"No Bull" Discussion on Genetic Markers

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Summary

Beef cattle production is a highly competitive activity and wise use of genetic resources and management options are required to ensure long-term profitability. Along with other food animal industries, genetic improvement provides one such opportunity for improved productive and economic efficiencies. Regardless of genetic merit, animal cohorts must be appropriately managed in order to maximize income over costs. Genetic markers provide promise for assisting with both these endeavors, namely increasing rates of genetic gain and providing new management opportunities. However, like all emerging technologies, they require considered use in order to provide economic benefits to the investor. This paper outlines some of the characteristics of these new and emerging genetic technologies.

Alternative Opportunities for Genetic Markers

Livestock industries are typically characterized by several different production tiers that vary in many of their attributes including investment opportunities for the application of new technology. It is important for producers to recognize their own particular production circumstances when considering adopting new technology for the purpose of increased profitability. Broadly speaking, producers belong to either the seedstock (or bull selling) sector or the so-called commercial (or bull buying) sector. The seedstock sector can be further partitioned into a few nucleus herds that lead genetic change and many multiplier herds that produce and market sons from outstanding industry bulls. The commercial sector can be partitioned into cow-calf herds, backgrounding operations, feedlots, processors, retailers, and so on down the production chain to consumers.

In the nucleus sector, the principal role for genetic markers is to increase the rate of genetic gain. The rate of genetic gain in the nucleus sector dictates the rate of genetic gain in the entire seedstock sector. The rate of genetic gain in the seedstock sector can be characterized for some traits by inspecting the graphs of genetic trends in EPDs that are published by Breed Associations in their sire catalogs and on their websites. These trends are incomplete for two reasons. First, they don't characterize all the economically-relevant traits. For example, fertility, longevity, and disease resistance traits tend to be under-represented in these catalogues. Second, they typically don't characterize the most important genetic change – that is the trend in overall profitability that would result from the simultaneous trends in the components of profit such as sale weights, calving ease, cow mature size, etc.

The annual rate of genetic change in the seedstock sector is dictated by three interacting components. These are the intensity of selection, the generation interval (or average age of parents when offspring are born), and the accuracy of selection. Annual advance from selection will be maximized when a few of only the very best candidates are selected and used widely at an early age. In practice, the accuracy of selection of young animals is limited for many of the economically-relevant traits, either because the traits have low heritability (e.g., reproductive traits), or they can only be measured late in life (e.g., longevity and carcass attributes) or under challenging conditions (e.g., disease or nutritional stress).

Genetic markers provide two novel opportunities for influencing seedstock gain. First, they provide an approach for parentage identification, allowing young candidate bulls to be tested competitively in multi-sire settings, and then resolving the offspring paternity at a later date. The candidate bulls could then be selected

on the basis of their progeny test performance. This approach can considerably reduce the cost of progeny testing. The technique is widely used in the New Zealand sheep industry, but to date, has seen only limited use in the U.S. beef industry.

The second novel opportunity for genetic markers to influence seedstock gain has long been promised but has yet to demonstrate impact. This involves the use of markers as an alternative to progeny testing in order to increase selection accuracy at a young age. There are at least four reasons why currently available genetic marker tests have not demonstrated the utility they have promised in this area. First, these marker tests have not been inexpensive. Some breeders have argued that they would be better to invest in additional phenotypic data collection (e.g., ultrasound scanning or progeny testing) than to invest in genetic markers. Second, these markers have not been comprehensively available for the entire portfolio of economically-relevant traits. There are no validated markers for cattle fertility or longevity, for example. Third, the traits for which these markers have been available (primarily tenderness and marbling) are traits that are subject to market failure along the production chain. That is, cow-calf producers do not receive incentives or clear market premiums for producing calves that they believe have a greater propensity for producing tender, marbled beef. This is partly attributable to the lack of a reliable live animal prediction for future tenderness and marbling, and partly due to the fact that only a minor proportion of genetic variation for these traits can be accounted for using all the currently known markers. Fourth, the impact of selection using markers cannot currently be reflected in breed association published trends, because the marker data is not unbiasedly available for inclusion in national evaluation systems. Whereas industry guidelines exist for the uniform collection of other performance traits, and the recording of information on entire cohorts is required, there is no industry body actively promoting the collection of molecular testing information. Accordingly, along with the expensive cost of testing, only test results from animals with favorable markers tend to be widely publicized, for obvious reasons.

Genetic markers also provide opportunities for testing and preselection of young bulls being sold from the seedstock to the unregistered commercial cow-calf sector. For example, the national crop of young bulls could be tested at birth and the genetically inferior half discarded as breeding candidates on the basis of the results. This activity would have no impact on the long-term industry rate of genetic gain, as that is solely dictated by the selection practices of a small number of nucleus breeders that provide new recruits to the existing team of outstanding bull fathers. Testing all potential breeding bulls prior to sale would increase the cost of every bull marketed for breeding by twice the cost of the test, to pay for the bulls own test as well as the test on the bull that was rejected for sale on the basis of the inferior test result. However, a major determinant of seedstock profitability is the proportion of young bulls that can be sold for breeding. This has led to undesirable practices such as feedlot finishing of young bulls and early calving of seedstock herds to ensure that as many bulls as possible can be finished for sale as breeding prospects. It may be that some of these bulls are being tested using current genetic markers, but it is unclear as to the subsequent fate of bulls that demonstrate inheritance of the unfavorable alleles. Bull buyers might like to ask bull breeders that advertise the genetic testing of their bulls as to the fate of those individuals that exhibit below-average test results. There is no industry benefit from investing in the research and application of genetic markers if all tested animals are going to be used for breeding, regardless of the test result. A return on investment requires that the purchase of new information, such as genetic marker tests, result in a different portfolio of bulls being used than would otherwise have been the case if the testing had not been undertaken. Further, the increase in productivity of the offspring of the selected bulls must exceed the costs of the genetic testing. Finally, the beneficiary of the extra productivity (e.g., feedlot manager or processor) must be prepared to pass at least some of the rewards along the production chain back to the bull breeder who incurred the testing costs.

The commercial sector has two opportunities for using genetic markers for management purposes. First, the cow-calf producer can use markers for determining paternity in order to aid in the management of their

bull battery. This can be enlightening, for example, when producers include yearling and mature bulls in a multi-sire breeding group, because in that circumstance, the yearling bulls will typically sire few if any of the offspring. In any bull team, paternity results often demonstrate that a few dominant sires produce most of the calves, and a few sires produce few if any offspring. Paternity testing gives producers valuable information that enables them to remove ineffective bulls from their bull team without compromising their overall calving rate. If possible, yearling bulls are best mated to cows in the absence of older bulls. Paternity determination using genetic markers relies on a process of exclusion to assign paternity. Small marker panels will have lower power and may result in more than one bull from a portfolio of possible sires as being identified as the potential sire. Larger panels allow near-perfect determination of paternity. In practice, it is important that all potential candidate sires are collected for DNA sampling and that DNA samples are not contaminated with samples from other animals, nor labeled in an ambiguous manner.

Backgrounders and feedlot managers could also use genetic markers for marker-assisted management which refers to the process of using DNA marker information to make decisions on how to manage a particular animal or a specific group of animals, to optimize performance. This has been undertaken for many years using cattle sex as a genetic marker. Steers and heifers finish at different rates and in some circumstances it is profitable to manage, feed, and harvest the animals in lines distinguished by sex. Of course in the case of sex, no genetic testing is required to distinguish the alternate alleles. The extent to which marker-assisted management can be implemented using current markers and the economic benefits relative to costs, has not been well characterized from a scientific viewpoint as evidenced by the lack of published results on this topic.

The same principle could also be used in feedlots for two purposes. Cattle could be fed and managed separately according to their propensity to marble. Genotypes that are not likely to produce high levels of marbling might be better finished on different regimes. Further, cattle could be fed and managed to reduce the variation in predicted harvest date. It is problematic for large beef feedlots to partially harvest pens of cattle for several reasons. The act of sorting a pen and removing some animals will in the short-term reduce the performance of the remaining animals from the pen. The ability of the feedlot manager to spread their overhead costs requires that pens be near capacity and half empty pens can only attract half the yardage fees of full pens. It makes economic sense for feedlots to keep pens full and to harvest the entire pen when its value over costs is maximized. Any presorting of lines to reduce the variation in the pen at the end of the feeding period will therefore be beneficial. It would be a relatively straightforward matter for a feedlot manager to undertake their own marker-assisted management trial to determine the practical feasibility of marker-assisted management within the context of their own procurement and management circumstances.

Nature and Scope of Available Genetic Markers

So-called causal polymorphisms that represent a change in the triplet code and therefore a change in amino acid sequence have long been believed to be ideal candidates for genetic testing, but relatively few such DNA changes have been found to influence productivity. Two notable exceptions are DGAT1 and GHR, that are known to influence lactational productivity in cattle. Many causal tests have been found for recessive genetic defects and other simply inherited characteristics such as coat color or double muscling.

Most genetic tests are based on DNA sequence that demonstrates an association with some aspect of productivity. In some cases, these associations are based on polymorphisms in or near genes that are believed to be involved in the process that influences a particular trait. Calpain and calpastatin are two such genes that are determinants of post-mortem tenderness and both genes have nearby single nucleotide polymorphisms (SNPs) that can be used to predict tenderness. Such genes may be subject to patents, royalties, and exclusive licensing, which can limit the ability of alternative companies to provide the same test. Companies may test for several SNPs in one gene,

and provide a test score that represents the collective result of all the tests. The association of such SNPs may differ from one breed to another and this further complicates interpretation. For example, tests in *Bos taurus* breeds (such as British or Continental breeds) may have different associations in *Bos indicus* (Brahman, Brahman crosses, or eared breeds).

Genetic tests may be marketed following discoveries anywhere in the world. Some research has been rigorous and included substantial testing, whereas others may be based on superficial results. This places the onus on the user to investigate the nature of the underlying research results if one wants to determine the likely confidence that can be associated with the marketing claims. This leads us to the topic of gene discovery and validation of genetic testing. Only three commercially-available genetic tests have been extensively researched and validated in U.S. beef cattle populations. Two of these tests are competing products for tenderness (GeneSTAR Tenderness and the tenderness component of the Igenity Profile) that are based on three SNPs. The third test is for marbling/ Quality Grade and is known as GeneSTAR Quality Grade (See Table 1).

Discovery and Validation of Trait Associations with Genetic Markers

Many scientific endeavors have been undertaken with a view to discover genes that influence productive traits. Such research has spanned many species and discoveries in one species (e.g. mice) have often led to findings in livestock species such as cattle. Until recently, experimentation has been based on microsatellite rather than SNP markers and these were much more costly, perhaps requiring genotyping investments of several thousand dollars per animal. Accordingly, most researchers limited their studies to as few animals as possible and often used crosses between disparate breeds that were known to vary for the attribute of interest. There are two consequences of such an approach to gene discovery. First, the experiments tend not to be very powerful, and can thus only find some of the largest genes that are associated with the investigated trait. A consequence of low power is that any associations that are discovered tend to be those that for chance reasons appear to be larger than they really are. Accordingly, validation experiments in new populations tend to find

Table 1. Commercially-available tests for beef cattle in the United States (as of March 2008). The three bold outlined cells represent tests that have been independently validated by the NBCEC.

Trait	Bovigen www.bovigen.com	Igenity www.igenity.com	MMI www.metamorphixinc.com
Quality grade	**GeneSTAR Quality Grade**	Igenity profile - quality grade	Tru-Marbling
Tenderness	**GeneSTAR Tenderness**	**Igenity Profile - Tenderness**	Tru-Tenderness
Other	Feed efficiency	Igenity profile – Yield grade, Fat thickness, Marbling, Hot carcass weight, Ribeye area, Heifer pregnancy rate, Stayability, Calving ease, and Docility	Average daily gain (not yet commercially available)
Results reported as	No. of stars and "Genetic Progeny Difference" (GPD)	1-10 scale (with 10 being the best)	"Molecular Genetic Value" (MGV)

smaller effects than the original research. Second, the discoveries may be of limited value in purebred populations, because the favorable allele may already be common among seedstock animals.

Validation is a critical activity to gain confidence in a particular gene test in a particular population under defined management protocols. The National Beef Cattle Evaluation Consortium (NBCEC) has provided validation services to companies marketing DNA tests, on behalf of the beef industry. The DNA testing companies pay for the cost of genotyping, while the NBCEC undertakes the statistical analysis. Validation is not simply a repeat of the gene discovery process, but a determination of the strength of support for the testing companies published claims based on independent data. The principal datasets used for validation have been the carcass and DNA samples collected for the National Cattlemen's Beef Association, funded Carcass Merit Project (CMP). These data have allowed validation of marker claims for carcass traits such as marbling and tenderness. A major problem with validation in practice is having access to an existing dataset that is independent of information used in gene discovery. The traits of the most interest for genetic markers include characteristics that are difficult to measure, such as feed efficiency, or disease resistance. Accordingly, any tests claimed to influence these traits cannot be validated without first creating a validation population. This lack of resource populations represents the major limitation to validation.

Validation has been useful in demonstrating to testing companies that certain tests do not perform in the manner they had believed. Tests that have been placed through validation protocols prior to market offering have in some cases failed validation, and some companies have acted responsibly by withdrawing their plans to market those tests.

The Future of Genetic Markers – On the Eve of a Revolution

Genetic testing is now undertaken almost exclusively using systems that test for the presence of alternative single nucleotide polymorphisms (SNP). These markers have been discovered through comparison of sequence information and occur whenever an individual is identified with a sequence that differs from the base sequence. There are likely to be many millions of SNPs spread along the genome. Some advantages of these markers include the fact that they exist throughout the genome, they can be reliably assessed, the testing process can be automated, and a huge number of alternative SNPs can be tested in a single chemical reaction. In humans, panels of more than 500,000 such tests can be assessed in one individual for around $300. In cattle, a panel of 50,000 markers became available in January 2008, and can be assessed for between $200 and $300 depending upon economies of scale dictated by the number of samples of interest.

The development of these relatively cheap, comprehensive panels of SNP markers has opened the door for a new approach to genetic testing. The old approach, described above, involved at least three steps. First gene discovery endeavors were undertaken to identify regions of the genome that appeared to be associated with differences in animal performance. Second, these regions were fine-mapped, using more markers, often in conjunction with candidate gene knowledge, to identify a small portfolio of SNPs markers for patenting and marketing. Third, the small panel of SNPs was made available, typically under some trademarked brand, with or without validation. Only the largest, most significant associations were likely to make it through this process to market. The gene tests were marketed independently of national evaluation systems, and no attempts were made to capture the genotypic results of tested animals. As shown in Table 1, the results of tests are reported on scales different for each company and different from the EPD scales that would be used for conventional analysis of similar traits. Tests are targeted at only a few specific traits, and are relatively expensive (~$30-60) when considered within the entire context of beef cattle improvement.

The new approach is known as genomic selection, and it is in its infancy. It involves the use of a large number of markers (e.g. 50,000) that can be simultaneously applied to predict the merit for many different traits. The results will be presented in the form of EPDs. The approach relies on a two-step analysis.

First, the effects of all the thousands or tens of thousands of SNPs are characterized in a so-called training data set. This analysis effectively derives an EPD for every chromosome fragment. Second, new animals are assessed with the dense SNP panel, with the results used to identify the chromosome fragments inherited by that animal. An overall measure of the merit of that animal is obtained by adding up the EPD for each of the chromosome fragments it inherited. This measure of merit is known as a genomic EPD. Currently, the principal training sets being used for these endeavors are represented by animals from the U.S. Meat Animal Research Center (notably the Cycle VII animals) and sires that have been widely used by various breed associations and have reliable EPDs based on conventional data analysis. Genomic selection is not currently being marketed by any testing companies, but this situation is likely to change rapidly if the scientific validation of the approach provides promising results.

Given the rate at which new tests are coming to market and the changes taking place in testing providers, readers requiring details as to specific tests and companies are encouraged to use web sources such as those maintained at University of California, Davis (http://animalscience.ucdavis.edu/animalbiotech) or by the National Beef Cattle Evaluation Consortium (http://www.nbcec.org).

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