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Association of *MC4R*, *FABP3* and *DGAT1* gene polymorphisms with reproductive traits in two domestic pig lines

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ABSTRACT. *MC4R*, *FABP3* and *DGAT1* are important genes related to productive and reproductive traits in pigs, such as food intake, growth rate, back fat thickness reduction and lactation. Selection of pigs for lean meat production may lead to losses in reproductive charcters because reduction of backfat thickness and low feed intake during the lactation phase can affect reproduction. To examine this possibility, we evaluated possible associations of SNPs in the *MC4R* (SNPg.1,578C>T), *FABP3* (SNPg.240T>C) and *DGAT1* (SNPg.9,422C>T) genes with the number of weaned piglets, birth weight, total weight of litter at birth, total litter weight at weaning, age at first mating and parity interval in 227 animals of two maternal lines: European and European/Chinese pigs. Specific fragments of the genes under study were amplified by Multiplex

ARMS-PCR and genotyped in an automatic sequencer. SNPs in the *MC4R* (SNPg.1,578C>T) and *FABP3* (SNPg-240T>C) genes showed three genotypic variations in both lines, while the SNP (SNPg.9,422C>T) of the *DGAT1* gene had three genotypic variations in European swine and two genotypic variations (homozygotes) in the European/Chinese breed. Polymorphisms of MC4R, FABP3 and DGAT1 genes evaluated by variance analysis had significant associations with the reproductive traits age at first mating and parity interval, while only the *DGAT1* gene in the European line was significantly associated with the production traits mean weight at weaning and total litter weight at birth andnd weaning.

Key words: Genetic association; Genotyping; Maternal line; SNPs; *Sus scrofa domesticus*

INTRODUCTION

The use of genetic markers from genes related to economically important traits is common in breeding programs (Coutinho et al., 2010; Ferreira et al., 2017). The selection of animals with desirable alleles and the identification of genes that act on these traits at birth can lead to higher genetic gains by decreasing the generation interval (Goddard and Hayes, 2009) and increasing the accuracy of the genetic value differences with the use of the expected differences in progeny aided by genomics (DEP-AG), for example. Single nucleotide polymorphisms (SNPs) are the best genetic variation resource for population studies. Advantages of SNP markers include the availability of fast, reliable and reproducible protocols at a relatively lower cost (Lenstra et al., 2012).

SNPs have been widely used in association studies with economically important traits in pigs. Several SNPs in the melanocortin 4 receptor (*MC4R*), the fatty acid, muscle and heart binding protein (*FABP3*) gene and the gene encoding the enzyme acyl-CoA: diacylglycerol acyltransferase (*DGAT1*) are related to meat quality traits in pigs (Cui et al., 2011; Tyra et al., 2011; Zhang et al., 2014), but no records exist of studies with SNPs in these genes in swine in relation to reproductive traits. The selection of pigs for lean meat production may have led to losses in the reproductive indexes because the reduced backfat thickness and low feed intake during the lactation phase affect reproduction after weaning. Along this line, we evaluated possible associations of SNPs in the *MC4R* (SNPg.1.578C>T), *FABP3* (SNPg.240T>C) and *DGAT1* (SNPg.9.422C>T) genes with production and reproduction traits in two distinct commercial lines.

MATERIAL AND METHODS

Sampling and description of the phenotypic data

The procedures described in this study were approved by the Ethics Committee on Animal Use of the Federal University of Uberlândia (protocol number 031/15). Two commercial lineages were chosen, with the Large White x Landrace Danish (European line - LWLD) being the female of the most commercialized Danish commercial line in Brazil

today and also representing the "universal" maternal line. While the other female lineage chosen has two Chinese breeds in its composition, in addition to the Large White and Landrace races, which makes it totally different from the universal lineage and interesting as an object of study. Blood from live animals was collected by puncturing the upper part of the ear onto sterile filter paper - FTA (Whatman, 55 mm) of two genetic groups (GG) of swine formed by commercial maternal lines as follows: LWLD with 113 animals, resulting from the crossing of Large White and Danish Landrace and LLJM with 114 animals from two European breeds (Large White and Landrace) crossed with two Chinese breeds (Jiaxing and Meishan).

DNA extraction and Multiplex Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR)

DNA was extracted from 227 blood samples in FTA cards and manipulated using the WhatmanTM FTATM for blood DNA (2015) protocol, which was modified by the MEDGEN Tecnologia Avançada em DNA Laboratory, Uberlândia - MG. The protocol used is described below: 25% of the paper circle containing the blood sample was transferred from the carton envelope to a 1.5-mL microtube, and then 150 µL of TLN (nuclear lysis buffer), 5 µL of 10% SDS (sodium dodecyl sulfate), 2 µL of proteinase K (20 mg / mL) and the sample was then incubated overnight at 60°C.

After incubation, the samples were centrifuged for 10 min at 2,862 µg, and the supernatant was transferred to a new 1.5-mL tube (pellet discarded). Then 30 µL of saturated NaCl (6 M) was added, the samples vortexed and centrifuged for 10 min at 2,862 µg. The supernatant was transferred to a new 1.5 ml tube and 300 µL of absolute ethanol were added, and the sample mixed by inversion, and a period of 20 to 30 min was expected for precipitation of the DNA. The samples were then centrifuged for 15 m at 10,500 µg to give a light colored pellet. The supernatant was discarded and the tubes inverted onto paper towel until the samples were dried completely, approximately 30 m. The DNA was resuspended in 100 µL ultrapure H_2O (MilliQ).The quality of the genomic DNA was verified using agarose gel electrophoresis, and the concentration of DNA was determined using a NanoDrop spectrophotometer (Thermo Scientific) and adjusted to 20 ng/µL. The primers used for amplification (Table 1) were designed using the DNA sequences deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) for each of the genes: *MC4R* (GenBank accession No.AF087937), *FABP3* (GenBank accession No. JN646857) and *DGAT1* (GenBank accession no.GI47522917).

To amplify the fragments of interest of the genes under study, the extracted DNA samples were subjected to the ARMS-PCR technique.

The ARMS-PCR mixture comprised 2.0 µL of 10X buffer, 0.6 µL of $MgCl_2$ (50 mM), 0.4 µL dNTPs (200 µm), 0.8 µL M13-NED (100 pmol), 0.8 µL M13-FAM (100 pmol), 2 pmol of each primer, 2U Platinum® Taq DNA polymerase (Life Technologies, CA, USA), and 20 ng DNA in a 20-µL reaction volume. Amplification was carried out on a Veriti® ThermalCycler (Life TechnologiesTM, CA, USA) as follows: 94°C for 30 s (denaturation), 58°C for 30 s (annealing) and 72°C for one min (extension). This cycle was repeated 15 times, followed by a second programme: 94°C for 30 s (denaturation), 53°C for 30 s (annealing), 72°C for one min (extension). This cycle was repeated 25 times, and then the samples were held at 72°C for 10 min for final extension.

Table 1. Primers designed to amplify target regions of *MC4R*, *FABP3*, and *DGAT1* genes.

Gene Symbol	Gene name	Chromosome/ polymorphism location	Primer set	Size (pb)
MC4R	Melanocortin4 receptor	SSC1 Éxon 2 (C/T; 1,578)	F*: 5' TAC CCT GAC CAT CTT GAT TG 3' FMUT*: 5' ACT CAT CGG AAT CGT ATG GAG TGC ATA AAT CAG GGG ATC 3' R*: 5' GTC AAG ATG CTA CCG TTC GAG TGC ATA AAT CAG GGG ATT 3'	196
FABP3	Fatty acid binding protein 3	SSC6 5'UTR (T/C; -240)	F*: 5'GTC AAG ATG CTA CCG TTC CTA GCC CAG CCT CAC CAT GGT 3' FMUT*: 5' ACT CAT CGG AAT CGT ATG CTA GCC CAG CCT CAC CAT GGC 3' R*:5' TGA GTC CCC ATT CAC TTC GAT G 3'	183
DGAT1	Diacylglycerol acyl transferase	SSC4 Éxon 17 (C/T; 9,422)	F*: 5'GTC AAG ATG CTA CCG TTC AGC CAG CGC CCC CGG TCC 3' FDMUT*: 5'ACT CAT CGG AAT CGT ATG AGC CAG CGC CCC CGG TCT 3' R*: 5' CTG TGC CTG CCT GCC ATC: 5'	143

*F: forward primer ; R: reverse primer; RMUT: reverse primer for nucleotide exchange; FMUT: forward primer for nucleotide exchange.

Genotyping

A total of 1 μL of the Multiplex-PCR products was diluted in 8 μL of Formamide Hi-Di™ (Applied Biosystems, CA, USA) and 0.3 μL of LIZ® 600 (Applied Biosystems, CA, USA). All samples were denatured at 95°C for 5 min, cooled for 10 min at -20°C and genotyped using the ABI 3500 automated sequencer (Applied Biosystems, CA, USA). The amplified fragments were analyzed with the GeneMaker® software (SoftGenetics LLC® genotyping version 3.0) for data acquisition and the nucleotide characterization of each marker, providing a genetic profile of each animal.

Statistical analyses

To check for Hardy-Weinberg equilibrium, a Chi-squared test was performed from the binominal expansion described by Falconer and Mackay (1996). The phenotypic measurements of the number of weaning piglets (NWP), mean birth weight (MBW), mean weight at weaning (MWW), total litter weight at birth (TWLB), total litter weight at weaning (TWLW), age of first mating (IPC) and parity interval (PI) were evaluated in a mixed model by applying a repeated measures analysis in terms of time. In this case, the MIXED procedure of SAS (Statistical Analysis System, v.9.3, Cary, NC) was used with the repeated option when the density function was used with continuous random variables. The order of parity was considered a repeated measure. The data were analyzed with the following generalized linear model:

$$Y_{ijkl} = \mu + (gen) + NB + OP + NWP + AFM + PI + e \qquad \text{(Eq. 1)}$$

In which:
Y = dependent variable (MBW, MWW, TWLB, TWLW);
μ = the general average of the population for the trait;
(gen) = random effect of the individual, nested in the genotype (AA, AB, BB);
NB = fixed effect of the number of live births;

OP = fixed effect of the order of parity (1, 2, 3 and 4);
NWP = fixed effect of the number of weaning piglets;
AFM = fixed effect of age at first mating;
PI = fixed effect of the parity interval;
e = random error.

In the case of presenting density functions in discrete random variables, the SAS procedure GENMOD was used with the repeated option. The order of delivery was considered a repeated measure. The data were analyzed with the following generalized linear model:

$$Y_{ijkl} = \mu + I\,(gen) + NB + NS + MM + MBW + MWW + OP + NWP + AFM + e \quad \text{(Eq. 1)}$$

In which:
Y = dependent variable (number of piglets weaned, age at first mating, parity interval);
μ = the general average of the population for the traits;
I (gen) = random effect of the individual, nested in the genotype (AA, AB, BB);
NB = fixed effect of the number of live births;
NS = fixed effect of the number of stillbirths;
MM = fixed effect of the number of mummified piglets;
MBW = fixed effect of mean birth weight;
MWW= fixed effect of mean weight at weaning;
OP= fixed effect of the order of parity (1, 2, 3 and 4);
NWP = fixed effect of weaning number;
AFM = fixed effect of age at first mating;
e = random error.

The Akaike (AIC) and Bayesian Schwarz (BIC) information criteria were used to choose the most appropriate (co)variance of the most appropriate residues (Wolfinger, 1993). The allelic substitution and dominance effects were estimated using the same mixed model but including the effect of genotype as a covariate. For the allelic substitution effect, the genotypes were indicated as 0, 1 and 2 for AA, AB and BB, respectively, corresponding to the number of B genes in the genotype; for the dominance effect, the genotypes were indicated as -1.0 and +1, corresponding to the values designated -a = -1, d = + a = +1 for complete dominance, such that the degree of dominance = d / a = 1.

In the genetic model, in which the genome incorporates the effects of dominance, this dominance is of the complete type and in the positive or favourable allele (d / a = +1), that is, the genotypic value of the heterozygous individual is identical to that of the favourable homozygote. For the estimation of dominance effects, the assigned values (-a, d and + a) for the AA, AB and BB genotypes were calculated as deviations from the mean of the homozygous genotypes.

RESULTS

The productivity data were obtained between the years 2012 and 2014 until the fourth parity of 227 female pigs. The descriptive statistics of the data evaluated are shown in Table 2.

Table 2 - Descriptive statistics of the productivity data of female swine for two genetic groups (Large White x Landrace -LWLD and Large White x Landrace x Jiaxing and Meishan -LLJM) of pigs.

Trait*	Parameters Number of observations	Mean	Standard deviation
	LWLD		
AFM (days)	97	238.10	7.64
NWP (No.)	418	11.28	3.67
PI (days)	338	148.19	10.50
MBW (kg)	437	1.49	0.06
MWW (kg)	442	5.56	0.78
TWLB (kg)	441	19.33	5.14
TWLW (kg)	435	60.63	24.86
	LLJM		
AFM (days)	93	238.87	7.19
NWP (nº)	415	11.58	3.29
PI (days)	339	148.56	12.44
MBW (kg)	438	1.48	0.08
MWW (kg)	425	5.77	0.67
TWLB (kg)	445	19.22	4.18
TWLW (kg)	425	66.04	21.49

AFM = age at first mating; NWP = piglets weaning number; PI = parity interval; MBW = mean birth weight; TWLB = total litter weight at birth; TWLW = total litter weight at weaning. ** LWLD = Large White and Landrace; LLJM = Large White, Landrace, Jiaxing and Meishan

The relationship of the possible associations and the F-test estimates related to the effects of allelic (α) and dominance (d) substitutions between the SNPs of the *MC4R* (SNPg.1,578C>T), *FABP3* (SNPg.-240T>C) and *DGAT1* (SNPg.9,422C>T) genes identified in the LWLD and LLJM pigs with the MBW, NWW, TWLB, TWLW, NWP, AFM, and PI traits are described in Tables 3 and 4.

Table 3 -Least Square Means (LSMEANS) and standard deviation for the mean birth weight (MBW), mean piglet weaning weight (NWW), total litter weight at birth (TWLB), and total litter weight at weaning (TWLW), piglet weaning number (NWP), age at first mating (AFM), and parity interval (PI), as well as the *MC4R* (SNPg.1,578C>T), *FABP3* (SNPg-240T>C), and *DGAT1* (SNPg. 9,422C>T) genes, in the LWLD (Large White x Landrace) and LLJM (Large White x Landrace x Jiaxing and Meishan) genetic groups of pigs.

Gene	Genotype	Trait						
		LWLD						
		MBW	MWW	TWLB	TWLW	NWP	AFM	PI
	CC (8)	1.47±0.01	5.27±0.33	18.01±0.16	56.87±2.04	10.30±0.89	251.80±4.66	146.70±3.66^{b}
	CT (56)	1.49±0.01	5.33±0.27	17.97±0.08	58.34±0.86	10.16±0.68	252.84±3.98	144.06±2.5^{a}
MC4R	TT (46)	1.48±0.01	5.33±0.30	17.88±0.09	57.99±0.95	10.54±0.74	250.96±4.11	144.82±2.60^{ab}
	TT (3)	1.50±0.02	5.39±0.36	18.01±0.25	54.26±4.73	9.67±0.93	250.95±5.29	141.65±4.41
	TC (39)	1.48±0.01	5.35±0.28	17.92±0.14	53.37±3.85	10.02±0.47	254.17±4.41	147.71±2.56
FABP3	CC (71)	1.49±0.01	5.35±0.27	17.99±0.13	52.76±3.74	10.17±0.43	253.59±4.44	145.76±2.58
	CC (94)	1.48±0.01	5.20±0.27^{a}	18.16±0.19	47.11±4.47^{a}	10.15±0.43	252.18±3.84^{a}	144.62±2.47^{a}
	CT (10)	1.48±0.01	5.39±0.26^{ab}	17.92±0.19	50.64±4.24^{ab}	10.08±0.59	251.71±4.04^{a}	143.50±3.15^{a}
DGAT1	TT (9)	1.50±0.01	5.55±0.27^{b}	18.21±0.20	52.75±4.47^{b}	9.83±0.70	263.29±4.20^{b}	147.80±3.33^{b}
		LLJM						
	CC (13)	1.51±0.02	5.86±0.28	18.73±0.46	61.78±2.21	9.46±0.55^{a}	243.70±1.91^{a}	152.65±2.71^{b}
MC4R	CT (60)	1.49±0.01	6.09±0.24	18.35±0.28	62.85±1.74	10.17±0.44^{b}	238.96±1.47^{b}	146.59±2.05^{a}
	TT (30)	1.48±0.02	6.18±0.26	18.01±0.35	62.60±1.91	10.04±0.48^{b}	239.96±1.60^{b}	146.18±2.26^{a}
	TT (2)	1.52±0.04	6.29±0.35	17.93±1.34	71.55±6.14	10.43±1.03	232.00±4.22^{a}	146.07±5.51^{ab}
FABP3	TC (21)	1.49±0.02	5.94±0.15	17.74±0.37	63.72±2.94	10.21±0.35	241.43±1.30^{b}	145.33±2.41^{a}
	CC (85)	1.50±0.01	5.98±0.15	18.46±0.25	63.04±2.91	10.27±0.28	241.98±0.65^{b}	147.45±2.06^{b}
	CC (112)	1.48±0.01	5.88±0.11	18.19±0.25	63.82±1.85	9.95±0.42	239.79±1.73	145.94±2.22
DGAT1	TT (2)	1.46±0.03	5.99±0.27	19.01±0.98	66.69±3.93	9.76±0.94	234.07±4.71	145.08±5.85

a, b different letters in the same column, within each gene, indicate statistically significant differences (P <0.05) by the Tukey-Kramer test.

Table 4 - Estimates of allelic substitution and dominance effects and their respective p-values associated with the *MC4R* (SNPg.1,578C>T), *FABP3* (SNPg-240T>C) and *DGAT1* (SNPg.9,422C>T) genes in the Large White and Landrace (LWLD) and Large White, Landrace, Jiaxing, Meishan (LLJM) genetic groups in terms of the mean birth weight (MBW), mean weight at weaning (MWW), total litter weight at birth (TWLB), total weaning weight at weaning (TWLW), piglet weaning number (NWP), age at first mating (AFM), and interval between parities (PI).

	LWLD				LLJM			
Traits	**α**	**P**	**d**	**P**	**A**	**P**	**d**	**P**
	MC4R (SNPg.1578C>T)				*MC4R* (SNPg.1578C>T)			
MBW	-0.0036	0.4081	0.0076	0.1777	-0.0159	0.0943	0.0024	0.8002
MWW	0.0363	0.6169	0.0407	0.5984	0.0350	0.5412	0.0633	0.4211
TWLB	-0.0573	0.2996	0.1415	0.0533	-0.2580	0.0174	0.0481	0.7570
TWLW	0.0440	0.9523	0.5321	0.5639	13.427	0.1565	0.1832	0.8028
NWP	0.2286	0.2416	-0.3936	0.1288	0.2380	0.0638	0.3937	0.1094
AFM	-19.549	0.0438	24.687	0.0395	-25.151	0.0149	-29.686	0.0400
PI	0.9567	0.3311	11.027	0.4150	-30.477	0.0285	-29.265	0,1253
	FABP3 (SNPg.-240T>C)				*FABP3* (SNPg.-240T>C)			
MBW	0.0009	0.8824	-0.0041	0.4901	0.0040	0.7071	-0.0082	0.4120
MWW	0.0117	0.8690	-0.1084	0.3222	-0.0262	0.6876	0.0272	0.7989
TWLB	0.0503	0.4374	-0.1096	0.2290	-0.0953	0.3369	-0.1840	0.3132
TWLW	-0.6336	0.5220	-19.028	0.2717	-0.4556	0.5630	-0.4260	0.7054
NWP	0.0932	0.6780	-0.1349	0.5960	0.4101	0.0087	0.2324	0.3486
AFM	-0.1073	0.9198	0.4553	0.7132	16.076	0.0841	21.260	0.1967
PI	0.0421	0.9715	18.890	0.1978	20.449	0.1109	-43.459	0.0386
	DGAT1 (SNPg.9422C>T)				*DGAT1* (SNPg.9422C>T)			
MBW	0.0061	0.1942	-0.0028	0.7834	0.0049	0.7963	-	-
MWW	0.1525	0.0174	0.1114	0.4233	0.0296	0.7976	-	-
TWLB	0.0030	0.9590	-0.1209	0.3627	0.0895	0.7617	-	-
TWLW	17.400	0.0242	18.049	0.2806	0.7788	0.5737	-	-
NWP	-0.3214	0.1311	-0.6921	0.1592	-0.2295	0.5075	-	-
AFM	48.952	0.0001	-26.056	0.2417	-21.286	0.4328	-	-
PI	12.810	0.2506	-16.310	0.5289	-0.0256	0.9927	-	-

α (effect of gene substitution), d (dominance effect) and P ($P < 0.05$).

MC4R (SNPg.1,578C>T)

For the *MC4R* gene, a 196 bp region, which composes exon 2, was amplified. For SNPg.1,578C>T, three genotype patterns (CC, CT and TT) were identified in the two genetic groups evaluated. A higher frequency of the T allele (0.67 and 0.58) was observed in relation to the C allele (0.33 and 0.42) for LWLD and LLJM, respectively. The most frequent genotype for the LWLD group was the TT homozygote (45%), and the LLJM group had a higher genotypic frequency for the heterozygote CT (49%). The probability of proportion test did not reject the null hypothesis that the observed genotypic frequencies are in Hardy-Weinberg equilibrium at a significance level of 5%. This result indicates that the SNPg.1,578C>T of the *MC4R* gene is in equilibrium in the LWLD population studied.

The mean values of traits evaluated with SNPg.1,578C>T were significant in the LWLD and LLJM genetic groups; the number of weaned piglets (NWP) and age at first mating (AFM) were only significant in the LLJM genetic group (Table 3). The mean values of NWP, AFM and PI among the genotypes were significantly different ($P<0.05$), and CC animals presented the highest averages for AFM and PI in both genetic groups as well as a lower mean for NWP in the LLJM group.

FABP3 (SNPg-240T>C)

The amplified region of the *FABP3* gene has 183 bp and composes part of the 5'UTR (5 'Untranslated Region). For SNPg-240T>C of the *FABP3* gene, a higher frequency of the C allele (0.80 and 0.88) was observed in relation to the T allele (0.20 and 0.12) for the LWLD and LLJM groups, respectively. Regarding the genotypes, the three genotype patterns were observed, but the CC homozygote showed a higher frequency in the two genetic groups analyzed (64% LWLD and 77% LLJM). For the probability of proportion test, the null hypothesis was not rejected; the observed genotypic frequencies are in Hardy-Weinberg equilibrium at a significance level of 5%. This result indicated that SNPg.-240T>C is in equilibrium in the LWLD.

There was a significant association ($P < 0.05$) between the SNPg-240T>C and the AFM and PI reproductive traits only in the LLJM genetic group (Table 3).

DGAT1 (SNPg.9,422C>T)

For the *DGAT1* gene, a 143 bp region that composes exon 17 was amplified. For SNPg.9,422C>T, a higher frequency of the C allele (0.88 and 0.98) was observed in relation to the T allele (0,12 and 0.02) for the LWLD and LLJM groups, respectively. The mean weaning weight (MWW), total litter weight at weaning (TWLW), age at first mating (AFM) and parity interval (PI) traits were significantly different only in the LWLD genetic group ($P < 0.05$) among the genotypes, whereas homozygous animals for the T allele showed the highest averages (Table 3).

DISCUSSION

MC4R (SNPg.1,578C>T)

The age at first mating is extremely important in farm stock since the gilts represent a large proportion of the animals; whereas the first parity may influence the reproductive efficiency of the herd (Cavalcante Neto et al., 2008; Rotava, 2014) and can increase the number of non-productive days. According to Whittmore and Kyriazakis (2006), selection of genotypes for lean meat and increased prolificity may have caused negative consequences, especially in primiparous females, mainly by the reduction of lipid deposition. Likewise, the increase in the number of piglets born per parity directly affects birth weight. The greater variability of birth weight in litters reduces the vitality of piglets with low birth weight, which results in higher pre-weaning mortality. In addition, the *MC4R* gene plays important roles in energy homeostasis control, body weight regulation, and fat mobilization (Kim et al., 2000; Mackowski et al., 2005; Piórkowska et al., 2010). This outcome may be a possible explanation for lower NWP and higher PI since SNPg.1.578C>T, when homozygous for the C allele, may be related to lower feed intake during the lactation period, requiring mobilization of adipose tissue for milk production and causing changes in milk production and composition. However Zhao et al. (2012), reported different deposition in the fat and lean in two divergent Banna mini-pig inbred lines does not result from their feed intake, but rather from their genotypes for fatness traits, influenced by feed nutrients serum triacylglycerol and free fatty acid and the expression

levels of genes and proteins which participate in the serum triacylglycerol synthesis process and the energy metabolism.

Szyndler-Nędza et al. (2013), by studying SNPg.1.426G>A in the *MC4R* gene with the milk composition of Large White and Polish Landrace breeds, found a significant influence on fat ($P \leq 0.001$), solids ($P \leq 0.001$), lactose content ($P \leq 0.001$) and protein content in milk ($P \leq 0.05$).

In relation to the PI trait in the homozygous animals for the CC genotype in the *MC4R* gene, these animals presented a mean of 2 and 6 days, respectively, in the LWLD and LLJM groups (Table 3), resulting in a smaller number of piglets weaned per sow per year and reflecting economic losses. The variables that regulate PI are the lactation period and weaning interval; the latter can be influenced by the lactation period and the nutritional status of the female. According to Pinheiro (2014), for high reproductive performance of the herd, diets high in quantity and quality are necessary and fundamental to the maintenance of a corporal score during gestation, in addition to a feeding management that allows low loss in the corporal condition during lactation. However, lines present in pig farms currently have low feed intake during the lactation period, which may lead to worsening of body conditions, compromising ovulation and the onset of the next oestrus cycle. Growth and carcass traits are important in the pig industry, and the melanocortin-4 receptor (*MC4R*) gene expressed in the nervous system is responsible for the regulation of food intake, energy balance and body weight (Choi et al., 2016). Fan et al. (2009) identified variation in the porcine *MC4R* gene the SNP p.Asp298Asn influences growth rate over the pigs lifetime, while the SNP p.Arg236His appears to impact on growth rate after weaning (in this case when fed ad libitum at the fastest-growing stage) in em pigs Berkshire and Yorkshire (B · Y) intercross breed resource family. As reviewed by Benoit et al. (2000), food intake may be influenced by changes in signalling in the central nervous system by the *MC4R* gene. In the association analysis performed by Houston et al. (2004), the *MC4R* gene was significantly associated with mean daily gain, daily feed intake and backfat thickness. Hirose et al. (2014), by evaluating the effects of candidate genes on production traits in Duroc pigs, detected positive correlations between the mean daily weight gain and backfat thickness of SNPs in the LEPR (c.2002T), *MC4R* (c.1426G) and PIK3C3 (c.2604C) genes.

To understand how SNPg.1,578C>T in the *MC4R* gene is related to reproductive traits, it is emphasized that the profile of the swine has changed in the last years through the selection of less backfat thickness and the higher production of lean meat. Thus, adult females have a larger size and weight, requiring more time to reach maturity and more energy and nutrients to supply the maintenance; furthermore, perhaps more importantly, the voluntary consumption of feed of these animals has decreased over the time of selection (Schenkel, 2007). As a result, current genetics are more prone to a loss in body reserves during lactation, reflecting possible reproductive losses, whereas, in the first farrowing, the females have fewer body reserves, presenting higher requirements for maintenance, producing more milk and eating less feed (Schenkel, 2007).

The T allele of SNPg.1,578C>T in the *MC4R* gene with the TWLB trait showed a complete dominance effect on the C allele since the CT presented a TWLB value similar to that of the TT homozygote. For the AFM trait, we observed an over-dominance effect of the C allele in the LWLD genetic group because the value of the CT genotype was higher than that of the CC genotype. However, the IPC trait in the LLJM genetic group had a degree of partial dominance of the T allele (Table 4). Through the estimates obtained in the LWLD

genetic group, the presence of the T allele increased the TWLB to 0.1415 kg, and the C allele increased the AFM to 2.4667 days. However, it is important to note that, in the AFM trait, the dominance effect was negative in the LLJM genetic group (d = -2.9686), and the negative value of d always indicates the dominance of the gene that contributes less. Likewise, a significant effect ($P < 0.05$) of allelic replacement (C > T) was observed for AFM in both populations and for TWLB and PI only in the LLJM group, indicating that the C allele is related to these traits, with a decrease of -1.9549 and -2.5151 days for AFM in the LWLD and LLJM genetic groups, respectively, and a decrease of -0.2580 kg and -3.0477 days for TWLB and PI, respectively, in the LLJM genetic group. In this case, the negative values of allelic substitution may be favourable to the reproductive traits from the point of view of selection since the animals begin their reproductive life earlier and present a smaller number of non-productive days, which leads to an increase in the number of weaned piglets/nut/year. However, for the TWLB production trait in the LLJM genetic group, this effect was unfavourable since the presence of the T allele caused a reduction in the total weight of piglets at birth.

However, genetic factors (e.g., hyperprolificity, uterine capacity, and placental efficiency) and maternal factors (e.g., nutrition) have a greater influence on piglet weight at birth (Panzardi et al., 2009). Holanda et al. (2005), by analysing a database where females were counted until the fourth delivery, identified a positive effect ($P < 0.05$) of the mean birth weight on the age of the mother at birth and the litter size. The authors observed that the largest litters occurred in females of 3.12 years of age. Certainly, younger females tend to have smaller litters compared to adult females. In addition, the demand for nutrients for the growth and maintenance of primiparous females during the gestation and lactation periods is higher than that in adult females. One of the problems that occurs with hyperprolific females is the lack of uniformity of litters, which results in greater weight variability among piglets.

FABP3 (SNPg-240T>C)

The *FABP3* gene is associated with fat content in the carcass, intramuscular fat and meat quality, and its main functions are the regulation of fatty acid uptake and intracellular transport (Chmurzyńska, 2006; Hong et al., 2015). Adipose tissue may influence reproductive traits because it plays an endocrine role, such as the metabolism of sex hormones (Pinto, 2014). It is likely that variants closely linked to the *FABP3* gene facilitate the transport of lipids from the diet to the metabolic processes involved in these two traits, contributing to a satisfactory body condition. Thus, the action of SNPg. -240T>C when homozygous for the T allele decreased AFM and PI, resulting in favourable action of these traits from an economic point of view. The LLJM group has, in its genetic constitution, Chinese pig breeds, which have a higher fat content in the carcass compared to that of the LWLD group (European pig breeds), causing a significant change in the action of the *FABP3* gene in this population. Zang et al. (2017) corroborate in their studies that animals from crosses (Landrace X Yorkshire; Duroc x (Landrace X Yorkshire)) tend to have a higher content of fatty acids and, consequently, higher intramuscular fat content. Chen et al. (2014) analyzed the *FABP3* gene polymorphism by PCR-RFLP in six distinct populations (Yanan, Jinhua, Duroc, Landrace, Yorkshire, and Duroc x (Landrace x Yorkshire)), and, as a result of this study, we found a high prevalence of alleles in homozygotes, suggesting that

the *FABP3* gene can be used as a genetic marker to increase intramuscular fat content. These results corroborate with Gerbens et al. (1999), which detected homozygous genotypic classes of H-FABP RFLP for the intramuscular fat content, backfat thickness. In contrast, Sweeney et al. (2012), studying the breed Largue White identified gp-634 SNP that was associated with a linear increase in intramuscular fat, evidenced in 27% heterozygous (CA) and 38% in homozygous (CC).

The estimate of allelic substitution for the number of copies of the C allele of SNPg-240T>C in the *FABP3* gene demonstrated that this allele is related to the NWP variable ($P<0.05$) in the LLJM genetic group (Table 4). However, heterozygous animals had, on average, 0.4101 weaned piglets/farrowing, while CC homozygotes had 0.8202 more weaned piglets/farrowing. Therefore, homozygous animals for the favourable allele are, on average, superior to animals that have no copy of that allele. Williams et al. (2009) studied the influence of the birth weight of piglets and demonstrated that the *FABP3* gene is expressed ten-fold higher in adipose tissue than in skeletal muscle, and this increase is reached soon after birth, regardless of birth weight, whereas the expression in adipose tissue decreases with age in low birth weight piglets (0.800 kg - 1.330 kg), which may represent a developmental difference among these animals.

The dominance effect of this gene was significant for the PI trait in the LLJM group ($P<0.05$). The T allele had partial dominance in relation to the C allele, and animals with the presence of the dominant allele showed an estimate of -4.3459 days between one and the next. Possibly, the presence of this allele, in combination with other genes, can regulate the lipid metabolism involved in these two traits, which is susceptible to stimuli related to nutrient availability and production (Lehninger and Nelson, 2006), such as lactation.

DGAT1 (SNPg.9,422C>T)

The *DGAT1* gene acts on the metabolism of diacylglycerol and is involved in the synthesis of triglycerides, the intestinal absorption of fat by the small intestine and the physiological process of lactation (Li et al., 2012). Because of these physiological functions, several studies have linked polymorphisms in the *DGAT1* gene with milk production and composition traits in dairy cattle (Grisart et al., 2004), meat quality in commercial pigs (Zhang et al., 2014) and fat content in beef cattle (Thaller et al., 2003). SNPg.9,422C>T identified in our study may have resulted in higher quality milk production in females with the TT genotype, resulting in heavier piglets at weaning, with a total litter weight at weaning (TWLW) of 5.5 kg that is higher compared to the other genotypes, but the females obtained higher PI. Possibly, these animals lost greater lipid reserves during the lactational period, which may be related to lower food intake, as reflected by genetic selection for lean meat, since the reduction of backfat thickness and low consumption during lactation can affect reproduction, decreasing the reproductive indexes as PI. These animals require a high nutritional demand during lactation due to the large number of piglets per calf while directly stimulating milk production.

However, for SNPg.9,422C>T in the *DGAT1* gene and for SNPg-240T>C in the *FABP3* gene in the LLJM genetic group, no associations were found among the genotypes and the MBW, PMD, PTLN, PTLD, NDES, IPC or IDP (Table 4) traits. These results may be due to the assumption that these maternal strains have undergone selection pressure for certain economic traits, such as litter size, meat production per year, average daily weight

gain (GMPD) and the number of piglets weaned per year. These results can also be attributed to differences in the frequencies of the alleles of these genes in the studied population, the frequency of founder animals, the statistical models used and the genotype and environment interactions. Therefore, it is presumed that there is an association between the SNPs evaluated in this work that are present in the *MC4R*, *FABP3* and *DGAT1* genes and the productivity traits in the lineages selected for hyperprolificity.

Estimates of the allelic substitution effect of SNPg.9,422C>T on the *DGAT1* gene for the MWW, TWLW and AFM (Table 4) traits were significant ($P < 0.05$) in the LWLD genetic group. The presence of the T allele were, on average, 0.1525 and 1.7400 kg in MWW and TWLW, respectively; this result equalled more per delivery, and, in homozygous animals, these values were duplicated. Likewise, for the AFM trait, the heterozygous animals presented an average of 4.8952 days more to reach sexual maturity. The dominance effect of this gene in the LWLD genetic group did not show significant effects ($P > 0.05$) on the studied traits. For the LLJM genetic group, it was not possible to calculate the dominance effect of the *DGAT1* gene on the traits evaluated in our study because only the homozygous genotypes were present.

CONCLUSIONS

The LWLD population showed greater variability of the genotypes. The polymorphisms of MC4R, FABP3 and DGAT1 genes evaluated by variance analysis had significant associations with the reproductive traits AFM (age at first mating) and (PI) parity interval. However, the *DGAT1* gene was significantly associated only in the LWLD genetic group with the MWW and TWLW production traits. This fact may have occurred due to the genetic selection for lean meat, since reduction of backfat thickness and low feed consumption during lactation can affect reproduction and weaning, decreasing the reproductive indexes. For most of the polymorphisms, it was possible to identify the association from the characteristics of reproduction and production. Therefore these can be used as markers for selection in the genetic improvement of swine.

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