

# Effect of thermal stress on the expression of HSP<sub>70</sub>, HSP<sub>90</sub> and on physiological parameters of Dairy Gyr young bulls in Brazil.

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## Summary

The Heat Shock Proteins (HSP) is a group of proteins that play a key role in cell protection against the effects of thermal stress. The present work investigated the effect of thermal stress on the expression of HSP<sub>70</sub>, HSP<sub>90</sub> and on the physiological parameters: rectal temperature (RT), heart rate (HR) and respiration rate (RR) in young bulls of the Dairy Gyr breed in Brazil. The animals were taken to the cattle shed in two periods: a) early in the morning and b) -after submitted to the sun during two hours (from 11:00 to 13:00hs). Following identification, RT, HR and RR were recorded and blood samples for HSP analyzes collected, by period (before and after exposure to the sun). For analysis of the expression of HSPs, the genetic material was extracted from leukocyte. Statistical analyzes were performed using the SPSS program, version 16.0. The air temperature was higher in the afternoon (25 x 40 p < 0, 001). Although the elevation of the temperature in the afternoon was sufficient to significantly alter the RT and the expression of HSP<sub>90</sub>, RR, HR and HSP<sub>70</sub> expression were not significantly affected. Possibly the time of sun exposure and environmental temperature were not enough to alter all the physiological parameters, because the Dairy Gyr is a zebu breed adapted to the tropical climate.

*Keywords: adaptation, Bos indicus, heat shock protein and heat stress.*

## Introduction

Selection for high milk production in cattle has led to productive and reproductive problems mainly when animals are submitted to thermal stress (Joksimović-Todorović, et al, 2011). Santana Jr et al (2015) related that in the Dairy Gyr population in Brazil, there is a trend of increase in the production levels with consequent loss in thermotolerance. Associated with this, it is mentioned that the effects of global warming should contribute to change the selection objectives and that more adapted species and breeds tend to be more used for food production (Hoffmann, 2010).

Some cellular mechanisms are activated during the process of thermal stress. Cellular tolerance to heat stress is mediated by a group of proteins called Heat Shock Proteins (HSP). HSPs can be grouped into families: HSP<sub>27</sub>, HSP<sub>47</sub>, HSP<sub>60</sub>, HSP<sub>70</sub>, HSP<sub>90</sub> and HSP<sub>110</sub>

according to their amino acid sequences and their molecular weights (Jäättelä & Wissing, 1992). These proteins act in cellular protection against temperature, preventing protein denaturation and blocking apoptosis (Basiricò, et al, 2011). Polymorphism for alleles of HSP<sub>70</sub> and HSP<sub>90</sub> were described in taurine, zebu and cross breeds with significant effect for heat tolerance (Basiricò, et al, 2011; Charoensook et al, 2012; Deb et al, 2013 and Bhat S., et al, 2016).

Among the cattle breeds, *Bos indicus*, presents a better response to thermal stress than *Bos taurus* (Beatty et al, 2006; Hansen, 2004). The Dairy Gyr breed has the first breeding program of a zebu breed for milk purpose in the world, having started progeny test in Brazil in 1985 (Vercesi Filho et al, 2010). It is the genetic base most used in cross-breeding schemes with taurine breeds for the production of the dairy population which are responsible for most of the milk produced in the country (Madalena, 2001).

The present study aimed to investigate the effects of the exposure of young bulls of the Gir Leiteiro race to thermal stress in the expression of HSP70 and HSP90 and in the physiological parameters.

## **Material and methods**

### **Data collection**

A total of 53 Dairy Gyr young bulls, candidates for the progeny test, aged 22-46 months, were included. The animals were kept at the School Farm of the Faculdades Associadas de Uberaba (FAZU), in the municipality of Uberaba-MG.

The animals were taken to the cattle shed in different periods: a) early in the morning and b) -after submitted to the sun during two hours (from 11:00 to 13:00hs - afternoon). Following identification RT, HR and RR were recorded and blood samples for HSP analyzes collected, by period.

### **Laboratorial analyses**

Blood was collected from the caudal vein under sterile conditions and kept on ice. The samples were centrifuged for plasma collection and leukocyte separation. The total RNA of leukocytes was isolated using PurelinkRNAMiniKit(Invitrogen, Carlsbad, CA) and stored at -80 ° C until the reverse transcription and PCR (RT-PCR) were performed.

After RNA extraction and cDNA synthesis, the samples were submitted to polymerase chain reactions (PCR) and subsequent analysis of the amplified gene fragments. The expression levels of the HSPs by period were determined by PCR using the genes HSP70, HSP90 and GAPDH (as a house keeping gene), the primers oligonucleotides are described in Table 1. Each gene was amplified in a separate reaction and each reaction performed in triplicate.

### **Statistical analyses**

Statistical analyzes were performed using the SPSS program, version 16.0. The distributions of the physiological (RT, RR, HR, HSP<sub>70</sub> and HSP<sub>90</sub>) and environmental (temperature and humidity) variables, did not meet the assumption of normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene test). The comparisons of the variables between the periods (morning and afternoon) were performed by the Wilcoxon

signed-rank test (paired difference test) for the paired periods per animal at the 95% confidence level.

## Results and Discussion

The descriptive statistics of the variables and the results of the statistical analysis are presented in table 1.

Table 1 - Descriptive statistics and statistical analysis

Periods	Estatistics	T	H	RT	RR	HR	HSP70	HSP90
Morning	N	53	53	53	53	53	49	47
	Mean	25.05	71.64	38.98	33.77	83.66	14.24	32.29
	SD	1.90	7.74	0.33	5.89	12.40	8.88	31.07
	Median	24.90	72.00	38.90	32.00	80.00	14.12	21.85
Afternoon	N	53	53	53	53	53	51	52
	Mean	40.29	30.92	39.23	35.02	84.45	23.50	44.50
	SD	1.37	2.38	0.33	7.52	13.89	23.52	33.90
	Median	40.30	30.50	39.20	32.00	80.00	14.46	36.79
<b>Wilcoxon signed-rank test</b>								
Negative rank	N	0	53	7	21	23	19	15
	Mean Rank	0	27	17.07	21.69	22.35	19.42	19.53
	Sum of rank	0	1431	119.50	455.50	514.00	369.00	293.00
Positive rank	N	53	0	42	27	24	27	31
	Mean Rank	27	0	26.32	26.69	25.58	26.37	25.42
	Sum of rank	1431	0	1105.50	720.50	614.00	712.00	788.00
Morning - afternoon	Z	- 6.33	- 6.33	- 4.94	- 1.37	- 0.53	- 1.87	- 2.70
	P	<0.001	<0.001	<0.001	0.172	0.594	0.061	0.007

SD- Standard Deviation; T- Air Temperature °C, H- Relative Humidity%; RT- Retal Temperature; RR- Respiration Rate, HR- Heart Rate, HSP<sub>70</sub>- Heat Shock Proteins 70 , HSP<sub>90</sub>- Heat Shock Proteins 90

Air temperature and humidity were significantly different between periods. Thus the temperature and humidity index (THI) in the morning was 73.87 indicating mild stress and 86.55 in the afternoon which indicates moderate stress. According to Hansen (2004) *Bos indicus* cattle present higher thermo-tolerance than *Bos taurus*. Consequently, these climatic parameters were not sufficient to significantly alter RR and HR in the Dairy Gyr breed. However, the RT and the expression of HSP<sub>90</sub> had a significant increase between the periods and the expression of HSP<sub>70</sub> presented a tendency to increase (p = 0.061) which may be indicative of low heat stress. The measures of HSP<sub>s</sub> expression showed a large individual variation that can be verified by the standard deviations of these traits (Table 1). Various environmental factors besides thermal stress can alter the expression pattern of this protein such as infection, inflammation, exercise, exposure of the cell to toxins, etc (Archana, et al, 2017). Therefore, repeated measurements on the same animal are indicated so that these environmental effects can be minimized.

## Conclusion

Although the average temperature in the afternoon reached 40C, it was not enough to significantly alter all the traits studied, which confirms the thermotolerance of the Dairy Gyr breed. To minimize environmental effects it is recommended that repeated measurements be made mainly for the expression of HSPs

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