PE4.117 Association of SNPs with carcass and meat quality traits in Nellore cattle 438.00

Fernanda Rezende (1) frezende@usp.br, JBS Ferraz(1), MN Bonin (1) JP Eler (1), TM Nishihara (1) MV Gallo (1) RA Silva (1), ARS Macedo(1), JC Amaral (1), GCR Oliveira (1), FV Meirelles (1)
(1)University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil

Abstract - In the current study, SNPs in the bovine calpain, calpastatin, corticotrophin-releasing hormone (CRH), acyl-CoA:diacylglycerol acyltransferase (DGAT), fatty acid binding protein 4 (FABP4), leptin (LEPTIN), melanocortin-1 receptor (MC1R), neuropeptide Y (NPY), stearoyl-CoA desaturase (SCD), mitochondrial transcription factor A (TFAM) and uncoupling proteins (UCP) genes were evaluated for associations with carcass and meat quality traits. In total, 22 SNPs were evaluated for associations with data from 671 Nellore bulls' carcass traits (hot carcass weight (HCW), rib eye area (REA) and backfat (BF)) and meat quality traits (Warner-Braztler shear force measured after 7, 14 and 21 days of ageing (WBSF7, WBSF14 and WBSF21), total lipids (LIPIDS) and cholesterol (CHOLESTEROL) content in 100 g of beef samples aged for 7 days). Estimates of allelic and genotypic frequencies for each marker were performed by PROC FREQ, SAS. Genetic markers effects were estimated by PROC MIXED, SAS. Significant or suggestive effects of at least two markers were detected on each analyzed trait. Based on that, these genetic markers can be used as auxiliary tools on selection process for carcass and meat quality trait in Nellore beef cattle.

F. M. Rezende is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (phone: +551935654105; e-mail: frezende@usp.br) J. B. S. Ferraz is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: jbferraz@usp.br) M. N. Bonin is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: marinabonin@usp.br) J. P. Eler is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: jopeler@usp.br) T. M. Nishihara is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: tha_mith@msn.com) M. V. Gallo is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: mayaragallo@gmail.com) R. A. Silva is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: raquel_as@ig.com.br) A. R. S. Macedo is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: ana.macedo@usp.br) J. C. Amaral is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: juliacamargo@dr.com) G. C. R. Oliveira is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: gaby_usp@yahoo.com.br) F. V. Meirellers is with University of Sao Paulo, 13635-900 Pirassununga, SP, Brazil (e-mail: meirellf@usp.br)

Index Terms - Bos indicus, genetic polymorphisms, genetic markers, lipid profile.

I. INTRODUCTION

RIB eye area and backfat measured at Longissimus dorsi muscle are indicators of noble cuts and edible carcass portion yields. Tenderness is an important beef trait, because it is associated with consumer's satisfaction. Additionally, the quantity and quality of lipids and cholesterol are becoming major concerns to consumers because of excessive consumption of high density calorie food has harmful effects on human health mainly on increasing cardiovascular diseases. The detection of genetic polymorphisms associated to genes that are involved with economically relevant traits is a very important auxiliary tool for selection in beef breeding programs, especially for traits difficult or expensive to measure. There are a lot of polymorphisms described in literature that have effects on beef cattle carcass and meat quality and composition traits. Among those, polymorphisms resulting of mutations on a single nucleotide (SNP) on calpain-calpastatin system genes were related to variations on meat tenderness [5, 12, 13, 17]. [20] suggest that a non-conservative aminoacid substitution associated to DGAT enzyme can influence fat deposition on meat. Other polymorphisms linked to FABP4 and TFAM genes had, also, revealed effects on backfat and marbling deposition in F2 animals Wagyu x Limousin [8, 10]. Despite of some studies mention the influence of polymorphisms associated to stearoyl-CoA desaturase (SCD) on beef fatty acids composition [9, 19] no effect was reported on the association of those polymorphisms on backfat deposition of beef cattle. To reinforce that, [14] described no correlation between a polymorphism linked to SCD gene and backfat deposition on Duroc swine. Physiologic regulation of feed intake, growth and energetic metabolism is controlled for many genes. A complex system involving corticotrophin-releasing hormone (CRH), leptin (LEPTIN) e neuropeptide Y (NPY) showed be associated on appetite regulation [3, 6]. Additionally, significant effect of polymorphisms on these genes were detected on carcass and meat quality

traits [3, 21, 4, 16, 18]. Additionally, melanocortin-1 receptor (MC1R) e uncoupling proteins (UCP) genes were considered as candidates genes for traits related to carcass and meat quality traits [7]. The objectives of this study were to estimate allele and genotypic frequencies of polymorphisms on CALPAIN, CALPASTATIN, CRH, DGAT, FABP4, LEPTIN, MC1R, NPY, SCD, TFAM and UCP genes and evaluate associations among these SNPs with hot carcass weight, rib eye area, backfat, tenderness, lipids and cholesterol levels in a Nellore beef cattle population raised in Brazil.

II. MATERIALS AND METHODS

A. Population

Phenotypic and genotypic information of 671 Nellore bulls was collected. All animals are progenies of bulls selected for production and reproduction traits. The animals were raised under pasture conditions until 18 months of age and, after, fed in feedlots until slaughter between 21 and 27 months of age. The slaughter occurred in six different dates, always in the mornings, and after, approximately, 16 hours of fastening. All animals were perfectly identified for measurements and sample collection.

B. Phenotypic traits

Animals were evaluated for carcass and meat quality traits. Carcass traits evaluated were hot carcass weight (HCW, kg), backfat (BF, mm) and rib eye area (REA, cm^2). Meat tenderness was measured as Warner-Bratzler shear force after 7, 14 and 21 days of ageing (WBSF7, WBSF14 and WBSF21, kg). To measure Warner-Bratzler shear force, steaks were cooked and sheared as described by [1]. 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 were considered as beef tenderness. Determination of total lipids was based on methodology described by [2]. Cholesterol extraction and quantification was made according to method described by [15], which promote cholesterol degradation by cholesterol oxidase enzyme. This reaction produces hydrogen peroxides that for second reaction turn out color. Color intensity is ready on spectrophotometer and it is directly proportional to the sample cholesterol content. Descriptive statistics of evaluated traits are described in Table 1.

C. 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 [11]. Genotyping process was carried out on laboratories located in USA and licensed by Merial/Igenity®, company that has the licenses to explore commercially the analyzed genetic markers. Animals were genotyped for 7 markers on LEPTIN gene, 3 on CALPAIN gene, 3 on TFAM gene, 2 on CALPASTATIN gene and for markers associated to the genes, CRH, DGAT, FABP4, MC1R, NPY, SCD e UCP. The markers were identified from Marker1 to Marker22 in this study.

D. Statistical analysis

Allelic and genotypic frequencies for each marker were estimates by simply count of different alleles and genotypes, using procedure FREQ from SAS. Markers' effects on analyzed 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 and analysis date (for lipids and cholesterol), besides, as covariates, the genotypes' effects observed to each different genetic markers, slaughter age, backfat (for WBSF, lipids and cholesterol), pH24 and sample temperature (for WBSF) and, as random effects, sire and residual effects. Statistic analyses were performed by PROC MIXED, SAS. An F-statistic was considered significant or suggestive for markers effect if the nominal P-value was of $P<0.05$ or $0.05<P<0.20$, respectively.

III. RESULTS AND DISCUSSION

A. *Allelic and genotypic frequencies*

Allelic and genotypic frequencies of genetic markers on analyzed population are showed on Table 2. Polymorphisms identified as MARK1, MARK3, MARK6, MARK7, MARK8, MARK9, MARK10, MARK11, MARK13, MARK14, MARK15 , MARK16, MARK17, MARK18 and MARK19, initially discovered in Bos taurus, showed to be almost fixed once frequencies for one of their alleles were too high on analyzed population, as can be observed on Table 2. In some of these polymorphisms, one of the alleles was verified only on heterozygosis. However, for MARK2, MARK4, MARK5, MARK12, MARK20, MARK21 and MARK22 there were observed variability on allele frequencies.

B. *Association analysis*

On Table 3 are described p-values observed on association analysis of genetic markers on carcass and meat quality traits analyzed. As it is showed on Table 3, MARK1 and MARK6 had significant effect on HCW and suggestive effects were observed for MARK7 and MARK9 on this trait. Significant effects of MARK13, MARK15 and MARK16 and suggestive effects of MARK1, MARK2, MARK3, MARK7, MARK8 and MARK14 were detected on REA. Only MARK1 had significant effect on BF. However, for MARK8, MARK14, MARK17, MARK21 and MARK22 were verified suggestive effects on BF. Significant or suggestive effects of MARK1, MARK2, MARK4, MARK5 and MARK13 on WBSF measured after 7, 14 and 21 days of ageing were described on Table 3. Also on WBSF7, MARK9 and MARK10 had significant effects and suggestive effect was described for MARK19. As well, suggestive effects of MARK7, MARK8, MARK10, MARK13 and MARK21 on WBSF14 were observed. On WBSF21, significant effect was related for MARK19 and suggestive effects for MARK7 and MARK9. On LIPIDS, significant effects were verified simply for MARK1 and MARK15. On another hand, on CHOLESTEROL, two genetic markers (MARK17 and MARK18) had significant effects and for seven markers (MARK1, MARK2, MARK9, MARK14, MARK16, MARK19 and MARK21) suggestive effects were described.

IV. CONCLUSIONS

DNA polymorphisms for which significant or suggestive allelic substitution effect were detected can be used as additional or auxiliary criteria on selection process of carcass and meat quality traits in Nellore cattle, raised under tropical pasture conditions.

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Table 1. Number of observations and descriptive statistics for carcass and meat quality traits measured on Nellore beef cattle raised in Brazil

Traits	N	AVG	SD	CV	MIN	MAX
SLAUGHTER_AGE, days	640	729.78	36.91	5.06	631.00	810.00
PH24	667	5.96	0.25	4.23	5.20	6.99
REA, cm^2	668	73.35	7.03	9.59	56.00	101.00
BF, mm	666	4.39	2.00	45.65	1.00	15.00
WBSF7_TEMP, °C	656	18.24	1.45	7.94	13.40	23.00
WBSF7, kg	671	5.93	1.45	24.37	1.82	9.99
WBSF14_TEMP, °C	666	18.14	1.62	8.95	3.85	23.00
WBSF14, kg	671	4.95	1.27	25.65	1.38	9.34
WBSF21_TEMP, °C	667	17.75	1.49	8.40	13.60	20.90
WBSF21, kg	671	4.41	1.12	25.32	1.61	8.53
LIPIDS, g/100g	589	2.19	0.65	29.59	0.96	4.60
CHOLESTEROL, mg/100g	627	56.42	8.26	14.64	28.76	83.95

N = number of observations; AVG = average; SD = standard deviation; CV = coefficient of variation; MIN = minimum; MAX = maximum.

Table 2. Allelic and genotypic frequencies of some genetic markers on a Nellore beef cattle population

Polymorphisms	N	Allelic frequencies		Genotypic frequencies		
		p	q	Homozygous 1	Heterozygous	Homozygous 2
MARK1	640	f(C)=0.78	f(G)=99.22	0.00	1.56	98.44
MARK2	637	f(C)=17.74	f(T)=82.26	3.14	29.20	67.66
MARK3	631	f(A)=7.77	f(G)=92.23	0.32	14.90	84.79
MARK4	633	f(C)=63.27	f(G)=36.73	39.65	47.24	13.11
MARK5	639	f(C)=37.01	f(T)=62.99	13.30	47.42	39.28
MARK6	640	f(C)=98.67	f(T)= 1.33	97.34	2.66	0.00
MARK7	637	f(A)=99.37	f(G)=0.63	98.90	0.94	0.16
MARK8	639	f(C)=99.61	f(G)= 0.39	99.22	0.78	0.00
MARK9	640	f(D)=2.58	f(I)=97.42	0.16	4.84	95.00
MARK10	639	f(A)=0.55	f(G)=99.45	0.00	1.10	98.90
MARK11	641	f(C)=93.29	f(T)=6.71	86.90	12.79	0.31

MARK12	635	f(A)=78.82	f(G)=21.18	60.63	36.38	2.99
MARK13	620	f(A)=99.92	f(T)=0.08	99.86	0.16	0.00
MARK14	631	f(C)=98.49	f(T)=1.51	96.99	3.01	0.00
MARK15	619	f(C)=89.26	f(T)=10.74	79.00	20.52	0.48
MARK16	640	f(C)=1.64	f(T)=98.36	0.00	3.28	96.72
MARK17	640	f(C)=99.45	f(T)=0.55	98.91	1.09	0.00
MARK18	592	f(C)=99.24	f(T)=0.76	98.42	1.52	0.00
MARK19	622	f(C)=93.73	f(T)=6.27	88.42	10.61	0.96
MARK20	637	f(A)=87.36	f(C)=12.64	76.30	22.14	1.57
MARK21	606	f(A)=65.02	f(G)=34.98	43.56	42.90	13.53
MARK22	639	f(C)=80.36	f(G)=19.64	63.69	33.33	2.97

Homozygous 1 = homozygous for allelic frequency equal p; Homozygous 2 = homozygous for allelic frequency equal q

Table 3. Observed P-values on association analysis of genetic markers and carcass and meat quality traits measured on Nellore beef cattle

Polymorphisms	Traits							
	HCW	REA	BF	WBSF7	WBSF14	WBSF21	LIPIDS	CHOLESTEROL
MARK1	0.0038	0.0705	0.0289	0.1410	0.0148	0.0431	0.0003	0.1497
MARK2	0.5758	0.1887	0.3079	0.0110	0.0080	0.0002	0.3330	0.1888
MARK3	0.5731	0.1012	0.5436	0.6058	0.5143	0.4779	0.2273	0.6409
MARK4	0.8827	0.4260	0.2513	0.1391	0.0219	0.0012	0.3474	0.7774
MARK5	0.9265	0.4842	0.2599	0.1560	0.0188	0.0029	0.3070	0.7931
MARK6	0.0405	0.5507	0.5636	0.9235	0.7992	0.2518	0.9464	0.5990
MARK7	0.0622	0.1743	0.5622	0.5936	0.0731	0.1628	0.4755	0.2231
MARK8	0.3201	0.0946	0.1582	0.5646	0.1759	0.8418	0.6414	0.5097
MARK9	0.1791	0.4635	0.6658	0.0096	0.6012	0.1317	0.4131	0.0630
MARK10	0.2614	0.4249	0.3056	0.0445	0.1087	0.7213	0.2104	0.6483
MARK11	0.5581	0.9983	0.9674	0.2293	0.3428	0.9573	0.9617	0.6184
MARK12	0.6348	0.6331	0.7677	0.7577	0.4868	0.6835	0.7255	0.4242
MARK13	0.4563	0.0152	0.6401	0.1784	0.0570	0.0052	0.2612	0.8482
MARK14	0.5615	0.1385	0.1559	0.7141	0.2353	0.4936	0.3338	0.1914
MARK15	0.5133	0.0226	0.2732	0.7721	0.4149	0.3695	0.0462	0.6730
MARK16	0.3779	0.0312	0.2345	0.6269	0.2046	0.4579	0.2964	0.1939
MARK17	0.6292	0.6955	0.0667	0.3359	0.3280	0.9089	0.3936	0.0088
MARK18	0.6564	0.7147	0.9385	0.8749	0.9484	0.5629	0.9207	0.0184
MARK19	0.8281	0.8283	0.4532	0.1840	0.2226	0.0357	0.4274	0.1081
MARK20	0.4851	0.9442	0.8136	0.7756	0.9073	0.7298	0.2361	0.2360
MARK21	0.3964	0.7026	0.1174	0.2209	0.1674	0.3601	0.3349	0.0886
MARK22	0.5746	0.8014	0.1132	0.6036	0.7825	0.6143	0.3517	0.7706