The association of \textit{RNF34} 3′UTR-588 G>A and \textit{RNF128} I1-2380C>T with carcass and meat quality traits of Chinese Simmental-cross steers

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Simple Summay: An experiment was performed to investigate the role of single nucleotide polymorphisms of the gene \textit{RNF34} 3′UTR-588 G>A and \textit{RNF128} I1-2380C>T with carcass and meat quality traits of Chinese Simmental-cross steers. Sequencing and restriction enzyme digestion was performed to detect genotypes of \textit{RNF34} 3′UTR-588 G>A and \textit{RNF128} I1-2380C>T. The associations of novel single nucleotide polymorphisms in intron regions of the \textit{RNF128} gene and in the 3′UTR region of \textit{RNF34} and meat quality traits of Chinese Simmental-cross steers were analyzed. Statistical analyses revealed that SNP of \textit{RNF128} was significantly associated with dressed weight, forepaw weight, carcass depth, carcass brisket depth, hind legs length (P<0.05), etc. And \textit{RNF34} were significantly associated with testis weight, kidney weight, tare weight (P<0.05), etc. Our findings suggest that polymorphisms in \textit{RNF34} and \textit{RNF128} might be important genetic factors that influence carcass and meat quality in beef cattle. Thus, they might be useful markers for meat quality traits in
ABSTRACT

Background:

An experiment was conducted to investigate the role of single nucleotide polymorphisms of the gene RNF34 3′UTR-588 G>A and RNF128 I1-2380C>T with carcass and meat quality traits of Chinese Simmental-cross steers.

METHOD:

We performed sequencing and restriction enzyme digestion to detect genotypes of RNF34 3′UTR-588 G>A and RNF128 I1-2380C>T. Then, we analyzed the association of novel single nucleotide polymorphisms in intron regions of the RNF128 gene and in the 3′UTR region of RNF34 and meat quality traits of Chinese Simmental-cross steers.

RESULTS:

Statistical analyses revealed that SNP of RNF128 (I1-2380C>T) was significantly associated with dressed weight, forepaw weight, carcass depth, carcass brisket depth, hind legs length (P<0.05), etc. And RNF34 (3′UTR-588 G>A) were significantly associated with testis weight, kidney weight, tare weight (P<0.05), etc.

CONCLUSION:

Our findings suggest that polymorphisms in RNF34 and RNF128 might be important genetic factors that influence carcass and meat quality in beef cattle. Thus, they might be useful markers for meat quality traits in future marker-assisted selection programs in beef cattle breeding and production.

Keywords: Cattle; RNF128; RNF34; Single nucleotide polymorphisms
INTRODUCTION:

With the rapid development of molecular biology techniques, identification of single nucleotide polymorphisms has been widely used to study the effects of genetic mutations on animal performance, which in turn could be used for a molecular marker-assisted approach to breeding and production.

*RNF34* and *RNF128* play an important role in many biological processes. Several papers showed that E3 ubiquitin-protein ligases are master regulators of energy metabolism and adaptive thermogenesis in brown fat cells. And *RNF34* is a bona fide E3 ubiquitin-protein ligase for PGC-1α and negatively regulates brown fat cell metabolism. *RNF34* binds to the C-terminal region of PGC-1α and targets it for degradation independently of the previously identified N-terminal phosphor degron motif [1,2]. In brown fat cells, knockdown of *RNF34* has several effects including increased endogenous PGC-1α protein levels, increased uncoupling protein 1 (UCP1) expression and increased oxygen consumption[3,4]. However, the opposite effects are observed in brown fat cells that ectopically express wild-type *RNF34* instead of its ligase activity-defective mutant form [5]. Interestingly, cold exposure and β3-adrenergic receptor signaling, conditions that induce PGC-1α expression, suppress *RNF34* expression in brown fat cells, indicating a physiological relevance for this E3 ligase in the thermogenesis process[6] [7]

The *RNF128* gene plays an important role in a series of cellular pathways and processes such as DNA repair, cell cycle regulation, apoptosis, and inflammatory response [7]. T-cell activation is tightly regulated in order to avoid autoimmunity. GRAIL protein, encoded by *RNF128* and related to energy metabolism in T-cells, is an E3 ubiquitin-protein ligase associated with T-cell tolerance. Interestingly, ubiquitination and degradation of CD40L by *RNF128* is one cause of T-cell incompetence[8,9]. In recent years, expressions of *RNA34* and *RNA128* showed a significant difference between adult cattle...
and younger ones[2].

Therefore, it is possible that the RNA34 and RNA128 maybe candidate genes influencing carcass and meat quality of beef cattle. But, at present, only a little information is available on the genetic polymorphism of bovine RNA34 and RNA128 genes and the effect of the genetic variants of RNA34 and RNA128 genes remain inconclusive. Hence, in the present study, SNPs of RNF34 and RNF128 were examined with respect to their association with carcass and meat quality traits in Chinese Simmental-cross steers. The results of this study may provide useful evidence to MAS in the process of pure breeding, crossbreeding, and the preservation of important genetic resources.

MATERIALS AND METHODS

Ethics statement

Animal experiments were conducted in strict accordance with the guidance for the care and use of laboratory animals by the Jilin University Animal Care and Use Committee (permit number: SYXK (Ji) 2008-0010/0011). All production traits were measured with standardized methods.

Materials

The animals of Simmental-cross steers for this study were taken from the Inner Mongolian Baolongshan cattle farm. Blood samples (10 mL each) were collected from the jugular vein using an anticoagulant (Acid citrate dextrose, ACD) followed by storage at -80°C. DNA was extracted from 1 mL of extracted whole blood using a DNA extraction kit (Tiangen, Beijing, China) according to the manufacturer’s protocol.
Methods

- **Trait measurements**

  Carcasses were stored in refrigerated rooms at temperatures ranging from 0 to 4°C for 24 h before the carcass and meat traits were measured. Trait measurements were made based on the GB/T17238-1998 cutting standards for fresh and chilled beef of China (China Standard Publishing House).

  Final body weight, living QIB, and ribeye area were recorded before slaughter. All visceral indicators, including the weight of the spleen, large intestine, small intestine, heart, liver, kidney and fat belly, were weighed after slaughter. Other carcass properties were also recorded, including the carcass weight, slaughter rate, net weight of bone, head weight, tare weight, fat color score, hind legs circumference, hind legs width, and carcass brisket depth etc. The described measurements were determined strictly according to established measurement standards. Carcass traits were shown in table1.

- **Primers and PCR amplification**

  Primers were designed based on bovine RNF34 and RNF128 sequences (ENSTA) using Primer 5 software. Primer sequences are as follows. *RNF34*-Forward: 5'-CGGGCTGTTTCCCAGGTTCT-3';
  
  *RNF34*-Reverse: 5'-CCCAATGATGTTGAAACGCAGA-3';

  *RNF128*-Forward: 5'-GAGCAAACAGAGGCTTACACAAC-3';

  *RNF128*-Reverse: 5'-TCAGTCTTACCTCTTGCCACTAG-3'. The primers were synthesized (Sangon, Shanghai, China).

  The PCRs were performed using the following cycling conditions: 95 °C for 5 min followed by 30
cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s with a final extension step at 72 °C for 10 min, as shown in Fig.1.

- **SNP detection and genotyping**

Restriction endonuclease Bpu1102 was used to distinguish the genotypes of the PCR products of RNF34 gene. PCR products were digested with restriction endonuclease Spe I for genotyping of RNF128 gene. Restriction digestion reactions were conducted at 37 °C for 6 h. PCR digestion products were resolved on 3% agarose gels to distinguish the bands representing three different genotypes.

Genotypes and gene frequencies are shown in table 2.

- **Statistical analysis**

SPSS 13.0 was used to calculate the relationship between genotype and production traits. Genotype frequencies were calculated and were analyzed by significance testing. The genotypic effects of the RNF34 gene and RNF128 gene were determined using the general linear model (GLM) of SPSS.

The fixed model was as follows: $Y_{ijkl} = u + f_{ys} + m_k + e_{ijkl}$, where $Y_{ijkl}$ is the observed value of $l$th individual from the breed $I$, of genotype $k$, in the $j$th farm-year-season; $u$ is the least square means of the observed values; $f_{ys}$ is the effective value of the $j$th farm-year-season; $m_k$ is the effective value of the genotype $k$; and $e_{ijkl}$ is the random residual effect corresponding to the observed value.

**RESULTS**

- **PCR amplification**
The PCR amplification products observed (intron 1) were consistent with the expected target fragments with good specificity. The PCR products were directly analyzed by restriction enzyme digestion as well as sequencing reactions.

- **Restriction endonuclease analysis and sequencing of different genotypes**

For SNP detection and genotyping, PCR amplification was first conducted on a mixture of randomly selected DNA samples followed by DNA sequencing that was performed by Sango (Shanghai, China). It was discovered that there was a single nucleotide polymorphism site at nucleotide 588 in the RNF34 gene 3'UTR region (Figure 1). To allow for genotype identification, PCR products from 255 samples were digested with the Bpu1102 restriction enzyme and resolved on a 3% agarose gel. As shown in table 1, a single band indicated a homozygous AA genotype; two bands indicated a homozygous GG genotype, and three bands indicated a heterozygous AG genotype. Among the 255 Simmental-cross steers, allele G had a frequency of 0.5882 at the 3'UTR-G588A polymorphism site, whereas allele A, had a frequency of 0.4118. The results from multi-comparison statistical analyses showed that there was no significant difference in the genotype distributions (Table 2, P>0.05).

For RNF128, the sequencing results indicated that there was a single nucleotide polymorphism of I1-2380 C> T in the first intron region. Following Spe I restriction, the following frequencies were found for the 213 samples, TT genotype, 0.5305; TC genotype, 0.0657; CC genotype, 0.4038; T allele, 0.5634; and C allele, 0.4366.

- **Association analyses of RNF34 and RNF128 polymorphisms with carcass and meat quality traits**
Associations of *RNF34* polymorphisms with carcass traits were analyzed by one-way ANOVA. Statistical analyses revealed that *RNF34* 3′UTR (c.+588 G>A) had a significant association with the carcass and meat quality traits, including tare weight, kidney weight, testis weight, fat color score (P<0.05), as shown in table 3.

Associations of *RNF128* gene polymorphisms with carcass traits were analyzed by one-way ANOVA and LSD to allow for multiple comparisons to be conducted with respect to production traits. As shown in table 4, there were significant differences between the different genotypes, involving dressed weight, forepaw weight, carcass depth, carcass brisket depth, the thickness of waist flesh, slaughter PH(P<0.05), and lung, trachea, hind legs length(P<0.01).

**DISCUSSION**

Meat quality is commercially important for the animal husbandry industry and is affected by the genetic background of the animals as well as management, nutrition and meat processing. Although, previous studies mainly focused on *RNF34* and *RNF128* were associated with cell differentiation and apoptosis[10]. *RNF34* gene transcripts are highly enriched in BCB oocytes, suggesting that *RNF34* may be involved in oocyte apoptosis [11,12]. And *RNF34* plays an important role in the regulation of NOD1, RNF34 , NF-κB pathways, which supports the idea that *RNF34* is a negative regulator of the NOD1 pathway through direct interaction and ubiquitination of NOD1[13,14] [14] . *RNF128*, an E3 ubiquitin-protein ligase, utilizes a unique single transmembrane protein with a split-function motif and is an important gatekeeper of T-cell unresponsiveness. Although it may play a role in other CD4 T-cell functions including activation, survival, and differentiation, GRAIL is most well characterized as a
negative regulator of T-cell receptor responsiveness and cytokine production[15,16]. But new important roles of \textit{RNF34} and \textit{RNF128} were founded in the present research.

In this study, SNPs of \textit{RNF34} and \textit{RNF128} were founded to play important roles in meat quality traits and growth traits. SNP of \textit{RNF34} 3'UTR-588 G>A suggested that the AA genotype was significantly associated with testis weight and fat color score. The AG genotype was significantly associated with kidney weight. Furthermore, the average production data for cattle of genotype AA were lower than for those for cattle with genotypes AG or GG in pH after acid exhausted and fat color score.

The \textit{RNF128} gene I1-2380C>T was founded different genotypes exited significantly influence with the carcass traits. The TT genotype was significantly associated with carcass weight, the net weight of bone, bullwhip, mesenteric fat weight, and thickness of waist flesh. The TC genotype was significantly associated with slaughter PH. The CC genotype was significantly associated with hind leg circumference. The LSM of the carcass weight, the net weight of bone, bullwhip weight, mesenteric fat weight, the thickness of waist flesh, and hind leg circumference for the TC genotype was higher than that of the TT or CC genotypes. The LSM of slaughter PH for the TT genotype was higher than that for the TC or CC genotypes. So we can select the excellent meat traits by genotypes of \textit{RNF34} and \textit{RNF128}. Thus, this study supports the development of a novel theory about the cultivation of excellent beef using molecular biology techniques.

\textbf{Conclusion:} Our findings suggest that polymorphisms in \textit{RNF34} and \textit{RNF128} might be important genetic factors that influence carcass and meat quality in beef cattle. Thus, they might be useful markers for meat quality traits in future marker-assisted selection programs in beef cattle breeding and production.
CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

ACKNOWLEDGMENTS

This work was supported by the Jilin Scientific and Technological Development Program (20130522084JH). This study was funded by the National Natural Science Foundation of China (No. 31660669) and the National Natural Science Foundation of China (No. 31372278).

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| Traits | RNF128 |       |       | RNF34 |       |       |
|--------|--------|-------|-------|-------|-------|-------|
|        | N      | Mean±SD         |        | N     | Mean±SD         |
| GW (Kg) | 213   | 506.5117±55.17678 | 255   | 509.5020±56.54048 |
| CW (Kg) | 213   | 268.5399±34.32056 | 255   | 269.7686±34.93046 |
| DP (%)  | 213   | 52.9379±1.9399901 | 255   | 52.873255±2.0756458 |
| NWB (Kg)| 213   | 19.7546±3.12495  | 255   | 19.7715±3.09257  |
| HW (kg) | 213   | 23.4677±2.29767  | 255   | 23.6072±2.33130  |
| FW (kg) | 213   | 6.0470±0.69623   | 255   | 6.0660±0.69769   |
| WH(kg) | 213   | 2.8099±0.37400   | 255   | 2.8207±0.37256   |
| PW(kg) | 213   | 39.2985±4.58529  | 255   | 39.6396±4.61396  |
| LI (kg) | 213   | 0.6104±0.14412   | 255   | 0.6130±0.14707   |
| SI(kg) | 213   | 9.1315±1.05708   | 255   | 9.1348±1.06245   |
| RW (kg) | 213   | 7.1634±0.79451   | 255   | 7.1755±0.78780   |
| OW (kg) | 213   | 3.6385±0.51115   | 255   | 3.6673±0.51533   |
| HW (kg) | 213   | 1.9730±0.28008   | 255   | 1.9816±0.28032   |
| LW (kg) | 213   | 6.5086±0.68182   | 255   | 6.5322±0.70268   |
| LT(kg) | 213   | 3.3238±0.40177   | 255   | 3.3412±0.39546   |
| KW(kg) | 213   | 1.2521±0.18142   | 255   | 1.2583±0.17756   |
| SW (kg) | 213   | 0.9036±0.17818   | 255   | 0.9080±0.18084   |
| BP (kg) | 213   | 0.3982±0.06107   | 255   | 0.3996±0.06006   |
| TW (kg) | 213   | 0.7026±0.15175   | 255   | 0.7026±0.15179   |
| OT (kg) | 213   | 1.4447±0.20326   | 255   | 1.4577±0.21152   |
| MFW(Kg) | 213   | 4.8448±1.07669   | 255   | 4.8787±1.10583   |
| SO(kg) | 213   | 2.4969±0.89609   | 255   | 2.5258±1.10853   |
| KFW(Kg) | 213   | 6.2017±2.01893   | 255   | 6.2885±2.06872   |
| GF (kg) | 213   | 0.8062±0.36322   | 255   | 0.8000±0.35013   |
| CL(cm) | 213   | 136.3697±7.52682 | 255   | 136.7598±7.39467 |
| CD(cm) | 213   | 63.7838±3.11876  | 255   | 63.9665±3.20093  |
| CCD(cm) | 213   | 64.5668±3.46723  | 255   | 64.8087±3.61591  |
| HLC(cm) | 213   | 49.5137±3.65334  | 255   | 49.4153±3.58996  |
| HLL(cm) | 213   | 44.6179±2.70399  | 255   | 44.6279±3.58996  |
| HLL(cm) | 213   | 78.0042±2.97953  | 255   | 78.1290±2.96754  |
| TMT(cm) | 213   | 18.1082±1.76469  | 255   | 18.1342±1.74472  |
| TL(cm) | 213   | 7.2878±0.75732   | 255   | 7.3126±0.76909   |
| BFT(cm) | 213   | 1.3142±0.45328   | 255   | 1.3197±0.46186   |
| FCR (%) | 213   | 61.7475±10.52322 | 255   | 62.1734±10.69373 |
| SpH    | 213   | 5.9675±0.36119   | 255   | 5.9675±0.35840   |
| PpH    | 213   | 5.4259±0.29706   | 255   | 5.4124±0.29315   |
| SF(Kg) | 213   | 4.5386±1.43806   | 255   | 4.5466±1.44030   |
| MBS    | 213   | 5.1556±0.71330   | 255   | 5.126±0.71532    |
| EMA    | 213   | 83.7820±12.18710 | 255   | 83.5498±12.33113 |
| Trait | Mean1 | SEM1 | Mean2 | SEM2 |
|-------|-------|------|-------|------|
| FCC   | 213   | 5.9434±0.9984 | 255   | 5.9409±1.01585 |
| FCI   | 213   | 83.3721±8.02517 | 255   | 83.2598±8.07216 |
| FCS   | 213   | 2.3582±0.82061  | 255   | 2.3031±0.83160  |

Traits: GW, Gross weight; CW, Carcass weight; DP, dressing percentage; NWB, net weight of bone; HW, head weight; FW, forepaw weight; WH, Weight heels; li, large intestine; SI, small intestine; RW, rumen weight; OW, omasum weight; HW, heart weight; LW, liver weight; LT, Lung, trachea; KW, kidney weight; SW, spleen weight; BP, bull penis; TW, testes weight; OT, ox tail; MFW, mesenteric fat weight; SO, Stomach oil; KFW, kidney fat weight; GF, Genital fat; CL, carcass length; CD, carcass depth; CCD, Carcass chest depth; HLC, hind leg circumference; HLW, hind leg width; HLL, hind leg length; TMT, thigh meat thickness; TL, thickness of loin; BFT, backfat thickness; FCR, fat coverage rate of carcass; SPH, slaughter PH; SF, shearing force; MBS, marbling score; EMA, Eye muscle area; LSM, least square mean; SE, standard error of mean.
Table 2. Genotypic frequencies for SNPs in *RNF128* and *RNF34* genes of different cattle populations

| Population       | RNF34 gene-3′UTR-588G>A | RNF128 gene I1-2380C>T |
|------------------|--------------------------|------------------------|
|                  | Number | Allele frequency | Genotype frequency | Number | Allele frequency | Genotype frequency |
| Chinese Simmental cattle | 255      | G (0.5882) | GG (0.2823) | 213      | T (0.5634) | TT (0.5305) |
|                   | A (0.4118) | GA (0.6118) | AA (0.1059) | C (0.4366) | TC (0.0657) | CC (0.4038) |
Table 3. RNF34 3’UTR (c.+588 G>A) association with the carcass and meat quality traits

| Trait      | RNF34 | Gentlytypes(3UTR-588G>A) |
|------------|-------|---------------------------|
|            | AA    |                           |
|            | N     | LSM | SE  | AG | LSM | SE  | GG | LSM | SE  |
| GW (Kg)    | 27    | 519.00 | 58.48 | 156 | 511.76 | 56.61 | 72 | 501.04 | 55.40 |
| CW (Kg)    | 27    | 275.85 | 35.15 | 156 | 270.81 | 35.66 | 72 | 265.22 | 33.15 |
| DP (%)     | 27    | 53.09 | 2.02 | 156 | 52.83 | 2.27 | 72 | 52.88 | 1.62 |
| NWB (Kg)   | 27    | 19.78 | 1.97 | 156 | 19.81 | 3.41 | 72 | 19.69 | 2.71 |
| HW (kg)    | 27    | 23.90 | 2.00 | 156 | 23.72 | 2.32 | 72 | 23.25 | 2.39 |
| FW (kg)    | 27    | 6.21  | 0.69 | 156 | 6.06  | 0.72 | 72 | 6.03  | 0.65 |
|WH(kg)     | 27    | 2.89  | 0.40 | 156 | 2.81  | 0.38 | 72 | 2.80  | 0.34 |
| PW(kg)     | 27    | 40.93 | 4.35 | 156 | 39.92 | 4.62 | 72 | 38.55 | 4.53 |
| LI (kg)    | 27    | 0.61  | 0.12 | 156 | 0.62  | 0.15 | 72 | 0.61  | 0.15 |
| SI(kg)     | 27    | 9.18  | 1.38 | 156 | 9.13  | 1.01 | 72 | 9.12  | 1.05 |
| RW (kg)    | 27    | 7.18  | 0.85 | 156 | 7.21  | 0.80 | 72 | 7.09  | 0.75 |
| OW (kg)    | 27    | 3.63  | 0.48 | 156 | 3.64  | 0.53 | 72 | 3.74  | 0.50 |
| HW (kg)    | 27    | 1.97  | 0.26 | 156 | 1.99  | 0.27 | 72 | 1.96  | 0.30 |
| LW (kg)    | 27    | 6.47  | 0.55 | 156 | 6.57  | 0.76 | 72 | 6.48  | 0.63 |
| LT(kg)     | 27    | 3.34  | 0.34 | 156 | 3.37  | 0.39 | 72 | 3.28  | 0.42 |
| KW(kg)     | 27    | 1.31a | 0.15 | 156 | 1.26ab | 0.19 | 72 | 1.23b | 0.15 |
| SW (kg)    | 27    | 0.85  | 0.18 | 156 | 0.92  | 0.18 | 72 | 0.91  | 0.18 |
| BP (kg)    | 27    | 0.41  | 0.06 | 156 | 0.41  | 0.06 | 72 | 0.39  | 0.05 |
| TW (kg)    | 27    | 0.68ab | 0.18 | 156 | 0.72a | 0.14 | 72 | 0.67b | 0.16 |
| OT (kg)    | 27    | 1.48  | 0.20 | 156 | 1.47  | 0.21 | 72 | 1.43  | 0.22 |
| MFW(Kg)    | 27    | 4.94  | 1.52 | 156 | 4.92  | 1.12 | 72 | 4.76  | 0.88 |
| SO(kg)     | 27    | 2.72  | 1.35 | 156 | 2.57  | 0.86 | 72 | 2.35  | 0.89 |
| KFW(Kg)    | 27    | 6.12  | 2.26 | 156 | 6.38  | 2.02 | 72 | 6.15  | 2.12 |
| GF (kg)    | 27    | 0.82  | 0.37 | 156 | 0.81  | 0.36 | 72 | 0.78  | 0.32 |
| CL(cm)     | 27    | 136.04 | 6.80 | 156 | 137.00 | 7.45 | 72 | 136.51 | 7.56 |
| CD(cm)     | 27    | 63.96 | 3.72 | 156 | 64.06 | 3.24 | 72 | 63.77 | 2.95 |
| CCD(cm)    | 27    | 65.89 | 4.13 | 156 | 64.82 | 3.64 | 72 | 64.39 | 3.32 |
| HLC(cm)    | 27    | 49.76 | 2.64 | 156 | 49.33 | 2.95 | 72 | 49.47 | 4.95 |
| HLV(cm)    | 27    | 45.02 | 2.25 | 156 | 44.45 | 2.75 | 72 | 44.87 | 2.89 |
| HLL(cm)    | 27    | 78.96 | 2.86 | 156 | 78.11 | 3.06 | 72 | 77.85 | 2.77 |
| TMT(cm)    | 27    | 18.06 | 1.73 | 156 | 18.22 | 1.61 | 72 | 17.97 | 2.02 |
| TL(cm)     | 27    | 7.15  | 0.69 | 156 | 7.36  | 0.77 | 72 | 7.28  | 0.79 |
| BFT(cm)    | 27    | 1.33  | 0.55 | 156 | 1.30  | 0.44 | 72 | 1.37  | 0.49 |
| FCR (%)    | 27    | 63.85 | 13.84 | 156 | 62.21 | 9.95 | 72 | 61.46 | 11.00 |
| SPH        | 27    | 5.90  | 0.36 | 156 | 5.97  | 0.27 | 72 | 5.99  | 0.35 |
| PPH        | 27    | 5.37ab | 0.30 | 156 | 5.39a | 0.27 | 72 | 5.48b | 0.33 |
| SF(Kg)     | 27    | 4.56  | 1.47 | 156 | 4.62  | 1.45 | 72 | 4.39  | 1.41 |
| MBS        | 27    | 5.11  | 0.70 | 156 | 5.09  | 0.69 | 72 | 5.21  | 0.77 |
| EMA        | 27    | 83.56 | 11.01 | 156 | 83.17 | 12.36 | 72 | 84.36 | 12.83 |
|   | FCC | 5.96 | 1.13 | 156 | 5.95 | 1.02 | 72 | 5.92 | 0.96 |
|---|-----|------|------|-----|------|------|----|------|------|
| FCI| 27  | 84.34| 8.70 | 156 | 82.93| 8.53 | 72 | 83.57| 6.78 |
| FCS| 27  | 2.15^a| 0.72 | 156 | 2.24^a| 0.84 | 72 | 2.50^b| 0.82 |
**Table 4. Association of RNF128 SNPs with carcass and meat quality traits in Simmental-cross steers**

| Trait      | RNF128 Genotypes(I1-2380C>T) |
|------------|-------------------------------|
|            | TT N LSM SE TC N LSM SE CC N LSM SE |
| GW (Kg)    | 113 507.31^a 56.85 14 542.21 36.79 86 499.65^A 53.57 |
| CW (Kg)    | 113 268.82^b 34.88 14 286.64^a 25.13 86 265.23^b 34.26 |
| DP (%)     | 113 52.92 2.05 14 52.82 2.04 86 52.98 1.79 |
| NWB (Kg)   | 113 19.88^b 3.34 14 21.14^a 2.01 86 19.37^b 2.93 |
| HW (kg)    | 113 23.47 2.46 14 24.43 1.67 86 23.31 2.15 |
| FW (kg)    | 113 6.07^a 0.71 14 6.49 0.60 86 5.95^A 0.67 |
| WH(kg)     | 113 2.80^A 0.38 14 3.13 0.47 86 2.77^A 0.33 |
| PW(kg)     | 113 39.54 4.84 14 40.71 3.90 86 38.75 4.31 |
| LI (kg)    | 113 0.60 0.12 14 0.60 0.14 86 0.62 0.17 |
| SI(kg)     | 113 9.13 1.09 14 9.46 1.18 86 9.09 0.99 |
| RW (kg)    | 113 7.13^a 0.81 14 7.66 0.68 86 7.13^a 0.77 |
| OW (kg)    | 113 3.64^a 0.51 14 3.98 0.58 86 3.59^A 0.48 |
| HW (kg)    | 113 1.99 0.31 14 2.01 0.15 86 1.94 0.26 |
| LW (kg)    | 113 6.56 0.71 14 6.55 0.59 86 6.44 0.65 |
| LT(kg)     | 113 3.31^A 0.40 14 3.64 0.40 86 3.30^A 0.39 |
| KW(kg)     | 113 1.26 0.20 14 1.29 0.15 86 1.23 0.16 |
| SW (kg)    | 113 0.91 0.18 14 0.89 0.16 86 0.90 0.18 |
| BP (kg)    | 113 0.40^b 0.06 14 0.43^a 0.07 86 0.39^b 0.06 |
| TW (kg)    | 113 0.70 0.14 14 0.75 0.18 86 0.71 0.16 |
| OT (kg)    | 113 1.43^a 0.21 14 1.57 0.14 86 1.44^a 0.19 |
| MFW(Kg)    | 113 4.88^b 1.13 14 5.43^a 1.01 86 4.71^b 0.99 |
| SO(kg)     | 113 2.51 0.85 14 2.67 0.97 86 2.45 0.95 |
| KFW(Kg)    | 113 6.22 2.12 14 5.87 1.84 86 6.23 1.93 |
| GF (kg)    | 113 0.80 0.33 14 0.86 0.41 86 0.81 0.39 |
| CL(cm)     | 113 136.11 7.92 14 138.46 4.15 86 136.37 7.44 |
| CD(cm)     | 113 63.74^a 3.33 14 65.75 2.87 86 63.52^a 2.78 |
| CCD(cm)    | 113 64.62^a 3.85 14 66.71 2.74 86 64.15^A 2.90 |
| HLC(cm)    | 113 49.22^a 3.14 14 51.32^b 2.85 86 49.60^b 4.29 |
| HLW(cm)    | 113 44.50 2.43 14 45.39 2.18 86 44.64 3.11 |
| HLL(cm)    | 113 77.87^A 3.03 14 80.61 2.98 86 77.76^A 2.74 |
| TMT(cm)    | 113 18.02^A 1.69 14 19.40 1.04 86 18.07^A 1.89 |
| TL(cm)     | 113 7.33^b 0.79 14 7.71^a 0.68 86 7.17^b 0.70 |
| BFT(cm)    | 113 1.30 0.46 14 1.39 0.37 86 1.32 0.46 |
| FCR (%)    | 113 62.16 11.46 14 65 11.44 86 60.68 8.95 |
| SPH        | 113 6.03^A 0.36 14 5.99^AB 0.32 86 5.89^B 0.35 |
| PPH        | 113 5.43 0.34 14 5.43 0.22 86 5.42 0.25 |
| SF(Kg)     | 113 4.48 1.41 14 4.52 1.76 86 4.62 1.44 |
| MBS        | 113 5.14 0.72 14 5.36 0.75 86 5.14 0.71 |
| EMA        | 113 83.60 12.48 14 84.00 9.94 86 83.99 12.25 |
| Trait | Unchanged | Change | Mean | SE | SF | SE |
|-------|-----------|--------|------|----|----|----|
| FCC   | 113       | 6.06   | 1.00 | 86 | 1.00 |
| FCI   | 113       | 84.02  | 7.02 | 86 | 82.78 |
| FCS   | 113       | 2.35   | 0.86 | 86 | 2.35 |

Traits: GW, Gross weight; CW, Carcass weight; DP, dressing percentage; NWB, net weight of bone; HW, head weight; FW, forepaw weight; WH, Weight heels; SI, small intestine; RW, rumen weight; OW, omasum weight; HW, heart weight; LW, liver weight; LT, Lung, trachea; KW, kidney weight; SW, spleen weight; BP, bull penis; TW, testes weight; OT, ox tail; MFW, mesenteric fat weight; SO, Stomach oil; KFW, kidney fat weight; GF, Genital fat; CL, carcass length; CD, carcass depth; CCD, Carcass chest depth; HLC, hind leg circumference; HLW, hind leg width; HLL, hind leg length; TMT, thigh meat thickness; TL, thickness of loin; BFT, backfat thickness; FCR, fat coverage rate of carcass; SPH, slaughter PH; PPH, PH after acid exhausted; SF, shearing force; MBS, marbling score; EMA, Eye muscle area; FCC, Flesh color (color card); FCI, Flesh (flesh-colored instrument); FCS, fat color score; LSM, least square mean; SE, standard error of mean.

**RNF04 gene 3′ UTR-588G>A**

**RNF128 gene II-2380C>T**

**Fig 1:** Identification of bovine *RNF34* gene 3′UTR-588G>A and *RNF128* gene II-2380C>T by sequencing and Restriction enzyme digestion analysis of PCR products.
Highlights

1. RNF34 3'UTR-588 G>A association with carcass and meat quality traits.

2. RNF128 I1-2380C>T association with carcass and meat quality traits.

[17]3. RNF34 and RNF128 might be important genetic factors that influence carcass and meat quality.