UNIVERSIDADE ESTADUAL DE MARINGÁ

CENTRO DE CIÊNCIAS AGRÁRIAS

DEPARTAMENTO DE ZOOTECNIA

TÍTULO

Autor(a):

Orientador(a):

Coorientador(a):

MARINGÁ

Estado do Paraná

Dezembro – 2022

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Trabalho de Conclusão de Curso apresentado, como parte das exigências para obtenção do título de ZOOTECNISTA da Universidade Estadual de Maringá - Área de Concentração: Produção Animal.

MARINGÁ

Estado do Paraná

Dezembro - 2022

“Sua capacidade de alcançar o sucesso depende do seu propósito e da sua força de vontade”

Alexandre Lacava

Aos meus pais, .... e ...., que me deram a base para que eu pudesse me tornar o que sou hoje.

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ÍNDICE

|  |  |  |
| --- | --- | --- |
|  |  | Página |
| LISTA DE TABELAS ............................................................................ | viii |
| LISTA DE FIGURAS .......................................................................... | ix |
| TÍTULO .............................................................................................. | 1 |
| Resumo ...................................................................................................Abstract ...................................................................................................Introdução ...............................................................................................Material e Métodos .................................................................................Resultados ...............................................................................................Discussão ................................................................................................Conclusões ..............................................................................................Referências ............................................................................................. | 1 |
| 2 |
| 3 |
| 5 |
| 9 |
| 10 |
| 12 |
| 13 |
| Normas da Revista Brasileira de Zootecnia ............................................ | 16 |

LISTA DE TABELAS

|  |  |
| --- | --- |
|  | Página |
| Tabela 1 - Sequências de primers utilizados na reação em cadeia da polimerase PCR em tempo real (RT-qPCR) ........................................................................... | 8 |
| Tabela 2 - Consumo de ração (CR) e ganho de peso (GP) de frangos de corte de 42 dias .................................................................................................................. | 9 |

LISTA DE FIGURAS

|  |  |
| --- | --- |
|  | Página |
| Figura 1 - Gel de agarose 1% para verificar a integridade do RNA total das amostras de fígado, baço, bursa de Fabricius e timo ............................................. | 7 |
| Figura 2 - Expressão do gene proteína do choque térmico 27 kDA (*HSP27)* no fígado, baço, bursa de Fabricius e timo de frangos de corte machos com 42 dias de idade ................................................................................................................ | 10 |

**TÍTULO**

**RESUMO -** Este estudo teve como objetivo avaliar o efeito do estresse por calor sobre o desempenho e a expressão do gene proteína do choque térmico 27 kDA (*HSP27)* no fígado, baço, bursa de Fabricius e timo de frangos de corte com 42 dias de idade. O experimento foi conduzido em delineamento inteiramente casualizado com dois tratamentos: conforto térmico (CT) e estresse por calor (EC). Aos 41 dias de idade, 40 aves foram submetidas ao EC de 38ºC por 24 horas, e o restante das aves (40 aves) permaneceram em CT. Observamos que as aves sob EC apresentaram menor consumo de ração e perda de peso corporal. Não houve efeito do EC sobre a expressão do mRNA *HSP27* em nenhum dos órgãos avaliados. Esses resultados sugerem que o EC prejudicou o desempenho das aves, possivelmente em função da menor disponibilidade de energia para os mecanismos moleculares direcionados a proteção do organismo contra o EC, a qual pode ter resultado na ausência de expressão do mRNA *HSP27.*

**Palavras-chave:** desempenho animal, proteínas do choque térmico, temperatura ambiente

TITLE

**ABSTRACT -** This study aimed to evaluate the effect of heat stress on the performance and heat shock protein 27 kDA gene expression (*HSP27*) in the liver, spleen, bursa of Fabricius and thymus of 42-day old broiler chickens. The experiment was conducted in a completely randomized design with two treatments: thermal comfort (CT) and heat stress (EC). At 41 days of age, 40 poultry were submitted to EC of 38ºC for 24 hours, and the remaining poultry (40 birds) remained in CT. We observed that the birds under HS presented lower feed intake and loss of body weight. There was no effect of EC on the expression of mRNA *HSP27* in any of the evaluated organs. These results suggest that EC harmed the poultry performance, possibly due to the lower availability of energy to the molecular mechanisms directed to the protection of the organism against EC, which may have resulted in the absence of mRNA *HSP27*expression.

**Keywords:** animal performance, heat shock proteins, ambient temperature

# Introdução

O estresse por calor em aves é uma das principais preocupações da indústria avícola, uma vez que causa alta mortalidade e/ou baixa produtividade, especialmente durante as estações mais quentes do ano (Mazzi et al., 2003).

Em todos os tecidos dos animais vivos são sintetizadas proteínas de baixo peso molecular que possuem funções específicas no crescimento celular e na reversão ou prevenção de danos causados por um estresse. Essas proteínas, cuja síntese é aumentada quando a célula é submetida a uma condição estressante, são denominadas proteína do choque térmico (heat shock protein – HSP) (Hernandes et al., 2002).

A resposta de diversos organismos ao choque térmico é um dos sistemas genéticos mais altamente conservados até hoje conhecidos, e, embora as proteínas do estresse não estejam entre as mais abundantes, elas compreendem uma das famílias de proteínas mais conservadas encontradas em diferentes organismos (Parsell e Lindquist, 1993)

Em 1962, descobriu-se que a exposição de células de glândulas salivares de *Drosophila busckii* ao calor produzia o surgimento em cromossomos de um novo padrão de espessamento, que representava sítios específicos de transcrição para a síntese de proteínas (Ritossa, 1962). O estresse térmico ou químico induzia nas *Drosophilas* a expressão de genes, até então quiescentes, os quais faziam com que as células estressadas fabricassem grandes quantidades de uma determinada classe de proteínas, que foram chamadas de heat shock proteins – HSPs (Meyer e Silva, 1999), sendo verificado posteriormente que essa resposta é um fenômeno praticamente universal entre todos os seres vivos.

A aquisição de termotolerância pode estar relacionada com aumentos nos níveis de algumas HSPs. A exposição dos indivíduos à hipertermia pode originar no organismo uma resposta rápida e transitória em nível transcricional e traducional, a qual foi considerada por Budon (1986), como um mecanismo responsável pela sobrevivência celular durante o período de estresse. Entre as HSPs, a que se apresenta em maiores níveis de expressão a condições estressantes, é a HSP70, sendo a HSP mais estudada até o momento (Beere et al., 2000; Mayer et al., 2005; Gupta et al., 2007). Diversas HSPs tem sido descritas em eucariontes, sendo classificadas de acordo com seu peso molecular (kDa), em diferentes grupos: HSP110, HSP90, HSP70, HSP60 e um complexo grupo com peso molecular entre 15 e 30 kDa, designadas como HSPs de baixo peso molecular.

Em frangos de corte muitos são os trabalhos tentando relacionar os níveis de expressão dos genes *HSPs* com a tolerância ao calor, entretanto esse mecanismo ainda não é bem compreendido (Xie et al., 2014; Rimoldi et al., 2015). Nesse sentido, o presente estudo foi desenvolvido para testar a hipótese de que o estresse por calor pode influenciar a expressão do gene proteína do choque térmico 27 kDa (*HSP27*) em diferentes órgãos de frangos de corte, na tentativa de proteger as células contra danos oxidativos induzidos pelo estresse por calor. Pra testar essa hipótese nós avaliamos o os efeitos do estresse por calor (38°C por 24 horas) sobre o desempenho animal (consumo de ração e ganho de peso) e a expressão do gene *HSP27* no fígado, baço, bursa de Fabricius e timo de frangos de corte de 42 dias de idade.

# Material e Métodos

Este trabalho foi conduzido de acordo com as especificações do Comitê de Ética no Uso de Animais (CEUA nº4000170615) da Universidade Estadual de Maringá.

*Animais e desenho experimental*

O experimento foi conduzido na Fazenda Experimental de Iguatemi da Universidade Estadual de Maringá, em um delineamento inteiramente casualizado com dois tratamentos referentes ao ambiente de conforto térmico (CT) e ambiente de estresse por calor (EC), e quatro repetições por tratamento.

Para a realização desse experimento, um total 80 frangos de corte machos (Cobb 500) (*Gallus gallus*) de 21 dias de idade foram utilizados. Esses animais foram divididos em dois grupos ambientes experimentais diferentes: ambiente de conforto térmico e ambiente de estresse por calor. As aves foram criadas em gaiolas coletivas (10 aves por gaiola), em salas climatizadas em ambiente de conforto térmico (de acordo com o manual da Cobb) até os 41 dias de idade, quando então as 40 aves que estavam no ambiente EC foram submetidas ao estresse térmico de 38ºC por 24 horas, e o restante dos animais permaneceram em conforto térmico. Todos os animais foram abatidos por deslocamento cervical aos 42 dias.

Durante todo o período experimental os animais tiveram livre acesso à agua e a ração. A dieta foi formulada para atender suas exigências nutricionais (Rostagno et al., 2011). A ração foi formulada com milho e farelo de soja contendo em sua composição 19,70% de proteína bruta e 3170 kcal/kg de energia metabolizável (EM).

*Desempenho animal: consumo de ração (CR) e ganho de peso (GP)*

Para calcular o ganho de peso dos animais do estresse as aves foram pesadas no inicio (41 dias) e no fim do período de estresse (42 dias). O consumo de ração foi calculado como a diferença entre a quantidade de ração oferecida no início (41 dias) e as sobras ao final do período experimental (42 dias). Para calcular o ganho de peso dos animais do conforto, as aves foram pesadas no início (21 dias) e no final (42 dias) do período experimental, e o ganho de peso foi calculado como peso final – peso inicial/21. O consumo de ração desse grupo experimental foi calculado como a quantidade de ração ofertada aos 21 dias – sobras de ração no final do período experimental (42 dias) / 21. O ganho de peso e o consumo de ração foram corrigidos para a mortalidade.

*Expressão gênica – Extração de RNA total*

Para as análises de expressão gênica a ave foi considerada como uma unidade experimental (n = 12). Ao final do período experimental proposto, os frangos foram eutanaseados por deslocamento cervical, e as amostras de fígado, baço, bursa de Fabricius e timo foram coletadas de seis animais de cada tratamento. As amostras foram coletadas e conservadas em nitrogênio líquido, e subsequentemente foram armazenadas em freezer a - 80ºC até o momento da extração de RNA.

O RNA total foi extraído dos tecidos com uso do reagente TRIzol® (Invitrogen, Carlsbad CA, USA) de acordo com as normas do fabricante, na proporção de 1000 µL para cada 100 mg de tecido. Após a extração do RNA total, a concentração (ng/ µL) do mesmo foi mensurada via espectrofotômetro Nanodrop 2000 (Thermo Fischer Scientific) no comprimento de onda de 260 nm. A integridade do RNA total foi avaliada em gel de agarose 1%, corado com SYBR™ Safe DNA Gel Stain (Invitrogen™, Carlsbad CA, USA), e visualizado no fotodocumentador para gel de eletroforese (L-PIX TOUCH – Loccus Biotecnologia) sob luz ultravioleta (Figura 1).



Figura 1 – Gel de agarose 1% para verificar a integridade do RNA total das amostras de fígado (1, 3), baço (5, 7, 9), bursa de Fabricius (11, 2) e timo (4, 6, 12).

*Tratamento do RNA total com DNase I*

 Essa reação foi realizada para evitar a contaminação das amostras com DNA genômico. Para essa reação, 1 µg de RNA total foi tratado com o kit DNase I amplification grade (Invitrogen, Carlsbad CA, USA), de acordo com as instruções do fabricante.

*Síntese de DNA complementar (cDNA)*

 Logo após o tratamento do RNA total com DNase I, foi realizada a síntese do cDNA, utilizando o kit SuperScript™ III First-Strand Syntesis Super Mix (Invitrogen Corporation, Brasil) de acordo com as normas do fabricante. As amostras de cDNA foram armazenadas em freezer a -20ºC até o momento do uso.

*Reação em cadeia da polimerase em tempo real – PCR em tempo real*

 Para as reações de PCR em tempo real foi utilizado o composto fluorescente SYBR Green (SYBR® Green PCR Master Mix – Applied Biosystems, USA). O primer para amplificação do gene *HSP27* foi desenhado de acordo com Zhao et al. (2014) e a sequência desse gene está depositada no site www.ncbi.nlm.nih.gov com o número de acesso NM\_205290.1 (Tabela 1). O gene β-actina (número de acesso L08165.1), foi utilizado como controle endógeno. Todas as análises foram realizadas em um volume de 25 µL e em duplicatas.

Tabela 1 -Sequências de primers utilizados na reação em cadeia da polimerase PCR em tempo real (RT-qPCR)

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Tamanho doamplicon (pb) | Temperatura deanelamento (ºC) | Sequências dos primers (5’🡪3’) |
| *HSP27* | 158 | 60 | F: ACACGAGGAGAAACAGGATGAG |
|  |  |  | R: ACTGGATGGCTGGCTTGG |
| *β-actina* | 130 | 60 | F: GCCAACAGAGAGAAGATGAC |
|  |  |  | R: CACCAGAGTCCATCACAATAC |

pb - tamanho do amplicon em pares de bases; F – forward; R – reverse; *HSP27* – proteína do choque térmico 27 kDA

*Análise estatística*

O método 2-∆CT foi utilizado para a análise de expressão relativa do gene *HSP27* e os resultados foram expressos como unidade arbitrária (UA). O procedimento UNIVARIATE foi aplicado para verificar a normalidade dos dados. No modelo foi considerado o efeito do ambiente. Os dados foram avaliados pela ANOVA e as médias comparadas utilizando o teste de F (P<0,05) (SAS Inst. Inc., Cary, NC, USA).

**Resultados**

*Desempenho animal: consumo de ração (CR) e ganho de peso (GP)*

Na tabela 2 são apresentados os resultados de desempenho animal (consumo de ração e ganho de peso). Observamos efeito do ambiente sobre o consumo de ração (P=0,0104) e ganho de peso (P<0,0001). Os animais submetidos ao ambiente de estresse por calor apresentaram menor consumo de ração, e também significativa perda de peso corporal, quando comparado com os animais que permaneceram no ambiente de conforto térmico.

Tabela 2 **-** Consumo de ração (CR) e ganho de peso (GP) de frangos de corte de 42 dias

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  *Ambiente* |  |  |  |
|  |  | Conforto térmico |  |  Estresse por calor |  |  |
|  |  | Média | DP |  | Média | DP |  | *Valor de P* |
| CR (kg) |  | 0,122a | 0,017 |  | 0,083b | 0,020 |  |  0,0104 |
| GP (kg) |  | 0,077a | 0,014 |  | -0,034b | 0,023 |  | <0,0001 |

a-b - Valores médios na mesma linha com letras diferentes sobrescritas são significativamente diferentes pelo Teste de F (P<0,05). Os resultados são apresentados como média ± desvio padrão.

*Expressão gênica*

 Os resultados do efeito do ambiente sobre a expressão do gene *HSP27* são apresentados na Figura 2. Não foi observado efeito significativo do ambiente sobre a expressão do gene *HSP27* no fígado, baço, bursa de Fabricius e timo (P>0,05).

Figura 2 – Expressão do gene proteína do choque térmico 27 kDA (*HSP27)* no fígado, baço, bursa de Fabricius e timo de frangos de corte machos com 42 dias de idade.

Os resultados são expressos como unidade arbitrária (UA), e são apresentados como média±desvio padrão.

# Discussão

Como era esperado, em nossos resultados observamos que o estresse por calor reduziu o consumo de ração acompanhado pela perda de peso corporal em frangos de corte com 42 dias de idade. Este resultado pode estar relacionado ao efeito anorexígeno que o estresse por calor promove através do aumento na expressão do gene grelina no proventrículo e jejuno das aves (Song et al., 2012). O estresse também pode ocasionar menor desempenho nas aves através de diversas outras mudanças fisiológicas e metabólicas como o maior nível de corticosterona (Quinteiro Filho et al., 2012), que acelera a degradação de proteínas corporal (Yunianto et al., 1997; Lin et al., 2006), e a redução de energia disponível para a renovação das células intestinais que prejudica o processo de absorção, com prejuízos diretos sobre o desenvolvimento dos músculos e ossos (Porto et al., 2015).

Durante o curso da evolução, o organismo dos animais das mais diversas espécies, desenvolveram mecanismos capazes de proteger suas células contra danos causados por agentes estressores, que podem alterar a sinalização e a transdução de sinal para o DNA e alterar a expressão de diferentes genes.

O estresse fisiológico e metabólico provocado pela alta temperatura ambiental tem sido associado com a maior produção de espécies reativas de oxigênio (ROS) (Marklund e Marklund, 1974) e mudanças nas atividades das principais enzimas antioxidantes (Pamok et al., 2009; Tan et al., 2010). O ROS pode danificar diretamente as proteínas do organismo animal através de processos conhecidos como carbonilação, glutationilações, formação de ligações dissulfeto e através de degradação e formação de agregados proteicos (revisado por Sharma et al., 2012). As células do organismo animal muitas vezes são capazes de se protegerem das injúrias causadas pelo ROS através do desencadeamento de certos mecanismos de defesa, como o sistema antioxidante envolvendo as enzimas superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx), bem como proteínas do choque térmico (HSPs), que são responsáveis por proteger os peptídeos nascentes e prevenir a degradação irreversível de proteínas celulares, incluindo as próprias enzimas antioxidantes.

Por várias décadas, o papel citoprotetor das HSPs em diferentes tecidos sob a condição de estresse tem sido discutido (Lindquist e Craig, 1988; Guo et al., 2007; Hao et al., 2012), uma vez que a ruptura da homeostase celular causada por diferentes agentes estressores pode alterar o perfil de expressão dos genes que codificam as proteínas HSPs. A proteína do choque térmico 27 kDA (HSP27) pertencente a família das HSPs de baixo peso molecular, foi preliminarmente caracterizada pela sua resposta ao choque térmico como uma proteína chaperona que facilita o renovelamento adequado de proteínas danificadas (Revisado por Vidyasagar et al., 2012). A continuação dos estudos sobre a HSP27 mostrou que a mesma pode atuar como um antioxidante, através da redução nos níveis de ROS e de ferro intracelular, e por proporcionar aumento nos níveis de glutationa intracelular, além de atuar como um fator anti-apoptótico (Mayes e Bukau, 2005; Wang et al., 2009).

Neste trabalho, não observamos efeito estatístico significativo do ambiente sobre a expressão do gene *HSP27* nos diferentes órgãos avaliados. Esse resultado sugere que nessa situação de estresse por calor, a redução do consumo de ração pode ter influenciado a ativação da via transcricional do gene *HSP27,* provavelmente em função da menor disponibilidade de energia necessária para desencadear os mecanismos moleculares direcionados a proteção do organismo contra os efeitos deletérios do estresse por calor, afetando assim de maneira direta o desempenho animal.

# Conclusões

Esses resultados sugerem que o estresse por calor prejudica o desempenho dos animais, possivelmente em função da menor disponibilidade de energia para os mecanismos moleculares direcionados a proteção do organismo contra o estresse por calor, a qual pode estar relacionada à ausência da ativação da via transcricional do gene *HSP27* observada neste estudo.

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