EVALUATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS ON MEAT QUALITY, NEUROENDOCRINE AND METABOLIC TRAITS

Mirele D. Poleti[1], Bárbara Silva[1], Cristina T. Moncau[1], Vanessa A.M. Silva[1], Alessandra F. Rosa[1] and Júlio César de C. Balieiro[1]

[1]Department of Basic Science, College of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo State, Brazil

Abstract – Exploration of the Hypothalamic-pituitary-adrenal (HPA) axis is an important point to the study of stress and welfare in animals. The present work on 50 Nellore cattle identified single nucleotide polymorphisms (SNPs) in glucocorticoid (GR) and mineralocorticoid (MR) receptors genes, central genes of the HPA axis, in order to understand individual differences in physiological responsiveness of cattle at slaughter. Associations between SNPs and meat quality traits also were evaluated. There were five SNPs identified by DNA sequencing, three in the MR gene and two in the GR gene. Plasma ACTH and cortisol were determined in the feedlot and at slaughter. Glycogen and lactate content were measured in *Longissimus dorsi* muscle collected 1 hour *postmortem*. All SNPs found were associated with at least one of traits analysed. SNPs in MR gene had more association with meat quality parameters, mainly cooking loss. The SNPs found in the GR gene were more associated with neuroendocrine function.

Key Words – HPA axis reactivity, stress, tenderness

I. INTRODUCTION

In response to stressful situation the hypothalamic-pituitary-adrenal (HPA) axis is activated stimulating the secretion of glucocorticoids hormone, as cortisol, for maintenance of metabolic homeostasis. Cortisol exerts its effects through mineralocorticoid (MR) and glucocorticoid (GR) receptors, which determine the sensibility of the stress system and promote the termination of the stress response [1].

Various studies have shown that stress responsiveness and aggressiveness is highly variable among individuals [1,2,3]. The resilience or vulnerability of any one individual to stressful situation depend genetic predisposition and early life experiences [4].

In humans, gene variants in MR and GR have been associated with variation in stress responsiveness [5].

In livestock, the stress has serious economic implications and in meat quality can result in higher ultimate pH or faster early *post mortem* pH decline with consequent dark cutting condition [6]. Thereby, it is possible observe that many polymorphisms are being described by relating physiological variations with HPA axis. However, this is well documented in humans. We propose an innovative context to genetic variability related stress responsiveness in cattle at slaughter and their possible implications with meat quality traits.

The aim of this work was identify single nucleotide polymorphisms (SNP) in glucocorticoid and mineralocorticoid receptors genes as potential candidates for different stress responses observed in animals and evaluate the relationship with meat quality.

II. MATERIALS AND METHODS

Fifty cattle of the Nellore breed with approximately 2 years old were randomly chosen and used in this experiment. They were feedlot finished and slaughtered in accordance with Brazilian legislation.

The genomic DNAs were isolated by standard techniques from total blood collected. The MR and GR gene primers were designed to amplify the exon 1 and 2, respectively. Polymerase chain reactions (PCR) of genomic DNA fragments were performed and the fragments were sequenced directly by DYEnamic ET Terminator Cycle Sequencing (GE Healthcare) with an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems).

Electropherogram were analysed with Phred/ Phrap and Consed softwares.

At slaughter the pH and temperature of carcass were measured at 1 and 24 hours *postmortem*. Samples of *Longissimus dorsi* (LD) muscle were collected 45 minutes *postmortem* for lactate and glycogen determination. Carcasses were split, weighed and then chilled at 0-2ºC before processing on the following day after slaughter. At 24 hours *postmortem*, four steaks of two inches thick each were removed from LD muscle between the 12th and 13th rib. The steaks were vacuum packaged and kept at 2-4°C for until 14 days for subsequent Warner-Bratzler Shear Force (WBSF) determination. At 1st, 7th and 14th days, the samples were removed of cold chamber and after 20 min. bloom time, LD color was evaluated objectively by using a portable spectrophotometer. After, steaks were cooked in electric broiler cookery to an internal temperature of 40°C, flipped, and cooked to a final internal temperature of 71°C. Steaks were stored overnight at 8°C, subsequently eight 1.27 cm diameter cores were removed from each steak parallel to the fiber direction. Each core was sheared once perpendicular to the muscle fibers using the WBSF equipment with a crosshead speed of 250 mm/min. Color measurements were expressed in the form of L*, a* and b* (black to white, green to red, and blue to yellow, respectively).

Blood samples for hormonal measurements were collected at the start in the feedlot (*in vivo*) and during exsanguinations at slaughter (*postmortem*). Immediately after collected they were centrifuged and the plasma divided into aliquots and storage at -20°C until analysis. The cortisol and adrenocorticotropic hormone (ACTH) were measured by an immunoenzymatic kit from Monobind Inc. and BioAmerica, respectively. For glycogen and lactate determination approximately 0.5g of LD muscle were homogenized in 2.5 mL of 0.6M percloric acid for 1 min. The lactate and glycogen content were assessed with assay EnzyChrom™ Glycogen Assay Kit (BioAssay Systems, Hayward, USA) and EnzyChrom™ L-Lactate Assay Kit (BioAssay Systems, Hayward, USA).

Analysis of individual associations for each marker with meat quality and neuroendocrine traits were evaluated using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). The following model was used:

$$Y_{ijkl} = \mu + S_i + C_j + \beta_1\left(I_{ijk} - \bar{I}\right) + e_{ijkl}$$

where Y_{ijkl} = observed values for quantification obtained for different traits; μ = common constant for all observations; S_i = is the random effect from sire (with mean 0 and variance σ^2_S); C_j = is the fixed effect of slaughter year j; b_1 = regression coefficient, included in model as covariate; I_{ijk} = alleles favorable number (0, 1 or 2 favorable alleles for MR or GR); $\bar{I}$ = is mean of favorable alleles for MR or GR; e_{ijkl}= random effect associated at Y_{ijkl} (with mean 0 and variance σ^2_e).

III. RESULTS AND DISCUSSION

A total of 50 animals were used for sequencing and identifying of polymorphisms. We found five SNPs in the studied genes, being three in MR gene and two in GR gene. Results for genotypic and allelic frequencies are presented in Table 1.

The GR1 and MR1 SNPs result in non-synonymous mutations, whilst the others SNPs result in synonymous mutations. In GR1, the CAG → CGG codon variation results in a glutamine to arginine change and in the MR1 TCC → CCC codon variation results in a serine to proline change.

Genetic variation can be derived from a wide range of different change in the DNA sequence and the SNPs are currently the most studied. SNPs in MR and GR gene have been found and associated with several modes of HPA axis reactivity in human [5].

Table 1 Genotypic and allelic frequency for SNPs found in GR and MR genes

Gene	Chromossome Position	Genotypic frequency			Allelic frequency	
GR1	53,945,411	AA 0.78	AG 0.22	GG ---	A 0.89	G 0.11
GR2	53,866,113	TT 0.06	TC 0.32	CC 0.62	T 0.42	C 0.58
MR1	10,410,611	TT 0.06	TC 0.34	CC 0.60	T 0.22	C 0.78
MR2	10,411,070	TT 0.04	TC 0.28	CC 0.68	T 0.18	C 0.82
MR3	10,411,322	GG 0.86	GA 0.14	AA --	G 0.93	A 0.07

The estimative of means, standard deviation, minimum and maximum measurements recorded for meat quality and temperament (cortisol and ACTH) are presented in Table 2.

Table 2 Estimative of Means, Standard Deviation (SD), minimum value (Min.) and maximum value (Max.) for neuroendocrine, metabolic and physical-chemistry traits in meat samples

Variable[1]	Mean	SD	Min.	Max.
pH 1h	6.70	0.20	6.05	7.04
pH 24h	5.66	0.35	5.20	6.70
WBSF_1d (kg)	9.50	2.02	3.35	15.65
WBSF_7d (kg)	8.08	1.98	3.60	13.88
WBSF_14d (kg)	6.81	2.00	3.82	13.23
CL_1d	24.52	5.66	13.57	42.34
CL_7d	25.09	5.96	15.98	43.84
CL_14d	24.66	4.41	13.42	33.61
L*1D	35.87	4.90	24.12	44.47
a*1D	16.04	3.10	10.37	23.23
b*1D	14.68	4.27	7.71	22.23
L*7D	36.90	4.64	27.93	45.53
a*7D	15.22	2.85	9.33	19.88
b*7D	13.99	3.28	7.53	19.52
L*14D	37.59	5.21	26.37	46.01
a*14D	15.24	2.75	9.17	19.56
b*14D	13.89	2.81	8.06	17.47
Lactate (µmol/g)	17.66	7.14	4.22	36.15
Glycogen (µmol/g)	14.15	6.98	1.71	30.79
Cortisol_IV	3.87	3.76	1.14	27.60
Cortisol_PM	5.99	2.39	2.56	14.34
ACTH_IV	12.72	9.71	0.32	38.43
ACTH_PM	11.41	8.45	0.59	35.18

[1] pH 1h and 24h = intramuscular pH obtained at 1 hour and 24 hour *postmortem;* WBSF = *Warner-Bratzler* Shear Force measured at 1, 7 and 14 days *postmortem*;, CL = cooking loss measured at 1, 7 and 14 days *postmortem;* L*,a* and b* were color measurements realized after 20 min bloom time at 1, 7 and 14 days *postmortem;* Cortisol_IV and ACTH_IV = concentration determined from blood collected during routine processing (*in vivo*); Cortisol_PM and ACTH_PM = concentration determined from blood collected immediately after exsanguinations (*postmortem*).

The pH values decrease from 1 hour *postmortem* to 24 hours, however some animals had pH ultimate greater than 6.0, suggesting the possible incidence of DFD (*Dry, Firm and Dark*) meat.

The DFD meat results from cattle with lower muscle glycogen stores at the slaughter [6]. The results demonstrated a curvilinear relationship between glycogen and pH ultimate (data not demonstrated). In relation the WBSF values, were observed that meat increased the tenderness during ageing period, as expected.

All these results can to be influenced by stress at slaughter. At slaughter, the animals showed higher plasma cortisol levels and lower plasma ACTH levels. These difference hormone profiles express the HPA axis regulation; suggest that the synthesis and secretion of corticosteroids is primarily mediated by action of ACTH.

The estimate of effects of substitutions gene of each polymorphism found for the different traits are shown in Table 3. Significant effects of substitution ($P<0.05$) were observed for three SNP in MR gene. The SNP for MR1 was associated with ACTH *in vivo* (ACTH_IV). The MR2 showed significant effect for pH 1 hour *postmortem* (pH_1h), while MR3 was presented relationship with muscle lactate (LACT). The last two marker (MR2 and MR3) also were associated significantly for cooking loss at 7^{th} and 14^{th} days of ageing (CL_7D, CL_14D).

For the markers related to the glucocorticoid receptor (GR1 and GR2), substitution effects significant were found in GR1 for cortisol levels *postmortem* (CORT_PM, $P<0.01$), ACTH *in vivo* (ACTH_IV, $P<0.01$) and cooking loss at 7^{th} days of ageing (CL_7D). For GR2 was observed substitution effects significant for muscle lactate levels (LACT).

The results suggest that these SNPs are related central of HPA axis and seem to be promising in studies of the genetic factors that may affect meat quality traits. However, these SNPs will be evaluated in a larger population.

IV. CONCLUSION

The markers in MR and GR genes can contribute for increased our knowledge about the interaction between genetic and pre-slaughter environmental factors on meat quality

Table 3 Estimates (EST) of mean effect of substitution gene (b_{1i}) for neuroendocrine, metabolic and physical-chemistry traits in meat samples by MR and GR markers

Variable[1]	Mean effect of substitution														
	MR1			MR2			MR3			GR1			GR2		
	Est	SE	Pr > |t|	Est	SE	Pr > |t|	Est	SE	Pr > |t|	Est	SE	Pr > |t|	Est	SE	Pr > |t|
CORT_IV	1.03	0.93	0.27	1.37	1.01	0.18	-0.74	1.67	0.66	0.88	1.34	0.52	0.27	0.83	0.75
CORT_PM	-0.35	0.57	0.54	-0.22	0.62	0.73	-0.70	1.00	0.49	2.12	0.74	0.01	-0.52	0.50	0.31
ACTH_IV	4.89	2.31	0.04	-0.43	2.64	0.87	-7.60	4.15	0.08	8.41	3.25	0.01	-1.42	2.14	0.51
ACTH_PM	3.39	1.81	0.07	2.39	2.02	0.25	3.38	3.32	0.32	2.31	2.68	0.39	-2.13	1.64	0.20
LACT	-1.84	1.15	0.12	-1.53	1.26	0.23	-4.78	1.97	0.02	2.52	1.65	0.13	-2.28	0.99	0.03
GLIC	0.77	1.32	0.56	1.95	1.47	0.19	4.30	2.29	0.07	0.63	1.89	0.74	0.60	1.19	0.62
L*1D	0.06	0.90	0.95	1.17	1.04	0.27	0.62	1.72	0.72	-0.98	1.28	0.45	0.36	0.81	0.66
a*1D	0.10	0.43	0.81	0.45	0.47	0.35	0.86	0.76	0.27	-0.66	0.61	0.29	0.07	0.39	0.85
b*1D	-0.26	0.45	0.57	0.37	0.54	0.50	0.32	0.88	0.72	-0.73	0.64	0.26	0.06	0.41	0.89
L*7D	-0.68	0.85	0.43	0.78	1.00	0.44	1.37	1.63	0.41	-1.01	1.22	0.41	0.87	0.77	0.27
a*7D	0.26	0.60	0.67	0.75	0.64	0.26	0.70	1.06	0.52	-1.00	0.85	0.25	0.83	0.52	0.12
b*7D	0.13	0.64	0.84	0.92	0.69	0.19	1.21	1.11	0.29	-1.04	0.90	0.26	0.90	0.56	0.12
L*14D	-1.02	1.03	0.33	0.71	1.21	0.56	2.84	1.79	0.12	-1.10	1.49	0.46	0.90	0.93	0.34
a*14D	0.11	0.59	0.85	0.74	0.65	0.27	1.12	1.00	0.27	-0.91	0.82	0.28	0.77	0.51	0.14
b*14D	-0.46	0.61	0.46	0.57	0.71	0.43	1.51	1.06	0.16	-0.97	0.87	0.27	0.84	0.54	0.13
pH_1h	-0.07	0.05	0.16	-0.11	0.05	0.05	-0.12	0.09	0.21	-0.13	0.07	0.09	0.04	0.05	0.45
pH_24h	0.01	0.08	0.95	-0.12	0.10	0.23	-0.20	0.16	0.21	0.17	0.12	0.16	-0.09	0.07	0.22
CL_1D	0.10	1.44	0.94	1.60	1.55	0.31	1.86	2.53	0.47	-2.41	1.98	0.23	0.72	1.25	0.57
CL_7D	0.76	1.49	0.61	3.42	1.62	0.04	8.96	2.43	0.00	-4.58	1.93	0.02	1.21	1.32	0.37
CL_14D	1.37	1.04	0.20	2.67	1.08	0.02	4.06	1.79	0.03	-0.15	1.52	0.92	0.68	0.95	0.48
WBSF_1D	0.62	0.44	0.17	0.90	0.47	0.07	1.04	0.80	0.20	-0.36	0.65	0.58	-0.61	0.39	0.13
WBSF_7D	0.37	0.47	0.44	0.48	0.52	0.36	0.42	0.86	0.63	-0.11	0.68	0.87	-0.37	0.42	0.38
WBSF_14D	-0.15	0.45	0.75	-0.50	0.51	0.34	-1.01	0.82	0.23	0.22	0.65	0.74	0.40	0.40	0.33

[1] pH 1h and 24h = pH obtained at 1 hour and 24 hour *postmortem;* WBSF = *Warner-Bratzler* Shear Force measured at 1, 7 and 14 days *postmortem*; CL = cooking loss measured at 1, 7 and 14 days *postmortem;* L*,a* and b* colour measurements realized after 20 min bloom time at 1, 7 and 14 days *postmortem;* CORT_IV and CORT_PM= plasma cortisol concentration (*in vivo* and *postmortem*); ACTH_IV and ACTH_PM = plasma adrenocorticotropic hormone concentration (*in vivo* and *postmortem*).

ACKNOWLEDGEMENTS

The authors thanks to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

REFERENCES

1. De Kloet, E.R. (2005). Stress and the brain: from adaptation to disease. Nature Reviews Neuroscience. 6:463-475.
2. DeRijk, R.H., de Kloet, E.R. (2008). Corticosteroid receptor polymorphisms: determinants of vulnerability and resilence. European Journal Pharmacology. 853:303-311.
3. Muráni, E., Ponsuksili, S., D'Eath, R.B., Tuner, S.P., Kurt, E., Evans, G., Thölking, L., Klont, R., Foury, A., Mormède, P., Wimmers, K. (2010) Association of HPA axis-related genetic variation with stress reactivity and aggressive behavior in pig. BMC Genetics. 11:74-84
4. McEwen, B.S., Stellar, R. (1993) Stress and the Individual: mechanisms leading to disease. Arch Intern Med. 153:2093-2101.
5. DeRijk, R.H. (2009). Single nucleotide polymorphisms related to HPA axis reactivity. Neuroimmunomodulation. 16:340-352.
6. Bourguet, C., Deiss, V., Gobert, M., Durand, D., Boissy, A., Terlouw, E.M.C. (2010). Characterising the emotional reactivity of cows to understand and predict their stress reactions to the slaughter procedure. Applied Animal Behaviour Science.125:9-21.