UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE JABOTICABAL

PROGRAMAÇÃO TÉRMICA FETAL: efeitos sobre a temperatura de preferência e características morfofisiológicas de frangos de corte

Viviane de Souza Morita
Bióloga

2015

UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE JABOTICABAL

PROGRAMAÇÃO TÉRMICA FETAL: efeitos sobre a temperatura de preferência e características morfofisiológicas de frangos de corte

Viviane de Souza Morita

Orientador: Profa. Dra. Isabel Cristina Boleli

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Zootecnia

2015

Morita, Viviane de Souza
M862p Programa térmica fetal : efeitos sobre a temperatura de preferência e características morfofisológicas de frangos de corte / Viviane de Souza Morita. – – Jaboticabal, 2015
xix, 114 p. ; 28 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2015
Orientadora: Isabel Cristina Boleli
Banca examinadora: Kênia Cardoso Bícego, Elisabeth Gonzáles, **Silvana Martinez Baraldi Artoni, Vanessa Michalsky Barbosa**
Bibliografia

1. Adaptação epigenética. 2. Aves. 3. Estresse térmico. 4. Incubação. 4. Termotolerância. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 591.3:636.6

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

DADOS CURRICULARES DO AUTOR

VIVIANE DE SOUZA MORITA – nascida em 26 de abril de 1985, na cidade de Jaboticabal (SP), filha de Valter Morita e Vilma Oliveira Souza Morita, ingressou no curso de Ciências Biológicas da Faculdade de Ciências Agrárias e Veterinárias/Unesp - Câmpus de Jaboticabal em março de 2003, graduando-se em janeiro de 2007. Em março de 2009, ingressou no Curso de Pós-Graduação em Zootecnia, em nível de Mestrado, na Faculdade de Ciências Agrárias e Veterinárias/Unesp - Câmpus de Jaboticabal, obtendo o título de mestre em fevereiro de 2011, no mês de março do mesmo ano, iniciou o curso de Doutorado na mesma Instituição, sob orientação da Profa. Dra. Isabel Cristina Boleli.

"Quando você quer alguma coisa, todo o universo conspira para que você realize o seu desejo."

Paulo Coelho

OFEREÇO

Aos meus queridos pais, Vilma e Valter
Ao meu irmão, Junior
Ao meu noivo, Geziel
Por toda dedicação, compreensão, incentivo, apoio, ajuda e amor incondicional.
Obrigada por estarem sempre ao meu lado. Sem vocês, eu não teria alcançado esta vitória na minha vida.
E ao meu avô, Arthur
Por todos os ensinamentos, dedicação, amor e zelo.
Obrigada por estar sempre presente em minha vida, mesmo que em pensamento.
Amo muito todos vocês!

DEDICO

À Profa. Dra. Isabel Cristina Boleli,
Pelos 12 anos de ensinamentos, orientação, apoio, amizade, incentivo e exemplo de profissionalismo.
Eu só tenho a lhe dizer: Muito obrigada por tudo!

AGRADECIMENTOS

A Deus, pelo dom da vida e por ter permanecido sempre ao meu lado, me iluminando, e me dando força e sabedoria.

À Faculdade de Ciências Agrárias e Veterinárias/ Unesp – Campus de Jaboticabal e ao Programa de Pós-graduação em Zootecnia pela oportunidade oferecida para a realização do curso.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp), pela concessão da bolsa de doutorado (proc. 2011/18373-2), da bolsa BEPE (proc. 2012/22133-0) e pelo auxílio de pesquisa concedido (proc. 2011/24156-4).

À banca de qualificação, Profas. Dras. Luciane Helena Gargaglioni Batalhão, Hirasilva Borba e Lizandra Amoroso e ao Prof. Dr. Alex Sandro Maia, pelas considerações.

Ao professor João Ademir, pelo auxílio nas análises estatísticas.

À Profa. Dra. Laura Nakaghi e equipe, pela paciência e cooperativismo.

Aos companheiros de trabalho e grandes amigos: Tamiris, João, Vitor, Eduardo, Mariana, Paula, Rachel, Evandro, Gisele, Joice, Diego, Sarah, Tocha e Liliana. Agradeço a todos vocês pela valiosa colaboração na realização desse trabalho, pelo apoio nas horas mais difíceis e pelo companheirismo nos momentos de descontração.

Ao Edmar e Sr. Orandi, pela confecção das lâminas histológicas, pela ajuda e pela amizade ao longo desses anos.

Aos funcionários da FCAV/UNESP: Clara, Wagner, Euclides e Caíque (Departamento de Morfologia e Fisiologia Animal), Izildo, Robson e Vicente (Setor de Avicultura), Oswaldo e Helinho (Fábrica de Ração), a todos, muito obrigada pela importante colaboração na realização deste trabalho.

Às minhas amigas Mariane, Fernanda, Camila, Marília, Flávia, Vânia e Tânia, pela felicidade compartilhada, por torcerem por mim e estarem sempre ao meu lado.

Ao Dr. Henry van den Brand, da Wageningen University, por todo o aprendizado, paciência, apoio, experiência e por ter me acolhido tão bem no ADP (Adaptation Physiology Group).

Aos amigos queridos que ficaram em Wageningen: Ampai, Novi, Sofie, Juncai, Carol, Carolina e Michiko, pelo aprendizado, pelos momentos inesquecíveis e pelas alegrias compartilhadas.

Aos funcionários da Wageningen University: Nanette, Ger, Rudi, Marcel, Monique e Ilona, por toda ajuda, zelo e apoio dispensados.

À toda minha família e à família do meu noivo, que sempre me apoiaram em todas as minhas decisões. Obrigada pela confiança, orações e pelo amor que sempre tiveram.

São pessoas assim tão especiais que me ajudaram a superar todos meus obstáculos e todas minhas dificuldades.

A todos aqueles que, direta ou indiretamente colaboraram com a realização deste trabalho, meus sinceros agradecimentos.

Enfim, agradeço a todos que estão ou que estiveram em minha vida durante este período, agradeço até mesmo as pessoas que me fizeram passar por grandes dores, pois com cada decepção, cresci e me fortaleci.

SUMÁRIO

LISTA DE TABELAS

LISTA DE FIGURAS

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo n° 021086/11 do trabalho de pesquisa intitulado **"Programação térmica fetal: efeitos sobre a temperatura de preferência e características morfofisológicas de frangos de corte"**, sob a responsabilidade da Profª, Drª. Isabel Cristina Boleli está de acordo com os Princípios Éticos na Experimentação (COBEA) e foi aprovado pela COMISSÃO ÉTICA NO USO DE ANIMAIS (CEUA), em reunião *ordinária* de 07 de Outubro de 2011.

Jaboticabal, 11 de Outubro de 2011.

Prof. Dr. Jeffrey Frederico Lui
Presidente - CEUA

Med. Vet. Maria Alice de Campos
Secretária - CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n - CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209 2600 - fax 16 3202 4275 - www.fcav.unesp.br

PROGRAMAÇÃO TÉRMICA FETAL: EFEITOS SOBRE A TEMPERATURA DE PREFERÊNCIA E CARACTERPISTICAS MORFOFISIOLÓGICAS DE FRANGOS DE CORTE

RESUMO – Estresse por calor durante a criação altera o desempenho e as características morfofuncionais de frangos de corte. Programação térmica fetal tem se mostrado efetiva na indução de características adaptativas ao calor na incubação, aumentando a termotolerância das aves no período pós-eclosão, por meio de adaptação epigenética. O presente estudo analisou os efeitos da exposição à temperatura de incubação fria ou quente durante a fase fetal sobre as características morfofisiológicas de pintos na eclosão e de frangos criados sob temperatura de preferência, recomendada para a linhagem ou alta. No Capítulo 1 é apresentada uma abordagem teórica sobre o tema. No Capítulo 2 são abordados os efeitos da temperatura fria ou quente durante a fase fetal sobre parâmetros de incubação (temperatura da casca dos ovos e eclodibilidade), temperatura de preferência e características do pinto recém-eclodido (peso corporal e dos órgãos, características da pele e das penas, temperatura superficial corporal e retal e concentração plasmática de T3, T4 e GH). Para isso, ovos férteis de matrizes de frangos de corte (Cobb 500®) foram incubados a partir do 13° dia à temperatura fria (36°C), controle (37,5°C) ou quente (39°C). Os dados mostram que exposição ao frio diminuiu a temperatura superficial corporal, a vascularização da pele e a concentração plasmática de T3 e GH. Por outro lado, exposição ao calor aumentou a eclodibilidade dos ovos, a temperatura superficial corporal e a vascularização dérmica, reduziu a espessura da pele e aumentou a temperatura de preferência dos pintos. No Capítulo 3 foi estudado se a exposição à temperatura fria ou quente de incubação altera a temperatura de preferência e resposta ao estresse dos frangos ao longo da criação. Para isso, foi adotado o mesmo protocolo de incubação utilizado no Capítulo 1. Após a eclosão, as aves foram alojadas em câmara climática com temperatura ajustada para os valores recomendados para a linhagem. Semanalmente, durante as seis semanas de criação, foi determinada a temperatura de preferência dos frangos e resposta ao estresse térmico. De acordo com os resultados, exposição ao calor aumenta a temperatura de preferência das aves somente durante as três primeiras semanas de idade. Além disso, temperatura quente de incubação minimiza e/ou retarda os efeitos ,do estresse térmico. Nos Capítulos 4 e 5 são analisados os efeitos da temperatura fria ou quente de incubação sobre as características ósseas (composição mineral, propriedades biomecânicas e características físicas) e desempenho e sobre as características da pele, peso corporal e do empenamento, temperatura superficial corporal e retal e concentração plasmática de T3, T4 e GH, respectivamente, de frangos criados em temperatura ambiente de preferência, recomendada para a linhagem ou alta. Nesse caso, foi utilizado um delineamento experimental 3x3 (incubação: temperatura fria, controle ou quente; criação: temperatura de preferência, recomendada ou alta). No Capítulo 4 foi verificado que criação sob temperatura de preferência não teve influência sobre as características ósseas dos frangos. Além disso, também foi observado que a exposição ao calor na fase fetal não minimiza e/ou evita os efeitos deletérios da alta temperatura de criação. O Capítulo 5 mostra que a exposição à temperatura fria ou quente durante a fase fetal não alterou as características dos frangos criados em temperatura de preferência, recomendada ou alta. Ademais, criação sob temperatura alta aumentou a temperatura superficial corporal e reduziu os níveis plasmáticos de T3, T4 e GH, o peso corporal e do empenamento e a espessura da pele. No Capítulo 6 foi estudado se pintos de ovos grandes, com maior produção de calor metabólico, são mais afetados quando expostos à temperatura de incubação quente na fase fetal do que os pintos de ovos pequenos. Para isso, foi adotado um delineamento experimental 2x2 (ovo: pequeno ou grande; incubação: temperatura da casca do ovo (TCO) controle ou TCO quente, por 12 hrs diárias, do 13° até o 18°dia de incubação). De acordo com os resultados, não houve diferença nas respostas dos pintos de ovos pequenos ou grandes frente à exposição à EST quente.

Palavras-chaves: adaptação epigenética, aves, estresse térmico, incubação, termotolerância

THERMAL PROGRAMMING DURING FETAL DEVELOPMENT: EFFECTS ON PREFERRED AMBIENT TEMPERATURE AND MORPHOPHYSIOLOGICAL CHARACTERISTICS OF BROILERS

ABSTRACT - Heat stress changes the performance and morphological and functional characteristics of broilers. Thermal programming during fetal development has been shown to be effective in the induction of adaptive characteristics to heat, increasing the thermotolerance of the birds during the post-hatching period by epigenetic adaptation. This study examined the effects of exposure to cold or hot incubation temperature during the fetal stage on the physical and physiological characteristics of chicks, at hatching, and chickens reared under preferred, recommended or high temperature. In Chapter 1 we present a theoretical approach to the topic. In Chapter 2, the effects of cold or hot temperature during the fetal stage on incubation parameters (temperature, eggshell and hatchability), preferred ambient temperature and characteristics of newly hatched chicks (body and organs weight, skin and feathering characteristics, body surface and rectal temperature and plasma T3, T4 and GH concentrations) were examined. For this, hatching eggs from a commercial broiler breeder flock (Cobb 500®) were exposed from day 13 of incubation to cold (36°C), control (37.5°C) or hot (39°C) temperature. The data show that exposure to cold reduced body surface temperature, skin vascularity and plasma T3 and GH concentrations. Moreover, exposure to hot increased eggs hatchability, body surface temperature and dermal vascularity, reduced skin thickness and increased thermal preference of the chicks. In Chapter 3 was studied whether exposure to cold or hot incubation temperature alters preferred ambient temperature and response to heat stress of broilers along rearing phase. For this, we adopted the same incubation protocol used in Chapter 1. After hatching, the birds were housed in climatic chamber with temperature set to the recommended values for the line. Weekly, during the six weeks of rearing, thermal preference and response to heat stress of birds were analyzed. According to the results, exposure to hot incubation temperature increases the preferred ambient temperature only during the first three weeks of age. Furthermore, hot incubation temperature minimizes and/or retards the effects of heat stress. In Chapters 4 and 5 were analyzed the effects of cold or hot incubation temperature on bone characteristics (mineral composition, biomechanical properties and physical characteristics) and performance and on skin characteristics, body and feathering weight, body surface and rectal temperature and plasma T3, T4 and GH concentration, respectively, of chickens reared at preferred, recommended or high temperature. In this case, a 3x3experimental design was used (incubation: cold, control or hot temperature; rearing: preferred, recommended or high temperature). In Chapter 4 it was found that rearing under preferred temperature had no influence on the bone characteristics of chickens. Furthermore, it was also observed that exposure to hot temperature during fetal life does not minimize and/or avoid the negatives effects of high environmental temperature. Chapter 5 shows that cold or hot incubation temperature did not alter broilers characteristics reared under preferred, recommended or high temperature. Moreover, high temperature increased body surface temperature and reduced plasma T3, T4 and GH levels, body and feathering weight and skin

thickness. In Chapter 6 was studied whether chicks from large eggs, with higher metabolic heat production, are most affected when exposed to hot incubation temperature during fetal stage than chicks of small eggs. For this, a 2x2 experimental design was adopted (egg: small or large; incubation: eggshell temperature control or hot, for 12 hrs daily, from the 13th to the 18th day of incubation). According to the results, there was no difference in the responses of chicks from small or large eggs submitted to hot EST.
Keywords: birds, epigenetic adaptation, incubation, heat stress, thermotolerance

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

O Brasil ocupa, atualmente, a posição de terceiro maior produtor e a de maior exportador mundial de carne de frangos, tendo atingido a produção de 12,30 milhões de toneladas em 2013 (UBABEF, 2014). Essa alta produtividade avícola foi propiciada pela seleção genética dos frangos para maior e mais rápida taxa de crescimento e melhor conversão, (Sherwood, 1977; Havenstein et al., 1994a,b) que possibilitou aumento de 50 a 60 vezes no peso corporal da aves entre a eclosão e a idade de abate (Havenstein et al., 2003 a). Incremento da taxa de crescimento, entretanto, resultou em aumento na produção de calor metabólico (Janke et al., 2004), o que torna os frangos altamente sensíveis à alta temperatura ambiente (Havestein et al., 2003b), uma vez que o processo de seleção genética não envolveu melhora no sistema de controle de temperatura corporal dessas aves.

Quando submetidas à condições de temperatura ambiente diferentes daquelas recomendadas para a linhagem, as aves, como animais homeotérmicos, dependem de mecanismos físicos, comportamentais e endócrinos (Yahav et al., 1996) para balancear a produção e a dissipação de calor, e manter sua temperatura corporal constante (Yalçin et al., 1997). Tais mecanismos envolvem alterações hemodinâmicas periféricas (Wolfenson, 1983; Yahav et al. 1998), padrão de empenamento (Wylie et al., 2001), temperatura corporal (Richards et al., 1971; Cahaner et al., 1995; Zhou e Yamamoto, 1997; Deeb e Cahaner, 1999), níveis hormonais circulantes (Dahlke et al., 2005; Willemsem et al., 2010), dentre outras alterações. A utilização desses mecanismos na tentativa de manter a homeotermia corporal gera gastos energéticos para as aves, comprometendo seu desempenho produtivo, aumentando a incidência de doenças metabólicas e bem-estar (Furlan e Macari, 2002).

Programação térmica pré-natal tem sido investigada por vários autores quanto a seu potencial uso como forma de manejo para aumentar a termotolerância das aves no período pós-eclosão, por meio da adaptação epigenética (Decuypere, 1984; Tzschentke et al., 2001; Janke et al., 2002; Tzschentke and Basta, 2002; Moraes et al., 2003, 2004; Yahav et al., 2004a,b; Collin et al., 2005, 2007; Yalçin et al., 2008b; Piestun et al., 2008; Tona et al., 2008; Willemsen et al., 2010; Shinder et al., 2009;

Walstra et al., 2010) A indução de adaptação epigenética de longa duração pela temperatura de incubação parece envolver alterações no sistema termorregulador imaturo e em importantes sistemas de controle fisiológicos (Dorner, 1974; Minne and Decuypere, 1984; Arjona et al., 1988; Tzschentke et al., 2004; Yahav, 2009). Tais alterações fisiológicas podem mudar o limiar de resposta termorregulatória das aves, , reduzindo sua sensibilidade térmica à variações de temperatura ambiente no período pós-eclosão (Decuypere and Michels, 1992; French, 2000; Walstra et al., 2010), e alterar sua temperatura ambiente de preferência, como registrado em perus, patos (Nichelmann, 2004; Tzschentke, 2007) e poedeiras (Walstra et al., 2010) na fase de inicial de criação, fase de transição da ectodermia para homeotermia das aves precoces (Whittow and Tazawa, 1991; Nichelmann and Tzschentke, 2002). Contudo, diferenças na intensidade, duração e período de realização da manipulação térmica e nos resultados obtidos indicam a existência de diferentes janelas para indução epigenética de alteração da termotolerância das aves, as quais precisam ser melhor estabelecidas e seus efeitos melhor entendidos, antes de serem propostas como mecanismos de modulação termorregulatória.

Atualmente é conhecido que os fetos são altamente responsivos à variações ambientais, o que tem alavancado as pesquisas dos efeitos da manipulação do ambiente de desenvolvimento fetal sobre seu fenótipo pós-nascimento. No que se refere á frangos de corte, não é conhecido se manipulação contínua da temperatura do ambiente de desenvolvimento fetal influencia o fenótipo dos pintos na eclosão, quais alterações fenotípicas ocorrem, se as alterações são de curta ou longa duração.. O entendimento de tais mecanismos é de suma importância para a cadeia de produção avícola, uma vez que pode contribuir para a determinação do potencial uso da manipulação térmica contínua na fase fetal na indução epigenética de maior termotolerância de frangos ao calor.

Considerando que os eixos neuroendócrinos já encontram-se funcionalmente estabelecidos no início da fase fetal (Jenkins e Porter, 2004), nossa hipótese é que manipulação contínua da temperatura de incubação durante a fase pode induzir alterações fenotípicas nos pintos, as quais podem permanecer após a eclosão por curta ou longa duração e alterar suas características morfofisiológicas e comportamentais, influenciando seu crescimento e desenvolvimento frente ou não à estresse por calor.

2. REVISÃO DE LITERATURA

Sensibilidade e resposta dos frangos de corte ao calor

A seleção genética para melhorias na eficiência alimentar, aumento do peso ao abate e diminuição do tempo de crescimento e idade para abate em frangos de corte não foi acompanhada de eficiência ou melhoria no processo de termorregulação, o que tornou as aves altamente sensíveis à temperaturas acima da termoneutra. Dessa forma, pode-se dizer que a temperatura do ambiente no qual as aves são criadas é um dos fatores determinantes do sucesso ou fracasso de um empreendimento avícola (Macari et al., 2004). Quando a temperatura ambiente de criação está acima da termoneutra, os mecanismos de controle térmico dos frangos na maioria das vezes não são suficientes para a manutenção da temperatura corporal, o que obriga o produtor a utilizar mecanismos exógenos (externos) para auxiliar na redução do calor ambiente, como por exemplo a utilização de galpões climatizados ou câmaras climáticas. Entretanto, a adoção de tais medidas encarecem a produção e expõem as aves à risco de morte, e o produtor à altas perdas econômicas ou perda total de produção, em caso de ocorrência de pane no sistema elétrico que abastece a região onde a granja está localizada. Sendo assim, é de extrema importância a busca por alternativas que aumente a tolerância térmica das aves frente à altas temperaturas ambiente.

Quando as aves são criadas em temperaturas dentro da sua zona de conforto térmico, a fração de energia metabolizável gasta para termogênese é mínima e a energia líquida de produção é máxima (Macari et al., 2004). Segundo a União Brasileira de Avicultura (UBA, 2009), a faixa de temperatura recomendada para aves adultas é de 21 a 23ºC, com umidade relativa entre 65% e 70%. Entretanto, quando a temperatura ambiente ultrapassa os valores recomendados, as aves entram em situação de estresse térmico, e na tentativa de impedir um aumento excessivo da temperatura corporal, elas aumentam a dissipação de calor e diminuem sua produção metabólica através de mecanismos físicos, comportamentais e endócrinos (Geraert et al. 1996; Yahav et al., 1996).

A manutenção da temperatura corporal depende do balanço entre a produção e a dissipação de calor (Yalçin et al., 1997). Considerando que a temperatura do ambiente esteja elevada (estresse por calor), uma reduzida perda/produção de calor da ave para o ambiente ocorrerá, de forma que quanto mais elevada a temperatura ambiental, menor será a perda calórica, uma vez que o calor é transferido do meio mais quente para o mais frio (La Scala, 2003). Para aumentar a dissipação de calor para os tecidos, onde ele é dissipado, mecanismos de perda de calor sensível e latente são utilizados pelas as aves. A dissipação de calor sensível ocorre por meio de mecanismos não evaporativos, ou seja, por condução, convecção e radiação, sendo a diferença entre a temperatura da pele do frango e o ambiente, o principal fator que interfere nos processos de perda de calor (Macari et al., 2002). Sendo assim, na tentativa de aumentar a dissipação de calor, a ave procura maximizar a área de superfície corporal, agachando, mantendo as asas afastadas do corpo e exibindo alterações hemodinâmicas periféricas, bem como vasodilatação periférica (Wolfenson, 1983; Yahav et al., 1998; Macari et al., 2002). Segundo Richards et al. (1971), Cahaner et al. (1995), Zhou e Yamamoto (1997) e Deeb e Cahaner (1999), Macari et al. (2002), o aumento do fluxo sanguíneo para as regiões periféricas não cobertas por penas na tentativa de maior dissipação de calor, resulta, muitas vezes, em elevação da temperatura superficial da pele, sendo o inverso também verdadeiro, ou seja, frente à baixa temperatura, ocorre uma redução no fluxo sanguíneo cutâneo, a fim de reduzir a perda de calor.

É conhecido que nas aves adultas, as penas possuem importante papel termorregulatório. Em temperaturas ambiente frias, a cobertura de penas possibilita às aves a manutenção de sua temperatura corporal. Por outro lado, sob altas temperaturas, essa cobertura de penas dificulta a dissipação de calor, levando ao aumento da temperatura corporal da ave. Na tentativa de aumentar a dissipação, as aves tendem aumentar a ptiloereção (Macari et al., 2002). De acordo com Cahaner et al. (1993, 1994) e Cooper e Washburn (1998), empenamento e temperatura ambiente encontram-se inversamente relacionados em frangos adultos. Segundo os autores, o aumento da temperatura ambiente é acompanhado de redução no empenamento das aves, levando-as a um melhor desempenho, uma vez que o menor isolamento externo possibilita melhor troca térmica entre a ave e o ambiente, exigindo menor gasto energético na manutenção da homeostase térmica.

Outro mecanismo importante de perda de calor das aves quando expostas a temperaturas elevadas é o resfriamento evaporativo respiratório, na tentativa de estimular a perda evaporativa de calor (ofegação) e manter o equilíbrio térmico corporal (Silva et al., 2001; Macari et al., 2002). Entretanto, aumento na frequência respiratória pode resultar em hipertermia e no desenvolvimento de alcalose respiratória (distúrbio do equilíbrio ácido-base). Sendo assim, embora a perda de calor por meio do resfriamento evaporativo represente uma importante via de dissipação de calor sob altas temperaturas ambientais, a mesma pode gerar consequências indesejáveis, podendo comprometer a saúde da ave (Macari et al., 2002).

Dentre os mecanismos endócrinos utilizados sob alta temperatura ambiente, o principal utilizado pelos frangos é a redução dos hormônios tireoideanos circulantes, particularmente o T_3, conhecido pela sua ação termogênica (Yahav et al., 1996). Aves mantidas sob altas temperaturas apresentam níveis plasmáticos destes hormônios reduzidos, diminuindo a atividade da bomba de sódio e potássio e o consumo de oxigênio pelas células animais, ocasionando redução da taxa metabólica (Chen et al., 1994). Segundo Jonier and Huston (1957) e Etches et al. (1995), a redução dos níveis circulantes dos hormônios tireoideanos é decorrente da menor atividade apresentada pela tireóide sob alta temperatura ambiente, resultando em maior tolerância ao calor pelas aves.

Paralelamente à alteração da concentração dos hormônios da tireóide, sob estresse térmico, os frangos reduzem o consumo de alimento e a eficiência digestiva, como tentativa de reduzir a produção de calor endógena, o que resulta na redução no desempenho no ganho de peso e na conversão alimentar (Teeter et al., 1984; Bonnet et al., 1997; Lana et al., 2000; Souza, 2008).

Redução dos níveis circulantes de T3 e T4, bem como o menor consumo de ração, ambos resultantes da exposição à alta temperatura ambiente, podem levar o frango à uma alteração do crescimento e diferenciação da placa de condrócitos, reduzindo o comprimento, a largura e o peso dos ossos (Teeter et al., 1984; Yalçin et al., 1996; Bruno et al., 2000; Pelicano et al., 2005; Faria Filho, 2006; Shao et al., 2006). Tais alterações resultam no menor desenvolvimento ósseo, principalmente do fêmur e da tíbia, reduzindo o desempenho, e consequentemente, sua produtividade.

Diante do exposto, faz-se necessária a busca por alternativas que visem aumentar a termotolerância das aves, reduzindo os efeitos deletérios ocasionados pelo estresse térmico à sanidade e bem-estar das aves, bem como à produção avícola.

Programação térmica pré-natal no aumento da termotolerância

Várias estratégias têm sido utilizadas para prevenir o estresse calórico em aves domésticas, tais como: genética; manipulação dos níveis nutricionais da dieta e níveis de eletrólitos na água; restrição alimentar, utilizando períodos de jejum para reduzir o incremento calórico ocasionado pela dieta; manejo do ambiente de criação, através da utilização de programas de luz, controle da umidade e temperatura; condicionamento ao calor realizado em pintos nos primeiros dias de vida; e por fim, programação térmica pré- ou pós-natal (Lin et al., 2006).

Programação térmica pré-natal tem sido analisada como uma alternativa para melhorar a tolerância das aves a desvios da temperatura ambiente, principalmente às altas temperaturas. Estudos anteriores utilizando exposição à alta temperatura de maneira intermitente, em diferentes períodos do desenvolvimento *in ovo,* registraram aumento da tolerância ao calor quando os frangos foram desafiados termicamente na criação (Iqbal et al., 1990; Moraes et al., 2003; Yahav et al., 2004; Collin et al., 2005; Yalçin et al., 2008b; Piestun et al., 2008). Da mesma forma, Shinder et al. (2009) verificaram que exposição à temperatura mais fria na incubação aumentou a tolerância das aves à baixa temperatura ambiente, quando as mesmas foram desafiadas na criação. Contrariamente, contudo, Collin et al. (2007) não verificaram aquisição de termotolerância quando as aves foram desafiadas na 6ª semana de idade.

Os dados de literatura citados anteriormente indicam que uma melhora efetiva da termotolerância ocorre quando a temperatura de incubação é alterada durante determinados ‘períodos críticos’ do desenvolvimento in ovo, nos quais, segundo Yahav (2009), o limiar de resposta dos sistemas termorregulatórios pode ser alterado, resultando em alterações fisiológicas de longa duração e aumento da tolerância das aves à ambientes quentes ou frios.

Embora muitos estudos tenham sido realizados utilizando programação térmica pré-natal intermitente em diversos períodos de desenvolvimento para induzir o aumento da tolerância das aves no período de criação, pouco se sabe ainda sobre os efeitos da programação térmica na indução da termotolerância quando aplicada de forma contínua.

Programação térmica pré-natal e títulos hormonais

De acordo com Yahav (2004b), o período ideal para a realização da manipulação térmica coincide com o período que os eixos endócrinos já se encontram estabelecidos, principalmente o eixo hipotálamo-hipófise-tireóide (eixo HPT). Isso, segundo Mcnabb e Olson (1996), ocorre por volta do 13-14° dia de incubação.

Os hormônios tireodianos secretados têm influência significativa sobre o metabolismo, desenvolvimento e mecanismos de termorregulação dos pintos no período pós-eclosão, além de outras funções importantes e fundamentais para o seu crescimento, sobrevivência após o nascimento e desempenho (Jenkins e Porter, 2004). Willemsem et al. (2010) observaram redução nos níveis plasmáticos dos hormônios tireodianos em frangos de corte de ovos incubados à temperatura 2 e 3°C acima da usual do 16° ao 18° de desenvolvimento. Considerando que os hormônios tireodianos são termogênicos, principalmente o T3, esses autores afirmam que a redução nos níveis hormonais pode ter ocorrido na tentativa de reduzir o metabolismo, resultando em maior tolerância ao calor pelas aves.

Durante o período de desenvolvimento e maturação dos importantes sistemas fisiológicos, tais como o sistema termorregulatório e o eixo HPT, ambiente tem notável impacto na determinação das respostas dos respectivos sistemas do organismo, especialmente por meio da indução de mudanças na organização neuronal e padrão de expressão de genes relacionados (programação perinatal epigenética/funcional) (Decuypere e Michels, 1992; Tzschentke e Plagemann, 2006).

Doner (1976) propôs um conceito geral etiológico de programação pré-natal epigenética da função dos sistemas regulatórios fundamentais durante toda a vida. Em seu conceito, os hormônios têm papel decisivo como organizadores do sistema neuroendócrino e imune dependentes de fatores do meio ambiente, os quais regulam todos os processos fundamentais da vida. De acordo com o autor, durante os períodos críticos, hormônios, neurotransmissores e citoquinas estão envolvidos na diferenciação, maturação e programação funcional de seus próprios controladores no sistema nervoso central, dentro dos seus respectivos sistemas regulatórios fisiológicos. Dessa forma, atuam como efetores endógenos críticos que transmitem as informações ambientais para o genoma. Finalmente, passam a também agir como fatores epigenéticos. Por um lado, este mecanismo parece ser uma base provável da programação ou programação defeituosa na fase neonatal da ave, que, por exemplo, pode causar transtornos metabólicos e cardiovasculares, assim como transtornos comportamentais observados durante a fase de criação dos animais.

Programação térmica pré-natal e Desenvolvimento embrionário

O desenvolvimento embrionário das aves ocorre de maneira independente do animal que produziu o ovo, uma vez que todos os nutrientes necessários para que esse processo ocorra já se encontram presentes a partir do momento da oviposição (Everaert e Decuypere, 2013).

O processo de chocagem dos ovos de aves pode ocorrer de forma natural pela galinha, ou de maneira artificial, utilizando uma máquina incubadora. Independentemente do método utilizado, certos fatores físicos tais como temperatura, umidade, viragem e ventilação devem ser considerados (Willemsem, 2010). Dentre eles, a temperatura é considerada o fator ambiental mais crítico durante a incubação, podendo influenciar o desenvolvimento embrionário (Romanoff, 1960), a eclodibilidade (Deeming and Ferguson, 1991; Wilson, 1991; Decuypere and Michels, 1992), a qualidade do pinto recém-eclodido (Lourens et al., 2005, 2007; Hulet et al., 2007) e o desempenho pós-eclosão (Lundy, 1969; Decuypere, 1984; Wilson, 1991).

Durante o desenvolvimento embrionário, as aves necessitam de uma temperatura constante e adequada para manter suas funções metabólicas normais (Romijin e Lokhorts, 1955; Wilson, 1991). Temperatura adequada de desenvolvimento é aquela em que a eclodibilidade dos ovos, a qualidade do pintinho e seu potencial de desenvolvimento é máxima, sendo denominada temperatura ótima de incubação, e que é em torno de 37.5°C a 37.8°C.

Segundo Nichelmann (2004), a temperatura ambiente afeta a produção de calor e a temperatura do embrião, levando à manifestação de respostas endotérmicas, que podem iniciar aos 18 dias de embriogênese. Contudo, fetos entre 14 e 21 dias de desenvolvimento comportam-se como animais endotérmicos com capacidade de produzir calor quando a temperatura corporal é inferior ao ideal (Yahav et al., 2004b). Porém, em temperaturas corporais inferiores à 34ºC, a resposta endotérmica não é evidente devido ao fato dos neurônios termossensíveis não serem ativos nessa temperatura (Yahav et al., 2004b).

Incubação de ovos à baixa temperatura (36,7°C) durante as primeiras semanas de incubação pode resultar em pior eficiência alimentar, pois o aumento da produção de calor *in ovo* na fase seguinte induz crescimento compensatório ainda na incubação, e que continua até a terceira semana de idade pós eclosão (Geers et al., 1983). Além disso, segundo o autor, ela também gera menor peso embrionário ao décimo dia, baixa eclodibilidade, má qualidade dos pintos quando comparado com embriões incubados a 37,8°C.

Alta temperatura de incubação, por sua vez, pode ocasionar redução do peso corporal, do comprimento e tamanho relativo do coração, problemas locomotores, aumento da mortalidade embrionária na fase final e mau posicionamento, entre outras consequências. Thompson et al. (1976) testou diferentes temperaturas de incubação: (40,6; 43,3; 46,1 e 48,9ºC) no 16º dia de incubação, durante vários tempos de exposição. Segundo os autores, 40,6ºC por 24h não alterou a eclodibilidade, 43,3ºC por 6 horas a causou diminuição da eclodibilidade, que se tornou ainda mais severa a partir de 9 horas de exposição. Por sua vez, as temperaturas de 46,1ºC durante 3 horas e 48,9ºC por 1 hora provocaram 100% de mortalidade embrionária. Os pintos sobreviventes de ovos submetidos à elevadas temperaturas eram fracos e com alta incidência de defeitos de pernas.

Similarmente, Leandro et al. (2000) não encontraram diferenças entre a eclodibilidade, peso ao nascer e qualidade de pernas, em estudo com variação de temperatura de calor (40ºC) ou frio (32ºC) durante cinco horas, a partir do 16º dia de incubação. Porém os ovos expostos ao estresse pelo frio ou calor tiveram um período de incubação maior do que aqueles mantidos expostos à temperatura normal, sendo o aumento do tempo médio total de incubação de 10 e 8 horas para frio e calor, respectivamente. Tais resultados indicam que mesmo um breve período de variação da temperatura de incubação, quando aplicada em um período crítico de desenvolvimento é suficiente para determinar estresse nos embriões de frangos de corte.

Tzschentke and Halle,(2009), estudando manipulação térmica na incubação de ovos férteis (ROSS 308®), encontraram que a incubação em temperatura crônica elevada (1°C acima do padrão) nos últimos quatro dias da incubação não teve nenhum efeito sobre a taxa de nascimento e do sexo das aves ou qualidade dos pintos.

Embora muitos estudos sobre os efeitos da manipulação térmica na incubação estejam sendo realizados (Shinder et al. 2009; Tzschentke e Halle, 2009), pouco se conhece à respeito da ocorrência de adaptações embrionárias e fetais para dissipar ou reter calor quando submetidos à condições adversas de incubação.

3. OBJETIVOS

Objetivou-se com o presente estudo testar a hipótese de que programação térmica pré-natal, contínua, durante a fase fetal resulta em alterações morfofisiológicas e comportamentais de longa duração nos pintos, tornando-os mais termotolerantes à alta temperatura ambiente na criação.

4. REFERÊNCIAS BIBLIOGRÁFICAS

Arjona AA, Denbow DM, Weaver Jr. WO. Effect of heat stress early in life on mortality of broilers exposed to high environmental temperatures just prior to marketing. Poultry Science, v.67, n.2, p.226-231, 1988.

Barott HG. Effects of temperature, humidity, and other factors on hatch of hen's eggs and on energy metabolism of chicks embryo. U.S. Department of Agriculture Technical Bulletin, v.553, p.13-45, 1937.

Bonnet S, Geraert PA, Lessire M et al. Effect of high ambient temperature on feed digestibility in broilers. Poultry Science, v.76, n.6, p.857-863, 1997.

Bruno LDG, Furlan RL, Malheiros EB et al. Influence of early quantitative food restriction on long bone growth at different environmental temperatures in broiler chickens. Brazilian Journal of Poultry Science, v.41, p.389-394, 2000.

Cahaner A, Deeb N, Gutman M. Effects of the plumage-reducing naked neck (No) gene on the performance of fast growing broilers at normal and high ambient temperatures. Poultry Science, v.72, p.767-775, 1993.

Cahaner A, Yunis R, Deeb N. Genetics of feathering and heat tolerance in broilers. Pages 67-70 in: Proceedings of the 9th European Poultry Conference. Vol. 2. Glasgow, UK, 1994.

Cahaner A, Pinchasov Y, Nir I. Effect of dietary protein under high ambient temperature on body weight, breast meat yield and abdominal fat deposition, of broiler stock differing in growth rate and fatness. Poultry Science, v.74, n.6, p.968-975, 1995.

Chen BL, Zhang Z, Chen WB. The study of avian influenza: I. The isolation and preliminary serological identification of avian influenza virus in chicken. Chinese Journal of Veterinary Medicine, v. 20, p.3–5, 1994.

Collin A, Picard M, Yahav Y. The effect of duration of thermal manipulation during broiler chicks embryogenesis on body weight and body temperature of post hatched chicks. Animal Research, v.54, p.105–112, 2005.

Collin A, Berri C, Tesseraud S et al. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poultry Science, v.86, p.795-800, 2007.

Cooper M A, Washburn KW. The relationships of body temperature to weight gain, feed consumption, and feed utilization in broilers under heat stress. Poultry Science, v. 77, n. 2, p. 237-242, 1998.

Dahlke F, Gonzales E, Furlan R.L. et al. Desenvolvimento folicular de embriões de frangos de corte de diferentes genótipos expostos ao estresse térmico crônico. Ciência Rural, v. 38, n. 8, p. 2314-2320, 2008.

Decuypere E. Incubation temperature in relation to postnatal performance in chickens. Archiv fur Experimentalle Veterinar Medizin, v.38, p. 439-449, 1984.

Decuypere E, Michels H. Incubation temperature as a management tool: a review. World's Poultry Science Journal 48: 27-38, 1992.

Decuypere E and Bruggeman V. Endocrine aspects of development: new challenges for the control of incubation process. World's Poultry Science Journal, 2005.

Deeb N and Cahaner A. The effects of naked neck genotypes, ambient temperature, and feeding status and their interactions on body temperature and performance of broilers. Poultry Science, v.78, p.1341-1346, 1999.

Dörner G. Environment-dependent brain differentation and fundamental processes of life. Acta Biologica Et Medica Germanica, v.33, p.129–148, 1974.

Etches RJ. Reproduction in Poultry. CAB International, Wallingford, UK, 1996.

Faria Filho DE. Aspectos produtivos, metabólicos, econômicos e ambientais da nutrição protéica para frangos de corte expostos ao calor [tese]. Jaboticabal (SP): Universidade Estadual Paulista

French NA. Effect of short periods of high incubation temperature on hatchability and incidence of embryo pathology of turkey eggs. Brazilian Journal of Poultry Science, v.41, p.377-382, 2000.

Furlan RL and Macari M. Termorregulação. In: Macari, M; Furlan, L.R. and Gonzales, E. (Ed.) Fisiologia Aviária Aplicada a Frangos de Corte. Jaboticabal: FUNEP/UNESP, p. 209-230, 2002.

Halle I. and Tzschentke B. Influence of temperature manipulation during the last 4 days of incubation on hatching results, post-hatching performance and adaptability to warm growing conditions in broiler chickens. Japan Poultry Science Association, v.48, p. 97-105, 2011.

Havenstein GB, Ferket PR, Qureshi MA. Growth, livability and feed conversion of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science, v.82, p.1500-1508, 2003a.

Havenstein GB, Ferket PR, Qureshi MA. Carcass composition and yield of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science, v.82, p.1509-1518, 2003b.

Havenstein GB, Ferket PR, Scheideler SE et al. Growth, Livability, and Feed Conversion of 1957 vs 1991 broilers when fed “typical” 1957 and 1991 broiler diets. Poultry Science, v.73, p.1785-1794, 1994a.

Havenstein GB, Ferket PR, Scheideler SE et al. Carcass composition and yield of 1991 vs 1957 broilers when fed “typical” 1957 and 1991 broiler diets. Poultry Science, v. 73, p.1795-1804, 1994b.

Hulet RM, Gladys G, Meijerhof R et al. Influence of egg shell embryonic incubation temperature and broiler breeder flock age on post hatch growth performance and carcass characteristics. Poultry Science, v86, p.408-412, 2007.

Iqbal A, Decuypere E, Abd El Azim A, Kühn ER. Pre- and post-hatch high temperature exposure affects the thyroid hormones and corticosterone response to acute heat stress in growing chicken (Gallus domesticus). Journal of Thermal Biology, v.15, n.2, p.149-153, 1990.

Janke O, Tzschentke B, Hochel J. et al. Metabolic responses of chicken and Muscovy duck embryos to high incubation temperatures. Comparative Biochemical Physiology Part A Molecular & Integrative Physiology, v.131, p.741–750, 2002.

Janke O, Tzschentke B, Boerjan M. Comparative investigations of heat production and body temperature in embryo s of modern chicken breeds. Avian and Poultry Biology Reviews, v.15, p.191-196, 2004.

Jenkins SA, Porter TE. Ontogeny of the hypothalamo-pituitary-adrenocortical axis in the chicken embryo: a review. Domestic Animal Endocrinology, v.26, p.267–275, 2004.

Lana GRQ, Rostagno HS, Albino LFT et al. Efeito da temperatura ambiente e da restrição alimentar sobre o desempenho e a composição da carcaça de frangos de corte. Brazilian Journal of Poultry Science, v.29, p.1117-1123, 2000.

Leandro NSM, Gonzales E, Varoli Jr JCV. et al. Incubabilidade e qualidade de pintos de ovos de matrizes de frangos de corte submetidos estresse de temperatura. Brazilian Journal of Poultry Science, v.2, p.39-44, 2000.

Lin H, Sui SJ, Jiao HC et al. Impaired development of broiler chickens by stress mimicked by corticosterone exposure. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, v.143, p.400–405, 2006.

Lourens A, van den Brand H, Heetkamp MJW et al. Effects of eggshell temperature and oxygen concentration on embryo growth and metabolism during incubation. Poultry Science, v.86, p.2194-2199, 2007.

Lourens A, van den Brand H, Meijerhof R et al. Effect of eggshell temperature during incubation on embryo development, hatchability, and posthatch development. Poultry Science, v. 84, p.914-920, 2005.

Lundy H. A review of the effects of temperature, humidity, turning and gaseous environment in the incubator on the hatchability of the hen's egg. In: The fertility and hatchability of the hen's egg (Carter TC, Freeman BM, eds). Edinburgh: Oliver and Boyd; 143-176, 1969.

Macari M, Furlan RL, Maiorka A. Aspectos fisiológicos e de manejo para manutenção da homeostase térmica e controle de síndromes metabólicas. In: MENDES AA, NÄÄS IA, MACARI M. (Eds.) Produção de frangos de corte. Campinas: FACTA, p.137-155, 2004.

Mcnabb FMA, Olson JM. Development of thermoregulation and its hormonal control in precocial and altricial birds. Poultry & Avian Biology Reviews, v.7, p. 111-125, 1996.

Moraes VMB, Malheiros RD, Bruggeman V e tal. Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. Journal of Thermal Biology, v. 28, p.133-140, 2003.

Nichelmann M. and Tzschentke B. Ontogeny of thermoregulation in precocial birds. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, v.131, p.751–763, 2002.

Nichelmann M. Perinatal epigenetic temperature adaptation in avian species: Comparison of turkey and Muscovy duck. 29:613–619, 2004.

Nichelmann M, Höchel J, Tzschentke B. Biological rhythms in birds- development, insights and perspectives. Comparative Biochemical Physiology Part A Molecular & Integrative Physiology, v.124, p.429–437. 1999.

Pelicano ERL, Souza PA, Souza HBA et al. Carcass and cut yields and meat qualitative traits of broilers fed diets containing probiotics and prebiotics. Brazilian Journal of Poultry Science, v.7, p.169-175, 2005.

Piestun Y, Shinder D, Ruzal M et al. Thermal manipulations in late term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. Poultry Science, v.87, p.1516-1525, 2008.

Romanoff AL (1960). The Avian Embryo; Structural and Functional Development. New York: Macmillan.

Romijn C and Lokhorst W. Chemical heat regulation in the chicken embryo. Poultry Science, v.34, p.649-654, 1955

Shao ZH, Hsu CW, Chang WT. Cytotoxicity induced by grape seed proanthocyanidins: Role of nitric oxide. Cell Biology and Toxicology, v.66, p.149–158, 2006.

Shinder D, Rusal M, Giloh M, Yahav S. Effect of repetitive acute cold exposures during the last phase of broiler embryogenesis on cold resistance through the life span. Poultry Science, v.88, p.636–646, 2009.

Silva N, Junqueira VCA, Silveira NFA. Manual de métodos de análise microbiológica de alimentos. 2.ed. São Paulo:Varela. 2001. 317p.

Tazawa H, Wakayama H, Turner JS. et al. Metabolic compensation for gradual cooling in developing chick embryos. Comparative Biochemical and Physiology, v.89A, p.125–129, 1988.

Teeter RG, Smith MO, Murray E. Force feeding methodology and equipment for poultry. Poultry Science, v.63, n.4, p.573-575, 1984.

Thompson JB, Wilson HR, Voitle RA. Influence of high temperature stress of 16-day embryos on subsequent hatchability. Poultry Science, v.55, p.892–894,1976.

Tona K, Onagbesan O, Bruggeman V. et al. Effects of heat conditioning at d 16 to 18 of incubation or during early broiler rearing on embryo physiology, post-hatch growth performance and heat tolerance. Archiv fur Geflugelkunde, v.72, p.75-83, 2008.

Tzschentke B. and Nichelmann M. Influence of prenatal and postnatal acclimation on nervous and peripheral thermoregulation. Annals New York Academy Sciences, v.813, n.15 p.87-94. 1997.

Tzschentke B, Basta D, Nichelmann M. Epigenetic temperature adaptation in birds: peculiarities and similarities in comparison to acclimation. News Biomededical Science, v.1, p.26-31, 2001.

Tzschentke B and Basta D. Early development of neuronal hypothalamic thermosensitivity in birds: Influence of epigenetic temperature adaptation. Comparative Biochemical Physiology Part A Molecular & Integrative Physiology, v.131, p.825–832, 2002.

Tzschentke B, Basta D, Janke O et al. Characteristics of early development of body functions and epigenetic adaptation to the environment in poultry: Focused on development of central nervous mechanisms. Poultry and Avian Biology Review, v.15, p.107–118, 2004.

Tzschentke B. Attainment of thermoregulation as affected by environmental factors. Poultry Science, v.86, p.1025-1036, 2007.

Tzschentke B and Halle I. Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio and later performance in male and female broiler chicks. British Poultry Science, v.50, p.634-640, 2009.

Walstra I, Ten Napel J, Kemp B. et al. Temperature manipulation during layer chick embryogenesis. Poultry Science, v.89, p.1502-1508, 2010.

Whittow GC and Tazawa H. The early development of thermoregulation in birds. Physiological and Biochemical Zoology, v.64, p.1371-1390, 1991.

Willemsen H, Swennen Q, Everaert N. et al. Effects of dietary supplementation of methionine and its hydroxy analog DL-2-hydroxy-4-methylthiobutanoic acid on growth performance, plasma hormone levels, and the redox status of broiler chickens exposed to high temperatures. Poultry Science, v.90, p.10, p.2311-2320, 2011.

Wilson HR. Interrelationships of egg size, chick size, posthatching growth, and hatchability. World's Poultry Science Journal, v.47, p.5-20, 1991.

Wolfenson D. Blood flow through arteriovenous anastomoses and its thermal function in the laying hen. Journal of Physiology, v.334, p.395–407, 1983.

Wylie LM, Robertson, GW, Macleod MG et al. Effects of ambient temperature and restricted feeding on growth of feathers in growing turkeys. British Poultry Science, London, v.42, p.449-455, 2001.

Yahav S, Collin A, Shinder D et al. Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. Poultry Science, v.83, p.1959-1963, 2004a.

Yahav S, Rath RS, Shinder D. The effect of thermal manipulations during embryogenesis of broiler chicks (Gallus domesticus) on hatchability, body weight and thermoregulation after hatch. Journal of Thermal Biology, v.29, p.245-250, 2004b.

Yahav S. Alleviating heat stress in domestic fowl- Different strategies. Journal of World Poultry Science, v.65, p.719–732, 2009.

Yahav S and Hurwitz S. Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poultry Science, v.75, n.3, p.402-405, 1996

Yahav S, Luger D, Cahaner A et al. Thermoregulation in naked neck chickens subjected to different ambient temperatures. British Poultry Science, v.39, p.133-138, 1988.

Yahav S, Shamay A, Haberfeld A et al. Induction of thermotolerance in chickens by temperature conditioning: heat shock protein expression. Annals New York Academy of Science, p.628-636, 1996.

Yahav S, Straschnow A, Luger D et al. Ventilation, sensible heat loss, broiler energy, and water balance under harsh environmental conditions. Poultry Science, v.83, p.253-258, 2004.

Yalçin S, Settar P, Ozkan S et al. Comparative evaluation of three commercial broiler stocks in hot versus temperate climates. Poultry Science, v.76, p.921-929, 1997.

Yalçın S, Çabuk M, Bruggeman V et al. Acclimation to heat during incubation: 3. Body weight, cloacal temperatures, and blood acid-base balance in broilers exposed to daily high temperatures. Poultry Science, v.87, p.2671–2677, 2008.

Zhou W.T., Yamamoto, S. Effects of environmental temperature and heat production due to food intake on abdominal temperature, shank skin temperature and respiration rate of broilers. British Poultry Science, v.107, n.6, p.107-114, 1997.

CAPÍTULO 2 - INCUBATION TEMPERATURE DURING FETAL DEVELOPMENT INFLUENCES MORPHOPHYSIOLOGICAL CHARACTERISTICS AND PREFERRED AMBIENT TEMPERATURE OF BROILER HATCHLINGS

VS Morita,[*] VR Almeida,[*] JB Matos Junior,[*] TI Vicentini[*] H van den Brand[‡] and IC Boleli[*1]

[*] *Department of Animal Morphology and Physiology, Sao Paulo State University, Access road Professor Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, Sao Paulo, Brazil and* [‡] *Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, PO Box 338, 6700 AH, Wageningen, the Netherlands*

ABSTRACT - Skin and feather characteristics, which play a critical role in body temperature maintenance, can probably be affected by incubation circumstances, such as incubation temperature. However, no study assessed the influence of incubation temperature during fetal period on morphometric and vascular development of the skin, feather characteristics, and its relation with hormones and preferred temperature in later life. Broiler breeder eggs were exposed to low (36°C), control (37.5°C), or high (39°C) temperatures, from day 13 of incubation to hatch. Heat-exposed hatchlings had higher temperature in the head, neck, and back surface, and thinner and more vascularized skin than cold exposed and control hatchlings. No differences were found among treatments for body weight, total feather weight, number and length of barbs, barbules length, and plasma T4 concentration. Cold-exposed hatchlings showed lower plasma T3 and GH, as well as, low T3/T4 ratio and lower vascularity in the neck, back, and thigh skin compared to control hatchlings. On the other hand, heat-exposed hatchlings had lower skin thickness and higher vascularity, and preferred a higher ambient temperature than hatchlings of both other incubation conditions. We conclude that changes in the skin thickness and vascularity reveal, additionally to changes in thyroid and growth hormone, as embryonic strategies to cope with high and low incubation temperatures. Through these mechanisms heat exposure during incubation is an environmental factor that exert early-life influences on ambient temperature preference of broiler hatchlings.

Key words: chickens, embryonic development, incubation temperature, skin characteristics, thermal preference

1. INTRODUCTION

In precocial birds, such as broilers, the quality and survival of the neonate depend on maternal investment on egg characteristics and incubation temperature (Lourens et al., 2005; Nangsuay et al., 2011, 2013), as well as on the care during the early post-hatching period. Under natural conditions, neonates require the maternal presence to maintain body temperature during the transition from ectothermy to homeothermy. Thermal discomfort may influence the health and well-being of the animal, as well as their growth and survival. The tegument plays a key role in homeostasis, as changes in dermal blood flow and surface temperature are involved in adjusting the body temperature. In addition, feathering aids in development of homeothermy by providing a natural insulation layer. Natural feathering alterations occur as birds exchange down feathers by adult contour feathers with changing metabolism (Furlan and Macari, 2002). Feathering pattern modifications may also occur in response to excessive cold or heat to prevent or enhance heat dissipation, respectively (Wylie et al., 2001).

In commercial broiler production, maternal egg incubation is replaced by artificial incubation, during which temperatures are remained constant to ensure high hatchability (Lourens et al., 2005). However, several studies have shown that 1-2°C intermittent increase in the usual incubation temperature (37.5-37.8°C) can alter offspring phenotype by improving thermal tolerance trough adulthood (Moraes et al. 2003; Yalçin and Siegel, 2003; Collin et al, 2005; Yalçin et al, 2005; Collin et al, 2007, Piestun et al, 2008, 2009). Additionally, utilizing constant temperature alteration, Dahlke et al. (2008) found that a 1°C increase in incubation temperature after day 7 increased feather follicle density on neonate backs. Scott et al. (2014) showed that an incubation temperature 1°C below usual (37.5^{o}C) during the first three days, and 1°C above usual during the last three days of incubation also increased feather follicle density on the back and thighs of 22-day old chickens.

Skin and feather alterations parallel with or be preceded by hormonal fluctuations. In mammals, skin characteristics are influenced by thyroid and growth hormones (Zouboulis, 2004). In birds, it is known that T3 and T4 hormones influence cell growth in feather follicles (Spearman, 1971). It has been reported that changes in incubation temperatures influence plasma thyroid hormone and growth hormone concentrations in neonate chickens (Willemsen et al., 2010), so it can be suggested that incubation temperature indirect affects feather follicle development via changes in thyroid hormone and growth hormone concentrations.

Morphophysiological adjustments related to homeothermy may finally translate into behavioral changes, such as the search for sites with appropriate temperatures. Indeed, turkey hatchlings from eggs incubated at 38.5°C from d 7 of incubation to hatch, preferred higher temperatures than hatchlings from eggs incubated at 37.5^{o}C (Nichelman and Tzschentke, 1999). Additionally, layer chicken hatchlings obtained from eggs incubated at 40°C, 4 h/d from day 14 to 18 of incubation preferred 1°C lower ambient temperatures until 7 days after hatching than hatchlings incubated at a constant eggshell temperature of 37.8^{o}C (Walstra et al., 2010).

Despite this vast number of studies to effects of incubation temperature, no attempts have been made: (i) To assess potential effects of incubation temperature on neonate skin thickness and vascularity as well as feather morphological characteristics; (ii) To associate changes in skin and feather characteristics with underlying hormonal fluctuations; (iii) To associate physiological changes with different temperature preferences after hatching.

We hypothesize that continuous exposure to cold or heat during the last days of incubation can induce a cascade of morphophysiological changes, which in turn, affects hatchling thermal preference. To test this hypothesis, we investigated whether a continuously higher or lower temperature, starting at d 13 of incubation affects skin and feather characteristics, body surface and rectal temperatures, plasma thyroid hormone and growth hormone concentrations, and preferred ambient temperature after hatching.

2. MATERIALS AND METHODS

Incubation

All experimental procedures used in this study were approved by the local Ethics Committee for use of animals in research (Fcav/Unesp, Jaboticabal, Brazil, protocol nº 021086/11).

A total of 780 Cobb 500 hatching eggs from a broiler breeder flock (aged 59 weeks) were obtained from a commercial hatchery (Globoaves, Itirapina, São Paulo, Brazil), weighed, and distributed homogeneously over six incubators (Premium Ecológica). Incubation temperature for all incubators was set at 37.5°C until d 12 of incubation. From d 13 until hatching, incubator temperature was lowered to 36ºC, increased to 39ºC or maintained at 37.5ºC. Per incubation temperature profile two repetitions (two incubators) were used. Relative humidity in all incubators was maintained at 60% throughout incubation. Egg rotation (45° of rotation/ two hours) was maintained until d 18 of incubation. At day 18 of incubation, all eggs were moved to hatching baskets, which were placed in the same incubators. Thermal programming from day 13 of development onward was determined based in the knowledge that functional maturation of the hypothalamo-pituitary-tyroid axis is established around 13-14 days of incubation (Jenkins and Porter, 2004).

Eggshell temperature, incubation duration and hatchability

Within each incubator, at five eggs, thermistors (Type T; Alutal, place, country) was attached in the eggshell covered with styrofoam and pasted with regular cello tape (3M) to register eggshell temperature (EST). Additionally, air temperature (AT) was registered in the central region of the incubator EST and AT were measured every 30 min from onset of incubation until d 19 of incubation and stored in data loggers connected to a computer for further analysis. Incubator thermostat temperature was measured daily, at 8 AM and 5 PM for the same period as EST and AT.

From day 19 of incubation (456 hours of incubation), the incubators were checked every three h to determine individual incubation time (h) per chick. Incubation time corresponded to the number of hours between the start of the incubation and hatching. After day 22 (528 hours of incubation), unhatched eggs were analyzed on fertility or moment of mortality. Clear eggs and eggs containing dead embryos were opened to investigate whether eggs were fertile or the moment of embryonic mortality.

Hatchability was calculated as the number of hatched chicks relative to the number of fertile eggs.

Body surface and rectal temperatures

After drying (3 h after hatch), all the hatchlings were sexed by examining the feather, according to the manual of chickens management (Cobb, 2008). Male hatchlings were weighed and 24 chicks per treatment (12 chicks/incubator) with an average BW (48.00±1.00g) were selected. Infrared thermal images of these 24 chicks per treatment were collected for determination of surface temperature of the head (cranial), neck, back (thoracic-dorsal), breast (chest-ventral) and thigh, using an infrared thermography camera with a precision of ± 0.7°C and spectrum range of 7.5 – 13 µm (FLIR E40, Stockholm, Sweden). To obtainment and analyze of thermography images, it was adopted methodology proposed by Nääs et al. (2010). For a better visualization of the images and to avoid visual interference from the surrounding area, a cardboard was placed behind each chick. The thermal camera was placed approximately 1 m from the bird and the image was registered using an angle of 90° from the chick surface. These procedures were utilized in an attempt to have a complete image filling within the entire angle of sight and to minimize errors due to angle distortion, respectively. As proposed by Cangar et al. (2008), it was used 0.95 as the emissivity coefficient (ε). The surface temperature of each of the five regions corresponded to the average temperature measured at several randomly chosen points in the head (13 points), neck (10 points), back (10 points), breast (12 points) and thigh (8 points), according adapted methodology of Nääs et al. (2010). Each thermal image was analyzed using software provided by the camera manufacturing company (FLIR Tools, Stockholm, Sweden). Rectal temperature was measured of the same chickens, using a digital thermometer (MedeQCO, Geschwenda, Alemanha) with an accuracy of ± 0.1 ° C.

Residual yolk and yolk free body weight and skin and feather analysis

Immediately, after blood collect, residual yolk was removed and hatchling yolk free body mass (YFBM) was obtained. Skin samples were removed using scissors and forceps from the neck (10x15 mm), back, and breast (between the insertion point of insertion of the wings) (20 x 25mm), and external region of the thigh (10x15 mm). All skin samples were immediately fixed in Bouin for 24 h at room temperature, washed in distillated water, and processed according routine method for light microscopy. Briefly, skin samples were dehydrated in a series of increasing ethanol concentrations (70%, 80%, 90%, 95% and 100%), diaphanized in ethanol-xylene solution (1:1) and xylene (100%, 3x), and embedded in a mixture of xylene-paraffin (1:1) followed by paraffin, approximately 45 min in each solution. Semi-serial 6-µm-thick cross-sections were prepared and stained with Masson`s Trichrome for morphological analysis (5 cross-sections/slice). Measurements of epidermis, dermis, and total skin thickness, and blood vessel perimeter and number of blood vessels per area (area: 50.700μm^2) in the dermis were realized in images obtained and examined using an image capture and analysis system (Leica, QWin, Wetzlar, Germany). Thirty measurements of each variable were considered per body region per hatchling in each slice. Feather weight was determined after manually removal of all the down feathers of the chickens from the whole body Feather weight was calculated as the difference between body weight with and without feathers. Feather weight was expressed in gram and in percentage related YFBM. Additionally, feather samples were excised from back, breast, and dorsal region of the neck (between the point insertion of the wings) and utilized for obtaining barbule length, and barb number, length, and width, using an image capture and analysis system (Leica- QWin, Wetzlar, Germany). A total of 15 feathers per body region per hatchling, and 10 barbs and 10 barbules per feather were measured.

Plasma hormone

After obtaining body surface and rectal temperature, the 24 chicks/treatment were killed by cervical dislocation to obtain blood samples, yolk free body weight and skin and feathering characteristics. Blood samples were collected by branchial venipuncture (approximately 1.5 ml of blood) and stored in eppendorf tubes containing EDTA. Blood was centrifuged at 2,000 rpm for 10 min at 4ºC to obtain plasma. Plasma was stored at –70°C until analyses. Plasma samples were analyzed for growth hormone (GH, ng/dl), 3,5,30-tri-iodo-l-thyronine (T_3, ng/ml) and 3,5,30,50-tetraiodo-l-thyronine (T_4, ng/dl). The total concentrations of T3, T4, and GH were measured by radioimmunoassays (RIA), with kits “Coat-a-count” (T3 AccuBind ELISA KIT, Monobind cod 125-300B; T4 AccuBind ELISA KIT, Monobind cod 225-300B; and chicken growth hormone ELISA kit, MyBioSource, cod MBS266317), using a gamma radiation counter C12 DPC.

Thermal preference test

In one- and two-day old male chicks a thermal preference test was executed, based on the methodology of Myhre et al. (1975) and Walstra et al. (2010). Two test chambers with similar dimensions (length x width x height: 160 x 60 x 50 cm), thermal gradient system, temperature registration, and bird location registration were used. Each test chamber existed of a thin wall of aluminum, a thicker wall of medium-density fiberboard (MDF), a roof of transparent acrylic, and a grating floor of aluminum over an aluminum tray. Two electrical resistances (1,000 W each) were fixed in the floor, arising a thermal gradient from 19ºC to 40ºC along the chamber length. Ambient temperature and bird position in the test chambers were registered by twelve thermal sensors and twelve infra-red sensors, respectively, along the chambers length. Data of temperature and bird position were obtained and stored each minute during the whole measuring period. Both test chambers were used simultaneously. During each test, two chicks were put together in each test chamber. A preliminary study had shown that using one chick per test results in immobility of the chicks and absence of exploration of the thermal gradient within the chambers. When two chicks were used at the same time, such lack of behavior did not occur. During the first 30 min of the test, chicks were positioned in the middle of the chambers and had the opportunity to explore them. After completion of this first period, the chicks were repositioned in the central region of the chamber and a new period of 60 minutes followed, during which the temperature and chick position within the chamber were registered. After the tests, preferred ambient temperature was established for each chick based on the ambient temperature in which the bird remained for a longer time. In case the chick remained a similar time at two or more temperatures, the average ambient temperature was calculated. The rectal temperature of the chicks was measured immediately before the thermal preference test.

Statistical Analyses

All variable determined were subjected to statistical analysis, using SAS 9.2 software package (SAS Institute, 2009). Model assumptions were proven for both means and residuals. Hatchability was tested with a logistic procedure with Treatment as factor. Temperatures (ambient, EST, skin surface, rectal) and skin, feather, and hormonal characteristics were subjected to a GLM procedure using Treatment (cold: 36ºC; control: 37.5ºC; and heat: 39ºC) as fixed factor. Means were compared after correction with Tukey for multiple comparisons. Data are expressed as means ± SE and means are considered different at $P<0.05$. For all variables the individual egg or chick was considered as the experimental unit. Only male chickens were used, whereas female chickens were removed from the experiment.

3. RESULTS

Induced changes in EST during fetal development

Figure 1A-C shows variations in thermostate temperature (TT), AT, and EST. In all incubation treatments, TT and AT were kept at 37.5°C until d 12 of incubation. From d 13 to 19 of incubation, treatments were changed into cold, control, and heat incubation. During this period, average EST of eggs incubated under control and cold conditions was similar and significantly lower ($P<0.05$) than the EST of eggs exposed to heat (Figure 2A). In the cold treatment, there was an average difference of 1.30°C between EST and AT, whereas this difference was reduced to zero during heat incubation ($P<0.05$). The difference between EST and AT of the control group was intermediate both other groups (give difference) and not significant from them.

Incubation duration, hatchability and hatchling body weight

No significant effect of incubation treatments could be observed on hatchling body weight (low = 48.50g, control = 48.94g, high = 48.98g, $P=0.81$, CV=6.58%). However, duration was significantly longer for the cold temperature treatment compared to the control and heat temperature treatments, which were similar (cold = 520h, control = 509h heat=503 h, $P<0.0001$, CV= 2.94%). Hatchability in the cold and control treatments was similar, but lower than in the heat treatment (cold = 67.6%, control = 68.1%, heat = 75.7%, P<0.001).

Body surface and rectal temperatures

Head (P=0.002) and back surface (P=0.03) temperatures increased with incubation temperature (Table 1), whereas neck surface temperatures were significantly higher in hatchlings obtained from the heat incubation treatment compared to cold and control treatments, which did not differ from each other. Rectal temperature and thigh and breast skin temperature were not affected by incubation treatment (P>0.05).

Feathering weight and characteristics

Incubation treatments had no effect on the absolute and relative feathering weight, as well as on barb number, length, and width, and barbule length (Table 2).

Skin thickness and vascularity

Heat incubation treatment resulted in lower skin and dermis thicknesses in all analyzed body regions, compared to cold and control incubation treatments, which did not differ from each other (Table 3). Incubation treatments had no significant effect on epidermis thickness.

Blood vessel number and perimeter in the dermis were evaluated as a measure of skin vascularity in the neck, back, breast, and thigh (Table 3). Number of blood vessels in the neck area of hatchlings obtained from the cold incubation treatment was lower (p=0.04) compared to control and heat treatments, which did not differ from each other. In all other regions, number of blood vessels did not significantly differ among incubation treatments. Incubation treatment had a significant influence on blood vessel perimeters (P≤0.05) in all analyzed body regions, but effects differed per region (Table 3). Neck and back blood vessel perimeters in the dermis were lower in the cold incubated hatchlings compared to control and heat incubated hatchlings. In the breast dermis, blood vessel perimeters were lower for the cold and control incubated hatchlings compared to the heat incubated hatchlings (P=0.03), whereas for the tight dermis the perimeter of blood vessels increased with incubation temperature.

Plasma hormones

PlasmaT3, T3 to T4 ratio and GH levels were significantly lower ($P < 0.05$) in hatchlings obtained from the cold incubation treatment compared to heat and control treatments, which did not differ from each other (Figure 3). Plasma T4 levels did not differ among treatments.

Preferred ambient temperature

Hatchlings obtained from heat treated eggs preferred a higher ambient temperature ($P<0.05$) compared with hatchlings from the two other incubation treatments, which did not differ from each other (Figure 4).

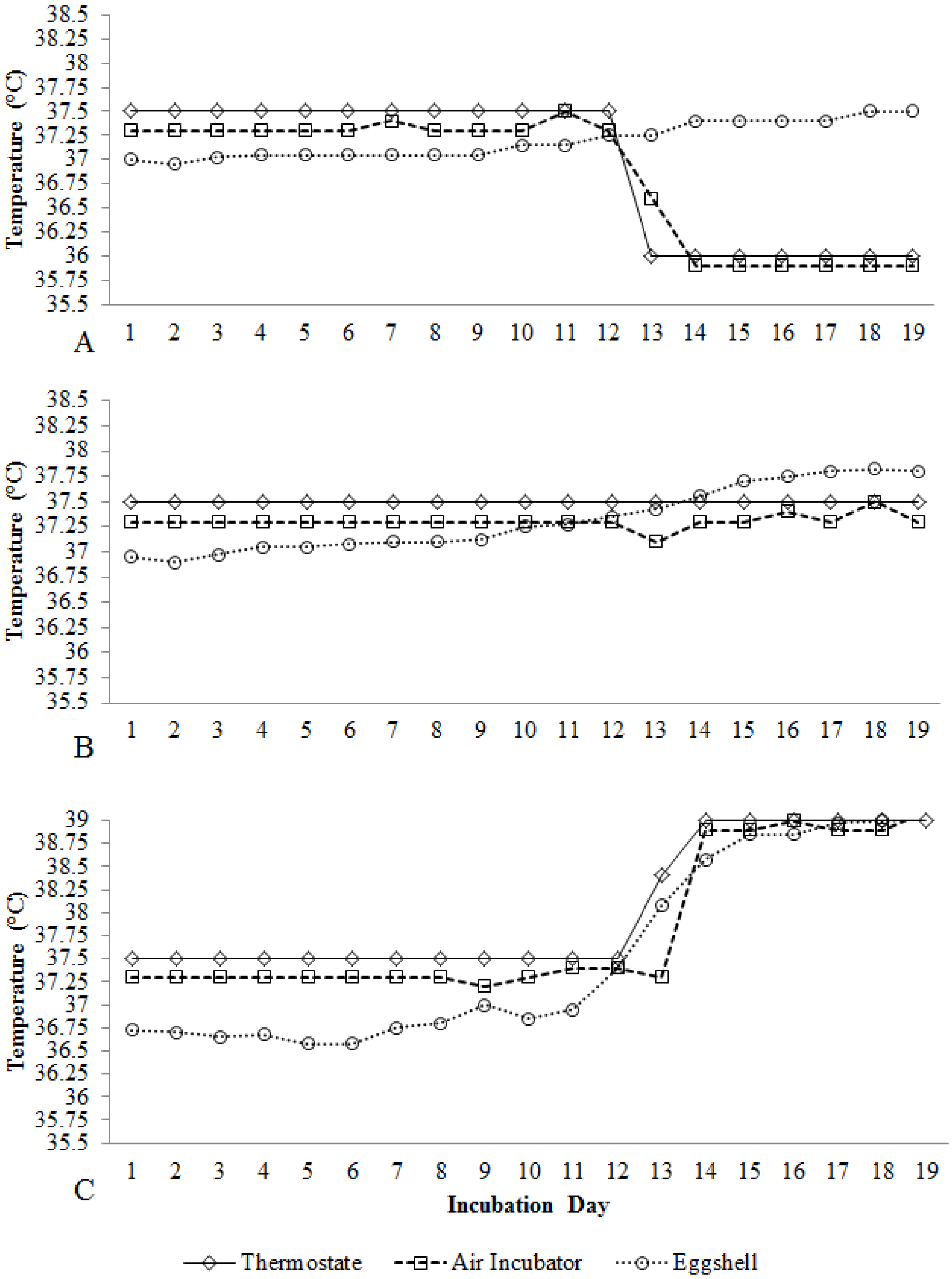

Figure 1. Thermostate, incubator air, and eggshell temperatures in cold (A), control (B), and heat (C) incubation treatments.

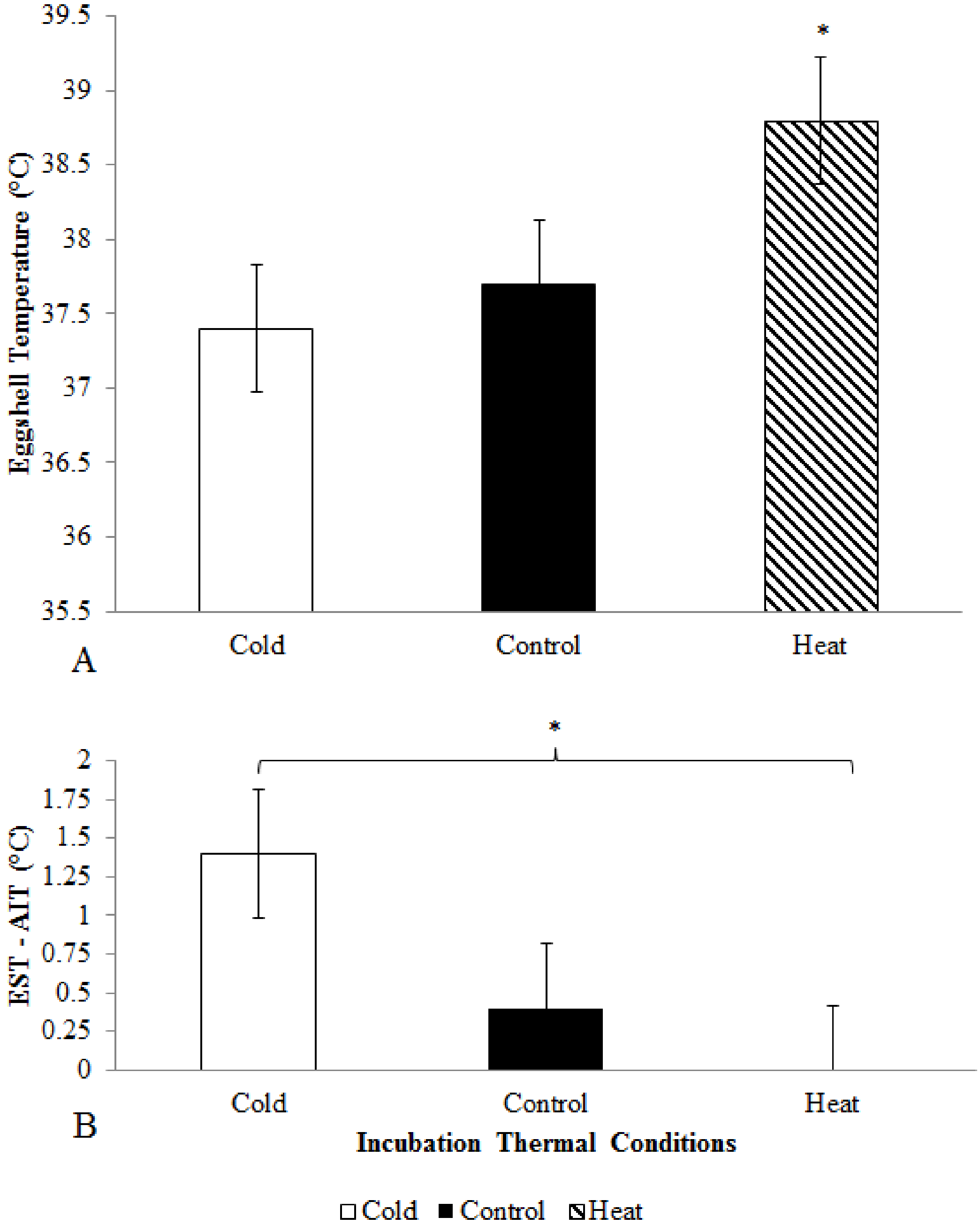

Figure 2. A. Average eggshell temperatures from d 13 to 19 of incubation in the cold (36°C), control (37.5°C), and heat (39°C) treatment. B. Average differences between eggshell (EST) and incubator temperatures (IT) from d 13 to 19 of incubation in the cold (36°C), control (37.5°C), and heat (39°C) treatment. *: indicate difference among the means (*P*< 0.05), Data are expressed as mean ± SD, n =10.

Table 1. Body surface and rectal temperatures of hatchlings, obtained from eggs exposed to cold (36°C), control (37.5°C), or heat (39°C) temperature from d 13 to 19 of incubation (means).

Variables	Incubation Thermal Condition				
	Cold	Control	Heat	*P value*	CV(%)
N=24					
Rectum	38.0	37.9	38.2	0.63	9.22
Head	32.1 ^{c}	33.7 ^{b}	34.3 ^{a}	0.002	3.80
Neck	30.6 ^{b}	31.5 ^{a}	32.0 ^{a}	0.04	3.94
Back	30.8 ^{c}	31.9 ^{b}	32.4 ^{a}	0.03	4.90
Thigh	31.3	31.5	31.8	0.18	5.01
Breast	30.1	30.7	30.9	0.69	4.28

$^{a-c:}$ Values within a row lacking a common superscript differ (P< 0.05).

Table 2. Feathering characteristics and total weight of hatchlings obtained from eggs exposed to cold (36°C), control (37.5°C) or heat (39°C) temperature from d 13 to 19 of incubation.

Variables		Incubation Thermal Condition			*P value*	CV(%)
		Cold	Control	Heat		
N=24						
Weight	g	1.94	1.70	1.58	0.44	39.36
	%*	3.72	3.24	2.98	0.43	39.45
	% **	3.88	3.70	3.47	0.52	22.27
Neck	Barb number	11.80	12.89	12.29	0.11	2.29
	Barb length (mm)	15.69	14.94	14.27	0.48	8.94
	Barb width (mm)	1.53	1.56	1.61	0.81	9.33
	Barbule length (mm)	1.19	1.17	1.13	0.42	4.21
Back	Barb number	13.97	14.08	14.05	0.32	6.55
	Barb length (mm)	14.06	13.89	13.95	0.36	8.67
	Barb width (mm)	1.76	1.71	1.70	0.12	8.3
	Barbule length (mm)	1.53	1.48	1.50	0.38	18.69
Breast	Barb number	15.97	14.67	15.61	0.25	5.65
	Barb length (mm)	12.23	11.05	11.94	0.44	9.25
	Barb width (mm)	2.00	1.82	1.80	0.20	7.0
	Barbule length (mm)	1.83	1.38	1.38	0.24	21.70

* calculated in relation to pre-incubation egg weight, ** calculated in relation to body weight.

Table 3. Skin, epidermis, and dermis thickness (µm) and blood vessel number (counts per field) and perimeter (µm) of hatchlings obtained from eggs exposed to cold (36°C), control (37.5°C), or heat (39°C) temperature from d 13 to 19 of incubation.

		Incubation Thermal Conditions			*P value*	CV (%)
		Cold	Control	Heat		
N=24						
Neck	Skin	432.81 [a]	469.82 [a]	360.85 [b]	0.004	8.48
	Epidermis	14.49	20.40	16.48	0.27	6.02
	Dermis	418.34 [a]	449.43 [a]	343.72 [b]	0.004	8.59
	Number	1.67 [b]	2.00 [a]	2.33 [a]	0.04	17.66
	Perimeter	47.39 [b]	54.88 [a]	55.42 [a]	0.05	9.52
Back	Skin	479.38 [a]	443.81 [a]	370.54 [b]	0.006	4.98
	Epidermis	12.31	16.38	16.67	0.18	10.84
	Dermis	462.79 [a]	427.50 [a]	353.80 [b]	0.004	5.25
	Number	1.98	2.14	2.26	0.07	8.23
	Perimeter	53.15 [b]	64.71 [a]	69.15 [a]	0.05	14.46
Breast	Skin	563.01 [a]	535.22 [a]	474.52 [b]	0.005	8.51
	Epidermis	20.92	17.68	15.88	0.08	4.11
	Dermis	542.09 [a]	527.66 [a]	458.53 [b]	0.002	9.17
	Number	1.45	1.59	1.62	0.09	15.09
	Perimeter	51.10 [b]	56.98 [b]	65.57 a	0.03	9.05
Thigh	Skin	387.29 [a]	392.46 [a]	338.75 [b]	0.009	4.57
	Epidermis	18.26	17.32	16.51	0.55	2.52
	Dermis	368.38 [a]	375.29 [a]	323.32 [b]	0.02	4.86
	Number	1.15	1.47	1.62	0.14	18.90
	Perimeter	44.11 [c]	54.48 [b]	69.80 [a]	<0.001	3.70

a-c: Values within a row lacking a common superscript differ ($P \leq 0.05$).

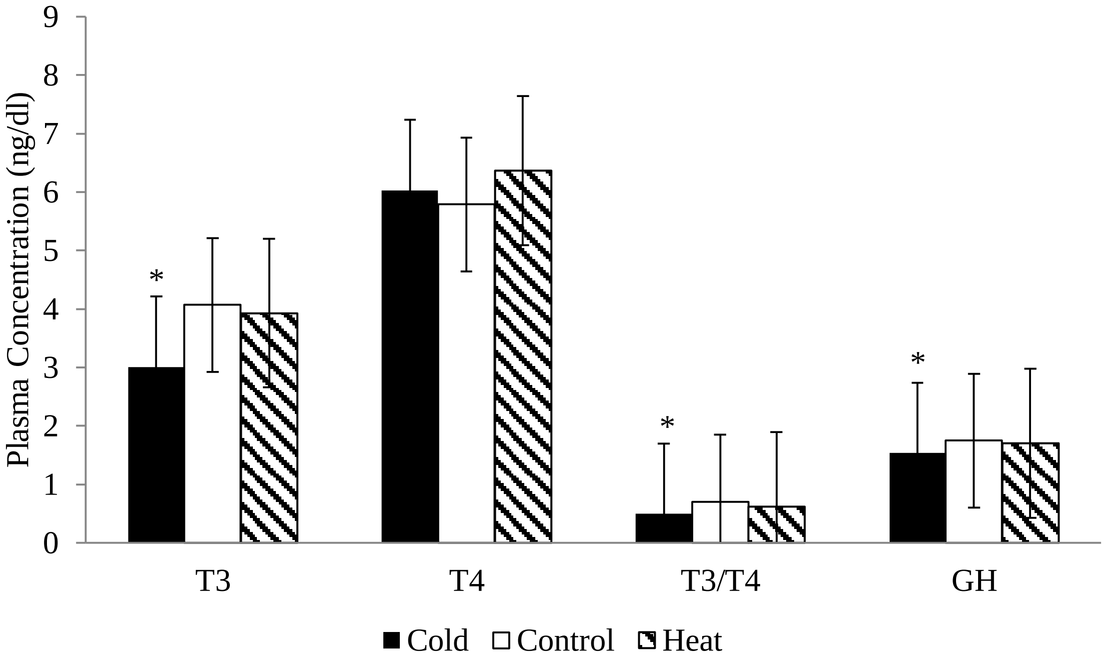

Figure 3. Plasma concentrations of triiodothyronine (T_3, ng/ml), thyroxine (T_4, ng/dl), T3/T4 ratio and growth hormone (GH) of hatchlings obtained from eggs exposed to cold (36°C), control (37.5°C), or heat (39°C) temperatures from d 13 to 19 of incubation *: mean differs from others (*P*< 0.05), Data are expressed as mean ± SD, n =24.

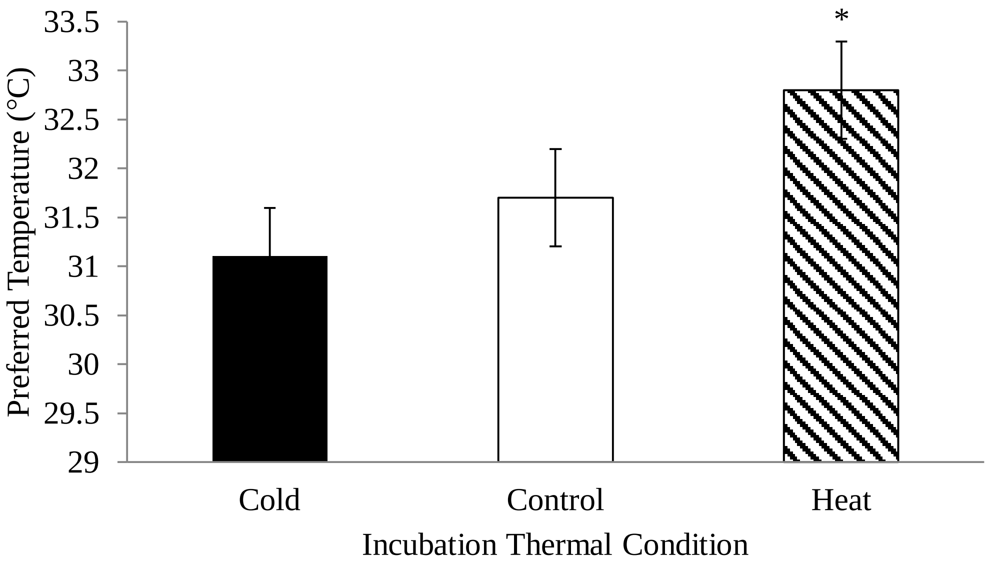

Figure 4. Preferred ambient temperature of hatchlings submitted to cold (36°C), control (37.5°C) or heat (39°C) incubation treatments during fetal development. *: mean differs from others (*P*< 0.05); n =12. Data are expressed as mean ± SD, n=16.

4. DISCUSSION

<u>Heat vs control incubation treatment</u>

The present study analyzed whether continuous temperature changes during the second half of incubation (d 13 to hatching) can induce morphophysiological alterations which, in turn, affect hatchling thermal preference. The used heat treatment (39.0^{o}C) caused a higher hatchability, hatchling head and breast surface temperatures, breast and thigh dermal vascularization and the reduction of skin thickness in all analyzed body regions compared to the used control treatment (37.5^{o}C). Additionally, the heat treatment increased hatchling's preferred ambient temperatures by approximately 1°C. No differences were observed in rectal temperature, body weight, feather morphology and plasma hormone concentrations (T3, T4, and GH) between the heat and control treatment.

Regarding the heat treatment, the current work differs from previous ones in two main points. Firstly, the current study demonstrated an increase in hatchability and no alterations in hormone concentrations between heat and control treatment, whereas other studies have reported decreased hatchability (Lourens and Van Middelkoop, 2000; Lourens et al., 2005; Collin et al., 2007; Piestun et al., 2008; Willemsen et al., 2010) and lower T3 plasma concentrations after high incubation temperatures (Piestun et al., 2008a;. Willemsem et al., 2010). These discrepancy results may be explained by no differences between AT and EST. Incubating eggs at an AT matching EST, as occurred throughout our heat treatment, improved hatchability without alter plasma hormones rates and incubation period. Secondly, studies have not analyzed changes to skin thickness and vascularization in relation to incubation temperature. The alterations that we observed in these parameters in association with higher preferred ambient temperatures constitute a novel finding. The skin plays a key role in heat maintenance or dissipation. The thinner and more vascularized tegument observed in the current work after heat incubation treatment apparently represents an adaptive embryonic response that would drive greater heat loss. This idea is supported by the increase in surface body temperature in the head and back of hatchlings, without a change in internal body temperature. Altogether, these morphophysiological changes may help to maintain body temperatures at normal or at least not lethal levels, even during high ambient temperatures in later life. Consequently, growth processes can be maintained as well, driven by the unchanged levels in thyroid and growth hormones. These hormones are directly involved in chick embryo development and the stable levels we observed may have contributed to the lack in alterations in incubation duration and body weight at hatching. On the other hand, a thinner and more vascularized tegument, leading to greater heat loss, may also have driven the choice for a higher ambient temperature, as a behavioral adjustment that helps hatchlings maintain homeothermy, which is in confirmation with Nichelmann et al. (1999).

Cold vs Control incubation treatment

The cold incubation treatment, on the other hand, resulted in reduced head, neck, and back surface temperatures, reduced neck, back and thigh dermal vascularization, decreases in T3 and GH plasma concentrations, and longer incubation duration compared to the control incubation treatment. The cold incubation treatment did not have an effect on body weight at hatch and preferred ambient temperature in day-old chickens compared to the control treatment. These findings are in agreement with previous works with low incubation temperature, which showed ducklings require longer incubation times and broiler hatchlings present lower T3 and T3/T4 ratios (DuRant et al., 2010). Willemsem et al. (2009) reported no changes in hormone concentrations of broiler chicks submitted during embryo development to intermittent periods at low temperatures. These previous results cannot be directly compared to ours as the period, frequency, and intensity of temperature alterations were very distinct. The reduction we observed in plasma T3 and T3/T4 ratios after cold treatment may result from a decrease in liver deiodinase activity which, in turn, impairs the conversion of T4 to T3 and increases incubation times (Darras et al., 2006).

Thyroid hormone levels are considered good indicators of an animal's metabolic state (Todini, 2007), whereas GH levels are indicators of growth rates. We observed that reductions in T3 and GH occurred along with longer incubation duration, in the cold treatment. These findings indicate that, at lower temperatures, metabolic and growth rates slow down, delaying embryonic development. Moreover, we report that, in direct opposition to the heat treatment, cold treatment resulted in hatchlings with lower neck, back, and thigh dermal vascularity. Considering that the heat exchange occurs from the hot to cold environment (La Scala, 2003), the large differences in AT and EST in this case, evidenced that reduced AT resulted in increased heat loss. Increased heat loss as well as lower metabolic rates may, in turn, have elicited physiological adjustments, such as the lower vascularization observed. Hatchling surface temperature was indeed lower, compared to controls, in areas with lower vascularization, indicating less heat exchange. Moreover, these chicks preferred ambient temperatures equal to those chosen by control birds.

Altogether our results are consistent with the hypothesis that incubation temperature is an important factor influencing hormone levels and morphophysiological characteristics associated with hatchling behavioral adjustments. Changes to peripheral vascularization and blood flow have been extensively described for adult animals subjected to thermal stress (Van Handel-Hruska et al, 1977; Wolfenson, 1983). Our findings demonstrate for the first time, that precocial birds in the late embryonic stage already react with adaptive adjustments to heat and cold incubation temperatures.

5. REFERENCES

Cangar O, Aerts JM, Buyse J, Berckmans D. Quantification of the spatial distribution of surface temperatures of broilers. Poultry Science, v.87, p.2493-2499, 2008.

Collin A, Picard M, Yahav S. The effect of duration of thermal manipulation during broiler chicks embryogenesis on body weight and body temperature of post hatched chicks. Animal Research, v.54, p.105–112, 2005.

Collin A, Berri C, Tesseraud S et al. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poultry Science, v.86, p.795-800, 2007.

Dahlke F, Gonzales E, Furla RL et al. Desenvolvimento folicular de embriões de frangos de corte de diferentes genótipos expostos ao estresse térmico crônico. Ciência Rural, v. 38, n. 8, p.2314-2320, 2008.

Darras VM, Verhoelst CH, Reyns et al. Thyroidhormone deiodination in birds. Thyroid, v.16, p.25–35, 2006.

DuRant SE, Hepp GR, Moore IT et al. Slight changes in incubation temperature affect early growth and stress endocrinology in wood duck (Aix sponsa) ducklings. The Journal of Experimental Biology, v.213, p.45–51, 2010.

Furlan RL and Macari M. Termorregulação. In: Macari, M; Furlan, L.R. and Gonzales, E. (Ed.) Fisiologia Aviária Aplicada a Frangos de Corte. Jaboticabal: FUNEP/UNESP, p. 209-230, 2002.

Jenkins SA. and Porter TE. Ontogeny of the hypothalamo-pituitary-adrenocortical axis in the chicken embryo: a review. Domestic Animal Endocrinology, v.26, n.4, p.267-75, 2004.

La Scala Junior N. Aspectos físicos da incubação. In: Macari, M. and Gonzales, E. (Ed.). Manejo da incubação: incubação artificial. Jaboticabal: FACTA, p.353-360, 2003.

Lourens A and van Middelkoop JH. Embryo temperature affects hatchability and grow-out performance of broilers. Avian and Poultry Biology Reviews, v.11, p.299–301, 2000.

Lourens A, van den Brand H, Meijerhof R et al. Effect of eggshell temperature during incubation on embryo development, hatchability, and posthatch development. Poultry Science, v.84, p.914-920, 2005.

Moraes VMB, Malheiros RD, Bruggeman V et al. Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. Journal of Thermal Biology, v.28, p.133-140, 2003.

Myhre K, Cabanac M, Myhre G. Thermoregulatory behavior and body temperature in chicks of willow grouse (Lagopus lagopus lagopus). Poultry Science, v.54, p.1174–1179, 1975.

Nääs IA, Romanini CEB, Neves DP et al. Broilers surface temperature distribution of 42 day old chickens. Scientia Agricola, v.67, n.5, p.497-502, 2010.

Nangsuay A, Meijerhof R, Ruangpanit Y. et al. Energy utilization and heat production of embryos from eggs originating from young and old broiler breeder flocks. Poultry Science, v. 92, p: 474–482, 2013.

Nangsuay A, Ruangpanit Y, Meijerhof R, Attamangkune S. Yolk absorption and embryo development of small and large eggs originating from young and old breeder hens. Poultry Science, v.90, p.2648–2655, 2011.

Piestun Y, Shinder D, Ruzal M et al. Thermal manipulations in late term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. Poultry Science, v.87, p.1516-1525, 2008.

Piestun Y, Halevy O, Yahav S. Thermal manipulations of broiler embryos—The effect on thermoregulation and development during embryogenesis. Poultry Science, v.88, p.2677–2688, 2009.

Richards SA. The significance of changes in the temperature of the skin and body core of the chicken in the regulation of heat loss. The Journal of Physiology, v.216, n.1, p.1-10, 1971.

SAS-Institute Inc. User´s guide: statistics, version. 9.1 edition. Cary: SAS Institute, 2009.

Scott KP, Martin JC, Duncan SH, Flint HJ. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. FEMS Microbiology Ecology, v.87, n.1, p.30-40, 2014.

Scott J, Costa MJ, Oviedo-Rondón E. Incubation temperature profiles affect broiler feathering. Proceedings of the 2014 International Poultry Scientific Forum, Atlanta, GA, USA, 2014.

Spearman RIC. Integumentary system. In: Bell, D.J. and Freeman, B.M. (Ed.) Physiology and Biochemistry of the Domestic Fowl. London and New York: Academic Press, n.2, p.603-620, 1971.

Todini L. Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. Animal, v.1, n.7, p.997-1008, 2007.

Van Handel-Hruska JM, Wegner TN, Nordstrom JO. Avian cardio-vascular responses following high environmental temperature acclimation. Federation American Society Experimental Biology, v. 36, p.524, 1977.

Walstra I, Ten Napel J, Kemp B, van den Brand H. Temperature manipulation during layer chick embryogenesis. Poultry Science, v.89, p.1502-1508, 2010.

Willemsen H, Kamers B et al. High- and low-temperature manipulation during late incubation: effects on embryonic development, the hatching process, and metabolism in broilers. Poultry Science, v.89, p.2678–2690, 2010.

Wolfenson D. Blood flow through arteriovenous anastomoses and its thermal function in the laying hen. The Journal of Physiology, v.334, p.395–407, 1983.

Wylie LM, Robertson GW et al. Effects of ambient temperature and restricted feeding on growth of feathers in growing turkeys. British Poultry Science, London, v.42, p.449-455, 2001.

Yalçin S and Siegel PB. Developmental stability of broiler embryos in relation to lenght of egg storage prior to incubation. Journal of Poultry Science, v.40, p.298-308, 2003.

Yalçin S and Ozkan S et al. Pre- and post-natal conditioning induced thermotolerance on body weight, physiological responses, and relative asymmetry of broilers originating from young and old breeder flocks. Poultry Science, v.84, p.967–976, 2005.

CAPÍTULO 3 - TEMPERATURE OF FETAL ENVIRONMENT ALTERS THERMAL PREFERENCE AND RESPONSE TO HEAT STRESS OF BROILERS CHICKENS ALONG REARING PHASE

VS Morita,[*] VR Almeida,[*] JB Matos Junior,[*] TI Vicentini,[*] H van den Brand[‡] and IC Boleli[*1]

[*] *Department of Animal Morphology and Physiology, Sao Paulo State University, Access road Professor Paulo Donato Castellane,s/n, 14884-900, Jaboticabal, Sao Paulo, Brazil and [‡]Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, PO Box 338, 6700 AA, Wageningen, the Netherlands*

ABSTRACT - The current study aimed to evaluate how long temperature manipulation of fetal environmental may alters the thermal preference throughout the rearing phase and how this manipulation may affects response to thermal challenge, metabolism and performance of broiler chickens. Eggs were exposed to low (36ºC) or high (39ºC) temperature instead of control temperature (37.5ºC) from day 13 of incubation until hatching. Cold-treatment chickens showed lower plasma T3 and GH levels at day 1 of age and lower T3 level at day 42. Preferred ambient and rectal temperature, T4 level, performance and response to thermal challenge were not altered in these birds. On the other hand, heat-treatment chickens exhibited high preferred ambient and rectal temperature during the first two weeks of age, low plasma T3 level at days 21 and 42 and late response to thermal challenge. However, these birds have no variation of T4 and GH level and performance. In conclusion, exposure to heat temperature during fetal development promoted long-lasting changes in the thermoregulatory system of chickens retards the thermal challenge response and consequently, the termotolerance of these birds. Moreover, the ambient preferred temperature by heat-treatment, but not by cold- and control-birds, correspond those recommended for strain under study, indicating that broiler chickens eggs, when incubated at a temperature equal to or less than usual (≤37.5 ° C), may be reared, up to 21 days of age, in temperature 1°C below to the recommended in the rearing protocol.

Keywords: broiler chicken, fetal development, preferred ambient temperature, thermal challenge, thermal manipulation

1. INTRODUCTION

Environmental temperature during poultry rearing is one of the physical factors that determines phenotypic expression of the birds' genetic potential for production and growth. Indeed, variations on the recommended temperature for a given broiler strain affect performance, decreasing production of meat and other poultry products, causing great economic losses (Geraert et al., 1996; Yahav et al., 1996).

Manipulation of environmental conditions during birds' fetal development has been shown to produce long-lasting effects on the resulting phenotypes, including physiological modifications needed to develop heat tolerance. Thus, thermal manipulation at incubation may be a potential mechanism to modulate performance, health and well-being. In fact, several studies have observed effects of thermal manipulation at incubation on various birds post-hatch characteristics including higher muscle growth (Halevy et al., 2001; Piestun et al., 2009; Loyau et al. 2013), increased gastro-intestinal growth (Uni et al., 2001), and improved heat tolerance when chickens are thermally challenged (Yahav et al., 2004, 2005; Collin et al., 2005; Yalçin et al., 2008; Piestun et al., 2008, Collin et al., 2011; Druyan et al., 2012).

Some studies have shown that prenatal thermal manipulation also influences the preferred temperature of poultry production. Eggs from ducks and turkeys incubated for the final week at low (34.5°C) or high (38.5°C) temperatures decreased and increased their preferred ambient temperature until they reached 10 days of age, respectively (Nichelmann, 2004). Tzschentke (2007) verified that continuous exposition to high temperature (38.5°C) from day 28 of incubation also increased the ducklings preferred temperature until they reached 10 days of age. In contrast, Walstra et al. (2010), showed that intermittent exposure of eggs of layer hens to high eggshell temperature (40°C) for a short periods at the end of incubation decreased their preferred temperature in the first week of rearing. In another study, we found that applying high temperature (39°C) during broiler chickens fetal development (day 13-21) also increased hatchlings preferred ambient temperature (Chapter 2). Therefore, continuous or intermittent pre-natal thermal manipulation alters thermal preference in turkeys, duck, layer and broiler chicks in their initial phase of rearing, a period in which these precocial birds acquires body temperature control (Nichelmann and Tzschentke, 2002). However, we still do not know how thermal manipulation affects the preferred temperature and the response to thermal challenge of precocial birds throughout the rearing period.

Therefore, our objective at the present study were: (i) to establish for how long temperature manipulation during fetal development affects the thermal preference of broiler chickens throughout the rearing phase; (ii) whether body temperature is associated with these birds thermal preference; and (iii) how temperature manipulation affects their response to thermal challenge, their metabolism and performance. The answers to these questions may contribute to further the current understanding on the effects of thermal manipulation of fetal environment on the post-hatching ontogenetic development and their potential use to improve poultry production.

2. MATERIALS AND METHODS

Incubation

All experimental procedures used in this study were approved by the local Ethics Committee for use of animals in research (Fcav/Unesp, Jaboticabal, Brazil, protocol nº 021086/11).

A total of 900 Cobb500® hatching eggs from broiler breeder flocks at 59 weeks of age were obtained from a commercial hatchery (Globoaves, Itirapina, São Paulo, Brazil), weighed and distributed homogeneously by mean weight (67-72g) in six incubators (150 eggs each). The temperature set point profiles for all incubators were adjusted to 37.5°C until day 12 of incubation. From day 13 until hatching, the incubators had the temperature diminished to 36ºC, increased to 39ºC or maintained at 37.5ºC, being two repetitions (two incubators) per temperature. The relative humidity into the incubators was maintained at 60% during all incubation period until hatching, independent of the incubation temperature. Egg rotation (45° of rotation/ two hours) was maintained until day 18 of incubation. Thermal programming from day 13 of development onward was determined based in the knowledge that functional maturation of the hypothalamo-pituitary-tyroid axis is established around 13-14 days of incubation (Jenkins and Porter, 2004).

Rearing

After hatching, 115 male chicks from each incubation treatment were housed in five boxes (2.50 x 1.50 m, N = 23) in a climatic chamber maintained at thermoneutral temperature recommended for the rearing manual (Cobb 500). The temperature and relative humidity inside the chamber were registered three times a day during the experimental period, and for this we used two digital termohigrometers located at equidistant points of the chamber. The first to the sixth week of the experiment, the average weekly values of temperature and RH, obtained by the average of the minimum and maximum values were 33.3 °C, 29.8 °C, 27.3 °C, 25.4 °C 23.0 °C 21.0 °C and 65%, 76%, 68%, 57%, 65%, 51%, respectively. The automatic management of monoblock, ventilator and heating lamps was utilized to maintain the climatic chamber temperature similar with the recommended by Cobb. Throughout the experimental period, water and feed were ad libitum. The diet of the chickens consisted of two feed types based on corn and soybean meal : (1-21 days: / kg = 2883, PB = 21:27) and growth (22-42 days: / kg = 3121, PB = 18.86),, formulated according with requirements established for broiler chickens under tropical conditions by Rostagno et al. (2011). The chicks received vaccine against Marek and Bouba Avian disease still in the hatchery, against infectious bursal disease (intermediate strain Lukert) and Newcastle (La Sota strain) on the 8th day of age and against Gumboro disease (strong strain Australia v-877) on the 18th day.

Thermal preference test

The thermal preference test used in the present study were based in the methodology of Myhre et al. (1975) and Walstra et al. (2010). Two test chambers with similar dimensions, thermal gradient system, and register of temperature and bird location were used, according to described in the Chapter 2. Briefly, each test chamber presents a thermal gradient from 19ºC to 40ºC along of its length, gradient of ambient temperature and bird position registered by twelve thermal sensors and twelve infra-hot sensors, respectively, positioned in opposite sides along of the chambers length, and data of temperature and bird position obtained and stored each minute during all experimental period, by means of a software developed for monitoring of the temperature gradient and bird dislocation.

Thermal preference tests were simultaneously realized in the two test chambers at day 1-2, 7-8, 14-15, 21-22, 28-29 and 35-36 of age, using two chickens (n= 12 birds/treatment), according Chapter 2. Following methodology used in the Chapter 2, during the first 30 minutes of the test, birds were positioned in the central region of the chambers, and had the opportunity to explore them. After completion of the first period, the birds were repositioned in the central region of the chambers, and a new period of 60 minutes followed, during which the temperature gradient and bird dislocation and position into the chambers were registered. After the tests, preferred ambient temperature was established for each bird with base in the ambient temperature in which the bird remained for a period longer than 25±3 minutes If the bird remained similar time at two or more temperatures, the mean thermal temperature was calculated. The rectal temperature of the birds was measured immediately before the thermal preference tests.

Plasma hormone

Blood samples were collected by branchial venipuncture at day 21 and 42 of age and stored in eppendorf tubes containing EDTA. Blood was centrifuged at 2,000 rpm for 10 min at 4ºC to obtain plasma. Plasma was stored at –70°C until analyses. Plasma samples were analyzed for growth hormone (GH, ng/dl), 3,5,30-tri-iodo-l-thyronine (T_3, ng/ml) and 3,5,30,50-tetraiodo-l-thyronine (T_4, ng/dl). The total concentrations of T3, T4, and GH were measured by radioimmunoassays (RIA), with kits “Coat-a-count” (T3 AccuBind ELISA KIT, Monobind cod 125-300B; T4 AccuBind ELISA KIT, Monobind cod 225-300B; and chicken growth hormone ELISA kit, MyBioSource, cod MBS266317), using a gamma radiation counter C12 DPC.

Performance

As performance parameters were evaluated live body weight (g) and feed intake (g). Body weight was registered at day 1, 21 and 42 of age. In turn, feed intake (g) was determined for initial (day 1-21) and growth (day 22-42) period.

Thermal challenge test

In the present study, the thermal challenge tests were performed based on the methodology of Walstra et al. (2010). For this, two acrylic climate chambers (side x side x height: 80 x 80 x 80 cm) equipped with resistance, fans and humidifiers, allowing variation of 15°C to 40 °C were used. The tests, performed on days 3- 4, 8-9, 15-16, 22-23, and 29-30 used the same chickens used in the thermal preference tests of the previous day, totalizing 12 chickens/ treatment /age. During the tests, the birds were kept 45 min under preferred ambient temperature (obtained in the previous day). After, the temperature of the chamber was adjusted to 5°C above the preferred temperature (thermal challenge), and in the subsequent 45 min, the chamber temperature was return to thermal preference of the chickens. The average time that the thermostat chamber leads to increase or decrease its temperature by 5 °C degrees (about 4 minutes), did not enter the recording of 45 minutes of each interval. Rectal temperature, measured with a digital thermometer, and respiratory movements, obtained through observation of movements per minute, were recorded at the end of each 45 min, in the other words, before thermal challenge, in the thermal challenge, and after thermal challenge. After the tests, the chickens used were identified to not to be reused in the preference tests followed by thermal challenge on the next weeks. This exclusion was necessary to prevent adaptation of birds to the chambers and prevent thermal conditioning to high temperatures during thermal transition of the birds, which occurs approximately up to the 10th day post-hatch (Tazawa et al., 1988; Nichelman and Tzschentke, 2002).

Statistical Analyses

Data were assessed with one-way analysis of variance (ANOVA) using the General Linear Models procedure of SAS 9.2 software package (SAS Institute, 2009). Preferred ambient and rectal temperature, hormone rates and performance results were tested for differences among thermal incubation conditions (cold: 36ºC; control:

37.5ºC; and heat: 39ºC), within each age. We examined the differences among the rectal temperatures and respiratory movements registered at the thermal challenge, before and after it in each age for each thermal incubation conditions. The differences between the rectal temperatures and respiratory movements registered at the thermal challenge and that before it (Δ1) or between the challenge and that after it (Δ2) were also examined in each age for each thermal incubation conditions. When more than 2 means were involved, in case of significant differences (at 5%), the means were compared by Tukey test.

3. RESULTS

Effect of temperature of fetal environment on the thermal preference and association with body temperature

The thermal preference test was performed at d 1-2, 14-15, 21-22, 28-29, 35-36 post-hatch. However, the data obtained at d 35-36 were not analyzed, because the chickens did not show enough mobility to determine their preferred temperature. We also analyzed rectal temperature at the same days to verify if thermal preference was associated with the body temperature of these chickens. Chickens from the heat incubation treatment preferred a significantly higher ambient temperature and had higher rectal temperatures until d 14-15, compared to cold and control treatments, among which no difference was observed (Figure 1A). After this age, no significant difference in thermal preference and rectal temperatures was verified among chickens obtained from different incubation treatments.

Effect of temperature of fetal environment on plasma hormone levels

Plasma T_3 and GH levels were significantly lower ($P< 0.05$) at d1 in chickens from the cold incubation treatment in comparison to heat and control-treatment, between which there were no significant differences (Table 2). Moreover, T3 levels were also lower at d21 and 42 for chickens obtained from the heat incubation treatment and for chickens from the cold and heat incubation treatments, respectively. Plasma T_4 did not change in the chickens of all temperatures and at all ages.

Effect of temperature of fetal environment on performance of broiler chickens

Body weight and food intake were evaluated as performance parameters (Table 2). Incubation treatments did not significant affect body weight at d1, 21 and 42. Food intake was evaluated on two periods: 1-21 and 22-42 days. Chickens from the heat incubation treatment showed lower food intake than the cold incubation treatment but only for the period of 1-21 days. Cold and control incubation treatments did not altered the food intake in both periods tested.Chickens belonging to the cold- and control-treatments present food intake changes

Effect of temperature of fetal environment on the response to thermal challenge

Broiler chickens were submitted to thermal challenge at d 2-3, 15-16, 22-23 and 29-30 post-hatch to evaluate if incubation treatments during fetal development affected their response to high ambient temperatures. Cold- and control-treatments chickens showed, in all analyzed ages, an increased rectal temperature during thermal challenge. The rectal temperatures then reduced when the birds were exposed back to their preferred temperature after thermal challenge. However, only birds at d8-9 reduced their rectal temperatures to similar values presented before the challenge (Figure 2A and B). Heat- treatment chickens showed similar results for the ages d2-3 and d22-23 and d29-30, although none of them presented a total return of the rectal temperatures to the values presented before the thermal challenge. However, heat-treatment chickens at d8-9 and 15-16 did not exhibit difference in rectal temperature when challenged (Figure 2C).

The differences between the rectal temperatures registered at the thermal challenge and that before it (Δ1) or between the challenge and that after it (Δ2) are shown in figure 3A. Heat-treatment chickens showed lower Δ1 and Δ2 at d8-9 and 15-16 in comparison with cold and control chickens, among which there were no difference.

Respiratory movements of cold and control chickens increased during thermal challenge at d22-23 and 29-30, and remained high after it (Figure 4A and B). Heat-treatment chickens showed similar data at d29-30 only (Figure 4C). At all other ages the chickens of all groups did not show significant differences of respiratory movements in response to thermal challenge.

The differences between the respiratory movements registered at the thermal challenge and that before it (Δ1) or between the challenge and that after it (Δ2) are shown in Figure 5. Heat-treatment chickens showed significantly lower Δ2 at d22-23 and 28-29 compared with chickens from the other two incubation conditions, which were similar. At all other ages the chickens of all groups did not show significant differences Δ1 and Δ2 obtained respiratory movements in response to thermal challenge.

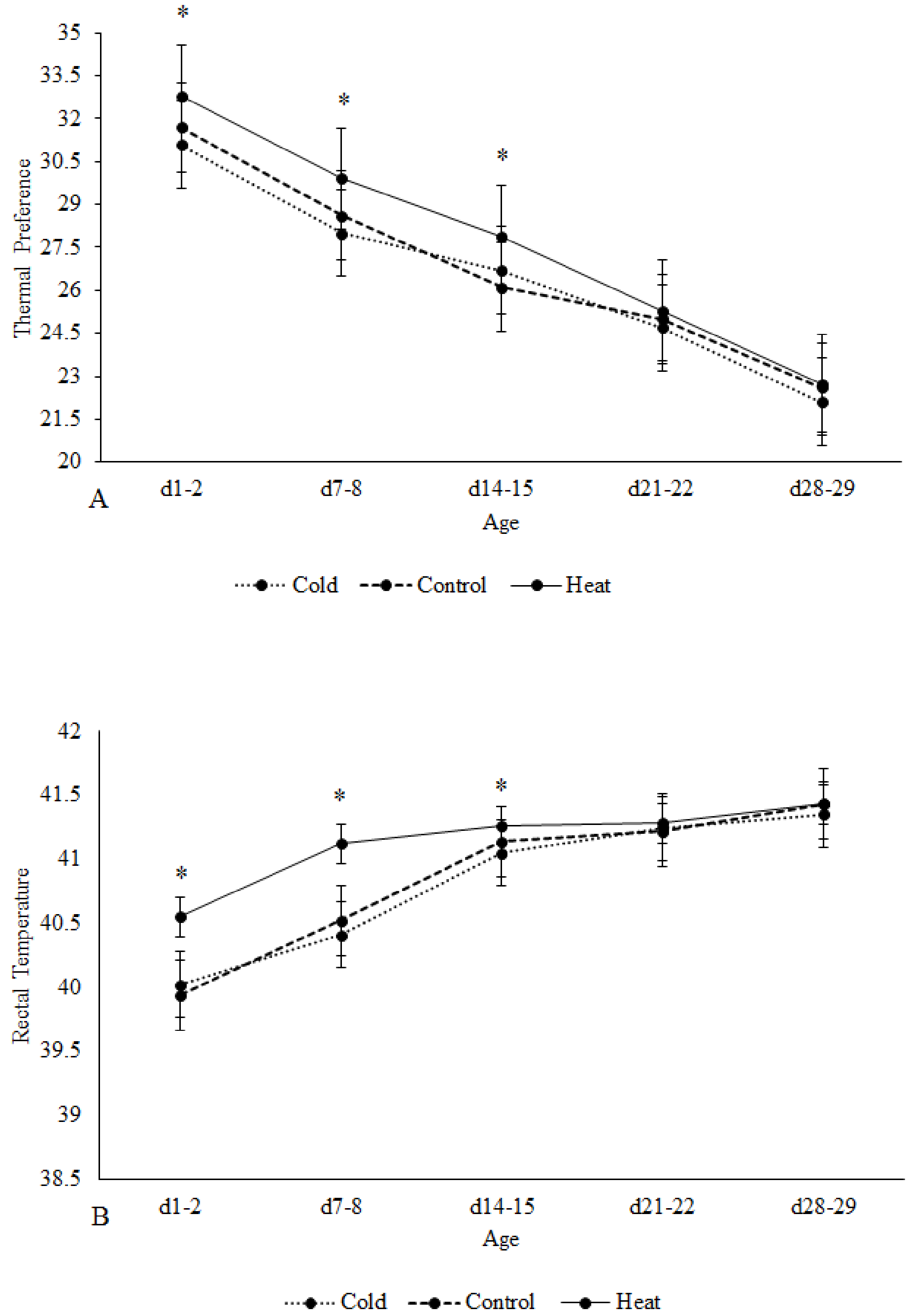

Figure 1. Thermal preference (A) and Rectal temperature (B) of broiler chickens obtained from eggs submitted to cold (36°C), control (37.5°C) or heat (39°C) incubation treatments during fetal development, according with age. *: means obtained from heat-treated chickens differ from the other groups at same age (*P*< 0.05). Data are expressed as mean ± SD, n =12.

Table 2. Effect of temperature of fetal environment on the levels of triiodothyronine (T3, ng/ml), thyroxine (T4, ng/dl) and growth hormone (GH) and performance of broiler chickens

	Incubation Thermal Condition				
	Cold	Control	Heat	*P-value*	CV (%)
T3 (ng/dl)					
d1	3.01 [b]	4.07 [a]	3.93 [a]	0.0119	21.04
d21	1.78 [a]	1.69 [a]	1.45 [b]	0.0002	25.17
d42	1.22 [b]	1.34 [a]	1.18 [b]	0.0314	32.18
T4 (ng/dl)					
d1	6.03	5.89	6.37	0.1215	26.22
d21	7.13	6.73	7.79	0.1541	8.01
d42	9.11	9.30	9.45	0.2004	11.16
GH (ng/dl)					
d1	1.54 [b]	1.75 [a]	1.70 [a]	0.0024	32.33
d21	3.80	4.56	3.67	0.2822	41.17
d42	1.96	1.40	1.49	0.2516	44.69
Body Weight (g)					
d1	48.50	48.94	48.98	0.8173	6.58
d21	810.30	787.60	790.83	0.2356	6.40
d42	2325.44	2325.28	2245.76	0.1540	5.24
Food Intake (g)					
d1-21	941.27	922.22	836.40	<0.0001	4.1
d21-42	2491.68	2471.44	2416.32	0.8786	12.87

Plasma concentrations of T3, T4 and GH as well as body weight (BW) were measured at d1 and d21 and d42 days of age. Food Intake was measured for the periods of 1-21 and 22-42 days. Broiler chickens tested belonged to the cold (36°C), the control (37.5°C) or heat (39°C) treatments referring to the temperatures of incubation during fetal development. a-b: different letters (rows) indicate significant differences among treatments (Tukey, P<0.05).

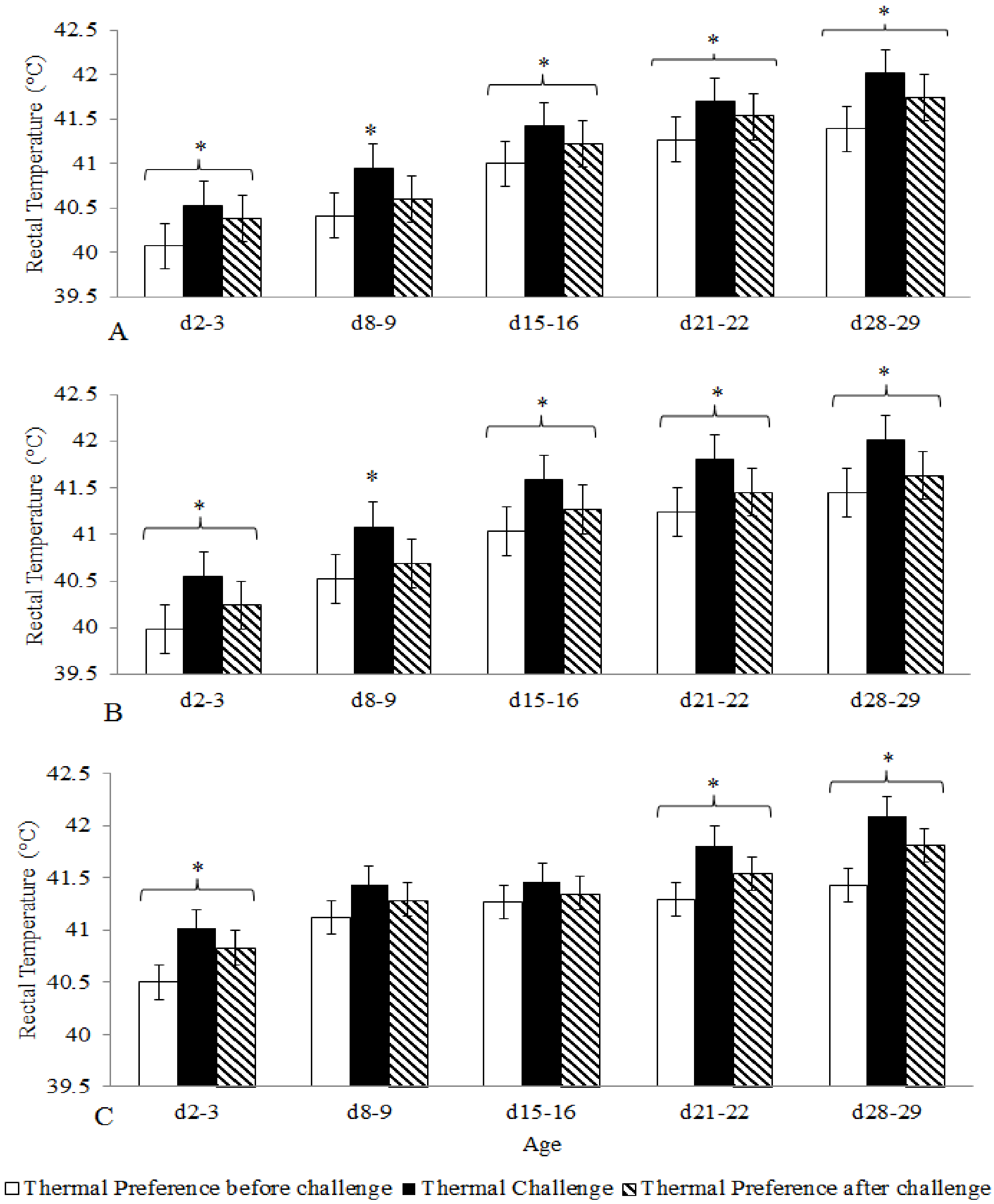

Figure 2. Rectal temperature of broiler chickens submitted to cold (36°C, A), control (37.5°C, B) or heat (39°C, C) incubation treatments during fetal development obtained under thermal preference (before thermal challenge), at 5°C above thermal preference (thermal challenge), and upon return to thermal preference (after thermal challenge), according to age. *: above the bar indicates the means that different from others in the same age, and above the bracket indicates difference among the means in the same age ($P < 0.05$). Data are expressed as mean ± SD, n=12.

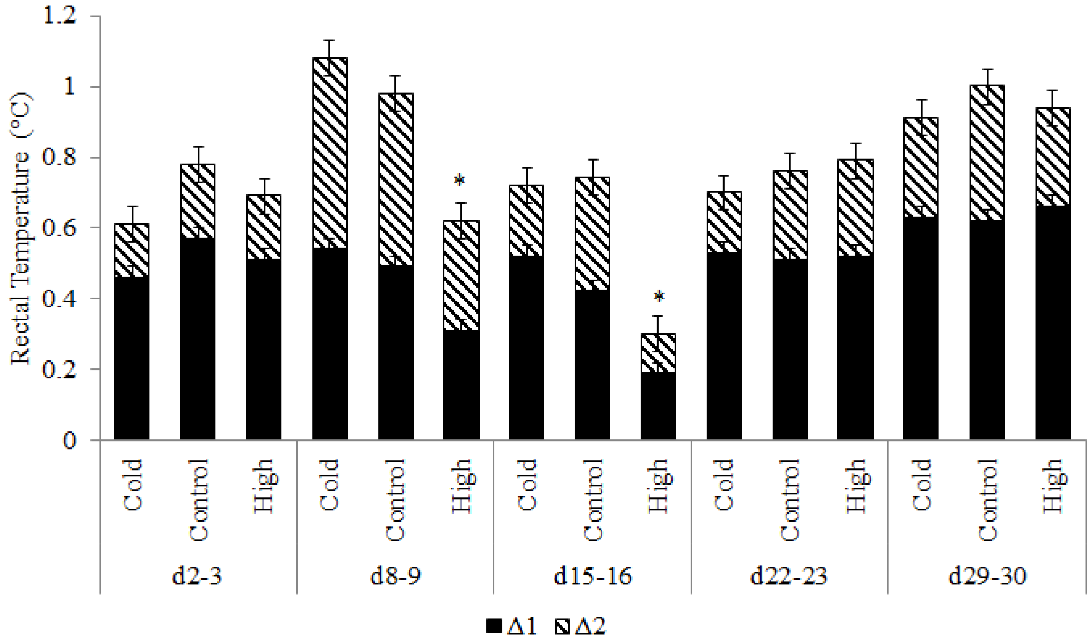

Figure 3. Average variation of rectal temperature of broiler chickens obtained from the eggs submitted to cold (36°C, A), control (37.5°C, B) or heat (39°C, C) incubation treatments during fetal development obtained: Δ1. between at 5°C above thermal preference (thermal challenge) and under thermal preference (before thermal challenge), Δ2. between at 5°C above thermal preference (thermal challenge) and upon return to thermal preference (after thermal challenge), according to age. *: above the bar indicates the means that different from others in the same age (*P*< 0.05). Data are expressed as mean ± SD, n=12.

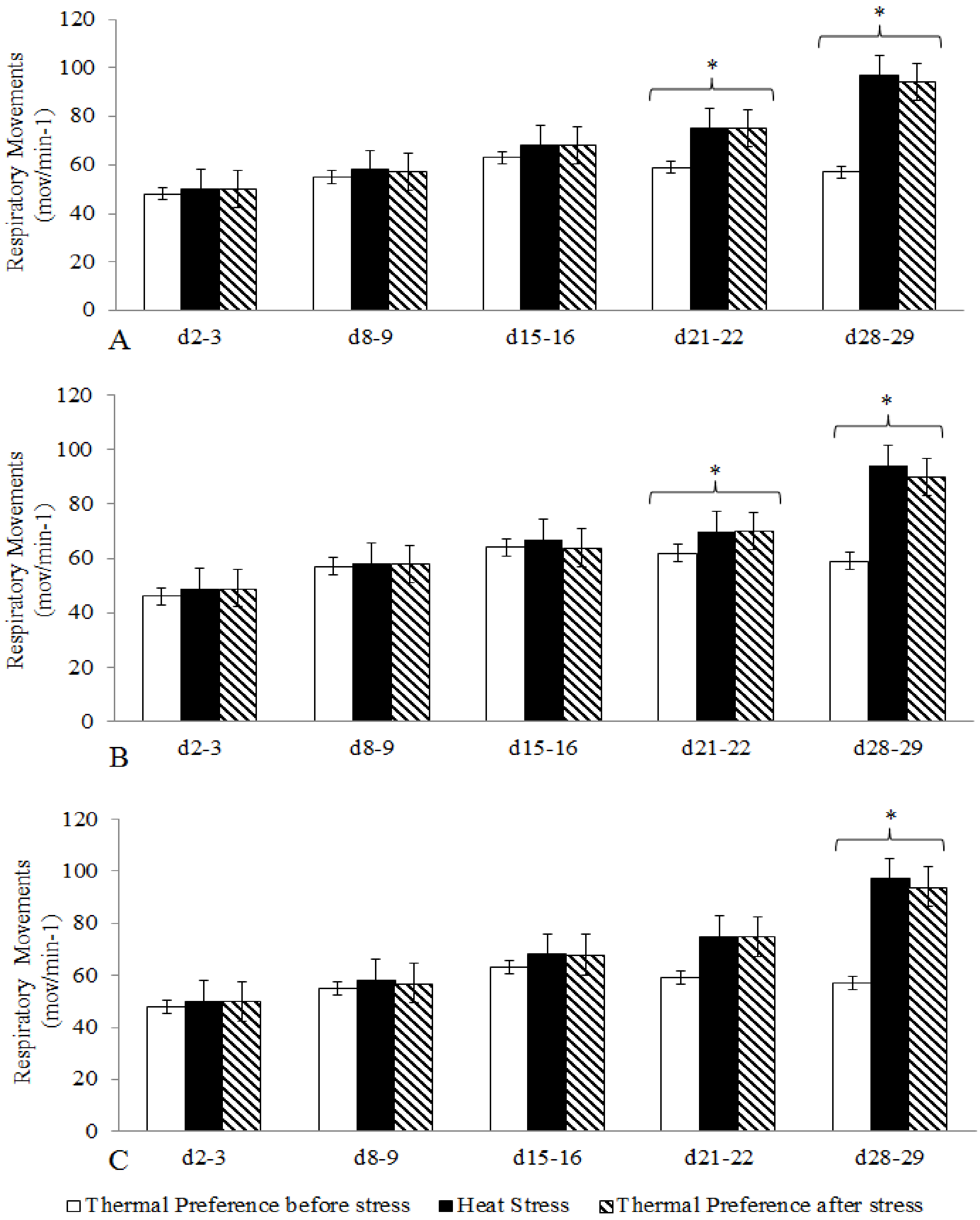

Figure 4. Breathing movements (mov/min) of broiler chickens obtained from eggs submitted to cold (36°C, A),control (37.5°C, B) or heat (39°C, C) incubation treatments during fetal development, obtained under thermal preference (before thermal challenge), at 5°C above thermal preference (thermal challenge), and upon return to thermal preference (after thermal challenge), according to age. *: above the bracket indicates difference among the means in the same age (*P*< 0.05). Data are expressed as mean ± SD, n=12.

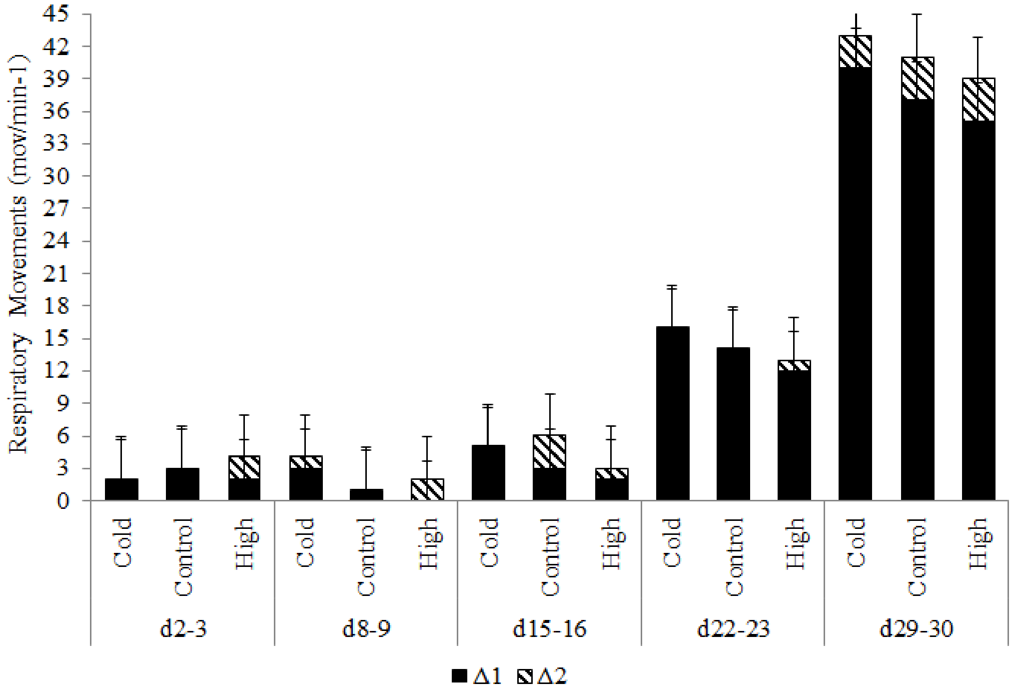

Figure 5. Average variation of respiratory movements of broiler chickens obtained from the eggs submitted to cold (36°C, A), control (37.5°C, B) or heat (39°C, C) incubation treatments during fetal development obtained: Δ1. between at 5°C above thermal preference (thermal challenge) and under thermal preference (before thermal challenge), Δ2. between at 5°C above thermal preference (thermal challenge) and upon return to thermal preference (after thermal challenge), according to age. Data are expressed as mean ± SD, n=12.

4. DISCUSSION

Here we investigated how thermal manipulation affects the preferred temperature, the metabolism, performance and the response to thermal challenge of precocial broiler chickens during rearing period.

Up to 14-15 days of age, in comparison with the recommended ambient temperature in the rearing profile for the studied strain, our data have shown that this temperature coincide with the preferred temperature by chickens from the heat incubation treatment and it is 1°C bellow the preferred temperature by cold- and control treatments. Indeed, the chickens from the heat incubation treatment preferred a significantly higher ambient temperature and had higher rectal temperatures than those from the other groups. These data suggest that egg incubation at high temperature affects the final rectal temperature of the resulting chickens and this combination determines the thermal preference of these birds. Rectal temperature has been shown to reflect heat exchanges between the animal and the environment. Because the difference between the surface and ambient temperatures is the driving force for sensible heat exchange (Yahav et al., 2005), the chickens with high rectal temperatures possibly opted for a high thermal preference to avoid heat loss, which would be greater if they had preferred the same temperature as the control group. Indeed, hatchlings obtained from the heat incubation treatment have thinner and more vascularized skin, which may allow greater heat exchange (Chapter 2). This hypothesis is supported by data showing that the differences obtained between rectal temperature and environment was same among the three treatments for whole period studied. Furthermore, according De Basilio et al. (2003), rectal temperature is considered to be an effective indicator of later heat tolerance, so this difference observed among chickens from the heat incubation treatment and the other groups may indicate a carry-over effect of fetal thermal programming in postnatal life.

Our findings partially agree with those obtained by Nichelmann (2004) and the Tzschentke (2007). Nichelmann (2004) exposed eggs from ducks and turkeys to high temperature (38.5 °C) for the final week of incubation. These birds also presented increased rectal temperature at 1 day of age and preferred higher ambient temperatures until 10 days of age. Tzschentke (2007) found that continuous exposure to high temperature (38.5 ° C) from day 28 of incubation also increased the ducklings preferred temperature until 10 days of age. However, our findings contrast to the results obtained by Nichelmann (2004) when the eggs were incubated at a low temperature (34.5 °C). In this case, the birds preferred lower ambient temperatures. Our data also differs from those presented by Walstra et al. (2010) who exposed eggs of layer hens intermittently to high eggshell temperature (40°C) and found that the birds did not change their rectal temperature and preferred lower ambient temperature. These discrepancies may be due to the different bird species and chicken strains and the thermal manipulation protocol used.

We also investigated whether fetal environmental temperature produced metabolic effects by assessing the levels plasma hormones of the birds. Thyroid hormones and GH are indicators of metabolic status and animal growth rate, respectively (Todini, 2007). Here we show that chickens obtained from the cold incubation treatment chickens presented reduced levels of T3 and GH at 1 day old but, at 42 days, only the T3 levels remained reduced. On the other hand, chickens from heat incubation treatment only showed reduction in the T3 concentrations and only at 21 and 42 days. The low levels of T3 and GH presented by the chickens from cold-treated eggs may be because the exposure of eggs to low temperatures slows fetal development and increases the duration of incubation, as suggested in the Chapter 2. In this study we found that, for the period of 1-21 days of age, chickens from the heat incubation treatment consumed less food than the cold-treatment. The initial growth period of birds is characterized by the maturation of the thermoregulatory system (Whittow and Tazawa, 1991; Nichelmann and Tzschentke, 2002). Therefore, the difference between these groups may be related to these birds feeding requirements to sustain their metabolic heat production and maintain their body temperature. Thus, the low T3 levels presented by the chickens from the heat incubation treatment seem to be a thermoregulatory mechanism used by these birds to reduce the metabolism and consequently heat production. Our results agree with those obtained by Willemsem et at. (2011) who found that, continuous thermal programming using temperature 2 and 3°C higher than the standard incubation temperature of broiler chicken eggs for the period d16-18.5, also found that heat reduces the chickens thyroid hormone plasma levels.

Although reduction of thyroid hormone levels causes reduction of food intake, body weight and temperature (Alva-Sanchez et al. 2012), the lower levels of T3 found here did not affect these variables. In contrast, Willemsem et at. (2011) found that incubation of broiler chickens 3°C above the standard incubation temperature lowered these birds body weights throughout the rearing period.

Here we show that chickens from the cold and the control incubation treatments responded to thermal challenge by first increasing rectal temperature, then reducing it never to pre-challenge temperatures in all analyzed ages. Chickens from both treatments showed increased respiratory rate in response to thermal challenge and did not restore the movements presented pre-challenge. In contrast, chickens from the heat incubation treatment also increased their rectal temperature when challenged, but only at days 2-3, 21-22 and 28-29. They also varied their respiratory frequency in response to thermal challenge but only on days 28-29 and with less intensity than the other treatments. Nevertheless, just like the chickens from the cold and control incubation treatments, these birds also did not restore their respiratory rate 45 min post thermal challenge to the rate pre-challenge. The later response and lower intensity of the heat treatment group indicates that continuous exposure to high temperature during fetal stage promoted long-lasting changes in the thermoregulatory system of chickens, including improving their coping ability to high temperature exposure in the rearing phase. Willemsen et al. (2010) observed similar for the results regarding the behavior of rectal temperature in response to thermal challenge for chickens from eggs exposed continuously 2 °C above the standard incubation temperature. Piestun et al. (2008), on the other hand, did not find thermotolerance when they exposed chicks derived from eggs treated continuously (day 7 to 16 of incubation) with high incubation temperatures (39.5C) to a thermal challenge (35°C, 4h) at d35.

Various studies involving intermittent thermal manipulation of broiler chicken eggs have shown that these birds do not improve their thermotolerance in response to thermal challenge (Yahav et al., 2004a; Collin et al., 2005b; Collin et al., 2007). However, Piestun et al. (2008) did report a successful thermotolerance of broiler chickens when the eggs were exposed to intermittently thermal manipulation (12h / ED, 39.5°C) from day 7 to 16. Indeed, utilizing similar incubation protocol, Loyau et al. (2013) also did not find effect of incubation thermal manipulation on increase of the thermotolerance when the chickens were challenged. These discrepancies probably reflect the specific experimental conditions used to test thermotolerance by these studies.

Altogether, our data have shown that the preferred temperatures by heat treatment, but not by cold and control birds, correspond those recommended for strain under study, indicating that eggs broiler chickens incubated at a temperature equal to or less than usual (≤37.5 ° C) must be reared, up to 21 days of age, in temperature 1°C below to the recommended in the rearing protocol. These findings are extremely important for the broiler poultry production, since they indicate that the protocol may be adapted to allow the chickens to be reared in their temperature preference, promoting the well-being of themselves and may lead to an improvement of production. However, more studies are needed to evaluate whether the lower ambient temperatures by chickens from the control incubation treatment in the initial phase results in improved performance of these birds.

5. REFERENCES

Collin A, Picard M, Yahav Y. The effect of duration of thermal manipulation during broiler chicks embryogenesis on body weight and body temperature of post hatched chicks. Animal Research, v.54, p.105–112, 2005.

Collin A, Berri C, Tesseraud S et al. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poultry Science, v.86, p.795-800, 2007.

Collin A, Bedrani L, Loyau T et al. Embryo acclimation: an innovative technique to limit mortality during thermal stress in chicken. INRA Productions Animales, v.24, n.2, p.191-197, 2011.

De Basilio V, Requena F, León A et al. Early age thermal conditioning immediately reduces body temperature of broiler chicks in a tropical environment. Poultry Science, v.82, p.1235-1241, 2003.

Druyan S, Piestun Y, Yahav S. Heat stress in domestic fowl: genetic and physiological aspects. In: Josipovic S. & Ludwig E. (Ed.) Heat Stress: Causes, treatment and Prevention. NovaScience Publishers Inc. NY USA. In press., 2003.

Halevy O, Krispin A, Leshem Y et al. Early-age heat exposure affects skeletal muscle satellite cell proliferation and differentiation in chicks. American Journal of Physiology, v.281, p.302-309, 2011.

Hammond CL, Simbi BH, Stickland NC. In ovo temperature manipulation influences embryonic motility and growth of limb tissues in the chick (Gallus gallus). The Journal of Experimental Biology, v.210, p.2667-2675, 2007.

Jenkins SA and Porter TE. Ontogeny of the hypothalamo-pituitary-adrenocortical axis in the chicken embryo: a review. Domestic Animal Endocrinology, v.26, n.4, p.267-75, 2004.

Loyau T, Berri C, Bedrani L et al. Embryo thermal manipulations modifies the physiology and body compositions of broiler chickens reared in floor pens without altering breast meat processing quality. Journal of Animal Science, v.91, n.8, p.3674-3685, 2013.

Myhre K, Cabanac M, Myhre G. Thermoregulatory behavior and body temperature in chicks of willow grouse (Lagopus lagopus lagopus). Poultry Science, v.54, p.1174–1179, 1975.

Nichelmann M and Tzschentke B. Ontogeny of thermo-regulation in precocial birds. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, v.131, p.751–763, 2002.

Nichelmann, M. Perinatal epigenetic temperature adaptation in avian species: Comparison of turkey and Muscovy duck. Journal of Thermal Biology, v.29, p.613–619, 2004.

Piestun Y, Shinder D, Ruzal M et al. Thermal manipulations in late term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. Poultry Science, v.87, p.1516-1525, 2008.

Piestun Y, Harel M, Barak M et al.Thermal manipulations in late term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. Journal of Applied Physiology, v.106, p.233- 240, 2009.

Rostagno HS, Albino LFT, Donzele JL et al. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3.ed. Viçosa, MG: UFV, Departamento de Zootecnia, 2011. 252p.

SAS-Institute Inc. User´s guide: statistics, version. 9.1 edition. Cary: SAS Institute, 2009.

Tazawa H, Wakayama H, Turner JS, Paganelli CV. Metabolic compensation for gradual cooling in developing chick embryos. Comparative Biochemical and Physiology, v.89A, p.125–129, 1988.

Tzschentke B. Attainment of thermoregulation as affected by environmental factors. Poultry Science, v.86, p.1025-1036, 2007.

Uni Z, Gal-Garber O, Geyra A et al. Change in growth and function of chick small intestine epithelium due to early thermal conditioning. Poultry Science, v.80, p.438-445, 2001.

Walstra I, Ten Napel J, Kemp B et al.Temperature manipulation during layer chick embryogenesis. Poultry Science, v.89, p.1502-1508, 2010.

Whittow GC and Tazawa H. The early development of thermoregulation in birds. Physiological Zoology, v.64, p.1371-1390, 1991.

Willemsen H, Li Y, Willems E et al. Intermittent thermal manipulations of broiler embryos during late incubation and their immediate effect on the embryonic development and hatching process. Poultry Science, v.90, p.1302–1312, 2011.

Yalçın S, Çabuk M, Bruggeman V et al. Acclimation to heat during incubation: 3. Body weight, cloacal temperatures, and blood acid-base balance in broilers exposed to daily high temperatures. Poultry Science, v.87, p.2671–2677, 2008.

Yahav S, Collin A, Shinder D, Picard M. Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. Poultry Science, v. 83, p.1959-1963, 2004.

Yahav S, Shinder D, Tanny J, Cohen S. Sensible heat loss: the broiler's paradox. World's Poultry Science Journal, v.61, p.419-434, 2005.

CAPÍTULO 4 - PREFERRED REARING TEMPERATURE DOES NOT IMPROVE BROILER BONE CHARACTERISTICS

VS Morita,[*] VR Almeida,[*] JB Matos Junior,[*] TI Vicentini,[*] GL Zanirato[*] and IC Boleli[*1]

[*] *Department of Animal Morphology and Physiology, Sao Paulo State University, Access road Professor Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, Sao Paulo, Brazil*

ABSTRACT - The present study evaluated whether broiler femoral and tibiotarsal development could be improved if birds were reared under their preferred temperature and whether continuous hot incubation temperature during the fetal period improves bone characteristics of broilers reared under heat stress, when assessed at slaughter age. Broilers incubated from day 13 until hatching under cold (36ºC), control (37.5°C) or hot (39ºC) temperature were reared under recommended, preferred or high temperatures. Rearing under the preferred temperature had no effect on broiler body weight or femoral and tibiotarsal characteristics. High rearing temperature, on the other hand, decreased the body weight, mineral contents of both bones, as well as femoral strenght and tibiotarsal rigidity. Regarding incubation temperature effects, egg exposure to hot temperature during the fetal period decreases femoral and tibiotarsal size and weight at slaughter age. In conclusion, rearing under the preferred broiler temperature has no effect on bone characteristics and the negative effects of high rearing temperature on bone development are not minimized by continuous high temperature during the fetal period, which in turn, negatively affects these variables.
Key words: femur, heat stress, incubation temperature, thermal preference, tibiotarsus

1. INTRODUCTION

The greater weight gain and rapid growth of modern strains of broilers cause adverse effects on the development and growth of the skeletal system (Thorp and Waddington, 1997; Bessi., 2006; Shim et al., 2012) in approximately 3% of birds, interfering with their welfare, health and performance (Kestin et al., 1999; Danbury et al., 2000). These bone disorders lead to bird death or disposal, which represents great economic losses (Cook, 2000) for the poultry industry worldwide. Therefore, the establishment of new technical management strategies would greatly maximize production by minimizing losses.

Poultry leg problems could be related, among other factors, to ambient temperature changes (Groves and Muir, 2014). High temperature during the rearing period has been shown to reduce the length, width and weight of the long bones of broiler legs (Yalçin et al., 1996), as well as the mineralization of tibiotarsi in Japanese quail *(Coturnix coturnux japonica)* (Sahin et al., 2006). Such effects have been associated with lower feed intake, which can lead to changes in broiler growth and differentiation of chondrocyte plates, reducing bone length, width and weight (Teeter et al., 1984;. Yalçin et al., 1996; Shao et al., 2006).

Incubation temperature could also interfere with bone growth (Hammond et al., 2007; Oviedo-Rondon et al., 2008; Al-Musawi et al. 2012, Van der Pol et al., 2014), since bone development and growth begins *in ovo*, and this effect, in turn, depends on the intensity, duration and time of temperature change in relation to the usual temperature (Groves and Muir, 2014). This highlights the potential use of incubation temperature manipulation in the modulation of bone characteristics during post-hatch growth. Increased (40 °C) and decreased (35 °C) temperatures from day 10 of incubation onward have been demonstrated to increase and decrease, respectively, the long bone length in broilers (Murray and May, 1972), suggesting that bone development is positively affected by incubation temperature. In turn, high temperature (39°C) throughout the last four days of incubation decreased the weight and length of hatchling femura and tibiotarsi (Oviedo-Rondon et al., 2008). Van der Pol et al. (2014) also verified a reduction of femur length in broiler hatchlings from eggs incubated at high temperature (39.4 °C) during the entire incubation period, and similarly to Oviedo-Rondon et al. (2008), did not find an effect of lower temperature on bone growth.

Previous studies in our laboratory have revealed for the first time in the literature that the incubation temperature during fetal development affects the skin thickness and vascularity, as well as the thermal preference of hatchlings. Incubation temperatures 1.5 °C above the usual (37.5 °C) reduce skin thickness and increase skin vascularity, while increasing the preferred ambient temperature. On the other hand, temperatures 1.5 °C below the usual temperature reduce skin vascularization but do not alter the hatchling´s thermal preference (Chapter 2), suggesting a change in birds´ heat tolerance. In addition, we also found that the effect of incubation temperature on thermal preference occurs for up to 14 days, and that chicks from eggs incubated at high temperature prefer an ambient temperature that coincides with the temperature recommended for the strain (Cobb® Rearing Manual). Also, chicks incubated under the usual temperature or below prefer lower temperatures than those recommended (Chapter 2 and 3). These studies led us to question whether incubation temperatures 1.5°C above or below the usual temperature during the fetal phase and rearing at the preferred temperature would alter broiler bone characteristics. The influence of incubation temperature on thermal preference was also studied by Nichelmann (2004), Tzschentke (2007) and Walstra et al. (2010) for turkeys, ducks and laying, however, these authors did not analyze whether rearing these birds under the preferred temperature influences their bone development.

Therefore, there is a lack of data in the literature regarding the effects of continuous incubation temperature during the fetal phase and rearing under preferred thermal conditions on the bone characteristics of broilers. These data are very important because they can indicate a new thermal management technique for incubation and rearing which would minimize problems associated with changes in bone characteristics. This study examined whether: (i) broilers from eggs incubated at hot or cold temperatures and reared under their preferred temperature present better femoral and tibiotarsal development than broilers incubated at the usual temperature and reared at the recommended temperature and (ii) continuous hot incubation temperature during the fetal stage minimizes or avoids the effects of high rearing temperature on broiler bone characteristics at slaughter age (42 days).

2. MATERIAL AND METHODS

Incubation

All experimental procedures used in this study were approved by the local Ethics Committee for use of animals in research (Fcav/Unesp, Jaboticabal, Brazil, protocol nº 021086/11).

A total of 4500 Cobb500® hatching eggs from broiler breeder flocks at 59 weeks of age were obtained from a commercial hatchery (Globoaves, Itirapina, São Paulo, Brazil), weighed and distributed homogeneously by mean weight (67-72g) in six vertical incubators (Premium Ecológica). The temperature profile for all incubators were adjusted to 37.5°C until day 12 of incubation. From day 13 until hatching, the temperature was reduced to 36ºC, increased to 39ºC or maintained at 37.5ºC, with two repetitions (two incubators) per temperature. The relative humidity in the incubators was maintained at 60% during the entire incubation period until hatching, independent of the incubation temperature. Egg rotation (45° of rotation/ two hours) was maintained until day 18 of incubation. Thermal programming from day 13 of development onward was determined based in the knowledge that functional maturation of the hypothalamo-pituitary-tyroid axis is established around 13-14 days of incubation (Jenkins and Porter, 2004).

Starting at 456 hours of incubation, the incubators were checked every three hours. After drying (3 h after hatch), all the hatchlings were sexed by examining the feathers, following the manual of chicken management (Cobb®, 2008).

Rearing

Male hatchlings were weighed and homogeneously distributed by the average weight (50.98g) into different rearing temperatures [temperature recommended for the strain (Cobb® Rearing Manual), preferred ambient temperature, as measured in the Chapter 3, and high temperature (± 5 °C above the recommended temperature)], with five replicates of 23 birds per treatment. Although the average difference between the recommended and preferred temperatures was found to be around 1°C, in the current study, this variation was approximately 2°C, because of limitations inherent to the thermal chamber.

The chicks were reared from 1 to 42 days of age in three thermal chambers equipped with heaters, monoblocks and exhaust fans, with automatic temperature controls and a daily light regime (light: dark = 22h: 2h, with the dark period occurring between 4 and 6 am). Table 1 presents data on the average temperature of the climatic chamber during the rearing period. Throughout the trial period, the birds received water and feed ad libitum. The diet during the experimental period consisted of two types of feed, both based on corn and soybean meal: early (1-21 days: / kg = 2883, PB = 21:27) and growth (22-42 days: / kg = 3121, PB = 18.86), formulated following the requirements established for broiler chickens under tropical conditions by Rostagno et al. (2011). The chicks received vaccines against Marek and Bouba Avian disease while in the hatchery, against infectious bursal disease (intermediate strain Lukert) and Newcastle (La Sota strain) on the 8th day of age and against Gumboro disease (strong strain Australia v-877) on the 18th day.

Table 1. Average rearing temperatures by incubation temperature and age.

	Rearing Temperatures (°C)								
	Recommended[1]			Preferred [2]			High[3]		
	Incubation Temperatures (°C)								
Week	Cold[4]	Control[5]	Hot[6]	Cold[4]	Control[5]	Hot[6]	Cold[4]	Control[5]	Hot[6]
1[a]	33.07	33.07	33.07	31.20	31.20	33.07	37.96	37.96	37.96
2[a]	29.89	29.89	29.89	28.00	28.00	29.89	34.56	34.56	34.56
3[a]	28.12	28.12	28.12	26.30	26.30	28.12	32.66	32.66	32.66
4[a]	25.13	25.13	25.13	24.88	24.88	25.13	30.74	30.74	30.74
5[a]	22.63	22.63	22.63	23.24	23.24	22.63	27.60	27.60	27.60
6[a]	21.15	21.15	21.15	20.65	20.65	21.15	26.8	26.8	26.8

[1]Recommended: recommended for the strain, according to the Cobb® Rearing Manual.
[2]Preferred: as measured for broiler chickens in the Chapter 3.
[3] High: 5ºC above recommended temperature.
[4, 5, 6]Cold, Control and Hot: 36ºC, 37.5ºC and 39ºC from d 13 of incubation, respectively.

Bone collection

Ten chickens were selected at random from each rearing temperature on day 42 of age. Chicken body weight was recorded and then were killed by cervical dislocation.

Legs were removed and dissected, and the fibula and cartilage were also removed. After this, the bones were kept refrigerated at 20°C for further analyses.

Bone morphological characteristics: femoral and tibiotarsal weight, length, width and density

The bones of the left side of the body were weighed on a digital scale (Marte, 0.001) and their length (cm) was obtained using a digital calliper. Diaphysis width (mm) was measured by wrapping a nylon cord around the diaphysis and measuring that length using a a digital calliper. Bone density was determined by dividing the weight (mg) by the length (mm), as described by Seedor (1993).

Bone biomechanical characteristics: strenght, maximum flexion and rigidity of femura and tibiotarsi

Femura and tibiotarsi were evaluated for the following mechanical properties: strength (N), maximum flexion (mm) and rigidity (N / m). For this, bones from the left side of the body were used to perform analyses of strenght, maximum flexion and rigidity through mechanical tests (three point bending and axial) in universal machine EMIC®, DL 3000 model. The bones were supported by two supports (two points), and the support distance was set according to the size of the smallest bone. The force was applied to the bone at a third point, which was the geometric mean between the two supports (middle third of the bone). The test was destructive and recorded the maximum force allowed. The 2000N load was applied at a rate of 5 mm / min to determine bone strenght, maximum flexion and rigidity. These variables express bone strenght at the head and middle third of the bone. Results were recorded on a computer system belonging to the equipment itself, which provides the values of force x deformation. To calculate the rigidity, we determined the part of the maximum force curve (N) x deformation (mm) on the body of the elastic phase (straight part of the curve). These data were analyzed using a linear equation (1st degree, $y = a.x + b$) that best fit the points. In this equation, the slope of the curve is given by a constant, which is obtained by multiplying the x variable which expresses the bone stiffness (slope of the curve in the elastic phase).

Bone chemical composition of femura and tibiotarsi

Femura and tibiotarsi of the right side of the body were tested for calcium, phosphorus, and ash. The analysis of mineral samples of the different treatments were performed in the animal nutrition laboratory at FCAV / UNESP, Jaboticabal, Sao Paulo, Brazil, according to the methodologies described by Silva and Queiroz (2002), and expressed as a percentage of defatted dry matter.

Statistical Analyses

Data were subjected to analysis of variance using the General Linear Model procedure (GLM) of the SAS ® software (SAS Institute, 2002). For significant effects ($p = 0.05$), we compared means using the Tukey test. Bone length, width and density data were correlated with bone strength utilizing the Pearson's R Correlation Test.

3. RESULTS

Body weight

At 42 days of age, there was significant main effect for rearing temperature ($P<0.0001$) on chicken body weight. Chickens reared at high temperature had lower body weight in comparison with chickens from the other two rearing temperatures (Figure 1A). We did not observe significant effects of incubation temperature ($P=0.2253$) on body weight (Figure 1B).

Bone morphological characteristics: femoral and tibiotarsal weight, length, width and density

Incubation and rearing temperatures had a significant effect ($P<0.05$) on bone morphological characteristics (Table 2). Hot incubation temperature decreased the length and weight of broiler femura and tibiotarsi relative to control, while cold incubation did not have any effect on bone characteristics ($P>0.05$). Furthermore, high rearing temperature decreased the length, width, weight and density of both bones relative to our control temperature (recommended), while the preferred temperature did not have any effects.

Bone biomechanical characteristics: strenght, maximum force deformation and rigidity of femura and tibiotarsi

As shown in Table 3, incubation temperature had no significant effect ($P>0.05$) on femoral and tiobiotarsal biomechanical characteristics. Rearing treatment had a significant effect ($P<0.05$) on femoral strength and tibiotarsal rigidity, which were lower in high-rearing temperature compared to the other two rearing-temperatures (which, in turn, did not differ from each other). There was a significant interaction ($P <0.05$) of incubation and rearing temperature on tibiotarsi strenght: high-rearing temperature reduced tibiotarsal strenght, and this reduction was higher in chickens from hot relative to control-incubation temperatures (Table 3 and Figure 2).

Correlation analyses of the femoral variables showed strong positive correlations between strenght and length (r=0.97), strenght and width (r=0.98) and strenght and density (r=0.97). Regarding the tibiotarsus, we also observed strong positive correlations between strenght and length (r=0.92), strenght and width (r=0.97) and strenght and density (r=0.97).

Bone chemical composition of femura and tibiotarsi

Table 4 lists bone chemical composition for both bones. There was a significant effect ($p<0.05$) of rearing treatment on the ash, calcium and phosphorus contents of both bones, which were lower in chickens reared under high temperatures.

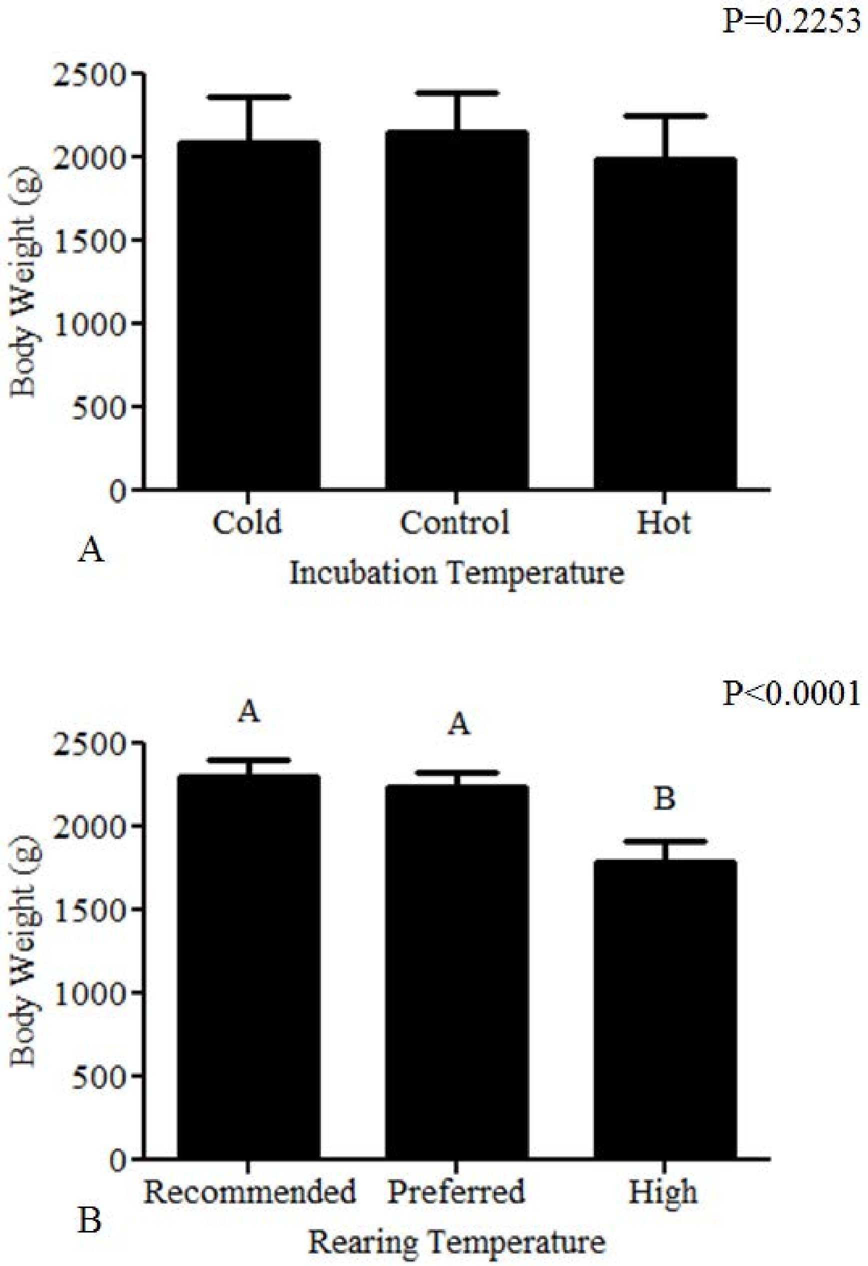

Figure 1. Body weight of broiler chickens from eggs incubated at cold (36°C), control (37.5°C) and hot (39°C) temperatures and reared at recommended (obtained from the Cobb® Rearing Manual), preferred (Chapter 3) or high (5ºC above preferred temperature) temperatures. [A-B:] different letters indicate significant differences among treatments (Tukey, $P<0.05$).

Table 2. Effect of incubation and rearing temperatures on morphological characteristics (length, width, weight and density) of femura and tibiotarsi in broiler chickens at 42 days of age.

	Femura				Tibiotarsi			
	Length (mm)	Width (mm)	Weight (g)	Density (mg/cm)	Length (mm)	Width (mm)	Weight (g)	Density (mg/cm)
Incubation Temperature (IT)								
Cold	68.14 ^{A}	7.29	6.45 ^{A}	94.60	90.41 ^{A}	5.72	8.51 ^{A}	94.03
Control	68.30 ^{A}	7.13	6.38 ^{A}	93.30	91.67 ^{A}	5.55	8.46 ^{A}	92.50
Hot	63.40 ^{B}	6.90	6.00 ^{B}	94.50	84.93 ^{B}	5.41	7.89 ^{B}	93.00
Rearing Temperature (RT)								
Recommended	69.30 ^{A}	7.64 ^{A}	6.90 ^{A}	100.33 ^{A}	92.18 ^{A}	5.89 ^{A}	9.20 ^{A}	99.80 ^{A}
Preferred	69.27 ^{A}	7.62 ^{A}	6.78 ^{A}	100.02 ^{A}	90.49 ^{A}	5.83 ^{A}	8.92 ^{A}	98.60 ^{A}
High	60.42 ^{B}	6.33 ^{B}	5.36 ^{B}	89.00 ^{B}	82.52 ^{B}	5.06 ^{B}	7.15 ^{B}	86.00 ^{B}
P-values								
IT	0.0130	0.1634	0.0141	0.1216	0.0127	0.1280	0.0139	0.1547
RT	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	<0.0001
IT x RT	0.5835	0.1083	0.6375	0.2272	0.5665	0.5824	0.6299	0.1541
CV (%)	4.18	10.08	12.96	9.19	4.96	12.13	14.07	7.95

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A-C:}$ different letters within column indicate significant differences among treatments (Tukey, P<0.05).

Table 3. Effect of incubation and rearing temperatures on biomechanical characteristics of femura and tibiotarsi of broiler chickens at 42 days of age.

	Femura			Tibiotarsi		
	Strenght (N)	MFD (mm)	Rigidity (N/mm)	Strenght (N)	MFD (mm)	Rigidity (N/mm)
Incubation Temperature (IT)						
Cold	170.84	2.61	64.88	201.11	2.53	79.49
Control	173.27	2.63	66.72	206.79	2.53	81.74
Hot	165.92	2.59	64.20	200.67	2.50	74.27
Rearing Temperature (RT)						
Recommended	181.42 A	2.72	67.48	206.82	2.60	83.39 A
Preferred	176.01 A	2.80	65.00	208.71	2.58	84.77 A
High	160.11 B	2.40	66.93	199.06	2.41	70.15 B
P-values						
IT	0.2700	0.0832	0.9008	0.0290	0.1371	0.4753
RT	0.0020	0.1438	0.2333	0.0211	0.0811	0.0225
IT x RT	0.1276	0.1952	0.1845	0.0114	0.3779	0.1599
CV (%)	20.19	20.87	33.01	25.11	13.89	36.91

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. A-C: different letters within column indicate significant differences among treatments (Tukey, P<0.05).

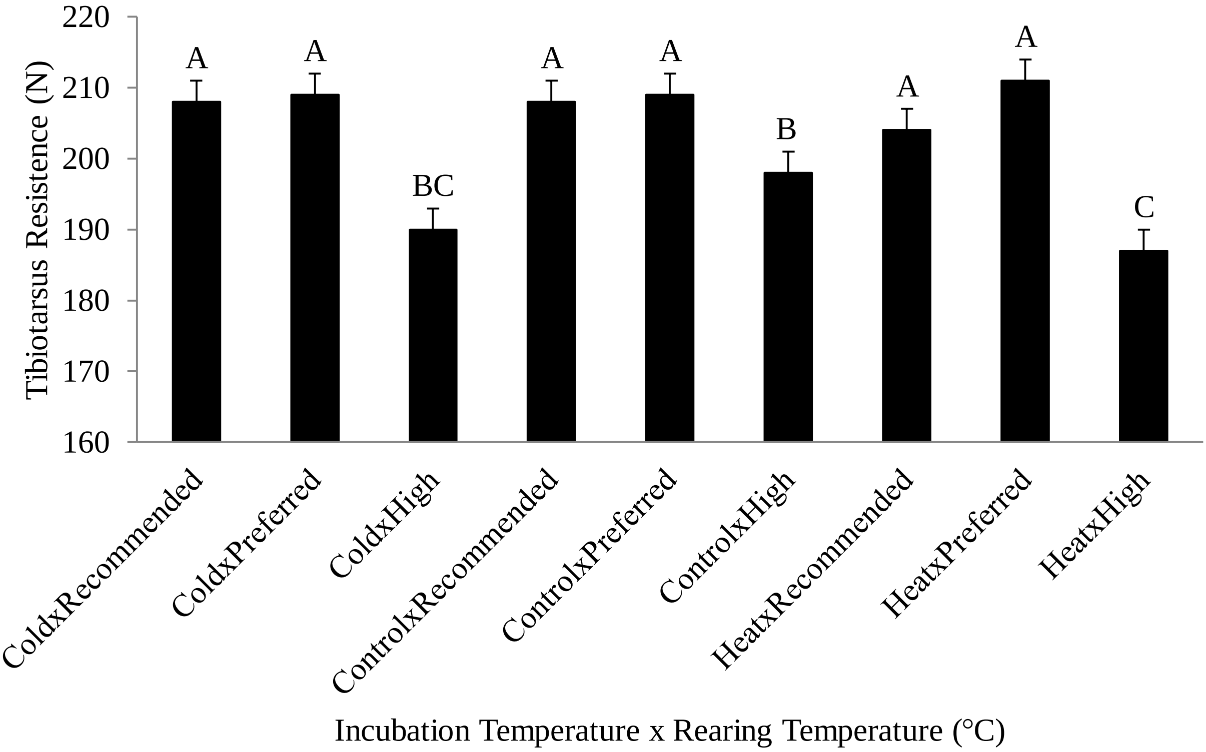

Figure 2. Interaction between incubation and rearing temperatures for tibiotarsus strenght. Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. A-C: different letters indicate significant differences among treatments (Tukey, P<0.05).

Table 4. Effect of incubation and rearing temperatures on ash, water, fat, calcium and phosphorus content (% dry matter) of femura and tibiotarsi of broiler chickens at 42 days of age

	Femura				Tibiotarsi			
	Ash	Ca	P	Ca/P	Ash	Ca	P	Ca/P
Incubation Temperature (IT)								
Cold	41.02	14.96	7.42	2.02	41.91	14.83	7.32	2.03
Control	40.59	14.88	7.34	2.03	42.78	14.76	7.21	2.05
Hot	41.45	15.15	7.66	1.98	42.46	15.02	7.15	2.09
Rearing Temperature (RT)								
Recommended	41.83 ^{A}	15.36 ^{A}	7.61 ^{A}	2.03	43.18 ^{A}	15.15 ^{A}	7.49 ^{A}	2.02
Preferred	41.68 ^{A}	15.50 ^{A}	7.88 ^{A}	1.96	42.82 ^{A}	15.42 ^{A}	7.36 ^{A}	2.07
High	39.54 ^{B}	14.02 ^{B}	6.90 ^{B}	2.03	40.98 ^{B}	14.05 ^{B}	6.68 ^{B}	2.08
P-values								
IT	0.1848	0.3858	0.5040	0.1776	0.8312	0.5777	0.5984	0.1545
RT	0.0018	0.0302	0.0193	0.1255	0.0312	0.014	0.0097	0.3009
IT x RT	0.1060	0.5225	0.6141	0.1299	0.4525	0.1544	0.4511	0.4551
CV (%)	8.19	7.82	7.67	8.01	6.13	6.39	7.14	7.91

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A-C:}$ different letters within column indicate significant differences among treatments (Tukey, P<0.05).

4. DISCUSSION

The present study investigated whether rearing under the preferred temperature improves the femoral and tibiotarsal development of broiler chickens and whether continuous hot incubation temperature during the fetal stage improves bone characteristics at the market age of broilers reared under heat stress. As observed in a previous study from our group (Chapter 3), cold and control incubation temperature broilers prefer rearing temperatures approximately 1 °C below the temperature recommended for the strain (Cobb® rearing manual), while broilers from hot incubation temperature prefer rearing temperatures that coincide with the recommended temperatures.

Contrary to our expectations, our results show that broilers raised under the preferred temperature did not show an improvement in either femoral or tibiotarsal weight, size, ash content, Ca, P and Ca/P, or bone biomechanical properties or total body weight. On the other hand, high ambient temperature rearing reduced total body weight, as well as weight, size and mineral contents of both bones, femoral strenght and tibiotarsal rigidity. Moreover, a main effect of incubation temperature indicated that hot incubation temperature reduced the size, weight and density of broiler femura and tiobiotarsi at 42 days of age. Finally, fetal development under cold conditions did not affect bone characteristics of the broilers at slaughter age.

A possible explanation for the lack of effect observed with the preferred rearing temperature may be that the preferred temperature was different among groups only during the first three weeks of rearing.

Broilers subjected to high rearing temperatures use physiological control mechanisms of body heat production and loss to support homeothermy (Yalçin et al., 1997). The present work is part of a research project studying the effects of incubation temperatures on broiler fetal development. One of the results from this project (Almeida et al., Submitted) revealed that high rearing temperature reduced feed intake, which indicates a reduction in metabolism. This reduction in feed intake may explain the reduced body weight, bone growth and mineralization, as well as the mechanical properties observed in the birds studied in the current work. Bone growth occurs by intramembranous and endochondral processes (Howlet et al., 1980). The fact that the birds reared under high temperature in our study showed a reduction in both the length and diameter of the femoral and tibiotarsal diaphysis shows that high rearing temperature affected both forms of bone growth. The reduction of body weight, femural and tibiotarsal length and tibiotarsal density of broilers reared under high temperature observed at 42 days of age was also observed by Bruno et al. (2000). However, our data showed a smaller reduction in these values, which may be explained by the higher temperature used in that study (3 °C to 9 °C above the control temperature, beginning from the 5th day of age).

Bone biomechanical properties are determined by bone size and chemical composition. Bone rigidity and strenght to compression are determined by the inorganic components, whereas elasticity and strenght to tractile forces are determined by the organic components (Pauwels, 1980). Our data suggest that the reduction of the femoral and tibiotarsal mechanical properties found in broilers raised under high temperature may have been the result of the lower organic and inorganic composition of the bones. Strong positive correlations between bone strenght and bone length and bone strenght and bone under heat conditions was found in the current study and also reported by Yalçin et al. (1997). This indicates that bones with reduced length or width may also present with lower bone strenght. In this study, we also observed a correlation between bone strenght and bone density (estimated using the Seedor index, Seedor et al., 1991), which was also found by Bruno et al. (2000).

With regard to incubation temperature, the use of hot incubation temperature did not minimize or prevent the negative effects on bone development exerted by high rearing temperature. In fact, independently of rearing temperature, hot incubation temperature reduced femoral and tibiotarsal size, weight and density in broiler chickens at 42 days of age, without interfering with bone composition and biomechanical properties. Similar data were found in hatchlings by Oviedo-Rondon et al. (2008), using high temperature from the 17th day of incubation. By contrast, Hammond et al. (2007) showed an increase in bone size using temperature 1 °C above the usual from the 4th to the 7th day of incubation. However, these authors did not evaluate the long-term effects of incubation temperature on bone characteristics. In the study by Almeida et al. (submitted), incubation temperature did not affect feed intake, suggesting that the negative effects of high incubation temperature on the leg bone characteristics mentioned above involved factors other than nutritional deficiency. Furthermore, for the first time in the literature, the current study reveals that fetal development under cold temperature does not affect bone characteristics of the broilers by slaughter age.

In sum, our data showed that: (i) broilers reared under their preferred temperature presented similar weight and bone development than broilers reared under the recommended temperature, and (ii) hot incubation temperature during the fetal phase did not minimize or avoid the adverse effects of high rearing temperature, and led to negative effects on bone characteristics. We conclude that neither the preferred rearing temperature nor hot incubation temperature confer any visible advantages for bone characteristics and total bird weight at slaughter age, in these experimental conditions.

5. REFERENCES

Al-Musawi SL, Stickland NC, Bayol SA. *In ovo* temperature manipulation differentially influences limb musculoskeletal development in two lines of chick embryos selected for divergent growth rates. The Journal of Experimental Biology, v. 215, p.1594–1604, 2012.

Bessi W. Welfare of broilers: A review. World's Poultry Science Journal, v.62, p.455–466, 2006.

Bruno LDG, Furlan RL, Malheiros EB et al. Influence of early quantitative food restriction on long bone growth at different environmental temperatures in broiler chickens. Brazilian Journal of Poultry Science, v.41, p.389-394, 2000.

Cobb-Vantress 2008. Broiler Management Guide. Siloam Springs, Arkansas: Cobb-Vantress.

Cook JKA. Avian pneumovirus infections of turkeys and chickens. Vet. J., 160: 118-125

Danbury TC, Weeks CA et al. Self selection of the analgesic drug carprofen by lame broiler chickens. Veterinary Record 146: 307-311.

Groves P, Muir WA. Meta-analysis of experiments linking incubation conditions with subsequent leg weakness in broiler chickens, PloS One, v.9, n.7, 2014.

Hammond CL, Simbi BH, Stickland NC. *In ovo* temperature manipulation influences embryonic motility and growth of limb tissues in the chick (Gallus gallus). Journal of Experimental Biology, v.210, p.2667-2675, 2007.

Howlett CR. The fine structure of the proximal growth plate and metaphysis of the avian tibia: Endochondral osteogenesis. Journal of Anatomy, v.130, p.745–768, 1980.

Jenkins SA and Porter TE. Ontogeny of the hypothalamo-pituitary-adrenocortical axis in the chicken embryo: a review. Domestic Animal Endocrinology, v.26, n.4, p.267-75, 2004.

Kestin SC, Su G, Sorensen P. Different commercial broiler crosses have different susceptibilities to leg weakness. Poultry Science, v.78, p.1085-1090, 1999.

Nichelmann, M. Perinatal epigenetic temperature adaptation in avian species: Comparison of turkey and Muscovy duck. Journal of Thermal Biology, v.29, p.613–619, 2004.

Oviedo-Rondón EO, Small J, Wineland MJ et al. Effects of incubator temperature and oxygen concentration during the plateau stage of oxygen consumption on turkey embryo long bone development. Poultry Science, v.87, p.1484–1492, 2008

Pauwels F. Biomechanics of the Locomotor Apparatus. New York: Springer-Verlag (1980).

Rostagno HS, Albino LFT et al. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3.ed. Viçosa, MG: UFV, Departamento de Zootecnia, 2011. 252p.

Sahin N, Onderci M, Sahin K et al. Dietary arginine silicate inositol complex improves bone mineralization in quail. Poultry Science, v.85, p.486–492, 2006.

SAS Institute. (2002). SAS User's Guide. Cary, NC: SAS Institute Inc.

Seedor JG. The biophosphanate alendronate (MK-217) inhibit bone loss due to ovariectomy in rats. Journal of Bone and Mineral Research, v.93, n.4, p.265-270, 1993.

Shao ZH, Chang WT, Ballock RT. Thyroid hormone and the growth plate. Reviews in Endocrine and Metabolic Disorders, v.7, p.265-271, 2006.

Shim MY, Karnuah AB, Anthony NB et al. The effects of broiler chicken growth rate on valgus, varus and tibial dyschondroplasia. Poultry Science, v.91, p.62–65, 2012.

Silva DJ and Queiroz AC. Análise de alimentos: métodos químicos e biológicos. 3.ed. Viçosa: UFV, 2002. 165p.

Teeter RG, Smith MO, Murray E. Force feeding methodology and equipament for poulty. Poultry Science, v.63, n.4, p.573-575, 1984.

Thorp BH and Waddington D. Relationships between the bone pathologies, ash, and mineral content of long bones in 35-day-old broiler chickens. Research in Veterinary Science, 62:67–73, 1997.

Tzschentke B. Attainment of thermoregulation as affected by environmental factors. Poultry Science, v.86, p.1025-1036, 2007.

Van der Pol CW, van Roovert-Reijrink IA, Maatjens CM et al. Effect of eggshell temperature throughout incubation on broiler hatchling leg bone development. Poultry Science, v. 93, n.11, p.2878-2883, 2014.

Walstra I, Ten Napel J, Kemp B, van den Brand H. Temperature manipulation during layer chick embryogenesis. Poultry Science, v.89, p.1502-1508, 2010.

Yalçin S, Ozkan S, Settar P et al. Influence of ambient temperature and genotype on bone parameters and incidence of leg disorders of male and female broilers. World's Poultry Congress, 1996, New Delhi, India. Proceedings… New Delhi, India, 1996. v. II, p.577-80.

Yalçin S, Settar P, Ozkan S, Cahaner A. Comparative evaluation of three commercial broiler stocks in hot versus temperate climates. Poultry Science, v.76, p.921-929, 1997.

CAPÍTULO 5 - TEMPERATURE DURING FETAL DEVELOPMENT HAS NO LONG – TERM EFFECT ON SKIN THICKNESS AND VASCULARITY OF BROILERS

VS Morita, VR Almeida, JB Matos Jr, TI Vicentini and I.C. Boleli[1]

[*] *Department of Animal Morphology and Physiology, Sao Paulo State University, Access road Professor Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, Sao Paulo, Brazil*

ABSTRACT – Incubation temperature above or below the usual during fetal development induces morphological and physiological changes, which influence ambient temperature preference of broiler hatchlings. Here we investigated the long-term and adaptive nature of these incubation temperature-induced changes under distinct rearing conditions. Broilers from eggs exposed to cold (36ºC), control (37.5°C) or hot (39ºC) temperatures during fetal phase (from day 13 of incubation to hatching) were reared under recommended, preferred or high temperatures. No differences were found among incubation temperatures for broiler skin and feathering characteristics, body weight, feed intake, body surface and rectal temperatures, and plasma T4 e GH levels at days 21 and 42. However, hot incubation temperature decreased the plasma T3 levels in both ages. Regarding the rearing temperatures, rearing under preferred temperature had no effect on the analyzed variables. High rearing temperature, on the other hand, increased body surface temperature and decreased plasma T3 and GH levels, body weight, total feathering weight, feed intake, and skin thickness and vascularity at day 21 of age. These characteristics, except for skin vascularity, were maintained until 42 days of age. Additionally, in this age, T4 concentration was also reduced. It is concluded that, independent of the rearing temperature, high incubation temperature induced long-term effect only on the plasma T3 levels, and was not effective to avoid or minimize the effects of the hot rearing temperature on the broiler body growth.

Key words: broilers, heat stress, performance, skin, thyroid hormone

1. INTRODUCTION

Heat exposure during growth phase affects the broiler performance and survival, resulting in shortage and increased cost of the broiler meat production. The negative effect of heat on broiler chicken production makes important the development of procedures that improve long-term thermotolerance of these birds.

Incubation temperature manipulation during critical periods of the *in ovo* development has been studied by many authors as to induce morphological, physiological and/or behavioral changes that reduce or avoid the adverse effects of poultry exposure to rearing temperature variations or that until improve their performance (Decuypere, 1984; Tzschentke et al., 2001; Janke et al., 2002; Tzschentke and Just, 2002; Yahav et al., 2004a,b; Piestun et al., 2008; Walsta et al., 2010; Piestun et al., 2013). In previous studies developed in our laboratory, it was verified that hatchlings from incubation under high temperature (39°C) during the fetal phase show thinner and more vascularized tegument. It was also observed that hatchlings from incubation under low temperature (36°C) present increased heat loss, as well as lower metabolic rates, that may, in turn, have elicited physiological adjustments, such as the reduced skin vascularity (Chapter 2). Additionally, in other study, we found that hatchlings from eggs incubated at hot temperature prefer higher rearing temperatures in the two first weeks of age, compared to hatchlings from eggs incubated at cold and control (37.5°C) temperatures (Chapter 3). These findings apparently represents an adaptive fetal response driving to a greater heat loss, that may help the body temperature maintenance at normal or at least not lethal levels, even preferring higher ambient temperature, as a behavioral adjustment that helps hatchlings maintain the homeothermy. The incubation temperature-induced changes reported in our studies cited above suggest the potential use of the temperature manipulation during fetal phase to increase the heat tolerance of broilers. However, an adaptive response implies in the occurrence of long-term changes (Decuypere and Michels, 1992, French, 2000), which have not been evaluated in our studies, but need to be investigated.

The purpose of the present study was to determine (i) whether continuous temperature manipulation during fetal phase induces long-term changes in the tegument, body rectal and surface temperatures, and hormone levels in broiler chickens reared under preferred, recommended and high temperatures, and (ii) whether temperature manipulation influences the thermotolerance of broiler to heat rearing conditions.

2. MATERIAL AND METHODS

Incubation

All experimental procedures used in this study were approved by the local Ethics Committee in Animal Use (CEUA, protocol nº 021086/11). A total of 4,500 fertile eggs from 59 weeks old broiler breeders (Cobb 500®) were obtained from a commercial hatchery (Globoaves, Itirapina, São Paulo, Brazil). The eggs were weighed and distributed homogeneously by mean weight (67-72g) in six vertical incubators (Premium Ecológica). Incubation temperature for all incubators was set at 37.5°C until day 12 of incubation. From day 13 until hatching, incubator temperature was lowered to 36ºC, increased to 39ºC or maintained at 37.5ºC. Per incubation temperature profile, two repetitions (two incubators) were used. Relative humidity in all incubators was maintained at 60% throughout incubation. Egg rotation (45° of rotation each two hours) was maintained until day 18 of incubation. At day 18 of incubation, all eggs were moved to hatching baskets, which were placed in the same incubators. Thermal changes from day 13 of incubation onward was determined based in the knowledge that functional maturation of the hypothalamo-pituitary-tyroid axis is established around 13-14 days of incubation (Jenkins and Porter, 2004). Starting at 456 hours of incubation, the incubators were checked every three hours. After drying (3 h after hatch), all hatchlings were sexed by examining the feathers, following the manual of broiler management (Cobb, 2008®).

Rearing

Male hatchlings were weighed and homogeneously distributed by the average body weight (50.98g ± 2.00) into three thermal chambers, whose temperatures was adjusted to the recommended for the strain (Cobb® Rearing Manual), preferred (measured in the Chapter 3) and high (± 5°C above the recommended temperature), with five replicates of 23 birds per treatment. All thermal chambers were equipped with heaters, monoblocks and exhaust fans, and automatic control of temperature and daily lighting program (2L:22D, with the dark period occurring between 4 and 6h am). Table 1 presents data on the weekly average temperature of the climatic chambers during the rearing period. Although the difference between the recommended and preferred average temperatures determined in our previous study has been around 1°C (Chapter 3), in the current study, the difference between the two temperatures was approximately 2°C, because of limitations inherent to thermal chambers.

Throughout the trial period, the birds received water and feed *ad libitum*. Broiler were fed a starter diet (2,883 kcal of EM/kg, 21.27% CP) from d 1 to 21 of age, and a grower diet (3,121kg of EM/kg, 18.86% CP) from d 22 to 42. Both diet were based on corn and soybean meal, and formulated following the requirements established for broiler chickens under tropical conditions by Rostagno et al. (2011). The chicks received vaccines against Marek and Bouba Avian disease while in the hatchery, against infectious bursal disease (intermediate strain Lukert) and Newcastle (La Sota strain) on the 8th day of age and against Gumboro disease (strong strain Australia v-877) on the 18th day.

Table 1. Average rearing temperatures by incubation temperature and age.

	Rearing Temperatures (°C)								
	Recommended[1]			Preferred [2]			High[3]		
	Incubation Temperatures (°C)								
Week	Cold[4]	Control[5]	Hot[6]	Cold[4]	Control[5]	Hot[6]	Cold[4]	Control[5]	Hot[6]
1[a]	33.07	33.07	33.07	31.20	31.20	33.07	37.96	37.96	37.96
2[a]	29.89	29.89	29.89	28.00	28.00	29.89	34.56	34.56	34.56
3[a]	28.12	28.12	28.12	26.30	26.30	28.12	32.66	32.66	32.66
4[a]	25.13	25.13	25.13	24.88	24.88	25.13	30.74	30.74	30.74
5[a]	22.63	22.63	22.63	23.24	23.24	22.63	27.60	27.60	27.60
6[a]	21.15	21.15	21.15	20.65	20.65	21.15	26.8	26.8	26.8

[1]Recommended: recommended for the strain, according to the Cobb® Rearing Manual.
[2]Preferred: as measured for broiler chickens in the Chapter 3.

[3] High: 5ºC above recommended temperature.

[4, 5, 6]Cold, Control and Hot: 36ºC, 37.5ºC and 39ºC from d 13 of incubation, respectively.

Body surface and rectal temperature

At 21 and 42 days of age, ten broiler chickens (two birds per replication) were selected at random from each rearing temperature to determine regional surface and rectal temperatures. For the first, infrared thermal images were obtained of body of each bird, using a infrared thermographic camera with precision of ± 0.7°C and spectrum range of 7.5 – 13 µm (FLIR E40, Stockholm, Sweden). To obtain and analyze the infrared thermographic images, it was adopted methodology proposed by Nääs et al. (2010). For a better visualization of the images and to avoid visual interference from the surrounding area, a cardboard was placed behind each chick. The thermal camera was placed approximately 1m from the bird and the images were registered using an angle of 90° from the chick surface. These procedures were utilized in an attempt to have a complete image filling within the entire angle of sight and to minimize errors due to angle distortion, respectively. As proposed by Cangar et al. (2008), it was used 0.95 as the emissivity coefficient (ε). From each bird, it was analyzed the surface temperature of the head (cranial), neck, back (thoracic-dorsal), breast (chest-ventral) and thigh. The regional surface temperatures corresponded to the average temperature obtained from measurements realized at several randomly chosen points in each region: head (13 points), neck (10 points), back (10 points), breast (12 points) and thigh (8 points), according adapted methodology of Nääs et al. (2010). Each thermal image was analyzed using software provided by the camera manufacturing company (FLIR Tools, Stockholm, Sweden). The rectal temperature of these birds was measure using a digital thermometer (MedeQCO, Geschwenda, Germany, with a ± 0.1°C accuracy) inserted into the cloaca about three inches.

Plasma hormone

At 21 and 42 days of age, two birds per replication, totalizing ten broiler chickens per treatment, had two blood samples was drawn from brachial vein (approximately 1.5 ml of blood per sample) and stored in eppendorf tubes containing EDTA (10µl anticoagulant/1.5ml blood). Blood samples were maintained at room temperature (~25ºC) during 2 hours for plasma separation. Then, blood samples were centrifuged at 3,000 rpm for 10 min at 4ºC.. Plasma (supernatant) was collected and stored at –70°C until analyses. Plasma samples were analyzed for growth hormone (GH, ng/dl), 3,5,30-tri-iodo-l-thyronine (T_3, ng/ml) and 3,5,30,50-tetraiodo-l-thyronine (T_4, ng/dl). The total concentrations of T3, T4, and GH were measured by radioimmunoassays (RIA), with kits "Coat-a-count" (T3 AccuBind ELISA KIT, Monobind cod 125-300B; T4 AccuBind ELISA KIT, Monobind cod 225-300B; and chicken growth hormone ELISA kit, MyBioSource, cod MBS266317, respectively), using a gamma radiation counter C12 DPC.

Skin and feather analysis

Immediately after blood collection, skin samples were removed from the neck (10x15 mm), back, and breast (between the insertion point of insertion of the wings) (20x25 mm), and external region of the thigh (10x15 mm), using scissors and forceps. All skin samples were immediately fixed in Bouin`s solution for 24h at room temperature, washed in distillated water, and processed according routine method for light microscopy. Briefly, skin samples were dehydrated in a series of increasing ethanol concentrations (70%, 80%, 90%, 95% and 100% 2x), diaphanized in ethanol-xylene solution (1:1) and xylene (100%, 3x), and embedded in a mixture of xylene-paraffin (1:1) followed by paraffin, approximately 45 min in each solution. Semi-serial 6-µm-thick cross-sections were prepared and stained with Masson`s Trichrome for morphological analysis (5 cross-sections/sample). The data of the thickness of epidermis, dermis and total skin as well as the number of blood vessels/area (area: 50.700µm^2) present in the dermis were obtained from histological sections, using a capture and image analysis system (Leica , Qwin , Wetzlar, Germany). Thirty measurements of each variable were considered per body region per bird.

Total feather weight of each bird was determined after manually removal of all feathers, and corresponded to difference between the body weight with and without feathers. Additionally, feather samples were excised from dorsal region of the neck, and back and breast (between the point insertion of the wings) and utilized for obtaining barbule length, and barb number, length, and width. All measurements were obtained using an image capture and analysis system (Leica- QWin, Wetzlar, Germany). A total of 15 feathers per body region per bird, and 10 barbs and 10 barbules per feather were measured.

Performance

As performance parameters were evaluated live body weight (g) and feed intake (g). Body weight was registered at day 21 and 42 of age. Feed intake (g) was determined for initial (day 1-21) and growth (day 22-42) period.

Statistical Analysis

All data were subjected to analysis of variance using the General Linear Model procedure (GLM) of SAS ® (SAS Institute, 2002). For all variables, we analyzed the effects of the incubation temperatures (I), rearing temperatures (R) and interactions among them (IxR), using the following model: $Y_{ijk} = \mu + I_i + R_j + (IxR) + e_{ijk}$, where Y is the dependent variable, μ is the overall mean, and e_{ijk} is the residual error term. In case of significant effect (5%), the comparison among means was performed by Tukey test.

3. RESULTS

Body surface and rectal temperatures

As shown in the Tables 2 and 3, surface temperatures at different body regions and rectal temperature at 21 and 42 days of age were not influenced by incubation temperature, but the first had significant effects of rearing temperature ($P<0.05$) in both ages, where surface temperature of head, neck, back and thigh were higher in broilers reared at high temperature than broilers reared under recommended and preferred temperatures, which were similar ($P>0.05$).

Plasma hormones

Table 4 shows the influences of incubation and rearing temperatures and interaction between them on plasma thyroid hormones and growth hormone (GH). Incubation and rearing temperatures had a significant effect ($P<0.05$) on plasma hormone concentrations at 21 and 42 days of age. At 21 days, plasma T3 concentration was lower in broilers from eggs incubated in comparison with broiler from control and cold incubation temperatures, and there was no difference between these two groups ($P>0.05$). Furthermore, plasma T3 and GH concentration was lower in broiler reared under high temperature than in broiler reared under recommended and preferred temperature, which were similar. These alterations were maintained until 42 days of age, when T4 concentration became also lower in broiler reared under high temperature.

Skin characteristics

Tables 6 and 7 present the results of skin thickness and vascularity obtained for 21 days old broilers, whereas Tables 8 and 9 had the results for 42 days old broilers. Skin characteristics were not affected by incubation temperatures, but was influenced by rearing temperature at 21 and 42 days of age. At 21 days of age, skin and dermis thickness of the neck, back and breast, as well as the dermis blood vessel number and perimeter of the breast were lower ($P<0.05$) in broiler from high rearing temperature in comparison with broiler from recommended and preferred rearing temperatures, which were similar (Tables 6 and 7). At 42 days, in turn, only the skin and dermis thickness of the neck and thigh was lower ($P<0.05$) in broilers reared under high than in the broilers reared under the other temperature conditions, which presented no difference temperature ($P>0.05$). No significant ($P>0.05$) effect of rearing temperature on the skin vascularity of the distinct regions of the body was observed (Tables 8 and 9).

Body weight, feathering characteristics and feed intake

In the Table 5 the results of body weight, feed intake, and feathering characteristics at 21 and 42 days of age are presented. Body and total feathering weight had no significant effect ($P>0.05$) of incubation temperature at 21 and 42 days of age, but they were altered by rearing temperature in the both analyzed ages, and were lower ($P \leq 0.05$) in broilers reared under high than under recommended and preferred temperatures, between which no difference was observed ($P > 0.05$). Regarding the feed intake, there was no significant ($P > 0.05$) effect of incubation temperature and interaction between incubation and rearing temperature on the analyzed periods, but occurred significant influence of rearing temperature on the two periods (1-21 and 22-42 days of age), where broilers reared under high temperature had lower ($P \leq 0.05$) feed intake than broilers reared under preferred and recommended temperatures, which were similar ($P > 0.05$) .

Table 2. Effect of incubation and rearing temperatures on surface temperature of different body regions and on rectal temperature in 21 days old broiler chickens.

	Body Regions					
	Head	Neck	Breast	Back	Thigh	Rectal
Incubation Temperature (IT)						
Cold	32.88	32.52	31.73	31.91	31.44	41.10
Control	32.71	32.41	31.78	31.70	31.55	41.03
Hot	33.24	32.49	31.62	31.76	31.58	41.25
Rearing Temperature (RT)						
Recommended	32.57^{B}	32.15^{B}	31.26^{B}	31.42^{B}	31.10^{B}	41.21
Preferred	32.41^{B}	32.09^{B}	31.37^{B}	31.56^{B}	31.25^{B}	41.05
High	33.75^{A}	33.25^{A}	32.50^{A}	32.41^{A}	32.22^{A}	41.13
P-values						
IT	0.5930	0.3721	0.1800	0.2810	0.9591	0.6401
RT	0.0271	<0.0001	0.0209	<0.0001	<0.0001	0.5352
IT x RT	0.3902	0.5932	0.1546	0.7213	0.2251	0.1952
CV (%)	12.01	10.33	9.18	33.29	21.66	5.15

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A-C:}$ different letters within column indicate significant differences among treatments (Tukey, $P < 0.05$).

Table 3. Effect of incubation and rearing temperatures on surface temperature of different body regions and on rectal temperature in 42 days old broiler chickens.

	Body Regions					
	Head	Neck	Breast	Back	Thigh	Rectal
Incubation Temperature (IT)						
Cold	32.61	32.42	31.46	31.86	31.90	40.60
Control	32.48	32.15	31.83	32.06	31.96	40.93
Hot	32.74	32.31	31.65	31.97	32.07	41.05
Rearing Temperature (RT)						
Recommended	31.87 [B]	31.65 [B]	30.86 [B]	31.02 [B]	31.13 [B]	40.70
Preferred	32.04 [B]	31.59 [B]	30.95 [B]	31.39 [B]	30.97 [B]	40.95
High	33.85 [A]	33.54 [A]	32.90 [A]	33.49 [A]	33.05 [A]	40.90
P-values						
IT	0.1661	0.2535	0.1581	0.1841	0.1012	0.1543
RT	0.0300	0.0235	0.0386	0.0001	0.0139	0.3567
IT x RT	0.1334	0.3923	0.1223	0.1931	0.1573	0.6116
CV (%)	24.04	20.53	19.78	21.52	19.66	6.76

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. [A-C:] different letters within column indicate significant differences among treatments (Tukey, $P<0.05$).

Table 4. Effect of incubation and rearing temperatures on plasma concentrations of triiodothyronine (T_3, ng/ml), thyroxine (T_4, ng/dl), T3/T4 ratio and growth hormone (GH) in 21 and 42 days old broiler chickens.

	D21			D42		
	T3 (ng/ml)	T4 (ng/ml)	GH (ng/ml)	T3 (ng/ml)	T4 (ng/ml)	GH (ng/ml)
Incubation Temperature (IT)						
Cold	1.52^{A}	7.02	3.36	1.13^{B}	9.41	1.41
Control	1.47^{A}	6.69	3.32	1.30^{A}	9.32	1.29
Hot	1.26^{B}	7.09	3.29	0.90^{B}	8.84	1.24
Rearing Temperature (RT)						
Recommended	1.64^{A}	6.95	4.01^{A}	1.31^{A}	9.22^{A}	1.62^{A}
Preferred	1.77^{A}	6.88	3.43^{B}	1.25^{A}	9.28^{A}	1.29^{B}
High	1.00^{B}	7.01	2.57^{C}	0.70^{B}	8.68^{B}	0.92^{C}
P-values						
IT	0.0098	0.1682	0.1529	<0.0001	0.1054	0.3021
RT	0.0010	0.4549	0.0077	0.0021	0.0252	0.0144
IT x RT	0.1128	0.2720	0.1965	0.1039	0.2001	0.1588
CV (%)	22.79	8.48	34.24	24.46	10.01	30.44

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A-C:}$ different letters within column indicate significant differences among treatments (Tukey, P<0.05).

Table 5. Effect of incubation and rearing temperatures on body and total feathering weight in 21 and 42 days old broiler chickens

	D21				D42			
	Body (g)	Feathering (g)	Feathering (%)	Feed intake (g)	Body (g)	Feathering (g)	Feathering (%)	Feed intake (g)
Incubation Temperature (IT)								
Cold	754.34	30.42	4.02	941.27	2147.25	104.73	4.85	2492.68
Control	786.47	30.21	3.85	922.23	2265.88	107.15	4.73	2471.44
Hot	707.08	26.74	3.75	870.78	1952.62	88.04	4.46	2550.20
Rearing Temperature (RT)								
Recommended	796.24 ^{A}	32.57 ^{A}	4.09	946.17	2298.83 ^{A}	114.38 ^{A}	4.98	2736.09
Preferred	765.34 ^{A}	28.92 ^{B}	3.75	954.58	2112.07 ^{B}	97.47 ^{B}	4.60	2781.98
High	636.23 ^{B}	23.72 ^{C}	3.71	823.53	1805.34 ^{C}	78.52 ^{C}	4.32	1996.32
P-values								
IT	0.2253	0.1406	0.2780	0.1508	0.1545	0.1075	0.0989	0.1041
RT	<0.0001	0.0002	0.0986	<0.0001	<0.0001	0.0252	0.1235	<0.0001
IT x RT	0.4349	0.6596	0.3826	0.3845	0.0728	0.7583	0.2489	0.1161
CV (%)	6.40	12.91	15.21	4.1	6.84	12.10	14.00	10.2

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A-C:}$ different letters within column indicate significant differences among treatments (Tukey, $P<0.05$).

Table 6. Effect of incubation and rearing temperatures on skin, epidermis, and dermis thickness (µm) in 21 days old broiler chickens.

	Neck			Back			Breast			Thigh		
	Epidermis (µm)	Dermis (µm)	Skin (µm)	Epidermis (µm)	Dermis (µm)	Skin (µm)	Epidermis (µm)	Dermis (µm)	Skin (µm)	Epidermis (µm)	Dermis (µm)	Skin (µm)
Incubation Temperature (IT)												
Cold	31.38	522.18	554.87	18.83	496.73	608.13	26.78	606.16	655.20	21.83	469.44	490.85
Control	30.13	553.75	576.95	18.84	486.81	598.65	24.03	643.24	646.18	20.54	479.19	501.13
Hot	30.99	534.81	558.43	19.02	463.54	587.61	24.76	630.02	655.06	20.07	471.26	461.93
Rearing Temperature (RT)												
Recommended	29.60 ^{B}	576.31 ^{A}	597.16 ^{A}	21.38 ^{A}	520.11 ^{A}	624.99 ^{A}	28.04 ^{A}	631.91 ^{A}	661.83 ^{A}	20.52	492.28	500.56
Preferred	30.08 ^{B}	549.30 ^{A}	565.43 ^{A}	20.92 ^{A}	509.20 ^{A}	625.64 ^{A}	26.65 ^{A}	649.71 ^{A}	683.73 ^{A}	20.16	499.54	522.83
High	32.51 ^{A}	478.61 ^{B}	509.97 ^{B}	15.40 ^{B}	432.99 ^{B}	556.43 ^{B}	21.52 ^{B}	591.91 ^{B}	613.43 ^{B}	21.79	437.49	458.44
P-values												
IT	0.2818	0.2620	0.8139	0.6023	0.5978	0.6809	0.8951	0.8955	0.6558	0.0717	0.1017	0.9040
RT	<0.0001	<0.0001	0.0161	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0383	0.1673	0.6170	0.7401
IT x RT	0.6749	0.6542	0.6870	0.5372	0.4986	0.7685	0.6299	0.7126	0.1253	0.1200	0.2110	0.7662
CV (%)	3.04	2.78	12.75	5.26	15.14	12.27	6.62	6.41	14.77	22.03	22.52	32.05

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A\text{-}C:}$ different letters within column indicate significant differences among treatments (Tukey, $P<0.05$).

Table 7. Effect of incubation and rearing temperatures on skin blood vessel number (counts per field) and perimeter (µm) in 21 days old broiler chickens.

	Neck		Back		Breast		Thigh	
	Number	Perimeter	Number	Perimeter	Number	Perimeter	Number	Perimeter
Incubation Temperature (IT)								
Cold	3.20	47.61	2.87	51.12	8.33	53.12	3.13	51.51
Control	3.20	50.78	3.00	55.89	8.27	56.52	3.00	49.78
Hot	3.00	53.01	2.80	57.06	8.40	57.10	3.20	52.05
Rearing Temperature (RT)								
Recommended	3.13	50.95	2.93	52.93	8.47^{A}	58.80^{A}	3.27	50.08
Preferred	3.20	46.78	2.90	58.23	8.90^{A}	58.16^{A}	3.00	49.57
High	3.13	48.51	2.87	60.30	7.80^{B}	55.29^{B}	2.87	51.67
P-values								
IT	0.4503	16.60	0.4607	0.2980	0.2662	0.4983	0.9004	0.3484
RT	0.7631	0.7130	0.1028	0.0974	0.0348	0.0469	1.000	0.3872
IT x RT	0.0986	0.5400	0.2597	0.1897	0.9388	0.1256	0.5312	0.9082
CV (%)	16.13	9.97	6.19	16.14	17.35	7.07	23.97	18.00

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A\text{-}C:}$ different letters within column indicate significant differences among treatments (Tukey, $P<0.05$).

Table 8. Effect of incubation and rearing temperatures on skin, epidermis, and dermis thickness (µm) in 42 days old broiler chickens.

	Neck			Back			Breast			Thigh		
	Epidermis (µm)	Dermis (µm)	Skin (µm)	Epidermis (µm)	Dermis (µm)	Skin (µm)	Epidermis (µm)	Dermis (µm)	Skin (µm)	Epidermis (µm)	Dermis (µm)	Skin (µm)
Incubation temperature (IT)												
Cold	40.05	672.56	713.92	24.21	738.60	763.81	32.67	795.64	835.93	27.00	587.46	628.67
Control	41.24	702.58	742.98	23.84	718.68	742.53	30.73	806.28	865.04	25.90	616.41	642.09
Hot	41.12	733.36	645.81	24.65	711.73	736.38	31.18	819.05	850.28	26.06	599.50	625.53
Rearing temperature (RT)												
Recommended	39.66^{B}	736.21^{A}	775.16^{A}	27.30	769.54^{A}	798.85^{A}	36.52^{A}	835.47^{A}	872.02^{A}	25.53^{C}	629.71^{A}	639.20^{A}
Preferred	41.99^{A}	721.87^{A}	763.59^{A}	25.47	738.03^{A}	763.50^{A}	33.01^{A}	830.77^{A}	865.31^{A}	26.08^{B}	635.45^{A}	661.38^{A}
High	41.00^{B}	605.49^{B}	449.15^{B}	20.20	670.21^{B}	694.24^{B}	26.92^{B}	766.31^{B}	793.26^{B}	27.36^{A}	551.19^{B}	584.91^{B}
P-values												
IT	0.3064	0.3245	0.0721	0.3733	0.7078	0.4196	0.1602	0.5493	0.1317	0.6784	0.5838	0.9438
RT	<0.0001	<0.0001	0.0182	<0.0001	0.0097	<0.0001	<0.0001	0.0391	<0.0001	0.0023	0.0016	0.0215
IT x RT	0.6981	0.7024	0.0186	0.3300	0.5017	0.3933	0.1429	0.5682	0.1877	0.8424	0.6457	0.9733
CV (%)	2.95	2.65	7.84	3.66	30.36	3.80	4.51	20.43	4.77	4.85	4.51	42.51

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A-C:}$ different letters within column indicate significant differences among treatments (Tukey, $P<0.05$).

Table 9. Effect of incubation and rearing temperatures on skin blood vessel number (counts per field) and perimeter (µm) in 42 days old broiler chickens.

	Neck		Back		Breast		Thigh	
	Number	Perimeter	Number	Perimeter	Number	Perimeter	Number	Perimeter
Incubation Temperature (IT)								
Cold	4.20	60.39	3.67	64.39	3.60	60.40	9.33	66.71
Control	3.87	62.14	4.00	58.86	3.40	61.27	9.67	62.89
Hot	4.10	63.33	3.90	57.40	3.50	68.72	10.00	69.67
Rearing Temperature (RT)								
Recommended	4.13	59.88	3.93	59.15	3.80	61.45	9.80	66.05
Preferred	4.30	59.39	3.60	60.01	3.40	63.32	10.50	65.49
High	3.80	63.91	3.80	61.50	3.27	62.96	8.87	63.99
P-values								
IT	0.6268	0.2258	0.4041	0.1061	0.7808	0.4776	0.4513	0.4687
RT	0.5806	0.3108	0.5064	0.4889	0.5340	0.5481	0.7656	0.3387
IT x RT	0.7823	0.9439	0.7114	0.6729	0.6462	0.0808	0.5028	0.4545
CV (%)	17.96	9.17	13.48	2.46	21.12	16.50	7.08	10.59

Cold, Control and Hot temperatures during fetal development: incubation at 36°C, 37.5°C and 39°C. Recommended: recommended for the strain, according to the Cobb® Rearing Manual. Preferred: as measured for broiler chickens in the Chapter 3; High: 5ºC above recommended temperature. $^{A\text{-}C:}$ different letters within column indicate significant differences among treatments (Tukey, P<0.05).

4. DISCUSSION

The current study investigated the long-term nature of the changes in the tegument, body surface temperature and hormone concentrations previously reported in hatchlings as a result of cold and hot incubation temperature during fetal phase (Chapter 2) for broiler reared under preferred, recommended and high temperatures, and whether these temperature manipulations influence the broiler tolerance to heat exposure during rearing.

Independent of rearing temperature, no differences were observed in the skin thickness and vascularity, surface temperature of the different body regions, rectal temperatures, body weight, feed intake, feathering weight and characteristics, and plasma T_4 and GH concentrations of the 21 and 42 days old broilers among incubation temperatures. However, plasma T_3 concentration was reduced by hot incubation temperature in both ages and by cold incubation temperature at 42 days of age compared to control incubation treatment. These results show that the changes in the tegument, and body surface and rectal temperatures previously observed in hatchlings as result of cold and hot incubation temperature (Chapter 2) have no long-term nature, unlike the reduction of plasma T_3 concentration, which was maintained up to 42 days of age. Therefore, we cannot fail to raise here the adaptative role of the changes in tegument thickness and vascularity and body surface and rectal temperatures induced by incubation temperature manipulation (Chapter 2) during the two first weeks of age, a times that incubation temperature manipulation interviewed in the broiler thermal preference during this period (Chapter 3). The adaptive role of these changes is also based on the fact that changes in the peripheral blood flow is one of sensible heat loss mechanisms used by birds for heat dissipation and body temperature maintenance under ambient temperature deviations (Wolfenson, 1983; Furlan and Macari, 2002), and the thermoregulatory system of the broiler chickens is functionally mature from the third week of rearing (Whittow and Tazawa, 1991; Nichelmann and Tzschentke, 2002). Although thyroid hormones influence feeding and metabolism during development post-hatch (MaNabb et al., 1998; Darras et al., 2006), reduction in the plasma T_3 concentration of the broilers by hot incubation temperature was not accompanied by reduction in feed intake and body weight (our data).

Concerning to rearing conditions, the present study shows that broilers chickens reared under high temperature presented increased surface temperature of the distinct body regions without rectal temperature alterations, and reduced plasma T_3 e GH concentration, body and feathering weight, and skin thickness and vascularity at 21 days of age, compared to broilers reared under recommended and preferred temperatures. It is shown that these changes continued to occur in 42 days-old broilers, except the change in the vascularity of the skin, and that plasma T4 concentration was also reduced. Preferred rearing temperature reduced plasma GH concentration in both analyzed ages and the body and feathering weight at 42 days of age. The absence of difference in the rectal temperature among the rearing temperatures in the present study shows that broilers were able to maintain their body temperature under heat conditions. Body temperature maintenance with or without ambient temperature changes depends on the balance between heat production and dissipation (Yalçin et al., 1997). For this, when exposed to heat stress, birds use mechanisms to reduce production (decreased food consumption, activity, etc.) and increase endogenous heat loss (increased respiratory movements, peripheral blood circulation and water consumption, ptiloerection, among others) (Bonnet et al. 1997; Lana et al., 2000; Souza, 2008).. Our findings shows that broilers raised under high temperature reduced the production and increased the loss of endogenous heat, decreasing feed intake and metabolism and increasing heat conduction, respectively, the two last demonstrated by the lower plasma concentration of thyroid hormones (T3 and T4) and by the increase of 3 to 4% (~1-1.2ºC) in surface temperature of the body regions, respectively, compared to broilers raised under recommended and preferred temperatures. Thyroid and growth hormones have important role in animal development (LUGER et al., 2001), influencing skin characteristics (Zouboulis, 2004) and food intake (Ryg and Jacobsen, 1982). Thus, the lowest rate of thyroid hormones and growth hormone in the blood seems to explain the skin thinner and less vascularized and the lower body weight and feathering registered for chickens raised under hot conditions. Our data are in agree with those results observed by Geyten et al. (1989), which also observed reduced feed intake with decreased plasma T3 and GH levels.

Researches have demonstrated that incubation temperature manipulation can induce long-term physiological changes (Minne and Decuypere, 1984) and improve thermotolerance of broilers to post-hatching heat exposure (Piestun et al., 2008a,b; Piestun et al., 2013) without to promote negative effects on performance (Piestun et al., 2008b, 2011). However, in contrast, long-term effects of incubation temperature manipulation on post-hatching phenotype that improve heat tolerance are not always observed. Collin et al. (2007), for example, reported that heat acclimation during incubation failed to improve long-term thermotolerance of broilers. In the current study, we reported only isolated effects of the incubation and rearing temperatures on the analyzed variables, whose occurrence indicates that continuously low or high incubation temperature during fetal phase had no effective role in increase or decrease, respectively, the broiler tolerance to heat exposure during rearing. In summary, the findings of this study show long-term effect of high incubation temperature only on the plasma T3 concentration, independent of the rearing temperature, but no effect of the temperature manipulation that lead to improved growth, development or heat tolerance of broilers was observed.

5. REFERENCES

Bonnet S, Geraert PA, Lessire M et al.Effect of high ambient temperature on feed digestibility in broilers. Poultry Science, v.76, n.6, p.857-863, 1997.

Decuypere E and Michels H. Incubation temperature as a management tool: a review. World's Poultry Science Journal 48: 27-38, 1992.

DuRant SE, Carter AW, Denver RJ et l. Are thyroid hormones mediators of incubation temperature-induced phenotypes in birds? Biology Letters, v.10, n.1, 20130950, 2014.

French NA. Effect of short periods of high incubation temperature on hatchability and incidence of embryo pathology of turkey eggs. Brazilian Journal of Poultry Science, v.41, p.377-382, 2000.

Furlan RL and Macari M. Fisiologia Aviária: Aplicada a frangos de corte. In: Macari, M.; Furlan, R.L.; Gonzales, E. Termorregulação**.** 2 ed. Jaboticabal: FUNEP, 2002. p. 209-230.

Havenstein GB, Ferket PR, Qureshi MA. Growth, Livability and Feed Conversion of1957 vs 2001 Broilers When Fed representative 1957 and 2001 Broiler diets. Poultry Science, v.82, p.1500-1508, 2003a.

Havenstein GB, Ferket PR, Qureshi MA. Carcass composition and yield of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science, v.82, p.1509-1518, 2003b.

Janke O, Tzschentke B, Hochel J, Nichelmann M. Metabolic responses of chicken and Muscovy duck embryos to high incubation temperatures. Comparative Biochemical Physiology Part A Molecular & Integrative Physiology, v.131, p.741–750, 2002.

Luger D, Shinder D, Rzepakovsky V et al. Association between weight gain, blood parameters, and thyroid hormones and the development of ascites syndrome in broiler chickens. Poultry Science, v.80, p.965-971, 2001.

Nääs IA, Romanini CEB, Neves DP et al. Broiler surface temperature distribution of 42 day old chickens. Scientia Agricola, v.67, n.5, p.497-502, 2010.

Nichelmann M. and Tzschentke B. Ontogeny of thermo-regulation in precocial birds. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, v.131, p.751–763, 2002.

Piestun Y, Shinder D, Ruzal M et al. Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermotolerance. Poultry Science, v.87, p.1516-1524, 2008a.

Piestun Y, Shinder D, Ruzal M et al. The effect of thermal manipulations during the development of the thyroid and adrenal axes on in-hatch and post-hatch thermoregulation. Journal of Thermal Biology, v.33, p.413-418, 2008b.

Rostagno HS, Albino LFT, Donzele JL et al. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais**.** 3.ed. –Viçosa, MG: UFV, DZO, 2011, 252p.

Ryg M and Jacobsen E. Seasonal changes in growth rate, feed intake, growth hormone, and thyroid hormones in young male reindeer. Canadian Journal Zoology, v.60, p.2941-2946, 1982.

Sas Institute. SAS user's guide: statistics. Cary, 2002. 466p.

Tzschentke B and Nichelmann M. Influence of prenatal and postnatal acclimation on nervous and peripheral thermoregulation. Annals New York Academy Sciences, v.813, n.15, p.87-94, 1987.

Van der Geyten S, Buys N, Sanders JP et al. Acute pretranslational regulation of type III iodothyronine deiodinase by growth hormone and dexamethasone in chicken embryos. Molecular and Cellular Endocrinology, v.147, p.49–56, 1999.

Walstra I, Napel J, Kemp B, van den Brand H. Temperature manipulation during layer chick embryogenesis. Poultry Science, v.89, p.502-1508, 2010.

Whittow GC and Tazawa H. The early development of thermoregulation in birds. Physiological and Biochemical Zoology, v.64, p.1371-1390, 1991.

Wolfenson D. Blood flow through arteriovenous anastomoses and its thermal function in the laying hen. Journal of Physiology, v.334, p.395–407, 1983.

Yahav S, Rath RS, Shinder D. The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. Journal of Thermal Biology, v.29, p.245-250, 2004.

CAPÍTULO 6 - EFFECTS OF EGG SIZE AND THERMAL PROGRAMMING ON HEAT PRODUCTION, INCUBATION PARAMETERS AND HATCHILING CHARACTERISTICS

VS Morita,[*] H van den Brand[‡] and IC Boleli[*1]

[*] *Department of Animal Morphology and Physiology, Sao Paulo State University, Access road Professor Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, Sao Paulo, Brazil and* [‡] *Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, PO Box 338, 6700 AH, Wageningen, the Netherlands*

ABSTRACT – To evaluate the effect of thermal programming and egg size on embryonic heat production, incubation parameters and hatchling characteristics, a total of 600 Cobb-500 hatching eggs were subjected to 4 treatments arranged in a 2 x 2 factorial randomized complete block design using 2 egg weights (56-59g and 70-73g, or small and large) and 2 eggshell temperature (EST) from E13-18 (37.5°C and 39°C). A significant effect of egg size was found for egg composition, hatchling characteristics and heat production. Large eggs had Yolk, albumen and eggshell weight, as well as, HP were higher in large eggs. Body weight, chick length and yolk free body, residual yolk, liver and heart weight were not influenced by EST, but they higher in chicks from large eggs. Rectal temperature was not influenced by EST and egg size, but the surface temperature of abdomen, back and neck were higher temperature of these parts is chicks from small eggs. A significant interaction between egg size and EST was found for weight loss. Large eggs exposed to 39oC for 12 h from ED13-18 lost more weight, while small eggs maintained at 37.5oC lost less weight than the other groups. Compared with the control EST, eggs from high EST had on average 4 h longer incubation period. It can be concluded that independent of EST, HP and egg size were directly related. Furthermore, although chicks from large eggs, which have greater availability of yolk, differ of chicks from small eggs in almost every physical and

physiological characteristic, they show the same reaction when exposed to thermal programming for 12 h from ED13-18.

Keywords: egg weight, embryo development, fetal development, high temperature

1. INTRODUCTION

Thermal programming is currently an useful tool to prepare chickens to high environmental temperatures in later life (broilers: Piestun et al., 2008; layers: Walsta et al., 2010). During thermal programming, eggs are exposed to higher incubation temperatures (39.0-39.5oC) during 12 h per day in the sensitive period, which last from embryonic day (ED) 7 to 18 (Yahav et al., 2009).

In various studies different starting moments are used varying from ED7 to ED18 and it has been shown that within this range the effects are not really different. Therefore, most studies currently use ED13-18 for 12 h per day. However, in all studies till know the egg composition is not taken into account. In almost all studies eggs from a prime flock (38-45 weeks) are used and egg weight is fixed in very narrow ranges. Nevertheless, it has been demonstrated that egg weight largely affect egg composition (Lourens et al., 2006, Nangsuay et al., 2011, 2013) and particularly the yolk size is important in this perspective. This means that the effect of thermal programming is probably affected by the egg size and particularly the yolk size, because yolk size largely determines heat production (Lourens et al., 2006; Nangsuay et al., 2013) and consequently the incubation temperature. Because of that our hypothesis is that chickens from larger eggs will have more effects of thermal programming than chickens of smaller eggs, which will be reflected in larger differences in physical and physiological characteristics of newly-hatched chicks between the control and programmed group. However, this is never investigated, although it can be of large importance both from a scientific point of view as well as from a practical point of view.

If egg size or yolk size has an effect on the results of thermal programming, programs need to be adjusted or even more, certain categories of eggs can be withdrawn from the thermal programming or not. In case we know the effects of egg size on results of thermal manipulation, possible differences in results among studies to thermal programming can be explained or at least ideas can be obtained for explanation. With this knowledge of fine tuning, hatcheries, particularly in regions with high temperatures, can be advised which temperatures during thermal programming should be used, or which eggs should not be used for thermal programming. In this way, treatments can be better adjusted to the needs of the embryos and consequently health and welfare can be further improved and energy expenditure (by heating the incubators) can be reduced. Therefore, the aim of this study was to investigate the effects of thermal programming for 12 hours from embryonic day 13 to 18 and egg size on heat production, incubation parameters and hatchling characteristics.

2. MATERIALS AND METHODS

To evaluate the effect of thermal programming and egg size on embryonic heat production, incubation parameters and hatchling characteristics, four experiments were carried out at facilities of Wageningen University, Wageningen, The Netherlands.

One hundred fifty fertile eggs from broiler breeder flocks (Cobb 500) of 38-41 weeks of age were used per batch. In batch 1 and 3 small eggs weighing 56-59g were obtained, whereas in batch 2 and 4, large eggs weighing 70-73g were used. In each batch, sixteen eggs were used for analysis of egg composition (egg, yolk, albumen and eggshell weight). Yolk and eggshell weight were measured. Albumen weight was calculated by subtracting the yolk and eggshell weight from the egg weight. The 134 eggs remaining were divided over two identical small open circuit climate respiration chambers (CRC; Lourens et al, 2006), separated into two treatments: thermal programming and control. In both chambers, the eggshell temperature (EST) was maintained at 37.5^{o}C from embryonic day (ED) 1 till ED12. From ED13 till ED18 the EST in the thermal programming chamber was increased till 39.0^{o}C for 12h/d. The rest of the day the EST was maintained at 37.5^{o}C. The machine temperature applied to obtain an EST of 37.5°C at ED 19 was maintained for the remaining incubation period. To measure EST, thermistors were attached to the eggshells of 5 individual, fertile eggs at the start of incubation in each CRC as described by Lourens et al. (2005). EST was measured every 30 sec and machine temperature (MT) was automatically adjusted every 5 min when the median EST drifted away from the EST set point. The MT was adjusted using the median EST of 5 eggs per CRC to avoid the low EST of eggs containing dead embryos from affecting the decision for the direction of the next MT step. In both treatments, the RH was kept at 55% and the eggs were turned each hour.

To determine HP, oxygen and carbon dioxide concentrations were measured every 9 min in both chambers and in fresh air. Carbon dioxide concentration was measured with a non-dispersive infrared CO analyzer (type Uras 3G, Hartmann and Braun, Frankfurt, Germany). Oxygen concentration was measured with a paramagnetic oxygen analyzer (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). The refreshed air volume was 2 L/min during the 18 d of incubation. Air volumes were measured with a Schlumberger G1.6 dry gas meter (Schlumberger, Dordrecht, The Netherlands). The HP was calculated per chamber as HP (kJ) = 16.18 × O_2 consumption (L) + 5.02 × CO_2 production (L) (Romijn and Lokhorst, 1961) and adjusted for fertility and embryonic mortality, based on the description of Lourens et al. (2006a). Values were expressed in milliwatts per living embryo (fertile egg).

Eggs were candled at ED13 and ED18, and twenty individual fertile eggs were weighed and weight loss (WL) was determined. Infertile eggs were removed and opened to identify true fertility and age of dead embryos as described by Lourens et al. (2006).

During ED19, 20 and 21, eggs were checked for hatching every six hours to determine incubation time (hours). Six hours old chicks were collected (n=15 per EST treatment), navel condition was scored as 1 (closed and clean navel area), 2 (black button up to 2 mm or black string), or 3 (black button exceeding 2 mm or open navel area) (Molenaar et al., 2010) and rectal temperature (RT) was recorded with a digital thermometer. Furthermore, surface temperature at different parts of the body (abdomen, back, leg and neck) were obtained using an infrared thermometer. Thereafter, chickens were weighed, measured and then killed by decapitation to obtain blood. After blood collection, chicks were opened to determine residual yolk (RY), yolk-free body (YFB), heart (HW) and liver (LW) weight. In the blood T3, T4 and corticosterone were determined.

Incubation parameters and hatchling characteristics were analyzed with the GLM procedure of the SAS 9.2 software package (SAS Institute, 2009) using model 1: Y_{ijk} =

$\mu + A_i + B_j + AB_{ij} + e_{ijk}$, where Y is the dependent variable, μ is the overall mean, A_i is EST (i= control or thermal programming), B_j is egg size (j= small or large), AB_{ij} is the interaction between EST and egg size egg size, and e_{ijk} is the error term.

For heat production, the CRC was used as the experimental unit in the statistical analyses. The HP per day from ED 7 to 18 was analyzed using the MIXED procedure of SAS 9.2 software package (SAS Institute, 2009) for repeated measurements. The model used was: $Y_{ijk} = \mu + A_i + B_j + C_k + \text{interactions} + e_{ijk}$, where Y is the heat production, μ is the overall mean, A_i is EST (i= control or thermal programming), B_j is egg size (j= small or large), C_k is embryonic day (k=ED 7 to ED18), and e_{ijk} is the residual error term. Distributions of the means and residuals were examined to check model assumptions. Least squares means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as least squares means. In all cases, a difference was considered significant at $P \leq 0.05$.

3. RESULTS

The weight of the egg constituents were influenced by the egg size (Table 1). Large eggs contained more yolk, albumen and eggshell ($P<0.001$) in comparison with small eggs. The effects of EST and egg size on embryonic HP are summarized in Figure 1. From ED12 onward, in eggs incubated at 37.5°C (control EST) and 39°C (thermal programming EST), HP was significantly higher in large eggs compared to small eggs ($P<0.001$). Heat production was not affected by EST ($P>0.05$) or the interaction between EST and egg size.

Table 2 shows the influences of EST and egg size and interaction EST x egg size on chick development. Body weight, chick length and yolk free body, residual yolk, liver and heart weight were not influenced by EST, but they had significant effect of egg size ($P<0.05$), which were higher in chicks from large in comparison from small eggs.

In the Table 3 the results of rectal temperature and surface temperature at different parts of the body are presented. Rectal temperature was not influenced by EST and egg size ($P>0.05$). However, the surface temperature of abdomen, back and neck were altered by egg size, where chicks from small eggs presented higher temperature of these parts than chicks from large eggs (P<0.05).

The results of weight loss, incubation duration and chick navel score are present in Table 4. The hatching rate of fertile eggs was 99.2% in small eggs and 98.5% in large eggs and were not affected by incubation treatments (data not shown). Significant interaction between egg size and EST was found for weight loss (P<0.001). Large eggs exposed to 39^{o}C for 12 h from ED13-18 (thermal programming EST) lost more weight, while small eggs maintained at 37.5^{o}C (control EST) lost less weight than the other groups. Effect of EST was verified on the incubation time (P<0.001). Eggs from thermal programming treatment had on average 4 h longer incubation period than eggs from control treatment. Thermal programming and egg size not altered chick navel condition (P>0.05).

Table 1. Effect of egg size (small and large) on yolk, albumen and eggshell weight (g).

Effect	Egg	Yolk	Albumen	Eggshell
Egg size				
Small	57.37 b	17.45 b	34.40 b	5.51 b
Large	71.43 a	21.73 a	41.86 a	6.84 a
Source of variation *(P-value)*				
P	<0.001	<0.001	<0.001	<0.001
CV (%)	3.72	2.88	2.81	8.24

a–b Means within a column lacking a common superscript differ (P ≤ 0.05).

Source of variation	7	8	9	10	11	12	13	14	15	16	17	18
EST	-	-	-	-	-	-	-	-	-	-	-	-
Egg size	-	-	-	-	-	-	*	*	*	*	*	*
EST x Egg size	-	-	-	-	-	-	-	-	-	-	-	-

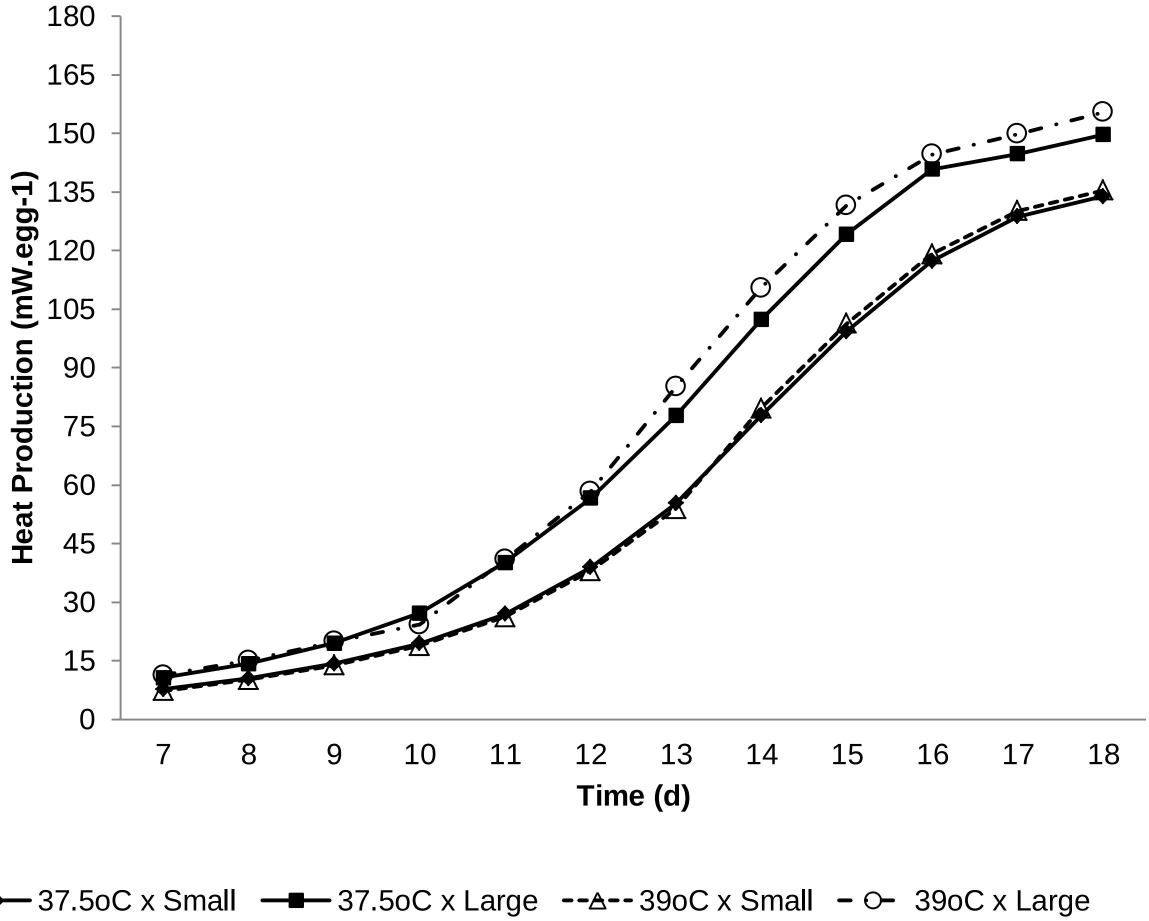

Figure 1. Heat production (mW.egg-1) in small (56-59g) and large (70-73g) eggs incubated at two EST settings (37.5^{o}C and 39^{o}C for 12 h from ED13-18), where *= P<0.05.

Table 2. Effect of EST (37.5^{o}C and 39^{o}C for 12 h from ED13-18) and egg size (small: 56-59g and large: 70-73g) on chick development. BW= body weight; CL= chicken length; YFB= yolk free body weight; RSY= residual yolk weight; LW= liver weight; HW= heart weight.

Effect	BW	CL	YFB	RSY	LW	HW
EST x Egg size						
37.5^{o}C x small	41.96	18.30	37.18	4.78	0.95	0.38
37.5^{o}C x large	51.40	18.85	40.21	11.18	1.11	0.49
39.0^{o}C x small	42.18	18.33	37.11	5.09	0.90	0.43
39.0^{o}C x large	51.69	18.83	42.90	8.79	1.04	0.48
Main effect						
EST						
37.5^{o}C	46.85	18.59	38.72	8.13	1.03	0.44
39.0^{o}C	47.05	18.57	40.03	6.94	0.97	0.45
Egg size						
Small	42.03 b	18.31b	37.03 b	4.98 b	0.92 b	0.41 b
Large	51.54 a	18.85 a	41.56 a	9.99 a	1.08 a	0.49 a
Source of variation *(P-value)*						
EST x Egg size	0.925	0.857	0.470	0.474	0.608	0.081
EST	0.430	0.982	0.495	0.580	0.059	0.406
Egg size	<0.001	0.002	0.027	0.011	<0.001	<0.001

a–b Means within a column and factor lacking a common superscript differ (P ≤ 0.05).

Table 3. Effect of EST (37.5°C and 39°C for 12 h from ED13-18) and egg size (small: 56-59g and large: 70-73g) on rectal temperature (RT) and surface temperature at different parts of the body (abdomen, back, leg and neck).

Effect	Rectal	Abdomen	Back	Leg	Neck
EST x Egg size					
37.5°C x small	38.54	34.10	34.30	33.44	33.50
37.5°C x large	38.87	32.83	32.93	32.60	32.63
39.0°C x small	38.66	33.68	33.68	33.08	33.37
39.0°C x large	38.62	32.25	32.51	32.63	32.26
Main effect					
EST					
37.5°C	38.7	33.5	33.5	33.0	33.0
39.0°C	38.6	33.0	34.0	32.8	32.8
Egg size					
Small	38.6	33.9 a	33.9 a	33.2	33.4 a
Large	38.7	32.5 b	32.7 b	32.6	32.4 b
Source of variation *(P-value)*					
EST x Egg size	0.169	0.818	0,752	0.661	0.701
EST	0.610	0.163	0.120	0.705	0.397
Egg size	0.281	<0.001	<0.001	0.152	0.002

a–b Means within a column and factor lacking a common superscript differ ($P \leq 0.05$).

Table 4. Effect of EST (37.5^oC or 39^oC for 12 h from ED13-18) and egg size (small or large) on egg weight loss (g), incubation time (hours) and chick navel score.

Effect	Weight loss (g)	Incubation time (h)	Navel score (%) 1 (good)	2 (moderate)	3 (bad)
EST x Egg size					
37.5^oC x small	7.4 d	508.40	21.4	50.0	28.6
37.5^oC x large	9.1 b	508.40	33.3	40.0	26.7
39.0^oC x small	8.2 c	504.80	33.3	33.3	33.3
39.0^oC x large	9.9 a	503.20	26.7	46.7	26.7
Main effect					
EST					
37.5^oC	8.3	508.1 a	27.3	45.0	27.6
39.0^oC	9.1	504.0 b	30.0	40.0	30.0
Egg size					
Small	7.8	506.2	27.3	41.6	31.0
Large	8.5	505.8	30.0	43.3	26.7
Source of variation *(P-value)*					
EST x Egg size	<0.001	0.223	0.131	0.428	0.218
EST	<0.001	<0.001	0.412	0.134	0.296
Egg size	<0.001	0.223	0.156	0.329	0.441

a–b Means within a column and factor lacking a common superscript differ (P ≤ 0.05).

4. DISCUSSION

This study was conducted to investigate the effects of egg size and thermal programming on heat production (HP), incubation parameters and hatchling characteristics. For this, eggs with two different sizes, small (weighing 56-59g) or large (weighing 70-73g), were obtained and incubated at 37.5^oC (control EST) or exposed at 39^oC for 12 hours from ED13-18 (thermal programming EST). Large eggs incubated at control or thermal programming EST produced, on average, 20 and 25 mW/egg more heat in comparison to small eggs maintained under same conditions from ED12 onward (Fig. 1 and 2). This is in accordance to the review by Wilson (1991), who concluded that embryo weight is not correlated with egg weight during the first half of the incubation period. Lourens et al. (2006) found similar results; however, the higher production of HP was seen from ED15 onward. This increase of metabolic heat production, verified from the second half of incubation period, suggests that the embryonic growth increases more in large eggs (Romijn and Lokhorst, 1956; Meijerhof and Van Beek, 1993 and Lourens et al., 2006). Furthermore, Etches (1996) says that embryonic HP is produce as by-product of the biochemical processes to transform egg nutrients into chick body tissue. This suggests that a higher amount of yolk present in large eggs which resulted in bigger chicks (in this study) seems to be related to the higher heat produced by these eggs.

The chick development was determined by length and body weight, as well as yolk free body, residual yolk, liver and heart weight of the newly hatched chicks (Willemsen et al., 2008). In the present study, chicks from large eggs hatched bigger than chicks from small eggs. This result is similar to the studies of Lourens et al. (2006) and Nangsuay et al. (2011), who demonstrated effect of egg size on chick development. Thermal programming had no effect on chick development. Although it is not significant, it is very interesting the results obtained for RSY. Under incubation at control temperature, the difference between chicks from large eggs and small eggs is on average 6.5 gram, while this difference under incubation at high temperature is only 3.7 grams. This difference suggests that with TM you indeed stimulate the yolk consumption, but particularly in large eggs, resulting in chick with same quality, once had not effect on navel score, and same BW than chicks from eggs maintained at control temperature, however obtained in shorter incubation time (present study).

Although several previous studies have analyzed body surface temperature of chicks, it is unknown the effect of thermal programming and egg size on surface temperature at different parts of the body. Our data showed that chicks from small eggs have surface temperature of back, abdomen, neck and leg higher than large eggs. The smaller temperatures found in chicks hatched from large eggs seem to be an adaptive response to higher HP of these eggs, in an attempt to avoid overheating.

Large eggs maintained at high incubation temperature have higher weight loss than small eggs under control temperature. The results are in agreement with previous experiments (Lourens, 2006) and seem to be related with the higher pore area and smaller thickness found in the eggshell of large eggs, additional to the higher amount of heat produced which need to be dissipated by these eggs.

In conclusion, our results indicate that, independent of EST, HP and egg size were directly related. Furthermore, although chicks from large eggs, which have greater availability of yolk, differ of chicks from small eggs in almost every physical and physiological characteristic, contradicting our hypothesis, they show the same reaction when exposed to thermal programming for 12 h from ED13-18.

5. REFERENCES

Etches RJ. Reproduction in Poultry. CAB International, Wallingford, UK, 1996.

Lourens A, van den Brand H, Meijerhof R, Kemp B. Effect of eggshell temperature during incubation on embryo development, hatchability, and post hatch development. Poultry Science, v.84, p.914-920. 2005.

Lourens A, Molenaar R, van den Brand H et al. Effect of egg size on heat production and the transition of energy from egg to hatchling. Poultry Science, v.85, p.770-776, 2006.

Meijerhof R and van Beek G. Mathematical modeling of temperature and moisture loss of hatching eggs. Journal of Theoretical Biology, v.165, p.27-41. 1993.

Molenaar R, Meijerhof R, Van Den Anker I et al. Effect of eggshell temperature and oxygen concentration on survival rate and nutrient utilization in chicken embryos. Poultry Science, v.89, p.2010-2021, 2010.

Morita VS, Boleli IC, Oliveira JA. Relationship between incubation temperature and egg size with heart hypoplasy in broiler chicks at hatching. International Journal of Poultry Science, v.11, p.761-768.

Nangsuay A, Ruangpanit Y, Meijerhof R, Attamangkune S. Yolk absorption and embryo development of small and large eggs originating from young and older breeder hens. Poultry Science, v.90, p.2648–2655, 2011.

Nangsuay, A, Meijerhof R, Ruangpanit Y et al. Energy utilization and heat production of embryos from eggs originating from young and old broiler breeder flocks. Poultry Science, v.9, n.2, p.474–482, 2013.

Piestun Y, Shinder D, Ruzal M et al. Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermotolerance. Poultry Science, v.87, p.1516-1525, 2011.

Romijn C and W Lokhorst. The caloric equilibrium of the chicken embryo. Poultry Science, v.35, p.829-834, 1956.

Walstra I, ten Napel J, Kemp B, van den Brand H. Temperature manipulation during layer chick embryogenesis. Poultry Science, v.89, p.1502-1508, 2010.

Willemsen H, Everaert N, Witters A et al. Critical assessment of chick quality measurements as an indicator of post hatch performance. Poultry Science, v.87, p.2358–2366, 2008.

Wilson HR. Physiological requirements of the developing embryo: temperature and turning. Pages 145-156 in Tullett SG (ed.), Avian incubation, Butterworth-Heinemann, London, UK, 1991.

Yahav S. Alleviating heat stress in domestic fowl - different strategies. Journal of World Poultry Science, v.65, p.719-732, 2009.