Differences in nutrient metabolism have been observed to occur in TM birds following heat stress (Loyau, et al., 2014, 2016; Al-Zghoul, et al., 2019a) and the current results may support similar adaptations. A corresponding trend between treatment interactions was also observed for BWG in the week following AHS and in p. major weights at processing, which appear to support a change in how the TM birds responded to dietary fat source. The application of TM during the second week of incubation also coincides with a period of intensive embryonic growth, during which the embryo primarily relies on yolk lipids to fuel its development (Moran Jr, 2007); high temperatures during this time may inadvertently alter the metabolic pathways of the developing embryo, with the consequences being amplified during rearing (De Oliveira, et al., 2008). Along with improved understanding of how TM alters

embryonic development, work concerning how TM may impact post-hatch nutrient metabolism and bird performance is also needed before it can be reliably implemented. As well as specific EST profiles, TM birds may benefit from specific dietary formulation (particularly following heat challenge). While dietary fat source as a main effect did not alter bird performance, the significant interaction with TM in the present results indicate a need for further work into how different heat stress interventions interact, particularly given the prevalence of fat supplementation during heat stress.

5.3 AvUCP and TBARS

Neither TM nor dietary fat source were shown to influence avUCP and TBARS concentrations in the current work. Interestingly, upregulation has been reported for avUCP in the liver of birds utilizing a TM profile similar to the one applied in the current trial (Al-Zghoul, et al., 2019b), however with different broiler strains than examined here. Differences in avUCP expression pattern were noted in that study between the strains during AHS and may be related to variations in EST during TM, as previously discussed. The discrepancy between liver and skeletal muscle tissue expression for avUCP has also been reported by other authors for various stimuli (Vianna, et al., 2001; Del Vesco, et al., 2013) and may represent different functions for avUCP dependent on tissue and treatment. Phylogenetic analysis of currently identified UCP suggests that birds have lost UCP1 and UCP2 or UCP3 at some point during evolution, which may have led to the development of avUCP serving multiple functions to compensate for these losses (Emre, et al., 2007). This hypothesis would explain the varied and frequently contradictory results for avUCP expression within the literature, as well as the similarities in proposed functions and homology with the three main mammalian UCP. While future work is needed to clarify the regulation and potential functions of avUCP, the current results do not

support a role for the protein in heat stress management through TM adaptation or dietary fatty acid profile.

In the current trial, avUCP was found to be downregulated by AHS concurrent with an increase in TBARS concentration, in agreement with previous studies (Mujahid, et al., 2006; Kikusato and Toyomizu, 2013). While avUCP may potentially contribute to regulation of oxidative stress, AHS appears to suppress this function and the heat stress interventions implemented here were not successful in enhancing avUCP mRNA expression. Higher inclusion levels of dietary fat may prove to be more effective at upregulating avUCP during AHS (Collin, et al., 2003a; Mujahid, et al., 2009) and future work should continue to explore the potential of avUCP to reduced oxidative stress during hyperthermia. Particularly in male birds, which exhibited increased levels of both avUCP expression and TBARS in the current results. Sexual dimorphism in broilers has gradually become more exaggerated with increased genetic selection for performance traits (Zuidhof, et al., 2014; Nogueira, et al., 2019) and may inadvertently result in an increased susceptibility to oxidative stress for fast growing males (Brothers, et al., 2019). As sex separate rearing is seldom used in broiler production, the results presented here suggest that heat stress interventions focused on male birds are likely to be successful for the entire flock.

The present study is the first to report differences between the sexes in avUCP expression and may explain some of the variations observed between other works. Studies that sampled only male birds or straight run flocks may not present an accurate picture of the factors influencing avUCP regulation. These results also bring into question what endocrinological factors may be influencing avUCP expression between the sexes. As broilers are still sexually immature at seven weeks of age, sex hormones are unlikely to be involved but work in broiler breeders may be able to verify this theory. Future research into the differences between the sexes observed here may

benefit from investigating hormones related to growth, such as thyroid hormone which has been observed to exert a strong influence on avUCP expression (Collin, et al., 2003b). The role of avUCP in metabolic differences between the sexes would also be of interests, as higher levels of expression have been noted in birds with poorer feed efficiency (Bottje, et al., 2006; Ojano-Dirain, et al., 2007). As female broilers tend to deposit more fat than males (Rondelli, et al., 2003), the dimorphism in avUCP expression may also be related to differences in lipid metabolism or specific enzymes related to lipogenesis. While the reasons behind sex differences in avUCP expression remain to be elucidated, this study clearly indicates a need for sex separate evaluation in future studies to help reduce the effects of sex differences on potential treatments.

5.4 Final overview

The present work contributes to the field of heat stress in broilers by providing an overview of heat stress interventions across multiple production levels, from hatchery to abattoir. An embryonic TM protocol was chosen here as the incubator is a more practical and cost-effective environment to implement TM, as compared to the grow-out house; however, high EST resulted in poor hatchability and broiler performance in the current trial. Previous work with the same TM profile had demonstrated improved heat tolerance without performance losses (Piestun, et al., 2008, 2011; Loyau, et al., 2013; Zaboli, et al., 2017) and the disparity observed here may be due to differences in machine design, flock age, or strain differences as discussed earlier. Measurement of additional parameters such as thyroid hormones and corticosterone levels would have enhanced the current results and perhaps further clarified the poor performance observed, however these were beyond the resources available. Along with these variables, further trials concerning TM would benefit from the inclusion of EST in addition to machine set points to standardize embryo exposure temperature and improve the repeatability of trial conditions.

The current results also demonstrated that dietary fat source may be less influential during heat stress at commercially relevant inclusion levels. Although previous work observed significant differences in bird performance and carcass composition associated with USFA under standard temperatures (Pinchasov and Nir, 1992; Crespo and Esteve-Garcia, 2001, 2002; Newman et al., 2002), these advantages were not apparent in the current trial. Higher inclusion levels of USFA as compared to SFA may have yielded different results (Wiseman and Salvador, 1991; De Witt, et al., 2009), however the inclusion level for the current trial (4.5%) was chosen to be commercially relevant. The addition of different fat sources at different inclusion rates, in conjunction of heat stress, might have enhanced the current trial but would have also limited treatment replication. The objective of the present work was to explore potential synergy between heat stress interventions and not examine nutrition alone. Although the combination of factors limited trial design, the work did highlight an interaction between TM and diet on bird performance following AHS. Other authors have suggested that lipid metabolism may be altered in TM birds following heat stress (Loyau, et al., 2014; Al-Zghoul, et al., 2019a) and further evaluation of enzymes involved in energy metabolism under similar conditions would be of value in validating these proposals.

Although neither of the heat stress intervention evaluated here were shown to alter avUCP mRNA expression, the current results are the first to report differences between the sexes. Sexual dimorphism for broilers in terms of oxidative stress has been previously reported (Brothers, et al., 2019), however further work is needed to determine if the higher avUCP expression noted for males here is associated with free radical management. Other work has suggested that bird age may also influence avUCP expression (Dridi, et al., 2004), however this variable was not examined in the current trial as the focus was primarily on AHS during the

finisher period. A systematic evaluation at of avUCP expression under standard rearing conditions may further highlight differences and shed more light on potential functions of this protein; at the very least such, work would establish a baseline of expression upon which experimental work could further expand. Until such work exists, future investigation into the role of avUCP should at a minimum consider sex as a relevant variable to help elucidate the true regulation and function of this protein.

In conclusion, heat stress remains a problem with no easy solutions. Genetic selection for high yield broilers will likely continue to push the physiological limits of the bird and reduce stress tolerance. Embryonic adaptations such as TM may reduce mortality losses associated with AHS but require further calibration before being commercially applicable. Dietary fat source does not appear to offer any advantages during AHS, however interactions with other heat stress interventions such as TM should be considered. Future work into novel proteins such as avUCP may offer a better understanding of the physiological response of the bird to AHS and contribute to the development of improved heat stress interventions.

5.5 References

- Al-Zghoul, M. B., A. R. e. S. Alliftawi, K. M. M. Saleh, and Z. W. Jaradat. 2019a. Expression of digestive enzyme and intestinal transporter genes during chronic heat stress in the thermally manipulated broiler chicken. *Poult. Sci.* 98:4113-4122.
- Al-Zghoul, M. B., H. Sukker, and M. M. Ababneh. 2019b. Effect of thermal manipulation of broilers embryos on the response to heat-induced oxidative stress. *Poult. Sci.* 98:991-1001.
- Bottje, W., N. R., Pumford, C. Ojano-Dirain, M. Iqbal, and K. Lassiter. 2006. Feed efficiency and mitochondrial function. *Poult. Sci.* 85, 8-14.
- Brothers, B. K., Z. Zhuo, M. Papah, and B. Abasht. 2019. RNA-seq analysis reveals spatial and sex differences in pectoralis major muscle of broiler chickens contributing to difference in susceptibility to wooden breast disease. *Front. Physiol.* 10:764.
- Cahaner, A., and F. Leenstra. 1992. Effects of high temperature on growth and efficiency of male and female broilers from lines selected for high weight gain, favorable feed conversion, and high or low fat content. *Poult. Sci.* 71:1237-1250.
- Collin, A., R. D. Malheiros, V. M. B. Moraes, P. Van As, V. M. Darras, M. Taouis, E. Decuypere, and J. Buyse. 2003a. Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. *Br. J. Nutr.* 90:261-269.
- Collin, A., M. Taouis, J. Buyse, N. B. Ifuta, V. M. Darras, P. Van As, R. D. Malheiros, V. M. B. Moraes, and E. Decuypere. 2003b. Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chicken. *Am. J. Physiol. Endocrinol. Metab.* 284:E771-E777.
- 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.
- Crespo, N., and E. Esteve-Garcia. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poult. Sci.* 80:71-78.

- De Oliveira, J., Z. Uni, and P. Ferket. 2008. Important metabolic pathways in poultry embryos prior to hatch. *Worlds Poult. Sci. J.* 64:488-499.
- 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.
- Del Vesco, A. P., E. Gasparino, A. R. O. Neto, R. M. Rossi, M. A. M. Soares, and S. C. C. da Silva. 2013. Effect of methionine supplementation on mitochondrial genes expression in the breast muscle and liver of broilers. *Livest. Sci.* 151:284-291.
- Dridi, S., Onagbesan, O., Swennen, Q., Buyse, J., Decuypere, E., Taouis, M., 2004. Gene expression, tissue distribution and potential physiological role of uncoupling protein in avian species. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 139, 273-283.
- Emre, Y., C. Hurtaud, D. Ricquier, F. Bouillaud, J. Hughes, and F. Criscuolo. 2007. Avian UCP: the killjoy in the evolution of the mitochondrial uncoupling proteins. *J. Mol. Evol.* 65:392-402.
- French, N. A. 1997. Modeling incubation temperature: the effects of incubator design, embryonic development, and egg size. *Poult. Sci.* 76:124-133.
- Hamidu, J. A., G. M. Fasenko, J. J. R. 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.
- 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.
- 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.

- Kikusato, M., and M. Toyomizu. 2013. Crucial role of membrane potential in heat stress-induced overproduction of reactive oxygen species in avian skeletal muscle mitochondria. *PLoS One* 8:e64412.
- Lekrisompong, N., H. Romero-Sanchez, P. W. Plumstead, K. E. Brannan, S. Yahav, and J. Brake. 2009. Broiler incubation. 2. Interaction of incubation and brooding temperatures on broiler chick feed consumption and growth. *Poult. Sci.* 88:1321-1329.
- 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. 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 Genomics.* 17:329.
- 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. 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.
- Meijerhof, R., and G. Van Beek. 1993. Mathematical modelling of temperature and moisture loss of hatching eggs. *J. Theor. Biol.* 165:27-41.
- 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.
- Moran Jr, E. T. 2007. Nutrition of the developing embryo and hatchling. *Poult. Sci.* 86:1043-1049.

- 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.
- Mujahid, A., K. Sato, Y. Akiba, and M. Toyomizu. 2006. Acute heat stress stimulates mitochondrial superoxide production in broiler skeletal muscle, possibly via downregulation of uncoupling protein content. *Poult. Sci.* 85:1259-1265.
- 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.
- Nogueira, B. R. F., M. P. Reis, A. C. Carvalho, E. A. C. Mendoza, B. L. Oliveira, V. A. Silva, and A. G. Bertechini. 2019. Performance, Growth Curves and Carcass Yield of Four Strains of Broiler Chicken. *Braz. J. Poult. Sci.* 21:1-8.
- Ojano-Dirain, C.P., N. R., Pumford, M. Toyomizu, and W. G. Bottje. 2007. Association of mitochondrial function and feed efficiency. *J. Poult. Sci.* 44, 221-237.
- 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., 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.
- 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.
- Rondelli, S., O., Martinez, and P. T. Garcia. 2003. Sex effect on productive parameters, carcass and body fat composition of two commercial broiler lines. *Braz. J. Poult. Sci.* 5:169-173.
- Velleman, S. G. 2015. Relationship of skeletal muscle development and growth to breast muscle myopathies: a review. *Avian Dis.* 59:525-531.

Vianna, C. R., T. Hagen, C.-Y. Zhang, E. Bachman, O. Boss, B. Gereben, A. S. Moriscot, B. B. Lowell, J. E. P. W. Bicudo, and A. C. Bianco. 2001. Cloning and functional characterization of an uncoupling protein homolog in hummingbirds. *Physiol. Genomics*. 5:137-145.

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

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. *Poult. Sci.* 93:2970-2982.

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