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GENOME-WIDE ASSOCIATION STUDY FOR MEAT QUALITY TRAITS IN A MULTI-BREED PIG POPULATION*

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Abstract

This study aimed at identifying genomic regions that affect nine pork quality traits in purebred and crossbred fatteners. A total of 259 fatteners represented by six purebreds/crosses were genotyped for 45556 SNP markers by Illumina Porcine SNP60 BeadChip. The following traits were recorded: glycogen potential (GP), glycogen content (GC), lactate content (LC), pH_{35}, pH_{24}, pH_{48}, drip loss after 48 hours (DL_{48}), colour lightness (L^*) and lean meat content (LMC). Multi-Locus Mixed Model methodology was applied to find associations between SNP markers and recorded traits. Several SNPs were found to be significantly associated with some pork quality traits: four SNPs (located on SSC7, SSC10 and SSC14) with GP, three SNPs (SSC10, SSC14) with GC, one SNP (SSC15) with DL_{48} and one SNP with pH_{48}. Genetic variation explained by significant SNPs ranged from 7.6% to 9.1%. Moreover, some genes (e.g. CAPN10, ALDH5A1, PASK, SNITA1 and MYH7B) located in the close vicinity to significant markers are proposed to be candidate genes explaining the genetic background of the traits studied.

Key words: pig, meat quality, genetic markers, GWAS

The muscle tissue of domestic animals is an important source of proteins for humans. Thirty three percent of the total consumed meat worldwide is pork (OECD/FAO, 2021). In Europe, besides a slight decrease in consumption from 34.4 to 33.4 kg *per capita* pork still remains the most frequently purchased meat (EC, 2020). An increasing number of consumers tend to prefer pork that is not only lean and safe but also has high quality (Henchion et al., 2014). Traditionally, the properties used to define the quality of fresh meat are those associated with its appearance, colour, texture (especially tenderness) and juiciness and are assessed by pH, colour, drip loss or water holding capacity (Troy and Kerry, 2010). According to Lebret and Candek-Potokar (2022), pork quality comprises a variety of attributes that are influenced by several factors ranging from genetic components to meat processing and preparation for consumption and their interactions as well. Pork quality is also an important economic trait for animal breeders and the meat industry. Meat quality is defined by many traits, but the most important are glycolytic potential (GP), pH and drip loss (DL) (Prange et al., 1977; Monin and Selier 1985). Since these traits are expensive and difficult to measure, they are usually not included in breeding index. These traits characterize most of the changes in glycogen metabolism, which affect technological processing of meat (Scheffer and Gerrard, 2007). In recent decades, with the advent of high-density SNP genotyping arrays, genome-wide association studies (GWAS) have become a useful tool for the detection of genome regions determining performance traits in livestock (Goddard and Hayes, 2009). To date, most of the loci involved in different traits in pigs are defined as QTL (quantitative trait loci). According to the latest version of the *Sus scrofa* 11.1 genome there are 31825 QTLs associated with 699 different traits in pigs that have been mapped and described in 730 published studies (Hu et al., 2019). QTLs, however, cover relatively large stretches of the chromosome without indicating specific genes engaged in the trait. Therefore, identifying genetic markers or genes participating in meat quality traits by the use

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of the GWAS approach has become effective method for analysing trait variation and its genetic architecture, including pork quality (Fontanesi et al., 2008; Piórkowska et al., 2017; Herault et al., 2018; Liu et al., 2020).

Lately Wang et al. (2023), using inter-cross pigs and almost 300 thousand markers, found 64 SNPs explaining 2.43 to 16.32% of phenotypic variation for several meat quality traits and suggested candidate genes involved in the variation of these traits.

The objective of this study was to extend such analysis in a different population of fatteners and to identify genomic regions and candidate genes that could be associated with the most important pork quality traits.

Material and methods

Animals

Initially, 283 fatteners were included in the study. Multidimensional Outlier Detection function (in SVS software, Golden Helix, USA) was applied to identify individuals being outside of the population cluster. This function calculates the inter-quartile range (IQR) distance around the centroid (median) of the study population cluster. Twenty four samples reached critical value 1.5 IQRs from the third quartile and they were excluded from the population. Thus, for further analysis, 259 fatteners representing six purebreds/crosses were included: 113 (Landrace × Yorkshire) × Duroc (L×Y)×D, 25 (Landrace × Yorkshire) × Hampshire (L×Y)×H, 18 Duroc (D), 49 Landrace (L), 33 Landrace × Duroc (L×D and 21 Landrace × Yorkshire (L×Y). Limited number of samples was caused by the availability of weaned piglets representing different purebred/cross and logistic requirements to uniform timing of fattening and time-consuming meat quality measurements (especially for GP). Selection of weaned piglets to experimental groups was random which highly decreased the chance of common sire for the breed/cross group. All fatteners were reared under the same environmental conditions in one farm. The maintenance, nutrition (complete feeds according to age) and slaughter conditions were also the same for all animals. All fatteners were genotyped in *RYR1* locus by the method described earlier (Kamiński et al., 2002) and were *RYR1*^{T}-allele free. The animals were slaughtered in autumn after transport (300 km) using an electric stunner (*Inarco, Stork*) in accordance with the technology applied at the meat plant. The fatteners were additionally standardized for hot carcass weight (85.51 kg with SD=3.44 kg) and lean carcass content (56.53% with SD=3.66%). All measurements of meat samples were done during the routine processing of meat in a commercial slaughter company which followed legal and ethical regulations provided in Directive 2010/63/EU.

Meat quality traits

Meat quality traits (Table 1) were measured using the same methodology as described earlier by Koćwin-Podsiadła et al. (2006). Briefly, fresh (up to 45 min immediately after bleeding) and chilled (after 24 h to 4°C) meat was evaluated on the m. *longissimus lumborum* (*LL*) (after the last rib), based on the following parameters:

- acidity of the muscle tissue (pH) measured directly in the LL muscle 35 min, 24 hours and 48 hours *post-mortem* (pH_{35}, $pH_{24,}$ pH_{48}, respectively), using a pistol pH-meter MASTER (Draminski, Poland). The pH-meter was calibrated prior to collecting the data with pH 4.64 and 7.00 buffer solutions (Mettler-Toledo, LLC, Columbus, Ohio, USA). Temperature compensation was undertaken in an automatic way due to technical capabilities of apparatus.
- colour lightness (L^*) of the muscle tissue was assessed 24 h (L^*_{24}) *post-mortem* with a Minolta portable chroma meter (model CR 310, Minolta, Osaka, Japan) using D65 illuminant and 50 mm orifice after 20 min of blooming. The apparatus was calibrated against white plate (illuminant D65 and 10 degree supplementary observer).
- drip loss, determined according to Prange et al. (1977), 48 h *post-mortem* (DL_{48})

The samples were cut from LL muscle 45 min *post-mortem* (immediately immersed into tubes with 1M $HClO_4$ and homogenised to inhibit glycolytic changes) and analysed for the glycolytic potential (GP) and content of glycogen and lactate. The glycolytic potential of the muscle tissue, measured in micromoles of lactate equivalent per 1 g of muscle tissue, was calculated according to the formula proposed by Monin and Sellier (1985): GP = 2([glycogen] + [glucose-6-phosphate] + [glucose]) + [lactate]. The content of glycogen was determined by the enzymatic method according to Dalrymple and Hamm (1973) and lactate content according to Bergmeyer (1974). A total of nine traits were analysed (Table 1). Those traits are considered the key quality attributes of pork used as criteria in the evaluation of faulty meat (Scheffer and Gerrard, 2007).

Differences between genetic groups of fatteners for pork quality traits were analysed by one-way analysis of variance using STATISTICA 13.1 (StatSoft, Tulsa, OK, USA). The model was as follows: $yi = \mu + ai + ei$ (yi – measured i^{th} trait, μ – overall population mean, ai – analysed factor effect of ith trait, ei – random error). The significance of differences between means was calculated using Tukey's test.

Genotyping and GWAS

Genomic DNA was isolated from the 300 µl of blood with the use of the Wizard Plus Megapreps DNA Purification System (Promega, USA). Genomic DNA was then used to genotype each pig by the Illumina Porcine SNP60 BeadChip (Ramos et al., 2009). Raw SNP data passed standard quality control in Illumina Genome Studio software (version 2011.1, Illumina, San Diego, CA) following the protocol provided by Illumina company (www.illumina.com/documents/products/technotes/technote_infinium_genotyping_data_analysis.pdf). Initial data

cleanup removed poorly performing SNPs or/and samples giving an insufficient number of informative genotypes. X chromosome markers were included in the analysis. All samples with individual genotypes had call rates over 99%. Monomorphic SNPs, SNPs which showed a significant (P<0.001) deviation from the Hardy–Weinberg equilibrium (HWE) and SNP markers with a Minor Allele Frequency lower than 0.01 were also removed from further analysis. Linkage disequilibrium pruning was applied (criteria: window size – 50; window increment – 5; r^2 threshold – 0.7) removing 20036 markers. After editing, 45556 SNP markers were used for final analysis.

Table 1. Means, standard error (in parenthesis) and significance of differences between genetic groups of fatteners for pork quality traits

Trait	Genetic group							
	L×D N=33	L×Y N=21	L N=49	(L×Y) × D N=113	D N=18	(L×Y) ×H N=25	Total N=259	P-value
Glycolytic potential (µmol/g)	137.16 BC (6.18)	137.49 BC (4.22)	144.89 C (5.52)	131.36 AB (2.00)	116.26 A (5.15)	220.54 D (10.94)	142.71 (2.54)	<0.001
Glycogen content (µmol/g)	50.57 BC (2.92)	49.95 BC (2.05)	52.78 C (2.93)	45.34 B (1.00)	36.91 A (2.90)	83.87 D (5.55)	50.92 (1.19)	<0.001
Lactate content (µmol/g)	36.09 A (2.05)	37.59 AB (1.90)	39.30 AB (1.47)	40.68 B (0.82)	42.43 B (1.70)	52.80 C (2.30)	40.88 (0.65)	<0.001
pH_{35}	6.54 B (0.03)	6.64 C (0.03)	6.61 C (0.02)	6.61 C (0.01)	6.59 BC (0.02)	6.37 A (0.03)	6.58 (0.01)	<0.001
pH_{24}	5.57 A (0.02)	5.57 A (0.03)	5.54 A (0.01)	5.71 B (0.01)	5.68 B (0.01)	5.58 A (0.02)	5.63 (0.01)	<0.001
pH_{48}	5.40 B (0.02)	5.41 B (0.02)	5.39 B (0.01)	5.51 C (0.01)	5.50 C (0.02)	5.31 A (0.01)	5.44 (0.01)	<0.001
Drip loss after 48 hours (%) DL_{48}	6.52 B (0.59)	6.67 B (0.48)	6.77 B (0.38)	5.01 A (0.21)	3.98 A (0.42)	7.02 B (0.56)	5.79 (0.17)	<0.001
Colour lightness L^*_{24}	53.39 A (0.50)	54.94 AB (0.64)	54.41 AB (0.53)	54.31 AB (0.27)	53.58 AB (0.45)	55.31 B (0.31)	54.31 (0.18)	<0.001
Lean meat content (%)	57.68 (0.76)	56.67 (1.19)	56.12 (0.58)	56.58 (0.30)	57.67 (0.43)	56.19 (0.48)	56.52 (0.23)	0.810

N – number of pigs. Means marked by different capital letters indicate significance of differences on P value.

Table 2. Significant SNP markers characterized by position on *Sus scrofa* chromosome (SSC), level of significance [$-\log_{10}$ (P-value)] and FDR, percentage of genomic variation for particular meat quality traits explained by the significant marker

SNP marker symbol	SSC	$-\log_{10}$ (P-value) for significant marker	FDR value	% of genetic variation explained	Trait
ALGA0039140	7	5.18	0.054	7.6	GP
ASGA0046337	10	5.65	0.025	8.5	GC
ASGA0046337	10	5.41	0.042	8.1	GP
ALGA0079424	14	5.94	0.038	9.0	GC
ALGA0079424	14	5.50	0.052	8.3	GP
MARC0064374	14	5.86	0.023	8.9	GC
MARC0064374	14	6.31	0.016	9.6	GP
H3GA0045199	15	5.97	0.096	8.3	DL_{48}
ASGA0077023	17	6.02	0.031	9.1	pH_{48}

GP – glycolytic potential; GC – glycogen content; DL_{48} – drip loss after 48 hours, pH_{48} – pH after 48 hours.

The associations between SNPs and recorded traits were based on the multi-locus mixed model (MLMM) methodology described by Segura et al. (2012). The multiple-step generalized least square procedure was employed to estimate the effects for single SNPs. Prior to each step, the genetic and residual variance components were estimated. Each significant SNP was then added to the model as a cofactor for the next step, and the P-values for all cofactors and variance components were re-estimated. According to Segura et al. (2012) for the forward regression, the following alternative stopping criteria were taken: ratio of genetic and phenotypic variances vs when a maximum number of forward steps is reached. As a variable, genetic group (pure or crossbreds) was included in the model. The Bayesian Information Criteria (BIC) was employed to choose the adequacy of the models. Moreover, the multiple Bonferroni criterion (mBonf) and false discovery rate (FDR) were applied to select the

best model (with the most loci for which all have P-values below the Bonferroni threshold). Finally, the following MLMM model was applied: y = Xβ+Zu+e, where y is a continuous variable (the pork quality trait), X is the matrix of fixed effects composed of 45556 SNP markers and one covariate (genetic group), Z is the incidence matrix relating random effects to each individual, which becomes an identity matrix, because one phenotypic measurement is performed per individual, e stands for the residual effects, and β and u are vectors representing coefficients of fixed and random effects. More detailed description of the MLMM algorithm of GWAS is given by Segura et al. (2012). Computations were performed using the Golden Helix SVS7 analysis software v. 8.9.1 (Bozeman, MT, USA). Q-Q plot for MLMM showed an inflation factor equal to 0.98 (data not shown).

Table 3. General characteristics of genomic regions involved in variation of meat quality traits

Significant marker	SSC	Distance between flanking markers (Mb)	Number of markers located between flanking markers	Number of genes* located between flanking markers	Trait
ALGA0039140	7	1.936	43	19	GP
ASGA0046337	10	1.048	30	13	GP/GC
ALGA0079424	14	0.817	25	0	GP/GC
MARC0064374	14	0.817	25	0	GP/GC
H3GA0045199	15	0.437	14	12	DL_{48}
ASGA0077023	17	1.914	19	38	pH_{48}

*genes coding proteins, SSC – *Sus scrofa* chromosome; GP – glycolytic potential; GC – glycogen content; DL_{48} – drip loss after 48 hours, pH_{48} – pH after 48 hours.

Table 4. Genes located in close vicinity of significant markers associated with glycolytic potential (GP) (SSC 7 and 14), glycogen content (GC) (SSC 10 and 14) and drip loss after 48 hours (DL) (SSC 15)

SSC	Position of the marker/gene	Gene/marker symbol/ trait	Gene function*	Reference(s)
1	2	3	4	5
7	18,279,643	*ALGA0039047*	–	
	19,244,607	*MRS2*	Magnesium ion transporter	Coudy-Gandilhon et al. (2019)
	19,289,385	*GPLD1*	Post-translation modifications	
	19,351,485	*ALDH5A1*	Energy metabolism	Tizioto et al. (2013)
	19,502,629	*ACOT13*	Energy production in mitochondria	
	19,568,837	*ALGA0039140* (GP)	–	
	19,609,358	*RIPOR2*	Communication between cells	Krivoruchko et al. (2022)
	20,216,211	*ASGA0031606*	–	
10	11,194,964	*ASGA0093887*	–	
	11,340,640	*TAF1A*	Component of transcription initiation complex	
	11,362,489	*MIA3*	Multifunctional	
	11,426,017	*ASGA0046337* (GC)	–	
	12,243,864	*ASGA0046443*	–	
14	93,146,090	*H3GA0041248*	–	
	93,230,657	*ALGA0079424* (GP, GC)	–	
	93,818,656	*MARC0064374* (GP, GC)	–	
	93,963,302	*ASGA0064938*	–	
15	139,192,454	*ASGA0070945*	–	
	139,457,866	*GPC1*	Cell division and growth regulation	Shi et al. (2021)
	139,557,644	*CAPN10*	Calcium-dependent cysteine proteases.	Ilian et al. (2004) Yang et al. (2008)
	139,606,775	AQP12	Cellular transport of water and glycerol	da Silva et al. (2020)

Table 4 – contd.

1	2	3	4	5
	139,610,624	*KIF1A*	Transports of membranous organelles	Martin et al. (2019)
	139,659,680	*H3GA0045199* (DL)		
	139,881,217	*MTERF4*	rRNA processing and regulation of transcription in mitochondrion.	
	139,887,439	*PASK*	Regulation of the insulin gene expression and glucagon secretion	Littiere et al. (2020)
	139,957,703	*HDLBP*	High density lipoprotein binding protein	
	140,060,304	*FARP2*	Enables guanyl–nucleotide exchange	Bolormaa et al., 2016
	140,097,188	*ASGA0084391*	–	
17	36,688,966	*ALGA0094454*	–	
	36,917,276	*SNTA1*	Syntrophin alpha 1	Eisinger et al. (2018)
	37,798,747	*ASGA0077023* (pH_{48})	–	
	38,362,158	*MYH7B*	Myosin heavy chain 7B	Lee et al. (2023)
	38,603,020	*ALGA0110267*	–	

SSC – *Sus scrofa* chromosome. Position of genes and markers were drawn from *Sus scrofa* genome assembly (Sscrofa11.1 Release 106). Significant markers were underlined in contrast to not underlined flanking markers.

*gene function definition based on ENSEMBL browser (www.ensembl.org).

Candidate genes were sought in the close vicinity to significant markers. Close vicinity means that neighbouring markers from the left and right sides of a significant marker were linked to each other by $R^2>0.5$. The genomic positions of candidate genes were assigned based on the *Sus scrofa* genome assembly database (Sscrofa11.1 Release 106) through the current NCBI (Warr et al., 2020). Candidate gene functions were found/suggested by an individual search in the PubMed database (https://pubmed.ncbi.nlm.nih.gov).

Results

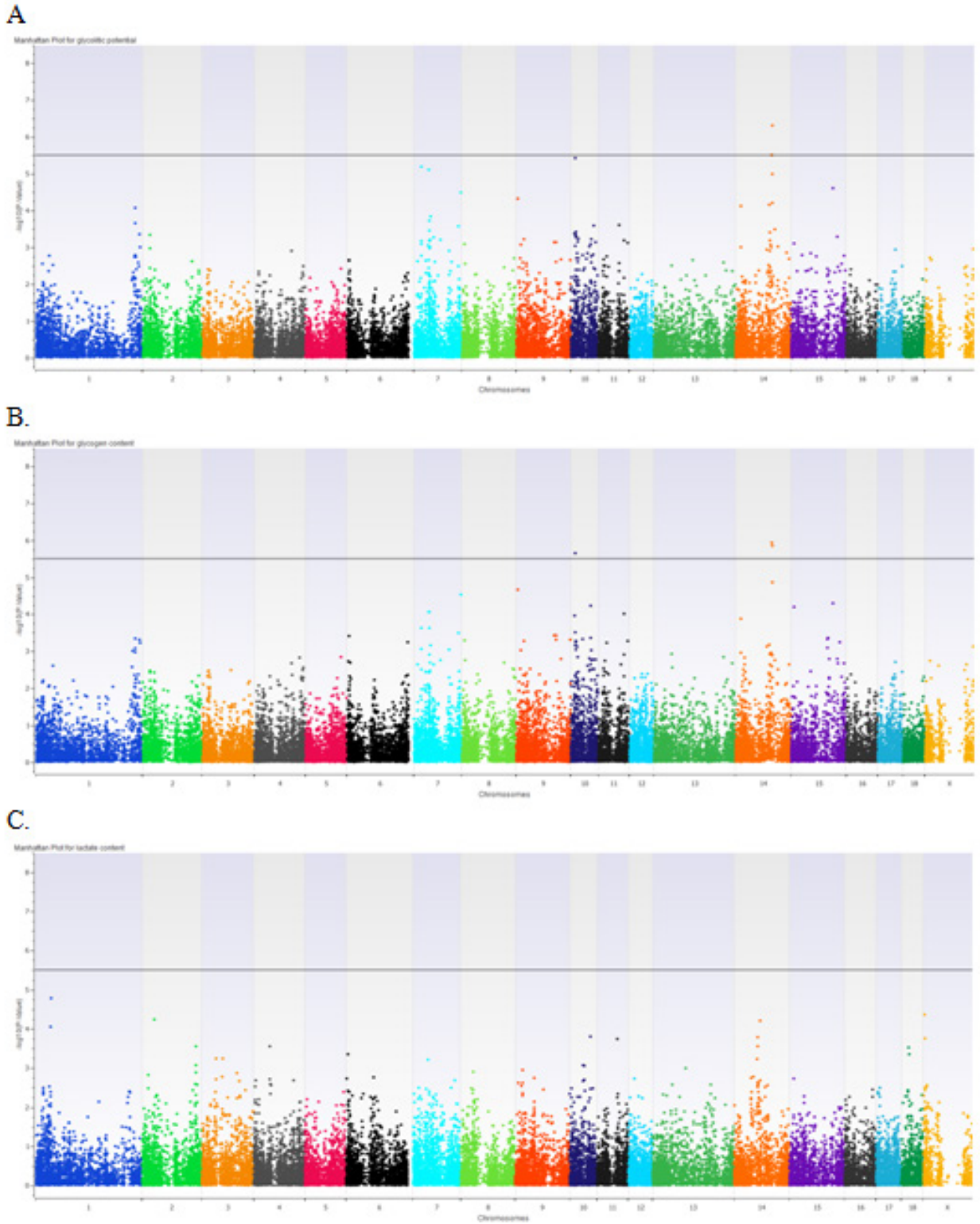
Meat samples were differentiated regarding the key meat biochemical indices and components, e.g. GP (glycogen and lactate measured 45 min. *post-mortem*) (Table 1). The highest values of the aforementioned traits were observed in (L×Y)×H fatteners (220.5, 83.87, 52.80 µmol/g, respectively). Both the lowest GP and GC were noted in meat samples of (L×Y)×D fatteners (116.26 and 39.97 µmol/g, respectively) while the lowest lactate concentration was found in samples of L, L×Y and L×D (Table 1). All analysed meat quality traits were significantly associated with the genetic group, except lean meat content. The lowest pH_{35} and pH_{48} were noted in (L×Y)×H fatteners (6.37 and 5.31, respectively) which resulted in an unfavourable drip loss measured 48 hour *post-mortem* (7.02%) and meat lightness (55.31). In comparison to other genetic groups, meat samples of D and (L×Y)×D fatteners were characterized by statistically significant higher pH_{48} ($P\leq0.001$) and lower DL_{48} ($P\leq0.001$). It should be noted that according to Bertram et al. (2000) the value of drip loss measured 48 hours *post-mortem* below 4% is considered as low.

An overview of all SNP effects for pork quality differences was presented in the Manhattan plots (Figure 1) and showed the $-\log^{10}$ (P-value*)* for the associations of 45556 SNPs.

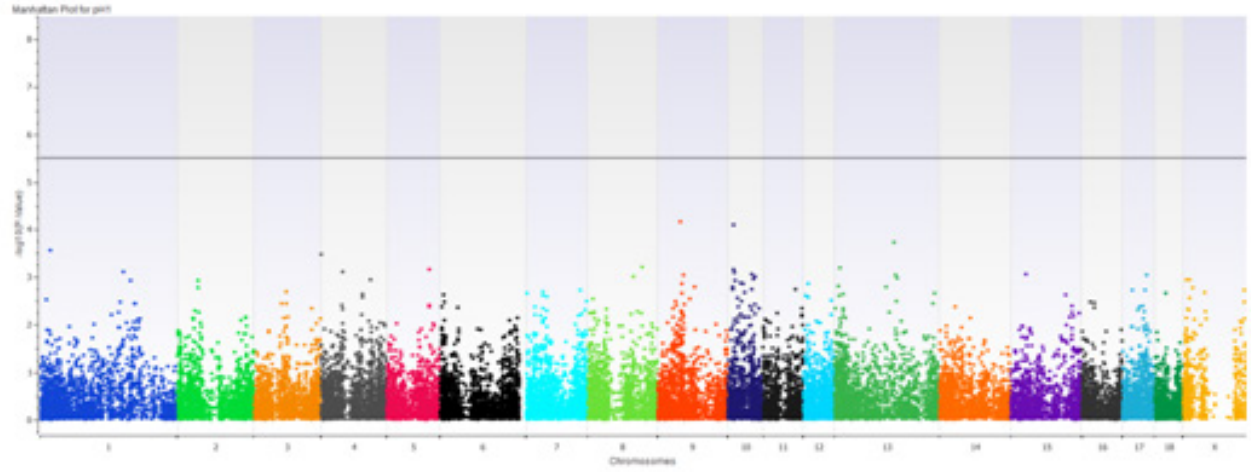
After FDR correction, 4, 3, 1 and 1 significant markers were found, for GL, GC, DL_{48} and pH_{48}, respectively (Table 2). The percentage of genetic variance explained by significant markers varied between 7.6% and 9.6% and was positively correlated with $-\log_{10}$ (P-value) (data not shown).

All significant markers were mapped in a specific location. For all significant SNP markers located on five chromosomes linkage analysis was conducted to find the chromosome subregion containing a significant marker flanked by two markers linked by r^2 greater than 0.5. In this way, the space was marked for seeking functional genes inherited (to some extent) together with a significant marker, and were, as could be expected, potentially involved in the genetic variation of the trait for which a significant marker was found. As it is shown in Table 3, the distance between markers flanking significant markers varied between 0.437 Mb and 1.936 Mb, although both pairs of flanking markers were linked by the same r^2 (0.52). This can be explained by local variation of linkage disequilibrium specific to each chromosome, breed and even population (Wang et al., 2013). The positions of flanking markers were then used to mark boundaries from both sides (directions) of a significant marker. All functional genes located between them were considered as candidate genes involved in the genetic variation of the trait. To ensure

whether the gene was functionally related to the trait, the PubMed database was searched using the following keywords: pork, meat, muscle, and e.g. lactate or glycogen, as specific terms referring to the trait for which a significant marker was found. Most of the genes located between flanking markers were not related to GP or GC or DL but some of them seem to be involved and these are presented in Table 4.



D.



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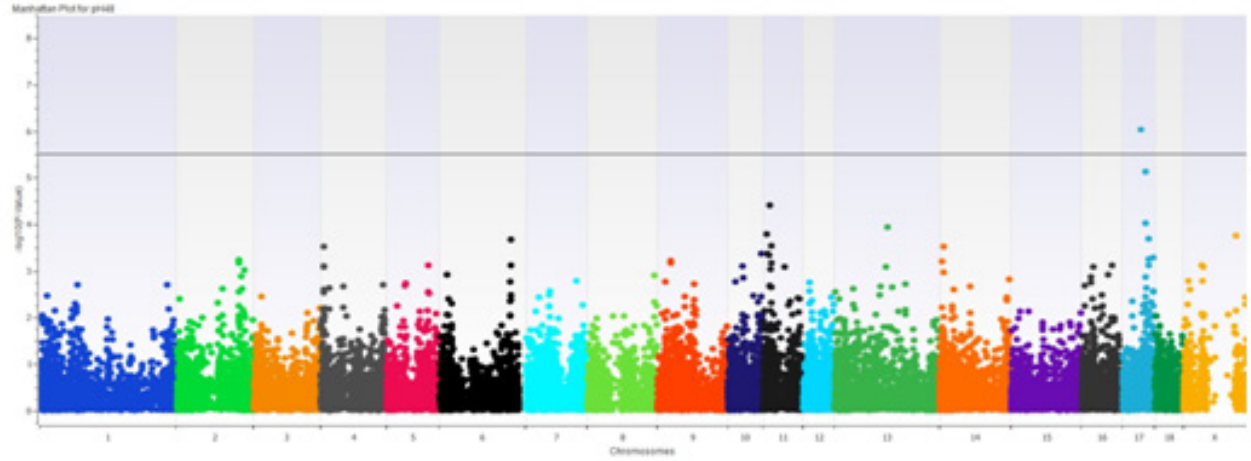
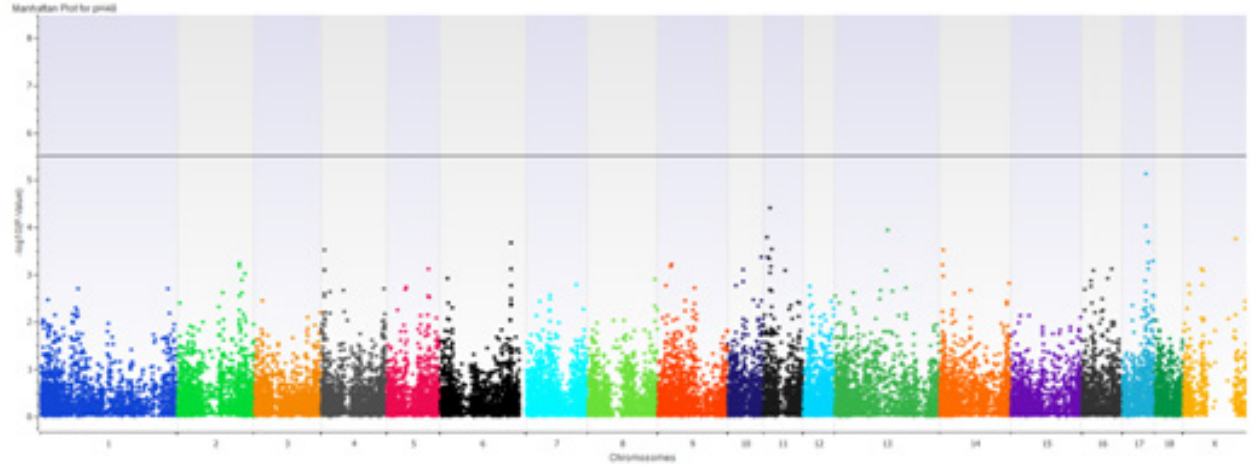


F.



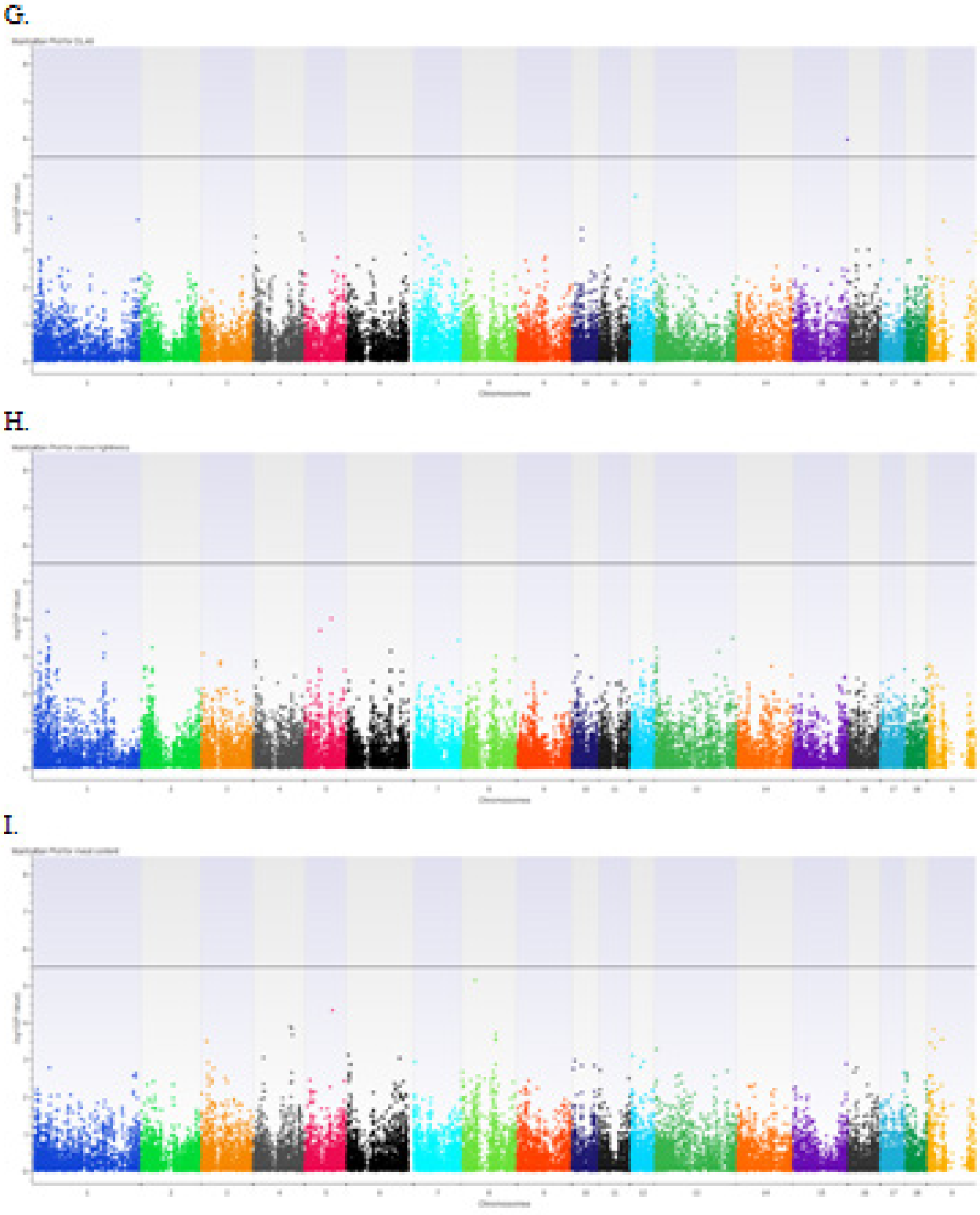


Figure 1. Manhattan plots showings the -log10 (P-value) for the associations of 45556 SNPs for: Glycolytic potential (A), Glycogen content (B), Lactate content (C), pH_{36} (D), pH_{24} (E), pH_{48} (F), DL_{48} (G), Colour lightness (H), Lean meat content (I)

Discussion

Over many decades, the genetic improvement of pork quality has been focused on the correlated response to the selection of recorded traits with measurements performed *in vivo*. Contrary to many other performance traits, in case of meat quality traits, direct selection is very difficult. Extraordinary opportunities appeared with the implementation of the so-called genomic selection introduced by Meuwissen et al. (2001) in dairy cattle, but this method requires a large reference population for traits of medium or low heritability. In spite of this limitation, data on traits and genotypes of SNPs were already used for smaller populations in attempts to identify single loci and their clusters determining pork quality (Fontanesi et al., 2008; Herault et al., 2018; Liu et al., 2020).

The majority of commercial pig populations are composed of different crossbreds. For instance, Liu et al. (2020) reported that in China, more than 70% of the pork produced originates from three-way crossbred Duroc × (Large White × Yorkshire) pigs. This corresponds to the structure of the research material used in the current study. Our analyses were carried out on both purebred and crossbred populations. Such an attitude allows for a better generalization of the results and extends their significance to *Sus scrofa* as a species being a subject of intense breeding programmes. The detection of genome regions determining pork quality traits was also carried out on mixed populations by other authors (Duan et al., 2009; Zhang et al., 2015).

Our results were obtained on relatively low number of samples if compared to other GWAS for pork quality traits. For example, Gao et al. (2021) and Zhong et al. (2022) used 582 and 1518 crossbred pigs, respectively. It has to be noted, however, that these teams analysed traits much simpler to measure (meat conductivity, marbling score, moisture, meat colour, pH and intramuscular fat) which were recorded by automatic measurements. GP measured in our experiment relies on sophisticated biochemical tests which limits the number of samples simultaneously analysed as it is also shown in GWAS for GP in which Xie et al. (2023) used 301 pigs.

In the studied populations four SNP markers with a significant effect on GL were identified, which are located on chromosomes 7, 10, and 14. This corresponds with results obtained by Fontanesi et al. (2008) for Italian Large White pigs.

It should be noted that in the present study, the significant SNPs explained a relatively high portion of genetic variance, ranging from 7.6% to 9.6% (for GP), from 8.5% to 9.0% (for GC), 8.3% for DL_{48} and 9.1% for pH_{48}. Significant SNP markers indicate the region in which QTL should exist. First of all, it was checked whether positions of significant SNP markers reside in already published QTL mapping positions in the AnimalQTL database for *Sus scrofa* (www.animalgenome.org). In this database, among 23 QTL for GP, three and one of them were also reported on SSC7 and SSC14, respectively, but in different genomic positions. Out of 27 QTL for DL mapped on SSC15 reported by Choi et al. (2011), one encompassed the significant marker H3GA0045199 and another was at a distance of 4 Mb (van Wijk et al., 2006). For GC, 18 QTL were reported in AnimalQTL db, but none of them correlated to locations on SSC10 and SSC14, in which significant markers for this trait were found by us. For pH measured in muscle or carcass, hundreds of QTL were mapped on different chromosomes, but on SSC17 only 12 QTL were identified, irrespective to how many hours after slaughter this parameter was measured. All 12 QTL were mapped distal (in 3' direction) to significant marker *ASGA0077023* in distance of about 31–32 Mb.

The most promising candidate gene for pork quality was found on chromosome 15: *CAPN10*, which codes an enzyme calpain 10. Calpains are cysteine proteinases that play an important role in *post-mortem* proteolysis of the structural muscle proteins that removes the linkage between myofibrillar structures, causing redistribution of extracellular water causing drip loss (Lian et al., 2013). Ilian et al. (2004) and Yang et al. (2008) reported that *CAPN10* might be a candidate gene for meat tenderization and water distribution in meat. Ilian et al. (2004) found that calpain 10 proteins were associated with the breakdown of certain myofibrillar linkage proteins including desmin in sheep *longissimus thoracis et lumborum*. Desmin is involved in organizing and maintaining the integrity and strength of the contractile myofibrils and the overall cytoskeleton structure of the skeletal muscle cells and its degradation is responsible for drip production (Huff-Lonergan and Lonergan, 2005). Another candidate gene for GP is *ALDH5A1* coding aldehyde dehydrogenase 5 (family member A1) which was found in a GWAS study for Nelore beef cattle as associated with energy metabolism and affecting meat quality traits: water holding capacity, fat colour parameters and tenderness (Tizioto et al., 2013).

On the contrary, there are no genes coding proteins between flanking markers on chromosome 14. This region contains only non-coding sequences. Because such DNA sequences may be involved in the regulation of other genes (Zhong et al., 2022), their potential influence on the analysed traits cannot be excluded. On chromosome 7, close to significant marker *ALGA0039140*, two genes are reported in the literature which indirectly can contribute to meat quality: *MRS2* and *RIPOR2*. *MRS2* codes for the magnesium transporter, is expressed in muscle and is responsible for whole-body magnesium homeostasis (Coudy-Gandilhon et al., 2019). *RIPOR2*, coding cell polarization regulator, has been found to be a marker for meat productivity in Merino sheep (Krivoruchko et al., 2022). Another candidate gene in this chromosome subregion – *ACOT13* codes for a protein which participates in general metabolism and regulation and therefore may also be involved in the variation of the GP. On chromosome 10, two genes were found (*TAF1A* and *MIA3*) which also participate in very basic cellular processes, and therefore they may contribute to GP, although in the

scientific literature there are no reports of such associations.

At a very close distance (ca. 60 kb) to marker *H3GA0045199* significantly associated with DL_{48}, the *AQP12* gene is located, which codes for aquaporin-12 – a protein facilitating water and glycerol movement across membranes (da Silva et al., 2020). Another gene located in the same subregion of SSC15 – *KIF1A* interacts with many proteins involved in the carcass composition and organ weight of Charolais cattle (Martin et al., 2019). In addition, *GPC1* gene might be engaged in drip loss because it has been found to be differentially expressed in *Longissimus dorsi* and to be associated with meat quality (Shi et al. 2021). Between significant marker *H3GA0045199* and flanking marker *ASGA0084391* there are four genes (*MTERF4, PASK, HDLBP, FARP2*) which play an important metabolic role and can indirectly influence drip loss as well. For example, FARP2 protein by binding to phospholipids and the cytoskeleton regulates integrin signalling and cell adhesion (Bolormaa et al. 2016) or PASK – serine/threonine kinase, is involved in the regulation of glucagon secretion and glycogen metabolism (Littiere et al., 2020).

In close vicinity to significant marker *ASGA0077023* (SSC17), among 38 genes coding for different proteins and located between flanking markers, at least three genes can be considered as candidate gene contributing to the variation of pH_{48}. First, *SNTA1*, coding syntrophin alpha 1 protein, which is expressed in muscle and adipocyte. Current data demonstrate beneficial effects of *SNTA1* deficiency in obesity in mice which is partly attributed to smaller adipocytes and reduced white adipose tissue mass. Higher SNTA protein in fat depots of obese mice may contribute to adipose tissue hypertrophy and ectopic lipid deposition (Eisinger et al., 2018). How these physiological effects can be associated with or dependent on pH is difficult to decipher since this aspect was not yet discussed in scientific literature reviewed by us.

Another gene lying between markers flanked significant marker *ASGA0077023* is *MYH7B* coding for one type of sarcomeres' myosin commonly known as molecular motor that contract muscle. Lee et al (2023) found that mutations in this gene are associated with hearing loss but also with an increase in the proportion of myosin heads that occupy the disordered-relaxed state, meaning more myosin heads are available to interact with actin. Their results provide the foundation for further study for the role of MYH7b outside the sarcomere, including the influence on pH in muscle.

The lack of papers confirming the involvement of a gene in the trait of interest does not eliminate this gene as a new candidate gene, especially if such a gene is proved to be expressed in muscle tissue. All genes with no direct link (based on literature) to the meat quality traits (*MRS2, ACOT13, TAF1A, MIA3, GPC1*, Table 4) are expressed in muscle (Expression Atlas, https://www.ebi.ac.uk/gxa/home). Genes are multifunctional (Espinosa-Cantu et al., 2020), and it can be assumed that not all their functions have already been elucidated. In future research candidate genes should be investigated in detailed expression studies to confirm or reject their actual role in the variation of meat quality traits.

Conclusion

Genome-wide analysis revealed SNP markers significantly associated with glycolytic potential, glycogen content, drip loss and pH of pork in population consisting of purebred and crossbred fatteners. Sequences located in the close vicinity of these markers were an indication of putative candidate genes which may contribute to the quality of pork and may better explain the complex molecular nature of key meat quality traits.

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