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IDENTIFICATION OF GENES HABOURING MUTATIONS AFFECTING EATING QUALITY TRAITS IN BEEF CATTLE USING BAYESIAN GENOMIC PREDICTION METHODS

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SUMMARY

Methods such as Genome-Wide Association Studies (GWAS) and Bayesian genomic prediction (BayesR) are commonly employed to enhance understanding of the genetic architecture of complex traits, identify genetic variants associated with these traits, and assist in breeding decisions and market allocation. This study aims to uncover genes harbouring variants influencing eating quality traits, including tenderness, juiciness, flavour, overall liking, and meat quality score (MQ4) in a large and diverse population of *Bos taurus indicus* cattle from Australia, the USA, and Ireland. The analysis involved 7,380 young males and females with phenotypic data and genotypes imputed up to 709,768 SNP (Illumina HD array). The BayesR approach was applied with a chain length of 40,000 iterations and a burn-in of 5,000 iterations. Notably, the findings highlight 324 SNPs with exceptionally high posterior inclusion probabilities (PIP > 0.9999 quantile for each trait), linked to 100 candidate genes. Among these, shared genetic signals across most of the traits within or close to genes such as *CAPN1*, *CAST*, *bta-mir-2407*, and *CCDC171* underscore their pivotal roles in meat quality across diverse populations. These insights contribute significantly to the global effort to enhance meat quality through genomics-driven cattle breeding programs.

INTRODUCTION

With global meat consumption projected to increase by 2030, especially in developing countries, the beef industry faces both a significant opportunity and a challenge to meet growing consumer demands. The Organisation for Economic Co-operation and Development (OECD) forecasts that this rise in demand will drive a need for more tailored beef products, opening avenues for beef consumer segmentation. Different criteria and/or perceptions are used to define meat quality (including sensory, nutritional, technological or hygienic quality (Geletu *et al.* 2021) and sustainability (Font-I-Furnols 2023)). Meat tenderness is considered the main sensory trait and is heavily influenced by consumer preferences and market demand (Warner *et al.* 2022).

Understanding the genetic basis of beef quality traits is essential for improving the efficiency and precision of cattle breeding programs. These traits are polygenic, shaped by many genes with small effects, as well as environmental factors (Arikawa *et al.* 2024), making them challenging to accurately identify and predict. The majority of studies have focused on using GWAS models to identify genomic regions associated with meat quality related traits like tenderness, important for beef quality in cattle populations (Medeiros de Oliveira Silva *et al.* 2017; Arikawa *et al.* 2024) but limited studies have utilized Bayesian methods for association analyses. Classical models, like GWAS, fit each SNP separately which can limit precision and statistical power due to multiple testing. On the other hand, Bayesian methods implicitly account for population structure and the multiple-testing problem inherent in classical single-marker GWAS (Wolc and Dekkers 2022).

This study leverages a genetically diverse dataset of *Bos taurus indicus* cattle from Australia (AUS), the USA, and Ireland (IR) to identify potential causal variants underlying eating quality traits. Results enhance understanding of genetic variation influencing beef cattle eating quality.

MATERIALS AND METHODS

Data collection. A total of 7,384 animals (3197, 1297 and 2900 from the USA, IR and AUS respectively) had 5 consumer meat eating quality traits; tenderness (TENDER), flavour (FLAVOUR), juiciness (JUICY), overall liking (OVERALL) and meat quality score (MQ4), where MQ4 is formed by weighting the four sensory scores (Watson *et al.* 2008). These records belong to 156 groups and grill cooking method. Each group had at least 5 animal records. In total across three countries there were 4552 males, 1625 steers, and 1188 females. Samples aged from 3 to 52 days with average (±SD) of 13.4 (3.1). A total of 5,532 animals were hormone growth promoter (HGP) free. Animals with carcass weights outside 2 times the interquartile range for each country were removed. Relaxed thresholds based on the distribution of 99.9% of the records were used to remove the possible outliers for 5 eating quality traits.

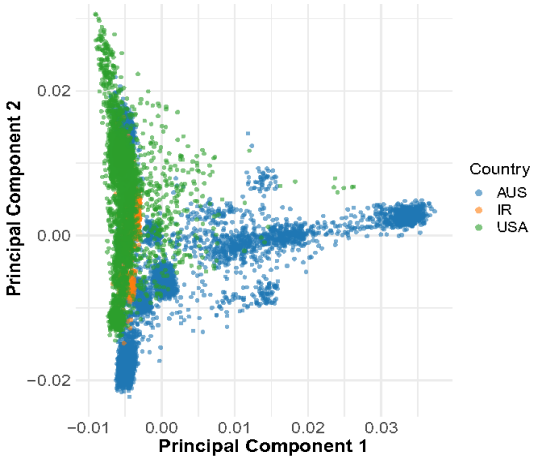

Figure 1. **Principal component analysis (PCA) results for genotyped animals** (Legends indicate the contributing countries, reflecting the genetic diversity of the dataset.)

Genotyping. Low-density genotypes from AUS (50k and 100k SNP chips) and imputed high-density genotypes from the USA and IR were available. Genotypes were imputed up to 709,768 SNPs (bovine high-density (HD) array) using findhap4 software (VanRaden *et al.* 2013). Imputation was performed separately for each country, utilizing a reference set of 4506 cattle from relevant breeds genotyped with the Bovine HD array (Hayes *et al.* 2023).

Bayesian genomic prediction. In the BayesR approach (Erbe *et al.* 2012), the following prediction equation was used to estimate the SNP effects for each of the five meat quality traits.

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Zg} + \boldsymbol{e}$$

where $\boldsymbol{y}$ is a vector of phenotypes, $\boldsymbol{b}$ includes the estimates of fixed class effects of contemporary group, SEX and HGP treatments, and covariates of days aged after slaughter, carcass weight, first 4 principal components (PCAs) from genotypes, heterosis, and country, $\boldsymbol{g}$ is a vector of $\boldsymbol{m}$ SNP effects with distribution $\boldsymbol{g} \sim \boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_i^2})$. The heterosis was defined as the regression of the trait on proportion of heterozygote loci across all loci for each animal. The genetic variance of the trait ($\boldsymbol{\sigma_g^2}$) was assumed $\boldsymbol{\sigma_i^2} = \{\boldsymbol{0}, \boldsymbol{10^{-4}\sigma_g^2}, \boldsymbol{10^{-3}\sigma_g^2}, \boldsymbol{10^{-2}\sigma_g^2}\}$. This setup allows the BayesR model to have a more flexible SNP effect distribution which is a mixture of four possible normal distributions, all with a mean of 0 but with different variances. $\boldsymbol{Z}$ is a matrix $\boldsymbol{n\ x\ m}$ of standardized genotypes and $\boldsymbol{e}$ is a vector of random residuals with $\boldsymbol{e} \sim \boldsymbol{N}(\boldsymbol{0}, \boldsymbol{\sigma_e^2})$. The posterior distributions were sampled using Marcov Chain Monte Carlo (MCMC) with Gibbs Sampling in GCTB (Zeng *et al.* 2018) with 40,000 iterations of which the first 5,000 are discarded as burn-in.

Functional analysis. We conducted a follow-up study identifying genes impacting eating quality in cattle. Top SNPs with Posterior Inclusion Probability (PIP) values above the 0.9999 quantile were used, focusing on strong associations. Genes within 10 kb of selected SNPs were analysed using *Bos taurus* ARS-UCD1.2 assembly as a reference for gene ontology enrichment analysis. Functional enrichment analysis was performed using DAVID 6.8 (Huang *et al.* 2009), with *Bos taurus* as background.

RESULTS AND DISCUSSION

Population overview. Figure 1 visualizes breed structure using PCA of imputed genotypes in GCTA (Yang *et al.* 2011b). In this figure, PC1 separates *Bos taurus* from *Bos indicus* animals, with Australian animals distributed along this axis. PC2 distinguishes different *Bos taurus* breeds, with Irish, some USA, and Australian animals lying along this axis. Animals used in this study were commercial animals and did not have an accurate breed assigned, so the labels in the figure refer to the contributing country rather than breed identities. (Figure 1).

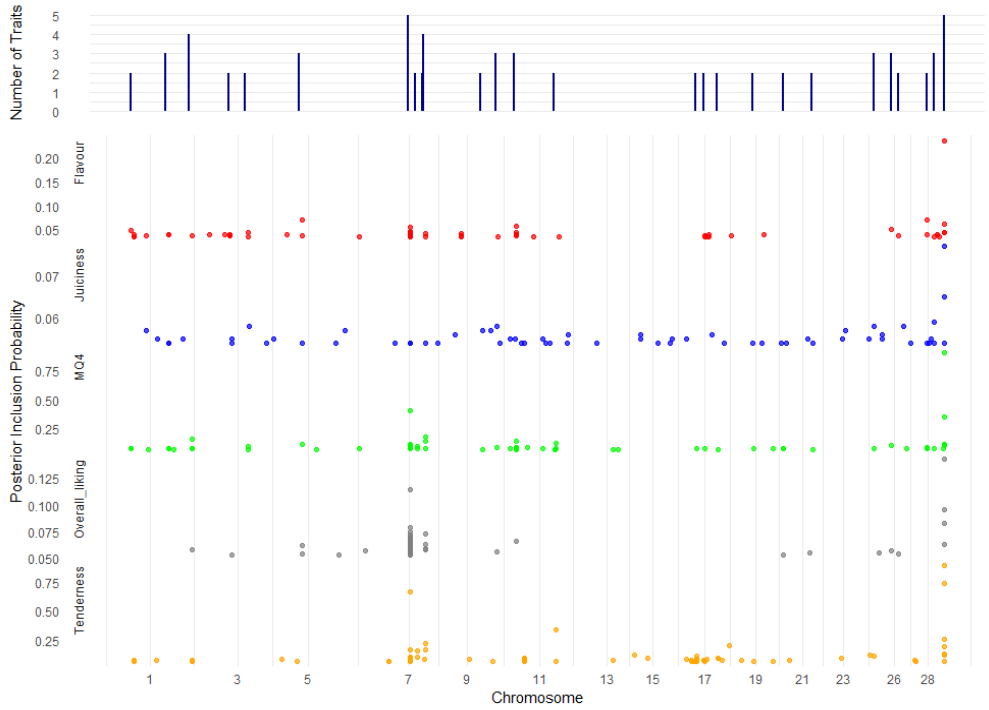

Figure 2. A bird's-eye view of the Manhattan plots showing only the top SNP (PIP>0.9999) for each of the meat quality traits. The lollipop heights in the top track are proportional to the number of traits each SNP is associated with.

Identification of potential candidate genes. Potential candidate genes were identified from top SNPs with PIP values above the 0.9999 quantile, yielding 324 SNPs across five traits. These SNPs clustered on several chromosomes, with notable associations to CAST and CAPN1 genes on chromosomes 7 and 29. Some SNPs were linked to multiple traits (Figure 2).

A total of 100 candidate genes located within a window of 10 kb upstream or downstream of the TOP-SNPs, including both novel genes and those previously reported in the literature. Among the novel candidates, *ANXA5* (Wang *et al.* 2024) and *BNC2* (Tan *et al. Unpublished data*) were highlighted for their potential roles in lipid metabolism and energy balance, contributing to marbling and tenderness. Additionally, *bta-mir-2407* was identified as a potential lipid related differentially expressed miRNAs (Li *et al.* 2022), further influencing meat quality. Several previously known genes were also confirmed, such as *CAPN1* and *CAST*, which are well-established for their involvement in post-mortem proteolysis and tenderness (Arikawa *et al.* 2024), as well as *TTLL5*, and *DOCK2,* which are recognized for their role in omega-3 and omega-6 fatty acids profile in muscle, and actin cytoskeleton remodelling, respectively (Lemos *et al.* 2016). The most strongly

enriched term identified by Gene Ontology (GO) enrichment analysis of genes associated with eating quality was "GTPase activator activity" (*p-value* < 8.0×10^{-5}).

CONCLUSION

This study provides a comprehensive overview of the genetic basis for meat eating quality traits in beef cattle by using relatively large and diverse population of *Bos taurus indicus*. The identification of novel genes, alongside the validation of established markers like *CAPN1* and *CAST*, highlights both new opportunities and robust evidence for improving eating quality through genetic selection.

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