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To cite this article: N Widyas *et al* 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **207** 012022

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Precaution in Introducing Double-Musclled Exotic Breeds into Indonesian Cattle Population

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Abstract. Indonesian has been striving to obtain meat self-sufficiency since the later decade and the most successful program so far is crossbreeding which was able to increase the national meat production. Recent attempt is to introduce double muscling (DM) sires as the male genetic resources in crossbreeding program. Before the program is implied, this paper will present a simulation study underlying the theoretical insight concerning the utilization of DM sires. This is important because DM was an expression of muscular hypertrophy (*mh*), a specific mutation in bovine MSTN gene which down-regulate the muscle fiber disposition; and thus, has specific inheritance and expression pattern.

Mating simulation with 10 DM sires and 1000 dams showed that the first-generation offspring (F1_Batch1) will all carry one copy of *mh*-gene. Assumption that 90% of F1_Batch1 were survived to adulthood with 50:50 sex ratio is made. The frequency of normal (+/+) individuals decreased whilst the frequency of heterozygous individuals carrying *mh*-gene mutations increased overtime for discrete and overlapping generations scenarios. There were new DM individuals emerging in overlapping-generation scenario. Calves carrying *mh*-gene were larger, gestation period was longer and prone to calving difficulties. DM dams also developed narrower birth canal due to the excess of muscle mass. Introduction of DM cattle is tempting to increase meat production. If DM cattle introduction is unavoidable, precautions are to be made regarding the reproduction of carrier cows. All F1 have to be slaughtered and must not be allowed to reproduce. Otherwise the *mh*-gene mutation would spread in the population and disturb the calf production. DM introduction will pose a major threat in the Indonesian cattle population especially from reproductive aspect.

1. Introduction

Indonesian has been striving to obtain meat self-sufficiency since the later decade and the most successful program so far is crossbreeding [1,2]. It has long been offered as a faster way to achieve genetic improvement in livestock, especially in large populations such as country level. Crossbreeding practice is conducted by introducing superior exotic breeds into the currently existing genepool of a species. Our local genetic resources are more robust to tropical environment [3,4] as a mechanism of adaptation, but less superior in producing meat and/or milk; therefore, crossbreeding is seen as a promising solution for the lack of meat production problem. The implementation of such program, however, need careful assessments and considerations since it bore the potential to disturb the local cattle population.



Indonesia have been importing male meat-type *Bos taurus* for artificial insemination (AI) semen source since early 1980's. Although far from being sufficient, crossbreeding program through AI technology managed to increase the population of meat-type cattle in Indonesia and thus boosting the national meat production [5]. Inspired by this success, Indonesian government now plan to introduce Double Muscling (DM) cattle through its semen and embryo transfer (ET) [6,7]. The conventional taurine is naturally of a bigger size compared to Indonesian locals which are mostly *Bos indicus* and *Bos javanicus* [3,4]. On the other hand, the DM cattle, aside from being naturally of taurian breed, it possesses the double muscling phenotype which is an expression of muscular hypertrophy (*mh*), a specific mutation in bovine myostatin (MSTN) gene [8–10]. The presence of this mutation is indeed causing DM cattle to develop more muscle both in mass and fiber size compared to conventional cattle, but its inheritance pattern is nowhere near genetically additive. Further, there are side effects which need to be considered when a decision to include DM cattle for national commodity is made especially from the female reproduction aspect. Introduction of DM cattle is tempting to increase meat production. However, thorough studies regarding this decision are to be made to avoid the unwanted effects. We conduct a literature and simulation study on the spreading of MSTN mutation in a hypothetical cattle population along with its inheritance pattern. We try to write this article in a practical way that it is readable and understandable to all of the stake holders.

2. The genetics of double-muscling trait

The *mh* allele is the causal mutation responsible for muscular hypertrophy; which phenotype manifestation is excess of muscles in both mass and numbers [9,11,12] resulting in cattle with extraordinary body conformation. This mutation occurred naturally in the form of 11-bp deletion in the MSTN gene and can be found in the most of the taurian breed populations such as Belgian Blue, Galician blond, Charolais, Angus and Piedmontese [8,9,11–14]. The frequency of the mutant allele, however, vary with regards to the historical aspects among populations [15]. The meat type DM cattle such as Belgian Blue (BB) were originally dual purpose breed in the beginning [16,17]. Selection program in favour of DM phenotype which was accumulated for generations had created a beef type Belgian Blue homozygous for the *mh*-allele (*mh/mh*) [8,14,17].

Preceding researches reported that there were two alleles: wild type (+) and mutated (*mh*) MSTN gene involved in the expression of DM [18]. It is therefore in the populations of dual-purpose BB and Piedmontese, there were three genotypes segregated at this particular locus: *+/+* (conventional cattle), *+/mh* and *mh/mh* [16,17,19]. Owing to the fact that the major cause of DM phenotype is a mutation in MSTN gene, the inheritance pattern of this trait does not strictly follow infinitesimal model as in complex traits. Complex traits behave in an additive manner following normal distribution because their expressions were governed by very many genes [20,21]. The DM phenotype, however, is affected by a single gene, thus its expression only depends on the number of *mh* allele present in an individual.

Straight and crossbreeding experiment had been conducted using DM individuals. In Piedmontese straight-breeding, homozygous *mh/mh* and heterozygous *+/mh* were significantly heavier and produce more carcass compared to their conventional counterpart [19]. In crossbreeding experiments, however, DM-sired cattle were not always superior in term of growth traits compared to the conventional-sired ones [22,23]. Scientific articles regarding the proportion of offspring exhibiting DM traits in the breeding experiment also existed. In DM × DM mating, the proportion of calves with DM phenotype were around 77 – 86%; whereas in DM × N (normal conventional cattle) mating were around 42 – 45% [24,25]. It is also important to notify that the phenotype of the heterozygote cattle could be anywhere between normal to heavily DM [11]. MSTN is not only functioned as a major gene controlling muscle growth which mutation can cause DM phenotype. MSTN also interacted with other genes; thus, mutation in MSTN could affect the product of the epistasis and causing large variation in the cattle's phenotype [11,12].

3. Simulation design

We conducted a simulation study using custom script in R programming language [26]. In this simulation, we set up a hypothetical base population with 10 DM sires homozygous *mh/mh* and 1000 normal local dams assumed to have *+/+* genotype. All sires and dams were mated at the age of two years old; after they were sexually matured. Two scenarios were made: 1) Discrete generations which followed the assumptions that the population only consisted of base generation and the first-generation offspring, and 2) overlapping generations; where the population also consisted individuals of the second generation as the offspring of the first generation. The second generation was therefore the offspring of the first-generation after they were sexually matured with the 10 DM sires in the base population while we avoid mating between related individuals. We run the simulation for five years or five consecutive mating cycles (batch) following **Figure 1**.

A					
Y0	Y1	Y2	Y3	Y4	Y5
Base	G1 Batch 1	G1 Batch 2	G1 Batch 3	G1 Batch 4	G1 Batch 5

B					
Y0	Y1	Y2	Y3	Y4	Y5
Base	G1 Batch 1	G1 Batch 2	G1 Batch 3	G1 Batch 4 G2 Batch 1	G1 Batch 5 G2 Batch 2

Figure 1. Simulation scenarios; A) discrete generations and B) overlapping generations

In the simulation, mating system were arranged to avoid inbreeding; dams' oestrus was synchronized, mating period was planned once a year resulting in calving at considerably close time range. We assume that sex ratio was 50:50 for male and female offspring and for both sexes, 90% of the calves survived until adulthood. Annual phenotypes, genotypes and allele frequencies were estimated and visualized.

4. Results and discussions

Population structure of the simulated population is presented in **Table 1**. Following the aforementioned scenarios, we can see the pattern of population expansion from the increasing numbers of individuals in the population over years. The number of individuals by sex differed. In the base population there were 10 sires and 1000 dams. As the mating cycle carried on and we limit the mortality by 10% of each cycle, the addition of male and female individuals became a linear function of the number of individuals from the base population.

We allow the simulation to run for five mating cycles and we record the frequency of every genotype (**Figure 2**). In **Figure 2A** we present the frequencies of the all three distinct genotypes under the discrete generation scenario, whereas in **Figure 2** we presented the same information under overlapping generations scenario. It is clearly visible that the frequency of normal (*+/+*) individuals decreased whilst the frequency of heterozygous individuals carrying *mh*-gene mutations increased overtime for both scenarios. The noticeable difference between discrete and overlapping scenario was concerning the frequency of the *mh/mh* genotype or the DM individuals. The number of the homozygous DM individuals was constant in the discrete scenario because there were only mating between DM sires and normal dams yielding in 100% heterozygous *+/mh* calf crop. On the other hand, in overlapping scenario, the heterozygous individuals in the first generation and first batch (Year 1; G1 Batch 1) was already matured at Year 3. The G1 Batch 1 females were then mated with unrelated DM

sire. This scenario yielded 50% *mh/mh* calves and 50% *+/mh* calves and thus, the frequency of *mh/mh* genotype increased.

Table 1. The simulated population structures

Year	Sex	No. of Individuals	
		Discrete	Overlapped
0	F	1000	1000
0	M	10	10
1	F	1450	1450
1	M	460	460
2	F	1900	1900
2	M	910	910
3	F	2350	2350
3	M	1360	1360
4	F	2800	2552
4	M	1810	1562
5	F	3250	2956
5	M	2260	1966

The frequency of allele *mh* and + during the simulation is presented in **Figure 3A** for discrete generations scenario and **Figure 3B** for overlapping generations scenario. Due to the nature of the simulated population, the frequency of allele *mh* increased and the frequency of allele + decreased overtime in both cases. In the scenario of overlapping generations, the decrease in + allele and the increase in *mh*-allele frequencies happened more rapid compared to the earlier scenario due to the presence of new *mh/mh* genotyped individuals confirming the results in **Figure 2**.

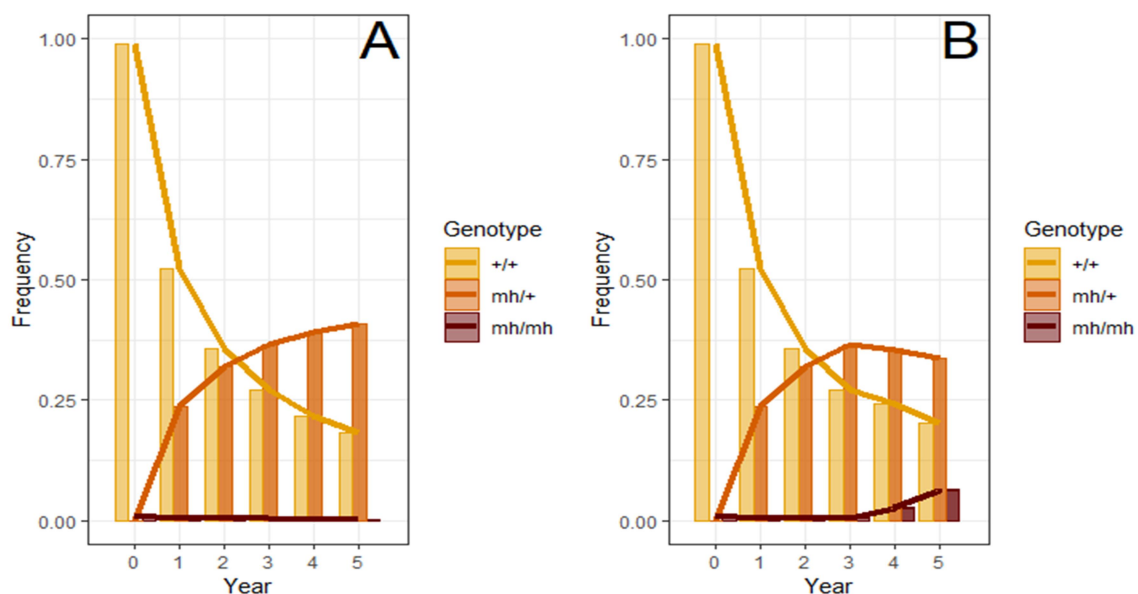


Figure 2. Genotype frequency in the simulated population: A) discrete generations and B) overlapping generations

Crossbreeding is mating between two genetically distinct breeds [20,21]. In this study we regarded the breeds as two populations: the DM population was homozygous for *mh*-allele whereas normal population only contained the + allele. The crossbreeding practice will create a new gene pool which contained both alleles and hence there will be three genotypes in the new admixed population.

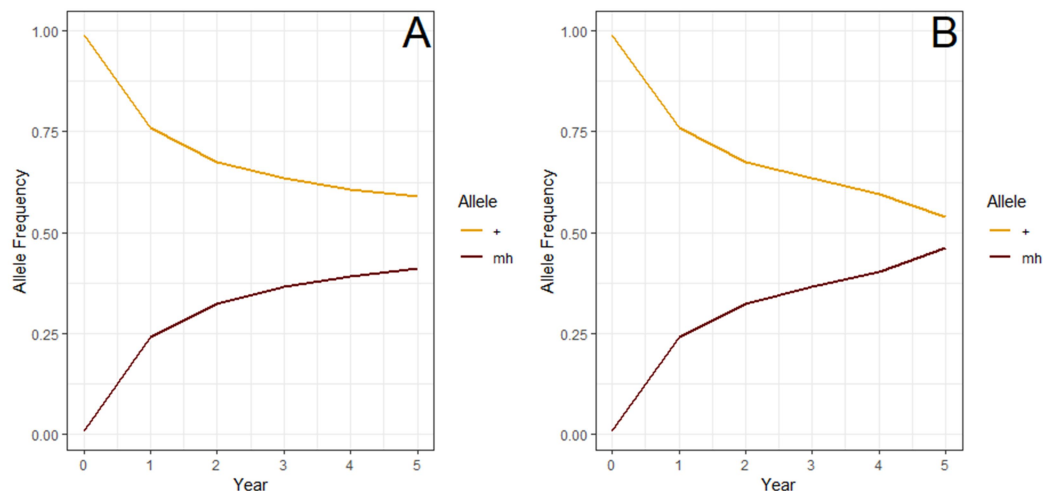


Figure 3. Allele frequency in the simulated population: A) discrete generations and B) overlapping generations

5. General Discussions

Cattle with DM phenotype produce considerably higher carcass percentage, more edible and premium cuts with lean and tender meat [12,14]. This merits however came with several physiological characteristics as summarized by Fiem et al [12] more specifically concerning the smaller skeletal systems, decreased internal organs and reduced skin weight compared to the normal cattle. These characteristics carried physiological consequences. DM cattle were prone to respiratory problems due to smaller lung and airway resistance. They have low endurance due to smaller heart and smaller digestive system [12,27].

From the reproductive aspects, DM cattle have narrower hip bone due to smaller skeletal system [12]; decreased pelvic area and pelvic opening compared to normal cattle and bigger foetal weight which often caused dystocia and perinatal mortality [12,19,28–30]. The reproduction problems were often solved by having caesarean surgery (C-section). Although the goal of C-section is to maintain future cow fertility and preserving calf life [31], C-section also brought problems to future reproductive efficiency of the cows. These problems including the prolonged calving interval as the result of longer interval from calving to first service and interval from first service to conception when compared to the cow with normal deliveries [32]. Higher calf mortality due to lower cow milk production also reported as the risk factors post C-section [33]. Previous reports mentioned that cows suffered more from post-surgery pain with side effects such as poor appetite, and uterine infection, thus reducing fertility as well as pregnancy rate [34]. Considering the poor sanitation and the fact that Indonesian smallholder farmers have less advanced relevant knowledge, post C-section risk as mentioned would be of a higher chance to occur. In addition to that, the cost for medicine and veterinary treatments increased. Therefore, instead of improving the reproductive aspects of DM cattle, this approach brought more problems such as longer calving interval, reduce in fertility, financially costly and laborious and posing welfare issues. In this regard, preserving cow fertility needs to be put as the highest priority for sustainable calf production.

From the simulation results, we can see that the crossbreeding between DM cattle and normal local cattle will produce homozygous offspring in generation 1 (G1). These cattle are potential as the meat

producer because they carry the *mh*-gene mutation which is expected to increase the muscle mass and volume and in the end the carcass percentage and quality will be improved. Results also showed that in the discrete generation scenario, G1 individuals were not allowed to reproduce and thus there will be no addition of homozygous DM individuals in the population except the 10 DM sires in the base population. This way, we can guarantee that the *mh*-mutation will not spread in the dam population. However, if the G1 individuals more specifically females were allowed to reproduce, as in the scenario with overlapping generation, there will be an increase in the number of homozygous DM individuals in the population. Based on the simulated population, by the end of five years interval, the population would consist of 33.60% heterozygote individuals of each sex; whereas the frequency of individuals homozygous for *mh* allele were 6.20 and 6.40% for female and male respectively. Earlier study mentioned that in the mating between DM sires and normal dams, 42-45% of the offspring were DM [24,25]. The results suggested that there were 33.6% heterozygote females; and 42-45% out of them (which was around 14-16%) were double muscled. Double muscled female cattle are prone to dystocia and all of the carriers were potential agents in spreading the *mh*-mutation. The inability to control the spread of *mh*-gene in the breeding population will carry reproductive consequences as mentioned before.

Lastly, we fully aware of the policy makers' expectation when the attempt to introduce DM cattle was brought to surface. The consideration to create a composite cattle breed by attempting to utilize the DM phenotype superiority, however, is an absurd idea as the expression of DM phenotype was only affected by the number of *mh* allele present and **not** by how many percent of DM parental breed proportion existed in an individual.

6. Conclusion

Creating a composite breed utilizing the superiority of DM cattle is not at all feasible. Crossbreeding program with DM cattle, anyhow, seems promising. If DM cattle introduction in Indonesian cattle population is unavoidable, precautions are to be made regarding the reproduction of carrier cows. All of F1 have to be slaughtered and must not be allowed to reproduce. Otherwise the *mh*-gene mutation would spread in the population and disturb the calf production. DM introduction will pose a major threat in the Indonesian cattle population especially on the reproductive aspect.

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