Simulating allele frequency changes in Indonesian goat crossbreeding scenarios

S Prastowo*, I F I Widowati, D M Nuraini and N Widyas

Animal Science Department, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia

Corresponding author: prastowo@staff.uns.ac.id

Abstract. This study was aimed to predict heterozygosity related to body weight (BW) trait from simulation of allele frequency changes in five crossbreeding scenario of Indonesian goat breed, namely Indonesian goat called Jawarandu (Etawah grade ♂< Kacang ♀), Boer (Boer ♂< Boer ♀), Boerja F1 (Boer ♂< Jawarandu ♀), Boerja F2 (Boer ♂< Boerja F1♀) and Boerja F3 (Boer ♂< Boerja F2 ♀). Allele frequency simulation was conducted, the heterozygosity were estimated and the associations to observed BW were measured from corresponding goat samples. BW (kg) data was retrieved from 1.5-year-old goats from the studied samples. In this study, allele that control BW trait was coded as “B” for high and “b” for low BW. The initial allele frequencies in Jawarandu were set as (B=0.45; b=0.55) and in Boer were (B=0.7; b=0.3). The simulation resulted in allele frequency in Boerja F1 were (B=0.57; b=0.43), F2 (B=0.64; b=0.36) and F3 (B=0.67; b=0.33). The estimated heterozygosity values were 0.54 0.42; 0.52; 0.47 and 0.44 in Jawarandu, Boer, F1, F2 and F3 respectively. Whereas the respective average of observed BW was 30.50±4.50; 49.50±5.90; 41.80±4.10; 41.60±3.60; and 39.80±3.20 kg. We confirmed that crossbreeding changes the heterozygosity in our simulation study.

1. Introduction

A strategy to improve local goat performance is by crossbreeding, which main benefit comes from the effect of heterosis. The occurrence of heterosis is determined by the combination of parental alleles. Crossbreeding increases heterozygosity and lowering homozygosity; hence the allele frequencies in the population will change. Indonesia has adopted crossbreeding system in order to improve the genetic status of their local livestock breeds. Crossbreeding in animal, is defined as the effort to improve local animal productivity by mate them with exotic breed. The benefit of crossbreeding mainly lies in the presence of heterosis [1,2]: the relative superiority of crossbred compared to their purebred parents [3]. The degree of heterosis is often indicated by the heterozygosity; which is the proportion of heterozygote genotypes in the population. Heterosis effect is expected from the crossbreeding method, by which the performance of its offspring(s) are relatively higher compared to the phenotype averaged from its parents.

Heterozygosity in different crossbreeding program would impact to the performance of crossbreed animals, and this is expected to improve the animal performance [4,5]. In local animal populations, normally there are still large room to improve its genetic potential either by pure line selection and/or crossbreeding program [6,7]. It is known that different allele of specific genes giving different
expression of phenotype [8,9]. As different allele expressed different protein, a different animal metabolism is the consequence, therefore different allele brings the different expression in different animal population. Allele with higher frequency tend to have more change to be inherited to the offspring, accumulated, then change the mean of traits in crossbreed population. Moreover, the interaction between allele, a mechanism of dominance and/or over dominance, would result in different expression of specific trait.

Currently, goat crossbreeding programs between Indonesian local breeds and Boer is a common strategy. Boer goat is a meat-type originated form African equipped with high growth rate and adaptable to hot tropical environment [10], diseases and has high reproductive performance as well as good in mothering ability [11]. Whereas Jawarandu is an Indonesian local breed, known as Bligon in some area, which is the descendant of Etawah grade and Kacang goats [12]. This breed is known to be robust to humid-tropical environment and also highly prolific [13]. The crossbreeding product between Boer bucks and Jawarandu does, namely Boerja [14] is potential as an improved meat-type goat breed [15,16]. Considering the aforementioned facts, this research was aimed to calculate the allele frequency changes and to estimate the heterozygosity of simulated crossbred population in Indonesian crossbreeding scenario which using female native goats and male Boer goats. The calculation will also be confirmed in real body weight (BW) phenotype data of crossbreed resulted from different crossbreeding scenario.

2. Materials and methods
The current study was divided into two parts, first a simulation to calculate allele frequency in five crossbreeding scenario using Indonesian and exotic goat, and secondly is to estimate the heterozygosity value. Since, the aim of goat crossbreeding is to produce meat-type goat, then BW trait will be an important trait to be considered. We use BW as the trait of interest within which its responsible alleles were simulated in this study. In the second part, heterozygosity values were associated with the observed BW from each crossbreed goat samples.

Five crossbreeding scenario namely Indonesian goat breed called Jawarandu (Etawah grade ♂ × Kacang ♀), Boer (Boer ♂ × Boer ♀), Boerja F1 (Boer ♂ × Jawarandu ♀), Boerja F2 (Boer ♂ × Boerja F1 ♀) and Boerja F3 (Boer ♂ × Boerja F2 ♀) were simulated to calculate allele frequency and heterozygosity. In this study, alleles that control BW trait were coded as “B” for high and “b” for low BW. The initial allele frequencies in Jawarandu goat were set up as (B=0.45; b=0.55) and in Boer were (B=0.7; b=0.3). Then, a punnet square (Figure 1) was draw to help the simulation. In this study,
the frequency of allele \( B = p \) or \( f(B) = p \) and allele \( b = q \) or \( f(b) = q \), then \( p + q = 1 \), therefore \( p^2 + 2pq + q^2 = 1 \) assuming the population is under Hardy-Weinberg equilibrium [17]. For the estimation process, a custom scripts were built in R programming language [18]. The heterozygosity (H) value was estimated by involving the frequency of both alleles with their three corresponding genotypes (BB, Bb and bb). Heterozygosity then calculated according to the following equation: \( p = P + \frac{1}{2} H \) and \( q = Q + \frac{1}{2} H \) [3]; where, \( p = f(B) \), \( q = f(b) \), \( P = f(BB) \), \( Q = f(bb) \), and \( H = f(Bb) + f(Bb) \).

In the second part, BW of crossbreed goat samples were measured at 1-1.5 y.o. The BW data was then associated to the simulated allele frequency and estimated H value.

3. Results and discussion

The allele frequency of crossbreeding scenario was tabulated in Table 1. We found that Jawarandu, the result of cross between Etawa grade and Kacang, has lowest frequency of B allele \( (p) \) and Boer has the highest one. In the other crossbreeding scenarios of Jawarandu does with Boer bucks (Boerja F1), then F2 and F3 we saw the increase trend of \( p \) and the decrease of \( q \). We saw that the higher Boer breed percentage in a crossbreed population resulted in a larger frequency of B allele (Figure 2). In H estimation, we found that Jawarandu has highest heterozygosity, while Boer has the lowest one.

| Crossbreed | p = f(B) | q = f(b) | P = f(BB) | Q = f(bb) | H = f(Bb) + f(Bb) |
|------------|----------|----------|-----------|-----------|-------------------|
| Jawarandu  | 0.45     | 0.55     | 0.18      | 0.28      | 0.54              |
| Boer       | 0.70     | 0.30     | 0.49      | 0.09      | 0.42              |
| Boerja F1  | 0.57     | 0.43     | 0.31      | 0.17      | 0.52              |
| Boerja F2  | 0.64     | 0.36     | 0.40      | 0.13      | 0.47              |
| Boerja F3  | 0.67     | 0.33     | 0.45      | 0.11      | 0.44              |

Figure 2. Allele frequency bar plot in each crossbreed scenario

We then tried to associate the change of allele frequency in different scenarios with the observed BW trait. In table 2, we saw that Jawarandu has lowest BW at 1.5 y.o, while Boer yield the highest BW. In crossbreed of Boer x Jawarandu in F1, F2 and F3 shown the improvement of BW compared to Jawarandu, however when it compared to Boer (Table 1), there is a decreasing trend. In the view of heterozygosity, the H value in Boerja F1, F2 and F3 shows a decrease trend (Table 1).
Crossbreeding between genetically different parental breed is considered as maximizing heterozygosity and, in contrast, inbreeding is reducing heterozygosity [19]. A crossbreeding scenario commonly aimed to introduce or to change the frequency of specific new gene which improve the productivity by utilizing heterosis effect [13]. Genetically, the heterosis is related to the non-additive effect which lies in dominance and over dominance mechanism. Heterosis has a positive effect because in the crossbred populations many genes are heterozygous; whereas the same genes were homozygous in the parental breeds. The amount of heterosis to be expected for a specific characteristic in a cross of two breeds depends of the number of loci involved, the differences between the two breeds and the relevant allele frequencies at these loci [20]. The effect of heterosis in crossbreeding program can be studied by simplifying the crossbreeding event into a single gene set up. The effect was then estimated by calculating the frequency or percentage of allele in a locus in a population level. Allele frequency reflecting gene(s) domination which control specific trait and value of heterozygosity. However, due to the heterosis effect is not additive component, therefore it can’t be inherited to the offspring. Heterosis mechanism mostly in the range interaction between the alleles within and/or between genes.

In this simulation study, the frequency of allele B (p and P) from Boerja F1 to F2 and F3 were showing increasing pattern (Table 1), however the H and BW means were decreasing (Table 2). The effect of heterozygosity in the crossbreeding simulation didn’t give improvement in BW. If BW trait is only affected by additive genes, we expect that we would have seen higher BW mean in a population with more B allele frequency; however, it doesn’t happen. In F2 and F3 populations where the B-allele frequencies were higher, the BW trait does not seem to be increasing. We believe that this could be an indicator that interaction(s) among alleles occurred in affecting the expression of BW trait; which by many is hypothesized as the mechanism underlying heterosis [21]. The optimal value of heterosis was obtained when the difference between parental breed is larger; or in other words when the heterozygosity is maximized [3]. We should also keep in mind that in reality BW is a polygenic trait, and there might be a different expression in the genes between individual which control BW. Moreover, environment factor also one of the components need to be considered to affect BW. Gene expression in this regard, affect to the different protein lead to different animal metabolism, then expressed in specific phenotype.

In agricultural settings, there are numerous examples in which heterosis has been harnessed to create more productive and more uniform products including livestock [22–26], but until now, its underlying mechanism is not clearly understand. Extensive genomic studies provide insights but no direct answers regarding the basis of heterosis. All modes of gene action: additivity, dominance, overdominance, and epistasis are observed at the molecular level, but the interpretation of those molecular effects to final phenotype remains complex and largely undefined [19]. Mostly, the heterosis mechanism at molecular level is relatively unknown. However, when considering the phenotype is the result of gene expression, heterosis mechanism can be related to the different of gene expression or its modification between parents vs. offspring [27]. This different in the gene expression included dominance, overdominance, and additive effects. In the modification of gene expression, qualitative or quantitative, seems the regulatory mechanism at post transcriptional level has specific role. It is known that small non-coding RNA, such as miRNA, involve in up or down regulated specific gene expression [28]. Previous study reporting the global pattern of miRNA expression in F1, F2 and their progenitor, its shown dominance and nonadditive expression pattern [8]. Another study

| Table 2. Body weight of Indonesia crossbreed goat |
|-----------------------------------------------|
| **Crossbreed** | **n (head)** | **Mean ± SD (kg)** |
| Jawarandu     | 8            | 30.50 ± 4.50      |
| Boer          | 26           | 49.50 ± 5.90      |
| Boerja F1     | 297          | 41.80 ± 4.10      |
| Boerja F2     | 168          | 41.60 ± 3.60      |
| Boerja F3     | 32           | 39.80 ± 3.20      |

Crossbreeding between genetically different parental breed is considered as maximizing heterozygosity and, in contrast, inbreeding is reducing heterozygosity [19].
also reporting the expression of miRNA in resulted hybrid might enhance gene expression, and could explain why the hybrid has better vigor compared to its parent [9].

The mating of unrelated individual often results in offspring with more vigor called heterosis. Heterosis could also be described as the phenomenon in which hybrids formed between individuals of the same or closely related species are more robust or vigorous than their parents [29]. There are two general model proposed of the way the heterosis is expressed. The first is an increase in size as the result of greater cell activities or greater of cell number, while the second way is by increase in biological efficiency in metabolism which support functions of growth, reproduction capacity, survival rate, and etc. These models, based on the two known underlying model namely dominance and over dominance [29]. In dominance mechanism, detrimental recessive alleles accumulated in the homozygous states during inbreeding, and this would reduce the vigor or inbreeding depression. The dominance hypothesis attributes the superiority of hybrids to the suppression of undesirable recessive alleles from one parent by dominant alleles from the other. Certain combinations of alleles that can be obtained by crossing two inbred strains are advantageous in the heterozygote. When inbred individuals are mated, their offspring become heterozygous at these loci and the detrimental alleles are covered by dominant alleles, thus relieving inbreeding depression and restoring vigor. Under the overdominance hypothesis, the products of heterozygous loci are more robust than those of homozygous locus. The overdominance hypothesis attributes the heterozygote advantage to the survival of many alleles that are recessive and harmful in homozygotes. It attributes the poor performance of inbred strains to a high percentage of these harmful recessives.

Heterosis has been of immense economic value in agriculture and has important implications regarding the fitness and fecundity of individuals in natural populations. Genetic models based on complementation of deleterious alleles, especially in the context of linkage and epistasis, are consistent with many observed manifestations of heterosis [19]. The amount of heterosis to be expected for a specific characteristic in a cross of two breeds depends of the number of loci involved and the differences between the two breeds in the relevant allele frequencies at these loci. It is important to emphasize that measures of heterosis are phenotype-dependent.

4. Conclusion
We confirmed that crossbreeding changes the heterozygosity value in our simulation study. Our attempt to associate this heterozygosity with BW trait in Indonesian crossbred goats yields in positive association results. For sustainable crossbreeding program, in all crossbreeding systems before animals are crossed, within line selection for the relevant traits and maintenance the pure line or breed are inevitable.

References
[1] Crow J F 2001 Heterosis Encycl. Genet. 933
[2] Oldenbroek K and Waaij L van der 2015 Textbook animal breeding. Animal breeding and genetic for BSc student. (Wageningen: Center for Genetic Resources and Animal Breeding and Genomics Group, Wageningen University and Reasearch Center, The Netherlands. Groen Kennisnet.)
[3] Bourdon R M 2014 Understanding Animal Breeding (Edinburgh Gate: Pearson Education Limited)
[4] Prastowo S, Widi T T S M T and Widyas N 2017 IOP Conference Series: Materials Science and Engineering 193 012028
[5] Buckley F, Lopez-Villalobos N and Heins B J 2014 Animal 8 122–33
[6] Widyas N, Nugroho T and Prastowo S 2017 IOP Conf. Ser. Mater. Sci. Eng. 193
[7] Nugroho T, Nurhidayati A, Ayuningtyas A I, Kustiyani C, Prastowo C, Prastowo S and Widyas N 2018 IOP Conference Series: Earth and Environmental Science 142 012010
[8] Zhou Y, Ren L, Xiao J, Zhong H, Wang J, Hu J, Yu F, Tao M, Zhang C, Liu Y and Liu S 2015 Sci. Rep. 5 13847
[9] Ding D, Wang Y, Han M, Fu Z, Li W, Liu Z, Hu Y and Tang J 2012 *PLoS One* **7** e39578
[10] Van Nickerk W A and Casey N H 1988 *Small Rumin. Res.* **1** 355–68
[11] Greyling J P C 2000 *Small Rumin. Res.* **36** 171–7
[12] Pandjono, Rusman and Budisatria I G S 2014 *Proceedings of the 16th AAAP Animal Science Congress Vol. II 10-14 November 2014* vol II (Yogyakarta: Gadjah Mada University) pp 973–5
[13] Wodzicka-Tomaszewska M, Djajanegara A, Gardiner S, Wiradarya T R and Mastika I M 1993 *Small Ruminant Production in the Humid Tropics* (Surakarta: Sebelas Maret University Press)
[14] Prastowo S, Nurhayat Y R and Widowati I F I 2019 *J. Ilmu-Ilmu Peternak.* **29** 65–74
[15] Shrestha J N B and Fahmy M H 2007 *Small Rumin. Res.* **67** 93–112
[16] Malan S W 2000 *Small Rumin. Res.* **36** 165–70
[17] Kinghorn B, van der Werf J and Ryan M 1999 *Animal Breeding Use of New Technologies* (Sidney: The Post Graduate Foundation in Veterinarian Science of the University of Sydney)
[18] R Core Team 2016 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
[19] Kaepepler S 2012 Heterosis: Many Genes, Many Mechanisms—End the Search for an Undiscovered Unifying Theory *ISRN Bot.*
[20] Waaïj K O en L van der 2014 *Animal Breeding and Genetics for BSc Student* (Netherland: Wegeningen University and Research Centre)
[21] Mackay T F C 2001 *Annu. Rev. Genet.* **35** 303–39
[22] Gama L T, Bressan M C, Rodrigues E C, Rossato L V, Moreira O C, Alves S P and Bessa R J B 2013 *Meat Sci.* **93** 98–104
[23] McDowell R E, Wilk J C and Talbott C W 1996 *J. Dairy Sci.* **79** 1292–12303
[24] Highfill C M, Esquivel-Font O, Dikeman M E and Kropf D H 2012 *Meat Sci.* **90** 881–6
[25] Gaughan J B, Mader T L, Holt S M, Josey M J and Rowan K J 1999 *J. Anim. Sci.* **77** 2398–405
[26] Prado I N, Prado R M, Rotta P P, Visantainer J V, Moletta J L and Perotto D 2008 *J. Anim. Feed Sci.* **41** 384–91
[27] Wang H, Fang Y, Wang L, Zhu W, Ji H, Wang H, Xu S and Sima Y 2015 *Sci. Rep.* **5** 8750
[28] Jonas S and Izaurralde E 2015 *Nat. Rev. Genet.* **16** 421–33
[29] Timberlake W E 2013 *Heterosis Brenner’s Encyclopedia of Genetics: Second Edition*