COMPARISON OF GENE EDITING VERSUS CONVENTIONAL BREEDING TO INTROGRESS THE *POLLED* ALLELE INTO THE TROPICALLY ADAPTED AUSTRALIAN BEEF CATTLE POPULATION

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SUMMARY

Breeding polled (hornless) cattle is a long-term solution to the costly and increasingly unacceptable cattle management practice of dehorning. This study simulated introgression of the *POLLED* allele into a tropically adapted Australian beef cattle population via conventional breeding or gene editing for multiple polled mating schemes and compared results to baseline selection on genetic merit using the Japan Ox Economic Index ($JapOx) alone, over the course of 20 years. Overall, our simulations show that given the limited number of polled Brahman sires, conventional breeding to increase the *POLLED* allele frequency will have to occur gradually to prevent major impacts on the rate of genetic gain. Furthermore, this study demonstrates how gene editing could help to ameliorate these impacts if a rapid decrease in *HORNED* allele frequency is required due to public pressure or legislation requiring the immediate cessation of dehorning practices.

INTRODUCTION

Dehorning is a standard cattle management practice to protect animals and humans from injury. It is an unpleasant, costly process subject to public scrutiny. Horns are inherited as an autosomal recessive trait (Long and Gregory 1978). However, the Brahman breed, which is most commonly used in extensive grazing systems in Northern Australia (Bunter *et al*. 2013), is predominantly horned. Therefore, decreasing *HORNED* allele frequency through conventional breeding strategies has been challenging (Prayaga 2007). Alternatively, the use of gene editing to produce high-genetic-merit polled sires has been proposed (Carlson *et al*. 2016). Although other genetic factors (i.e., scur and African horn) have been associated with the presence/absence of horns, these factors are believed to segregate independently so this study only modeled *HORNED* and *POLLED* alleles. The objective of this study was to simulate introgression of *POLLED* into a tropically adapted Australian beef cattle population via conventional breeding or gene editing for multiple polled mating schemes and compare to baseline selection on genetic merit, using the Japan Ox Economic Index ($JapOx) alone, over the course of 20 years (yr).

MATERIALS AND METHODS

Simulation. Geneedit.py (Cole and Mueller 2019) was used to simulate introgression of *POLLED* into the Australian Brahman population via conventional breeding or gene editing. Ten nucleus (seedstock) herds supplied bulls to 200 multiplier (commercial) herds. The seedstock base population was 15,000 cows and 40 bulls. The commercial base population was 35,000 cows and 800 bulls. True breeding values for $JapOx were determined by randomly sampling from a normal distribution, with a standard deviation (SD) of $34 for both the seedstock and commercial populations, and a mean of

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$34 for seedstock cows and $0 for commercial cows (Johnston and Graser, 2009). Base population bulls averaged 1 genetic SD higher than cows. The proportion of polled bulls was set to 30% heterozygous (Pp) and 2.6% homozygous (PP). PP bulls averaged 0.16 SD lower $JapOx than horned bulls, and the *HORNED* frequency for both base populations was set to 80% (Connors *et al*. 2018). Pre-weaning calf loss was set to 8% (seedstock) and 13% (commercial), and the dehorning mortality for both populations was 2% (Bunter *et al*. 2013).

To maintain a maximum population size of 3,000 (~1,800 breeding age) seedstock and 100,000 (~61,000 breeding age) commercial cows, cows were culled first by age (≥ 10 yr) and then at random. Both seedstock and commercial females had their first calf at age 3 and seedstock bulls were eligible for breeding at age 2. The seedstock population kept the top 5% of $JapOx 2-yr-old bulls for breeding to seedstock cows and the remainder were mated to commercial cows. To maintain a population size of 60 seedstock and 1,800 commercial bulls, bulls were culled first by age (≥ 5 yr) and then by $JapOx ranking. Ten replicates of each scenario were simulated for 20 yr, with overlapping generations as described previously (Cole 2015; Mueller *et al*. 2019).

Mating schemes. Each herd used a unique portfolio of sires and the maximum sire portfolio sizes were 6 and 10 bulls for seedstock and commercial herds, respectively. To model mating via natural service, each bull was limited to 35 matings per year and bulls within a sire portfolio were mated randomly to cows in all scenarios. Three mating schemes, 1 baseline (A) and 2 polled (B, C) were modeled. To establish a baseline and model current practice, scheme A used $JapOx as the sole sire selection criterion. In scheme B, PP bulls were preferentially selected for sire portfolios, and then both Pp and horned sires were used for the remaining sire portfolios. In contrast, in scheme C only PP bulls could be included in the sire portfolios and if the mating limit was reached then cows were left open. Scheme C models a potential situation if producers are prohibited from using sires that result in horned offspring.

Gene editing. Polled mating scheme C described above was also simulated with the addition of gene editing for polled. In these scenarios, gene editing was modeled as an added step to the elite sire production system proposed by Kasinathan *et al*. (2015), which combines the use of advanced reproductive technologies and somatic cell nuclear transfer cloning with embryo transfer. In the C-1% and C-10% scenarios, seedstock bull calves were sorted yearly on $JapOx and the top 1% or 10%, respectively, of Pp and horned bulls were cloned and then gene edited to be PP.

RESULTS AND DISCUSSION

HORNED **frequency.** The baseline scenario A did not result in a significant decrease of *HORNED* frequency in the Australian Brahman population after 20 yr (Figure 1), which is consistent with US dairy simulation results (Cole 2015; Mueller *et al*. 2019). The preferential selection of PP sires in scheme B, resulted in a significant decrease ($P \leq 0.05$) in *HORNED* frequency after 20 yr compared to baseline scheme A. However, after only 5 yr scheme C resulted in a significantly lower ($P \leq 0.05$) *HORNED* frequency (66%), than scheme B (74%). Both scenario C and C-1%, which included gene editing only the top 1% of seedstock bull calves per year, resulted in a similar ($P = 0.81$) rapid decrease in *HORNED* frequency to 10.2% after 20 yr. Additionally, scenario C-10% resulted in a slightly lower *HORNED* frequency (9.8%; $P \leq 0.05$) after 20 years than either scenario C or C-1%.
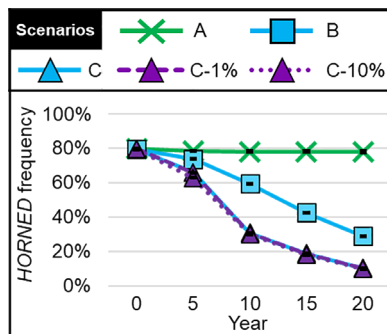

Figure 1. Average effect of each mating scenario on *HORNED* frequency. Error bars (black bars) represent SEM

Inbreeding. In all scenarios inbreeding increased less than 1% per generation. This level of inbreeding has been found to have relatively minor effects on traits of economic or biological significance in tropical beef cattle (Burrow, 1998). A limitation of the simulation is the assumption that all base population animals were initially unrelated, which is unlikely to be valid in a commercial setting.

Genetic gain. The greatest genetic gain ($JapOx) after 20 yr was achieved in baseline scheme A ($160). Selection of polled sires resulted in significantly slower ($P \leq 0.05$) rates of genetic gain ($JapOx) compared to baseline scheme A (Figure 2), which is consistent with previous findings in dairy (Spurlock et al., 2014; Mueller et al., 2019). However, the addition of gene editing to scheme C, scenarios C-1% and C-10% both resulted in significantly greater ($P \leq 0.05$) genetic gain than the polled conventional breeding scenarios B and C. Of the polled scenarios, C-10% resulted in the greatest genetic gain after 20 yr ($154), which was significantly higher ($P \leq 0.05$) than C-1% ($144). A limitation of the simulation is the assumption that true breeding values for $JapOx are known (i.e., breeding value accuracy = 1). Accuracies for cattle in commercial populations with little performance or pedigree information are likely considerably lower, decreasing the rate of gain.
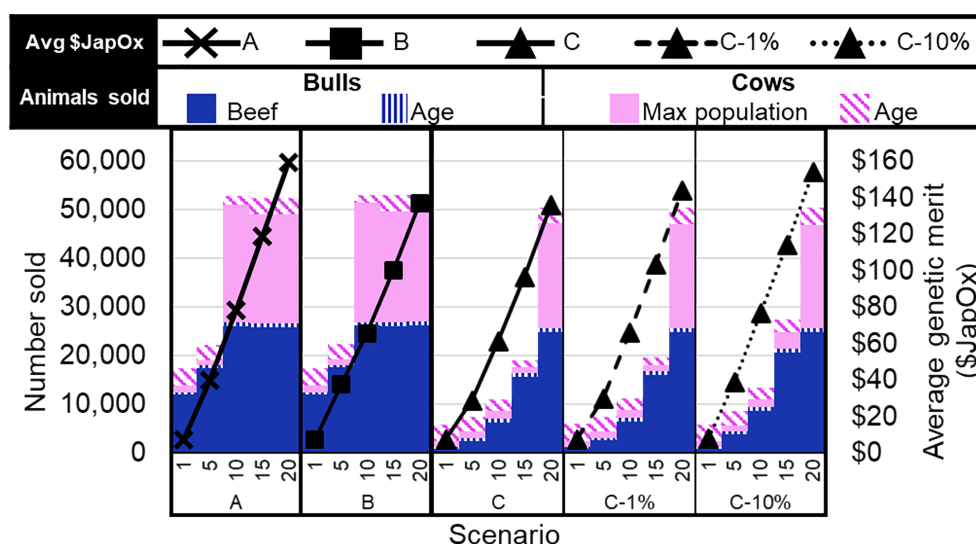

Figure 2. Average effect of each mating scenario on the number of animals sold per year by category on the primary y-axis and the average $JapOx per scenario on the secondary y-axis

Number of cows bred and animals sold for beef. After 10 yr of both scheme A (baseline) and B (preferential PP), the maximum multiplier cow population size was reached. Due to the delayed mating age (3 yr) there were ~61,000 cows bred in yr 10 and thereafter, and there were no cows left open in these mating schemes. Therefore, at maximum population size, scheme A and B resulted in ~26,000 steers sold for beef per year (Figure 2). In contrast, due to the limited number of PP sires available a significantly greater ($P \leq 0.05$) number of cows were left open yr 1 to 7 in scheme C, which resulted in a significantly smaller ($P \leq 0.05$) cow population size until yr 18. Consequently, scheme C resulted in significantly less ($P \leq 0.05$) total animals sold per year until yr 18 (Figure 2). The addition of gene editing only the top 1% of seedstock bull calves per year to this mating scheme (C-1%) resulted in similar numbers of total animals sold for beef per year. However, scenario C-10% resulted in significantly more ($P \leq 0.05$) total animals sold per year from yr 3-18 than either scenario C or C-1%. All 4 polled mating scenarios (B, C, C-1% and C-10%) resulted in significantly more (*P*

≤ 0.05) total animals sold for beef in year 20 than baseline scheme A, as a result of decreased calf loss due to less calves needing to be dehorned.

Scenarios. Preferential selection of PP sires (B) decreased *HORNED* frequency to 29% after 20 yr, whereas the obligatory use of only PP sires (C) decreased the frequency to 10% after 20 yr. The C-1% scenario, which added gene editing only the top 1% of seedstock bull calves per year to mating scheme C, resulted in similar *HORNED* frequency, genetic gain and number of total animals sold for beef per year to scenario C. However, gene editing the top 10% of seedstock bull calves per year (C-10%) resulted in significantly higher *POLLED* frequency, genetic gain and number of total animals sold for beef per year to scenario C.

Scheme C models a situation that could arise if producers are prohibited from using genetics that result in horned offspring. In this simulation cows were left open if no suitable PP sire was available. A more realistic alternative would be to use PP bulls from other less tropically adapted breeds, which could result in higher levels of mortality due to ill-adapted sires and progeny.

Regulatory considerations. Given recent developments outlined by the Australian Office of the Gene Technology Regulator (OGTR) it appears that animals modified using template-guided techniques, like the *POLLED* allele, will be regulated as genetically modified organisms (GMO) in Australia (Mallapaty, 2019). This is not the case in other countries (e.g., Brazil) and may effectively preclude the use of gene editing to introduce the *POLLED* into Australian cattle breeding programs.

CONCLUSIONS

Overall, our simulations show that given the limited number of polled Brahman sires, conventional breeding to increase *POLLED* frequency will have to occur gradually to prevent a major impact on the rate of genetic gain ($JapOx). Furthermore, this study demonstrates how gene editing could help reduce this loss if a rapid decrease in *HORNED* frequency is necessary due to public pressure or legislation ceasing dehorning practices immediately.

ACKNOWLEDGEMENTS

REFERENCES

Bunter K.L., Johnston D.J., Wolcott M.L. and Fordyce G. (2013) *Anim. Prod. Sci.* **54**: 25.

Burrow, H.M. (1998) *Livestock Production Science* **55**:227.

Carlson D.F., Lancto C.A., Zang B., Kim E.-S., Walton M., Oldeschulte D., Seabury C., Sonstegard T.S. and Fahrenkrug S.C. (2016) *Nat. Biotechnol.* **34**:479.

Cole J.B. (2015) *Genetics, selection, evolution : GSE* **47**: 94.

Cole J.B., and Mueller M.L. (2019) Geneedit.py. https://github.com/wintermind/gene-editing.

Connors N.K., Randhawa I.A.S. and Johnston D.J.. (2018) Brahman polled data. unpublished data.

Johnston D.J. and Graser H. (2009) Meat and Livestock Australia Ltd. ISBN: 9781741919202.

Kasinathan P., H. Wei T., Xiang, J.A., Molina J., Metzger D., Broek S., Kasinathan D.C., Faber and Allan M.F. (2015) *Scientific Reports* **5**: 8674.

Mallapaty S. (2019) *Nature*

Mueller,M.L., Cole J.B., Sonstegard T.S. and Van Eenennaam A.L. (2019) *J. Dairy Sci.* **102**: 4215.

Prayaga K.C. (2007) *Aust. J. Agric. Res.* **58**: 1.

Spurlock D.M., Stock M.L. and Coetzee J.F. (2014) *J. Dairy Sci.* **97**: 5265.