Acta Veterinaria Hungarica 66 (2), pp. 215–225 (2018)
DOI: 10.1556/004.2018.020

EFFECT OF SINGLE-NUCLEOTIDE POLYMORPHISMS ON THE BREEDING VALUE OF FERTILITY AND BREEDING VALUE OF BEEF IN HUNGARIAN SIMMENTAL CATTLE

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(Received 11 September 2017; accepted 12 February 2018)

The objective of this study was to estimate the effect of single-nucleotide polymorphisms (SNPs) on the breeding value of fertility (BVF) and the breeding value of beef (BVB) in Hungarian Simmental cattle. Genotypes were determined on a high-density Illumina Bovine DNA Chip. Data screening and data identification were performed by multi-locus mixed-model. Statistical analyses were carried out to find associations between individual genotypes and the investigated quality values. Three loci showed considerable association with BVF ($-\log_{10} P$ = 9.5, 9.9 and 14.5, respectively) on chromosomes 9, 28 and 29, respectively. The frequencies of their minor alleles (MAF) were 0.375, 0.355 and 0.354, respectively. Two loci showed association with BVB ($-\log_{10} P$ = 25.3 and 22.7) on chromosomes 2 and 11, respectively (their MAF were 0.438 and 0.229). The abovementioned loci provide a straightforward possibility to assist selection by molecular tools.

Key words: Hungarian Simmental cattle, SNP, breeding value of fertility, breeding value of beef

The first Western European cattle species such as the Simmental had arrived in Hungary in the 18th century with Bavarian and Swabian settlers. However, country-wide spread of the breed occurred only at the end of the 19th century. The complete control of livestock had been introduced in 1894 by starting herdbook registration, which led to the development of local varieties of the species (varieties of Bonyhád, Vas, Nógrád, Hont, etc.). The colour varieties ranged from light to dark yellow or red with irregular white patches. Targeted breeding work resulted in solid constitution, good carcass development and good milking ability. Very soon the Hungarian Simmental became the dominant breed in Hungary until the late 1970s. At present, the population is bred for dual purpose

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(both milk and beef production), and the Hungarian Simmental breed constitutes about 18% (65,000 animals) of the cattle population in Hungary.

For a long period, breeding value estimation of dual-purpose Hungarian Simmental cattle was based on dairy traits only. The main reason was the improvement of dairy traits which was set as a breeding goal. After fulfilling this demand, the improvement of beef traits also became important. In the first step (1992), the Association of Hungarian Simmental Cattle Breeders (AHSCB) decided to include beef traits (frame, muscularity, body conformation, udder conformation) in type classification. Later (from 1994) it was proposed to set up central self performance testing of sire candidates, which aided the selection of bulls with the best weight gain traits for artificial insemination. It is obvious that phenotypic data related to beef production give insufficient information on the progenies of sires, since the estimation of slaughter traits is not possible. On the other hand, requirements for slaughter traits and beef quality traits are becoming higher and higher in actual high-quality beef production. Compatibility can be ensured only if the Hungarian Simmental cattle population is selected for both slaughter traits and beef quality traits (Füller, 2010).

Nowadays, there is considerable interest in the application of genomic breeding value (BV) estimation to promote rapid and efficient selection in farm animals. In the past few years, advances in molecular genetics have enabled the application of DNA chip technique to pave the way for achieving different breeding objectives. The genome-wide association study (GWAS) based on the typing of single nucleotide polymorphisms (SNPs) by DNA chip technique is suitable for finding loci associated with beef quality and intramuscular fat content in different cattle breeds, since correlations among genetic background and breeding value of beef can be highlighted by statistical analysis.

Availability of genomic information on a large number of animals has changed dairy cattle breeding worldwide (Nayeri et al., 2016). In beef cattle (Picard et al., 2006; Allais et al., 2014) and dual-purpose cattle genomic selection can change selection progress, although the effective population number and the accuracy of genomic estimated breeding value are lower than in dairy cattle.

In Nordic Red cattle a 660-kilobase deletion has been found to be related to higher milk yield and lower fertility (Kadri et al., 2014). Minozzi et al. (2013) found no obvious common SNPs influencing the studied fertility traits in the Holstein breed, but a network analysis revealed interconnection of genes which influenced fertility.

Several genes involved in spermatogenesis have been identified (Li et al., 2012) on chromosomes (Chrs) 20 and 23 in Holstein cattle. In the case of Brahman and Tropical Composite bulls, markers on Chrs 2, 5, 14 and X were associated with testicular development, hormone levels and sperm quality (Fortes et al., 2012, 2013). Han and Peñagaricano (2016) have identified genomic regions on Chrs 5, 9, 13, 15, 21 and 25 harbouring genes related to sperm biology.

At present the breeding decisions in the AHSCB are based mainly on breeding values; however, some SNPs have been demonstrated to affect intramuscular fat content and meat quality traits in the Hungarian Simmental breed as well (Anton et al., 2008, 2013). Estimated breeding values (EBV) signify the level of the breeding potential of animals for specific traits. Indexes are scores of genetic merit combining the relative economic values of several EBV traits. Indexes take into account performance data collected on known relatives, relationships between performance traits, and the degree to which traits are inherited from one generation to the next.

The AHSCB has been taking measures to improve beef quality in recent years. For breeding purpose and market expectations, a new dual-purpose index has been developed where the weighting of breeding values is quoted as follows: 40% for milk, 30% for meat and 30% for fitness (Húth et al., 2013).

In this study, the association between phenotypic traits and genomic polymorphisms was investigated by SNP array covering the whole genome. We focused on *(i)* breeding value of fertility (BVF) that can be related to milk production, and *(ii)* breeding value of beef (BVB) that can be connected to meat production.

The authors will present some pinpointed DNA loci, which can be utilised by breeders in their selection plans to speed up the selection procedure and to reach their desired phenotypes suitable for current market demands.

Materials and methods

Evaluation of BVF

For bulls, there is no direct method for the estimation of fertility. In this case, bulls are scored on the basis of the fertility of their female offspring. Progeny testing is performed by considering the number of inseminations for the successful conception and non-return rate of heifers until 56 days (NR56). According to the results obtained by Komlósi and Húth (2016), the h^2 of the two traits of Hungarian Simmental heifers was 0.006, with a very strong genetic correlation (–0.95) between them. The h^2 for cow fertility traits varied between 0.018 and 0.041. Due to the low h^2 for heifer fertility and its low correlation with cow fertility ($r = 0.14$), the authors recommend that selection should be based on cows' NR56 and days open only for this breed.

Evaluation of BVB

In dual-purpose Hungarian Simmental bulls, the evaluation of BVB is based on net weight gain, lean meat % and EUROP conformation score of carcasses. Weighting of breeding values is quoted as follows: 22% for net weight

gain, 39% for lean meat production and 39% for S/EUROP conformation score of carcass muscularity.

BVB = 0.22 BV nwg + 0.39 BV lmp + 0.39 BV EUROP cs

BV nwg = Breeding value for net weight gain

BV lmp = Breeding value for lean meat %

BV EUROP cs = Breeding value for S/EUROP conformation score of carcass muscularity

Samples and genotyping

A total of 146 Hungarian Simmental bull samples from eleven farms were collected from the gene bank of the AHSCB and were stored in liquid nitrogen at –196 °C until DNA extraction. Samples were chosen considering the following criteria: *(i)* availability of blood, semen or DNA, *(ii)* having high or low values regarding BVF and BVB, *(iii)* maximising the representativeness of the population. The latest criterion is based on selecting animals which are the least related to each other based on their pedigree.

DNA typing was performed on high-resolution SNP chips developed for cattle (GeneSeek® Genomic Profiler™ High-Density; GGP HD150K). Genotyping was done by Neogen Europe Ltd., Scotland, UK. Performance data and breeding parameters of bulls were collected from the database of the AHSCB.

Data evaluation

Samples were excluded from analysis if the call rate was below 95%. Only SNPs having consistently high call rates (> 95%) were included in this study. Duplicated samples (Identity By Descent, IBD > 0.95) were excluded from the dataset. After excluding monomorphic loci and loci with an MAF < 0.05, the final dataset included 129 animals and 76,592 SNPs.

For data screening and identification of loci associated with BVF and BVB, multi-locus mixed-models were used. Phenotypic values (BVB and BVF) were left as they were, a continuous variable.

The genomic inflation factor, lambda value was calculated from the median of the distribution of the chi-square statistic from results divided by the median of the corresponding (ideal) chi-square distribution (Armitage, 1955). Lambda values were 1.06 and 1.09 for BVF and BVB, respectively. For the correction of population structure, genomic kinship matrix was used in a multi-locus mixed model (Segura et al., 2012).

We used the model $y = X\beta + Zu + e$, where y is the BVB or BVF, X is the matrix of fixed effects composed of SNPs and covariates (age and farm), Z is the matrix of random animal effects, e means the residual effects, and β and u are vectors representing coefficients of fixed and random effects, respectively.

The Ensemble cow UMD3.1 and Gene Ontology (GO) (Ashburner et al., 2000) databases were used to look for the surroundings of the most significant SNPs and for functional categories.

All data formatting, filtering and statistical analyses were performed by the SVS software (GoldenHelix, US).

Results and discussion

In Nelore cattle, the ASAP1 gene (ArfGAP with SH3 domain, ankyrin repeat and PH domain 1) was located on the bovine Chr 14 where there are reports of QTLs for meat production traits (Tizioto et al., 2012). Liu et al. (2015) studied 14 cattle breeds, including Simmental, for 16 SNPs of eight genes. They reported possible associations in the case of the Dragon Beef population.

In Aberdeen Angus sired beef cattle, the effects of μ-calpain (CAPN1 on Chr 29), calpastatin (CAST on Chr 7), leptin (LEP on Chr 24), growth hormone receptor (GHR on Chr 20) and acylCoA:diacylglycerol acyltransferase 1 (DGAT1 on Chr14) have been proven on milk and beef traits (Gill et al., 2009).

Five candidate genes have been under scrutiny (Ekerljung et al., 2012; Li et al., 2013) in relation to the variation in meat tenderness, pH, colour, marbling and water holding capacity where the Simmental breed was also included. The CAST gene had effects on tenderness, whereas the CAPN1 was associated with marbling and meat colour stability. Among the above-mentioned examples, the chromosomal localisation of our hits overlaps with Chr 29; however, according to the finer resolution, the rs137311103 locus (Table 1) is located more than 40 million bases from the CAPN1. A genome-wide association study on crossbred beef cattle has been conducted on residual feed intake (RFI) and several candidate genes have been identified to be associated with the RFI mechanism (Abo-Ismail et al., 2014).

Since we worked with breeding values derived from complex measurements, and not with traits listed in the articles cited above, it is not surprising that our findings reflect other, hitherto not reported regions.

According to the analysis outcome, several loci were identified to be associated with the breeding value of beef. Out of seven loci ($-\log_{10} P > 5$), two loci, located on Chrs 2 and 11, seem to be useful in the selection programme (Fig. 1, Table 1).

Breeding values collected from the database ranged from 80 to 130. The allelic pattern of the locus at Chr 2 has changed to homozygous in all animals with a BV higher than 110. The majority of animals with a BV lower than 102 were homozygous at the locus on Chr 11. Their minor allele frequencies (0.438 and 0.229) indicate the straightforward possibility to assist selection by molecular tools.

Table 1

List of loci associated with BVB or BVF, their genomic location and nearest genes

Marker ss ID	Chr	Position	$-\log_{10}$ P	$-\log_{10}$ P after Bonferroni correction	Nearest gene(s)	Associated with	MAF	FDR
rs41628842	2	111962847	25.3	20.4	ACSL3, RPS6, KCNE4	BVB	0.438	1.9e-21
rs133063240	11	27988487	22.7	17.8	PRKCE	BVB	0.229	4.9e-19
rs41656753	9	29910981	9.5	4.7	GJA1, TBC1D32, SNORA25	BVF	0.375	2.4e-3
rs42151703	28	42540318	9.9	5.1	GPRIN2, GDF2, GDF10	BVF	0.355	4.3e-6
rs137311103	29	3901625	14.5	9.7	FAT3, CHORDC1, HSP90	BVF	0.354	2.1e-10

BVF: Breeding Value of Fertility; BVB: Breeding Value of Beef; MAF: Minor Allele Frequency; FDR: False Discovery Rate

Looking at the UMD3.1 cow sequence in the Ensemble database there are three protein-coding sequences in the near vicinity of the locus rs41628842 (Chr 2). RPS6 (40S ribosomal protein coding sequence) is activated by L-Arg stimulating proliferation and migration of ovine trophectoderm cells (Kim et al., 2011). ACSL3 (long-chain-fatty-acid-CoA ligase 3) is involved in long-chain fatty acid uptake (Krammer et al., 2011), while KCNE4 is a potassium voltage-gated channel subfamily E regulatory subunit 4 (GO:0005249).

Locus rs133063240 on Chr 11 is located within the protein kinase C epsilon (PRKCE) gene and beside the S1 RNA Binding Domain 1 (SRBD1) gene. The differential expression of PRKCE protein and an elevation of $[Ca^{2+}]$ are important for the acquisition of luteolytic response to PGF2 alpha (Goravanahally et al., 2007). The SRBD1 gene has a predicted ability to bind nucleic acid (GO:0006139) and take part in metabolic processes.

In the case of BVF, association analysis was carried out to find correlations between SNP genotypes and breeding parameters data of bulls that had been collected from the database of the AHSCB.

Three loci showed considerable association with BVF on Chrs 9, 28 and 29 ($-\log_{10}$ P = 9.53, 9.94 and 14.55) respectively (Table 1). The frequencies of their minor alleles were 0.375, 0.355 and 0.354.

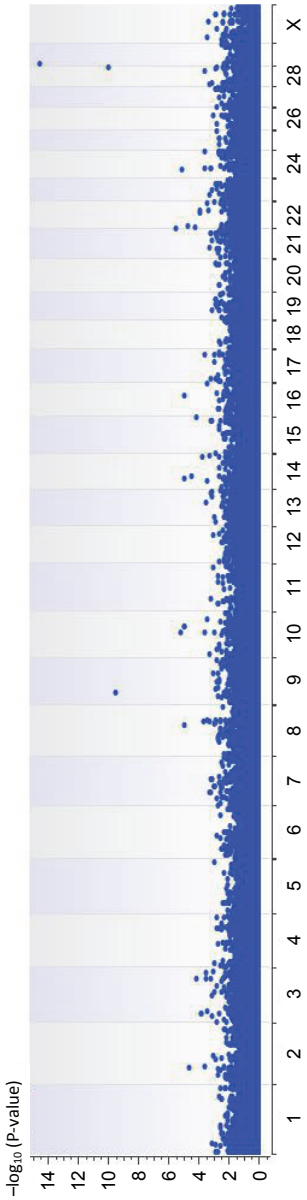

Fig. 1. Manhattan plot of SNPs regarding BVF. Loci on chromosomes 9, 28 and 29 display the highest $-\log_{10}$ P values (see dots > 8), which are associated with the breeding value of fertility in Hungarian Simmental cattle

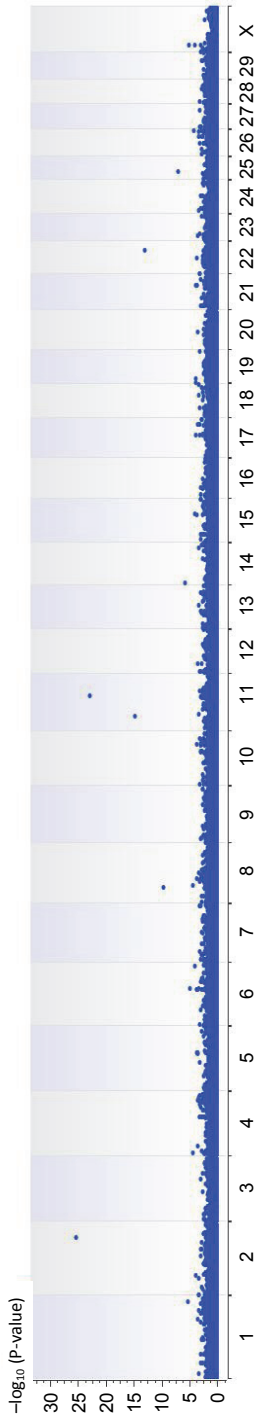

Fig. 2. Manhattan plot of SNPs regarding BVB. Loci on chromosomes 2 and 11 display the highest $-\log_{10}$ P values (see dots > 20), which are associated with the breeding value of beef in Hungarian Simmental cattle

The rs41656753 locus on Chr 9 is located near the GJA1 (gap junction protein alpha 1), TBC1D32 and SNORA25. GJA1 acts by enhancing intercellular electrical and chemical transmission, thus sensitising bladder muscles to cholinergic neural stimuli and causing them to contract, and it may play a role in cell growth inhibition. The expression of gap junction proteins may be important for trophoblast migration and fusion with maternal epithelial cells (Pfarrer et al., 2006), having an influence on *in utero* embryonic development. According to the GO molecular function terms TBC1D32 might play a role in left–right symmetry and embryonic digit morphogenesis (GO:0042733). SNORA25 (Small Nucleolar RNA) is a member of the H/ACA class of small nucleolar RNA that guides the sites of modification of uridines to pseudouridines (Kiss et al., 2004).

The annotated sequences closest to the rs137311103 locus on Chr 29 are FAT3 (FAT atypical cadherin 3) playing a role in homophilic cell adhesion via plasma membrane adhesion molecules and CHORDC1 (cysteine- and histidine-rich domain-containing protein 1) regulating centrosome duplication, probably by inhibiting the kinase activity. It is proposed to act as a co-chaperone for HSP90 and may play a role in the regulation of NOD1 via a HSP90 chaperone complex. CHORDC1 is involved in the stress response, prevents tumorigenesis and has the third and fourth highest reads per kilobase of transcript per million mapped reads in the human placenta and testis (Fagerberg et al., 2013).

The rs42151703 locus on Chr 28 is surrounded by GPRIN2 (G protein regulated inducer of neurite outgrowth 2), GDF2 (growth differentiation factor 2) having the predicted ability to regulate osteoblast differentiation, angiogenesis, apoptotic process and vasculogenesis. GDF10 (growth differentiation factor 10) is involved in osteogenesis and adipogenesis. It plays an inhibitory role in the process of osteoblast differentiation (Adoligbe et al., 2012).

Conclusions

The SNPs and their positions found in this study are different from other hits found in e.g. Nordic Red (Kadri et al., 2014), Holstein (Li et al., 2012), Brahman and Tropical Composite (Fortes et al., 2012, 2013). The similarity between the results is that the surrounding genes of SNPs have proven or predicted capacity of altering embryonic or cell developments. Molecular tests can provide facilities for direct selection among alleles of the highlighted SNPs; however, the benefits of given alleles depend on the breed itself and on economic goals. A possible marker-assisted selection (MAS) approach – such as selecting for favourable alleles at reported loci at chromosomes mentioned above – might be performed if higher fertility, weight gain and/or higher lean meat production are desirable.

Herewith we suggest that the results described here should be incorporated by the AHSCB into the evaluation process of BVF and BVB in current and future breeding programmes.

Acknowledgement

This work was supported by the Hungarian Scientific Research Fund (Project no. 111643).

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