RESULTS EVALUATION OF PREDICTION POLLED/HORNED CATTLE IN MEAT SIMENTAL

ZHODNOCENÍ VÝSLEDKŮ PREDIKCE BEZROHOSTI U MASNÉHO SIMENTÁLA

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ABSTRACT

The proposal of this project was to develop method to determinig polled or horned individuals in Meat Simenatal cattle breed. The main method was set up using recent literature as well as patent published in USA (US2005153328), togehter with microsatelite analysis published at the beginning of 90s‘. A single gene in cattle controls the horn development trait and the polled phenotype is dominant to the horned phenotype. Thus, hornless cattle may be either heterozygous (horned carriers) or homozygous for the polled allele and the ability to distinguish between carriers and non-carriers is crucial to breeding programs. The physical detection of horned or polled cattle is further complicated by the presence of scurs. Scurs are rudimentary horns that are usually small and loosely attached to the head but can be large and attached well enough to make them difficult to distinguish from horns (Brenneman et al., 1996). Total amount of 63 animals were analysed in 7 SNPs and 2 microsatelite loci. Five genotypes were known at the beginning because they were horned. We were able to established or predicted genotype in 28 animals in total of 58 (63 animals were used and 5 of them were horned, we had already known their genotypes). Prediction success of genotypes was to low (less than 50%) due to missing microsatellite loci used in recent experiments by the authors. For the further experimetns, some SNPs should be removed (for ex. MMBTA25303) and be replaced by another one. Also, 3 of 5 microsatelite loci should be added in experimet (for ex. ARO9, ARO24, SOD1MICRO2) for better analysis in families groups.

Key words: polled/horned cattle, SNPs, microsatelite, haplotype, DNA sequencing, PCR-RFLP

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INTRODUCTION

In the beef and dairy industries, horns on cattle are the cause of several economic and management problems. Horns pose hazards to animal handlers and also to other cattle causing large economic losses due to bruising. Difficulty in calving (dystocia) has been associated with horns and the widespread practice of dehorning young cattle has been shown to be stressful and reduce growth rates (Goonewardene et al., (1999).

In some breeds (e.g. Angus) the polled condition has been selected for but in others such as Hereford, the breed was established with the horned phenotype. There is increasing pressure from the live export and feedlot trades on producers to sell hornless cattle. Selective breeding with polled cattle is the means of introgressing the polled trait into horned cattle breeds.

A single gene in cattle controls the horn development trait and the polled phenotype is dominant to the horned phenotype. Thus, hornless cattle may be either heterozygous (horned carriers) or homozygous for the polled allele and the ability to distinguish between carriers and non-carriers is crucial to breeding programs. The physical detection of horned or polled cattle is further complicated by the presence of scurs. Scurs are rudimentary horns that are usually small and loosely attached to the head but can be large and attached well enough to make them difficult to distinguish from horns (Brenneman et al., 1996).

The subsequent mapping of five bovine Chr 1q12 microsatellite markers (ARO9, ARO24, TGLA49, SOD1MICRO2, BM6438) allowed the integration of the physical contig map with existing linkage maps of this region and also determined the exact order of these markers for the first time (Drögemüller, et al. 2005). Because these markers showed tight linkage to the polled phenotype, it is highly probable that the polled gene is located within this contig.

According to the newest studies and observations, we tried to test the usage of single nucleotide polymorphism (SNPs) for determining the genotype of a horned/polled ruminant subject. Even if the advantage of determining the SNPs variability instead of microsatellites is commonly known, it is not used routinely in this scope yet.

We based our study at the invention of the patent nos. WO2005052133 and US2005153328 (Denise S. K, et al. 2005) but during our study, several conditions were modified. As this discovery claims, the tested markers can be used to determine the genotype of all horned and polled animals and they provide a set of markers that can be used individually, or in combination to distinguish homozygous polled individuals from heterozygous polled animals. We made our pilot study with the aim to verify usefulness of its claims in Meat Simmental breed reared in the Czech Republic.

Genomic test to determine polled/horned cattle is based on analysis of microsatelites and of SNPs according to both patents mentioned above. This should be novel genomic test and lead to increasing of 7 SNPs either to increasing of costs for DNA testing as well.

According to newest scientific paper 7 SNPs and 2 microsatelites were used for prediction of genotype. Total 63 animals of Meat Simental were used in analysis.

MATERIAL AND METHODS

Experimental group

Total amount of 63 animals were chosen according to their phenotype, only 5 animals were horned to distinguish right haplotyne and microsatelite loci for detection horned allele. DNA was isolated from blood or hair using commercial kit JETQUICK Blood and Cell Culture DNA Spin Kit (Genomed), standard protocol was used.

PCR amplification

Selected primers demarked seven amplicons containing SNPs that were said to be useful in prediction of the horned/polled genotype. The primer (in all 32) and amplicon sequences (in all 16) were published in cited patents (here not shown). For the brief characterisation see Table 1.

SNP	MMBT25287	MMBT25303	MMBT25316	MMBT25314	MMBT25313	MMBT10493	MMBT25986
SNP-Allele 1	G	C	G	G	G	G	T
SNP-Allele 2	A	A	C	A	C	A	G
PCR (bp)	121	140	137	116	105	100	90
RFLP	x	x	Cfr I	Nsp I	x	Aci I	EcoR II

Tab. 1 List of selected SNPs loci

For microsatelite determination differend primers were used according to scientific papers. Sequences of BMT6438 loci primers were obtained to Bishop et al., 1994 and TGLA49 to Georges et al., 1992.

Reaction mix in total volume (25 µl) comprised of 50 ng of genomic DNA, 12,5 µl of Combi PPP Master Mix (Top Bio), forward and reverse primer (200nM each), and redistilled H_2O. The cycling conditions were the same for all seven amplified products and optimized for the PTC200 equipment (BioTech) as follows: 95/2 min; 30x (95/30s, 60/30s, 72/30s); 72/7min; 4/∞. The results of amplification were verified at 3% agarose gel stained with ethidiumbromide.

SNPs testing

Four markers were analysed by standard means of PCR-RFLP method but in loci where no restriction endonuclease recognized the polymorphic site, the automatic sequencing of amplified PCR fragment (3 amplicons) was applied. Standard protocol for automatic gene analyzer ABI PRISM 310 (Applied Biosystems) was used.

Microsatelite genotyping

Fragmentation analysis was used for detection alleles in both loci. Size of BMT6438 and TGLA49 was from 256 to 271, or 109 to 127, respectively. Detailed descriptions of the methods conditions are disposal at the author.

Results evaluation

The PowerMarker (Liu, et al., 2005) software for haplotype prediction was used. Microsatelite genotypes were drawn in pedigree and analyzed one by one.

RESULTS AND DISCUSSION

In the first step, the automatically generated haplotype combinations for each animal were compared with the haplotype table from the patent.

We intended to find out the likely haplotype combination of the animal and according to the table predict its´ genotype. However, using this straightforward technique of results´ interpretation proved to be not very sufficient and fallacious. One of the reasons is the frequent occurrence of haplotype combinations in our file that are not showed in patent table. As we worked with small file of relative animals of one breed, this is the predictable situation.

Using microsatellite genotypes pedigree trees were created for each family. If some cow (mother) was marked as a horned (at the phenotype level), there were not complications to distinguish right allele, which is responsible for horned cattle. Both heterozygote and homozygote genotypes were established. One condition had to be realized, at least 5 calves in one family. On the other hand, if only polled individuals (at the phenotype level) were occurred in family, separations of homozygote and heterozygote individuals were much more complicated. In most cases, it was impossible to establish genotype. It might be because of low variability of microsatellite genotypes in family.

The best option how to predict genotype in polled loci was to combine results from SNPs analysis together with results from microsatellite analysis. In some cases genotype was established but success of prediction was too low.

During defining of horned/polled genotype, we also come out from classical principles of hornlessness heredity. We took into account the tree possibilities: PP – double polled (homozygote), Pp – polled (heterozygote), pp – horned. We worked only with the given data from breeders and no checking of said phenotype was done. So we can expect the false assessment of the phenotype.

By analysis only with allele frequencies, we find out that one marker was fully monomorphic (MMBTA25303) in our file and four of them (MMBT25287, MMBT25313, MMBT 25314, MMBT10493) indicated the significant relation to the polled phenotype.

We were able to established or predicted genotype in 28 animals in total of 58 (63 animals were used and 5 of them were horned, we had already known their genotypes).

SUMMARY

According to newest literature, our experimet was to design well. We were able to established some genotypes and predict polled in individulas. Still, success of investigation is not good, and was to less than 50% of animals included in experimental group. See, that group was designed as well as possible according to information about individuals. It means, that in common situation the probability to distinguish right genotype in polled loci will be much more lower.

For the further experimetns, some SNPs should be removed (for ex. MMBTA25303) and be replaced by another one. Also, 3 of 5 microsatelite loci should be added in experimet (for ex. ARO9, ARO24, SOD1MICRO2) for better analysis in families groups.

No concrete number or name of animal was used in this publication because request of breeders or owner of animals. Presented data will be easily to abuse with using specific information about animals.

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