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1 **ASSOCIATION OF PREDICTED DELETERIOUS SINGLE**
2 **NUCLEOTIDE POLYMORPHISMS WITH CARCASS TRAITS IN**
3 **MEAT-TYPE CHICKENS**

4

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44 Association of predicted deleterious single nucleotide polymorphisms with carcass traits
45 in meat-type chickens
46
47 Key words: association study; missense SNPs; target sequencing; broilers
48
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55
56 ABSTRACT
57
58 In previous studies, we used genome wide association (GWAS) to identify
59 quantitative trait loci (QTL) associated with weight and yield of abdominal fat,
60 drumstick, thigh and breast traits in chickens. However, this methodology assumes that
61 the studied variants are in linkage disequilibrium with the causal mutation and
62 consequently do not identify it. In an attempt to identify causal mutations in candidate
63 genes for carcass traits in broilers, we selected 20 predicted deleterious SNPs within
64 QTLs for association analysis. Additive, dominance and allele substitution effects were
65 tested. From the 20 SNPs analyzed, we identified six SNPs with significant association
66 (p-value <0.05) with carcass traits, and three are highlighted here. The SNP
67 rs736010549 was associated with drumstick weight and yield with significant additive
68 and dominance effects. The SNP rs739508259 was associated with thigh weight and
69 yield, and with significant additive and allele substitution effects. The SNP

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70 rs313532967 was associated with breast weight and yield. The three SNPs that were
71 associated with carcass traits (rs736010549, rs739508259 and rs313532967) are
72 respectively located in the coding regions of the *WDR77*, *VWA8* and *BARL* genes. These
73 genes are involved in biological processes such as steroid hormone signaling pathway,
74 estrogen binding, and regulation of cell proliferation. Our strategy allowed the
75 identification of putative casual mutations associated with muscle growth.
76

77 ## BACKGROUND

78 Chicken is an important source of protein for human nutrition and a model system in
79 growth and developmental biology (Ellegren 2005). The complete genome sequence of
80 a Red Jungle Fowl female (*Gallus gallus gallus*), that is considered the ancestor of
81 domestic chicken (*G. g. domesticus*) (Abplanalp 1992; Cassoli 2007; Dodgson *et al.*
82 2011), was completed in 2004 (Hillier *et al.* 2004) and opened the opportunity to
83 explore the molecular control of complex phenotypes such as growth and muscle
84 deposition among other traits.

85 High throughput sequencing of several chicken lines allowed the identification
86 of millions of single nucleotide polymorphisms (SNPs) in the chicken genome (Rubin *et*
87 *al.* 2010; Boschiero *et al.* 2018) and develop high density SNP panels (Kranis *et al.*
88 2013). SNPs are the most common and frequent DNA variant, with approximately 5
89 SNPs per kilobase (kb) in chicken (Rubin *et al.* 2010). When located in coding and
90 regulatory regions of genes, they may affect traits of economic interest in animal models
91 and livestock species (Roux *et al.* 2014).

92 High-density SNP panels were used in genome wide association studies
93 (GWAS) to identify genomic regions associated with quantitative traits such as body

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94 weight (Gu *et al.* 2011a), fatness traits (Sun *et al.* 2013), breast and leg muscle weight,
95 wing weight (Xie *et al.* 2012), carcass and eviscerated weight (Liu *et al.* 2013).

96 GWAS relies on the linkage disequilibrium of the genetic variant present in the
97 SNP panel and the casual mutation, so further studies are necessary to identify the
98 mutation responsible for the phenotype of interest. In an attempt to solve this issue,
99 statistical evidence, such as association studies combined with functional annotations of
100 genes and genetic variants, is important to determine he causal mutation (Spain and
101 Barrett 2015). A SNP that occurs in coding regions can be classified as missense when
102 the coded amino acid is changed, or synonymous, when the coded amino acid remains
103 the same. Thereby, missense SNPs can be predicted as deleterious or tolerated by SIFT
104 tool [Sorting Intolerant From Tolerant, (Ng and Henikoff 2003)]. Changes at well-
105 conserved positions tend to be deleterious due to the assumption that important amino
106 acids will be conserved in the protein family. (Ng and Henikoff 2002, 2003). When a
107 SNP is predicted as a deleterious mutation, it means that the change of amino acids
108 probably affects the protein structure and function, and consequently, may potentially
109 alter the phenotype.

110 Using missense SNPs, some previous studies identified associations with body
111 weight at hatch, semi-eviscerated carcass weight, eviscerated carcass weight, leg muscle
112 weight and carcass weight (Wang *et al.* 2015), abdominal fat weight, body weight at
113 different ages and body size traits (Han *et al.* 2012). However, there are no studies in
114 the literature using predicted deleterious SNPs for association studies to identify casual
115 mutations. Therefore, in this study we used previously developed whole genome
116 sequence and GWAS information to identified predicted deleterious SNPs in QTL
117 regions. Furthermore, we tested the association of these SNP with carcass traits in order
118 to identify potential causal mutations in broilers.

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119

120 METHODS AND MATERIALS

121 **Ethics statement**

122 In this study, all experimental protocols that used animals were performed in
123 agreement with the resolution number 010/2012 approved by the Embrapa Swine and
124 Poultry Ethics Committee on Animal Utilization to ensure compliance with
125 international guidelines for animal welfare.

126

127 **Experimental Population**

128 The TT reference population used for this study was generated from an Embrapa
129 broiler line called TT. The TT line has been under selection since 1992, for many
130 generations and several traits, with the goals to increase body weight and carcass yield,
131 improve viability, fertility, hatchability, feed conversion, and reduce abdominal fat
132 (Rosário *et al.* 2009). The TT Reference Population is an expansion of the TT line and
133 was developed from crossing 20 males and 92 females (1:5) in five hatches, yielding
134 approximately 1,500 chickens (Cruz et al. 2015; Marchesi et al. 2017). From this
135 population we selected 237 offspring and 37 parental chickens (12 males and 25
136 females) for target sequencing. The offspring were selected based on the following
137 criteria: (1) descendent of one of the 14 parental males that we have the whole genome
138 sequencing data; (2) from families that have between 5 to 7 animals; (3) hatched on the
139 first three incubations.

140

141 **Phenotype measurement**

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142 Body weight at 42 days of age (BW42) was measured six hours after fasting.
143 Blood samples were collected for DNA extraction during the bleeding. After bleeding
144 feathers were removed mechanically following a hot water bath (60ºC for 45 s). The
145 carcass cuts as breast weight (BTW), thigh weight (THW), drumstick weight (DRW)
146 and abdominal fat weight (ABFW) were individually measured in grams. Drumstick
147 yield (DR%), abdominal fat yield (ABF%), thigh yield (TH%) and breast weight (BT%)
148 were estimated as a percentage of live body weight at 42 days of age (BW42). More
149 details about the slaughter and phenotypes measurements are available at (Venturini *et*
150 *al.* 2014; Cruz *et al.* 2015).

151

152 SNPs selection and custom amplicon design

153 Predicted deleterious SNPs were selected from whole genome re-sequence data
154 previously generated from 14 of the 37 parental animals of the population used in this
155 study (TT Reference population). Sequences were generated with an Illumina HiSeq
156 and SNP identified using SAMtools v.1.2 software (Li *et al.* 2009). Further details about
157 library preparation, sequencing and filtering are available in Moreira *et al.* (2015) and
158 Boschiero *et al.* (2018). SNP functional annotation was performed using VEP (Variant
159 Effect Predictor, McLaren *et al.* 2016) and deleterious prediction was based on SIFT
160 score prediction (Sorting Intolerant From Tolerant, Ng and Henikoff 2003). All the
161 SNPs identified are available at EVA-EMBL database
162 (https://www.ebi.ac.uk/ena/data/view/PRJEB25004).

163 In addition, a GWAS was performed by Moreira *et al.* (unpublished data) in the
164 same meat-type chicken population (TT), in which some QTLs associated with BTW,
165 BT%, THW, TH%, DRW, DR%, ABFW and ABF% traits were identified using high-
166 density SNP chip (600K) data.

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167 For SNPs selection, we overlapped all predicted deleterious SNPs identified in
168 parental animals with the genomic windows identified in GWAS analysis that explained
169 greater than 0.53 percent of the additive genetic variance associated with the studied
170 traits (Supplemental Material, Table S1). Afterwards, the overlapped SNPs were
171 analyzed with Tagger tool in Haploview software (Barrett *et al.* 2005) based on the
172 linkage disequilibrium. In existence of adjacent SNPs with $r^2 > 0.3$, just one SNP
173 remained to avoid the selection of markers in linkage disequilibrium and consequently,
174 markers accounting for the same effect.
175 We defined 150 bp around each predicted deleterious SNP as a target region,
176 with the variant located in the middle of the region. These regions were selected for
177 target sequencing, and the amplicons designed were performed using DesignStudio
178 online platform (Illumina Technology).
179

180 **Target sequencing**

181 Genomic DNA was extracted using PureLink® Genomic DNA kit (Invitrogen,
182 Carlsbad, CA, USA) and quantified using Qubit® 2.0 Fluorometer (Thermo Fisher
183 Scientific, Waltham, MA, USA). DNA integrity was evaluated in 1% agarose gel.
184 Library preparation was performed according to Truseq® Custom Amplicon Low Input
185 Kit Reference Guide (Illumina Technology). Libraries were quantified with quantitative
186 real time PCR, using KAPA® Library Quantification kit (KAPA Biosystem) and size
187 estimated using either Bioanalyzer® (Agilent Technologies) or Fragment Analyzer
188 (Advanced Analytical Technologies). Paired-end sequencing with a read length of 150
189 bp was performed on a MiniSeq™ (Illumina Technology).
190

191 **Sequencing data analyses, variant calling and functional annotation**

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192 The SNP calling was conducted for the 282 chickens (offspring and parental
193 generations). Raw sequencing data were aligned against the chicken reference genome
194 *Gallus_gallus*5.0 (NCBI) with BWA v.0.7.15 program, using BWA-MEM algorithm.
195 For the SNP calling, we used SAMtools v.1.3.1 program (Li *et al.* 2009), with *mpileup*
196 option (Li 2011), and mapping and base qualities (Phred) ≥ 20. The variant calling was
197 performed with all 282 animals together. After the initial variant identification, the
198 following filtering options were applied: INDEL removal, minor allele frequency
199 (MAF) ≥ 0.05, SNP call rate ≥ 0.7, biallelic locus, sequencing depth ≥ 15 and Phred
200 score quality ≥ 40.
201 After variant calling and filtration, the remainig SNPs were annotated using VEP
202 tool version 91 (McLaren *et al.* 2016) available on Ensemble v. 91 website (Zerbino *et*
203 *al.* 2018), and the SIFT score was predicted.
204
205 **Linkage disequilibrium analysis**
206 Linkage disequilibrium analysis was conducted to indentify SNPs in strong
207 linkage and avoid testing SNPs that capture the same effects. Predicted deleterious
208 SNPs were visualized in Haploview program (Barrett *et al.* 2005) and adjacent SNPs
209 with $r^2 > 0.8$ were considered having a strong LD, and consequently one of them was
210 excluded from association analyses. Haploview was also used to determine if the SNPs
211 were in Hardy-Weinberg equilibrium (HWE).
212
213 **Association analysis**
214 For association analysis, Proc Mixed Procedure was used on SAS 9.4 Studio
215 online platform (Statistical Analysis System Institute Inc., Cary, NC). Association
216 analysis was performed with all 20 predicted deleterious SNPs together for each carcass

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217 trait, and because of that correction for multiple tests was not necessary. The model
218 used was:

219 $$y = X\beta + Wa + Zu + e$$

220 Where y is the vector of observations for the measured phenotype; X is the
221 incidence matrix relating the fixed effects to **y**; β is the vector of fixed effects, which
222 included sex and incubation; W is the genotype matrix (coded as 0, 1 and 2; 0 and 2 for
223 homozygous and 1 for heterozygous) for all 20 deleterious SNPs and a is the vector of
224 SNPs fixed effects. Z is the incidence matrix relating u to y; u is the vector of the family
225 random effect; and e is the vector of residual effects. For the weight traits, BW42 was
226 used as a covariate. Association was considered significant at p-value < 0.05 for the F
227 test.

228 Orthogonal contrasts were used to compare the mean performance of one
229 homozygote against another and to estimate additive and allelic substitution effects.
230 Similarly, dominance effect was estimated through the orthogonal contrast of the mean
231 performance of the heterozygote against the mean performance of both homozygotes.
232 These analyses were performed under the same linear model detailed above with Proc
233 Mixed Procedure on SAS 9.4 Studio online platform, considering the SNPs that
234 presented the three genotypes (0, 1 and 2). Estimates and contrasts were set based on the
235 methodology defined by Falconer and Mackay (1996). Effects were considered
236 significant for p-value < 0.05 in the F test.

237

238 **Data availability**

239 Data and reagents are available upon request. Table S1 contain the characterization of
240 genomic windows identified in genome wide association analysis.

241

242 # RESULTS

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243 ## Phenotype measures

244 The summary statistics for BW42, THW, TH%, ABFW, ABF%, DRW, DR%,
245 BTW and BT% are given in Table 1.

246

247 Table 1. Number of animals (N), mean, standard deviation (SD), minimum and maximum values for body
248 weight at 42 days of age (BW42), thigh weight (THW), thigh yield (TH%), abdominal fat weight
249 (ABFW), abdominal fat yield (ABF%), drumstick weight (DRW), drumstick yield (DR%), breast weight
250 (BTW) and breast yield (BT%).

Phenotype	N	Mean	SD	Minimum	Maximum
BW42 (g)	237	2219.75	254.87	1310.00	2816.00
THW (g)	237	203.75	30.27	110.80	277.80
TH%	237	9.16	0.63	7.26	11.64
ABFW (g)	237	50.93	14.63	19.00	91.00
ABF%	237	2.29	0.61	1.01	4.25
DRW (g)	237	30.01	44.63	161.60	419.20
DR%	237	13.80	0.89	11.81	16.35
BTW (g)	237	495.39	61.93	260.00	660.00
BT%	237	22.32	1.31	18.28	26.51

251

252 ## SNP selection and amplicon design

253 The whole genome re-sequencing from 14 parental chickens of the studied meat-
254 type population identified approximately 11 million SNPs across the genome, and after
255 functional annotation 4,708 of them were predicted as deleterious SNPs. As the result of
256 the overlap between these 4,708 SNPs and the six selected genomic windows identified
257 in GWAS, 89 predicted deleterious SNPs were kept for the further analysis. After
258 linkage disequilibrium analysis ($r^2>0.3$), 20 uncorrelated SNPs remained for the
259 amplicon design using DesignStudio online platform. The final amplicon panel had
260 99% of coverage.

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261

262 ## Sequencing and variant calling

263 Libraries sequencing from MiniSeq produced an average number of raw reads of
264 298,791 per sample. The average of overall mapping rate of the raw reads against the
265 *Gallus_gallus*5.0 (NCBI) genome assembly was 99.74%. After variant calling, 1,957
266 variants (including SNPs and INDELs) were initially detected, and 195 SNPs remained
267 after filtration. The average depth of the remaining SNPs was 7,961 reads.

268 Functional annotation was performed for the 195 SNPs. As shown in Table 2, 29
269 SNPs were annotated as novel variants. From the 195 SNPs, 26% were in intronic
270 regions, 20% were classified as missense and 14% were synonymous variants.

271 As already mentioned, missense SNPs can be predicted as deleterious or
272 tolerated based on its SIFT score (Ng and Henikoff 2003). In our study, from the 56
273 missense SNPs identified, 26 were classified as tolerated, 21 as deleterious, and 9 had
274 no prediction (Table 2).

275

276 Table 2. Number of novel and existing variants, and classification of functional annotation of single
277 nucleotide polymorphisms (SNPs) performed with Variant Effect Predictor (VEP) online platform.

Variant	**Number of SNPs**
Novel	29
Existing	166

Variant	**SIFT Prediction**	**Number of SNPs**
	Deleterious	21
Missense	Tolerated	26
	No prediction	9
Intron		70
Intergenic		25

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Synonymous	45
5' URT	1
Downstream gene	32
Upstream gene	35
Splice	6

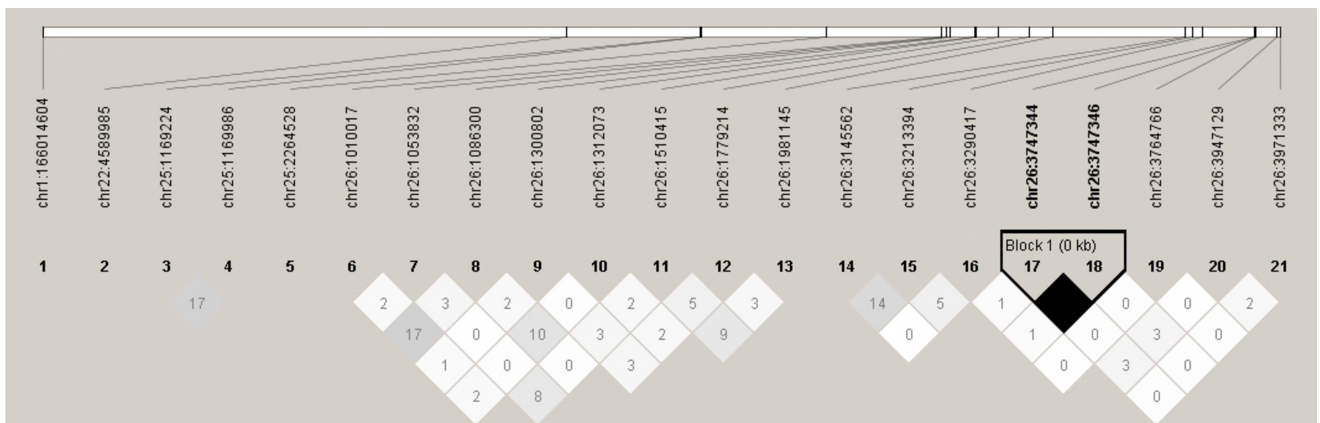
278

279

280 ## Linkage disequilibrium verification

281 As presented in Figure 1, only one region of adjacent SNPs had $r^2 > 0.3$ (r^2 =
282 1.0, black square – chr26:3747344 and chr26: 3747346). Thus, chr26:3747344 was
283 excluded, leaving 20 predicted deleterious SNPs for further analysis.

284



285

286 Figure 1. Linkage disequilibrium plot of the 21 predicted deleterious SNPs after filtering steps. The r^2
287 value is presented within each square. The gradient color also represents r^2 values, white is 0 and black is
288 1.

289

290 Detailed information of the 20 predicted deleterious SNPs (genome position,
291 SNP ID, located gene, alleles and genotypes frequencies, HWE test and SIFT score) is
292 presented in Table 3. Two SNPs did not have *rs* ID, and four genes were considered as
293 novel genes, therefore, the ensemble gene ID was also presented. Seven SNPs did not
294 have any animal genotyped with the alternative homozygous, and five SNPs were
295 significant for HWE test.

296 Table 3. Deleterious SNPs selected for the association analyses with carcass traits.

GGA	Position	SNP ID	Gene Symbol	Ensembl Gene ID	R/A	Allele Frequency		Genotype Frequency						HW p-value	SIFT
								HREF		HT		HALT			
						R	A	Freq	N	Freq	N	Freq	N		
1	166,014,604	rs739508259	*VWA8*	ENSGALG00000016955	G/C	0.588	0.412	0.364	86	0.449	106	0.186	44	0.6262	0.01
22	4,589,985	rs314536739	*ANXA4*	ENSGALG00000038783	C/T	0.738	0.262	0.531	126	0.413	98	0.054	13	0.4782	0.02
25	1,169,224	rs312547749	Novel Gene	ENSGALG00000027316	C/T	0.435	0.565	0.181	43	0.506	120	0.312	74	0.2798	0.00
25	1,169,986	rs737797683	*CRNN*	ENSGALG00000027316	G/C	0.808	0.192	0.666	158	0.282	67	0.050	12	0.2771	0.00
25	2,264,528	rs739048621	Novel Gene	ENSGALG00000014643	G/A	0.947	0.052	0.894	212	0.105	25	0.000	0	0.7875	0.00
26	1,010,017	c.482C>T	*MYBPH*	ENSGALG00000000164	C/T	0.764	0.236	0.616	146	0.295	70	0.088	21	0.0071*	0.04
26	1,053,832	c.383C>T	*CEPT1*	ENSGALG00000000142	C/T	0.941	0.059	0.881	209	0.118	28	0.000	0	0.7402	0.02
26	1,086,300	rs312325687	Novel Gene	ENSGALG00000000104	A/G	0.639	0.361	0.443	105	0.392	93	0.164	39	0.0124	0.01
26	1,300,802	rs314560661	*AHCYL1*	ENSGALG00000000329	T/C	0.941	0.059	0.881	209	0.118	28	0.000	0	0.7875	0.01
26	1,312,073	rs14297872	*STRIP1*	ENSGALG00000037995	C/T	0.932	0.068	0.865	205	0.135	32	0.000	0	0.6067	0.01
26	1,510,415	rs741234441	Novel Gene	ENSGALG00000000477	C/T	0.605	0.395	0.320	76	0.569	135	0.109	26	2.101E-6*	0.01
26	1,779,214	rs733369312	*PPP1R15B*	ENSGALG00000000611	C/A	0.624	0.376	0.299	71	0.649	154	0.050	12	5.457E-13*	0.00
26	1,981,145	rs731705610	*CNTN2*	ENSGALG00000000653	C/T	0.947	0.053	0.894	212	0.105	25	0.000	0	0.9367	0.02
26	3,145,562	rs738655377	Novel Gene	ENSGALG00000028858	A/G	0.863	0.137	0.725	172	0.274	65	0.000	0	1.0E-4*	0.00
26	3,213,394	rs736010549	*WDR77*	ENSGALG00000040864	A/T	0.810	0.190	0.628	149	0.362	86	0.008	2	0.1768	0.01

26	3,290,417	rs737237434	*DDX20*	ENSGALG00000001504	A/G	0.780	0.220	0.605	143	0.347	82	0.046	11	0.8671	0.01
26	3,747,346	rs14300225	*PTPN22*	ENSGALG00000021656	C/T	0.084	0.916	0.004	1	0.160	38	0.835	198	0.7153	0.00
26	3,764,766	rs739340698	*AP4B1*	ENSGALG00000035295	C/T	0.935	0.065	0.873	207	0.122	29	0.004	1	1.0	0.02
26	3,947,129	rs313532967	*BARL*	ENSGALG00000002170	A/G	0.736	0.264	0.493	117	0.485	115	0.021	5	5.410E-5*	0.00
26	3,971,333	rs741234600	*SYCP1*	ENSGALG00000002511	A/C	0.950	0.050	0.881	209	0.118	28	0.000	0	0.5256	0.03

297 GGA, *Gallus gallus* chromosome; R, reference allele; and A, alternative allele; HREF, homozygous of reference allele; HT, heterozygous; HALT, homozygous of alternative
298 allele; HW, Hardy-Weinberg. SIFT, score predicted in functional annotation; *Significant p-value <0.05.

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300 **Association analysis, additive and dominance effects**

301 Of the 20 predicted deleterious SNPs studied, six were significantly associated (p-
302 value<0.05) with at least one carcass trait. Three SNPs (rs737797683, rs313532967 and
303 rs741234600) were associated with breast traits; another three (rs739508259, rs312325687
304 and rs741234600) were associated with thigh traits; and one SNP (rs736010549) was
305 associated with drumstick weight. No SNP was associated with abdominal fat traits. Detailed
306 results for all association tests (p-values) are presented in Table 4.

307 Table 4. SNPs association analyses results for carcass traits.

308

SNP ID	N[a]	BW42	BTW	BT%	THW	TH%	DRW	DR%	ABFW	ABF%
rs739508259	234	0.2221	0.8332	0.8003	0.0098*	0.0146*	0.7289	0.6781	0.2133	0.1428
rs314536739	234	0.2297	0.2395	0.1313	0.1342	0.1393	0.4548	0.3859	0.1078	0.5586
rs312547749	234	0.3428	0.1884	0.2211	0.8707	0.7415	0.3945	0.3945	0.5279	0.5510
rs737797683	234	0.1798	0.0532	0.0346*	0.0573	0.0487*	0.1075	0.0959	0.7872	0.7077
rs739048621	234	0.6947	0.5004	0.5365	0.5458	0.5398	0.5261	0.5375	0.3414	0.4976
c.482C>T	234	0.6547	0.2735	0.3831	0.7965	0.6477	0.7273	0.8108	0.9809	0.8872
c.383C>T	234	0.0042*	0.8124	0.4050	0.3775	0.1619	0.3114	0.2233	0.9014	0.2758
rs312325687	234	0.1343	0.1050	0.1199	0.0354*	0.0220*	0.9174	0.9382	0.9506	0.9583
rs314560661	234	0.8758	0.2043	0.3390	0.3678	0.4045	0.4196	0.3606	0.5637	0.9232
rs14297872	234	0.0304*	0.5964	0.8217	0.2631	0.1209	0.4663	0.4815	0.8762	0.5523
rs741234441	234	0.2136	0.4656	0.4490	0.2668	0.2490	0.6970	0.7647	0.6032	0.9682
rs733369312	234	0.6968	0.9697	0.9677	0.1303	0.2975	0.1121	0.0505	0.6662	0.5475
rs731705610	234	0.1467	0.2037	0.1149	0.2577	0.1953	0.1164	0.1230	0.0669	0.1613
rs738655377	234	0.8992	0.1334	0.1881	0.1141	0.1789	0.8429	0.9745	0.4098	0.5450
rs736010549	234	0.9026	0.0677	0.1090	0.5474	0.6187	0.0038*	0.0052*	0.1523	0.3421
rs737237434	234	0.6837	0.1330	0.1863	0.0611	0.0885	0.5636	0.5137	0.8907	0.8882

rs14300224	234	0.8352	0.5980	0.4824	0.8976	0.9416	0.7702	0.6967	0.0934	0.1217
rs739340698	234	0.1450	0.2839	0.2143	0.4592	0.4109	0.4663	0.5506	0.7801	0.8390
rs313532967	234	0.6840	0.0144*	0.0234*	0.9630	0.9307	0.6828	0.5735	0.1644	0.1666
rs741234600	234	0.5306	0.0229*	0.0208*	0.0086*	0.0093*	0.2248	0.3559	0.3680	0.371

[a]Sample sizes. *Significant at $p < 0.05$. BW42: body weight at 42 days; BTW: breast weight; BT%: breast yield; THW: thigh weight; TH% thigh yield; DRW: drumstick weight; DR%: drumstick yield; ABFW: abdominal fat weight; ABF%: abdominal fat yield.

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312 Additive and dominance effects were estimated only for associated SNPs that
313 presented the three genotypes, consequently rs741234600 was not considered in these
314 analyses. We deemed significant effects with p-value <0.05. Allele substitution effect test was
315 performed only for significant additive effects (Table 5). Additive and allele substitution
316 effects for rs739508259 and rs312325687 associated with THW and TH% were significant.
317 Additive and dominance effects for rs736010549 associated with DRW and DR% were
318 significant, but not for allele substitution test.

319 Table 5. P-values, estimates and standard error for additive, dominance and allele substitution effects for SNPs with respective associated traits in broilers.

SNP ID	**Association**		**Additive Effect**		**Dominance Effect**		**Allele Substitution Effect**	
	Trait	**p-value**	**P**	**E (se)**	**P**	**E (se)**	**P**	**E (se)**
rs739508259	THW	0.0098**	0.0029*	-5.7594 (1.9068)	0.7616	-0.7945 (2.6155)	0.0062*	5.1219 (1.8496)
	TH%	0.0146*	0.0042*	-0.2479 (0.08523)	0.6723	-0.04971 (0.1174)	0.0064*	0.2268 (0.08213)
rs737797683	BT%	0.0346*	0.0104*	-0.4857 (0.1880)	0.2308	-0.2553 (0.2124)	0.0506	0.3041 (0.1547)
	TH%	0.0487*	0.1966	-0.1902 (0.1469)	0.0144*	-0.4123 (0.1673)	-	-
rs312325687	THW	0.0354*	0.0124*	6.6692 (2.6307)	0.9484	-0.1913 (2.9505)	0.0126*	-6.5551 (2.5853)
	TH%	0.0220*	0.0069*	0.3185 (0.1160)	0.9387	0.01018 (0.1323)	0.0063*	-0.3148 (0.1130)
rs736010549	DRW	0.0038*	0.0009*	-13.8438 (4.1322)	0.0016*	-13.8035 (4.3241)	0.2394	2.3175 (1.9641)
	DR%	0.0052*	0.0016*	-0.5862 (0.1836)	0.0017*	-0.6107 (0.1919)	0.3520	0.08240 (0.08834)
rs313532967	BTW	0.0144*	0.6572	-2.4332 (5.4758)	0.2326	6.9216 (5.7825)	-	-
	BT%	0.0234*	0.6708	-0.1085 (0.2550)	0.2634	0.3015 (0.2689)	-	-
rs741234600	BTW	0.0229*	-	-	-	-	0.0602	9.4399 (4.9996)
	BT%	0.0208*	-	-	-	-	0.0491*	0.4620 (0.2335)
	THW	0.0086*	-	-	-	-	0.0136*	10.0902 (4.0577)
	TH%	0.0093*	-	-	-	-	0.0121*	0.4598 (0.1819)

320 THW: thigh weight; TH% thigh yield; DRW: drumstick weight; DR%: drumstick yield; BTW: breast weight; BT%: breast yield. P, E and Se are p-values, estimates and
321 standard errors for the respective analysis. *Significant at $p < 0.05$.

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322 # DISCUSSION

323 The identification of genetic markers associated with carcass weight and yield traits
324 has been the focus of several studies due to the economic importance of these traits in broiler
325 production. With the main goal of finding putative causal mutations for carcass traits, this
326 study selected predicted deleterious SNPs present in QTLs regions to be evaluated as potential
327 causal mutations in our TT Reference Population.

328 The SNP rs739508259 is located in the von willebrand factor A domain containing 8
329 (*VWA8*) gene and within the GGA-1 at 166 Mb genomic window identified in the GWAS
330 analysis. This region was associated with DRW and DR%, explaining 3.20 and 2.79 of the
331 additive genetic variance, respectively. This SNP is a G>C nucleotide change with minor
332 allele (C) frequency of 0.42. The nucleotide change causes the amino acid substitution of
333 glutamine to histidine. The rs739508259 was associated with THW and TH% and had
334 significant additive and allele substitution effects for both traits. On average, for each C allele
335 in the animal's genotype, an increase of 5.12g was observed for THW and 0.24% for TH%,
336 compared to the GG genotype. The window on GGA-1 at 166 Mb also explained 0.20% and
337 0.14% of the additive genetic variance for THW and TH%, respectively. However, these
338 proportions were not enough to be considered significant (Additional File 1). Furthermore, it
339 is interesting to observe that SNP rs739508259 was not significantly associated with DRW
340 and DR% and this may be because we used a subset of 237 animals from the 1,408 animals
341 used in GWAS analysis or because the low number of animals used (237) in the association
342 analysis. Similar findings were also noted for other associated SNPs.

343 In mice *VWA8* gene is highly expressed in skeletal muscle, has ATPase domains,
344 mitochondrial targeting sequences and is a mitochondrial protein (Luo *et al.* 2017). More
345 studies are necessary to relate this gene with muscle growth in chickens.

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346 The predicted deleterious SNP rs736010549 is located in the WD repeat domain 77
347 (*WDR77*) gene. Furthermore, it is located within the GGA-26 at 3 Mb genomic window
348 identified in GWAS analysis, and this region was associated with breast weight (BRW) and
349 breast yield (BR%), representing 0.53 and 0.86 of the additive genetic variance, respectively
350 (Additional File 1). This polymorphism results in an amino acid change from serine to
351 cysteine (A/T allele substitution), with the minor allele (T) frequency of 0.19. This SNP was
352 associated with drumstick weight (DRW) and drumstick yield (DR%). It was also significant
353 for additive and dominance effects tests for both traits. On average, animals with TT genotype
354 had 13.8g more of DRW and 0.58 % more of DR% than AA animals. This SNP presented
355 complete dominance for both traits.

356 The WD repeat domain 77 (*WDR77*) gene belongs to the WD repeat proteins that is
357 characterized by multiple protein interaction capacity (Friesen *et al.* 2001). The protein p44
358 (also named as methylosome protein 50, MEP50) coded by *WDR77* and is an androgen
359 receptor (AR) coactivator by multiprotein complex formation (Hosohata *et al.* 2003). In
360 humans, p44 was associated with inhibition of prostate cancer cell growth as coactivator of
361 AR (Zhou *et al.* 2006; Gu *et al.* 2011b) and with breast cancer growth mediated through
362 estrogen and its receptor (Peng *et al.* 2010).

363 Several studies showed the inhibitory action of androgenic steroids in chicken growth
364 (Fennell *et al.* 1990; Fennell and Scanes 1992; Esquivel-Hernandez *et al.* 2016), which is a
365 possible consequence of the androgen receptor or estrogen receptor aromatization (Fennel *et*
366 *al.* 1996; Callewaert *et al.* 2010). Kong *et al.* (2017) studied different expressed (DE) genes in
367 a selected and unselected broiler breeds, and among their results, they suggested that inhibited
368 AR was predicted to be an effective regulatory factor for DE genes in selected breed,
369 corroborating previously cited studies. Our results suggest that SNP rs736010549 alter the
370 conformation of p44, decreasing the AR activation and so contributing to growth in chickens.

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371 The predicted deleterious rs313532967 is located in the bile acid receptor-like (*BARL*)
372 gene and within the GGA-26 at 3 Mb genomic window identified in GWAS analysis. This
373 region was associated with BTW and BT%, representing 0.53 and 0.86 of the additive genetic
374 variance respectively. This SNP is an A>G change, resulting in the amino acid change of
375 asparagine to serine, and the minor allele (G) frequency is 0.264. The HWE test was
376 significative, and this may be due to our finite population, or indicating that this locus may be
377 under selection or inbreeding. This SNP was associated with BTW and BT% and did not have
378 additive or dominant effects. Only five animals had GG genotype and this could help explain
379 the lack of additive and dominant effects.

380 The *BARL* gene have a DNA-binding domain of Farnesoid X receptor (FXR) family.
381 This domain in humans was intensively studied and when it is activated by bile acids it can
382 regulate bile acids synthesis, conjugation and transport, consequently impacting in lipid and
383 glucose metabolism (Claudel *et al.* 2005; Preidis *et al.* 2017). When bile acids are released in
384 the ileum, its induces the synthesis of fibroblast growth factor (FGF-19) which stimulates
385 hepatic protein and glycogen synthesis (Kir *et al.* 2011). In an interesting work in broilers, Lai
386 *et al.* (2018) demonstrated that dietary supplementation of swine bile acids for broiler
387 chickens influences their growth performance and carcass characteristics as reduction of
388 abdominal fat, increase of carcass weight, eviscerated weight and leg weight. Therefore, our
389 study indicates that *BARL* gene can be involved in growth and carcass development in
390 chicken.

391 The SNP rs741234600 is located in synaptonemal complex protein 1 (*SYCP1*) gene
392 and within the GGA-26 at 3 Mb genomic window identified in GWAS analysis. This region
393 was associated with BTW and BT%, representing 0.53 and 0.86 of the additive genetic
394 variance respectively. In our study, this SNP was associated with BTW, BT%, THW and
395 TH%, and is an A>C nucleotide change, resulting in the amino acid substitution of lysine to

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396 threonine, and the minor allele (C) frequency is 0.05. As the CC genotype (alternative
397 homozygous) was not present in the evaluated animals, only allele substitution effect was
398 performed. For each allele A, the animals had 0.46% more BT%, 10.09 g more THW and
399 0.45% more TH%.

400 The SNP rs737797683 is located in the cornulin (*CRNN*) gene, within the GGA-25 at
401 1 Mb genomic window identified in GWAS analysis. This region was associated with BT%,
402 BR% and ABF% representing 0.24, 0.24 and 0.23 of the additive genetic variance
403 respectively. This SNP was associated with BT% and TH%. This SNP is an G>C change,
404 resulting in the amino acid change of aspartic acid to histidine, and the minor allele (C)
405 frequency is 0.19. This polymorphism was significant for additive effect for BT% trait,
406 significant for dominance effect for TH%.

407 The SNP rs312325687 is located in the cryptochrome 4 (*CRY4*) gene and within the
408 GGA-26 at 1 Mb genomic window identified in GWAS analysis, previously associated with
409 ABFW and ABF%, representing 1.06 and 0.54 of the additive genetic variance respectively.
410 This SNP was associated with THW and TH% and is an A>G change, resulting in the amino
411 acid change of aspartic acid to glycine, being the minor allele (G) frequency equal to 0.36.
412 This polymorphism had significant additive and allele substitution effects for both traits.

413 *SYCP1*, *CRNN*, *CRY4* were not selected as candidate genes for muscle growth or
414 carcass development in this study because there is no information available in the literature to
415 support this. More studies with these genes are necessary to understand their relationship with
416 carcass and muscle growth.

417 It is pertinent to note that although rs738655377 was not significantly associated with
418 any of the phenotypes tested, none of the animal were homozygous for the alternative allele,
419 and the HWE test was significant. This observation provides evidence for a lethal

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420 polymorphism when in homozygosity. This variant is within a novel gene
421 (ENSGALG00000028858) that has gene ontology terms related to oxidoreductase activity.
422 In conclusion, our study identified 20 predicted deleterious SNPs in different QTLs
423 associated with carcass traits and succeeded in associating six of them with phenotypes
424 related to muscle growth. Three predicted deleterious SNPs associated were located in genes
425 that we consider candidate genes for carcass and muscle weight, and development. The main
426 limitation of our study is that it is difficult to determine if the identified mutations are the
427 causative mutation or are in linkage disequilibrium with the real causal mutation.
428

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439

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Table S1. Characterization of genomic windows identified in genome wide association analysis for the studied traits.

Trait	GGA_Mb[a]	Start – end positions (Chr_SNP)	SNPs[b]	%GV[c]	PPA (p>0)[d]
DRW	1_166	1_166000511 - 1_166999195	390	3.20	0.92
	26_3	26_3000141 - 26_3998650	998	0.22	0.94
DR%	1_166	1_166000511 - 1_166999195	390	2.79	0.93
	26_3	26_3000141 - 26_3998650	998	0.25	0.95
THW	1_166	1_166000511 - 1_166999195	390	0.20	0.71
	22_4	22_4000760 - 22_4676714	1035	0.54	0.95
	26_1	26_1002598 - 26_1999851	662	0.11	0.84
TH%	1_166	1_166000511 - 1_166999195	390	0.14	0.71
	22_4	22_4000760 - 22_4676714	1035	0.57	0.97
	25_1	25_1000996 - 25_1982441	691	0.11	0.85
	26_1	26_1002598 - 26_1999851	662	0.14	0.85
BRW	25_1	25_1000996 - 25_1982441	691	0.24	0.88
	25_2	25_2001192 - 25_2887176	512	0.81	0.88
	26_3	26_3000141 - 26_3998650	998	0.53	0.96
BR%	25_1	25_1000996 - 25_1982441	691	0.24	0.90
	25_2	25_2001192 - 25_2887176	512	0.6	0.84
	26_3	26_3000141 - 26_3998650	998	0.86	0.98
ABFW	26_1	26_1002598 - 26_1999851	662	1.06	0.95
ABF%	25_1	25_1000996 - 25_1982441	691	0.23	0.87
	26_1	26_1002598 - 26_1999851	662	0.54	0.92

[a]Map position based on Gallus_gallus-5.0 NCBI assembly; [b]Number of SNPs per region; [c]% of genetic variance explained by the window; [d]Posterior probability of association (PPA) as reported by Onteru et al. (2013). DRW: drumstick weight; DR%: drumstick yield; THW: thigh weight; TW%: thigh yield; BTW: breast weight; BT%: breast yield; ABFW: abdominal fat weight; ABF%: abdominal fat yield;