

## GENOMIC SELECTION OF NILI-RAVI BUFFALO: A CHOICE FOR BUFFALO BREEDERS

**M. Moaen-ud-Din\* and G. Bilal****ABSTRACT**

Among three well documented breeds of buffalo dairy breeds in Pakistan, Nili-Ravi is the best milk producer owing to its characteristic of disease and parasitic resistance, and better convertor of roughages into useful products than cattle. A selection program to enhance the genetic potential for milk production of Nili-Ravi using progeny testing program is going on. Traditional progeny testing program has made a remarkable improvement in the genetic potential of dairy cattle in the developed world. However, this program faces severe implementation issues in buffalo improvement due to limitation of resources and basic infrastructure. Simulated studies have shown the potential of genomic selection in shortening generation interval and increasing the accuracy of selection (especially young bulls) that can bring a relatively rapid genetic improvement. The current study intends to explore the application of genomic selection in a typical buffalo breeding perspective using Nili-Ravi in Pakistan as an example. The assumed size of the training population for genomic selection was 15860 present with BRI, Pattoki. Our calculations indicated that genomic selection can reduce the generation intervals in the male to male selection pathway from 9.5 years down to 3.3 years. It can result in almost 2 times increase in response to selection compared to that in a progeny

testing program. Furthermore, it reduced the costs of proving bulls by 88%. The present study suggests the initiation of the program of genomic selection for Nili-Ravi in Pakistan and may serve as an example for other developing countries. The findings of the current study may encourage the researchers and policy makers to use the genomic selection for improvement in the productivity of dairy buffalo of developing countries.

**Keywords:** developing country, genomic selection, Nili-Ravi buffalo

**INTRODUCTION**

Pakistan is among the top five milk-producing countries in the world. It has an annual gross milk production of about 46440 thousand tons. Buffalo contribute approximately 65% of the total milk production. Pakistan possesses 30.4 million head of buffalo (GOP, 2011). In this context, there are 3 established breeds of dairy cattle i.e. Nili-Ravi, Kundi and Azakeili. These breeds are very well adapted to the harsh climatic condition of the region and have beneficial disease and parasitic resistance characteristics. As far as economic importance of Nili-Ravi is concerned; it is the best milk producing breed in world under tropics and sub-tropics harsh climatic conditions

(Khan, 2007). The breed has been used for up gradation of other breeds i.e. Chinese indigenous buffalo (Yang, 2010), Bangladeshi indigenous buffalo (Khatun *et al.*, 2009). Moreover, some cross breeding experiments have also been conducted with Mediterranean region breeds of the world i.e. Mediterranean buffalo from Italy (Yang *et al.*, 2013).

Currently, progeny testing program is underway under the umbrella of the Buffalo Research Institute, Pattoki (BRI) (Figure 1). The salient objectives of the program are to maintenance of nucleus herd of superior germ plasm of Nili Ravi through registration and documentation of institutional and private Nili Ravi farms/herds; performance recording for genetic evaluation and identification of superior germ plasm. The main breeding objective is to improve milk yield. The strategy adopted is open nucleus herd. In the nucleus herd, 5 institutional (government) and 674 private farms are included under bull mother scheme. There are 15860 registered cows and currently there are 231 registered bull with 129 bulls under progeny testing.

However, the application of traditional progeny testing program under Pakistani conditions seems challenging due to small herd size and less awareness among farmer community and breed organizations regarding pedigree and performance recording of their animals. Moreover, typical progeny testing program, as being practiced in the developed countries, requires more time and expense which appears to be less optimistic enterprise under Pakistani conditions.

(Schaeffer, 2006) presented a compelling argument of why dairy cattle breeding organizations should consider replacing conventional progeny-testing schemes with breeding schemes that use genomic selection. Therefore, the current study

intends to explore the application of genomic selection in a typical developing country situation using Nili-Ravi buffalo in Pakistan as an example.

## MATERIALS AND METHODS

### Key assumptions

The main breeding objective of the proposed genomic selection scheme is to improve milk production as envisaged in the existing progeny testing program. The main genetic parameters are heritability and repeatability of 305-day lactation milk yield. Approximately, 10,000 cows are hypothesized to be improved over the period of 15 years employing all four paths of genetic selection given in Table 1. For this purpose 1548 bulls are to be tested taken from elite herd of 15860 cows considering 7% mortality, sex ratio of 0.50 and 0.6 calving rate. This many bulls are needed to be evaluated to get high accuracy. The crossbreeding of Nili-Ravi with any other breed is almost zero; therefore, the animals included in genetics improvement program are pure breed. The key assumption regarding cost calculations include cost for purchase of bulls (approximately 2000 US\$/bull), their feeding and management cost (4120 US\$/bull), semen storage and insemination costs (11000 US\$/bull) while 250 US\$ for genotyping per animal. Finally the generation interval was calculated based on age of sexual maturity in Nili-Ravi cows (3.7 Years) and age for start of semen collection from Nili-Ravi bulls (2.5 Years) in Pakistan.

### Development of reference population

There are 15860 registered cows under BRI progeny testing program (PTP). The cows will be evaluated for EBVs based on lactation records

Table 1. Rates of genetic gain from four selection paths from traditional progeny testing vs genomic selection.

Pathway	PTP						GS					
	Selection (%)	Intensity (i)	Accuracy (rTI)	Generation Interval (L)	i · rTI	Gain ( $\Delta G/Y$ )	Selection (%)	Intensity (i)	Accuracy (rTI)	Generation Interval (L)	i · rTI	Gain ( $\Delta G/Y$ )
Sire of bulls	5	2.06	0.99	9.5	2.04	46.8	5	2.06	0.75	3.3	1.54	102.1
Sire of cows	20	1.4	0.75	8.7	1.05	26.3	20	1.4	0.75	3.3	1.05	69.4
Dams of bulls	30	2.42	0.6	6.2	1.45	51.1	2	2.42	0.75	4.5	1.82	87.9
Dams of cows	70	0.5	0.5	5.3	0.14	10.3	85	0.27	0.5	4.5	0.14	6.5
Total				29.7	4.68	134.4				15.6	4.55	265.9

and all cows will also be investigated for genomic breeding value using Axiom® Buffalo Genotyping Array lower density. Based on estimated breeding value and genomic breeding value, top 50% (7930) of cows will be selected as GS herd and will then be investigated for genomic breeding value using Axiom® Buffalo Genotyping Array 90K. Top 70% of the GS herd will be selected as an elite herd. Bull calves (N=5551) of elite cows will be genotyped using Axiom® Buffalo Genotyping Array 90K chip to validate the chip results against phenotypic records of their dams. Top 5% bull calves (N=77) of the elite cows will be included in breeding program. The bulls included in breeding program will be tested against at least 30 daughter records and best one will retain in the herd (Sire of cows). The male bull calves will be investigated for genomic breeding value using Axiom® Buffalo Genotyping Array chip and will be included in breeding program (Sire of bulls). The daughters of elite cows (N=1548) will be sent back to test herd (Figure 3). The expected accuracy for reference population is calculated based on heritability estimates of 0.30 using method referred by (Goddard, 2009).

### Testing of existing bulls

There are 129 standing bulls with SPU Qadarabad. These bulls will be tested with Bovine Axiom® Buffalo Genotyping Array 90K Chip for further breeding decision. These bulls will be selected or discarded based on all available information including GBVs.

## RESULTS AND DISCUSSION

Since the advent of genome wide association studies (GWAS studies), SNP markers

have been used extensively in evaluating human diseases (Kimura and Muramatsu, 2002; Mishiro, 2004; Yang *et al.*, 2008; Ni *et al.*, 2012). SNP markers have also been used in ecological studies of wild animals to reveal ecologically important traits (Van Bers *et al.*, 2012). These studies are further extended to improve the production traits of domestic species. SNP chip of 60 K has been developed for chicken (Groenen *et al.*, 2011), 7 K SNP chip for Atlantic salmon (Karlsson *et al.*, 2011). Finally Axiom® Buffalo Genotyping Array is available for genomic selection of buffalo (Iamartino *et al.*, 2013).

A prediction equation is estimated based on reference population phenotypic data with genomic data in genomic selection programs that is used to predict breeding values in animals without phenotype data (Borner and Reinsch, 2012). Now it is possible to decide breeding value of very young bull (at birth) with great accuracy based on parents average. This has substantial implications for the design of breeding schemes (Schaeffer, 2006). There seems to be, potentially, no need to wait for record on milk production of candidate to achieve required accuracy rather young bulls with no progeny but having genomic information combined with the parent average can be used as potential sires as they have the required accuracy (the confidence level is about 72% without any daughter record) (Schaeffer, 2006). Eventually, the development of high throughput genotyping methods and reduced genotyping cost has made the application of genomic selection feasible.

The accuracy of genomic selection is an important aspect to be considered before actually going for it. The main determinants of accuracy are effective population size ( $N_e$ ), Number of records ( $N$ ) of reference population, and heritability for the trait (Goddard, 2009). In the current scenario, there

are no proven bulls with highly accurate EBVs that can be used as a reference population as opposed to the situation in most of the developed countries (Buch *et al.*, 2012). The improvement in the accuracy of genomic predictions with increasing size of the reference population is non-linear (Goddard, 2009). (Goddard 2009) found that as the reference population size increased from 500 to 100,000 animals, the accuracy raised from 0.42 to 0.98. However, it is dependent on how accurately the phenotypic measures reflect heritability (the true breeding value) of the animals (Daetwyler *et al.*, 2008; Goddard, 2009). As the heritability estimate for milk production is moderate, therefore, accuracy estimate is likely to be reasonable (Daetwyler *et al.*, 2008; Berry *et al.*, 2011). However, relatedness is another important factor and, for the same size of reference population, the greater the relatedness of the reference population to the population predicted, the more will be the accuracy of the genomic predictions (Habier *et al.*, 2007; Habier *et al.*, 2010).

(Schaeffer, 2006) suggested replacing conventional progeny-testing schemes with breeding schemes that use genomic selection. Following the Schaeffer calculations; we modified a simple four-pathway selection model for progeny testing with accuracies predicted by (Meuwissen Th, 2001; Goddard, 2009) of 0.75 and reduced the generation intervals in the male pathways from 9.5 years down to 3.5 years slightly lower than reported in Sahiwal cattle (Moaeen ud Din *et al.*, 2013).

The time required to implement four selection paths is reduced from 29.7 in a typical progeny testing program to 15.6 years with genomic selection by virtue of reduced generation interval especially in male pathways (Table 1). Moreover, there was slight difference to Sahiwal that may be attributed to difference in generation interval

in two species (Moaeen ud Din *et al.*, 2013). The results for similar simulation studies were reported by (Berry *et al.*, 2011) for Irish dairy cattle in which generation interval was reduced from 23.75 years in progeny testing to 9.53 years for genomic selection in cattle. The difference of our values might be because of breed and species difference. We used available literature estimates on Nili-Ravi buffalo to modify the proposed scheme of (Schaeffer, 2006). The additive genetic standard deviation used was 218 kg milk based on performance data already reported for Nili-Ravi buffalo (Khan *et al.*, 2007). The estimated rates of genetic gain and relevant parameters (calculated based on existing setup of Nili-Ravi buffalo genetic improvement program sketched in Figure 1) are given in Table 1. The proposed genomic selection scheme resulted in an increase in response to selection by a factor of approximately 2.0, compared to that in a progeny testing scheme. Our results are similar to the finding of Schaeffer (Schaeffer, 2006) and those reported in Sahiwal cattle (Moaeen ud Din *et al.*, 2013).

We calculated the cost and input efforts required to implement the genomic selection in Nili-Ravi buffalo within existing progeny testing scheme. Furthermore, progeny testing (PT) was compared with genomic selection (GS) (Figure 2). The details of these calculations are given in Figure 2. Briefly these include cost for purchase of bulls, their feeding and management cost, semen storage and insemination costs. Current calculations indicated that a cost of 40333 US\$ are required to prove one bull and cost per unit deviation is 0.630 M US\$ using genomic selection whereas, the cost of traditional progeny testing using four paths of selection (not present in current selection scheme) is 342400 US\$. The cost is much higher (20.88 M US\$) if selection is practiced for only two pathways as is the case with existing Nili-Ravi breeding

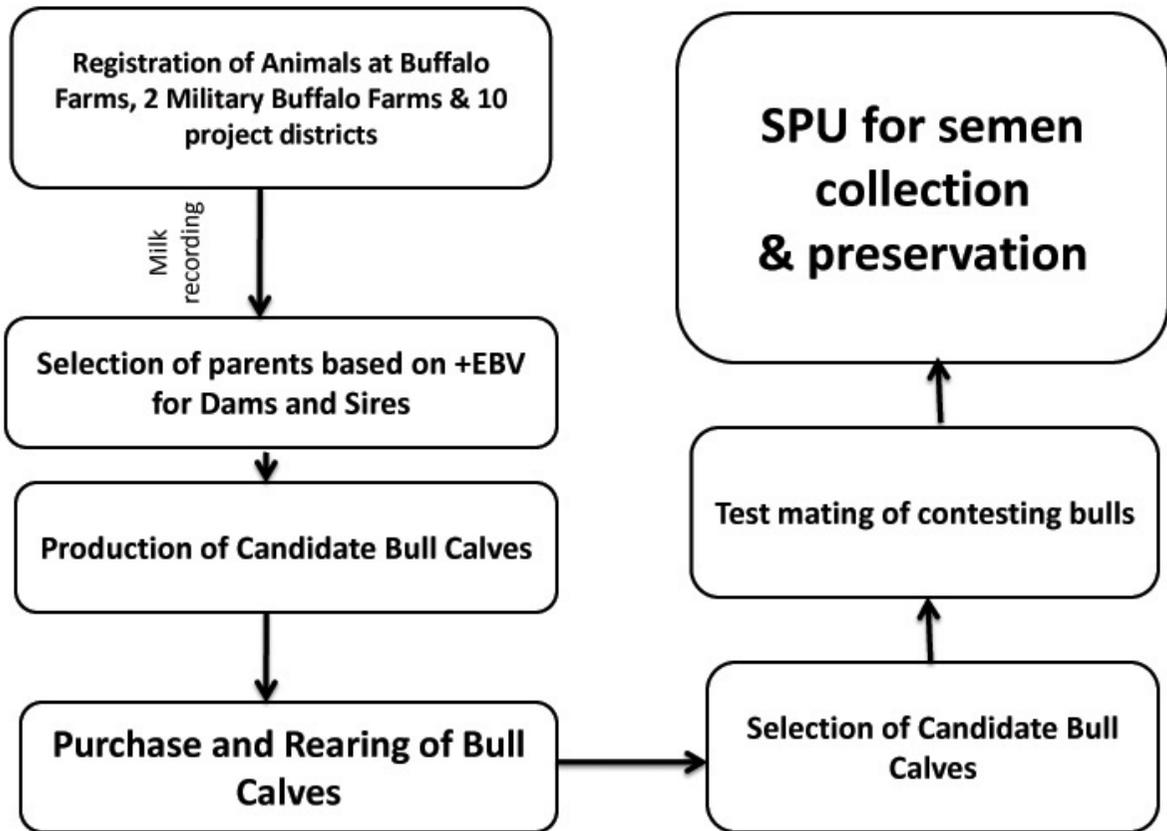


Figure 1. Progeny Testing Program underway for Nili-Ravi buffalo selection in Pakistan.

## PT VS GS in Nili-Ravi

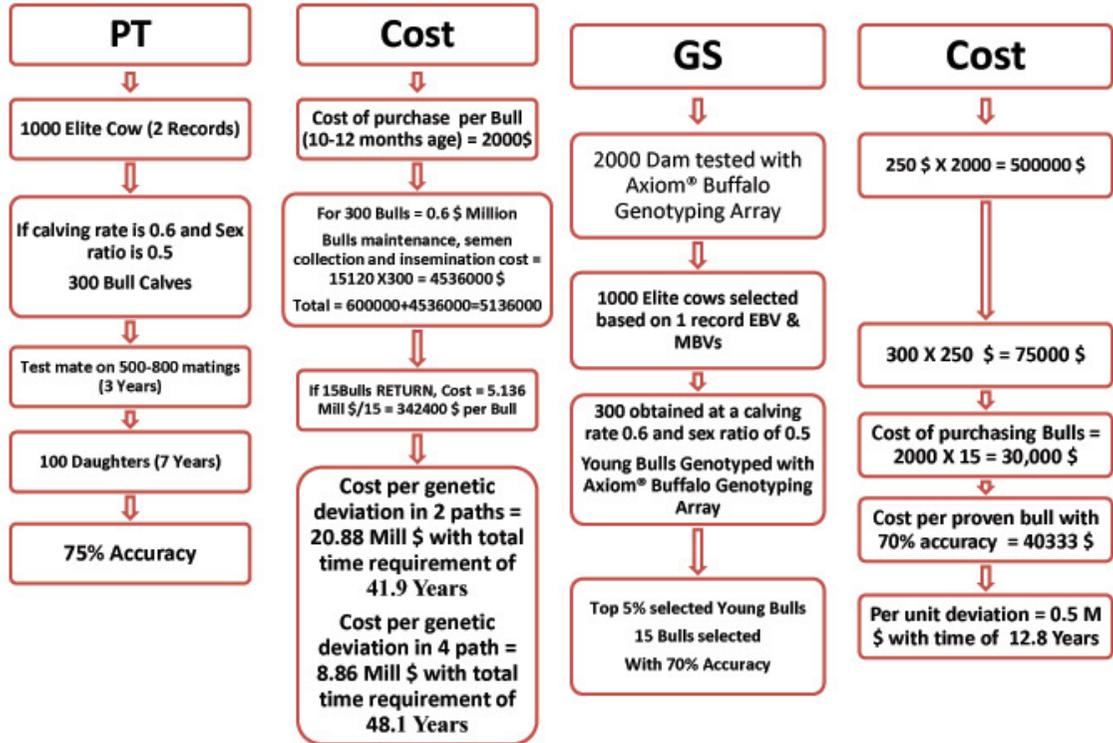


Figure 2. Progeny Testing (PT) VS Genomic Selection (GS) in Nili-Ravi buffalo.

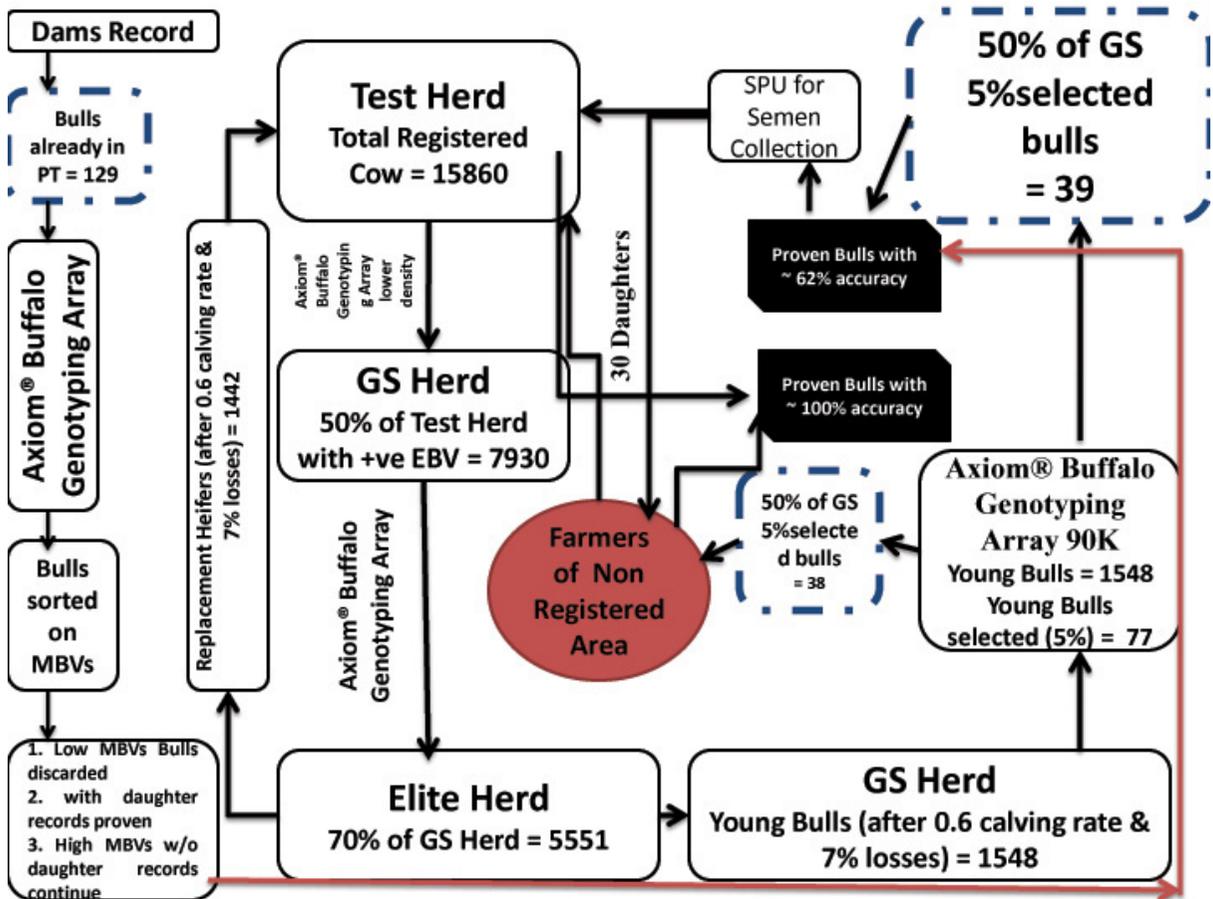


Figure 3. Genomic Selection Program for Nili-Ravi buffalo.

program (Figure 2). The use of genomic selection may potentially reduce the costs of proving bulls by 88% compared to progeny testing program. This is closer to the value reported by Schaeffer (92%) (Schaeffer, 2006) and that reported in Sahiwal (Moaeen ud Din *et al.*, 2013).

Application of genomic selection may enhance the rate of genetic gain and accuracy of selection in Nili-Ravi buffalo breed of Pakistan. Therefore, the idea of genomic selection presented in the current paper may have implications for the direction of research and policies regarding genetic improvement of dairy animals in developing countries.

#### **Potential limitations of application of GS**

As breeding scheme design under genomic selection is a new area of research so, it is especially uphill task in developing countries perspective. In the absence of a well structured reference population for genomic selection and lack of proven bulls and performance recording system, scope of genomic selection appears to be challenging under existing conditions. However, it could be a wise strategy to take advantage of genomic selection tools in a developing country situation because these are potentially expected to be more robust and less demanding as compared to tradition progeny testing program. The results from some recently published literature have shown promising effects of genomic selection on the rates of genetic gain in cattle (Hayes *et al.*, 2009; Humblot *et al.*, 2010; Pszczola *et al.*, 2011; Bouquet and Juga, 2013).

As opposed to developed countries, farmers in developing countries are less aware of the basic breeding principals, breeding schemes and technologies. There are no commercial companies involved in breeding schemes. So, it

could be a challenging task to implement genomic selection in absence of these factors. However, genomic selection coupled with current progeny testing program under government umbrella could be a better alternative to overcome the problems. It is worth mentioning that despite of the immense potential of genomic selection in improvement of dairy animals, it may not be considered an immediate alternative of traditional progeny testing program under the current circumstances. The performance recording of animals is almost always required, as suggested by the Punjab livestock breeding act 2012, for establishing and subsequent updating of reference population which is a pre-requisite of a genomic selection program.

#### **Implication**

The findings of the current study may encourage the researchers and policy makers to use the genomic selection for improvement in the productivity of dairy cattle of developing countries. The successful execution will revolutionize the genetic improvement in developing countries.

#### **REFERENCES**

- Berry, D.P., M.L. Bermingham, M. Good and S.J. More. 2011. Genetics of animal health and disease in cattle. *Irish Vet. J.*, **64**: 5.
- Borner, V. and N. Reinsch. 2012. Optimising multistage dairy cattle breeding schemes including genomic selection using decorrelated or optimum selection indices. *Genet. Sel. Evol.*, **44**: 1.
- Bouquet, A. and J. Juga. 2013. Integrating genomic selection into dairy cattle breeding programmes: a review. *Animal*, **7**: 705-713.
- Buch, L.H., M. Kargo, P. Berg, J. Lassen and

- A.C. Sorensen. 2012. The value of cows in reference populations for genomic selection of new functional traits. *Animal*, **6**: 880-886.
- Daetwyler, H.D., B. Villanueva and J.A. Woolliams. 2008. Accuracy of predicting the genetic risk of disease using a genome-wide approach. *PloS One*, **3**(10).
- Goddard, M. 2009. Genomic selection: prediction of accuracy and maximisation of long term response. *Genetica*, **136**: 245-257.
- Government of Pakistan, 2011. *Agricultural Statistics of Pakistan*. Agricultural statistics of pakistan. Statistics Division Pakistan, Bureau of Statistics, Pakistan
- Groenen, M.A., H.J. Megens, Y. Zare, W.C. Warren, L.W. Hillier, R.P. Crooijmans, A. Vereijken, R. Okimoto, W.M. Muir and H.H. Cheng. 2011. The development and characterization of a 60K SNP chip for chicken. *BMC Genomics*, **12**: 274.
- Habier, D., R.L. Fernando and J.C. Dekkers. 2007. The impact of genetic relationship information on genome-assisted breeding values. *Genetics*, **177**: 2389-2397.
- Habier, D., J. Tetens, F.R. Seefried, P. Lichtner and G. Thaller. 2010. The impact of genetic relationship information on genomic breeding values in German Holstein cattle. *Genet. Sel. Evol.*, **42**: 5.
- Hayes, B.J., P.J. Bowman, A.J. Chamberlain and M.E. Goddard. 2009. Invited review: Genomic selection in dairy cattle: progress and challenges. *J. Dairy Sci.*, **92**: 433-443.
- Humblot, P., D. Le Bourhis, S. Fritz, J.J. Colleau, C. Gonzalez, C. Guyader Joly, A. Malafosse, Y. Heyman, Y. Amigues, M. Tissier and C. Ponsart. 2010. Reproductive technologies and genomic selection in cattle. *Vet. Med. Int.*, **2010**: 192787.
- Iamartino, D., J.L. Williams, E.L. Nicolazzi, S. Biffani, F. Biscarini, R. Pasquariello, T. Sonstegard, C. Van Tassell, S. Schroeder, J. Reecy, E. Fritz, J. Knoltes, D.A.A. de Oliveira, M.G. Drummond, E. Bastianetto, A. Coletta, A. Ali and L. Ramunno. 2013. The buffalo genome and the application of genomics in animal management and improvement. *Buffalo Bull.*, **32**: 151-158.
- Karlsson, S., T. Moen, S. Lien, K.A. Glover and K. Hindar. 2011. Generic genetic differences between farmed and wild Atlantic salmon identified from a 7K SNP-chip. *Molecular Ecology Resources*, **11**: 247-253.
- Khan, M.S., F.U. Hassan, M.S. Rehman, A.U. Hyder and I.R. Bajwa. 2007. Genetic control of milk yield from lactations of different duration in Nili-Ravi buffaloes. *Archiv Für Tierzucht.*, **50**: 227-239.
- Khan, M.S. 2007. Genetic resources and diversity in dairy buffaloes of Pakistan. *Pak. Vet. J.*, **27**: 201-207.
- Khatun, M.R., M. Arifuzzaman and A. Ashraf. 2009. A comparative analysis on factors affecting calf mortality of buffalo in a breeding farm. *Pakistan Journal of Biological Sciences of Biological Sciences*, **12**: 1535-1538.
- Kimura, T. and M. Muramatsu. 2002. DNA chip and SNP. *Gan To Kagaku Ryoho*, **29**: 2573-2577.
- Meuwissen Th., M.E. 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, **157**: 1819-1829.
- Mishiro, S. 2004. DNA chip, expression profile, and SNP analyses applied for clinical gastroenterology. *Nihon Shokakibyō Gakkai Zasshi*, **101**: 121-126.
- Moaen ud Din, M., G. Bilal and H.M. Waheed. 2013. Genomic selection of Sahiwal cattle:

- A developing country perspective. *J. Dairy Sci.*, **96**: 442.
- Ni, Y., A.W. Hall, A. Battenhouse and V.R. Iyer. 2012. Simultaneous SNP identification and assessment of allele-specific bias from ChIP-seq data. *BMC Genet.*, **13**: 46.
- Pszczola, M., H.A. Mulder and M.P. Calus. 2011. Effect of enlarging the reference population with (un)genotyped animals on the accuracy of genomic selection in dairy cattle. *J. Dairy Sci.*, **94**: 431-441.
- Schaeffer, L.R. 2006. Strategy for applying genome-wide selection in dairy cattle. *J. Anim. Breed Genet.*, **123**: 218-223.
- Van Bers, N.E., A.W. Santure, K. Van Oers, I. De Cauwer, B.W. Dibbits, C. Mateman, R.P. Crooijmans, B.C. Sheldon, M.E. Visser, M.A. Groenen and J. Slate. 2012. The design and cross-population application of a genome-wide SNP chip for the great tit *Parus major*. *Mol. Ecol. Resour.*, **12**: 753-770.
- Yang, B.Z., X.W. Liang, J. Qin, C.J. Yang and J.H. Shang. 2013. Brief introduction to the development of Chinese dairy buffalo industry. *Buffalo Bull.*, **32**: 111-120.
- Yang, Y.C. 2010. Dairy buffalo breeding in countryside of China. *Ital. J. Anim. Sci.*, **6**: 25-29.
- Yang, H.H., N. Hu, P.R. Taylor and M.P. Lee. 2008. Whole genome-wide association study using affymetrix SNP chip: a two-stage sequential selection method to identify genes that increase the risk of developing complex diseases. *Methods Mol. Med.*, **141**: 23-35.