Hunting down genes

Here's how geneticists ran down the impact on one gene on carcass traits

There are many genes that influence appetite and metabolism in cattle. A detailed understanding of how these genes function both independently and coordinately with each other will allow cattle breeders and producers to understand the role genetics plays in carcass composition, and would also allow them to better predict when and how a particular animal will finish, maximizing that animal's profit potential. These predictive tools should ultimately remove a lot of the guesswork from breeding and finishing cattle. The science itself is still in the relatively early stages, but many of the genes involved are now known, and research is underway to determine if natural variations in these genes affect finishing characteristics, such as marbling or backfat. Some of this research takes place at the University of Saskatchewan in the genetics department of Animal Science under the supervision of Dr. Sheila Schmutz.

I recently completed a Master's of Science degree in Sheila's lab that focused on characterizing a potential appetite gene known as *Pro-Melanin-Concentrating Hormone*, or *PMCH*. *PMCH* was a good candidate to affect carcass traits in cattle, based on research done by others using rats and mice as models. Mammalian genes are generally very similar across species and generally have similar functions. Some genes code for more than one product and *PMCH* is no exception. There are three peptides encoded by *PMCH*. The most studied of these is Melanin-Concentrating Hormone, or MCH. In rodents MCH was shown to induce feeding as well as to slow metabolic activity. We hoped it might have similar effects in cattle.

My first task was to determine the nucleotide sequence of cattle *PMCH* and identify any existing variation within the gene amongst cattle of different *Bos taurus* breeds. A single-nucleotide polymorphism, or SNP, was discovered. A SNP occurs when one nucleotide is substituted for another in the DNA sequence at a defined position within the gene. In this case the two nucleotides are *A* and *T*. Mammals have two copies of each chromosome, one inherited from their father, and one inherited from their mother. A homozygous animal has two copies of the same nucleotide (or allele), one inherited from each parent. A heterozygous animal inherited one allele from their dam, and a different allele from their sire. The combinations of alleles a given animal can have are referred to as its genotype which, in this case, are *AA*, *AT*, or *TT*.

Most genes consist of coding regions, also called exons, whose nucleotide sequence determines the amino acids that make up the functional gene product, for example MCH. Introns are spans of nucleotides that separate the exons and do not code for anything. They may seem quite useless, but introns have important evolutionary roles, and can allow a single gene to produce variant end products in some species. Genes also have promoter regions that regulate the rate of transcription (expression) of the gene to alter the amount of functional peptides produced. This happens because there are proteins that can either repress or induce expression of a gene. These proteins recognize specific sequences of DNA and bind to them, which will aid or interfere with the binding of other proteins that are required for gene replication to take place. Nucleotide substitutions that occur in these promoter regions may change the binding site so that these proteins no longer recognize this site and do not bind. Alternatively, substitutions may introduce a new binding site that wasn't there before.

The *PMCH* SNP was found in the promoter region. This SNP was unique from many others as it possibly had a regulatory effect instead of changing an amino acid, for example, and was in relatively close proximity to the start of the gene and not thousands of nucleotides away. The *T* allele of the SNP was thought to introduce a binding site for a transcriptional repressor, while the *A* allele resulted in a DNA sequence that was not recognized by this repressor (Figure 1). The effects of this are likely decreased expression of the gene in the presence of the *T* allele due to repressor binding. In this instance there would ultimately be a decreased amount of functional peptide, MCH. When the *A* allele is present, it is likely that this repressor does not bind because the binding site is not recognized, ultimately allowing relatively higher expression of MCH.

Next was to figure out if the SNP in the promoter region of *PMCH* had any effect on carcass traits. Detailed carcass evaluations were available for two populations of crossbred *Bos taurus* animals of five major beef breeds. These animals were genotyped at the SNP, meaning that the alleles each animal carries were determined. This allowed us to compare the carcass measurements of each genotypic group. Statistical analysis was carried out comparing genotype with measureable carcass traits. Most interesting to us were traits directly related to fat production. It was found that the SNP did show a significant effect on backfat, measured as average fat and grade fat in two populations of crossbred cattle (Figure 2). Homozygotes with the *A* allele had greater backfat levels on average than homozygotes with the *T* allele. The heterozygous animals (*AT*) were found to have an intermediate level of backfat. This result suggests that down regulation of *PMCH* results in decreased backfat level, which would happen when there were one or two copies of the *T* allele. It could be postulated that this is because decreased expression of *PMCH* would result in decreased food intake and faster metabolic rate. The decrease in gene expression appears to be proportional to the number of *T* alleles present, which is reflected in the amount of backfat.

A consumer taste panel evaluated the steaks according to tenderness, palatability, and flavor. Statistical analysis was undertaken to see if genotype influenced the quality of steak based

on these consumer evaluations. The homozygous animals with the higher level of backfat, two *A* alleles, were also found to produce more tender and palatable steaks while the homozygous animals with less backfat, two *T* alleles, were less tender and not as palatable (Figure 3). Again, the heterozygous animals fell in between the two homozygous groups. This indicates that decreased expression of *PMCH* not only decreases backfat levels, but also appears to decrease both tenderness and palatability. As a result of this finding, the *A* allele was denoted as the favorable allele.

It was determined that approximately 70 per cent of cattle from these two populations carry the favorable *A* allele, while the remaining 30 per cent carry the less favorable *T* allele. The favorable homozygotes (with two *A* alleles) made up approximately 50 per cent of the populations, while the heterozygotes comprised about 40 per cent. The remaining approximately 10 per cent carried no copies of the favorable allele (they have two *T* alleles). Based on these figures, it appears as though cattlemen have been unknowingly selecting for cattle with the preferential allele, likely because this allele is associated with a desirable visual trait. Despite this, there are still many cattle without the favorable allele, which may limit the feedlot potential of these animals.

Selection of animals based on their *PMCH* genotype may allow breeders and producers to select and sort animals by genotype, in order to better predict finishing time as well as to generate a more consistent beef product. This tool is not yet available to producers, but one day will hopefully be used in conjunction with other genetic tests to improve carcass finishing predictions and potential.

— *Sarah Helgeson and Sheila Schmutz*

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