Breeding Focus 2016 – Improving Welfare

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Preface

The inaugural 'Breeding Focus' workshop was held in 2014 to outline and discuss avenues for genetic improvement of resilience. The Breeding Focus workshop was developed to provide a forum for exchange between industry and research across livestock and aquaculture industries. The objective of Breeding Focus is to cross-foster ideas and to encourage discussion between representatives from different industries because the challenges faced by individual breeding organisations are similar across species. This book accompanies the Breeding Focus 2016 workshop. The topic of this workshop is 'Breeding Focus 2016 - Improving welfare'.

> *"Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment."* (World Organisation for Animal Health 2008).

Animal breeding offers opportunities to improve the state of animals. Existing methodologies and technologies used in animal breeding can be used to improve welfare of animals on farm while maintaining productivity. Welfare and productivity are not necessarily in opposition because several welfare measures are genetically independent from productivity traits. Further, it is often economically beneficial to improve welfare traits. These aspects provide ample opportunities to improve both welfare and productivity through selective breeding.

The chapters of this book describe existing frameworks to define welfare of animals and outline examples of genetic improvement of welfare of farm animals. A reflection on ethical issues of animal breeding and welfare is presented and further avenues for genetic improvement of welfare are discussed.

We thank all authors for their contributions to this book and their presentations at the Breeding Focus 2016 workshop in Armidale. Each manuscript was subject to peer review by two referees. We thank all reviewers who generously gave their time to referee each book chapter. A special thank you goes to Kathy Dobos for looking after all details of organising this workshop and for her meticulous work on putting this book together.

Susanne Hermesch and Sonja Dominik

Armidale, September 2016.

Breeding polled cattle in Australia

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Abstract

Economic losses in beef cattle due to bruised meat can be largely attributed to the presence of horns. While dehorning practices can provide some economic improvement, it is more labour intensive and is likely to be subject to renewed animal welfare legislation in the future. Breeding naturally polled animals is the long term alternative to reducing economic loss while maintaining best practice animal welfare. The haplotype Poll test is aimed to estimate the Poll genetics of an animal, given the alleles observed at 10 microsatellites in the vicinity of the Poll locus on chromosome 1. The following provides a summary of the genetics of polled cattle and the test used to estimate Poll probability of beef cattle.

Introduction

Prior to the domestication/commercialisation of cattle, horns were important for animal survival; even after domestication horns were still a desired trait in most cattle breeding areas (particularly for draughting stock) until recently (Medugorac *et al.* 2012). Horned cattle in commercial scale operations (dairy and beef) are the major cause of bruising, hide damage and other injuries, particularly within yards, feedlots, and during transportation (Prayaga 2007). Currently, bruising injuries from horns is estimated to cost the Australian meat industry $30 million per year (CSIRO 2014), equivalent to approximately $4 per head at slaughter. In addition to economic costs of horned cattle, there is increased risk of injury to handlers, as cattle with horns assert more dominant behaviour and have a generally more aggressive temperament within yards (Anonymous 1974). Hence, removing the horn 'bud' (i.e. dehorning) from the animal at an early age (less than 6 months) is now commonly accepted management practice in modern cattle husbandry (Medugorac *et al.* 2012).

While common practice and commercially necessary, dehorning is a painful procedure regardless of the method used, and as such is likely to be subject to renewed animal welfare legislation in the near future (Capitan *et al.* 2009). Within Northern Australia, mustering practices often lead to calves being dehorned at up to 10 months of age, which can lead to larger wounds, longer wound healing time, and secondary infections; this may translate into short term weight loss and increased mortality rates (Prayaga 2007; Henshall *et al.* 2014). Studies into calf mortality within Australian production systems found that the incidence of calf deaths after 3 months of age was associated with the process of dehorning (Bunter *et al.* 2013). Thus,

while dehorning provides an economic improvement at the point of slaughter, it can also lead to economic loss from weight loss and mortality, along with labour costs for the process. Further to this, dehorning is generally perceived to be "treating the symptom, and not the cause", as it must be repeated for each generation (Capitan *et al.* 2009).

The alternative to dehorning of horned cattle is to breed polled cattle. Horns in cattle form as a free-floating bud, which later fuses to the skull to form as a fixed bony extension. However, horn development and morphology demonstrates significant polymorphism within the species (Medugorac *et al.* 2012). Scurs appear as small and only loosely attached horns, while polled cattle are naturally hornless (Seichter *et al.* 2012).

The genetics of polled cattle

At least three genes, Poll, Scur, and African Horn, have been associated with the presence/absence of horns, with phenotypes dependent on dominance between alleles, epistatic interactions and sex-influenced expression (Mariasegaram *et al.* 2012), though the following focuses exclusively on the Poll gene. The position of the Poll locus has been mapped to chromosome 1 (Georges *et al.* 1993) along with a series of ten microsatellite markers between 1,495,504 bp and 2,119,315 bp (Mariasegaram *et al.* 2012; Piper *et al.* 2014). These markers were identified as diagnostic for the Poll locus (POLL), though the exact gene responsible is still undetermined (Prayaga 2007). A commercial test based on a 202 base pair (bp) insertion-deletion event was developed specifically for *Bos Taurus* cattle (Medugorac *et al.* 2012). The Beef Cooperative Research Centre in Australia released a single marker DNA test for Polled in 2010, which could be applied to *Bos Indicus* cattle and their crosses. This test was based on a 303 bp allele at the microsatellite marker CSAFG29, which was strongly associated with the Polled phenotype in Brahman cattle (Mariasegaram *et al.* 2012). Pre-commercialisation testing of the CSAFG29 marker POLL test on various breeds in Australia demonstrated its limitations to assign POLL genotype accurately in some breeds. Limousin and breeds with high proportions of Angus content obtained genotype assignments with accuracies as low as ~39% (Henshall *et al.* 2014). Another allele within marker CSAFG29, of 305 bp in length, was discovered and was associated with both Polled and Horned alleles at the Poll locus (Henshall *et al.* 2011). This gave rise to an improved test, whereby two sources of the 305 bp allele at CSAFG29 are observed: one that forms a haplotype with the Horned allele at the Poll locus (prevalent in French Limousin) and one that forms a haplotype with the Polled allele at the Poll locus (prevalent in Angus) (Henshall *et al.* 2014). This newly improved test now provides POLL genotypes more accurately for more breeds, including Limousin and Angus crosses, and is based on ten microsatellite markers to form diagnostic POLL haplotypes rather than single marker results.

Summary of the diagnostic Poll haplotype test

Animal samples submitted for testing are usually hair, though previously stored DNA (e.g. semen) has been used (Henshall *et al.* 2014). Along with the sample, the animal has a designated

phenotype (Horned, Polled, Scurred, or Unknown) which can vary in its reliability/accuracy. Where available, sire samples with progeny tested Poll genotypes were included (Progeny-tested-PP or ptPP, ptPH, and ptHH) (Piper *et al.* 2014).

The haplotype diagnostic POLL test uses ten microsatellite markers identified within the Poll locus (Mariasegaram *et al.* 2012; Piper *et al.* 2014). Haplotypes from these 10 markers were estimated using the haplo.em function of the haplo.stats (Sinnwell *et al.* 2007) package in R (R Core Team 2014). Animals that could have multiple possible pairs of haplotypes were omitted. Assumptions of the phenotype based on the genotype are handled by a penetrance function to weight the estimations appropriately given there are possibilities of phenotyping errors, genotyping errors, and mislabelling errors (Piper *et al.* 2014). Assignment of haplotypes as either Horned or Polled is estimated using an MCMC sampler which applies the Metropolis-Hastings algorithm (Hastings 1970), thereby providing each haplotype with a Polled probability.

Using this method, haplotypes are assigned as Horned/Polled if they are (i) observed in Polled animals with homozygous haplotypes; (ii) observed within progeny tested animals; (iii) observed in Horned animals; or (iv) observed in Polled or Scurred animals, where the other haplotype is Horned (Henshall *et al.* 2014; Piper *et al.* 2014).

Improvements to the haplotypes Poll test

Originally, haplotype estimation was performed only once (by haplo.em) for each dataset; this led to some animals being assigned varying sets of haplotypes at each different 'batch' run, with no record of variability between runs. The MCMC sampler was then run for 600,000 iterations (with a burn-in of 600,000) over 8 chains which assigned haplotypes with a Polled probability, which was a time consuming process. Furthermore, uncommon haplotype pairs with a probability of less than 0.99 were dropped from the program and their probability of being polled was not estimated. Consequently, some animal's results would not be determined.

These issues have since been addressed to clarify uncertainty of Polled probabilities of haplotypes, and determine probable genotypes for animals that were dropped because of multiple pairs of haplotypes. First, haplotype estimation is performed for each of the 100 chains, meaning variable haplotype estimations are captured and can be considered for Polled probabilities. Those animals which received a haplotype pair probability of less than 0.99 are now re-estimated; their varied and/or multiple haplotype probabilities are accumulated over the 100 chains and the animal's mean Polled probabilities reported. This now provides more accurate estimations for those animals with multiple possible pairs of haplotypes, where they were previously not determined. Secondly, the MCMC sampler has been enhanced and is now run for 100,000 iterations (100,000 burn-in) on a single chain, which is repeated 100 times in parallel. This has significantly cut down computation time from >24 hours to less than 5 hours.

Current test statistics

The improvements above have enabled increased variability in haplotypes assigned, and increased number of animals assigned Polled probabilities. Now that animals with varied and/or multiple haplotypes are re-estimated, an additional 500 animals have had genotype probabilities assigned where previously they received no result. Furthermore, uncommon/rare haplotypes (probability <0.99) are now included in the test, increasing haplotype variability at each test run. Since implementing the changes mentioned above, the number of animals has grown from approximately 8000 to over 12000, and the number of haplotypes assigned have increased from approximately 1600 to over 1900. Despite the increase in haplotype variability, the first 200 haplotypes account for approximately 85% of assignments within the test, as seen in Figure 1; the remaining 1700 haplotypes, while only accounting for 15% of animal's haplotype assignments, provide a much more accurate assignment of Polled probability.

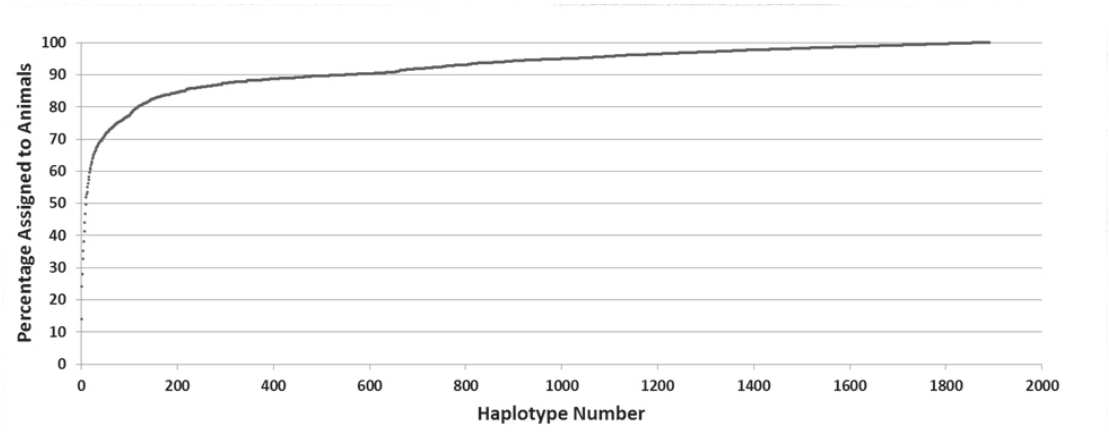

Figure 1. Current percentage of times each haplotype is assigned. NB. The first 200 haplotypes account for approximately 85% of haplotype assignments

Currently there are more than 35 breeds of animals tested, and their relative proportions can be seen in Figure 2. Brahman, Limousin, and Hereford animals make up 50% of all animals submitted thus far, which is not surprising given these breeds were targeted in the development of the test.

The various phenotypes submitted to the test are mostly 'Unknown', and approximately a quarter of all animals are submitted as 'Polled' animals, as seen in Figure 3. Less than 10% of animals are submitted as 'Horned', and less than 5% are 'Scurred'. This will have an impact on the test, as overall the phenotypes will drive haplotype Polled probabilities.

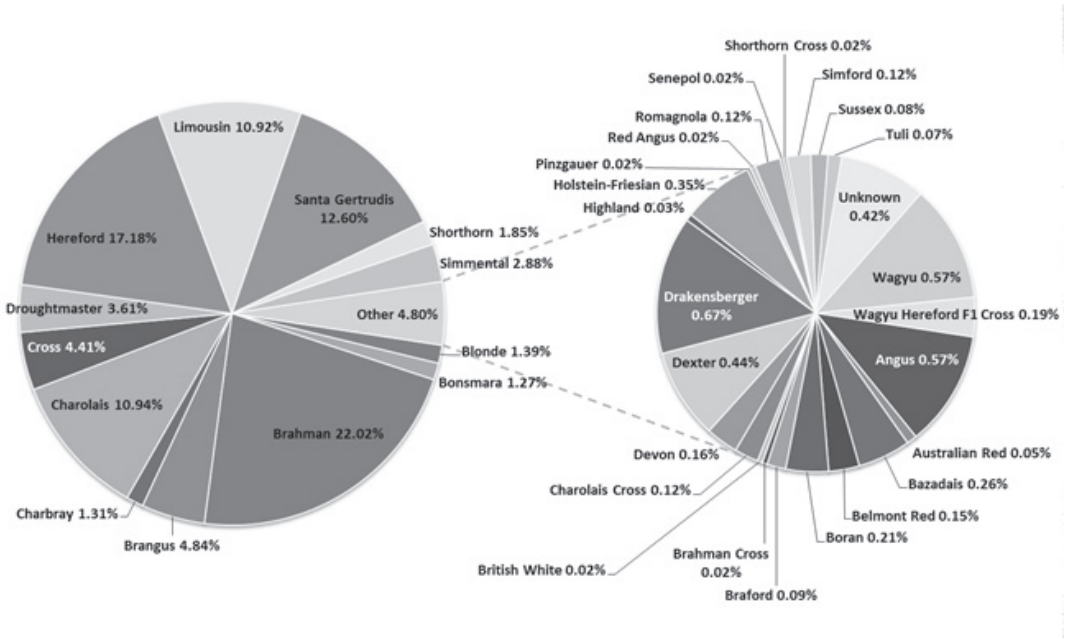

Figure 2. Current proportions of breeds included in the POLL haplotyping test

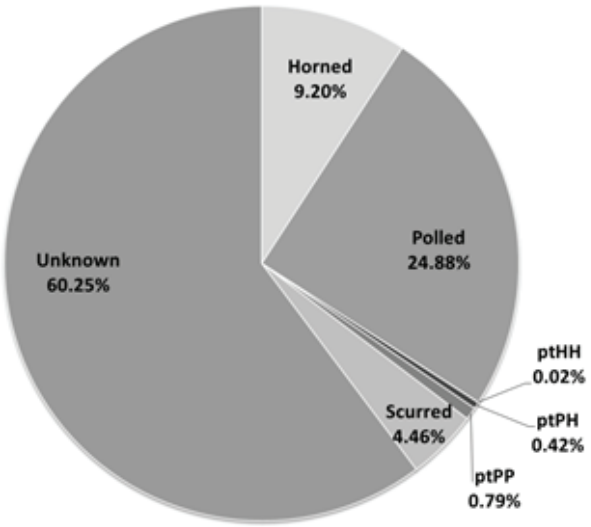

Figure 3. Current proportions of phenotypes submitted to the POLL haplotyping test

Impact on industry

The fastest way to increase the proportion of polled cattle is to exclusively use homozygous Polled bulls, ensuring all progeny will either be homozygous or heterozygous Polled (depending on the genetics of the cow/dam). The difficulty lies in determining whether a bull is homozygous or heterozygous Polled, which cannot be determined from phenotype alone and requires progeny testing. The haplotype Poll test may solve this problem, by providing an estimated Poll genotype with 98% probability. Though issues can arise when applying the test to largely untested breeds and when rare haplotypes are observed; in these cases, more data and further testing will increase the accuracy of the genotype predictions. The key to the success of the haplotype Poll test is dependent on the submission of more varied breeds and phenotypes. It is well understood that there is likely to be some ascertainment bias in the testing of animals (Henshall *et al.* 2011). Breeders are unlikely to submit horned animals as they are likely to be assigned as homozygous Horned, nor would they submit scurred animals as they are not likely to be homozygous Polled. While more commonly, a breeder will submit polled animals, seeking confirmation of a Polled homozygous phenotype for breeding or marketing purposes. The breeder is doing nothing wrong, though the cumulative effects of the biased phenotypes submitted to the test will affect the assignment of haplotypes to either Horned or Polled, and submission of animals with varied phenotypes is encouraged to enable more accurate estimation. Submission of Horned phenotypes will allow more accurate assignment of haplotypes as either Horned or Polled, which means the accuracy of the test as a whole will improve.

Conclusions

The haplotype POLL test is providing the beef cattle industry a tool with which breeders can actively and more accurately select sires of Polled genotype. This will inevitably speed up the increase in the proportion of polled cattle, and as such decrease the need for dehorning and/or economic losses from horned cattle. Future submissions of horned cattle will improve the accuracy of the test, which thus far has been dominated by Polled and Unknown phenotypes.

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