SKIN MELANIN AND PLASMA 25-HYDROXY-VITAMIN D_3 IMPROVE MEAT TENDERNESS IN NELLORE CATTLE

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Abstract – Vitamin D_3 is photosynthesized under skin by the UV rays from sunlight. The *Bos indicus* cattle have high skin melanin (MEL_S) concentrations, which influences the photosynthesis of vitamin D_3. A conversion from vitamin D_3 to 25-hydroxy-vitamin D_3 occurs in the liver. Vitamin D_3 metabolites can alter the plasma and muscle calcium and improve the meat tenderness. This work aimed to study the relationships between MEL_S, plasma 25-hydroxy-vitamin D_3 ($25\text{-}D_P$), plasma and muscle calcium, and meat tenderness [Myofibrillar Fragmentation Index (MFI) and shear force (SF) at 1, 7, and 14 days of aging] in Nellore cattle (n=86). The MEL_S, $25\text{-}D_P$, and plasma and muscle calcium were not correlated. However, MEL_S was correlated negatively with SF and $25\text{-}D_P$ was correlated positively with MFI. Also, plasma and muscle calcium was correlated positively with MFI and negatively with SF. Finally, higher MEL_S, $25\text{-}D_P$, and plasma and muscle calcium concentrations improved the beef tenderness.

Key Words – Calcium, Meat quality, UV radiation.

I. INTRODUCTION

Vitamin D_3 is photosynthesized under the skin by UV rays from sunlight. Either vitamin D_3 or its metabolites improves meat tenderness in *Bos taurus* cattle [1,2], but not in *Bos indicus* cattle [3,4]. Also, the *Bos indicus* and *Bos taurus* cattle differ due to the concentrations of skin melanin (MEL_S) [5], which can influence the photosynthesis of vitamin D_3 [6]. Higher concentrations of vitamin D_3 or its metabolites improves the absorption of calcium from diet by the intestine and increases the concentrations of plasma and muscle calcium [7,8]. This can enhance the action of the calpains enzymes and increase the myofibrillar fragmentation, leading to a tender meat [9]. Therefore, the objective of this work was to investigate the relationships between the skin melanin (MEL_S), plasma 25-hydroxy-vitamin D_3 ($25\text{-}D_P$), and plasma and muscle calcium concentrations, and meat tenderness in Nellore cattle.

II. MATERIALS AND METHODS

A total of 86 Nellore cattle with average weight of 516 ± 39 kg and average age of 24 months were used. Blood samples were immediately collected at the slaughter for the $25\text{-}D_P$ 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 Myofibrillar Fragmentation Index (MFI) and shear force (SF) analysis.

The quantification of MEL_S was performed following the procedure described for humans [10]. The $25\text{-}D_P$ concentrations were determined by Electrochemiluminescence Immunoassay (ECLIA) with the *Elecsys Vitamin D total* kit (Cobas®, Roche Diagnostics GmbH) [11]. Plasma calcium was analyzed by a colorimetric method using the *QuantiChrom™ Calcium Assay* kit (DICA-500, Biossay Systems). MFI analysis were conducted according to the procedure described previously [12], where meat samples were initially homogenized in MFI buffer at 22,500 rpm for 40 seconds using a Turratec TE-102 homogenizer. The SF determination was carried out following the recommendations of the AMSA [13]. Briefly, the steaks were cooked on electric oven until reaching internal temperature of 71ºC. After the cooking, the steaks were stored overnight at 2ºC. In the next day, six to eight cores were removed

from steaks parallel to the direction of the muscle fibers. Cores were then perpendicularly sheared by the Warner-Bratzler equipment.

A descriptive analysis was performed using PROC MEANS procedure, while the *Pearson* correlations were performed using the PROC CORR procedure. The correlation coefficients were considered significant when the probability value was lower than or equal 5% and suggestive when the probability value was between 5 and 7%.

III. RESULTS AND DISCUSSION

Descriptive analysis

Means and dispersions of the data were presented in Table 1. No work quantifying the MEL_S concentrations in cattle was found until now. Nevertheless, the means and dispersions for MEL_S for Nellore cattle in this work were close to those ones found for African and Indian people with dark skin [10].

Higher means and lower coefficients of variation for the 25-D_P concentrations were observed in other works [1, 14], where 25-D_P was analyzed by the Radioimmunoassay (RIA). There is report showing that the ECLIA method underestimates 25-D_P values when compared to RIA method [11].

Table 1 Simple statistics for MEL_S, 25-D_P, plasma and muscle calcium concentrations, and meat tenderness in Nellore cattle

Trait	n	Mean	SD	CV (%)	Min	Max
MEL_S (µg/mg)	86	14.5	4.15	28.7	7.6	32.9
25-D_P (ng/mL)	30	29.2	13.70	46.9	10.0	65.7
Ca_P (mg/dL)	86	11.7	1.01	8.6	9.4	14.2
Ca_M (mg/100 g)	43	2.6	1.04	39.9	0.3	4.5
MFI_{1d}	86	55.6	13.76	24.8	29.7	102.4
MFI_{7d}	86	77.8	20.15	25.9	31.0	125.1
MFI_{14d}	86	99.5	20.34	20.5	55.7	142.3
SF_{1d} (kgf)	86	8.7	2.20	25.3	3.0	15.3
SF_{7d} (kgf)	86	7.4	1.58	21.3	3.8	12.6
SF_{14d} (kgf)	86	5.9	1.42	23.9	3.5	10.8

Legend: SD = standard deviation; CV = coefficient of variation; Min = minimum; Max = maximum; MEL_S = skin melanin; 25-D_P = plasma 25-hydroxy-vitamin D_3; Ca_P = plasma calcium; Ca_M = muscle calcium; MFI = Myofibrillar Fragmentation Index; SF = shear force; 1d, 7d, and 14d = 1, 7, and 14 days of aging.

Because extracellular calcium is important for the vital functions of the animals [8], a lower variability was verified for the plasma calcium concentration. On the other hand, the muscle calcium concentrations had higher variation (39.9%) than those ones found by other authors [2]. Yet, means and coefficients of variation for the meat tenderness at different times of aging are close to those ones observed in *Bos indicus* cattle [3, 15, 16, 17].

Correlation analysis

There was no correlation (P≥0.34) between MEL_S, 25-D_P, and plasma and muscle concentrations (Table 2). This suggests that changes in MEL_S concentrations did not yield changes in 25-D_P, and plasma and muscle calcium concentrations.

Table 2 Correlation coefficients between MEL_S, 25-D_P, and plasma and muscle calcium concentrations in Nellore cattle

Trait	MEL_S	25-D_P	Ca_P	Ca_M
MEL_S		−0.03	0.10	−0.07
25-D_P			−0.16	−0.13
Ca_P				−0.05
Ca_M				

Legend: MEL_S = skin melanin; 25-D_P = plasma 25-hydroxy-vitamin D_3; Ca_P = plasma calcium; Ca_M = muscle calcium. No correlation coefficient was significant (P≥0.34)

The MEL_S concentrations were slightly and negatively correlated with SF values at 1 (r=−0.20; P=0.06) and 14 (r=−0.22; P=0.04) days of aging (Table 3). In turn, the 25-D_P concentrations was moderately and positively correlated with MFI values at 7 (r=0.34; P=0.06) and 14 (r=0.41; P=0.02) days of aging. It is possible to verify that MEL_S and 25-D_P concentrations appear to positively influence the meat tenderness. Higher myofibrillar fragmentations and lower shear force values were noted when higher MEL_S and 25-D_P concentrations were observed.

Darker skin is more protected against UV rays than lighter skin due to higher MEL_S concentrations in former [18]. A darker skin seems to mobilize higher antioxidants concentrations in order to neutralize the free radicals from oxidative processes in skin [19]. During oxidative stress, the endogen mechanism of protection such as apoptosis can occur in a higher frequency [18], what could lead to improved meat tenderness [20] in those animals with higher MEL_S concentrations.

Table 3 Correlation coefficients of MEL_S, 25-D_P, and plasma and muscle calcium concentrations with MFI and SF in steaks aged from Nellore cattle

Trait	Day	MEL_S	25-D_P	Ca_P	Ca_M
MFI	1	0.01	0.04	0.07	**0.29**
	7	0.06	**0.34**	**0.24**	0.23
	14	0.15	**0.41**	0.12	0.23
SF	1	**−0.20**	0.06	0.08	0.05
	7	−0.17	0.02	**−0.19**	−0.03
	14	**−0.22**	−0.04	−0.12	**−0.30**

Legend: Day = aging time in days; MEL_S = skin melanin; 25-D_P = plasma 25-hydroxy-vitamin D_3; Ca_P = plasma calcium; Ca_M = muscle calcium; MFI = Myofibrillar Fragmentation Index; SF = shear force. Correlation coefficients in bold were significant ($P \leq 0.07$)

Yet, the higher 25-D_P concentrations appear not to alter meat tenderness by modifying the plasma and muscle calcium concentrations, since no correlations were observed among the 25-D_P, and plasma and muscle calcium concentrations (Table 2). There is report that the most of the body cells, including the muscle, do not only have vitamin D receptors, but also have the capacity to convert 25-hydroxy-vitamin D_3 to 1,25-hydroxy-vitamin D_3 [21]. Effects such as increase of intracellular calcium and apoptosis were previously reported for the vitamin D_3 and its metabolites [7,21]. Improved meat tenderness was also observed when higher vitamin D_3 and/or its metabolites concentrations were found in plasma [1,14].

Plasma calcium concentrations were slightly correlated with MFI ($r=0.24$; $P=0.03$) and SF ($r=-0.19$; $P=0.07$) values at the day 7 of aging, while muscle calcium concentrations were moderately correlated with MFI values at the day 1 of aging ($r=0.29$; $P=0.06$) and SF at the day 14 of aging ($r=-0.30$; $P=0.05$) (Table 3). These correlations indicate that higher plasma and muscle calcium concentrations improve meat tenderness, leading to a higher myofibrillar fragmentation and lower SF values. During the meat aging, the protein degradation process is attributed to enhanced action of the calpains enzymes, which are calcium-dependent [9]. Higher plasma and muscle calcium concentrations could then improve the calpains activity and, thereafter, the meat tenderness [2].

IV. CONCLUSION

The higher MEL_S concentrations did not reduce the 25-D_P, and plasma and muscle calcium concentrations. However, the higher MEL_S concentrations improved meat tenderness, while the higher 25-D_P concentrations increased the myofibrillar proteolysis.

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