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Heat stress induces expression of *HSP* genes in genetically divergent chickens

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Abstract

Background

Chickens are animals that are sensitive to thermal stress, which may decrease their production level in terms that it affects feed intake and thus, decreasing body weight gain. The Heat Shock Factors (*HSF*) and Heat Shock Proteins (*HSP*) genes are involved in the key cellular defense mechanisms during exposure in hot environments. Aimed with this study to analyze the expression of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes in two local breeds (Peloco and Caneluda) and a commercial broiler line (Cobb 500®) to verify differences in resistance of these chicken to Heat stress treatment. Chicken were submitted to heat stress under an average temperature of 39˚C ± 1.

Results

Under stress environment, the *HSP70* and *HSP90* genes were more expressed in backyard chickens than in broiler. There was a difference in *HSP70* and *HSP90* expression between Caneluda and Cobb and between Peloco and Cobb under stress and comfort environment respectively. HSP70 expression is higher in local breeds during heat stress than in a commercial broiler line. No significant differences were observed in the expression of *HSF1* and *HSF3* genes between breeds or environments.

Conclusions

HSP70 and *HSP90* genes are highly expressed, *HSF1* and *HSF3* genes did not have high expression in all genetic groups. *HSP70* and *HSP90* are highly expressed in Peloco and Caneluda within heat stress, these breeds proved to be very resistant to high temperature.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Poultry is one of the main sectors of the agribusiness producing thousands of tons of meat per year. However, environments with high temperatures may cause negative impacts on broiler' physiology and production, leading to economic losses [1,2]. Broilers reached high levels of production due to genetic improvement, on the other hand, its metabolism become more accelerate, presenting poor thermoregulation, and as consequence being not well adapted to hot environments [3–5]. Differently, native backyard chickens are more adapted to environments in which they live, with rusticity that allow them to survive and reproduce constantly. These chicken are more resistant to high temperatures [6,7], however, they have low productive levels since they did not undergone genetic improvement and have low investment in breeding [8,9].

There are factors that act on defense mechanism against high temperatures. The ability of homeostasis can minimize extracellular damage [10] by altering gene expression in the presence of stress and returning to basal conditions after returning to thermal comfort conditions [11,12]. One of the defense mechanisms is the activation of more than 500 genes in the first ten minutes of exposure [13–15]. Among these genes are the *Heat Shock Factor* (HSF) leading the induction of gene expression [16,17] and the *Heat Shock Proteins* (HSP) that are some of the main defenses against heat stress [11,12].

The HSF1 and HSF3 are considered the main genes of HSF family in response to heat shock in chicken. Has been believed that induction of HSF1 and HSF3 in regulate HSP were bird-specific, however, a recent study has demonstrated that HSF1 and HSF3 have also regulate HSP70 expression in lizards and frogs [18]. *HSF1* is activated at low temperatures while *HSF3* continues to be activated at higher temperatures and longer exposure [19].The *HSP70* and *HSP90* genes are the most studied HSPs family and each has different functions [1]. The *HSP70* binds to newly synthesized proteins, preventing aggregation and assisting in folding [20,21], whereas *HSP90* interacts with proteins in older stages of folding, in addition to modifying the configuration of these proteins [22].

Many studies have reported genes related to heat stress in mammals [23,24], plants [25], fish [26] and broiler [27,28], but so far no studies have been identified the relation of chicken resistance to thermal stress with the expression of HSF's and HSP's genes. Aimed with this study to analyze the expression of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes in two local breeds of chickens and a commercial chicken line in order to verify the resistance of these birds to heat stress.

Material and methods

Ethical approval

Experiment procedures were approved by the Ethics Committee on Animal Use—CEUA of Universidade Estadual do Sudoeste da Bahia (UESB), protocol 109/2015.

Animals

In this study, we used 36 male and female chickens, being 12 chicks of each breed (Peloco and Caneluda (backyard breeds), and Cobb 500® (commercial line)). Commercial birds were acquired a week after the birth of backyard chicken in the Universidade Estadual do Sudoeste da Bahia (UESB), Itapetinga, and raised under the same environmental conditions from November 2 to December 2 of 2015 with an average temperature of 26.5˚C. The predominant climate of Itapetinga region is semi-arid, in which the temperature increases during the day and decrease during the night. Nutritional diet followed the requirements of the Brazilian

Table 1. Initial feed used in the production of chicks up to 30 days of age (ROSTAGNO, GOMES, 2011).

Corn	61.1%
Soybean Meal	35.0%
Dicalcium Phosphate	2.00%
Limestone	1.10%
NaCl	0.30%
Vitamin And Mineral Supplement	0.40%
Nutritional Levels	
Crude Protein	21.2%
Metabolizable Energy	2.89%
Calcium	1.01%
Phosphor Available	0.49%
Sodium	1.63%
Lysine	1.10%
Methionine + Cysteine	0.74%

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Tables for Poultry and Swine [29] and the feed was produced in the poultry sector of UESB (Table 1). All chickens were raised in semi-open stalls and lined with wood shavings (wood chips).

Heat stress and collect of tissue samples

At 30 days of age all chicks were transferred to the climatic chambers. Birds were housed in groups of up to 12 chicks per cage. Heat stress was performed in two stages so that all chicken had the same slaughter age (30 days). First, six chicks of Peloco breed and six chicks of Caneluda breed were subjected to heat stress under an average temperature of 39.5˚C and environmental relative humidity of 60% for 30 minutes. In the second stage, six chicks of Cobb 500® line were subjected to heat stress with the same conditions of temperature, humidity and time. During the heat stress period, animals had *ad libitum* access to food and water.

During the heat stress, chickens were constantly observed for behavioral changes, in order to avoid deaths caused by excessive temperature. The acute heat stress was determined at the moment that most of the chicken (±90%) were prostrate (lying with the abdominal faced down), and with increased respiratory rate. Control chickens (six chicks of each genetic group) were slaughtered at the second stage of the experiment at 4 am (local time) to ensure thermal comfort temperature (23˚C). All chicks (Heat stressed and comfort) were slaughtered by cervical dislocation.

After slaughter, samples of *Pectoralis major* muscle were collected, placed in cryogenic tubes, identified and stored in liquid nitrogen. Samples were transported to the Veterinary Genetics Laboratory at the Universidade Estadual de Santa Cruz (UESC), separated and stored in Ultrafreezer (-80˚C).

Extraction, quantification and quality of total RNA

For total RNA extraction, kit SV Total RNA Isolation System® (Promega Corporation, Madison, USA) was used according to manufacturer's protocol. The concentration and quality of RNA were verified by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc, Carlsbad, CA, USA) using the absorbance at 230, 260, 280nm. Besides, RNA integrity was analyzed by the presence of bands corresponding to 28S and 18S ribosomal RNAs observed through electrophoresis of 1 ug of RNA in 1% agarose gel stained with ethidium bromide.

Table 2. Description of *G. gallus* genes related to heat stress, reference genes for chickens and their specific primers used in RT-qPCR analyzes. The primers of *HSF3*, *HSP70* and *HSP90* genes were designed by ALMEIDA, (2007).

GENE	DISCRIPTION	SEQUENCE (5'-3')	FUNCTION
HSF1*	Heat shock factor protein 1	F: TGTGGCTGATTCTTGGCTTT	Heat shock response
		R: GAGGGAGACAGAGGGGTTTC	
HSF3	Heat shock factor protein 3	F: CGGAAGATGGAAATGGAGAG	Heat shock response
		R: TCAGGAAGCAGGAGAGGAGA	
HSP70	Heat shock protein 70kDa	F: ATTCTTGCGTGGGTGTCTTC	Heat shock response
		R: GATGGTGTTGGTGGGGTTC	
HSP90	Heat shock protein 90kDa	F: TGAAACACTGAGGCAGAAGG	Heat shock response
		R: AAAGCCAGAGGACAGGAGAG	
MRPS27**	Mitochondrial ribosomal protein S27	F: GCTCCCAGCTCTATGGTTATG	Reference gene
		R: ATCACCTGCAAGGCTCTATTT	
RPL5**	Ribosomal protein L5	F: AATATAACGCCTGATGGGATGG	Reference gene
		R: CTTGACTTCTCTCTTGGGTTTCT	
MRPS30**	Mitochondrial ribosomal protein S30	F: CCTGAATCCCGAGGTTAACTATT	Reference gene
		R: GAGGTGCGGCTTATCATCTATC	

*Primer drawn by the authors of this work;

**Reference Genes obtained in previous studies [31].

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Reverse transcription of mRNA

The commercial kit GoScript TM Reverse Transcription System (Promega Corporation, Madison, USA) was used for reverse transcription of mRNA. Up to five micrograms of total RNA from samples were mixed to 1μl of Oligo(dT) (500μg/ml) and heated in 70˚C for 5 minutes. After incubation, 4μl of 5X Reaction Buffer, 3.2μl $MgCl_2$, 1μl dNTP (0,5mM), 1μl of reverse transcriptase enzyme, 0.5μl of inhibitor of recombinant ribonuclease RNaseOUT (20units) and ultrapure water completing 15μl. This mix were added to RNA+OligodT mix completing a volume total of 20μl and incubated on a thermocycler. Anneal at 25˚C for 5 minutes; extend at 42˚C for one hour, and 70˚C for 15 minutes to inactivate the reverse transcriptase. After reverse transcription, cDNA was stored at -20˚C. The concentration of cDNA was measured by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc, Carlsbad, CA, USA) using the absorbance at 230, 260, 280nm.

Target gene selection and optimization of RT-qPCR

Four target genes involved in regulation of heat stress in *Gallus gallus* were selected to be evaluated in different genetic groups (Table 2). To obtain the standard curve, we used a cDNA pool of all treatments aiming to optimize and calculate the PCR efficiency. We used three cDNA concentrations (15, 45 and 135ng/μl) and three primer concentrations (200, 400, 800 mM).

RT-qPCR reaction conditions were set with initial denaturation temperature at 95˚C for two minutes, and 40 cycles of denaturation at 95˚C for 15 seconds. The extension temperature was individually standardized for each pair of primer for 60 seconds. At the end of amplification reaction, we included an additional step with gradual temperature increasing from 60 to 95˚C for dissociation curve analysis. Amplification of all genes was performed in duplicate in a 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Results were obtained by using the Sequence Detection Systems software (V. 2.0.6) (Applied Biosystems Foster City, CA, USA) that generated the cycle threshold (Ct) parameter. The Ct values of duplicates were obtained directly from the above program and used to calculate the average Ct

and standard deviation. PCR amplification efficiency was calculated for each reference gene using the following formula: $E = (10^{(-1/slope)-1})x100$ [30]. After efficiency analysis, the most appropriate annealing temperature and primer concentration were used in PCR reactions.

Real time quantitative PCR

The reaction of RT-qPCR was performed using SYBR Green detection kit with GoTaq qPCR Master Mix (Promega, Madison, WI, EUA), using specific primers. Gene amplification was performed in duplicate using the Real Time PCR 7500 Fast system (Applied Biosystems, Foster City, CA, EUA) and results were obtained with the *Sequence Detection Systems* program (V. 2.0.6) (Applied Biosystems, Foster City, CA, EUA) that generated the *cycle threshold* (Ct) parameters.

The Ct values were exported to Microsoft Excel to calculate the Ct mean, standard deviation and the standard curve for each gene. A negative control (ultra-pure water) also was added in each assay. The qPCR reaction conditions were defined as follow: Initial denaturation at 95˚C during ten minutes and 40 cycles of denaturation at 95˚C for 15 seconds. The extension temperature between 60 and 64˚C during one minute was ideal for all primers. Ct values of control wells were excluded from subsequent analyzes as well as the undetectable values.

Statistical analysis of target genes

To perform the statistical analysis, %QPCR_MIXED [32] was used in the statistical software SAS® 9.0. This macro performs analyzes by mixed linear models of RT-qPCR data. The program normalizes the data using the ΔΔCT method [33], thus generating *Fold Change*, which is the value of the relative expression between the control and the treatment [34].

In order to determine if there was difference between treatments (genetic groups and environment), contrasts were made between the factors comparing them to each other. Within this statistical model, the effects of genetic groups (Caneluda, Cobb and Peloco) and environment (comfort and thermal stress) were considered fixed, and the Genes factor was considered random. In this way it is possible to test the linear combinations between the levels of these factors (Comfort X Stress); (Cobb X Caneluda, Caneluda X Peloco and Cobb X Peloco) and also the variability between the genes in each treatment/breed, besides the interaction effects. Were considered different contrasts those that obtained p-value ≤ 0.05.

Results

Efficiency and specificity of primers

Prior to performing expression analysis of the interest genes, we performed efficiency test. The annealing temperature of 62˚C was determined as optimal for all primers. The amplification efficiency varied between 93% and 105% corresponding to slope between -3.49 and -3.20. The coefficient of determination (R^2) values were higher than 0.99 (Table 3). The primers specificity was evaluated through the dissociation curve, which showed only one peak indicating no primer dimers were detected and presenting excellent performance (Fig 1).

Descriptive statistics of target genes

Descriptive statistics were performed using BestKeeper tool [27]. It is possible to notice an expression variability through quantification cycles in four target genes, which was grouped into two categories (strong and moderate). Three genes (*HSP70*, *HSF1* and *HSP90*) had strong mRNA expression, with Ct values varying between 16 and 27 cycles, and one gene (*HSF3*) with moderate expression with 34 cycles [35] (Table 4).

Table 3. Parameters of the specific primers of genes related to thermal stress and reference genes for broilers obtained from the analysis of efficiency curve in RT-qPCR.

GENE	AT (˚C)	[CDNA]	[PRIMER]	EFFICIENCY (%)	R^2	SLOPE
HSF1	62	45ng/μl	800mM	93	0.996	-3.494
HSF3	62	45ng/μl	800mM	101	0.999	-3.300
HSP70	62	45ng/μl	400mM	105	1	-3.199
HSP90	62	45ng/μl	400mM	102	0.999	-3.266
MRPS27	62	45ng/μl	800mM	105	0.998	-3.201
RPL5	62	45ng/μl	800mM	102	0.999	-3.284
MRPS30	62	45ng/μl	800mM	105	0.999	-3.207

AT = Annealing Temperature; *SLOPE* = Slope of the Line; R^2 = Coefficient of Determination; [CDNA] = cDNA Concentration; [PRIMER] = Primer Concentration

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Relative expression of target genes

According to previous analysis [31], the MRPS27, RPL5 and MRPS30 genes were considered stable under genetic groups and environment factors which was performed a normalization factor by geometric mean. Therefore, these genes were used to normalize the relative expression of target genes.

Comparing environments (stress X comfort), *HSF1* and *HSF3* genes were not significantly different among the three genetic groups. On the other hand, the *HSP70* genes had high expression and were statistically different within the three genetic groups (Table 5/Fig 2).

In thermal stress analysis, comparisons between genetic groups (Caneluda X Cobb, Caneluda X Peloco and Cobb X Peloco) were not significant for *HSF1*, *HSF3* and *HSP70* genes. In contrast, *HSP90* gene had a difference in relative expression in Cobb line compared to Caneluda and Peloco. In the comparison between Caneluda X Peloco, none of the analyzed genes showed a significant difference in relative expression (Table 6/Fig 3).

Breeds were also compared within thermal comfort. Only the *HSP70* and *HSP90* genes had statistically significant relative expression comparing Cobb X Caneluda and Cobb X Peloco. Comparing Caneluda X Peloco, none of the four genes showed difference in relative expression (Table 7/Fig 4).

Comparing the genetic groups without considering environments, it was possible to notice that the commercial line Cobb 500® had different relative expression in relation to the native breed Peloco (Peloco X Cobb) for all genes. While comparing Cobb 500® to Caneluda (Caneluda X Cobb), there was only a significant difference for *HSP70* gene. In the comparison between Caneluda X Peloco, none of the four genes showed a significant difference in relative expression (Table 8/Fig 5).

Discussion

High temperatures can cause several damages to livestock production, especially in poultry farming causing financial losses. In addition to the technological mechanisms that try to alleviate the thermal stress in chicken, there are physiological factors that decrease the effects of heat. According to DE NADAL et al., (2011) [10] exposure to thermal stress can promote expression of genes related to survival while not expressing less essential genes, resulting in the rapid expression of Heat Shock Factors (HSF) and Heat Shock Protein (HSP) [36,37]

In this study, *HSF1* and *HSF3* genes showed low relative expression in all treatments (heat stress and thermal comfort), and had a difference in expression for Cobb compared to Peloco. These genes are not well expressed in acute thermal stress. *HSF1* is activated in medium heat

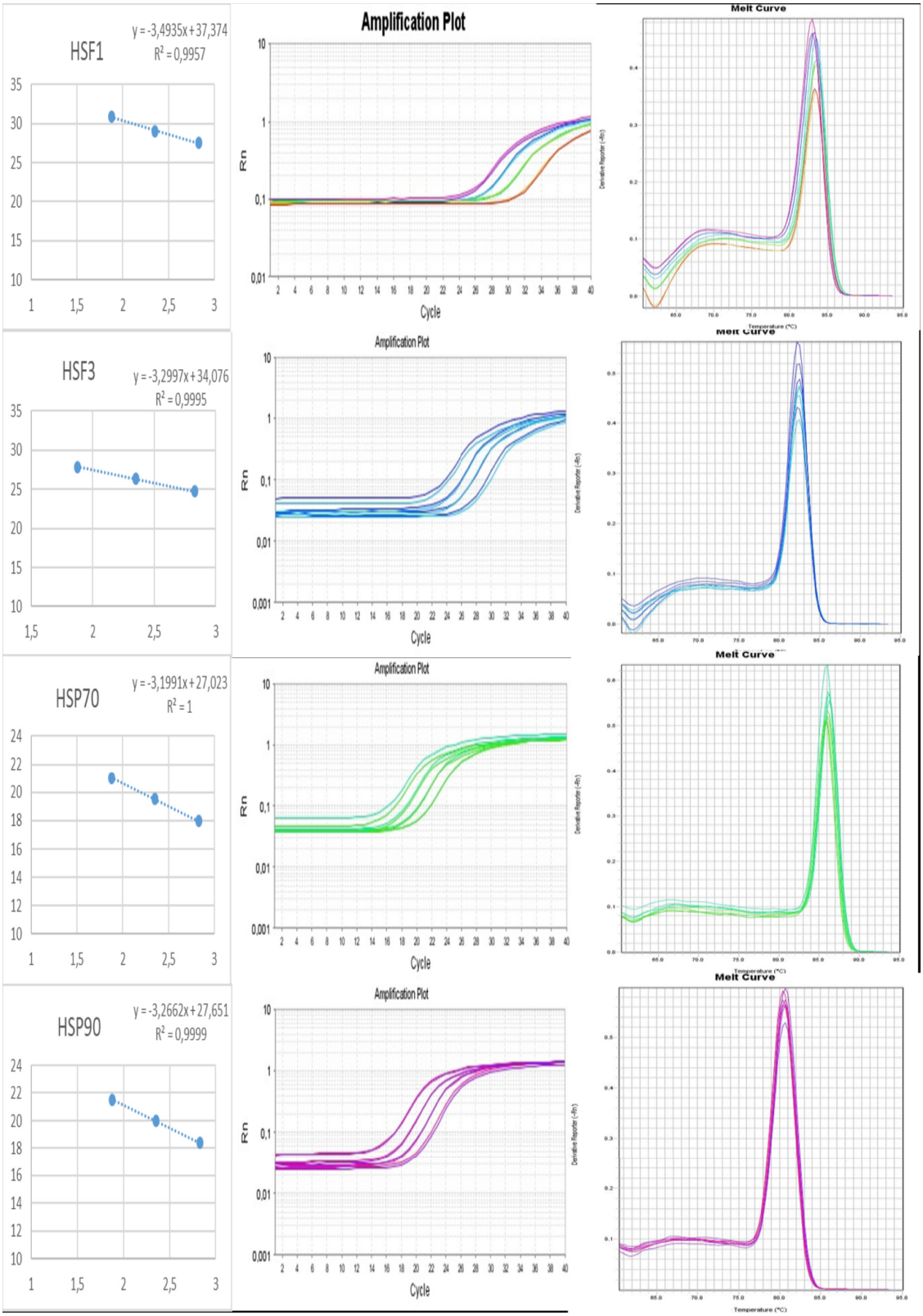

Fig 1. Regression curves, amplification and dissociation of the efficiency test for the 4 target genes (*HSF1*, *HSF3*, *HSP70* and *HSP90*) from broilers derived from RT-qPCR reactions. All dissociation curves show only one peak.

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Table 4. Descriptive statistics and expression levels of target genes related to heat stress in broilers (n = 36).

n = 36	HSF1	HSF3	HSP70	HSP90
geo Mean [Ct]	29.15	26.12	20.97	20.26
ar Mean [Ct]	29.19	26.16	21.16	20.33
min [Ct]	26.49	23.39	16.47	17.86
max [Ct]	33.92	30.25	26.35	24.96
std dev [± Ct]	**1.26**	**1.07**	**2.44**	**1.35**
CV [% Ct]	**4.33**	**4.08**	**11.54**	**6.66**
coeff. of corr. [r]	0.746	0.319	0.251	0.177

Abbreviations: [Ct] Cycle threshold; geo Mean [Ct]: Geometric mean of Ct; ar Mean [Ct]: Arithmetic mean of Ct; Min [Ct] and Max [Ct]: Ct threshold values; std dev [±Ct]: Standard deviation of Ct; CV [% Ct]: Coefficient of variation of Ct levels expressed as a percentage; SD and CV are indicated in bold.

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stress whereas *HSF3* is activated in chronic thermal stress [19,37]. This suggests that *HSF3* may play an important role in long periods of heat stress in chicken [36].

Some studies in humans [38] and plants [39–41] have shown that different types of stress can promote the HSF family genes expression. The response of HSF genes during thermal stress may be involved in expression of HSP's genes [1,42], however, this mechanism is not yet well known [43]. *HSF1* induces only *HSP70* [44] whereas *HSF3* promotes the expression of all HSPs in chicken [45]. In addition, PRAKASAM et al. (2013) [46] have demonstrated that *HSF3* is also involved in the expression of IL-6 pyrogenic cytokine during thermal stress.

Heat shock proteins produce responses to temperature rise and are driven by some factors besides heat, such as microbial infection, tissue trauma and genetic injury [47]. In this study, *HSP70* and *HSP90* genes had a significant difference in relative expression in all comparisons, especially while comparing native chicken to commercial line Cobb, since the last one is more sensitive to heat stress [48–52].

In comparison between thermal comfort and heat stress, Caneluda and Peloco had high expression of *HSP70* while Cobb had medium relative expression in a thermal stress environment. Even with high expression of *HSP70* gene, the local breeds remained comfortable during the thermal stress, while commercial line chicken showed a great level of discomfort, suggesting that the genes played a protective role. The *HSP90* gene had medium expression in the three genetic groups. In heat environments, *HSP70* gene expression plays a better role in cellular functions than *HSP90* [47].

Within the thermal comfort environment, Cobb chicken had higher expression of *HSP70* and *HSP90* than Caneluda and Peloco, even though the expression of these genes has been

Table 5. Relative expression analysis of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes in the different genetic groups of chicken comparing the comfort and thermal stress environments.

Gene	Comparison between treatment within genetic groups					
	Caneluda		Cobb		Peloco	
	Comfort X Stress		Comfort X Stress		Comfort X Stress	
	FC	*p-value*	FC	*p-value*	FC	*p-value*
HSF1	1.42	0.23	-1.17	0.58	-1.04	0.88
HSF3	1.03	0.90	-1.29	0.26	-1.35	0.19
HSP70	**15.71**	**<.0001**	**7.54**	**<.0001**	**22.67**	**<.0001**
HSP90	**5.92**	**<.0001**	**4.92**	**<.0001**	**5.01**	**<.0001**

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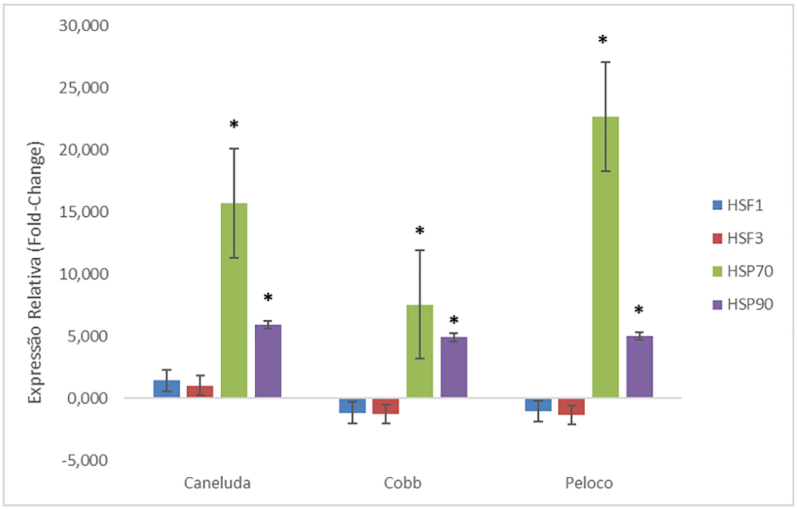

Fig 2. Relative expression analysis of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes in different chicken genetic groups comparing comfort and thermal stress environments. *p-Value <0.05.

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low. As commercial chicken are genetically improved for production traits [3], the maintenance characteristics are decreased and these chickens are not adapted to warm environment conditions [8]. In this way, breeding environment could influence the expression of these genes in terms that in production environment the temperature was high and not controlled. Besides that, natural selection has been acting in Peloco and Caneluda chickens making them resistance to high temperatures from tropical weather, which seems have a negative correlation between production traits and heat resistance.

In relation to heat stress environment, only *HSP90* gene had significant expression in Caneluda animals compared to Cobb (p-value = 0.0004) and in Peloco chicken also compared to Cobb line (p-value = 0.0003). Comparing Caneluda and Peloco, there was no significant difference in *HSP90* expression. Caneluda and Peloco are extensively reared animals, being more adapted to the warm environment and able to stay under thermal stress easily than Cobb, therefore, these wild chicken are more resistant to heat, even at temperatures higher than they are used to.

In gene expression analysis without considering heat stress and thermal comfort, there was difference in expression only in the comparison between Cobb and Peloco for all evaluated genes. The *HSF1*, *HSF3* and *HSP70* genes were more expressed in Peloco, while *HSP90* was more expressed in Cobb. In comparison between Caneluda X Cobb there was no significant

Table 6. Relative expression analysis of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes comparing the different chicken genetic groups within the thermal stress environment.

Gene	Comparison between genetic groups under heat stress					
	Stress		Stress		Stress	
	Cobb X Caneluda		Caneluda X Peloco		Cobb X Peloco	
	FC	*p-value*	FC	*p-value*	FC	*p-value*
HSF1	-1.00	1.00	1.51	0.16	1.51	0.16
HSF3	1.02	0.92	1.44	0.11	1.47	0.09
HSP70	1.51	0.26	-1.24	0.56	1.22	0.58
HSP90	**-2.14**	**<.0001**	-1.03	0.89	**-2.20**	**<.0001**

https://doi.org/10.1371/journal.pone.0186083.t006

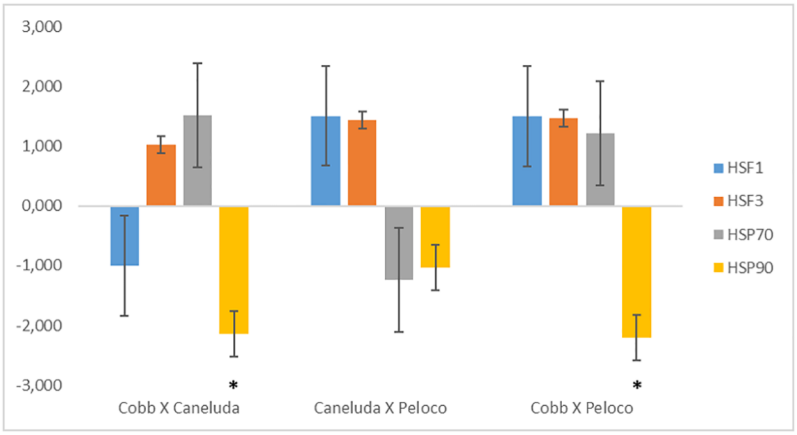

Fig 3. Relative expression analysis of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes comparing the different chicken genetic groups within the thermal stress environment. *p-Value <0.05.

https://doi.org/10.1371/journal.pone.0186083.g003

Table 7. Relative expression analysis of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes comparing the different chicken genetic groups within the thermal comfort environment.

Gene	Comparison between genetic groups under heat comfort					
	Comfort		Comfort		Comfort	
	Cobb X Caneluda		Caneluda X Peloco		Cobb X Peloco	
	FC	*p-value*	FC	*p-value*	FC	*p-value*
HSF1	1.65	0.08	1.02	0.94	1.69	0.07
HSF3	1.36	0.18	1.04	0.88	1.41	0.13
HSP70	**3.15**	**<.0001**	1.16	0.68	**3.67**	**<.0001**
HSP90	**-1.78**	**0.01**	-1.22	0.34	**-2.16**	**<.0001**

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difference in expression of HSF's genes, however, the *HSP70* gene was more expressed in Caneluda and the *HSP90* more expressed in Cobb.

Some studies have shown changes in HSP expression from heart, liver, kidney, blood and muscle of broilers [1,53,54]. The results of *HSP70* and *HSP90* genes presented in this study are in agreement with those reported by LEI et al. (2009) [54], XIE et al., (2014)[1] and YU et al.,

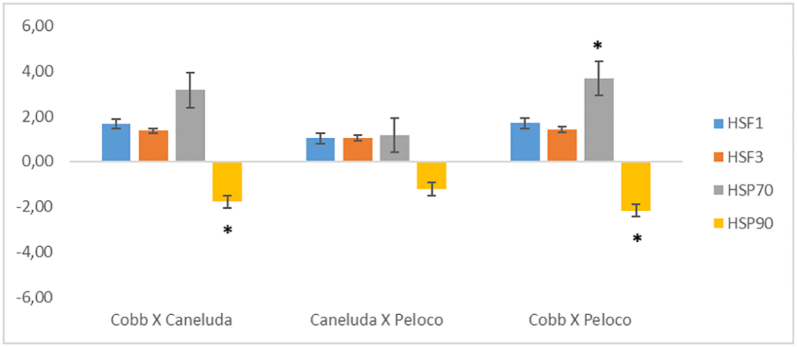

Fig 4. Relative expression analysis of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes comparing the different genetic groups of chicken within the thermal comfort environment. *p-Value <0.05.

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Table 8. Relative expression analysis of HSF1, HSF3, HSP70 and HSP90 genes comparing the different genetic groups of chicken without considering the environments (comfort and thermal stress).

Gene	Comparison between genetic groups without considering the environments					
	Cobb X Caneluda		Caneluda X Peloco		Cobb X Peloco	
	FC	*p-value*	FC	*p-value*	FC	*p-value*
HSF1	1.29	0.22	1.24	0.29	**1.59**	**0.02**
HSF3	1.18	0.31	1.22	0.21	**1.44**	**0.02**
HSP70	**2.18**	**<.0001**	-1.03	0.90	**2.12**	**<.0001**
HSP90	**-1.95**	**<.0001**	-1.12	0.44	**-2.18**	**<.0001**

https://doi.org/10.1371/journal.pone.0186083.t008

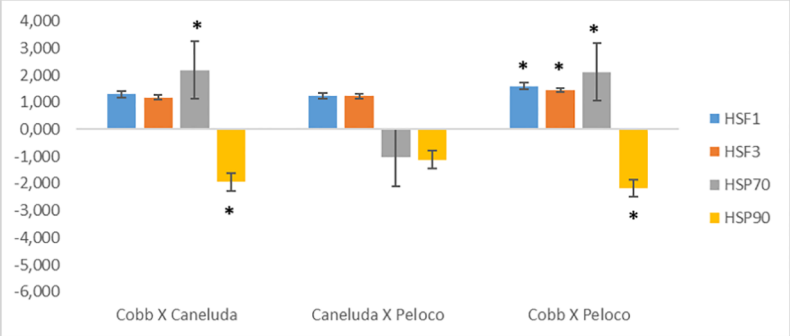

Fig 5. Relative expression analysis of *HSF1*, *HSF3*, *HSP70* and *HSP90* genes comparing the different genetic groups of chicken without considering the environments (comfort and thermal stress). *p-Value <0.05.

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(2008)[53]. *HSF1* and *HSF3* genes showed different results than those observed by XIE et al., (2014) [1], which reported high expression of these genes in chickens submitted to thermal stress. This inconsistency of results may have been due to the induction method at high temperatures and the time of exposure of the animals.

It is important to have more studies using these genetic groups to construct a molecular profile in relation to thermal stress, using these and other genes of the HSF's and HSP's family, besides genes that are directly and indirectly related to thermal stress in chickens.

Conclusion

Given the above, it can be stated that *HSP70* and *HSP90* genes are highly expressed in all evaluated genetic groups. The *HSF1* and *HSF3* genes did not have high expression in the studied genetic groups neither in comfort and stress environments, whereas *HSP70* and *HSP90* are highly expressed in Peloco and Caneluda within thermal stress, these breeds proved to be very resistant to high temperature.

Supporting information

S1 Table. Data of target gene expression. The file contains raw data of Target Genes expression as Ct. 32 samples, two factors and four target genes.
(XLSX)

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