Prospecting Genetic Markers Influence On Tenderness And Beef Fat And Cholesterol Content Of Nellore Cattle[1]

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Introduction

A large number of single nucleotide polymorphism (SNP), associated with genes which expressions are related on meat quality traits, were described on *Bos taurus* animals or on *Bos taurus* highly influenced breeds. However, in Brazil, more than 80% of the 200 million heads herd are represented by *Bos indicus* animals, especially by the Nellore breed, raised on very distinct production system than where markers were described. In this context, the present research had as objective identify, on a set of 95 genetic markers, initially described on *Bos taurus* animals, potential markers to be used as auxiliary tools on selection for tenderness, intramuscular fat and cholesterol content on Nellore breeding programs.

Material and methods

Samples description. Data on 641 Nellore bulls, raised under pasture conditions until 18 months of age and, after, fed in feedlots until slaughter, with ages varying from 21 to 28 months and live weights around 560 kg, were collected. All animals were half-sibs or full-sibs of bulls selected for growth and reproductive traits. The animals were slaughtered in six different dates in a commercial slaughter plant, always in the mornings, and after, approximately, 16 hours of fastening with free access to fresh water. Four samples of *Longissimus dorsi* muscle of each animal were collected. Three of them were aged for 7, 14 and 21 days for tenderness evaluation and the last one, after 7 days of ageing, was frozen under -18 ºC till intramuscular fat and cholesterol content analysis were done.

Data collection. To measure Warner-Bratzler shear force (WBSF7, WBSF14 and WBSF21, kg), steaks were cooked and sheared as described by AMSA (1995). From each steak were taken 8 sub-samples of ½" of diameter and the average of these measures in the Warner-Braztler Shear Force equipment was considered as beef tenderness. Determination of intramuscular fat (IMF, g/100g of meat) was based on methodology described by Bligh & Dyer (1959). Cholesterol extraction and quantification was made according to method described by Saldanha, Mazalli & Bragagnolo (2004), which promote cholesterol degradation by cholesterol oxidase enzyme (CHOLESTEROL, mg/100g of meat). Descriptive statistics of evaluated traits are described in Table 1.

Genotyping and polymorphisms. DNA was extracted from blood samples collected using EDTA vacuum tubes and impregnated on FTA cards by NaCl extraction and precipitation method described for Olerup & Zetterquist (1992). Genotyping process was carried out on laboratories located in USA and licensed by Merial/Igenity®, company that has the licenses

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to explore commercially the analyzed genetic markers. Animals were genotyped for markers in BETA_CASEIN, BETA_LACTOGLOBULIN, CALPAIN, CALPASTATIN, CRH, DGAT, FABP4, GHR, GNS, LEPTIN, MC1R, NPY, SCD, TFAM e UCP genes. Markers were identified from MARK_1 to MARK _25 in this study.

Table 1: Number of observations and descriptive statistics for analyzed meat quality traits

Traits	N	AVG	MIN	MAX	SD	CV
WBSF7	638	5.92	1.82	9.99	1.45	24.55
WBSF14	638	4.94	1.38	9.34	1.27	25.76
WBSF21	638	4.40	1.61	8.53	1.13	25.71
IMF	559	2.19	0.96	4.60	0.65	29.69
CHOLESTEROL	597	56.49	28.76	83.95	8.31	14.72

N = nº of observations; AVG = average; SD = standard deviation; CV = coefficient of variation; MIN, MAX = minimum, maximum.

Statistical analysis. Allelic and genotypic frequencies for each marker were estimates by simply count of different alleles and genotypes, using PROC FREQ from SAS. Markers' effects on meat traits were evaluated considering phenotypic information as dependent variable and genotypes effects observed to each different marker as covariates on a paternal half-sib structure. Statistic mixed model considered as fixed effects slaughter group (for WBSF) and analysis date (for IMF and CHOLESTEROL), besides, as covariates, the genotypes' effects observed to each different genetic markers, slaughter age (for WBSF), backfat, pH24 (for WBSF) and sample temperature (for WBSF) and, as random effects, sire and residual effects. Statistic analyses were performed by PROC MIXED, SAS. F-statistic was considered significant or suggestive for allelic substitution effect if the nominal P-value was of $P<0.05$ or $0.05\leq P\leq 0.25$, respectively.

Results and discussion

Allelic and genotypic frequencies. Number of genotyped animals and allelic and genotypic frequencies of genetic markers on analyzed population are shown in Table 2. From a set of 95 SNP initially discovered in *Bos taurus*, 81 SNP showed to be fixed or the frequencies for one of the alleles were too high, more than 95%, in the *Bos indicus* breed analyzed. However, for 14 polymorphisms there were observed variability on allele frequencies, what made possible study markers' influence on meat traits by allele substitution effect analysis.

Association analysis. Markers' effects estimations are described in Table 3, showing the association of the 14 genetic markers on analyzed meat traits. Those effects represent the substitution of one allele per other on each genetic marker. On the set of 14 markers on Table 3, only for MARK_8, MARK_12 and MARK_23 did not have any effect on analyzed traits. On another hand, MARK_18 was an important marker, as it was detected significant or suggestive effect of this mark on almost all traits, except for IMF. Polymorphisms MARK_2, MARK_20 and MARK_24 presented effects on three of five traits considered on this research. Analyzing Table 3, six markers had some effect detected on WBSF7. On WBSF14,

WBSF21 and CHOLESTEROL significant or suggestive effects of five markers were verified and, on IMF, four markers had their effects detected. Independent of ageing period, MARKER_18 and MARKER_24 were important for WBSF. Only MARKER_19 had influence on both, IMF and CHOLESTEROL.

Table 2: Allelic and genotypic frequencies of genetic markers on a Nellore cattle population

Polymorphisms	N	Allelic frequencies		Genotypic frequencies		
		p	q	Homo 1	Hetero	Homo 2
MARK_1	639	99.30	0.70	98.59	1.41	0.00
MARK_2	639	74.65	25.35	55.24	38.81	5.95
MARK_3	640	0.78	99.22	0.00	1.56	98.44
MARK_4	637	17.72	82.26	3.14	29.20	67.66
MARK_5	631	7.77	92.23	0.32	14.90	84.79
MARK_6	640	98.67	1.33	97.34	2.66	0.00
MARK_7	637	99.37	0.63	98.90	0.94	0.16
MARK_8	641	93.29	6.71	86.90	12.79	0.31
MARK_9	639	99.61	0.39	99.22	0.78	0.00
MARK_10	640	15.00	85.00	2.19	25.63	72.19
MARK_11	637	79.75	20.25	63.27	32.97	3.77
MARK_12	635	78.82	21.18	60.63	36.38	2.99
MARK_13	631	98.49	1.51	96.99	3.01	0.00
MARK_14	619	89.26	10.74	79.00	20.52	0.48
MARK_15	640	2.58	97.42	0.16	4.84	95.00
MARK_16	639	0.55	99.45	0.00	1.10	98.90
MARK_17	592	99.24	0.76	98.48	1.52	0.00
MARK_18	622	93.73	6.27	88.42	10.61	0.96
MARK_19	637	87.36	12.64	76.30	22.14	1.57
MARK_20	606	65.02	34.98	43.56	42.90	13.53
MARK_21	640	1.64	98.36	0.00	3.28	96.72
MARK_22	640	99.45	0.55	98.91	1.09	0.00
MARK_23	639	80.36	19.64	63.69	33.33	2.97
MARK_24	633	63.27	36.73	39.65	47.24	13.11
MARK_25	639	37.01	62.99	13.30	47.42	39.28

p=frequency of allele 1; q=frequency of allele 2; Homo 1=homozygous for allelic frequency equal p; Hetero=heterozygous; Homo 2=homozygous for allelic frequency equal q.

Table 3: Markers effects on tenderness after 7, 14, 21 days of ageing (WBSF7, WBSF14, WBSF21) and intramuscular fat (IMF) and cholesterol content

Polymorphisms	WBSF7	WBSF14	WBSF21	IMF	CHOLESTEROL
MARK_2	-0.03 (0.098)	-0.15†† (0.083)	-0.04*** (0.076)	-0.11* (0.044)	0.20 (0.432)
MARK_4	-0.25* (0.109)	-0.23* (0.093)	-0.29 (0.084)	-0.05 (0.050)	-0.53 (0.481)
MARK_5	-0.07 (0.162)	0.12 (0.138)	0.07 (0.125)	-0.09† (0.073)	-0.22 (0.721)
MARK_8	-0.16 (0.166)	-0.09 (0.145)	0.003 (0.130)	-0.02 (0.080)	-0.44 (0.747)
MARK_10	0.09 (0.120)	0.10 (0.103)	0.11† (0.093)	-0.02 (0.054)	-0.75† (0.582)
MARK_11	-0.12† (0.104)	0.01 (0.089)	-0.11† (0.081)	0.05 (0.048)	-0.09 (0.469)
MARK_12	0.04 (0.108)	-0.06 (0.092)	-0.04 (0.084)	-0.03 (0.051)	-0.47 (0.482)
MARK_14	-0.01 (0.146)	-0.10 (0.125)	-0.09 (0.111)	-0.14* (0.067)	-0.27 (0.664)
MARK_15	-0.60* (0.278)	0.09 (0.236)	-0.16 (0.219)	0.10 (0.123)	2.40* (1.129)
MARK_18	-0.21† (0.175)	-0.18† (0.149)	-0.22†† (0.134)	0.05 (0.078)	-1.50* (0.755)
MARK_19	-0.06 (0.132)	0.01 (0.113)	0.03 (0.102)	-0.07† (0.060)	-0.73† (0.561)
MARK_20	-0.11† (0.089)	-0.10† (0.076)	-0.05 (0.069)	-0.04 (0.041)	-0.73†† (0.387)
MARK_23	-0.06 (0.110)	-0.02 (0.094)	-0.03 (0.086)	0.05 (0.050)	-0.29 (0.493)
MARK_24	-0.11† (0.088)	-0.17* (0.075)	-0.19** (0.068)	-0.03 (0.040)	0.01 (0.387)

† 0.15<P≤0.25; †† 0.05<P≤0.15; * 0.01<P≤0.05; ** 0,001<P≤0,01; *** P≤0,001

Conclusion

On a set of 95 genetic markers, initially described on *Bos taurus*, most of them were fixed or the frequencies for one of the alleles were too high on analyzed Nellore animals. However, it was possible to identify some markers affecting tenderness, fat and cholesterol content, which can be use as auxiliary tools in a Nellore breeding program.

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