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Effect of essential oils blend inclusion on performance and *HSP70* gene expression in broilers chickens under chronic heat stress

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Abstract. The objective of this study was to evaluate the effects of the inclusion of a blend of essential oils (capsaicin, carvacrol, cinnamaldehyde and eugenol) in broiler submitted to thermal stress. Performance parameters and gene expression of heat shock protein 70 kDa (*HSP70*) were evaluated in the liver and jejunum. Regarding performance, animals subjected to chronic heat stress had lower feed intake and weight gain and higher feed conversion. The inclusion of 3% of the blend in the feed increased the *HSP70* gene expression in jejunum and liver of 42 days old broiler chickens.

Keywords: capsaicin, carvacrol, cinnamaldehyde, eugenol, thermal comfort

Efeito da inclusão do blend de óleos essenciais no desempenho e na expressão do gene *HSP70* em frangos de corte sob estresse térmico crônico

Resumo. O objetivo deste estudo foi avaliar os efeitos da inclusão de um blend de óleos essenciais (capsaicina, carvacrol, cinamaldeído e eugenol) em frangos de corte submetidos a estresse térmico. Foram avaliados os parâmetros de desempenho e expressão do gene da proteína de choque térmico 70 kDa (*HSP70*) no fígado e jejuno. Em relação ao desempenho, os animais submetidos ao estresse por calor crônico tiveram menor consumo de ração e ganho de peso e maior conversão alimentar. A inclusão de 3% do blend na ração aumentou a expressão do gene *HSP70* no jejuno e fígado de frangos de corte de 42 dias de idade.

Palavras-chave: capsaicina, carvacrol, cinamaldeído, eugenol, conforto térmico

Efecto de la inclusión de mezclas de aceites esenciales sobre el rendimiento y la expresión del gen *HSP70* en pollos de engorde bajo estrés por calor crónico

Resumen. El objetivo de este estudio fue evaluar los efectos de la inclusión de una mezcla de aceites esenciales (capsaicina, carvacrol, cinamaldehído y eugenol) en pollos de engorde sometidos a estrés térmico. Los parámetros de rendimiento y la expresión génica de la proteína de choque térmico 70 kDa (*HSP70*) se evaluaron en el hígado y el yeyuno. En cuanto al rendimiento, los animales sometidos a estrés por calor crónico tuvieron menor consumo de alimento y ganancia de peso y mayor conversión alimenticia. La inclusión del 3% de la mezcla en la ración aumentó la expresión del gen *HSP70* en yeyuno e hígado de pollos de engorde de 42 días de edad.

Palabras clave: capsaicina, carvacrol, cinamaldehído, eugenol, confort térmico

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Introduction

Of all the environmental factors that can harm the health of broilers, heat stress is the one that most negatively impacts the entire poultry production system. Faced with this problem, the search for alternative strategies that aim to minimize the negative effects of heat stress on animals through nutritional modulation has been increasingly constant. This study was proposed under the hypothesis that chronic heat stress can cause damage to the animal performance by inducing oxidative stress and damage to the intestinal surface, and that supplementation of the essential oils blend (capsaicin, carvacrol, cinnamaldehyde and eugenol) in broiler diets could minimize the negative effects of stress by acting in different ways in the animal's body. To test this hypothesis, broilers were exposed to chronic heat stress of 32º C from the 1st to the 42nd day of life, and in the same period to evaluate how feed additives could improve the animal's tolerance to heat stress, the same received diets with different levels of the blend of essential oils (capsaicin, carvacrol, cinnamaldehyde and eugenol), through performance evaluation and expression *HSP70* of 42-day-old broiler.

Materials y Methods

All animal experiments were approved by the Ethics Committee on Use of Production Animals of the Federal University of Viçosa, Brazil (n°. 49/2022). A total of 250 one-day-old male Cobb 500 chicks were used in the experiment. On the first day, the birds were weighed and distributed in a completely randomized design, with a 2 × 5 factorial arrangement with five replications and five birds/replicate. The first factor was temperature challenge: thermoneutral, following the recommendations for the Cobb 500 strain, and chronic heat stress, where the animals were exposed to heat stress of 32°C during all life. The second factor was the inclusion of essential oils blend in the diet: 0% (CTL), 0.75%, 1.5%, 2.25% and 3%. The birds were housed in suspended cages in a controlled environment under a 24 h light photoperiod. Birds had free access to feed and water throughout the experiment. Diets were based on corn and soybean meal. For performance analyses, each cage was considered an experimental unit (n = 5). Body weight gain (BWG) was determined as the difference in measurements at the beginning and end of the period (22 to 42 days). Feed intake (FI) was obtained by the difference between the amount of feed provided and consumed in the period from 22 to 42 days. The feed conversion ratio (FCR) was calculated as the ratio of FI to BWG. To the gene expression analysis, three inclusions of the blend of essential oils were considered: CTL, 1.5% and 3%. Five 42 days old broilers from each treatment were chosen, and their jejunum and liver were collected in TRIzol (Invitrogen, Carlsbad CA, USA), being subsequently stored in a -80°C freezer. Total RNA was extracted using the TRIzol reagent, following the manufacturer's recommendations. Total RNA was quantified using the NanoDrop 2000-c spectrophotometer (ThermoFisher Scientific), in wavelength of 260 nm. Total RNA was treated with the DNase I amplification kit grade (Invitrogen, Carlsbad CA, USA), according to the instructions of the manufacturer. Complementary DNA (cDNA) synthesis was performed using the SuperScript III First-Strand Synthesis SuperMix kit (Invitrogen Corporation, Brazil), according to the manufacturer's instructions. The chain reactions of real-time polymerase were performed using SYBR Green PCR Master Mix (Applied Biosystems, USA) following the recommendations of manufacturer. The *HSP70* (Gene ID: 423504) were designed based on the sequences of the genes filed with the NCBI www.ncbi.nlm.nih.gov. The β-actin gene (Gene ID: 396526) was used as an endogenous control. The relative quantification of gene expression was calculated using the $2^{-\Delta CT}$ method (Livak and Schmittgen, 2001). The results are expressed as arbitrary units (AU). All data were analyzed by two-way ANOVA, which considers the main effects (temperature challenge and diet) and the interaction between the factors. Means were compared by the Tukey test ($P < 0.05$) (SAS Inst. Inc., Cary, NC).

Results and Discussion

The performance results of the broiler chickens from 22 to 42 days old are presented in Table 1. In this study, five levels of inclusion of the blend of the oils essential were evaluated in animals challenged with thermal stress. No interaction effect between the evaluated parameters was observed. There was no effect of diet on performance parameters. High temperature environment negatively affected BWG, FI and FCR. Broiler are high metabolic heat production animals and reduction of feed intake is a mechanism for reducing metabolic heat production. Thermal stress also negatively impacts nutrient absorption, reducing the absorption surface area of the intestine, causing edemas, ruptures and apoptosis of intestinal cells (Chen *et al.*, 2021).

Table 1. Performance of broilers from 22 to 42 days

		FI (kg)		BWG (kg)		FCR	
		Mean	SE	Mean	SE	Mean	SE
TN	CTL	3.810	0.198	2.199	0.140	1.734	0.041
	0.75%	3.714	0.094	2.146	0.100	1.733	0.085
	1.5%	3.777	0.119	2.192	0.132	1.728	0.117
	2.25%	3.751	0.158	2.171	0.266	1.741	0.141
	3%	3.559	0.140	2.143	0.237	1.672	0.130
HSC	CTL	2.549	0.096	1.361	0.098	1.877	0.080
	0.75%	2.308	0.187	1.183	0.185	1.984	0.285
	1.5%	2.364	0.228	1.205	0.217	1.988	0.181
	2.25%	2.251	0.259	1.154	0.125	1.960	0.222
	3%	2.375	0.177	1.238	0.098	1.921	0.069
Main Effects							
Environment	TN	3.722[a]	0.160	2.170[a]	0.172	1.722[b]	0.103
	HSC	2.369[b]	0.207	1.228[b]	0.157	1.946[a]	0.176
Diet	CTL	3.179	0.681	1.780	0.456	1.806	0.096
	0.75%	3.011	0.754	1.665	0.527	1.858	0.238
	1.5%	3.070	0.764	1.698	0.547	1.858	0.198
	2.25%	3.001	0.816	1.662	0.571	1.851	0.210
	3%	2.967	0.642	1.690	0.507	1.796	0.164

[a,b]Means in the same column followed by different letters differ significantly by the Tukey test, $P < 0.05$.
Results are presented as mean and standard error, SE. FI = feed intake; BWG = body weight gain; FCR = feed conversion ratio. TN = thermoneutral environment; HSC = chronic heat stress environment.

High temperature environment increased *HSP70* gene expression in both organs (Figure 1a). HSP70 is a protein expressed in several organs, and the synthesis is triggered by a series of factors such as heat stress, due to this it can be used as a stress marker for poultry (Greene *et al.,* 2019). The control diet and the inclusion of 1.5% of the blend of essential oils in the diet, showed lower levels of *HSP70* gene expression in jejunum and liver (Figure 1b), indicating that animals that consumed these levels of inclusion had a better capacity to tolerate heat stress than animals that consumed 3% of inclusion. The use of essential oils is an interesting alternative to antibiotic growth promoters. Several beneficial effects have been described, such as antibacterial action, improvement of gastrointestinal tract enzymes and preservation of intestinal health (Abd El-Hack *et al.,* 2022).

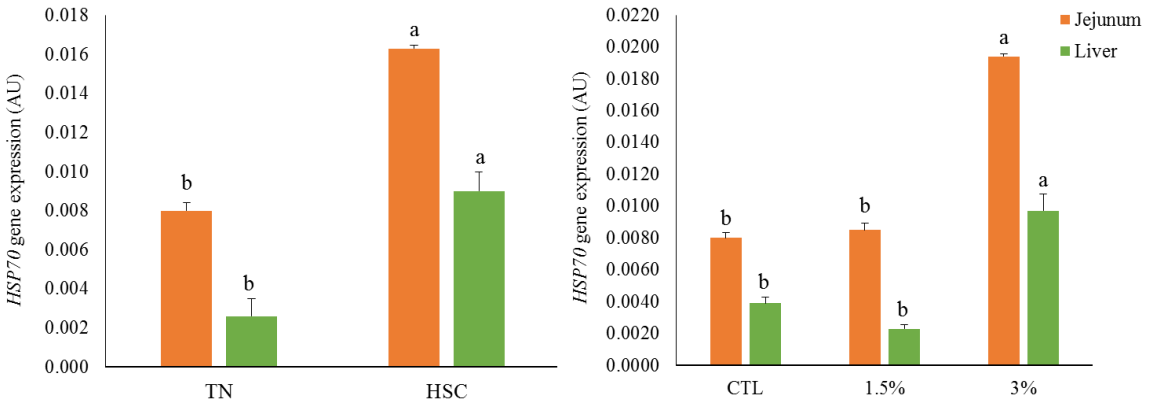

Figure 1. Effect of temperature challenge (a) and diet (b) on heat shock protein 70 kDa (*HSP70*) gene expression in the jejunum (in orange) and liver (in green) of 42 days old broiler. [a,b]Different letters represent significant differences by the Tukey test, $P < 0.05$. TN = thermoneutral environment; HSC = chronic heat stress environment.

Capsaicin is a compound derived from peppers and its use in poultry has been associated with anti-inflammatory and antioxidant improvements and modulation of intestinal motility, but, according to Bley *et al.* (2012) prolonged exposure to high doses of capsaicin can cause adverse effects on the organism

Conclusion

Our results show that different inclusions of the essential oil blend containing capsaicin, carvacrol, cinnamaldehyde and eugenol had no effect on the performance of 42-day-old broilers. However, the inclusion of 3% of the blend seems to impair the thermal stress tolerance.

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Conflicts of interest: The authors declare no conflicts of interest.

Literature Cited

Abd El-Hack, M. E., M. T. El-Saadony, A. M. Saad, H. M. Salem, N. M. Ashry, M. M. A. Ghanima, M. Shukry, A. A. Swelum, A. E. Taha, A. M. El-Tahan, S. F. AbuQamar, and K. A. El-Tarabily. 2022. Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. Poultry Science, 101: 101584. https://doi.org/10.1016/j.psj.2021.101584

Bley, K., G. Boorman, B. Mohammad, D. McKenzie, and S. Babbar. 2012. A comprehensive review of the carcinogenic and anticarcinogenic potential of capsaicin. Toxicologic Pathology, 40: 847-873. https://doi.org/10.1177/0192623312444471

Greene, E. S., H. Rajaei-Sharifabadi, and S. Dridi. 2019. Feather HSP70: a novel non-invasive molecular marker for monitoring stress induced by heat exposure in broilers. Poultry Science, 98: 3400-3404. https://doi.org/10.3382/ps/pez120

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, 25: 402-408. https://doi.org/10.1006/meth.2001.1262