DIACYLGLYCEROL ACYLTRANSFERASE 1 *(DGAT1)*, STEAROYL-COA DESATURASE 1 *(SCD1)*, AND FATTY ACID SYNTHASE *(FASN)* GENOTYPES AND THEIR ASSOCIATION WITH FAT PERCENTAGE AND MAJOR FATTY ACIDS IN COLOSTRUM AND MILK FROM HOLSTEIN × AUSTRALIAN FRIESIAN SAHIWAL COWS

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ABSTRACT

This study analyzed the association of bovine lipogenic gene markers – diacylglycerol acyltransferase 1 (*DGAT1*), stearoyl-CoA desaturase 1 (*SCD1*), and fatty acid synthase (*FASN*), with fat percentage and major fatty acids (myristic acid C14:0, palmitic acid C16:0, oleic acid C18:1n-9, and linoleic acid C18:2n-6) in 180 colostrum and milk samples collected from 45 Holstein × Australian Friesian Sahiwal (AFS) cows in a local dairy farm in Bay, Laguna. Genotypes for *DGAT1* (AK, KK), *SCD1* (AA, AV, VV), and *FASN* (CT, TT) were determined through polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) using DNA extracted from hair follicles. The AK genotype of *DGAT1* gene was associated (P<0.05) with higher fat content in colostrum and higher C14:0 in milk than for the KK genotype. The AV genotype of *SCD1* gene was associated (P<0.05) with the highest fat content and C18:1n-9 in colostrum and highest C16:0 and C18:0 in milk, compared to AA or VV genotypes. The CT genotype of the *FASN* gene was associated (P<0.05) with higher C14:0 in colostrum compared to CC genotype. This study showed polymorphisms in *DGAT1*, *SCD1*, and *FASN* genotypes and their significant associations with fat content and some major fatty acids in colostrum and milk.

Keywords: Colostrum and milk fats, fatty acid, gene markers, Holstein × AFS cows

INTRODUCTION

Fat percentage and fatty acid (FA) composition are important measures of the nutritional quality of milk fats from dairy cows. These may not only be used to improve the manufacturing and processing of dairy products (Timlin *et al.* 2021) but also provide a better understanding of their possible effects on human cardiovascular health (Chen and Liu, 2020).

While milk FAs may be affected by breed, stage of lactation, diet, and season, the genetic variation found in fat percentage and FA composition traits can be used for their improvement by selective breeding (Sanjayaranj *et al*., 2023). However, the high costs of FA

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analyses can limit their application in traditional selection programs. Many researchers have thus investigated the influence of lipogenic genes and their use in marker assisted selection (MAS) programs, where gene markers may improve the accuracy of selection for superior breeding stock for milk production (Mahmoudi and Rashidi, 2023).

Examples of lipogenic genes coding for key enzymes of lipid metabolism in the mammary glands and found to affect bovine milk yield and composition, and FA composition include diacylglycerol acyltransferase 1 (*DGAT1*), stearoyl-CoA desaturase (*SCD1*), and fatty acid synthase (*FASN*). DGAT1 is a key enzyme that catalyzes the last step in triglyceride synthesis (Grisart *et al.,* 2004) and had been compared for 38 *Bos indicus* and *Bos taurus* breeds (Kaupe *et al*., 2004). SCD1 is a rate-limiting enzyme responsible for the conversion of saturated FAs into monounsaturated FAs (Ntambi and Miyazaki, 2004) and was reported in Italian Holstein Friesian (Mele *et al.,* 2007). FASN is an enzyme that catalyzes the *de novo* synthesis of fatty acids in cells (Kale *et al.*, 2021) and had been compared for Japanese Black cattle, Holstein, Angus, and Hereford (Abe *et al.,* 2009).

Despite the growing interest on colostrum as a potentially beneficial food ingredient or functional food (Mehra *et al*., 2021; Bondoc *et al*., 2022; Ceniti *et al*., 2022), gene association studies with FAs in colostrum are uncommon. This is because the research focus in colostrum was more on its role in the development of the calf, and that surplus colostrum was formerly regarded as unmarketable for human food (O'Callaghan *et al.* 2020). In the Philippines, there is little information on the nutritional quality (based on FA composition) of both colostrum and milk from local dairy cattle herds. In this regard, the objective of this study was to determine the association of *DGAT1*, *SCD1*, and *FASN* genotypes with fat content and major FAs in colostrum and milk obtained from a local Holstein × Australian Friesian Sahiwal (AFS) dairy herd. The associations will provide insight into the underlying mechanism of lipogenic genes and polymorphisms that can be used for selection purposes in dairy cows.

MATERIALS AND METHODS

Colostrum and milk samples

A total of 180 colostrum and milk samples were collected from 45 Holstein × AFS cows that calved from 03 February 2020 to 21 February 2021 at the Real Fresh Dairy Farm in Bay, Laguna. Colostrum samples obtained within 24 hours after calving and raw milk samples later collected on the 30th, 60th, and 90th day of lactation on the same animal were placed in 500 mL plastic bottles, and immediately frozen at –20 °C until further analysis. Milk samples were collected on different days of lactation to determine their possible effects on fat content and major FAs. However, the fat content and major FAs were not significantly different in milk collected on the 30th, 60th, and 90th day of lactation. Hence, the type of milk in the statistical analysis was classified as colostrum or milk only. Average age at calving and number of lactations was 4.63 ± 2.92 years and 2.74 ± 2.22 lactations, respectively.

Determination of colostrum fats and fatty acid composition

Fat percentage of colostrum and milk samples was measured by a Fourier transformed infrared spectroscopy using the MilkoScan Mars (FOSS Analytical A/S, Hillerod, Denmark).

Fat extraction and the preparation of fatty acid methyl esters (FAMEs) were done

using the methods described by Bondoc and Ramos (2022). The FAMEs were analyzed by gas chromatography using a Shimadzu GC 2010 Plus - Capillary Gas Chromatograph System (Shimadzu Corporation, Kyoto, Japan) that is equipped with Flame Ionization Detector (FID) and AOC-20i autosampler. It used a FAMEWax (USP G16) capillary column (30 m, 0.32 mm ID, and 0.25 μm film thickness, Restek Corporation, U.S.). The injector port and FID temperatures were set to 125 °C and then increased to 240 °C at 3 °C per min and maintained for 5 min. Hydrogen gas was used as a carrier at 40 mL per min, while nitrogen was used as a makeup gas at 30 mL per min. Individual fatty acids were identified by comparing their retention times with known FAME standards.

Eighteen (18) fatty acids were determined as a percentage of total FAs (g/100 g of total fatty acids), including eight saturated FA (SFA) – lauric acid C12:0, myristic acid C14:0, pentadecanoic acid C15:0, palmitic acid C16:0, margaric acid C17:0, stearic acid C18:0, arachidic acid C20:0, behenic acid C22:0; six monounsaturated FA (MUFA) – myristoleic acid C14:1n-5, palmitoleic acid C16:1n-7, oleic acid C18:1n-9, trans-vaccenic acid C18:1n-7, eicosenoic acid C20:1n-11, C erucic acid 22:1n-9; and four polyunsaturated fatty acid (PUFA) –linoleic acid or LA C18:2n-6, alpha α-linolenic acid or ALA C18:3n-3, arachidonic acid or AA C20:4n-6, docosahexaenoic acid or DHA C22:6n-3.

DNA extraction and amplification, and genotyping

Genomic DNA was extracted from hair follicles using the NucleoSpin® Tissue Genomic DNA Extraction Kit (Machery-Nagel, Germany) following the supplier's protocol with some modifications. About 20-30 hair follicles (roots) were placed in a 1.5 mL microcentrifuge tube and added with180 μL Buffer T1, and then frozen with liquid nitrogen. The samples were freeze-thawed repeatedly (4 times) in water bath set to 56°C before adding 25 μL of the proteinase K solution. After incubated overnight at 56°C, the mixture was added with 200 μL Buffer B3, and incubated at 70°C for 10 min. Ethanol (210 μL) was then added to the hair follicle sample and transferred in a NucleoSpin® Tissue Column and centrifuged at 11,000 × g for 1 min. The flow-through (supernatant) was removed, and the residual fluid was vortexed and then mixed with 500 μL Buffer BW and centrifuged at 11,000 × g for 1 min. A second washing with 600 μL Wash Buffer B5 was performed, initially centrifuged at 11,000 × g for 1 min and then repeated at 11,000 × g for 5 min to remove the residual ethanol. The sample column was transferred into a fresh 1.5 mL microcentrifuge tube for the elution. About 50 μL Buffer BE (elution buffer preheated to 70°C) was added directly to the sample column. After 1 min of incubation at room temperature, the tube was centrifuged at 11,000 × g for 1 min to collect the purified DNA.

Genotyping for bovine *DGAT1*, *SCD1*, and *FASN* polymorphism was performed using the polymerase chain reaction – restriction fragment length polymorphism method (PCR-RFLP). Primer sequences were: *DGAT1* – K232A variant (Kaupe *et al.,* 2004) – *forward* 5'-GCACCATCCTCTTCCTCAAG-3' and *reverse* 5'-GGAAGCGCTTTCGGATG-3', *SCD1* (Barton *et al.,* 2010) – *forward* 5'-ATGTATGGATACCGCCCTTATGAC-3' and *reverse* 5'-TTCTGGCACGTAACCTAATACCCTAAGC-3', and *FASN* (Abe *et al*. (2009) – *forward* 5'-CTACCAAGCCAGGCAGGTC-3' and *reverse* 5'-GCCATTGTACTTGGGCTTGT-3'. Restriction enzymes were *Eae1*, *Fnu4HI*, and *Nci*I corresponding to *DGAT1* (exon 8 chromosome 14), *SCD1* (exon 5 chromosome 26), and *FASN* (exon 34 chromosome 19), respectively (Table 1).

The PCR amplification was carried out using a Veriti 96-well thermal cycler with

Table 1. List of primers and restriction enzymes used in the analysis of bovine *DGAT1*, *SCD1*, and *FASN* genes

Gene	Forward Primer	Reverse Primer	Product Size (bp)	Restriction Enzyme	Recognition Site	Chromosome (location)/ Reference sequence	Position of SNP polymorphism	Reference
DGAT1	GCA CCA TCC TCT TCC TCA AG	GGA AGC GCT TTC GGA TG	411	*Eae*I	5'…Y^GGCCR..3' 3'…RCCGG^Y..5'	14 (Exon 8)/ AY065621	10,433	Kaupe *et al.* (2004)
SCD1	ATG TAT GGA TAC CGC CCT TAT GAC	TTC TGG CAC GTA ACC TAA TAC CCT AAG C	~17 kbp	*Fnu4H*I	5'… GC^NGC...3' 3'… CGN^CG...5'	26 (Exon 5)/ AY241933	878	Barton *et al.* (2010)
FASN	CTA CCA AGC CAG GCA GGT C	GCC ATT GTA CTT GGG CTT GT	336	*Nci*I	5'… CC^SGG…3' 3'…GGS^CC…5'	19 (Exon 34)/ AF285607	16,039	Abe *et al.* (2009)

[*DGAT1*] Diacylglycerol acyltransferase 1, [*SCD1*] Stearoyl-CoA desaturase, [*FASN*] Fatty acid synthase

thermal profiles used for each primer pair. The amplified products were digested with *Eae1*, *Fnu4HI*, and *Nci*I for *DGAT1*, *SCD1,* and *FASN* respectively. The PCR products were separated by electrophoresis on 3% agarose gel for 30–45 min and visualized under UV transillumination (ENDURO™ GDS Gel Documentation System). The resulting PCR products and genotypes were validated by sending DNA samples to private laboratories for Sanger nucleotide sequencing. Sequence similarities with the corresponding regions of other cattle breeds were determined using the Basic Local Alignment Search Tool (BLAST) at the NCBI database.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) for the *DGAT1*, *SCD1*, and *FASN* genes and genotypes was analyzed using an online chi-square calculator (by Binkowski and Miks (2018)). The polymorphic information content (PIC) was estimated to measure the ability of each molecular marker to detect polymorphisms. The heterozygosity (H) in each gene marker was also calculated to determine the average frequency of heterozygous individuals.

The association of each lipogenic genotype (as a treatment) with fat percentage and proportion of major FAs was analyzed following the SAS GLM procedure (SAS Ver. 9.2, 2009) for unbalanced data using the statistical model: $y_{ijkl} = \mu + MType_i + (MType \times Genotype)_{ij} + Lact_k + e_{ijkl}$, where y_{ijkl} is fat content and major FAs with the highest proportion by weight of total fatty acids – C14:0; C16:0; C18:0, C18:1n-9, and C18:2n-6 (g/100 g of total FAs); μ is the overall mean, $MType_i$ is the i^{th} type of milk (i.e., colostrum and milk), $(MType \times Genotype)_{ij}$ is the interaction effect between the i^{th} milk type and the j^{th} genotypes (i.e., *DGAT1* – AA, AK, KK; *SCD1* – AA, AV, VV; *FASN* – CC, CT, TT), $Lact_k$ is the k^{th} covariate effect of number of lactations, and e_{ijkl} is the error term. Results on each lipogenic genotype effects were presented as least-square means ± standard errors, and compared between specific marker genotypes. Differences were considered significant at P value < 0.05 and compared separately for colostrum and milk.

RESULTS AND DISCUSSION

Table 2 shows that colostrum had significantly higher (P<0.05) percent fat than that in milk from Holstein × *AFS cows***.** The five major FAs with the highest proportions by weight of total FAs were oleic acid C18:1n-9, palmitic acid C16:0, stearic acid C18:0, C14:0 myristic acid, and linoleic acid C18:2n-6 – representing about 81.47% and 69.40% of total FAs in bovine colostrum and milk, respectively. Myristic acid and linoleic acid were significantly higher in colostrum than in milk (P<0.05). However, the proportion of oleic acid, palmitic acid, and stearic acid were similar between colostrum and milk (P>0.05).

Except for C12:0 and C16:1 n-7, the proportion of other FAs in bovine colostrum and milk (C15:0, C17:0, C20:0, C22:0, C14:1 n-5, C18:1 n-7, C20:1 n-11, C22:1 n-9, C18:3 n-3, C20:4 n-6 and C22-6 n-3) were less than one percent. C18:1 n-7, C22:1 n-9, and C22-6 n-3 were not detected in colostrum.

Total SFAs were about 1.25 times higher in colostrum than in milk due mainly to higher levels of palmitic acid in colostrum. Total MUFA, as well as total PUFA, were similar in colostrum and milk. The omega-6 FAs (i.e., linoleic acid C18:2 n-6 and arachidonic acid C20:4 n-6) were higher in colostrum (5.5%) than in milk (4.7%); while omega-3 FAs (i.e., α-linolenic acid C18:3 n-3 and docosahexaenoic acid C22:6 n-3) were lower in colostrum

(0.20%) than in milk (0.53%). Milk, compared to colostrum, may have the greater potential benefit on human health as it had the lower atherogenicity (1.05), thrombogenicity (1.95), and n-6/n-3 ratio (8.86); and higher PUFA/SFA ratio (0.14: 1), MUFA/SFA ratio (0.74: 1), health promoting index (0.91) and hypocholesterolemic/ hypercholesterolemic ratio (1.31: 1) – based on equations presented by Chen and Liu (2020).

Table 2. Fat percentage, major fatty acids, and fatty acid groups (g/100 g of total fatty acids) in colostrum and milk from Holstein × AFS cows.

	Colostrum	**Milk**
Fat percentage	3.29 ± 0.24[a]	2.64 ± 0.14[b]
Saturated FAs		
C12:0	1.51 ± 0.11	1.63 ± 0.07
C14:0	5.68 ± 0.26[a]	4.46 ± 0.15[b]
C15:0	0.52 ± 0.03[b]	0.62 ± 0.02[a]
C16:0	27.82 ± 0.74[a]	19.39 ± 0.43[b]
C17:0	0.47 ± 0.02	0.44 ± 0.01
C18:0	14.12 ± 0.69	13.26 ± 0.41
C20:0	0.38 ± 0.03[b]	0.48 ± 0.02[a]
C22:0	0.14 ± 0.03[b]	0.20 ± 0.02[a]
Monounsaturated FAs		
C14:1 n-5	0.24 ± 0.03[b]	0.42 ± 0.02[a]
C16:1 n-7	1.16 ± 0.06	1.12 ± 0.03
C18:1 n-9	28.35 ± 1.15	27.83 ± 0.67
C18:1 n-7	n.d.	0.19 ± 0.05
C20:1 n-11	0.36 ± 0.02[a]	0.25 ± 0.01[b]
C22:1 n-9	n.d.	0.14 ± 0.05
Polyunsaturated FAs		
C18:2 n-6, LA	5.50 ± 0.26[a]	4.46 ± 0.15[b]
C18:3 n-3, ALA	0.20 ± 0.03[b]	0.34 ± 0.02[a]
C20:4 n-6, AA	n.d.	0.23 ± 0.04
C22:6 n-3, DHA	n.d.	0.20 ± 0.02
Total SFA	50.64	40.48
Total UFA	35.81	35.42
Total MUFA	30.12	29.94
Total PUFA	5.70	5.48
n-3 (ALA +DHA)	0.20	0.53
n-6 (LA + AA)	5.50	4.70

n.d. – not detected.

[SFA] saturated fatty acids; [UFA] unsaturated fatty acids; [MUFA] monounsaturated fatty acids; [PUFA] polyunsaturated fatty acids; [LA] linoleic acid); [ALA] α-linolenic acid; [AA] arachidonic acid; [DHA] docosahexaenoic acid; [n-3] omega-3 fatty acids; [n-6] omega-6 fatty acids

Table 3. Gene and genotypic frequencies and measures of polymorphism of *DGAT1*, *SCD1*, and *FASN* genes in the local herd of Holstein × AFS cows.

Gene markers	No. of Holstein × AFS cows				Genotypic frequency			Gene frequency		HWE		Polymorphism	
										χ^2	P value	PIC	H
DGAT1	AA	AK	KK	Total	AA	AK	KK	A	K	18.223	0.0001	0.3623	0.4753
	0	35	10	45	0.15	0.48	0.37	0.39	0.61				
SCD1	AA	AV	VV	Total	AA	AV	VV	A	V	0.141	0.9321	0.3578	0.2938
	26	17	2	45	0.59	0.36	0.05	0.77	0.23				
FASN	CC	CT	TT	Total	CC	CT	TT	C	T	3.223	0.1996	0.3331	0.2776
	26	19	0	45	0.62	0.33	0.05	0.79	0.21				

Note: HWE – Hardy-Weinberg equilibrium, χ^2 – Chi-square value, PIC – Polymorphic information content, H – Heterozygosity
P value less than 0.05 suggests that the Holstein × AFS cows for a particular gene is not in a state of Hardy-Weinberg genetic equilibrium.

Gene and genotype distribution

In the local Holstein × AFS dairy herd, the AK and KK genotypes were found for the *DGAT1* gene (i.e., allele frequency of A is 0.39). The distribution of *DGAT1* genes and genotypes, however, was not consistent with the Hardy Weinberg equilibrium (Table 3). This could be due to sampling effect, breeding history, and selection pressure for other dairy cattle breeds. In contrast, the AA, AV, and VV genotypes were detected for the *SCD1* gene (i.e., allele frequency of A is 0.77). The CC and CT genotypes were identified for the *FASN* gene (i.e., allele frequency of C is 0.79). The distribution of both *SCD1* and *FASN* genes and genotypes was in Hardy Weinberg equilibrium.

Polymorphic information content (PIC) was highest (i.e., more informative) in *DGAT1* (0.3624), followed by *SCD1* (0.3578), and lowest in *FASN1* (0.3331). The differences, however, were small. The average frequency of heterozygous individuals was highest in *DGAT1* (0.4753), followed by *SCD1* (0.2938), and lowest in *FASN1* (0.2776).

Effects of lipogenic genotypes on colostrum fats and FA composition

The effects of lipogenic genotypes on fat content and FA composition in bovine colostrum are not commonly reported in the literature. In the current study on Holstein × AFS cows, the AK genotype of *DGAT1* gene was associated ($P<0.05$) with higher fat content in colostrum (3.54%) than for cows with KK genotype (2.75%), see Table 4. In terms of *SCD1* genotypes, the AV cows had the highest fat content (3.86%) and oleic acid C18:1n-9 (30.90%) in colostrum, compared to cows with AA (3.05% and 27.25%, respectively) or VV genotype (1.71% and 24.18%, respectively). In contrast, the CT genotype of the *FASN* gene was associated ($P<0.05$) with higher myristic acid C14:0 (6.10%) compared to cows with CC genotype (5.26%). Results on the lipogenic genotype effects suggest a possible role of *DGAT1*, *SCD1*, and *FASN* genes in the genetic variation of colostrum nutritional properties. However, selecting cows to produce colostrum with a particular fat content and FA composition may be impractical since colostrum yield is only 0.5% of the cow's annual milk production so that production of colostrum all year round in commercial quantities will be limited (O'Callaghan *et al*., 2020).

Association of lipogenic genotypes with milk fats and FAs

***DGAT1 genotypes*.** The AK genotype in the local herd of Holstein × AFS cows, was associated ($P<0.05$) with slightly higher myristic acid C14:0 (4.56%) than for cows with KK genotype (4.01%), see Table 4. The results are consistent with the findings of Schennink *et al.* (2007), who showed that the K allele was associated with a lower concentration of C14:0 in Dutch Holstein Friesian cows. In a similar finding for Chinese Holsteins, Li *et al*. (2021) reported a higher proportion of C14:0 (12.60%) in AK cows compared to cows with the KK genotype (12.09%). This implies that milk from KK cows appears to have greater health benefits than milk from AK cows since myristic acid – a saturated FA, is considered hypercholesterolemic and may raise both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations (Temme *et al*., 1997).

***SCD1 genotypes*.** The AV genotype was associated ($P<0.05$) with the highest palmitic acid C16:0 (21.30%) and stearic acid C18:0 (14.57%) in milk fats from Holstein × AFS cows compared to cows with AA (18.22% and 12.84%, respectively) and VV genotype (18.56% and 9.07%, respectively), see Table 4. Similar results were found by Mele *et al.* (2007), who showed that Italian Holsteins with the AV genotype had the highest proportion of C16:0

Table 4.Fat percentage and major fatty acids in colostrum and milk for *DGAT1*, *SCD1*, and FASN genotypes in Holstein × AFS cows.

	***DGAT1* genotypes**			***SCD1* genotypes**			***FASN* genotypes**		
	AA	**AK**	**KK**	**AA**	**AV**	**VV**	**CC**	**CT**	TT
Colostrum									
Fat percentage	-	3.54 ± 0.27^{a}	2.75 ± 0.50^{b}	3.05 ± 0.31^{b}	3.86 ± 0.38^{a}	1.71 ± 1.11^{c}	3.40 ± 0.32	3.18 ± 0.36	-
Major FAs, %									
C14:0	-	5.62 ± 0.29	5.74 ± 0.51	5.82 ± 0.33^{ab}	5.08 ± 0.41^{b}	6.94 ± 1.07^{a}	5.26 ± 0.33^{b}	6.10 ± 0.40^{a}	-
C16:0	-	28.12 ± 0.82	26.52 ± 1.47	27.88 ± 0.94^{ab}	26.81 ± 1.15^{b}	31.34 ± 3.02^{a}	27.01 ± 0.95	28.64 ± 1.16	-
C18:0	-	14.31 ± 0.77	13.56 ± 1.38	14.35 ± 0.88^{a}	14.69 ± 1.08^{a}	10.18 ± 2.82^{b}	14.83 ± 0.88	13.41 ± 1.07	-
C18:1 n-9	-	28.55 ± 1.26	27.78 ± 2.26	27.25 ± 1.45^{b}	30.90 ± 1.79^{a}	24.18 ± 4.68^{b}	29.00 ± 1.46	27.70 ± 1.77	-
C18:2 n-6	-	5.33 ± 0.30	5.91 ± 0.53	5.64 ± 0.34	5.16 ± 0.42	6.36 ± 1.10	5.50 ± 0.33	5.49 ± 0.40	-
Milk									
Fat percentage	-	2.61 ± 0.15	2.62 ± 0.29	2.74 ± 0.18^{a}	2.56 ± 0.22^{a}	1.43 ± 0.63^{b}	2.48 ± 0.18	2.79 ± 0.21	-
Major FAs, %									
C14:0	-	4.56 ± 0.17^{a}	4.01 ± 0.30^{b}	4.12 ± 0.19^{b}	4.81 ± 0.25^{a}	5.35 ± 0.62^{a}	4.37 ± 0.19	4.55 ± 0.23	-
C16:0	-	19.28 ± 0.48	19.72 ± 0.86	18.22 ± 0.55^{b}	21.30 ± 0.70^{a}	18.56 ± 1.75^{b}	19.32 ± 0.55	19.45 ± 0.67	-
C18:0	-	13.44 ± 0.45	12.71 ± 0.81	12.84 ± 0.51^{b}	14.57 ± 0.65^{a}	9.07 ± 1.63^{c}	13.32 ± 0.52	13.20 ± 0.62	-
C18:1 n-9	-	27.77 ± 0.74	28.53 ± 1.33	26.25 ± 1.45^{b}	30.90 ± 1.79^{a}	26.90 ± 2.70^{ab}	28.11 ± 0.86	27.55 ± 1.03	-
C18:2 n-6	-	4.54 ± 0.17	4.47 ± 0.31	4.64 ± 0.34^{b}	5.16 ± 0.42^{ab}	6.36 ± 1.10^{a}	4.67 ± 0.20	4.24 ± 0.23	-

Note: Least square means with different superscript letters within a row for the same candidate gene marker are significantly different (P<0.05).

(24.12%) and C18:0 (8.91%) compared to AA cows (23.92% and 8.70%, respectively) and VV cows (23.7% and 8.58%, respectively). Schennink *et al.* (2008) also reported that the V allele is associated with a higher C16:0 and C18:0 in milk fats from Dutch Holstein Friesian cows in comparison to the A allele. Palmitic acid, together with lauric acid (C12:0) and myristic acid (C14:0), add to the total dietary saturated FAs, which when consumed in high quantities have been shown in many epidemiologic, clinical, and animal studies to be associated with an increase in blood cholesterol and increased risk of atherosclerosis and coronary heart disease in humans. On the other hand, stearic acid – when compared with other saturated FAs, lowers LDL cholesterol (Mensink, 2005).

FASN genotypes. In our study, *FASN* genotypes in Holstein × AFS cows had no significant effect on fat content and major milk fatty acids ($P>0.05$). In contrast, The *FASN* gene had been associated with milk fats in Friesian-sired and Jersey-sired cows in New Zealand (Morris *et al*., 2007) and milk fatty acids (especially C14:0) in Chinese Holsteins (Li *et al*., 2016) and Simmental and Holstein × Simmental cows in Croatia (Mauric *et al.,* 2019).

CONCLUSION

The results on the lipogenic genotype effects suggest that DGAT1, SCD1, and FASN genes and genotypes may have an important role in the local marker assisted selection program for Holstein × AFS cows aimed at changing fat percentage and some FAs, and consequently, improve the nutritional quality of bovine colostrum and milk.

In a future local marker assisted selection program, the frequency of the desirable lipogenic gene or genotype that has been associated with better nutritional quality of bovine colostrum and milk should be increased. Moreover, the unbalanced distribution of the lipogenic genes for the local Holstein × AFS dairy herd in this study suggests that the size of population with individual records on fat percentage and major FAs should also be increased.

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REFERENCES

Abe T, Saburi J, Hasebe H, Nakagawa T, Misumi S, Nade T, Nakajima H, Shoji N, Kobayashi M, and Kobayashi E. 2009. Novel mutations of the FASN gene and their effect on fatty acid composition in Japanese black beef. *Biochem Genet* 47(5-6):397-411.

Barton L, Kott T, Bures D, Rehak D, R. Zahradkova R, Kottov B. 2010. The polymorphisms of stearoyl-CoA desaturase (*SCD1*) and sterol regulatory element binding protein-1 (*SREBP-1*) genes and their association with the fatty acid profile of muscle and subcutaneous fat in Fleckvieh bulls. *Meat Sci* 85(1):15-20.

Binkowski J and Miks S. 2018. Gene-Calc [Computer software]. Available from: http://gene-calc.pl/hardy-weinberg.

Bondoc OL, Ebron AO, Ramos AR, and Borja MAM. 2022. Composition, yield and freezing point of colostrum and milk from Holstein × Sahiwal cows in a commercial dairy farm in Bay, Laguna, Philippines. *Philipp J Vet Anim Sci* 48(1):70-81.

Bondoc OL and Ramos AR. 2022. Fatty acid composition and nutritional indices/ratios of colostrum and milk from Murrah and "Murrah × Carabao" crossbred buffaloes. *Philipp J Sci* 151(1):139-152.

Ceniti C, Costanzo N, Morittu VM, Tilocca B, Roncada P, and Britti D. 2022. Review: Colostrum as an emerging food: nutraceutical properties and food supplement. *Food Rev Int* 39(7):4636-4664.

Chen J and Liu H. 2020. Nutritional indices for assessing fatty acids: a mini-review. *Int J Mol Sci* 21(16):5695.

Grisart B, Farnir F, Karim L, Cambisano N, Kim JJ, Kvasz A, Mni M, Simon P, Frere JM, and Coppieters W. 2004. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. *Proc Natl Acad Sci* 101(8):2398-2403.

Kale DS, Singh J, Sathe YB, and Patil D. 2021. FASN gene and its role in Bovine milk production. *IJBTT* 11(1):20-25.

Kaupe B, Winter A, Fries R, and Erhardt G. 2004. DGAT1 polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. *J Dairy Res* 71(2):182-187.

Li C, Sun D, Zhang S, Yang S, Alim MA, Zhang Q, Li Y, and Liu L. 2016. Genetic effects of *FASN*, *PPARGC1A*, *ABCG2* and *IGF1* revealing the association with milk fatty acids in a Chinese Holstein cattle population based on a post genome-wide association study. *BMC Genetics* 17:110.

Li Y, Zhou H, Cheng L, Edwards GR, and Hickford JG. 2021. Effect of *DGAT1* variant (K232A) on milk traits and milk fat composition in outdoor pasture-grazed dairy cattle. *NZ J Agric Res* 64(1):101-113.

Mahmoudi P and Rashidi A. 2023. Strong evidence for association between K232A polymorphism of the DGAT1 gene and milk fat and protein contents: A meta-analysis. *J Dairy Sci* 106(4):2573-2587.

Mauric M, Masek T, Ljoljic DB, Grbavac J, and Starcevic K. 2019. Effects of different variants of the FASN gene on production performance and milk fatty acid composition in Holstein × Simmental dairy cows. *Vet Med (Praha)* 64(3):101-108.

Mehra R, Singh R, Nayan V, Buttar HS, Kumar N, Kumar S, Bhardwaj A, Kaushik R, and Kumar H. 2021. Nutritional attributes of bovine colostrum components in human health and disease: a comprehensive review. *Food Biosci* 40:100907.

Mele M, Conte G, Castiglioni B, Chessa S, Macciotta NP, Serra A, Buccioni A, Pagnacco G, and Secchiari P. 2007. Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. *J Dairy Sci* 90(9):4458-4465.

Mensink RP. 2005. Effects of stearic acid on plasma lipid and lipoproteins in humans. Lipids 40(12):1201-1205.

Morris CA, Cullen NG, Glass BC, Hyndman DL, Manley TR, Hickey SM, McEwan JC, Pitchford WS, Bottema CD, and Lee MD. 2007. Fatty acid synthase effects on bovine adipose fat and milk fat. *Mamm Genome* 18(1):64-74.

Ntambi JM and Miyazaki M. 2004. Regulation of stearoyl-CoA desaturases and role in

metabolism. *Prog Lipid Res* 43(2):91-104.

O'Callaghan TF, O'Donovan M, Murphy JP, Sugrue K, Mannion D, McCarthy WP, Timlin M, Kilcawley KN, Hickey RM, and Tobin JT. 2020. Evolution of the bovine milk fatty acid profile – from colostrum to milk five days post parturition. *Int Dairy J* 104:104655.

Sanjayaranj I, MacGibbon AKH, Holroyd SE, Janssen PWM, Blair HT, and Lopez-Villalobos N. 2023. Association of single nucleotide polymorphism in the DGAT1 gene with the fatty acid composition of cows milked once and twice a day. *Genes* 14(3):767.

Schennink AWM, Stoop WM, Visker MHPW, Heck JML, Bovenhuis H, van der Poel JJ, van Valenberg HJF, and van Arendonk JAM. 2007. *DGAT1* underlies large genetic variation in milk-fat composition of dairy cows. *Anim Genet* 38(5):467-473.

Schennink AWM, Heck JML, Visker MHPW, van Valenberg HJF, and van Arendonk JAM. 2008. Milk fatty acid unsaturation: genetic parameters and effects of Stearoyl-CoA Desaturase (*SCD1*) and Acyl CoA: Diacylglycerol Acyltransferase 1 (*DGAT1*). *J Dairy Sci* 91(5):2135-2143.

Statistical Analysis System [SAS]. 2009. SAS/STAT ® 9.2 User's Guide. 2nd ed. USA: SAS Institute, Inc. Cary, NC USA.

Temme EH, Mensink RP, and Hornstra G. 1997. Effects of medium chain fatty acids (MCFA), myristic acid, and oleic acid on serum lipoproteins in healthy subjects. *J Lipid Res* 38(9):1746-1754.

Timlin M, Tobin JT, Brodkorb A, Murphy EG, Dillon P, Hennessy D, O'Donovan M, Pierce KM, and O'Callaghan TF. 2021. The impact of seasonality in pasture-based production systems on milk composition and functionality. *Foods* 10(3):607.