Polymorphisms in Fatty Acid Desaturase 2 Gene Are Associated with Milk Production Traits in Chinese Holstein Cows

Mingxun Li 1,2, Qi Song Gao 1, Mengqi Wang 1, Yan Liang 1, Yujia Sun 2, Zhi Chen 1,2, Huimin Zhang 1,2, Niel A. Karrow 3, Zhangping Yang 1,2 and Yongjiang Mao 1,2,*

1 Key Laboratory of Animal Genetics & Breeding and Molecular Design of Jiangsu Province, Yangzhou University, Yangzhou 225009, China; limingxun@live.com (M.L.); MZ120181011@yzu.edu.cn (Q.G.); mengqi.wang.1@ulaval.ca (M.W.); 15755081060@163.com (Y.L); chenzhijerom@163.com (Z.C.); minmin-911@163.com (H.Z.); yzp@yzu.edu.cn (Z.Y.)
2 Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou 225009, China; ysunshine30@outlook.com
3 Center for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON N1G 2W1, Canada; nkarrow@uoguelph.ca
* Correspondence: cattle@yzu.edu.cn

Received: 26 February 2020; Accepted: 10 April 2020; Published: 12 April 2020

Simple Summary: Searching for causative polymorphisms underlying the variability of milk production traits and then incorporating them into breeding programs are very effective ways to improve the efficiency and reliability of conventional dairy cattle breeding. Fatty acid desaturase 2 (FADS2) plays a pivotal role in the biosynthesis of polyunsaturated fatty acids. Previous studies provided evidence that FADS2 was one of the most downregulated genes during negative energy balance in the liver of postpartum dairy cattle. Genes involved in the energetic pathways may influence other production traits, such as protein, fat and milk yields. Therefore, in the present study, we investigated the common genetic variants of the FADS2 gene in Chinese Holstein cows. Our results provided direct evidence that FADS2 was an interesting candidate for selection to increase milk production and improve resistance against mastitis.

Abstract: This study investigated the single nucleotide polymorphisms (SNPs) of Fatty acid desaturase 2 (FADS2) gene and further explored their genetic effects on conventionally collected milk production traits in Chinese Holstein cows using 18,264 test-day records of 841 cows. One missense mutation c. 908 C > T (SNP site in the complementary DNA sequence), which caused an amino acid change from alanine to valine (294Ala > Val), and two 3’ untranslated region (UTR) SNPs, c.1571 G > A and c.2776 A > G were finally identified. The SNP c.908 C > T was significantly associated with test-day milk yield, fat percentage and 305-day milk, fat and protein yield. In particular, the T allele of the SNP c.908 C > T showed a significant association with decreased somatic cell score (SCS) in the investigated population. Significant relationship between the SNP c.1571 G > A and 305-day milk yield showed that genotype GG was linked to the highest milk yield. Substituting the allele G for A at the c.2776 A > G locus resulted in a decrease of protein percentage. Our results demonstrated that FADS2 was an interesting candidate for selection to increase milk production and improve resistance against mastitis.

Keywords: fatty acid desaturase 2; single nucleotide polymorphisms; milk production traits; somatic cell score
1. Introduction

Advances in the determination of genetic variants and chromosomal regions influencing economically important traits provide new opportunities for the improvement of milk production traits in dairy cattle [1]. Searching for causative polymorphisms underlying the variability of milk production traits and then incorporating them into breeding programs are very effective ways to increase the efficiency and reliability of conventional dairy cattle breeding [2]. Genome-wide association studies (GWAS) and candidate gene approach are two main strategies for studying the genetic architecture of complex traits [3]. Both approaches have their advantages and limitations. Broadly, genome-wide association studies involve scanning common variation encompassing the entire genome, and as such can pinpoint genes regardless of whether their functions were known [4], but it is expensive and resource intensive, while the candidate gene approach is more powerful and more straightforward for the genetic dissection of complex traits, but it is limited by its reliance on existing knowledge about the molecular mechanisms that contribute to phenotype [5]. At present, many candidate genes involved in the development of dairy cow mammary gland and lactation processes have been identified as affecting milk production and composition, such as diacylglycerol acyltransferase 1 (DGAT1), stearoyl-CoA desaturase (SCD), fatty acid-binding protein-4 (FABP4) and fatty acid desaturase 2 (FADS2) [6–9].

The FADS2 gene encodes a crucial enzyme of long-chain polyunsaturated fatty acids (LC-PUFAs) biosynthesis able to catalyze the introduction of double bonds at the sixth carbon atom in a large spectrum of fatty acids [10,11]. Loss of FADS2 expression in the FADS2-deficient mouse impeded the processing of essential fatty acids linoleic (C18:2n-6) and α-linolenic acid (C18:3n-3) to n-6/n-3 LC-PUFAs, demonstrating that FADS2 is the only enzyme that catalyzes this pivotal step [12]. The FADS2-deficient liver exhibited severe changes in the phospholipid-bilayer structures of subcellular membranes, which disturbed the maturation of transcription factor sterol regulatory element-binding protein (SREBP1c), and therefore perturbed lipid metabolism [13].

The bovine FADS2 gene has been proposed as a candidate gene influencing milk fatty acids composition [9,14,15], a primary aspect of milk nutritional quality [16]. Bovine FADS2 is comprised of 12 exons encoding 359 amino acid chains and is located on bovine chromosome 29 (BTA29) in the region 29q17-29q18, a region associated with fatty acid content and respiratory diseases susceptibility. In addition to FADS2, FADS1 and FADS3 are also clustered at the same genomic locus. Multiple single nucleotide polymorphisms (SNPs) have been determined in the bovine FADS-gene cluster. Ibeagha-Awemu et al. described the genetic diversity within FADS1 and FADS2 genes, and demonstrated significant associations between three SNPs with two milk n-6 LC-PUFAs, dihomo-gamma-linolenic acid (DGLA, C20:3n-6) and arachidonic acid (ARA, C20:4n-6), and one n-3 LC-PUFA, eicosapentaenoic acid (EPA, C20:5n-3) of Canadian Holstein cows [9]. Takahashi et al. reported that the SNP rs211580559 in exon 7 of the FADS2 gene had a significant effect on intramuscular linoleic acid (LA, C18:2n-6) composition in Japanese Black Steers [17]. In a transcriptomic study, Wang et al. produced evidence that FADS2 was a strong candidate gene related to intramuscular fat deposition [18]. Recently, Proskura et al. revealed significant relationships between the SNP rs209202414 in intron 3 of the FADS2 gene and milk eicosatrienoic acid (ETA, C20:3n-3) and docosadienoic acid (DDA, C22:2n-6) in Jersey cows [19].

The precedent results revealed the associations of the FADS2 SNPs with milk fat traits in dairy cattle. However, much remains unknown about the effects of the FADS2 polymorphisms on routinely collected milk production traits [9,19]. Previous studies provided evidence that FADS2 was one of the most downregulated genes during negative energy balance in the liver of postpartum dairy cattle [20,21]. It is reasonable to assume that genes involved in the energetic pathways may influence other production traits, such as protein, fat and milk yields, which represent the principal breeding objectives of the current selection programs. Therefore, in the present study, we investigated the common genetic variants of the FADS2 gene in Chinese Holstein cows, explored the effects of genetic polymorphisms on milk production traits and further evaluated the average effects of allele substitution.
2. Materials and Methods

2.1. Animals and Milk Production Records

From June 2010 to December 2014, a total of 20,556 milk samples from 2558 lactations (lactations 1 to 5) of 841 cows reared in Yancheng city of China, were collected during monthly test-day milk recording. These cows were daughters of 162 sires, with 2 to 43 daughters per sire, housed in free stalls, milked three times per day and fed a total mixed ration (TMR). Milk samples were analyzed in the Dairy Herd Improvement laboratory of Shanghai Dairy Cattle Breeding Center using Milko-Scan FT6000 (Foss Electric, Denmark). Values of somatic cell count (SCC) were determined with a Fossmatic 5000 cell counter (Foss Electric, Denmark). Somatic cell score (SCS) were calculated using the formula: \[ SCS = \log_2(\frac{SCC}{100,000}) + 3 \] [22]. Only data with SCC between $1 \times 10^3$ and $5 \times 10^5$ were kept for further analyses. Following this criterion, a total of 18,264 test-day records were contained in this study.

2.2. DNA Extraction and SNP Genotyping

Blood samples were collected from above mentioned 841 Chinese Holstein cows. Genomic DNA was extracted from the white blood cells using a standard phenol-chloroform procedure with a slight modification in centrifugation speed and time [23,24]. The DNA concentration was measured by NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and evaluated for integrity by 1% agarose gel electrophoresis.

According to previous studies [9], three primer pairs were designed to screen genetic polymorphisms situated in the exon 7 and 3’ untranslated region (UTR) of \textit{FADS2} in Chinese Holstein cows (Table S1). DNA samples from 20 cows were utilized for PCR amplification and sequencing to identify SNPs. The PCR reactions were carried out in a PTC-200 DNA Engine cycler (Bio-Rad, CA, USA) using an optimal annealing temperature (Table S1) determined by a PCR temperature gradient. Twenty microliters of PCR amplicons were sequenced in Shanghai Sangon Company (Shanghai, China) using an ABI PRISM 3700 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). After sequencing, the forward and reverse sequences were assembled using the ContigExpress module in Vector NTI Advance 11.5 (Invitrogen, Carlsbad, CA, USA) to discover novel SNPs. Animal genotyping for the discovered SNPs was carried out with the MassARRAY system (Sequenom Inc., San Diego, CA, USA) [25], which uses a matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (MALDI-TOF).

2.3. Statistical Analyses

The allele and genotype frequencies were directly calculated. The linkage disequilibrium ($r^2$) were measured for all SNP’s pairs using the SHEsis software [26]. Association studies were carried out using the following linear model:

\[ y_{ijklmn} = \mu + \text{Sire}_i + T_j + \text{parity}_k + \text{DIM}_l + G_m + e_{ijklmn} \]  

(1)

where $y$ is the phenotypic value for the analyzed trait; $\mu$ is the overall mean; Sire$_i$ is the fixed effect of the $i$th sire; $T_j$ is the fixed effect of tested year and season of calving; parity$_k$ is the fixed effect of the parity (three classes: parity 1, 2 and 3–5); $\text{DIM}_l$ is the fixed effect of the stage of lactation (10 levels of 30 d each); $G_m$ is the fixed effect of the $m$th genotype; and $e_{ijklmn}$ is the random residual.

The allelic additive ($a$), dominance ($d$) and substitution ($\alpha$) effects were estimated using the equation: $a = (AA - BB)/2$, $d = AB - (AA + BB)/2$ and $\alpha = a + d (q - p)$, where AA and BB represent the phenotypic value of two homozygous genotypes, AB represents the phenotypic value of heterozygous genotype and $p$ and $q$ are the corresponding allele frequencies of A and B [27].
3. Results

3.1. SNP Detection

Investigation of the exon 7 and 3’ UTR sequences of FADS2 evidenced the presence of three SNPs, including one SNP c.908 C > T (rs211580559) located within the protein coding sequence, and two SNPs in the 3’ UTR, c.1571 G > A (rs210169303) and c.2776 A > G (rs207932003). Details of the three segregating SNPs including the location on BTA29 relative to FADS2 were illustrated in Figure 1. The SNP c.908 C > T was a missense mutation that caused an amino acid change from alanine to valine (p. Ala294Val). TargetScan analysis suggested that the SNP c.1571 G > A was located within the binding site for bta-miR-744. The presence of the minor allele A abolished the ability of miR-744 to bind FADS2.

![Figure 1. Schematic diagram of the fatty acid desaturase 2 gene with the localization of the three identified single nucleotide polymorphisms (SNPs).](image_url)

3.2. Genetic Diversity Analyses

The allele and genotype frequencies are presented in Table 1. The minor allele frequency (MAF) for the three segregating SNPs ranged from 0.107 (c.1571 G > A) to 0.407 (c.908 C > T). Linkage disequilibrium was measured by $r^2$ between all the SNP pairs. The $r^2$ values between c.908 C>T and c.1571 G > A, c.908 C > T and c.2776 A > G and c.1571 G > A and c.2776 A > G were 0.054, 0.152 and 0.024, respectively. No significant evidence of linkage disequilibrium was detected between the loci in the investigated population.

Table 1. The allele and genotype frequencies, and Hardy–Weinberg equilibrium test for the SNPs in the fatty acid desaturase 2 gene in Chinese Holstein cows.

| Locus       | Allele | Allele Frequency | Genotype | Genotype Frequency | Observed Count | Expected Count |
|-------------|--------|------------------|----------|-------------------|----------------|---------------|
| c.908 C > T | C      | 0.593            | CC       | 0.351             | 295            | 295.48        |
|             | T      | 0.407            | CT       | 0.484             | 407            | 406.31        |
|             |        |                  | TT       | 0.165             | 139            | 139.48        |
| c.1571 G > A| G      | 0.893            | GG       | 0.806             | 678            | 670.63        |
|             | A      | 0.107            | AG       | 0.174             | 146            | 160.74        |
|             |        |                  | AA       | 0.020             | 17             | 9.63          |
| c.2776 A > G| A      | 0.819            | AA       | 0.668             | 562            | 563.65        |
|             | G      | 0.181            | AG       | 0.301             | 253            | 249.69        |
|             |        |                  | GG       | 0.031             | 26             | 27.65         |
3.3. Associations of SNPs with Milking Traits and Somatic Cell Score

The effects of the investigated FADS2 SNPs on milk production traits are presented in Figure 2 and Table S2. The SNP c.908 C > T, a missense mutation within the FADS2 gene, was significantly associated with test-day milk yield, fat percentage and 305-day milk, fat and protein yield (Figure 2A–F). In particular, the TT cows yielded more milk, fat and protein for the entire 305-d lactation than CC animals. The milk from the CC cows was significantly richer in test-day fat (+1.19%) compared with CT genotype (4.26 vs. 4.21, Table S2). In the case of test-day milk yield, 305-day milk yield, and 305-day protein yield, the SNP c.908 C > T exhibited overdominance with the heterozygous genotype was greater than the two homozygous, confirmed by a [d/a] ratio higher than the threshold value of 1.2 [28]. This could be a result of heterozygotes experiencing advantageous effects, which is consistent with the highest genotype frequency exhibited by CT heterozygotes. A significant association was also observed between the c.908 C > T and SCS. In this case, the genotype TT was associated with the lowest SCS (Figure 2F). This result is supported by allele substitution analysis whereby substituting the allele T for C linked to a decrease of 0.05 of milk SCS to the allele T (Table S2). Protein sequence alignment of FADS2 showed that Val 294 was highly conserved among mammalian species including cattle, sheep, goat, pig, horse, human, rat, mouse, gorilla and dog (Figure 3).

![Figure 2](image-url)

**Figure 2.** The effects of investigated fatty acid desaturase 2 SNPs on milk production traits. (A–F) The SNP c.908 C > T was significantly associated with test-day milk yield, fat percentage and 305-day milk, fat and protein yield, and somatic cell score (n = 6553 for CC; n = 8667 for CT; and n = 3024 for TT). (G) The GG genotype at the c.1571 G > A locus was linked to the highest 305-day milk yield (n = 14578 for GG; n = 3313 for GA; and n = 373 for AA). (H) Substituting allele G for A at the c.2776 A > G locus resulted in a decrease of protein percentage (n = 12110 for AA; n = 5619 for AG; and n = 518 for GG). Different lowercase letters indicate significant differences between genotypes (p < 0.05); Different uppercase letters indicate significant differences between genotypes (p < 0.01). Data shown are means ± SE. The dotted line represents the overall mean.
whereas the G allele frequency at the c.1571G

4. Discussion

FADS2 plays a pivotal role in the biosynthesis of polyunsaturated fatty acids [29–32]. Genome-wide association studies (GWAS) have confirmed the effects of FADS2 genetic variations on diseases related to lipid metabolism [33,34]. In domestic animals, Zhu et al. reported that single nucleotide polymorphisms (SNPs) in FADS2 affected essential fatty acid content in muscle and the growth rate of early developing chickens [35]. Matsumoto et al. revealed that the FADS2 g.-823G > A had significant effects on several beef quality traits including beef marbling score [36]. Boschetti et al. demonstrated that FADS1/FADS2 genotypes are related to desaturating ability, with a significant impact on the PUFA content of chicken breast meat [37]. These findings confirmed that the FADS2 gene is a strong candidate gene affecting the fatty acid composition.

In the present study, we investigated the genetic diversity of three identified SNPs in the FADS2 gene and further explored their genetic effects on conventionally collected milk production traits in Chinese Holstein cows. Genotype distribution demonstrated that the allele frequencies at the c.908 C > T locus were in agreement with previous findings carried out in Canadian Holstein cows [9], whereas the G allele frequency at the c.1571G > A locus was slightly higher in our investigated Chinese Holstein population (0.893 vs. 0.731) [9]. This might be attributed to several reasons including different breeding objectives that favored the G allele in Chinese Holstein cows, and smaller population size or simply the greater number of farms involved in the previous study.

Significant associations were recorded between FADS2 polymorphisms and milk production traits. The SNP c.908 C > T was associated with higher milk, fat and protein yields, confirming an important role of the FADS2 gene in affecting milk production traits. Moreover, the T allele of the SNP c.908 C > T showed a significant association with decreased somatic cell score (SCS) in the investigated population. Currently, the dairy cows have been subjected to intense selection for milk yield which has adversely affected cow health [38,39]. The decreased genetic trend in cow health is becoming a major concern and the focus of selective breeding has shifted from a production-oriented to a more balanced breeding goal. Traits encompassing health, such as mastitis resistance, have now been incorporated into selection programs. Genetic evaluation and selection for decreased SCS can decrease mastitis incidence in dairy
cattle populations [39]. In previous studies, Vesna et al. have described that α-linoleic acid (ALA) supplementation decreased somatic cell count of dairy goats [40]. Greco et al. reported that altering the dietary n-6 to n-3 ratio influenced cow lactation performance and SCC to an LPS challenge [41]. Given these findings and the anti-inflammatory effects of n-3 fatty acids [42], it is reasonable to assume that the genes associated with n3/n6 PUFA profiles may have potential effects on immune responses and somatic cell count.

The SNP c.908 C > T was a missense mutation that caused an amino acid change from alanine to valine (294Ala > Val). Multiple alignments of FADS2 protein sequences demonstrated that Val 294 was highly conserved among mammalian species, implying its importance for FADS2 protein function. Takahashi et al. reported that the SNP c.908 C > T had a significant effect on intramuscular linoleic acid content in Japanese Black Steers [17]. However, in another study, this SNP has not exhibited a functional consequence, with no significant effects on any of the investigated milk fatty acid profiles in dairy cattle [9]. The Val and Ala are both hydrophobic amino acids with aliphatic side chain groups. This replacement in amino acids does not appear to cause the structural and functional change of FADS2 protein. Therefore, the biological mechanism of how this missense mutation affected milk production traits requires to be further elucidated.

The SNP c.1571 G > A resulted in a decrease of 305-day milk yield. In Canadian Holstein cows, this SNP has been associated with milk n-6 fatty acids, C18:2n10t12c and C18:2n6tt, before FDR correction [9]. However, in the present study, no significant association was found between the SNP c.1571 G > A and any of fat related traits, such as test-day fat content and 305-day fat yield. The SNP c.1571 G > A is located in the 3' UTR of FADS2 gene. There is conclusive evidence that 3' UTR sequences participated in gene expression regulation through different molecular mechanisms, including miRNA binding, polyadenylation and RNA stability [43]. Bioinformatics analysis suggested that the presence of the minor allele A abolished the ability of miR-744 to bind FADS2. These findings provide additional support to the proposition of FADS2 as a candidate gene for further research to unravel the mechanisms by which it influences milk production traits.

RNA-seq analysis showed that FADS2 was the most significantly downregulated gene in the liver of severe negative energy balance cows, indicating that FADS2 is a potential gene that may play a crucial role in metabolic adaptations to negative energy balance in high-yielding dairy cows [21]. Given that the milk synthesis is energetically costly [44,45], it is reasonable to hypothesize that genes involved in the energetic pathways can also affect milk production traits, such as protein, fat and milk yields. This relationship is partially confirmed by previous findings that the alanine variant of the DGAT1 p.Lys232Ala polymorphism and the tyrosine variant of the GHR p.Phe279Tyr polymorphism that were reported to have favorable effects on effective energy balance accumulating throughout the lactation period are associated with increased milk production [46–48]. These findings may explain why FADS2 polymorphisms were significantly associated with milk production traits.

5. Conclusions

To the best of our knowledge, this is the first study to estimate the effects of FADS2 polymorphisms on milk production traits in Chinese Holstein cows. Our results provided direct evidence that FADS2 was an interesting candidate for selection to increase milk production and improve resistance against mastitis. Specifically, the TT genotype at the c.908C > T locus was the most desirable to select for animals producing higher quality and healthier milk because that TT genotype was significantly associated with higher 305-day milk, fat and protein yield and a lower SCS. Further experimentations are required to validate the role of identified SNPs on milk production traits in other populations and breeds before applying them in gene-assisted selection in Holstein cow.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2615/10/4/671/s1, Table S1: The primers used for SNPs identification of bovine FADS2 gene. Table S2: Effects of FADS2 gene SNPs on milk production traits.
Author Contributions: Conceptualization, Y.M.; data curation, M.W. and H.Z.; formal analysis, M.L.; funding acquisition, M.L. and Y.M.; investigation, Y.S. and Z.C.; resources, Z.Y. and Y.M.; supervision, Z.Y.; validation, Q.G. and Y.L.; writing—original draft, M.L.; writing—review and editing, N.A.K. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by National Natural Science Foundation of China (31972555, 31702080, 31872324), Natural Science Foundation of the Jiangsu Higher Education Institutions of China (18KJA230003, 17KJB230005), and China Postdoctoral Science Foundation (2018M630614).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Macciotta, N.P.; Mele, M.; Conte, G.; Serra, A.; Cassandro, M.; Dal Zotto, R.; Borlino, A.C.; Pagnacco, G.; Secchiari, P. Association between a polymorphism at the stearoyl CoA desaturase locus and milk production traits in Italian Holsteins. *J. Dairy Sci.* 2008, 91, 3184–3189. [CrossRef] [PubMed]
2. Selvaggi, M.; Dario, C.; Normanno, G.; Celano, G.V.; Dario, M. Genetic polymorphism of STAT5A protein: Relationships with production traits and milk composition in Italian Brown cattle. *J. Dairy Res.* 2009, 76, 441–445. [CrossRef] [PubMed]
3. Amos, W.; Driscoll, E.; Hoffman, J. Candidate genes versus genome-wide associations: Which are better for detecting genetic susceptibility to infectious disease? *Proc. R. Soc. B Biol. Sci.* 2011, 278, 1183–1188. [CrossRef] [PubMed]
4. Hayes, B.J.; Bowman, P.J.; Chamberlain, A.; Goddard, M. Invited review: Genomic selection in dairy cattle: Progress and challenges. *J. Dairy Sci.* 2009, 92, 433–443. [CrossRef] [PubMed]
5. Zhu, M.; Zhao, S. Candidate gene identification approach: Progress and challenges. *Int. J. Biol. Sci.* 2007, 3, 420. [CrossRef]
6. Tzompa-Sosa, D.A.; van Valenberg, H.; Van Aken, G.; Bovenhuis, H. Milk fat triacylglycerols and their relations with milk fatty acid composition, DGAT1 K232A polymorphism, and milk production traits. *J. Dairy Sci.* 2016, 99, 3624–3631. [CrossRef]
7. Nafikov, R.; Schoonmaker, J.; Korn, K.; Noack, K.; Garrick, D.; Koehler, K.; Minick-Bormann, J.; Reecy, J.; Spurlock, D.; Beitz, D. Association of polymorphisms in solute carrier family 27, isoform A6 (SLC27A6) and fatty acid-binding protein-3 and fatty acid-binding protein-4 (FABP3 and FABP4) with fatty acid composition of bovine milk. *J. Dairy Sci.* 2013, 96, 6007–6021. [CrossRef]
8. Rincon, G.; Islas-Trejo, A.; Castillo, A.R.; Bauman, D.E.; German, B.J.; Medrano, J.F. Polymorphisms in genes in the SREBP1 signalling pathway and SCD are associated with milk fatty acid composition in Holstein cattle. *J. Dairy Sci.* 2012, 79, 66–75. [CrossRef]
9. Ibeagha-Awemu, E.M.; Akwanji, K.A.; Beaudoin, F.; Zhao, X. Associations between variants of FADS genes and omega-3 and omega-6 milk fatty acids of Canadian Holstein cows. *BMC Genet.* 2014, 15, 25. [CrossRef]
10. Park, W.J.; Kothapalli, K.S.; Lawrence, P.; Tyburczy, C.; Brenna, J.T. An alternate pathway to long-chain polyunsaturates: The FADS2 gene product Delta8-desaturates 20:2n-6 and 20:3n. *J. Lipid Res.* 2009, 50, 1195–1202. [CrossRef]
11. Nakamura, M.T.; Nara, T.Y. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu. Rev. Nutr.* 2004, 24, 345–376. [CrossRef] [PubMed]
12. Stoffel, W.; Holz, B.; Jenke, B.; Binczek, E.; Günter, R.H.; Kiss, C.; Karakesisoglou, I.; Thevis, M.; Weber, A.A.; Arnhold, S. Δ6-Desaturase (FADS2) deficiency unveils the role of ω3- and ω6-polyunsaturated fatty acids. *EMBO J.* 2008, 27, 2281–2292. [CrossRef] [PubMed]
13. Stoffel, W.; Hammels, I.; Jenke, B.; Binczek, E.; Schmidt-Soltau, I.; Brodesser, S.; Odenthal, M.; Thevis, M. Obesity resistance and deregulation of lipogenesis in Δ6-fatty acid desaturase (FADS2) deficiency. *EMBO Rep.* 2014, 15, 110–120. [CrossRef] [PubMed]
14. Xie, L.; Innis, S.M. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. *J. Nutr.* 2008, 138, 2222–2228. [CrossRef]
15. Li, M.; Lu, X.; Gao, Q.; Wang, M.; Arbab, A.A.I.; Sun, Y.; Chen, Z.; Zhang, H.; Karrow, N.A.; Yang, Z. A Functional 3′ UTR Polymorphism of FADS2 Affects Cow Milk Composition through Modifying Mir-744 Binding. *Animals* 2019, 9, 1090. [CrossRef]

16. Soyeurt, H.; Dardenne, P.; Gillon, A.; Croquet, C.; Vanderick, S.; Mayeres, P.; Bertozzi, C.; Gengler, N. Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. *Curr. Opin. Lipidol.* 2010, 21, 64–69. [CrossRef]

17. Takahashi, H.; Hayashi, M.; Ushizawa, K.; Nishino, K.; Haga, Y.; Saito, T.; Fujimori, Y.; Iwama, N.; Takeda, H.; Komatsu, M. Association of bovine fatty acid desaturase 2 gene single-nucleotide polymorphisms with intramuscular fatty acid composition in Japanese Black Steers. *Open J. Anim. Sci.* 2016, 6, 105–115. [CrossRef]

18. Wang, X.; Zhang, Y.; Zhang, X.; Wang, D.; Jin, G.; Li, B.; Xu, F.; Cheng, J.; Zhang, F.; Wu, S. The comprehensive liver transcriptome of two cattle breeds with different intramuscular fat content. *Biochem. Biophys. Res. Commun.* 2017, 490, 1018–1025. [CrossRef] [PubMed]

19. Proskura, W.S.; Liput, M.; Soborski, D.; Sobek, Z.; Yu, Y.H.; Cheng, Y.H.; Dybus, A. The effect of polymorphism in the FADS2 gene on the fatty acid composition of bovine milk. *Arch. Anim. Breed.* 2019, 62, 547–555. [CrossRef] [PubMed]

20. Fatima, A.; Waters, S.; O’Boyle, P.; Seoighe, C.; Morris, D.G. Alterations in hepatic mRNA expression during negative energy balance in postpartum dairy cattle. *BMC Genom.* 2014, 15, 28. [CrossRef]

21. McCabe, M.; Waters, S.; Morris, D.; Kenny, D.; Lynn, D.; Creevey, C. RNA-seq analysis of differential gene expression in liver from lactating dairy cows divergent in negative energy balance. *BMC Genom.* 2012, 13, 193. [CrossRef]

22. Wiggans, G.; Shook, G. A lactation measure of somatic cell count. *J. Dairy Sci.* 1987, 70, 2666–2672. [CrossRef]

23. Sambrook, J.; Russell, D.W. Purification of nucleic acids by extraction with phenol: Chloroform. *Cold Spring Harb. Protoc.* 2006, 1, pdb-prot4455. [CrossRef]

24. Li, M.; Sun, X.; Hua, L.; Lai, X.; Lan, X.; Lei, C.; Zhang, C.; Qi, X.; Chen, H. SIRT1 gene polymorphisms are associated with growth traits in Nanyang cattle. *Mol. Cell. Probes* 2013, 27, 215–220. [CrossRef]

25. Gabriel, S.; Ziaugra, L.; Tabbaa, D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr. Protoc. Hum. Genet.* 2009, 60, 2.12. 11–12.12. 18. [CrossRef]

26. Yong, Y.; Lin, H. SHEssis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res.* 2005, 15, 97. [CrossRef]

27. Falconer, D.; Mackay, T. *Introduction to Quantitative Genetics*, 4th ed.; Longman: Harlow, UK, 1996.

28. Stuber, C.W.; Edwards, M.; Wendel, J.F. Molecular Marker-Facilitated Investigations of Quantitative Trait Loci in Maize. II. Factors Influencing Yield and its Component Traits. *Crop Sci.* 1987, 27, 639–648. [CrossRef]

29. Merino, D.M.; Johnston, H.; Clarke, S.; Roke, K.; Nielsen, D.; Badawi, A.; El-Sohemy, A.; Ma, D.W.; Mutch, D.M. Polymorphisms in FADS1 and FADS2 alter desaturase activity in young Caucasian and Asian adults. *Mol. Genet. Metab.* 2011, 103, 171–178. [CrossRef]

30. Vaittinen, M.; Walle, P.; Kuosmanen, E.; Männistö, V.; Käkelä, P.; Ågren, J.; Schwab, U.; Pihlajamäki, J. FADS2 genotype regulates delta-6 desaturase activity and inflammation in human adipose tissue. *J. Lipid Res.* 2016, 57, 56–65. [CrossRef]

31. Lattka, E.; Illig, T.; Koletzko, B.; Heinrich, J. Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. *Curr. Opin. Lipidol.* 2010, 21, 64–69. [CrossRef]

32. Koletzko, B.; Reischl, E.; Tanjung, C.; Gonzalez-Casanova, I.; Ramakrishnan, U.; Meldrum, S.; Simmer, K.; Heinrich, J.; Demmelmaier, H. FADS1 and FADS2 Polymorphisms Modulate Fatty Acid Metabolism and Dietary Impact on Health. *Ann. Rev. Nutr.* 2019, 39, 21–44. [CrossRef]

33. Gillingham, L.G.; Harding, S.V.; Rideout, T.C.; Yurkova, N.; Cunnane, S.C.; Eck, P.K.; Jones, P.J. Dietary oils and FADS1-FADS2 genetic variants modulate [13C] α-linolenic acid metabolism and plasma fatty acid composition. *Am. J. Clin. Nutr.* 2012, 97, 195–207. [CrossRef]

34. Illig, T.; Gieger, C.; Zhai, G.; Römisch-Margl, W.; Wang-Sattler, R.; Prehn, C.; Altmaier, E.; Kastenmüller, G.; Kato, B.S.; Mewes, H.W. A genome-wide perspective of genetic variation in human metabolism. *Nat. Genet.* 2010, 42, 137. [CrossRef]

35. Zhu, S.; Tian, Y.; Zhang, S.; Chen, Q.; Wang, Q.; Han, R.; Kang, X. Adjacent SNPs in the transcriptional regulatory region of the FADS2 gene associated with fatty acid and growth traits in chickens. *Genet. Mol. Res. 2014, 13, 3329–3336. [CrossRef]
36. Matsumoto, H.; Nogi, T.; Tabuchi, I.; Oyama, K.; Mannen, H.; Sasazaki, S. The SNPs in the promoter regions of the bovine FADS2 and FABP4 genes are associated with beef quality traits. *Livest. Sci.* **2014**, *163*, 34–40. [CrossRef]

37. Boschetti, E.; Bordoni, A.; Meluzzi, A.; Castellini, C.; Dal Bosco, A.; Sirri, F. Fatty acid composition of chicken breast meat is dependent on genotype-related variation of FADS1 and FADS2 gene expression and desaturating activity. *Animal* **2016**, *10*, 700–708. [CrossRef]

38. Walsh, S.; Buckley, F.; Berry, D.; Rath, M.; Pierce, K.; Byrne, N.; Dillon, P. Effects of breed, feeding system, and parity on udder health and milking characteristics. *J. Dairy Sci.* **2007**, *90*, 5767–5779. [CrossRef]

39. Miglior, F.; Fleming, A.; Malchiodi, F.; Brito, L.F.; Martin, P.; Baes, C.F. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. *J. Dairy Sci.* **2017**, *100*, 10251–10271. [CrossRef]

40. Vesna, G.; Kompan, D. Milk yield, milk composition and somatic cell count of dairy goats given n-3 unsaturated fatty acids diet supplement. *Acta Vet-Beograd* **2012**, *62*, 281–287. [CrossRef]

41. Greco, L.; Neto, J.N.; Pedrico, A.; Ferrazza, R.; Lima, F.; Bisinotto, R.; Martinez, N.; Garcia, M.; Ribeiro, E.; Gomes, G. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on performance and inflammatory responses to a lipopolysaccharide challenge in lactating Holstein cows. *J. Dairy Sci.* **2015**, *98*, 602–617. [CrossRef]

42. Im, D.S. Omega-3 fatty acids in anti-inflammation (pro-resolution) and GPCRs. *Prog. Lipid Res.* **2012**, *51*, 232–237. [CrossRef]

43. Szostak, E.; Gebauer, F. Translational control by 3′-UTR-binding proteins. *Brief. Funct. Genom.* **2012**, *12*, 58–65. [CrossRef]

44. Council, N.R. *Nutrient Requirements of Dairy Cattle: 2001*; National Academies Press: Washington, DC, USA, 2001.

45. Mackle, T.; Kay, J.; Auldist, M.; McGibbon, A.; Philpott, B.; Baumgard, L.; Bauman, D. Effects of abomasal infusion of conjugated linoleic acid on milk fat concentration and yield from pasture-fed dairy cows. *J. Dairy Sci.* **2003**, *86*, 644–652. [CrossRef]

46. Oikonomou, G.; Angelopoulou, K.; Arsenos, G.; Zygiyiannis, D.; Banos, G. The effects of polymorphisms in the DGAT1, leptin and growth hormone receptor gene loci on body energy, blood metabolic and reproductive traits of Holstein cows. *Anim. Genet.* **2009**, *40*, 10–17. [CrossRef]

47. Grisart, B.; Coppieters, W.; Farnir, F.; Karim, L.; Ford, C.; Berzi, P.; Cambisano, N.; Mni, M.; Reid, S.; Simon, P. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res.* **2002**, *12*, 222–231. [CrossRef]

48. Blott, S.; Kim, J.J.; Moisio, S.; Schmidt-Küntzel, A.; Cornet, A.; Berzi, P.; Cambisano, N.; Ford, C.; Grisart, B.; Johnson, D. Molecular dissection of a quantitative trait locus: A phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *Genetics* **2003**, *163*, 253–266.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).