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BREEDING BUFFALOES IN GENOMICS ERA - ISSUES OF RECORDING AND EVALUATION

M. S. Khan, M. S. Rehman and F. Hassan

Department of Animal Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
Corresponding author: e-mail: drsajjad2@yahoo.com

ABSTRACT

Animal breeding is a very challenging discipline. Developments in the science of genomics are changing the face of animal breeding. While genomes of many farm animal species have either been sequenced or are in process of being sequenced, commercial companies are competing for efficiency and new innovations are being claimed every day. The DNA extracted from blood, hair or semen is genotyped for thousands of genetic markers (Single Nucleotide Polymorphisms). These represent places on the chromosomes where animals differ in the four nucleotides. With enough SNPs, association between SNP allele and quantitative alleles gives useful information for genetic evaluations. Thus estimating the genetic merit of an animal by assessing its own genetic make-up besides its own or relative's actual performance is becoming common. It is being suggested that the genomic evaluations will revolutionize breeding programs and the traditional progeny testing programs will change or may even become obsolete. Presently, SNP chips are being used for some of the *Bos taurus* dairy cattle breeds such as Holsteins and efforts are underway for other breeds to include more reference populations. Buffaloes on the other hand, are mainly raised in the developing world where capacities of the institutions for such science are limited. We have a long way to go to harvest any benefit from advancements in genomics. Reference populations will be needed to use such technologies in buffaloes and authentic performance and pedigree recording will be fundamental in these efforts. Progress in genomics and issues of implementing such a recording and genetic evaluation program in Pakistan are reviewed in this paper.

Key words: buffalo, milk, genomics, recording.

INTRODUCTION

Buffalo is generally found in Asia with small populations in other continents. How important it may be for any country, it does not fall in the "big five" category which consists of cattle, sheep, goat, pig and chicken (FAO, 2007). They have many similarities with cattle. The genetic control of economically important traits is quite similar to cattle. Most of the productive traits have a fairly good genetic control while most of the reproductive traits have a weak genetic control (Khan et al., 2007). Efforts to improve buffalo productivity however, lagged behind cattle but this also true for other genetic resources indigenous to the developing countries. Example of tangible genetic progress in any indigenous cattle or buffalo breed under a developing set up is difficult to find. List of things that are generally blamed include: poor genetic potential for traits like milk yield, small holder setup and lack of long term strategies and commitments (see Moioli et al., 2000, editors for Bled workshop proceedings). Animal recording has been limited to institutional herds and most productive buffaloes with the farmers have been excluded (FAO, 2007). Limited bull testing and very weak selection intensity impediment progress (Khan et al., 1999).

After the completion of human genome project in 2003, developments in the science of genomics have been very fast. The face of animal breeding is changing. While genomes of many farm animal species have either been sequenced or are in process of being sequenced, commercial companies are competing for efficiency and new innovations are being claimed every day. Animals are being tested for thousands of genetic markers like Single Nucleotide Polymorphisms (SNP-pronounced 'snips'), which represent places on the chromosomes where animals differ in the four nucleotides. With enough SNPs, association between SNP allele and quantitative alleles gives useful information for genetic evaluations. It is being suggested that the genomic evaluations will revolutionize breeding programs (Wiggans et al., 2009) and the traditional progeny testing programs will change or may even become obsolete (Schaeffer, 2008). Presently, SNP chips are being used for some of the *Bos taurus* dairy cattle breeds such as Holsteins, Jerseys and their crossbreds (Harris et al., 2008) and efforts are underway for other breeds to include more reference populations.

Buffaloes on the other hand are mainly raised in the developing world where capacities of the institutions for science of genomics are limited. Reference populations are needed to use such technologies in buffaloes and authentic performance and pedigree recording are fundamental in these efforts. The objective of this paper is to review the status of performance recording in buffaloes, especially in Pakistan and discuss

the issues of implementing state of the art recording and genetic evaluation program in Pakistan.

WHAT IS SNP TECHNOLOGY

An array of genetic markers viz., morphological, biochemical and DNA based has been used in various fields including animal genetics and livestock improvement. The diversification of the existing molecular markers has been an important development in the field of genetics over the past two decades (Schlötterer, 2004). A novel class of DNA markers namely SNP has recently become highly preferred in genomic association studies. The increase in the range of molecular markers partly stems from the realisation that no particular marker type is ideal for all situations, and SNP are no exception to this. SNP are single base-pair variations in DNA. The abundance of SNP in the genome makes it a powerful tool for genetic studies. Unlike micro- and minisatellites with multiple alleles, SNPs have only two alleles (biallelic). The level of heterozygosity can be increased by combining alleles of different SNPs into haplotypes. In recent times, various SNP databases have been constructed to assess the SNP data available in humans, animals and plants. SNPs can serve as genetic markers for genes of low penetrance for linkage studies in families, linkage disequilibrium in populations, and association and comparative genomic studies.

In fact, the more recent SNP concept has basically arisen from the recent need for very high densities of genetic markers for the studies of multifactorial diseases, and the recent progress in polymorphism detection and genotyping techniques. For SNP genotyping there are many techniques available. One key feature of most SNP genotyping techniques is the two-step separation: first is the generation of allele-specific molecular reaction products; and second separation and detection of the allele specific products for their identification. A very broad range of genotyping SNP techniques is now available as commercial kits.

APPLICATION OF SNP TECHNOLOGY IN ADVANCED COUNTRIES

In a recent review by Hayes et al. (2009) development in genomic selection for cattle breeding have been discussed. It was argued that there were two major developments that led to application of genomic selection in dairy cattle breeding. The recent sequencing of the bovine genome led to the discovery of many thousands of DNA markers, in the form of SNP and secondly, it was demonstrated that it was possible to make very accurate selection decisions when breeding values were predicted from dense marker data alone, using a method termed genomic selection. A prediction equation based on the SNP is first derived. The entire genome is divided into small segments, the effects of which are estimated in a reference population in which animals are both phenotyped and genotyped. In this way, the effects of all loci that contribute to genetic variation are captured, even if the effects of the individual loci are very small. In subsequent generations, animals can be genotyped for the markers to determine which chromosome segments they carry, and the estimated effects of the segments the animal carries can then be summed across the whole genome to predict the genomic estimated breeding values (GEBV). This could potentially lead to a doubling of the rate of genetic gain through selection and breeding from bulls at a very younger age through reduction in generation interval. Cost of progeny testing of bulls could be reduced tremendously.

Describing the developments in countries such as Australia, New Zealand, United States and the Netherlands, Hayes et al. (2009) presented technical details for estimating GEBVs. These are being summarized here with lesser technical details. For Australia a total of 798 Australian Holstein-Friesian bulls born between 1998 and 2003 and progeny tested were genotyped for 56,947 SNP by using the Illumina Bovine SNP50TM chip. A total of 730 of the 798 sires had greater than 90% of SNP genotyped. A total of 38,259 SNP satisfied all the SNP selection criteria. The implementation of genomic selection methodologies is more difficult if some animals have missing genotypes for some markers. The phenotypes used were deregressed Australian breeding values (ABV) for various traits and indexes. The breeding values were deregressed to remove the contribution from relatives other than daughters. To reduce the number of SNP to be considered in the prediction equations for computational tractability, the effect of each SNP in turn on each trait was tested. For each trait, the SNP that were significant at $P < 0.05$ were taken to the next stage. On the basis of the above one-SNP-at-a-time model, the significant SNP were chosen to be fitted simultaneously in another model. For some traits, all the SNP were fitted in the models for comparison. Two methods were used to derive the prediction equations. The first method used was a simple BLUP approach treating all SNP as having an effect that is sampled from the same normal distribution i.e., the effects of all SNP are assumed to be very small. The other approach was a Bayesian approach which uses the priors that many SNP are likely to have small individual effects on the trait and only a few will have a moderate to large effect.

The Bayesian method as compared to BLUP approach gave small increases in reliability for all traits except fertility, in the order of 2 to 7%. Interestingly, fitting all SNP in the Bayesian analysis, rather than preselected subsets, did not result in increased accuracy of the traits for which this was tried, and in some cases led to slightly decreased accuracy.

For New Zealand Holsteins, developments in genomic selection have been presented by Harris et al. (2008). Their reference population consisted of approximately 4,500 progeny tested bulls that were genotyped for the same SNP set as described above. To derive the prediction equations, wide range of methods including BLUP were tried. Reliabilities of GEBV for young bulls with no daughter information calculated in this way were in the range of 50 to 67% for milk production traits, live body weight, fertility, SCC, and longevity, compared with an average 34% for parental average breeding values. These reliabilities are generally greater than those achieved in the Australian data. Much larger number of bulls in the New Zealand reference population, as well as the fact that the New Zealand reliabilities were predicted rather than realized, could be the reasons. The Bayesian methods gave slightly greater (2 to 3%) reliabilities than the BLUP approach.

For US and Canadian Holsteins, VanRaden et al. (2009) reported improvement in reliabilities of GEBV for young bulls. The reference population from which the prediction equations were derived consisted of 3,576 bulls genotyped for 38,416 SNP with the Illumina Bovine SNP50TM chip. Prediction methods included a method similar to BLUP, which assumed a normal distribution for the marker effects, and a Bayesian method with a heavier tail before allowing for genes of the major effect. The calculations of GEBV, the parent average or polygenic effect from pedigree were combined with the genomic predictions by selection index to obtain the final GEBV. Averaged across traits, the GEBV had a reliability of 50%, compared with 27% from the parent average alone. Using BLUP rather than Bayesian approach gave only a slightly (1%) reduced reliability, as was observed in the Australian and New Zealand results.

In the Netherlands the reference population consisted of 1,583 bulls genotyped with a custom-made SNP chip containing 57,660 SNP, of which 46,529 SNP were used in subsequent analysis. They calculated the accuracy of GEBV by randomly dropping out 5% of the 429 bulls born between 1999 and 2003 from the reference population, calculating GEBV for these bulls, and then correlating them with the actual EBV of the bulls, which included progeny test information. This was repeated 20 times so that each bull was dropped out once and used as a reference bull in the other 19 runs. Their methodology for calculating SNP effects followed the Gibbs sampling scheme proposed by Meuwissen and Goddard (2004), implemented for single SNP rather than haplotypes (Calus et al., 2008). The increase in reliability of GEBV over parent average EBV at the time of birth was 33% (fat percentage), 19% (kilograms of protein), 15% (feet and legs), 13% (udder depth, SCS), and 9% (fertility). They concluded that having a larger number of bulls in their reference population would increase the reliability of GEBV in their selection candidates substantially.

In all these advanced countries, the reliabilities of GEBV were substantially greater than breeding values from parental averages. In all countries, the dairy cattle breeding companies are likely to take advantage of the GEBV both to improve rate of genetic gain and to reduce the cost of their breeding programs. The increase in reliability of breeding value as a result of including the genomic information was greater in the data from the United States and New Zealand than in the Australian data, most likely reflecting the large number of bulls those countries used in their reference populations. However, the method of calculating reliability of the GEBV differed between countries, making a direct comparison difficult.

A common finding was that the BLUP method, which assumes a normal distribution of marker effects, performed only slightly worse than the Bayesian methods, which use a prior allowing for genes of moderate to large effect. A conclusion from this common result would be that for most dairy traits, the assumption of the BLUP method, that there are many genes of small effect and few or none of moderate to large effect, might be close to reality. An alternative explanation might be that the SNP track large chromosome segments and that the effect of the chromosome segment is divided over many SNP. There were some individual SNP with large effects, however; for example, there is a polymorphism in the DGAT1 gene that has a large effect on fat percentage (Grisart et al., 2004), and this was detected by the surrounding SNP (VanRaden et al., 2008). In all countries, the final GEBV was calculated by combining the parental average breeding value from pedigree information with the breeding value from genomic information by using selection index theory. For example, the components could be weighted by their reliability. The advantage of using both sources of information is that any QTL not captured by the SNP effects may be captured by the parental average or polygenic breeding value. This may be particularly important to capture QTL at low frequency.

OPTIMIZING BREEDING PROGRAM DESIGN WITH GENOMIC SELECTION

As genomic selection allows prediction of accurate breeding values for young animals, it can affect breeding program to a great extent. The results from the summary of a recent review demonstrate that GEBV with very high accuracy can already be calculated for bull calves, at least for some traits. This reduces the generation interval by at least half. Further genetic gain can be made both by genotyping the elite bull dams and selecting a smaller number for mating to specific sires, and by screening very large numbers of bull calves with the markers to increase the selection intensity greatly. This can further reduce the generation interval. Schaeffer (2006) demonstrated that effect of genomic selection

might be to shift the structure of the dairy cattle breeding industry to a model similar to that used by the poultry, in which companies maintain a nucleus of elite animals. Another effect of genomic selection may be a more appropriate balance in the direction of genetic gain for certain traits like fertility. However, if small reference populations are used, the accuracy of selection on fertility will remain low. Another aspect is the impact of genomic selection on inbreeding. Reduced generation interval can potentially increase the rate of inbreeding. But it could be managed by screening a much larger number of selection candidates for bull teams to restrict the contribution of any one sire family to the selected bulls, such that inbreeding could be maintained at an acceptable level.

DEVELOPMENTS IN BUFFALO GENOMICS

Over the years a limited research progress has been seen regarding buffalo genomics. Today we have more precise information about evolutionary history and genetic variability in water buffaloes. Moreover, the construction of genetic maps and comparative mapping to find out conserved regions on the buffalo genome helped scientists to understand coding region sequences of the genes. In Pakistan few studies of Karyotyping, genetic variability or phylogenetic analyses among river buffaloes (Sajid, 2005; Abbas, 2007) have been performed in the Department of Animal Breeding and Genetics, a pioneer department in this discipline in the country. A brief update of molecular research work regarding water buffalo is summarized here.

Genetic variation among various buffalo breeds using microsatellite loci have been studied in different countries (Backer et al., 1997; Kumar et al., 2006). Those results were helpful in developing rational breeding and conservation strategies for indigenous buffalo populations. Few phylogenetic studies based on microsatellite loci indicated that *Bos taurus* and *Bos indicus* grouped first, followed by *Bos frontalis* and *Bos grunniens*. The *Bison bison* branched off next and *B. bubalis* and *S. caffer* emerged as the two most divergent species from the *Bos* clade (Ritz et al., 2000) while phylogenetic analysis based on amplified fragment length polymorphism (AFLP) fingerprinting of bovid species, including African and water buffalo, revealed three tree reconstructions: African buffalo with water buffalo, ox with zebu, and bison with wisent (Buntjer et al., 2002). The domestication of water buffalo using mitochondrial D-loop DNA sequence determined on 80 water buffaloes revealed that domestication which occurred on the Indian subcontinent about 5,000 years ago and on the South-East Asian mainland, these populations interbred with wild buffaloes and/or domestic animals from China (Kierstein et al., 2004).

In few more studies, polymorphisms in the major histocompatibility complex have been seen in water buffalo breeds (Sena et al., 2003), the complete coding region sequence of the river buffalo *SRY* gene has been determined (Parma et al., 2004), analyses of sequences and expression profiles of buffalo interleukin-12 (*IL12*) revealed significant sequence identity with bovine *IL12* and functional cross-reactivity with bovine immune cells (Premraj et al., 2006). A full-length cDNA of interleukin-18 (*IL18*) of the Indian water buffalo was determined, revealing a very similar amino acid sequence (99% and 95% identity) to cattle and sheep, respectively (Chaudhury and Bera, 2005).

The genetic maps using somatic cell hybrid panel (El Nahas et al., 1996) and FISH-mapping techniques (Iannuzzi et al., 2003) have been constructed. The first genetic map for river buffalo with only 54 loci, mostly assigned by FISH, was reported by Iannuzzi (1998). In this first genetic map, at least one bovine molecular marker was assigned to each river buffalo chromosome or chromosome arm. Improved genetic maps with 99 (El Nahas et al., 2001) and 293 (Iannuzzi et al., 2003) loci were later established. The later map included 171 type-I loci and 122 type-II loci, which were mostly microsatellites. Of the 293 assigned loci, 247 were assigned by FISH (Iannuzzi et al., 2003).

An advanced river buffalo cytogenetic map including 309 loci has also been presented. Of these loci, 186 are type-I and 124 type-II. Although some chromosome bands are still without markers, specifically along chromosomes 1q, 3q, 7, 9, 12, 21, and 24, this cytogenetic map covers all chromosomes and chromosome regions, improving our knowledge on the river buffalo genome, especially considering that a linkage map is still lacking in this species and preliminary radiation hybrid (RH) maps have only recently been performed for some river buffalo chromosomes (Amaral et al., 2007; Strafuzza et al., 2007).

The comparative mapping studies have also been performed between river buffalo and other related species (cattle, sheep, and goat), as well as between river buffalo and humans in order to detect conserved chromosome segments and syntenies. These studies revealed high levels of homology among autosomal chromosomes of bovids. Indeed, the same chromosome banding patterns and gene order among all autosomes (or chromosome arms) of cattle, river buffalo, sheep, and goat have so far been found (Iannuzzi et al., 1999, 2001; Di Meo et al., 2006).

CHALLENGES

SNP associations estimated for one population may not produce accurate estimates for another population. Harris et al. (2008) reported that SNP estimates calculated from a Holstein-Friesian reference population did not produce accurate GEBV in Jersey bulls, and vice versa. Genomic selection relies on the phase of linkage disequilibrium (LD) between markers

and QTL being the same in the selection candidates as in the reference population. However, as the two populations diverge, this is less and less likely to be the case, especially if the distance between markers and QTL is relatively large. The SNP are in LD with QTL within a breed, but the relationship does not hold across a breed. De Roos et al. (2008) analyzed the extent of LD within and between several beef and dairy breeds, and concluded that for breeds as divergent as the Holstein and Jersey, at least 300,000 SNP would be required so that markers could be discovered that would work across breeds. VanRaden et al. (2008) indicated limitations of SNP technology. Although there were appreciably gains in reliability for most traits in Holsteins, results for Jersey and Brown Swiss populations indicated prediction equations developed within one breed (such as Holstein) were not accurate when applied to genotypes from another breed because generations of recombination and drift change marker-QTL associations. It is assumed that the same mutations affecting production traits are polymorphic in different breeds which may be true for some but not for all mutations (Hayes et al., 2009). Use of a multibreed reference population may be a solution so that all the genetic variants are captured. The genotype x environment interaction may also reduce the accuracy of predicted GEBV when the chromosome segment effects are estimated from animals in another population (Hayes et al., 2009).

Now if there has to be reference populations for harvesting benefits of genomic science, where should these be constructed and what structure should they have is the question to be answered. In the absence of these populations, genomic evaluations would not be possible. Such simulation studies are missing for buffaloes. Yet, developments in dairy cattle could help in finding the direction.

Buffaloes are generally raised under smallholder set up where implementation of any genetic improvement program is not easy both due to lack of realization of importance of recording as well as need for it. Farmers know everything about their animals, although, not in comparison to other farmers. Recording of buffaloes is therefore mainly done in the institutional herds and on a few military farms. Apart from these, buffaloes at farmer level are being recorded under the progeny testing program of Buffalo Research Institute but quality and quantity needs improvement. Artificial insemination is limited to 5-10% of the buffaloes and rate of pregnancy through frozen semen is less than any satisfactory level (Khan, 2000). Experience of developing a recording setup for Kundhi buffaloes in Sindh province has been described by Ghaffar et al. (2008). Although, contractual recording was opted for this program, as opposed to a permanent structure, maintenance of accuracy and authenticity was not easy. Change in the project scope hampered the earlier attempts and therefore it may be fairy to conclude that project based initiatives are less likely to succeed. Farmers based recording set up could be an option, but this option is yet to be tried under our setup where political influences are likely to affect its success.

The option for developing the reference populations for buffaloes seems to be in expanding the recording efforts to big selected breeders and the military farms apart from the current efforts under Buffalo Research Institute. It should however, be expanded to other districts of Punjab but with breeders who are willing to participate. The guidelines to record different attributes are available for application in buffaloes (Moioli et al. 2000).

Conclusions: New developments in genomics are being practically utilized to benefit cattle farmers in advanced production set-ups. Gains in reliabilities of breeding values are appreciably high. Reduction in generation intervals reduces the cost of evaluating animals and can appreciably enhance the genetic gain. The technology therefore offers new opportunities to develop management systems to optimize the production environment based on an animal's genotype. These developments can potentially change the face of buffalo breeding. However, it requires collaborative efforts of various institutions across buffalo raising countries. The roadmap available through cattle studies can help shorten the time to achieve the sustainable utilization of buffaloes. As a first step however, reference buffalo populations (discovery populations) recorded for ancestry and performance will be needed under different production setups to harvest developments in the science of genomics for genome enabled evaluation of buffaloes.

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