ASSOCIATIONS OF POLYMORPHISMS IN THE *MC1R* AND *DBP* GENES WITH MEAT COLOR IN NELLORE CATTLE MAY BE RELATED TO SKIN PIGMENTATION AND PLASMA 1,25-DI-HYDROXY-VITAMIN D_3

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Abstract – Skin melanin (MEL_S) regulates plasma 1,25-di-hydroxy-vitamin D_3 (1,25-D_P), which can influence the plasma and muscle calcium in domestic animals. Either 1,25-D_P or calcium could affect meat color by protecting or inducing the meat oxidation, respectively. This work aimed to investigate the associations of single nucleotide polymorphisms (SNPs) in the melanocortin-1 receptor [*MC1R*; rs109688013 (C/T) and rs110710422 (G/−)] and vitamin D_3-binding protein [*DBP*; rs135330728 (T/C)] genes with MEL_S, 1,25-D_P, plasma and muscle calcium, and meat color (*L, *a**, and *b** values at 1, 7, and 14 days of aging). Nellore cattle (n=86) were used for the genotyping and traits measurement. In the *MC1R* gene, the rs109688013 SNP allele T was fixed (100%), while the rs110710422 SNP allele G and its deletion (−) had a frequency of 97.7 and 2.3%, respectively. *MC1R* SNPs resulted in *Extension* locus (*E*/*E* = T/T + G/G and *E*/*e* = T/T + G/−), which was associated with 1,25-D_P and *b** values at the day 1. In the *DBP* gene, the rs135330728 SNP alleles C and T had a frequency of 73.8 and 26.2%, respectively. *DBP* SNP was associated with MEL_S, and *L** and *a** values at the day 7. Associations of the *Extension* locus and *DBP* SNP with the meat color seem to be a consequence of the differences in 1,25-D_P and MEL_S.**

Key Words – Meat quality, UV radiation, SNP.

I. INTRODUCTION

Meat color is a visual aspect associated with freshness, flavor, tenderness and safety, which can influence in the purchase decision from consumers [1]. Higher concentration of vitamin D_3 and its metabolites in plasma from animals could have a beneficial effect on the meat color [2] due to an anti-oxidative capacity related to increased antioxidant enzymes concentration [3]. On the other hand, higher concentration of plasma and muscle calcium had a detrimental effect on the meat color [2] due to the meat oxidation caused by the increase of free radicals [4]. In these cases, the skin pigmentation would play a major role in the regulation of vitamin D_3 and calcium [5]. The concentration of skin melanin (MEL_S) is principally influenced by melanocortin-1 receptor (*MC1R*) [6]. Individuals containing higher concentrations of MEL_S absorb more UV rays from sunlight and photosynthesize less vitamin D_3 under skin [5,7]. Photosynthesized vitamin D_3 is transported through the bloodstream by the vitamin D_3-binding protein (*DBP*) to the liver, where is converted into 25-hydroxy-vitamin D_3, whose is posteriorly transported to the kidneys, where is finally converted into the active metabolite 1,25-di-hydroxy-vitamin D_3 [8]. Thus, this work aimed to investigate if MEL_S, plasma 1,25-di-hydroxy-vitamin D_3 (1,25-D_P), plasma and muscle calcium, and meat color are associated with the single nucleotide polymorphisms (SNPs) in the *MC1R* and *DBP* genes.

II. MATERIALS AND METHODS

Animals and sampling

Nellore cattle (n=86) with average weight of 516 ± 39 kg and average age of 24 months were used. At slaughter, the blood samples were immediately collected for the genotyping, 1,25-D_P, and plasma calcium analysis. Pieces of skin from dorsal region located close to tail were collected during the skinning to quantify the melanin concentration. Samples from the *Longissimus lumborum* muscle were immediately taken at the slaughter for the muscle calcium analysis. After 24 hours post-

mortem (pm), steaks from that same muscle were removed from the carcasses and aged for 1, 7, and 14 days pm for the meat color measurements.

DNA extraction and genotyping

Genomic DNA was extracted from blood using a salting out procedure. Genotyping of SNPs marker for the *MC1R* and *DBP* genes were carried out by Real-Time Polymerase Chain Reaction (RT-PCR). The oligonucleotide primers were designed based on dbSNP published on the *NCBI* website. In the *MC1R* gene, the rs109688013 (C/T) and rs110710422 (G/−; where – means the deletion of the nucleotide G) SNPs, located at chromosome 18 and exon 1, were chosen. The combination of the nucleotides in the *MC1R* SNPs resulted in alleles of the *Extension* locus (*E* = T+G and *e* = T+−). In the *DBP* gene, the rs135330728 (T/C) SNP, located at chromosome 6 and exon 13, was also selected. The RT-PCR was conducted in 10 μL reaction volumes using 20 ng of genomic DNA, 0.25 μL Custom Taqman® SNP Genotyping Assays (Applied Biosystems), 5 μL Taqman® Universal PCR Master Mix (Applied Biosystems).

Phenotypic traits

The quantification of MEL_S was conducted following the procedure described for humans [9]. The 1,25-D_P concentrations were determined by Radioimmunoassay (RIA) [10] preceded by a separation of the fractions of vitamin D through the High-Performance Liquid Chromatography (HPLC) [11]. Plasma calcium was determined by a colorimetric method using the *QuantiChrom*^{TM} *Calcium Assay* kit (DICA-500, Biossay Systems). Color parameters (*L**, *a**, and *b** values) were measured in the surface of the steaks using a CM-2500d Minolta Chroma Meter with a 30-mm aperture, a D_{65} light source, and a 10° observer.

Statistical analysis

The MEL_S, plasma calcium, and color parameters were analyzed with a mixed model that included fixed effects of sex condition, slaughter group, additive (included into the diet) and genotype, and random effects of sire and error. The age of the animals at the slaughter was included in model as a covariate. A simplified model including the effects of genotype and error was used to analyze the 1,25-D_P and muscle calcium, because of the lower sample size. The data were analyzed by the PROC MIXED procedure of the SAS. When significant ($P<0.05$) or suggestive ($0.05 \leq P \leq 0.08$) differences for *F* test were detected, the least squares means were separated by the *t-Student* test.

III. RESULTS AND DISCUSSION

Genotypic and allelic frequencies

In the *MC1R* gene, the rs109688013 SNP allele T was found to be fixed in the population. Yet, in the rs110710422 SNP of those same gene, the genotype G/G and G/− had a frequency of 95.4 and 4.6%, respectively. In this case, the allele G and the deletion had a frequency of 97.7 and 2.3%, respectively. Because the rs109688013 SNP allele T was fixed, it was possible to obtain the genotypic and allelic frequencies for the *Extension* locus. The genotypes *E*/*E* (T/T + G/G) and *E*/*e* (T/T + G/−) had also a frequency of 95.4 and 4.6%, respectively. Thus, the frequency of alleles *E* and *e* were of 97.7 and 2.3%, respectively.

In the rs135330728 SNP at the *DBP* gene, the allele C was the most frequent (73.8%) and the allele T was the less frequent (26.2%). In 52.3 and 43.0% of animals were respectively observed the genotypes C/C and C/T, while only in 4.7% of animals was observed the genotype T/T.

Association of the Extension locus and DBP SNP with MEL_S, 1,25-D_P, and plasma and muscle calcium

There was no association ($P \geq 0.10$) of the *Extension* locus with MEL_S, and plasma and muscle calcium concentrations (Table 1), but an association between *Extension* locus and 1,25-D_P concentrations was detected ($P<0.01$). Animals with genotypes *E*/*E* had higher ($P<0.01$) 1,25-D_P concentrations than animals with genotypes *E*/*e*.

The allele *e* leads to a change of amino acids, which is responsible for the premature appearance of the stop codon and for the loss of protein functionality [12]. However, the allele *E* is dominant on the allele *e*, making the stop codon without effect [6]. The lack of differences in

MEL_S concentrations due to the genotypes of the *Extension* locus could then be explained. In turn, the association between *Extension* locus and 1,25-D_P concentrations with no change in MEL_S concentrations suggests that *Extension* locus may have a direct effect on the 1,25-D_P concentrations.

Table 1 Effect of the *Extension* locus and *DBP* SNP on the MEL_S, 1,25-D_P and plasma and muscle calcium in Nellore cattle

Trait	Genotype			*P*
Extension locus (*MC1R* gene)				
	E/*E* (n=82)	*E*/*e* (n=4)	*e*/*e* (n=0)	
MEL_S (n=86)	14.4 (0.46)	13.6 (2.19)	-	0.71
1,25-D_P (n=30)$^{\varphi}$	32.1 (2.13)^{a}	14.8 (5.43)^{b}	-	<0.01
Ca_P (n=86)	11.7 (0.11)	12.6 (0.55)	-	0.10
Ca_M (n=43)$^{\lambda}$	2.6 (0.16)	3.1 (0.60)	-	0.37
rs135330728 SNP (*DBP* gene)				
	C/C (n=45)	C/T (n=37)	T/T (n=4)	
MEL_S (n=86)	14.0 (0.61)^{b}	14.3 (0.66)^{b}	19.1 (2.04)^{a}	0.07
1,25-D_P (n=30)§	29.3 (3.38)	29.8 (3.65)	31.5 (6.33)	0.95
Ca_P (n=86)	11.5 (0.17)	11.8 (0.18)	12.3 (0.54)	0.28
Ca_M (n=43)$^{£}$	2.8 (0.24)	2.5 (0.23)	2.2 (0.52)	0.39

Legend: Least squares means (standard error); MEL_S = skin melanin (μg/mg); 1,25-D_P = plasma 1,25-di-hydroxy-vitamin D_3 (pg/mL); Ca_P = plasma calcium (mg/dL); Ca_M = muscle calcium (mg/100 g); φ = C/T (n=6) and T/T (n=24); λ = C/T (n=4) and T/T (n=39); § = C/C (n=14), C/T (n=12) and T/T (n=4); £ = C/C (n=19), C/T (n=20) and T/T (n=4). ^{a,b}Least squares means followed by different letters among the genotypes differ statistically by the *t-Student* test (P≤0.03).

At the *DBP* gene, an association was found (P=0.07) between the rs135330728 SNP and MEL_S concentrations (Table 1). Animals with genotypes T/T had higher MEL_S concentrations (P≤0.03) than animals with genotypes C/C and C/T, which did not differ (P=0.81). Nevertheless, no association of that SNP was observed (P≥0.22) with 1,25-D_P, and plasma and muscle calcium concentrations. No association between the rs43338560 and rs43338565 SNPs, located in the intronic region of the *DBP* gene, with plasma vitamin D_3 concentrations in Holstein cattle was also observed [13], although associations between *DBP* SNPs and plasma vitamin D_3 metabolites concentrations were reported in humans [7].

The *Bos indicus* cattle were naturally selected for several years to support high levels of UV radiation under tropical climate. Interesting associations of the *Extension* locus and *DBP* SNP with MEL_S and 1,25-D_P concentrations may point that the selection of alleles for the MEL_S concentrations could indirectly select alleles for the 1,25-D_P concentrations and vice versa.

Association of the Extension locus and DBP SNP with beef color

Differences in *L** and *a** values for the aged steaks were not observed (P≥0.23) due to the genotypes of the *Extension* locus, but differences (P=0.04) in *b** values were found at 1 day pm (Table 2). Steaks from animals with genotypes *E*/*E* had higher (P=0.04) *b** values than steaks from animals with genotypes *E*/*e*. A more yellow meat in animals with genotypes *E*/*E* could be a result of the higher 1,25-D_P concentrations in those animals (Table 1). An anti-oxidative effect of vitamin D_3 on the beef color with an increase of *a** and *b** values was previously reported [2].

Table 2 Effect of the *Extension* locus and *DBP* SNP on the meat color parameters in Nellore cattle (n=86)

Trait	Day	Genotype			*P*
Extension locus (*MC1R* gene)					
		E/*E* (n=82)	*E*/*e* (n=4)	*e*/*e* (n=0)	
*L**	1$^{\lambda}$	23.5 (0.62)	22.2 (2.44)	-	0.58
	7	30.8 (0.37)	31.2 (1.82)	-	0.83
	14	27.8 (0.41)	26.7 (2.00)	-	0.58
*a**	1$^{\lambda}$	22.4 (0.53)	20.6 (1.50)	-	0.23
	7	18.1 (0.30)	16.6 (1.38)	-	0.29
	14	21.4 (0.31)	22.1 (1.54)	-	0.66
*b**	1$^{\lambda}$	14.7 (0.36)^{a}	12.1 (1.29)^{b}	-	0.04
	7	16.2 (0.23)	14.6 (1.06)	-	0.13
	14	17.1 (0.27)	17.3 (0.90)	-	0.77
rs135330728 SNP (*DBP* gene)					
		C/C (n=45)	C/T (n=37)	T/T (n=4)	
*L**	1§	24.0 (0.74)	23.1 (0.79)	23.1 (2.31)	0.69
	7	31.3 (0.49)^{a}	29.9 (0.54)^{b}	32.9 (1.70)^{a}	0.07
	14	27.8 (0.56)	27.8 (0.61)	28.2 (1.94)	0.97
*a**	1§	22.0 (0.62)	22.7 (0.65)	22.3 (1.46)	0.52
	7	17.8 (0.37)^{ab}	18.5 (0.40)^{a}	15.7 (1.28)^{b}	0.08
	14	21.2 (0.42)	21.7 (0.47)	20.4 (1.48)	0.55
*b**	1§	14.2 (0.49)	14.8 (0.52)	16.0 (1.26)	0.23
	7	16.5 (0.29)	16.0 (0.32)	15.1 (1.01)	0.36
	14	17.0 (0.31)	17.5 (0.33)	15.9 (0.84)	0.10

Legend: Least squares means (standard error); Day = aging day; *L** = lightness (dark to pale); *a** = chroma (green to red); *b** = chroma (blue to yellow); λ = C/T (n=6) and T/T (n=79); § = C/C (n=44), C/T (n=37) and T/T (n=4). ^{a,b}Least squares means followed by different letters among the genotypes differ statistically by the *t-Student* test (P≤0.06).

The rs135330728 SNP, in the *DBP* gene, was associated (P≤0.08) with the *L** and *a** values at 7 days pm (Table 2). Overall, the animals with

genotypes C/T had darker and redder steaks ($P \leq 0.06$) than the animals with genotypes T/T. This could be related to the differences in MEL_S concentrations observed in those animals (Table 1), where the lowest MEL_S concentrations were found in the animals with genotypes C/T. The MEL_S concentrations were correlated positively with *L** values and negatively with *a** values (data not published), indicating that higher MEL_S concentrations seem to be related to a worsened meat color by possibly increasing the water loss and the discoloration. A more pigmented skin could be linked to a more unprotected antioxidant defense system of muscle due to greater mobilization of antioxidants to neutralize the free radicals from oxidative processes in skin [14].

IV. CONCLUSION

The *Extension* locus was associated with 1,25-D_P concentrations, while the *DBP* SNP was associated with MEL_S concentrations. Both of them were also associated with meat color parameters and these associations could be attributed to the differences in 1,25-D_P and MEL_S concentrations.

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REFERENCES

1. Girolami, A., Napolitano, F., Faraone, D. & Braghieri, A. (2013). Measurement of meat color using a computer vision system. Meat Science 93: 111-118.
2. Lobo-Jr., A. R., Delgado, E. F., Mourão, G. B., Pedreira, A. C. M. S., Berndt, A. & Demarchi, J. J. A. A. (2012). Interaction of dietary vitamin D_3 and sunlight exposure on *B. indicus* cattle: Animal performance, carcass traits, and meat quality. Livestock Science 145: 196-204.
3. Hamden, K., Carreau, S., Jamoussi, K., Miladi, S., Lajmi, S., Aloulou, D., Ayadi, F. & Elfeki, A. (2009). 1α,25 Dihydroxyvitamin D_3: Therapeutic and preventive effects against oxidative stress, hepatic, pancreatic and renal injury in alloxan-induced diabetes in rats. Journal of Nutritional Science and Vitaminology 55: 215-222.
4. Lawrence, T. E., Dikeman, M. E., Hunt, M. C., Kastner, C. L. & Johnson, D. E. (2003). Effects of calcium salts on beef *Longissimus* quality. Meat Science 64: 299-308.
5. Harris, S. S. & Dawson-Hughes, B. (1998). Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white. American Journal of Clinical Nutrition 67: 1232-1236.
6. Olson, T. A. (1999). Genetics of colour variation. In R. Fries, & A. Ruvinsky, The genetics of cattle. New York: CABI Publishing.
7. Engelman, C. D., Fingerlin, T. E., Langefeld, C. D., Hicks, P. J., Rich, S. S., Wagenknecht, L. E., Bowden, D. W. & Norris, J. M. (2008). Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. The Journal of Clinical Endocrinology and Metabolism 93: 3381-3388.
8. Omdahl, J. L., Morris, H. A. & May, B. K. (2002). Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. Annual Review of Nutrition 22: 139-166.
9. Alaluf, S., Atkins, D., Barrett, K., Blount, M., Carter, N. & Heath, A. (2002). Ethnic variation in melanin content and composition in photoexposed and photoprotected human skin. Pigment Cell Research 15: 112-118.
10. Hollis, B. W., Kamerud, J. Q., Kurkowski, A., Beaulieu, J. & Napoli, J. L. (1996). Quantification of circulating 1,25-dihydroxyvitamin D by radioimmunoassay with ^{125}I-labeled tracer. Clinical Chemistry 42: 586-592.
11. Horst, R. L., Littledike, E. T., Riley, J. L. & Napoli, J. L. (1981). Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals. Analytical Biochemistry 116: 189-203.
12. Rouzaud, F., Martin, J., Gallet, P. F., Delourme, D., Goulemot-Leger, V., Amigues, Y., Ménissier, F., Levéziel, H., Julien, R. & Oulmouden, A. (2000). A first genotyping assay of French cattle breeds based on a new allele of the *extension* gene encoding the melanocortin-1 receptor (Mc1r). Genetics Selection Evolution 32: 511-520.
13. Ferreri, M., Gao, J., Ren, G., Chen, L., Su, J. & Han, B. (2011). Variability of osteocalcin status in Chinese Holstein cattle: Do phylogeny, vitamin D or gene polymorphisms matter? Asian-Australasian Journal of Animal Sciences 24: 173-180.
14. Horak, P., Sild, E., Soomets, U., Sepp, T. & Kilk, K. (2010). Oxidative stress and information content of black and yellow plumage coloration: an experiment with greenfinches. The Journal of Experimental Biology 213: 2225-2233.