SUMMARY

LoinMAX[TM] and MyoMAX[TM] were both validated before commercial release. The validation process consisted of confirming the phenotype in commercial flocks and evaluating correlations of the phenotype with other important traits such as lamb survival. The effects of LoinMAX[TM] and MyoMAX[TM] were consistent in different genetic backgrounds and had no negative effect on other production traits. Therefore we were confident that the tests would be useful in selection for meat yield in a commercial sheep breeding programme. The strategies by which commercial breeding programmes are now using LoinMAX[TM] and MyoMAX[TM] depends on the frequency of the alleles in their flocks.

Prospects for genome wide selection in the New Zealand livestock industries

K.G. DODDS, P.R. AMER[1], R.J. SPELMAN[2], J.A. ARCHER and B. AUVRAY

AgResearch, Invermay Agricultural Centre, Mosgiel
[1]AbacusBio Limited, Dunedin
[2]Livestock Improvement Corporation, Hamilton

ABSTRACT

A number of DNA marker applications aid genetic selection within subpopulations of New Zealand's livestock farming industries. Advances in genomic tools have opened the possibility of genotyping individuals for a large set of markers, enough to have a large proportion of the genome 'tagged' with at least one of these markers. This creates opportunities for genome wide selection (GWS) – the selection of breeding animals based on very large numbers of marker genotypes. There are a number of requirements for GWS to work, including the existence of genotyping technology to provide genotypes at a sufficient density, tractable statistical methods for estimating marker effects to use in prediction models and suitably phenotyped populations which when genotyped allow estimation of relationships between markers and traits. Additional phenotyped populations are required to validate their predictive ability and demonstrate economic benefits to breeders and to the commercial tier. A high density genotyping platform is available for cattle, but may need further developments. Plans are underway for a similar platform for sheep, but not for deer. The dairy industry is well placed to implement GWS, as there is a large bank of DNA samples of industry sires representing most of the genome variation present. Genotyping these animals will allow marker effects to be estimated and the reduced generation interval arising from selecting young sires should easily overcome any decrease in selection accuracy compared with progeny testing. Industry structures are likely to evolve to allow a return to entities undertaking GWS. GWS will be most beneficial for traits with low heritability, that cannot be measured on selected parents and/or that are expensive to measure. Genes of known effect may continue to be accounted for individually, particularly if they exhibit non-additive effects.

Keywords: genome; marker assisted selection; dairy; beef; sheep; deer.

INTRODUCTION

The last century saw selection in livestock breeding programmes advance from ad-hoc visual and family-based procedures to the use of quantitative information to provide selection rankings based on predictions of genetic merit. New statistical models that incorporate information from multiple relatives and multiple traits and their implementation using computers have enabled this development. The focus has been on a relatively small number of highly heritable traits that are relatively simple to record. Most applications have used the additive infinitesimal model, whereby a trait is assumed to be influenced by an infinite number of genes each with an infinitely small, non-interacting effect. While this has been and remains a powerful tool for industry improvement, the limited breadth of focus has constrained the potential overall benefits.

The end of the last century saw the advent of molecular marker maps for livestock which

enabled the detection of quantitative trait loci (QTL) – specific genomic regions that are shown to have variants causing phenotypic differences in a trait (Haley, 1995). These QTL can then be incorporated into selection programs by tracing the variants in families using markers (marker assisted selection; MAS) or by directly genotyping for either the causative genomic difference, if known, or a very accurate marker for the causative genotype (genotype assisted selection; GAS). Although MAS does not require the causative mutation to be identified, it does require that the association between QTL and marker alleles be established for each family or population (if consistent for all families in the population) in which it is applied. The cost effectiveness of MAS and GAS has been hampered by the cost and difficulty of discovering usable genetic variants, and in some cases by the costs of DNA testing the selection candidates.

The start of this century has seen a further advancement of molecular tools, with large amounts of genomic sequence becoming available for many species, including sheep and cattle. For example, there is currently a programme to produce a (near) complete genome sequence for cattle (Kappes *et al.*, 2006), following on from similar efforts in humans and other species. One application of this sequence information is the development of molecular markers at a very much higher density than was previously available. These markers are generally base changes at a particular genome position, known as single nucleotide polymorphisms (SNPs). At a time when large numbers of SNPs are being discovered in farmed livestock species, the development of technologies for genotyping vast numbers (in the 100,000s for humans) of these markers in a single assay has opened up the possibility of using markers across the whole genome (genome wide selection; GWS) to 'tag' most of the genetic variants contributing to trait differences.

This paper discusses what impact GWS might have in the main NZ livestock breeding industries. An overview of GWS technology is given, followed by a discussion of its potential applications in the New Zealand dairy, beef, sheep and deer industries. These applications are considered in terms of the characteristics of each of the industries. Finally, we discuss some general issues and summarise the findings.

GENOME WIDE SELECTION

GWS requires a marker set that covers the genome with sufficient density that one or more informative combinations of markers can capture most of the information associated with each genetic variant affecting traits of interest. An estimation step is required to quantify the effect each tiny section of the genome, defined by the markers, has on these traits in a relevant set of animals. We will refer to this set of animals as the 'training set'. Ideally, the training set would be made up of animals from earlier generations of the population where GWS is to be applied. In this way, the genetic variants and estimated marker effects will be characteristic of the population. It is also likely that many appropriate phenotypes have already been recorded in the population although a downside would be the absence of phenotypes for traits which have not historically been recorded, because they have been deemed intractable or non cost effective for conventional quantitative genetic selection systems. The training set should be designed to ensure no confounding of environments and genotypes, *e.g.* regional (environmental) differences in trait values may be incorrectly attributed to regional differences in bloodlines. In addition, the training set should match the breed in which GWS is to be applied. The estimation step depends on availability of tractable statistical methods that can handle prediction with such high-dimensional data. Meuwissen *et al.* (2001) have suggested some possible methods.

GWS can be applied without knowledge of the genetic relationship among the candidates for selection. Because GWS uses markers as proxies for the actual genetic variants, and because most of the variants are tagged by these markers, then most of the genetic information about an individual is provided by these markers. This means GWS can be applied without pedigree information and therefore enlarges the population to which it could be applied.

GWS can be applied to unphenotyped animals. Once the marker-phenotype relationships have been determined in the estimation step, GWS would normally be applied to individuals without phenotype records of their own or of their progeny. This allows individuals to be selected at a younger age than previously practiced, particularly when progeny testing was used. In this way, the generation interval within a breeding program is limited only by the earliest possible breeding age of selection candidates. It also enlarges the population to which genetic selection could be applied.

GWS has high accuracy. Previous theoretical studies (Meuwissen *et al.*, 2001; Schaeffer, 2006) indicate that the accuracy of selection with GWS is comparable to that obtained by testing moderate to large numbers of progeny. This means that the

shorter generation intervals can be achieved without a large trade-off in the rate of genetic progress per generation due to reduced accuracy. Non-optimal resources may result in some reduced accuracy for earlier applications of GWS (Hayes & Goddard, 2007).

GWS IN DAIRY CATTLE

The NZ dairy industry (Table 1) is characterised by the wide-spread use of a few highly selected sires following progeny testing. This industry structure is favourable for GWS, because of the scope to decrease the generation interval by eliminating the need for progeny testing. Schaeffer (2006) has presented a theoretical analysis of the advantages of GWS for dairy cattle based on the Canadian Holstein population. The proposed application of GWS proceeds as follows. The initial step involves estimating the marker effects by genotyping 2500 sires previously progeny tested. Then a two-stage selection process is implemented to select 1000 bull dams, with the first stage using conventional methods to select 2000 cows and the second stage using GWS. Twenty bulls are then selected from the 500 male progeny of these cows, again using GWS. These bulls are then used on the general population from one year of age (for two to three years), without progeny testing, with the best few used to mate selected bull dams for the next year. This scheme is predicted to cost less than 10%, and result in more than double the genetic gain per year, compared to the scheme involving progeny testing used currently. This is assuming slightly more conservative accuracies than those of Meuwissen *et al.* (2001). The increased genetic gain comes mainly from the reduced generation interval for sires which easily outweighs a slightly lower selection accuracy.

Similar arguments will apply to the NZ dairy industry, although a scheme is more likely to cost around 50% of the current system than the 10% given by Schaeffer (2006). LIC has commenced the research phase of GWS with comparable numbers of bulls to that used in the study of Schaeffer (2006). CR Delta, who owns Ambreed, the second largest dairy AI company in New Zealand, have also commenced a GWS research project. There is currently a 10,000 marker platform (SNP chip) for cattle (http://www.affymetrix.com/support/technical/data sheets.affx), which is expected to increase to 25,000 SNPs by early 2007. In addition, a 60,000 SNP panel is planned for release late in 2007 by Illumina. These approach or exceed the 30,000 evenly spaced markers required (Hayes *et al.*, 2006) to capture the same information as in the study of Meuwissen *et al.* (2001). A resource for the estimation step could be readily assembled, as the dairy industry have collected and stored DNA samples of sires used in the LIC breeding scheme since the early 1980's. A first application is likely to be artificial insemination (AI) companies using GWS to select among progeny test candidates, thereby reducing the number needing to be progeny tested for the same genetic gain (same generation intervals and accuracies following progeny testing). This could occur with the current marker resource, and would have the added benefit that the methodology could be demonstrated before commercial farmers are presented with GWS-, non progeny tested selected bulls. This appears to be the strategy being adopted by Holland Genetics (owned by CR Delta) (http://www.hg.nl/news/pressreleases-bericht.jsp?id=6933).

Table 1: Attributes of New Zealand livestock industries of importance to the application of genome wide selection.

Attribute	Dairy	Beef	Sheep	Deer
Population Size (millions) [1]	5.1	4.4	40.0	1.7
Breeding females (millions) [1]	4.1	1.3	29.6	0.8
Annual slaughter (millions) [2]		3.7	29.9	0.4
Annual slaughter (000 tonnes) [2]		641	538	23
Animal value (female at normal age of first breeding; $NZ) [3]	992	626	87	184
Number of main breeds (>5% of nucleus population)	2	5	5	2
Number of tiers in breeding structure	2	3	3	3
Dissemination of genetic gain to commercial tier	AI	Multipliers	Multipliers	Multipliers
Current reliance on progeny testing	High	Moderate	Moderate	Low
Proportion of economic genetic variation in unrecorded traits	Low	High	Moderate	Low
Commercial structures that motivate investment	AI companies	Not currently	Some large scale breeding corporations	Not currently
SNP-chip availability (years from now)	0-2	0-2	2-10	5-15

[1]Source: http://www.stats.govt.nz
[2]Source: http://www.nzmeatstats.co.nz
[3]Source: http://www.ird.govt.nz

The analysis of Schaeffer (2006) essentially assumes that GWS is based on the same traits as are available with initial progeny test results. Additional gains could be made by incorporating information on traits such as longevity and fertility that would not normally be available until after selection of bulls for widespread usage. Currently the majority of information for both of these traits is collected after the sire has had one to two years of wide-spread use and thus the majority of his direct genetic contribution is completed before accurate estimates are available. There could be some other variations to the scheme presented. For example GWS could be applied to all candidate bulls without first selecting their dams. This would more than halve the amount of genotyping required, but would require more cows to be contract mated.

GWS IN BEEF CATTLE

The New Zealand beef industry (Table 1) is essentially a three-tier system (Garrick, 1997), with the top two tiers being registered herds that supply bulls to the tier below. The breeders in the top tier drive the direction of genetic change through their own selection and through extensive importation of semen. Almost all commercial breeding cows are naturally mated. Selection is based on liveweight traits (Garrick, 1997) and visual criteria related to aesthetics and functionality, with the majority of performance records available for individual selection candidates themselves prior to practical breeding ages. The industry population structure is further fragmented through a larger number of significant breeds than the two dominant breeds present in the dairy industry (Charteris & Garrick, 1996). Inserting GWS into this current industry structure will not have the large economic advantages that have been predicted for dairy industries, because it will have less impact on generation intervals and it would not be used as an alternative to expensive progeny testing. Therefore the application of GWS to the beef industry would need to be associated with a change in breeding objectives and industry structure in order to be cost effective. Traits such as meat quality, fertility and survival are currently considered not worth selecting on, because of one or more of: low heritability, expense of measurement and expense and consequent increased generation intervals for progeny testing. This is in spite of a very substantial amount of genetic variation in overall profitability associated with them. A five-fold increase in the efficiency of selecting these traits (*i.e.* even if less than quarter that shown for dairy

cattle by Schaeffer (2006) is possible) could result in this becoming economically feasible.

There would need to be suitable training sets developed. For meat quality traits this might be achieved by evaluating progeny from a set of sires chosen to represent the population. It could also be done by evaluating a random set of slaughter animals, thereby bypassing the costs of conducting a progeny test. Care would need to be taken to design a training set with no confounding of environments and genotypes. Lowly heritable traits such as fertility and survival would be best evaluated via progeny evaluations, to increase the 'genetic signal' against the background environmental noise in the training set. These traits often involve several years of recording on breeding cows, and therefore a *de-novo* training set would take some time to generate. The application of GWS to maternal traits, such as fertility and survival, is likely to result in the development of separate maternal and paternal breeds or lines. The ultimate aim of lines selected for maternal traits is to breed cows, while the paternal lines (perhaps bred without using GWS) supply bulls to sire animals for slaughter. The market for improved maternal beef genetics would be limited though, as over half the animals slaughtered for beef are sourced from dairy dams (Garrick, 1997).

An additional requirement to make GWS feasible is that there is a pathway to recover the costs of GWS. In this dairy industry it is likely that the AI companies would undertake GWS and recover the costs in the sale of semen. The use of AI is not widespread in the beef industry (Garrick, 1997), so an AI company would need to be confident of an upsurge in the use of AI before embarking on GWS to select their sires. From the producer's point of view, the value of the additional gains possible by using GWS-selected sires needs to outweigh direct and indirect (*e.g.* management) costs of using AI. It is unlikely that an individual breeder could undertake GWS, but it would be possible for cooperative or corporate farms. The genetics could be disseminated through a multiplier tier, by increased use of AI or by both these methods. For breeders to justify the expense of selecting for quality traits, there will need to be sufficient price advantage for higher quality products for the producers. There is unlikely to be much differentiation at the individual level, because of the measurement costs, and so improvement of quality traits would require vertically integrated structures involving cooperatives or corporate farms, producers, processors and marketers of an improved quality brand.

GWS IN SHEEP

As for beef cattle, the sheep industry (Table 1) is essentially a three tier system (Garrick *et al.*, 2000), with a top ('nucleus') tier of breeders supplying rams to a 'multiplier' tier which in turn supplies rams to the production tier. A difference though is the current absence of importation as a driver of genetic change. As the main product focus of the sheep industry is meat, the implications of GWS on this industry are similar to those for the beef industry. Use of GWS in the sheep industry is likely to have similar implications for the use of AI, selection of training sets, application to traits other than liveweight (*e.g.* fertility, survival and meat quality), and increased vertical integration in the industry.

However, there are some differences. One obvious difference is that sheep produce wool as well as meat, but this is unlikely to be of critical relevance when it comes to GWS (even for fine wool production), as wool prices are declining, measurements are available on individuals to be selected, and quantity and quality traits are moderately to highly heritable. A more important difference is that there has been more emphasis on reproduction and survival traits. This has resulted in a clearer delineation between maternal and sire breeding programmes and each of these breeding sectors is likely to remain separated in the application of GWS. It has also resulted in several solutions becoming available to increase prolificacy (such as the use of specific breeds, *e.g.* the Finn, or introgression of specific genes variants, *e.g.* the Inverdale), so that GWS for fertility in sheep would more likely be aimed at other components of this trait, *e.g.* hogget pregnancy, distribution of litter size to achieve high twinning rates, embryo survival or aseasonality. An additional difference compared to the New Zealand beef industry is that there has been greater emphasis on meat quality, due to a greater proportion of lamb than beef being sold as high quality cuts. Most terminal sire breeders include some measure of meat quality, most commonly ultrasonic muscle and fat depth, in their selection index. However, as with beef, it will be quality traits that are measured following slaughter where GWS will have the greatest opportunities. The greater focus on meat quality has also led to some closer associations between breeder and marketer (*e.g.* Anon, 2006) and such associations could be the vehicle for the application of GWS. There are a number of subsets of the sheep industry with unique trait recording such as parasite resistance, facial eczema resistance and CT scanning for carcass composition. Thus, GWS

creates the opportunity to spread knowledge and benefits of recording in these systems to a much wider base. Breeders could then sort sale rams according to the production system for which they are best suited. Sheep breeders seem to be more open to crossbreeding (for example, with recently imported breeds) or the use of non-registered animals than do beef breeders. This would give GWS more variation to operate on, but also means that the training set must be chosen carefully. For example a mixed breed training set is likely to give poor results if applied to a purebred population. Sheep have a shorter generation interval than cattle, and can be parents by one year of age. Therefore there is a shorter lag between using multipliers instead of AI than there is with cattle, and with AI being more difficult in sheep this may result in multipliers being the preferred method of disseminating the genetic improvement. A further difference between sheep and beef is that appropriate genomic resources are at an earlier stage of development for sheep, with development of a 20,000 SNP chip projected to start in July 2007 (http://www.sheephapmap.org/). The marker density required for sheep is probably similar to that for cattle, although this will not be clarified until the proposed SNP chip has been used. This will delay the introduction of GWS in sheep compared to cattle. Finally, the production value of a single sheep (or even a litter) is less than a beef animal, so if the costs per production animal and relative genetic gains are similar, GWS will be less attractive in sheep than with beef.

GWS IN DEER

The deer industry (Table 1) also has many similarities with the beef (and sheep) industries. It operates as essentially a three tier structure (Archer, 2003). Deer breeding is less developed than for the other industries with across herd evaluation being a recent innovation (http://www.deeresearch.org.nz), and being applied to liveweight only. AI has recently seen increased use, but is still likely to have its major application in the nucleus and multiplier levels rather than in commercial herds. However, while greater use of AI is unlikely to change the pathway of genetic improvement, it may alter ownership of the multiplier level, with commercial herds breeding their own sire stags by AI rather than purchasing from a specialist multiplier. Whether GWS is applied at the elite nucleus level only, or at the multiplier level (*i.e.* to select stags for use as sires in the commercial herd) will depend on the eventual cost. At the multiplier level, GWS could replace phenotypic recording and its associated

management complexity, making running a multiplier herd on commercial properties significantly easier. Application of GWS to antler traits is unlikely because of both their high heritability and limited size of the velvet and trophy industries. GWS would most likely be applied to traits such as fertility, calving date, disease resistance and meat quality, where phenotypic measurements for classic genetic evaluation are difficult, costly, measured too late in life or incur management penalties. As for sheep and beef, appropriate commercial relationships would need to be established to allow financial benefits from the improved genetics to be returned to the entity that undertakes GWS. Training sets need to be formed taking breed (Red, Elk/Wapiti or mixed) for GWS application into consideration. However the application of GWS in the deer industry is unlikely to occur for some time, due to the lack of an appropriate marker resource – there are currently no plans to generate marker sets of sufficient density to undertake GWS. The relatively small size of the industry means that development costs for GWS are proportionately very high relative to other livestock industries. This issue applies to all genetic technologies (*e.g.* single gene tests), and so the deer industry is likely to leverage off the technological developments from other species, despite the consequent time lag.

GENERAL ISSUES

This paper has considered possible implications of GWS being applied within the New Zealand breeding industries. All these industries, to a greater or lesser extent, import some of their genetic material. Therefore any gains made overseas by GWS will have an indirect effect on gains made here. The beef industry also has its genetic evaluation done mainly offshore. This is unlikely to be an issue for GWS, as it will be applied "in-house" and in parallel with normal genetic evaluation, with selected animals eventually being evaluated (by progeny information) as being superior to that predicted from earlier industry genetic evaluations. This will be a useful validation of whether GWS is working as expected.

We foresee that AI will be the main reproductive technology used to disseminate the improved genetic material. Other technologies exist, such as embryo transfer and cloning. However these are not economical in the production tier, and for a multiplier tier would involve a greater lag between selection and commercial production than would AI. These

technologies may find a place in the top tier though.

A number of genetic marker tests are already being used in the New Zealand industries (Campbell & McLaren, 2007). Although it might appear that a genome-wide technology would supersede the use of individually marked genes, the two strategies are likely to operate in parallel. Single gene tests are likely to remain sufficiently cheaper than genome tests for some time, and therefore can economically be applied to larger populations, *e.g.* in the multiplier tier. GWS is mainly seen as a method of improving additive effects, while the characterisation of single genes may reveal non-additive effects (dominance, imprinting, epistasis) which can be used to advantage in single gene MAS or GAS in conjunction with specifically targeted mating strategies. Presently it is unclear whether GWS can be applied in a manner which will take advantage of heterosis effects, such as those used by cross-breeding in beef and dairy production.

Genotyping costs have fallen dramatically over the last 20 years, and the costs of SNP chips are likely to follow this trend. A ten-fold price decrease might see GWS applied to sire selection from the multiplier tier and to dam selection in the nucleus tier. A further ten-fold price drop might see GWS applied to multiplier dams or even producer dams, particularly if it had proved effective for health and reproduction traits. There would also be the possibility of screening production populations for niche markets if there were differential preferences among consumers. SNP chips could also be used in production environments for parentage, or for heritability estimation or genetic evaluation using actual (rather than that expected from pedigree) proportions of genes shared between relatives (Visscher *et al.*, 2006).

CONCLUSIONS

Genome wide selection is likely to have a major impact on the New Zealand livestock industries in the next five to 20 years. Its first application will be in the dairy industry as cattle will be the first species to have sufficient marker resources and appropriate training sets, there are already structures that allow a return to the investor, and the technology is likely to be economically viable, mainly due to being able to dramatically reduce generation intervals. The main differences among the other species will be the availability of the genomic resource, which is imminent for beef, in the planning stages for sheep, but unplanned for deer. For these industries GWS would most likely

be motivated by its application to non-production traits such as fertility, survival, health and product quality. There will need to be greater vertical integration and greater use of AI in these industries

to allow a financial return to entities that invest in GWS. These facets may cause a barrier to the use of GWS until genotyping costs drop sufficiently to catalyse the changes needed.

Uptake of DNA testing by the livestock industries of New Zealand

A.M. CRAWFORD, R.M. ANDERSON and K.M. McEWAN

AgResearch, Invermay Agricultural Centre, Mosgiel

ABSTRACT

This paper describes, from the perspective of the main animal genotyping service laboratory, the uptake of DNA tests by the New Zealand livestock industries. We describe a rapid increase in the use of DNA testing over the last 3-4 years in New Zealand. In particular pedigree establishment and verification has shown significant uptake by deer, cattle and sheep breeders. The highest penetration of DNA testing has been in the deer industry where we estimate 30-40% of the recorded breeding population uses DNA testing to establish and verify pedigrees. The increases have mainly been due to an increase in the number of samples per client rather than an increase in the number of clients using the service.

Keywords: DNA; pedigrees; parentage testing; gene tests.

INTRODUCTION

The last fifteen years have seen the development of microsatellite based systems to identify pedigrees in a wide range of animal species including livestock and companion animals (Dodds *et al.*, 2005). More recently a large number of QTL, and in some cases the causative gene alteration, have been discovered for an array of productive traits in livestock (Andersson & Georges, 2004). The motivation for such work is to assist farmers and breeding companies to select for improved livestock and maximise genetic gain. This paper examines, from the perspective of one major DNA genotyping service company, *Genomnz*^TM, how widespread and rapidly DNA

testing in sheep, cattle and deer has been taken up by the livestock breeding industry in New Zealand.

MATERIALS AND METHODS

The commercial DNA tests offered by *Genomnz*^TM are listed in Table 1. Although our data covers most non-dairy livestock DNA testing in New Zealand we are only a minor provider of DNA testing to the dairy industry where GeneMark™ is the major provider. Two other service laboratories have been active during this time. Commercial DNA tests are provided by Lincoln University for footrot resistance and lamb survival, and between 1999 and 2003 a DNA testing service was provided by Signagen™ which has since ceased trading.

Table 1: Combined list of DNA tests offered by the *Genomnz*^TM service laboratory.

Species	Test	Total number of samples tested up to Jan 2007	Year test introduced
Sheep	Parentage identification	277,707	2000
	Inverdale gene test	10,642	1997
	Booroola gene test	8,747	1996
Cattle	Parentage verification and identification	15,597	1996
	Myostatin gene test	332	2001
	Calpain-1 gene test	290	2004
	BLAD gene test	232	2005
	CVM gene test	276	2005
Deer	Parentage identification	59,329	1996
	Wapiti hybrid test	~5000	1996
Salmon	Parentage verification	4,287	2003