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*Article*

# Single Nucleotide Polymorphisms in the Fatty Acid Binding Protein 4, Fatty Acid Synthase and Stearoyl-CoA Desaturase Genes Influence Carcass Characteristics of Tropical Crossbred Beef Steers

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**Abstract:** This study explored the identification of single nucleotide polymorphisms (SNP) in fatty acid binding protein 4 (*FABP4*), stearoyl-CoA desaturase (*SCD*), and fatty acid synthase (*FASN*) genes that may influence the carcass traits of tropical crossbred beef cattle. The hypothesis tested was that SNP in the *FABP4*, *SCD*, and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle. Fifty *Bos indicus* and *Bos taurus* crossbred steers were backgrounded on either buffel grass only, or buffel grass and desmanthus mixed pastures for 147 days and finished in a commercial feedlot for 110 days. Steers were slaughtered within 48 h of leaving the feedlot within a lairage period not exceeding 12 h and carcasses graded 12 h after slaughter. Next-generation sequencing of the *FASN*, *FABP4*, and *SCD* genes identified multiple SNP loci that were correlated and significantly associated with carcass traits. The *FABP4* g.44677205A>G locus was significantly associated with hump height and correlated with loin eye muscle area (EMA; $p < 0.05$). Polymorphism in the *SCD* gene g.21275851C>A locus was associated with subcutaneous fat depth and marbling score ($p < 0.05$). The CC genotype had a higher subcutaneous fat depth and marbling score ($p < 0.05$) than the AA genotype. Significant correlations were observed between carcass marbling score and subcutaneous fat depth within the *FASN* SNP locus ($p < 0.05$). Therefore, the hypothesis that SNP in the *FABP4*, *SCD*, and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle was accepted. These findings suggest that SNP in the *FABP4*, *SCD*, and *FASN* genes may be used in carcass grading and meat quality improvement through marker-assisted selection of northern Australian crossbred beef cattle.

**Keywords:** next-generation sequencing; backfat thickness; lipogenic genes; loin eye muscle area; marbling

## 1. Introduction

Observable and measurable carcass traits are important in assigning meat quality values. The postmortem measurements of subcutaneous fat depth and marbling are useful in carcass grading as indirect meat quality indicators [1,2]. Characteristics such as loin eye muscle area (EMA) and subcutaneous fat depth are indicative of the amount

of saleable carcass, while marbling score is a vital indicator of meat eating quality [3]. High beef quality standards are vital for consumer satisfaction, influencing the decision to re-purchase and maintain or increase the global market share of export-dependent beef industries [4,5]. Tropical beef cattle breeds (*Bos indicus*) are predominant in northern Australia (where over half of the Australian beef cattle herd is reared), due to their ability to adapt and survive in harsh environments characterized by poor feed quality, high ambient temperatures, and high parasite load [6]. However, tropical beef cattle have a low growth rate and produce comparatively tougher meat with lower fat content than the taurine breeds. Thus, they are crossed with the taurine breeds to improve growth rate and meat quality without compromising their ability to survive in harsh environments [7]. Therefore, the measure of tropical breed content, as determined by the hump height, is critical during carcass assessment [8].

Carcass traits are influenced by age, genetics, and management; hence, selective breeding to achieve long-term enhancement of economically important carcass traits is a relevant tool [3]. However, it is difficult to attain efficient genetic gain using traditional breeding methods since most meat quality measurements are obtained after animal slaughter, thus making it difficult to determine meat quality in a living animal [9,10]. This creates a substantial genetic lag when progeny phenotypic carcass traits are used to select breeding sires [3]. Advances in molecular genetics have led to the identification of candidate genes influencing meat quality traits [11], and single nucleotide polymorphisms (SNP) loci in genes have been evaluated for their potential use as genetic markers for marker-assisted selection aimed at improving meat quality [12,13]. The fatty acid binding protein 4 (*FABP4*), fatty acid synthase (*FASN*), and stearoyl-CoA desaturase (*SCD*) genes are among the candidate genes associated with carcass traits. The bovine *FABP4* gene encodes a cytoplasmic protein that binds long-chain fatty acids (FA) and other hydrophobic ligands [14]. The protein is involved in the regulation of lipid hydrolysis and intracellular fatty acids trafficking [14]; as a result, *FABP4* is considered a functional and positional candidate gene for fat content and distribution rate in the muscle [15,16]. Associations of SNP in the *FABP4* gene with marbling score and hot carcass weight in Holstein bulls [10], carcass weight, marbling score, and meat quality grade in Hanwoo cattle [17,18], and marbling score in Yanbian yellow cattle [19] have been reported. *FASN* encodes a multifunctional polypeptide of enzymes associated with fatty acid biosynthesis in the cytosol of animal cells [20] and is considered a candidate gene for marker-assisted selection and breeding for intramuscular fat (IMF) for improved meat quality and carcass grades [21]. Several *FASN* SNP have been associated with backfat thickness, loin eye muscle area (EMA), and IMF content in Qinchuan cattle [3] and with hot carcass weight in Beefmaster, Brangus, Bonsmara, Romosinuano, Hereford, and Angus beef cattle [22]. Polymorphisms of the *SCD* gene have been reported to be associated with marbling score, but not backfat thickness in Wagyu x Limousin cattle [23]. In Chinese Simmental cattle, SNP were associated with IMF, but not marbling score [24]. In contrast, neither backfat thickness nor IMF in Spanish commercial beef cattle [25] were associated with any SNP. Barendse et al. [16] reported that *SCD* g.2502C>G SNP was associated with muscle IMF in Angus, but not Hereford, Shorthorn, Murray Grey, Belmont Red, Brahman, and Santa Gertrudis cattle. These findings indicate that SNP-based genetic markers need to be tested and confirmed for the specific breeds of interest [26], hence the need for a targeted search for SNP in *FABP4*, *SCD,* and *FASN* genes in northern Australian crossbred tropical beef cattle and to determine their association with carcass traits. Therefore, this study aimed to detect SNP in the exons and introns of *FABP4*, *SCD,* and *FASN* genes of northern Australian tropical crossbred beef cattle and investigate their associations with carcass traits. It was hypothesised that SNP in the *FABP4*, *SCD,* and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle.

## 2. Materials and Methods

This study followed the Australian code of practice for the care and use of animals for scientific purposes [27] and was approved by the James Cook University Animal Ethics Committee (Approval Number 2639).

### *2.1. Animal Management*

Fifty tropical steers of crossbred *Bos indicus* and *Bos taurus* breeds were randomly selected and used for this study based on an a priori G-Power analysis to achieve 80% power with a 4.0 critical F-value for a large effect size and $\alpha$ of 0.05. Steers were backgrounded on buffel grass only or buffel grass and desmanthus pastures for 147 days as described previously [28] and finished in a commercial feedlot for 110 days. The steers were managed and transported according to approved Meat Standards Australia (MSA) protocols [29] and slaughtered in a commercial abattoir within 48 h of leaving the feedlot with a lairage period not exceeding 12 h.

### *2.2. Carcass Evaluation*

Carcasses were graded according to the Aus-Meat and MSA (Meat Standards Australia) grading standards [30] by an accredited meat grader at a commercial export-licensed abattoir in Queensland, Australia. The traits of interest were hot standard carcass weight (HCW), hump height, marbling score, subcutaneous rib fat (back fat) thickness, rump fat thickness at the P8 site, eye muscle area (EMA), meat colour, and MSA index. Carcass assessment was carried out at the 12th–13th rib interface 12 h after slaughter. The hump height, an indicator of tropical breed content of the carcass, was measured at the position of the greatest hump width by placing a ruler parallel to the surface of the sawn chine perpendicular to the first thoracic vertebrae and recorded in millimetres. Carcass marbling indicates the level of intramuscular fat content and was scored using the 100 (devoid) to 1100+ (abundant) reference standards, while subcutaneous fat thickness at the 12th rib and P8 site were measured in millimetres using a ruler grid. The EMA was the *M. longissimus dorsi* surface area at the 12th rib calculated in square centimetres and measured using a plastic grid. The meat colour was scored at the bloomed loin eye muscle using the 1 (light) to 7 (dark) scale colour reference standards. The MSA Index was calculated as the sum of the predicted eating quality scores for 39 MSA cuts weighted by their relative proportion of total carcass weight.

### *2.3. Blood Collection and Genomic DNA Extraction*

Blood was collected at the start of the backgrounding phase via caudal venipuncture into 4 mL EDTA-containing vacutainer tubes (BD, Sydney, Australia) and stored at −80 °C prior to analysis. Blood samples were later thawed at room temperature and an aliquot of 2 mL was used for genomic DNA extraction using NucleoSpin Blood Kit (Macherey-Nagel GmbH and Co. KG, Duren, Germany) according to the manufacturer's protocol. DNA yield and purity were quantified using a NanoDrop ND-1000 (Thermo Fisher Scientific Australia Pty Ltd., Victoria, Australia) and diluted to 50 ng/µL with nuclease-free water.

### *2.4. Primer Design*

All primers were designed using the Geneious Prime software version 2020.2.5 (Biomatters Ltd., Auckland, New Zealand) and synthesised at Integrated DNA Technologies Pte Ltd. (Queenstown, Singapore). *Bos taurus* (Hereford) sequences of *FASN*, *FABP4*, and *SCD* gene were obtained from the National Center for Biotechnology Information database (GenBank) and used as references. The 15 kb *SCD* sequence (NC_ 037353.1) was split into three fragments and the 18 kb long *FASN* (NC_ 037346.1) sequence was split into two overlapping fragments, while the 4 kb *FABP4* (NC_ 037341.1) gene was not fragmented before amplification (Table 1, Supplementary Figure S1).

**Table 1.** Primer sequences for target gene amplification.

| Gene [1] | Fragment | Primer | Sequence (5′ to 3′) | Annealing Temp (°C) | Product Size (bp) |
|---|---|---|---|---|---|
| *SCD* | 1 | Forward | GGAAGAAGACATCCGCCCTGAAAT | 60 | 5092 |
| | | Reverse | AGGAAGCGAGATTGGCACTGTATG | 60 | |
| *SCD* | 2 | Forward | GGAAGAAGACATCCGCCCTGAAAT | 60 | 10,177 |
| | | Reverse | TGCCTCTGAGGGGATCTATTTGGT | 60 | |
| *SCD* | 3 | Forward | ATGAGCCACACTGTGAACAAACCT | 60 | 2861 |
| | | Reverse | TTCTTTTTCTGGACAGGCAAGCCT | 60 | |
| *FASN* | 1 | Forward | TTGAGCTTCTGAGTATGATGGGAG | 68 | 7302 |
| | | Reverse | ACCATCTATTATGCCTCCCTCAAC | 68 | |
| *FASN* | 2 | Forward | CTATAAGATCGGTGAGTCCTTGCA | 68 | 8648 |
| | | Reverse | TAGTATTATTCACAGCTCCCTGGC | 68 | |
| *FABP4* | - | Forward | GCTAAGACTGCCTGTATGTTCCCC | 60 | 3041 |
| | | Reverse | ACCTAGAGAAATAGACAATCGCCC | 60 | |

[1] *SCD*, stearoyl-CoA desaturase; *FASN*, fatty acid synthase; *FABP4*, fatty acid binding protein 4.

### 2.5. Target Gene Amplification

Gene amplification, library preparation, normalization, sequencing, and data analysis procedures were carried out as described previously [31]. In brief, three diverse long-range polymerase chain reaction (PCR) approaches were tested to amplify the *SCD*, *FASN*, and *FABP4* genes, and the optimum combinations were selected. Platinum SuperFi PCR Master Mix and Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Scoresby, Australia) were used to amplify the *FABP4* and *SCD* genes, respectively, under similar PCR conditions. The amplification reactions were executed in a SimpliAmp Thermal Cycler (Thermofisher Scientific, Scoresby, Australia) in a total volume of 50 µL consisting of 25 µL of PCR master mix, 100 ng of DNA template, and 0.5 µM of each primer in a 3-step procedure. The conditions were as follows: single initial denaturation at 98 °C for 1 min, 35 cycles of denaturation, annealing, and extension at 98 °C for 15 s, 60 °C for 15 s, and 72 °C for 9 min, respectively, followed by a final extension at 72 °C for 9 min and a 4 °C hold. The *FASN* gene fragments were amplified using PrimeSTAR GXL Master Mix (TaKaRa Bio Inc., Kusatsu, Shiga, Japan) in a 2-step protocol. The amplification reaction mix consisted of 1.25 units of polymerase, 10 µL of 5× buffer, 0.2 µM of each primer, 200 µM of dNTP mixture, and 100 ng of DNA template in a total volume of 50 µL. The amplification reaction conditions included initial denaturation for 1 min at 98 °C and 30 cycles of denaturation and annealing combined with extension at 98 °C for 10 s and 68 °C for 10 min, respectively. The amplification products were visualised using gel electrophoresis in 0.8% agarose gel to evaluate success of the assay.

### 2.6. PCR Products Clean-Up

All PCR products were cleaned using the Sera-Mag SpeedBeads in a Zephyr NGS Workstation (PerkinElmer, Waltham, MA, USA) and quantified using the Promega dsDNA Quantifluor System Kit (Ref: E2670, 00002484139) on the PerkinElmer Enspire Workstation. The six amplification products from the three genes were pooled at 0.4 nM, normalised to 2 ng/µL and then diluted to 0.2 ng/µL with 10 mM Tris-HCl at pH 8.0 for library preparation.

### 2.7. Library Preparation and Sequencing

Libraries were prepared with the Nextera XT DNA Library Prep kit (Illumina, CA, USA) according to the manufacturer's instructions. Libraries were purified using 0.6× Sera-Mag SpeedBeads (Merck KGaA, Darmstadt, Germany) and washed twice with 80% ethanol to remove unincorporated adapters and fragments shorter than 250 bp length. The DNA libraries were analysed for fragment size and concentration using Agilent High Sensitivity D5000 reagents and ScreenTape on the Tape Station 4200 (Agilent Technologies, Santa Clara, CA, USA) and further concentration determined using the QuantiFluor® dsDNA

System (Promega, Madison, WI, USA). The fragment size and concentration data were used to normalise each library to 2 nM with 10 mM and pH 8.5 Tris-HCl, and samples of the different steers were then pooled together. A 10 pM input of the pooled samples and 10% PhiX spike-in were used for sequencing on the Illumina MiSeq benchtop sequencer with a 500-cycle MiSeq Reagent Nano Kit v2 (Illumina, Inc, San Diego, CA, USA).

### *2.8. Sequence Data Analysis and Calculations*

Sequence data were downloaded from the Illumina Dashboard-BaseSpace Sequence Hub (https://basespace.illumina.com/dashboard, accessed on 27 July 2021) and analysed using the Geneious Prime software with NC_ 037353.1, NC_ 037346.1 and NC_ 037341.1 as the *SCD*, *FASN,* and *FABP4* reference sequences, respectively. The retrieved reads were trimmed, and adapters were removed using the BBDuk trimmer. The Phred quality score was set at 20 to increase the probability of calling true SNP to 99%. All short reads of 20 bp or less were discarded and low-coverage regions were excluded during SNP calling. The allele and genotype frequencies were determined by direct counting, while the polymorphism information content (PIC) was determined using the GDIcall online calculator (http://www.msrcall.com/Gdicall.aspx) (accessed on 11 January 2022). Hardy–Weinberg equilibrium (HWE) and expected heterozygosity (He) were calculated as described previously [32].

### *2.9. Statistical Analysis*

Statistical data analyses were carried out using the R software version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Initial summary statistics were computed for means, standard deviations, distribution, and range to identify any outliers. The chi-square test was used to determine if the SNP were in HWE. Distance-based hierarchical clustering of the SNP loci was used to examine the degree of linkage disequilibrium between each pair of loci [33] and the results were presented as dendrograms and heatmaps. Spearman's correlation analysis was used to estimate the strength of relationships between genomic variants and carcass traits. Linear models were fitted to investigate whether a gene SNP was associated with carcass traits using generalized least squares linear procedures to compute group means, while significant differences between least-square means were compared using the Tukey-adjusted multiple comparisons test. Differences were declared significant at $p < 0.05$.

## 3. Results

### *3.1. Genetic Variants and Population Diversity*

In a targeted evaluation of the associations between SNP loci in the *FABP4*, *SCD,* and *FASN* genes with chiller-assessed carcass traits of tropical crossbred beef cattle, a total of 65 SNP loci were identified. These comprise 11, 27, and 27 SNP for the *FABP4*, *SCD,* and *FASN* genes, respectively (Supplementary Table S1). Twenty-eight SNP were considered novel since they were not found in the Bovine Genome Variation Database (BGVD) (http://animal.nwsuaf.edu.cn/code/index.php/BosVar, accessed on 28 January 2022). Only seven SNP were predicted to be non-synonymous amino acid substitutions (Table 2). The SNP were in HWE except for g.50786977A>G and g.50790973C>A in the *FASN* gene (Supplementary Table S1; $p \leq 0.04$). The MAF, He, and PIC for the SNP ranged between 0.11–0.47, 0.20–0.49, and 0.18–0.37, respectively, for all three genes. Correlation coefficients were used to measure the degree of linkage disequilibrium between each pair of loci depicted in Supplementary Figures S2–S4 and showed the presence of local patterns forming four clusters for each gene.

**Table 2.** *FABP4*, *SCD*, and *FASN* gene polymorphisms, protein-coding sequence positions, and non-synonymous amino acid substitutions.

| Gene [1] | SNP (dbSNP ID) [2] | PCS Position [3] | Amino Acid Substitution |
|---|---|---|---|
| *FABP4* | g.44677959T>C (rs110757796) | 220 | Isoleucine to Valine |
| *SCD* | g.21272422C>T (rs41255693) | 878 | Alanine to Valine |
| *FASN* | g.50784533C>G (rs481622676) | 2066 | Alanine to Glycine |
| | g.50786496A>G | 3145 | Serine to Glycine |
| | g.50788575T>C (rs41919993) | 4168 | Tyrosine to Histidine |
| | g.50790973C>A (rs109149276) | 5572 | Leucine to Isoleucine |
| | g.50794099T>C | 7277 | Isoleucine to Threonine |

[1] *FABP4*: fatty acid binding protein 4, *SCD*: stearoyl-CoA desaturase, *FASN*: fatty acid synthase. [2] SNP: single nucleotide polymorphism. Variant dbSNP ID are based on the Bovine Genome Variation Database (BGVD). SNP without dbSNP ID are not listed in BGVD. [3] PCS: protein-coding sequence.

### *3.2. Correlations between SNP and Carcass Traits*

The clustering patterns of the SNP loci are shown in the top panels of Figures 1A, 2A and 3A. The level of SNP heterozygosity and homozygosity varied widely between closely related individuals in the *SCD* gene compared to both *FABP4* and *FASN* genes. The correlation coefficients between the SNP and carcass traits are presented in the bottom panels of Figures 1B, 2B and 3B. Most of the SNP loci in the *FABP4* gene were in linkage disequilibrium ($p < 0.05$), but the correlations between SNP and carcass traits were observed in only three SNP. The g.44677205A>G (rs109388335), g.44677611G>C (rs41729172), and g.44679833A>G (rs133333024) SNP were all positively correlated with hump height ($p < 0.05$), while g.44677205A>G (rs109388335) was negatively correlated with EMA ($p < 0.05$). All SNP identified in the *SCD* gene were in linkage disequilibrium ($p < 0.05$), except g.21267896C>T (rs136334180) that was not correlated with g.21274479G>A (rs382184952) (Figure 2B). Polymorphisms on the g.21271392G>A (rs211294052) and g.21274479G>A (rs382184952) loci were positively correlated with HCW and EMA, respectively ($p < 0.05$). Moreover, g.21275851C>A (novel) was negatively correlated with P8 fat thickness, backfat thickness, and fat class ($p < 0.01$), while g.21273692T>C (rs208058585), g.21276141C>T (rs41255697), and g.21276672A>G (rs41255698) were negatively correlated with carcass marbling score ($p < 0.05$). Most SNP detected in the *FASN* gene were in linkage disequilibrium (Figure 3B; $p < 0.05$). Positive correlations were observed in g.50784533C>G (rs481622676) and g.50792445C>T (novel) SNP with carcass P8 fat and backfat thickness, while g.50786221A>G (rs518879624) and g.50787362C>T (novel) were negatively correlated with carcass marbling ($p < 0.05$). Several carcass traits were significantly correlated with each other. For instance, HCW was positively correlated ($\geq$30) with P8 fat, backfat thickness, EMA, and MSA index, but negatively correlated with meat colour scores ($p < 0.05$). A highly positive correlation ($\geq$80) between P8 fat with back fat and fat class was observed ($p < 0.001$). Hump height was negatively correlated with MSA index ($p < 0.001$), but there was no correlation between hump height and other carcass traits.

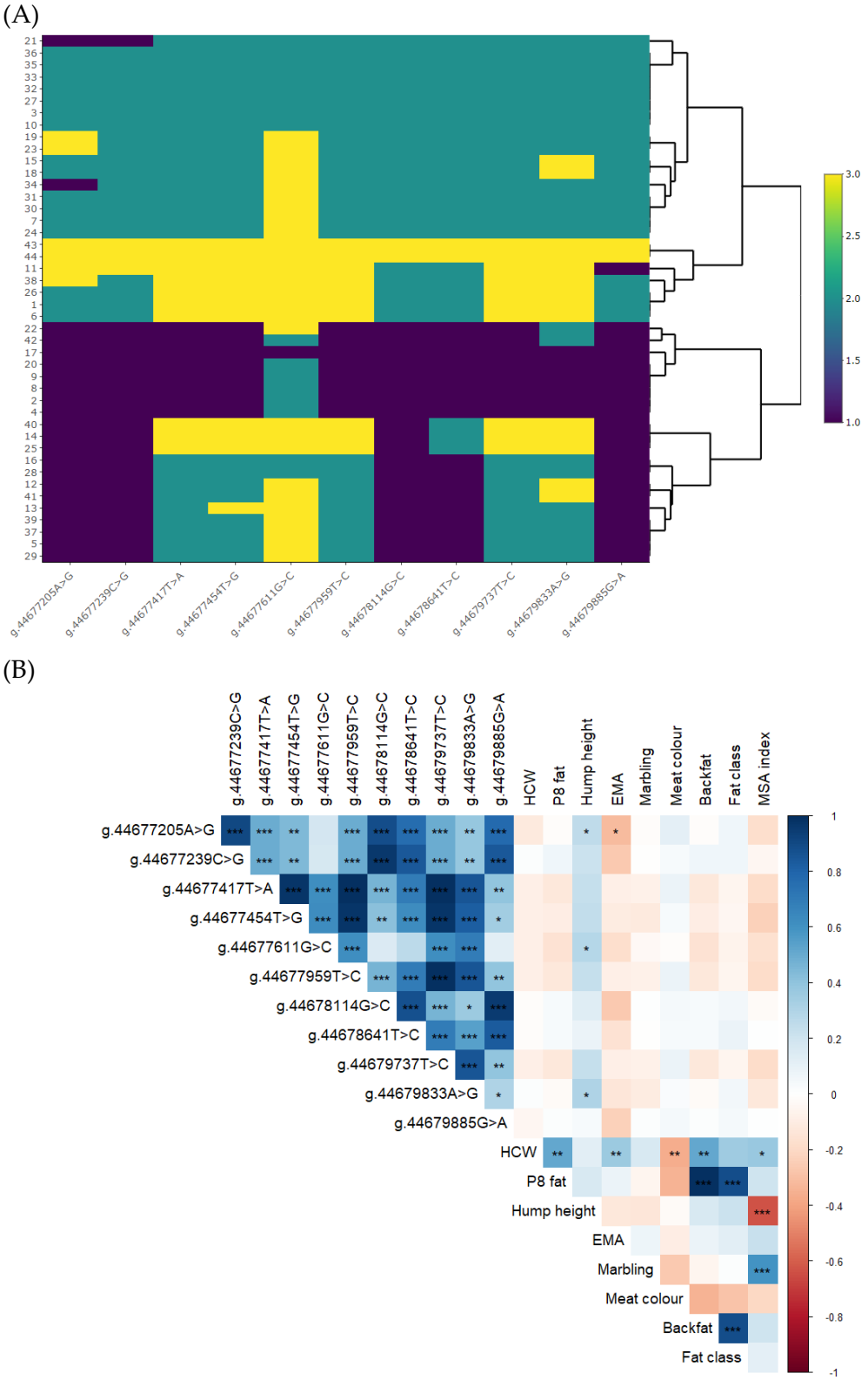


**Figure 1.** Single nucleotide polymorphisms on the *FABP4* gene. (**A**) Clustering map of genetic variants; homozygotes similar to the reference sequence (Hereford), heterozygotes, and alternative homozygotes. (**B**) Correlations between detected SNP and carcass traits. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

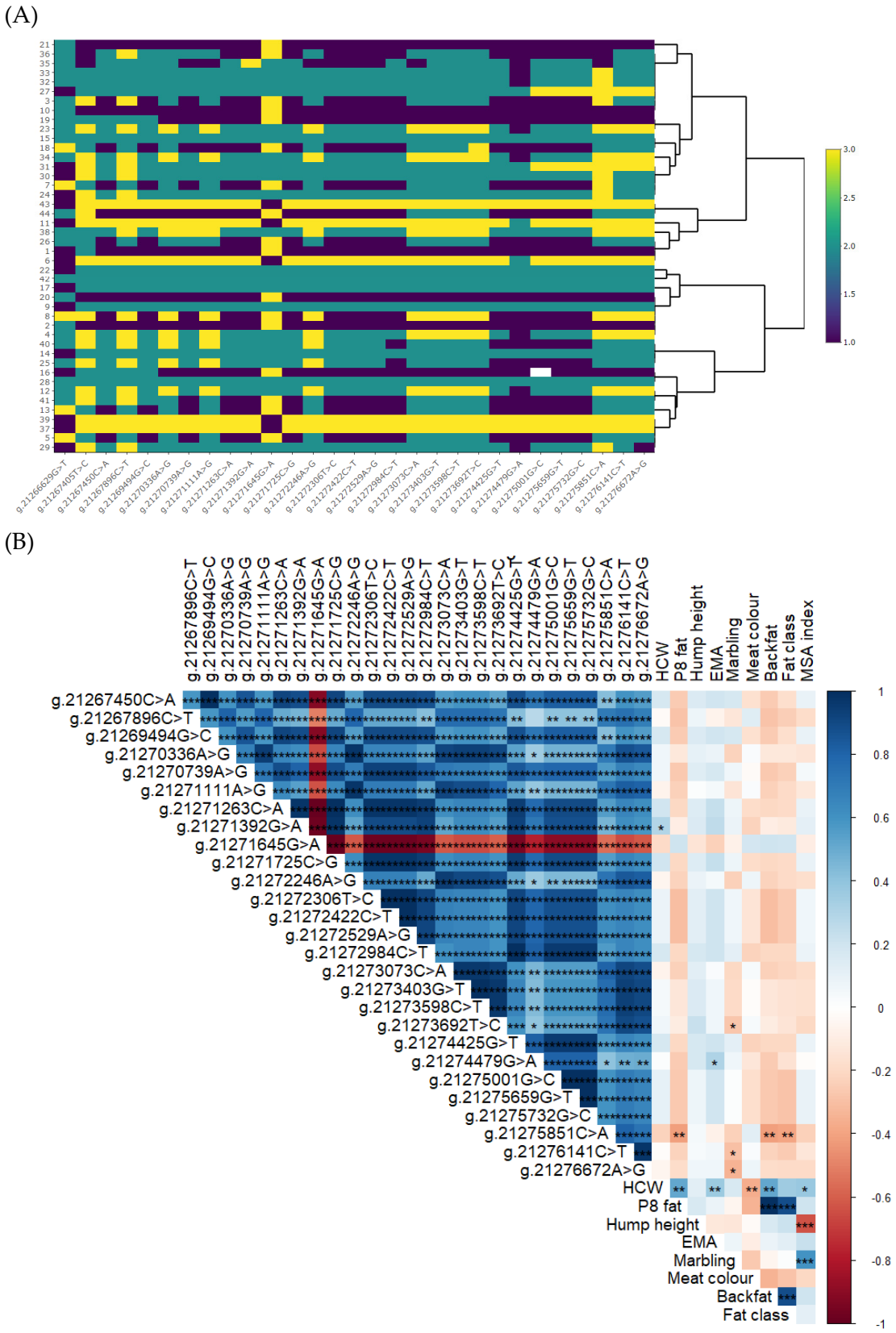


**Figure 2.** (**A**) Clustering map of genetic variants of the *SCD* SNP; homozygotes similar to the reference gene (Hereford), heterozygotes, and the alternative homozygotes. (**B**) Correlations between SNP and carcass traits. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

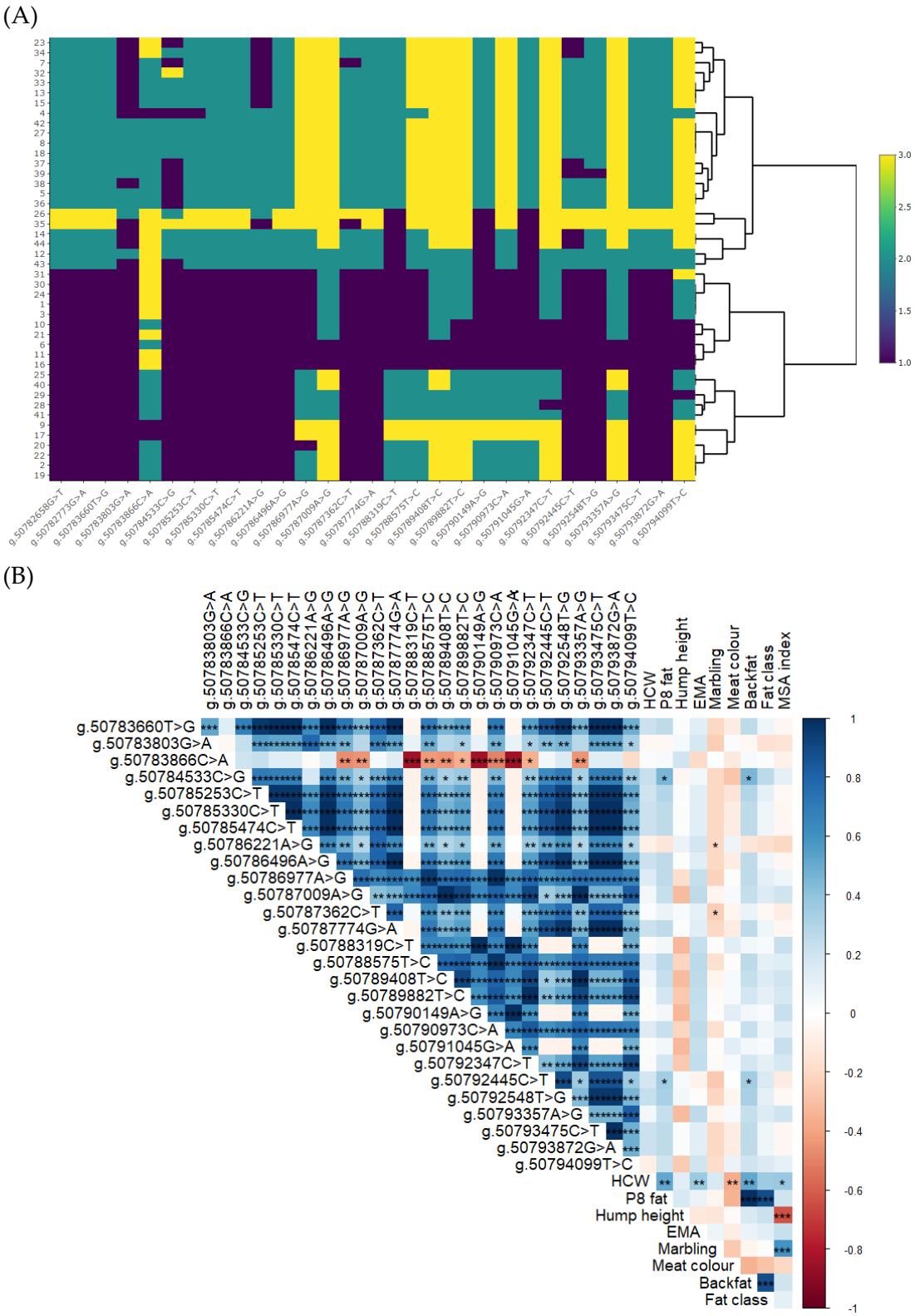


**Figure 3.** (**A**) Clustering map of genetic variants of the *FASN* SNP; homozygotes similar to the reference gene (Hereford), heterozygotes, and the alternative homozygotes. (**B**) Correlations between genes SNP and carcass traits. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

### *3.3. Associations between SNP and Carcass Traits*

Associations between *FABP4* g.44677205A>G (rs109388335), *SCD* g.21275851C>A (novel) and *FASN* g.50784533C>G (rs481622676) SNP with carcass traits are presented

in Table 3. The *FABP4* g.44677205A>G SNP had a significant association with hump height ($p < 0.01$) and tended to be associated with EMA ($p = 0.05$). Multiple genotype comparisons at the g.44677205A>G locus showed that the GG genotype had a higher hump height (125.8 ± 10.68 mm) compared to the AA (109.3 ± 13.39 mm) genotype ($p \leq 0.01$), but similar to the AG genotype ($p = 0.08$). The hump height difference between the AA and AG genotypes was insignificant ($p = 0.64$). EMA was significantly higher for the AA (88.4 ± 8.71 $cm^2$) than the GG (81.3 ± 3.88 $cm^2$) and AG (86.7 ± 4.93 $cm^2$) genotypes (Figure 4; $p \leq 0.03$), but genotypic differences between AA and AG were not significant ($p = 0.81$). The AA genotype of *SCD* g.21275851C>A SNP had the lowest P8 fat, marbling, backfat, and fat class, while the highest scores were observed for the CC genotype ($p < 0.02$). Multiple comparisons between *SCD* g.21275851C>A SNP variants and carcass traits indicated that P8 fat, marbling, backfat, and fat class scores were significantly different between the CC and AA genotypes (Figure 5; $p < 0.03$). No significant associations were observed between the *FASN* g.50784533C>G variants and the carcass traits measured ($p \geq 0.10$).

**Table 3.** Least square means ± SD of carcass traits due to SNP in *FABP4* g.44677205A>G, *SCD* g.21275851C>A, and *FASN* g.50784533C>G in northern Australian tropical crossbred beef cattle.

| **Variable** [1] | | | | | ***p*-Value** [2] |
|---|---|---|---|---|---|
| ***FABP4*** | | | | | |
| g.44677205A>G | Total (n = 44) | AA (n = 22) | AG (n = 16) | GG (n = 6) | |
| HCW (kg) | 329.9 ± 26.15 | 331.1 ± 20.06 | 333.0 ± 32.81 | 317.6 ± 27.26 | 0.16 |
| P8 fat (mm) | 16.7 ± 5.80 | 17.0 ± 5.96 | 17.0 ± 6.41 | 14.6 ± 3.27 | 0.66 |
| Hump height (mm) | 112.5 ± 15.42 | 109.3 ± 13.39 | 111.8 ± 17.5 | 125.8 ± 10.68 | <0.01 |
| EMA ($cm^2$) | 86.8 ± 7.26 | 88.4 ± 8.71 | 86.7 ± 4.93 | 81.3 ± 3.88 | 0.05 |
| Marbling (score) | 361.5 ± 79.77 | 372.7 ± 83.05 | 353.1 ± 81.79 | 343.3 ± 67.13 | 0.86 |
| Meat colour (score) | 2.3 ± 1.11 | 2.1 ± 1.18 | 2.3 ± 0.96 | 2.5 ± 1.38 | 0.70 |
| Back fat (mm) | 14.7 ± 5.80 | 15.0 ± 5.96 | 15.0 ± 6.41 | 12.6 ± 3.27 | 0.74 |
| Fat class (score) | 3.8 ± 0.65 | 3.9 ± 0.75 | 3.8 ± 0.62 | 3.8 ± 0.41 | 0.98 |
| MSA index | 51.11 ± 2.32 | 51.3 ± 2.28 | 51.2 ± 2.6 | 49.6 ± 0.42 | 0.39 |
| ***SCD*** | | | | | |
| g.21275851C>A | Total (n = 44) | CC (n = 9) | CA (n = 15) | AA (n = 20) | |
| HCW (kg) | 329.9 ± 26.15 | 333.0 ± 38.21 | 333.2 ± 23.32 | 326.1 ± 22.44 | 0.75 |
| P8 fat (mm) | 16.7 ± 5.80 | 19.6 ± 6.73 | 18.6 ± 5.03 | 14.0 ± 4.88 | 0.01 |
| Hump height (mm) | 112.5 ± 15.42 | 105.5 ± 12.61 | 116.6 ± 15.99 | 112.5 ± 15.68 | 0.24 |
| EMA ($cm^2$) | 86.8 ± 7.26 | 85.8 ± 5.49 | 88.2 ± 8.60 | 86.2 ± 7.07 | 0.82 |
| Marbling (score) | 361.5 ± 79.77 | 421.1 ± 75.9 | 338.0 ± 83.85 | 352.5 ± 67.74 | 0.02 |
| Meat colour (score) | 2.3 ± 1.11 | 2.2 ± 1.20 | 2.3 ± 1.23 | 2.3 ± 1.03 | 0.98 |
| Back fat (mm) | 14.7 ± 5.80 | 17.5 ± 6.73 | 16.6 ± 5.05 | 12.0 ± 4.88 | 0.01 |
| Fat class (score) | 3.8 ± 0.655 | 4.2 ± 0.667 | 4.0 ± 0.594 | 3.6 ± 0.598 | 0.02 |
| MSA index | 51.1 ± 2.32 | 52.3 ± 2.18 | 50.9 ± 2.82 | 50.7 ± 1.90 | 0.19 |
| ***FASN*** | | | | | |
| g.50784533C>G | Total (n = 44) | CC (n = 30) | CG (n = 12) | GG (n = 2) | |
| HCW (kg) | 329.9 ± 26.15 | 327.2 ± 23.24 | 329.5 ± 24.73 | 377.5 ± 40.31 | 0.62 |
| P8 fat (mm) | 16.7 ± 5.80 | 15.5 ± 5.10 | 18.4 ± 5.84 | 25.0 ± 9.90 | 0.10 |
| Hump height (mm) | 112.5 ± 15.42 | 110.2 ± 14.97 | 110.2 ± 15.60 | 130.2 ± 14.14 | 0.92 |
| EMA ($cm^2$) | 86.8 ± 7.26 | 86.5 ± 7.90 | 87.4 ± 6.05 | 88.5 ± 6.36 | 0.69 |
| Marbling (score) | 361.5 ± 79.77 | 380.2 ± 91.95 | 350.2 ± 62.68 | 345.2 ± 49.50 | 0.23 |
| Meat colour (score) | 2.3 ± 1.11 | 2.5 ± 1.12 | 1.9 ± 1.15 | 2.0 ± 0.710 | 0.31 |
| Back fat (mm) | 14.7 ± 5.80 | 13.5 ± 5.10 | 16.4 ± 5.84 | 23.0 ± 9.90 | 0.10 |
| Fat class (score) | 3.8 ± 0.650 | 3.8 ± 0.648 | 3.9 ± 0.669 | 4.5 ± 0.707 | 0.38 |
| MSA index | 51.1 ± 2.32 | 50.9 ± 2.01 | 51.4 ± 3.24 | 51.8 ± 1.22 | 0.75 |

[1] *SCD*, stearoyl-CoA desaturase; *FASN*, fatty acid synthase; *FABP4*, fatty acid binding protein 4; HCW, hot carcass weight; P8 fat; subcutaneous fat thickness at the rump site; EMA, loin eye muscle area; MSA index, Meat Standards Australia index. [2] ANOVA *p*-value.

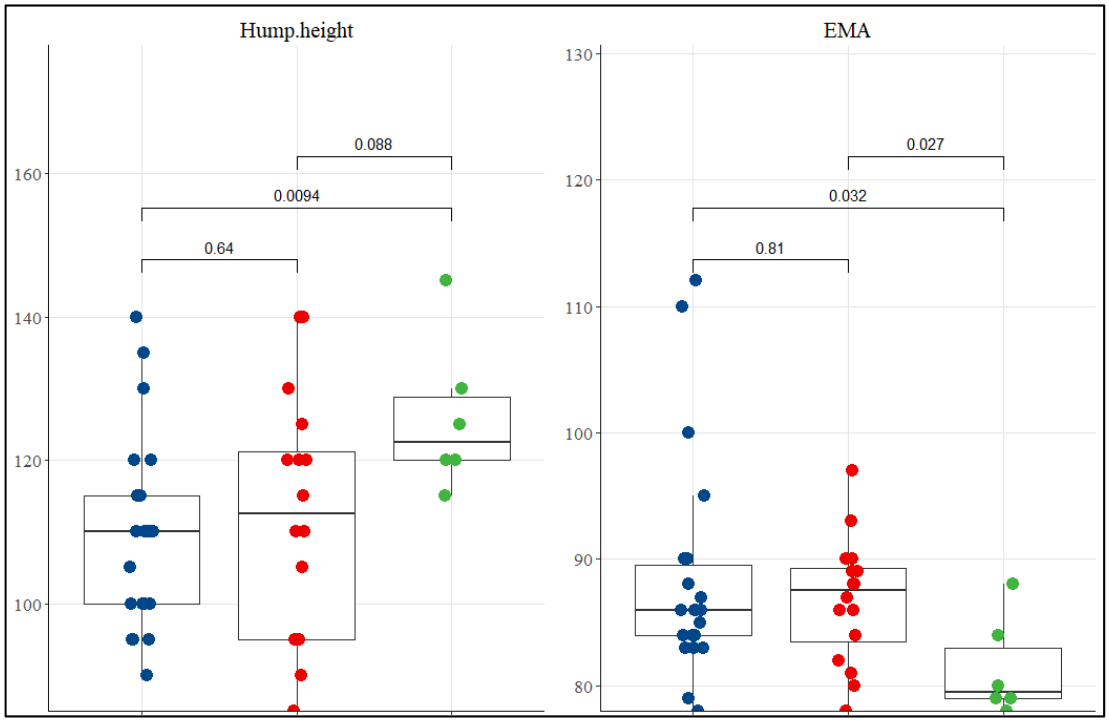


**Figure 4.** Multiple comparisons of hump height and loin eye muscle area (EMA) for *FABP4* g.44677205A>G genotypic variants AA (●), AG (●), and GG (●).

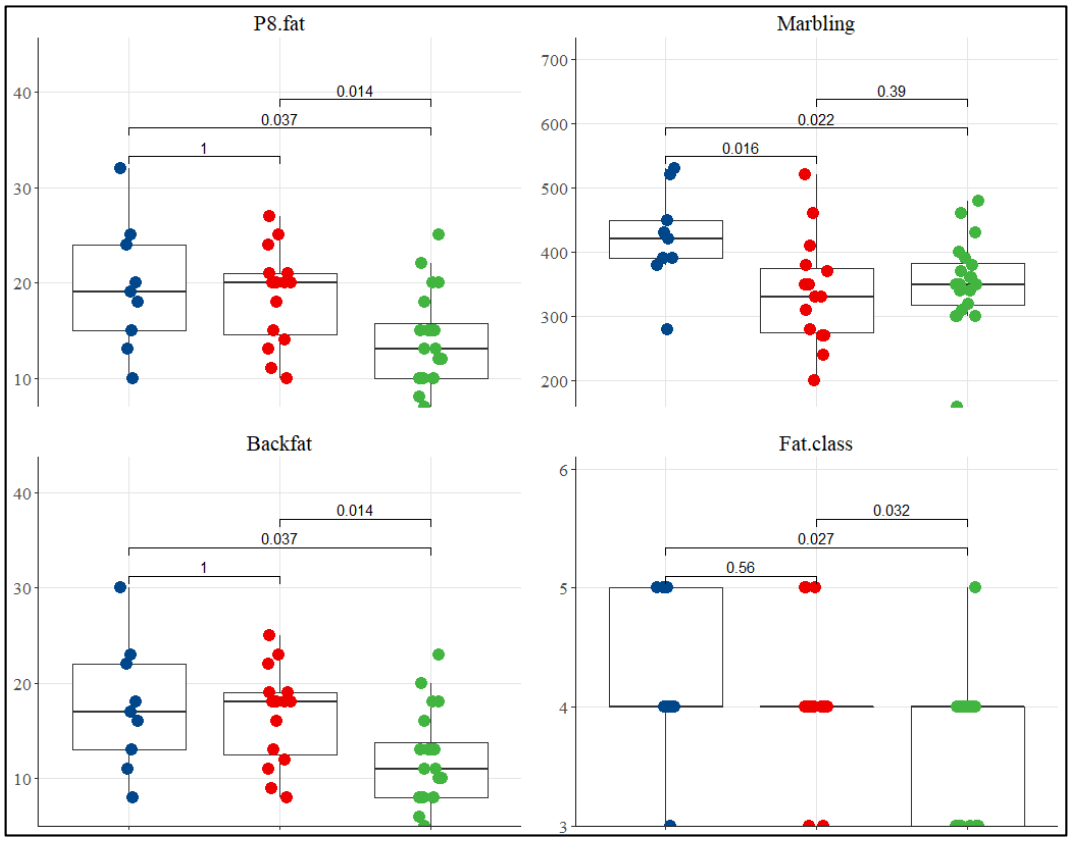


**Figure 5.** Multiple comparisons of P8 fat, marbling, backfat, and fat class of *SCD* g.21275851C>A genotypic variants CC (●), CA (●), and AA (●).

## 4. Discussion

Chiller-assessed carcass quality measurements are obtained after slaughter, hence the data cannot be used to make management decisions such as culling the inferior and breeding the superior performing animals [9,10]. Regions on chromosomes 1, 9, 14, 16, 19, 23, 26, and 29, had been previously identified to be associated with fatty acid composition and carcass traits in a cohort of *Bos indicus*, *Bos taurus,* and tropical composite beef cattle using high-density data [34]. Other studies reported that single allelic substitutions influence carcass traits and can be used to predict carcass quality in living animals [17,23]. In this study, a targeted next-generation sequencing technique was used to identify SNP in the *FABP4*, *SCD,* and *FASN* genes that may be used as molecular markers for carcass quality selection in northern Australian tropically adapted beef cattle. A marker PIC is one of the indicators of marker quality and it reflects the ability of a marker to detect polymorphisms between individuals in a population [35]. Markers with PIC values above 0.5 are deemed very informative, 0.25–0.50 are a bit informative, and below 0.25 are minimally informative [36]. In this study, all the markers were informative with the exception of only one marker at the g.50783803G>A locus that had a PIC below 0.25.

### *4.1. FABP4 Gene Polymorphisms*

The *FABP4* gene is a primary metabolic indicator of IMF deposition as it is located within the quantitative trait loci region that contributes to serum leptin, a protein involved in body fat regulation, and *FABP4* also encodes a protein involved in intracellular fatty acids trafficking [37,38]. Damon et al. [39] reported that *FABP4* protein level was positively correlated with fat cell count and lipid content in porcine. Genetic variants of the *FABP4* gene in cattle and their associations with meat and carcass traits have been reported [14,16,18,40]. The *FABP4* g.3691G>A SNP was associated with marbling score, while g.2834C>G was reported to be associated with HCW in Holstein bulls [10]. The g.3473T>A SNP was reported to have a significant effect on carcass weight, while g.3631A>G significantly influenced marbling score in Hanwoo cattle [17]. In another study, *FABP4* g.3691G>A SNP was reported to influence marbling score and meat quality grade in Hanwoo cattle [18]. Five SNP (g.3496A>C, g.3745T>C, g.3533A>T, g.3767T>C, and g.3711G>C) were associated with marbling scores in Yanbian yellow cattle [19]. However, these polymorphisms were not observed in this study. Furthermore, there were no correlations or associations between the identified *FABP4* SNP in the present study and carcass subcutaneous fat depth (P8 fat and backfat) or marbling score. These findings agree with a previous study [38] that reported no significant effect of g.44677959T>C (c.220) with subcutaneous fat nor marbling score in Japanese Black cattle. In contrast, Cho et al. [41] reported an SNP association with backfat thickness in Korean cattle, while Goszczynski et al. [2] reported an additive effect in cattle of varying breeds. Three SNP (g.44677205A>G, g.44677611G>C, and g44679833A<G) were positively correlated with hump height in this study, with the homozygous GG genotype having the highest and AA the lowest measurements. Hump height is used to estimate the amount of tropical breed content of the carcass, with high values indicating higher tropical breed content and, subsequently, lower meat eating quality [42]. Therefore, the correlations indicate that genetic variants of the three loci are influenced by breed, in agreement with previous studies [18,43]. Both EMA and subcutaneous fat depth indicate the amount of saleable meat from the carcass [3]. The negative correlation between g.44677205A>G and EMA, and the lack of correlation with subcutaneous fat depth, indicate that selecting for the A allele may increase the amount of carcass saleable meat without increasing the subcutaneous fat that ends up being trimmed off [44].

### *4.2. SCD Gene Polymorphisms*

The *SCD* gene is highly expressed in lipogenic tissues and encodes for an enzyme that desaturates SFA by introducing a *cis* double bond at the 9th and 10th carbon interface of the fatty acid [45]. The unsaturation of the fatty acid chain is a key determining factor of the melting temperature of triacylglycerols, thus influencing meat fat hardness [44]. Alleles as-

sociated with high *SCD* enzyme activity have been reported to be associated with increased marbling scores in Wagyu × Limousin cattle [23]. Similarly, three SNP were correlated with carcass marbling score in this study, where the C, T, and G alleles of the g.21273692T>C, g.21276141C>T, and g.21276672A>G loci, respectively, had favourably higher marbling scores. The missense mutation that causes substitution of valine (type V) to alanine (type A) at PCS position 878 (g.21272422C>T) had no effect on carcass traits measured in this study. These findings align with previous studies that reported no effect on backfat thickness in Spanish commercial beef and Chinese Simmental cattle [24,25]. In addition, the SNP was not associated with HCW, EMA, backfat thickness, and marbling score of Japanese black cattle reported by Ohsaki et al. [46]. More studies are required to determine the mechanism behind the effect of *SCD* SNP reported in the Wagyu x Limousin cattle [23] and lack of it in other cattle breeds [24,25,46]. Accordingly, the study of Li et al. [11] on the effect of SNP on carcass traits of beef cattle populations indicated no significant effect on marbling score and meat colour soon after slaughter. Genes responsible for fat deposition and metabolism are reported to influence meat colour since high fat levels accelerate lipid myoglobin oxidation and, subsequently, meat discoloration, since marbling influences visually assessed meat colour [47]. The lack of effect of *SCD* SNP on meat colour observed in this study may be due to the short period between slaughter and carcass assessments.

### *4.3. FASN Gene Polymorphisms*

The *FASN* gene encodes an essential homodimeric cytosolic enzyme critical for de novo lipogenesis by catalysing palmitic acid synthesis from acetyl-CoA and malonyl-CoA [48,49]. Palmitic acid is a predominant fatty acid in beef and is used as a substrate for the synthesis of other fatty acids through elongation and desaturation [5,50]. Fatty acid composition is reported to influence carcass traits [51,52]. The majority of association studies on *FASN* gene polymorphisms have focused on fatty acid composition [53–56] with little emphasis on carcass traits [3,40]. Polymorphism in the *FASN* thioesterase domain was found to have a significant effect on beef grade by influencing fat deposition in Korean cattle [48]. Oh et al. [57] examined the effect of five missense SNP in the *FASN* gene and reported an association of all the SNP with carcass backfat thickness and marbling score with no influence on carcass weight in Korean cattle. In another study, Raza et al. [3] reported associations between g.13192T>C with backfat thickness and EMA, and g.13232C>T with IMF in Qinchuan cattle. Polymorphism in *FASN* influenced HCW, but no associations with subcutaneous fat thickness and marbling score were observed in crossbred beef cattle [22], or with marbling score in the loin eye muscle of purebred American Angus bulls [58]. Matsuhashi et al. [56] reported no association between *FASN* g.16024A>G SNP with carcass weight and subcutaneous fat thickness of Japanese black cattle. In this study, polymorphisms in the g.50784533C>G and g.50792445C>T loci were correlated with subcutaneous fat depth, while g.50786221A>G and g.50787362C>T were correlated with marbling scores. The loci g.50787362C>T and g.50792445C>T are located in the intron, hence they may influence carcass fat content through regulation of alternative splicing or gene expression [59]. Furthermore, the allelic mutations in g.50784533C>G locus in exon 13 resulted in amino acid substitution from alanine to glycine at PCS position 2066 that encodes for malonyl-CoA-/acetyl-CoA-acyl carrier protein-transacylase (AT/MT) domain [48]. The AT/MT domain catalyses the transfer of acetyl-CoA to the acyl carrier protein and transacylates the malonyl-CoA to the acyl carrier protein, which is the initial step in de novo fatty acids synthesis [60]; thus, the SNP may influence fatty acids synthesis. The differences between studies may be due to breed effect or in the SNP studied [61,62]. Effects of genetic markers on phenotypic outcomes are often breed-specific, and may not be extrapolated to all cattle breeds [18,25,43]. Since marbling and MSA index scores were highly correlated, these findings indicate that SNP in the *FASN* gene may be used in marker-assisted selection for improved carcass grades and beef eating quality in northern Australian tropical crossbred cattle.

## 5. Conclusions

This study aimed to examine SNP present in the exons and introns of *FABP4*, *SCD*, and *FASN* genes of northern Australian tropical crossbred beef cattle and examine their associations with the chiller-assessed carcass traits. The results showed significant correlations between SNP in *FABP4* with hump height and loin eye muscle area, *SCD* SNP with carcass weight, loin eye muscle area, and marbling, while *FASN* SNP were correlated with subcutaneous fat thickness and marbling score. Therefore, the hypothesis that SNP in the *FABP4*, *SCD*, and *FASN* genes are associated with chiller-assessed carcass traits of tropically adapted northern Australian crossbred beef cattle was accepted. These findings indicate the involvement of these genes in carcass traits of the tropical crossbred beef cattle population of northern Australia and the potential to use SNP in carcass grade and meat quality improvement through marker-assisted selection.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12081171/s1, Figure S1: Gel image of the amplification products of the three target genes visualised in 0.8% agarose gel; Table S1: SNP genetic variants identified in the *FABP4*, *SCD*, and *FASN* genes; Figure S2: Correlation coefficients between all SNP in *FABP4* gene. The rectangle represents distance-based clustering of SNP loci; Figure S3: Correlation coefficients between all SNP in *SCD* gene. The rectangle represents distance-based clustering of SNP loci; Figure S4: Correlation coefficients between all SNP in *FASN* gene. The rectangle represents distance-based clustering of SNP loci.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (8th Edition, 2013), and approved by the James Cook University Animal Ethics Committee (Approval Number 2639 issued on 5 July 2019).

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**Data Availability Statement:** The data presented in this study are available from the corresponding author on request.

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