Original Article

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THE EFFECTS OF SOLUTE CARRIER FAMILY 27 MEMBER 1 (*SLC27A1*) GENOTYPES ON FAT CONTENT AND MAJOR FATTY ACIDS IN COLOSTRUM AND MILK FROM MURRAH AND "MURRAH × CARABAO" CROSSBRED BUFFALOES

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ABSTRACT

This study analyzed the effect of Solute carrier family 27 member 1 (*SLC27A1*) genotypes on fat content and major fatty acids (lauric acid C12:0, myristic acid C14:0, palmitic acid C16:0, stearic acid C18:0, and oleic acid C18:1n-9) in milk and colostrum of 46 Murrah and "Murrah × Carabao" crossbred buffaloes at the Philippine Carabao Center - University of the Philippines Los Baños dairy herd. The *SLC27A1* genotypes (CC and CT) were determined by the polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method using DNA extracted from hair follicles. This study found polymorphism in the *SLC27A1* gene that were consistent with Hardy Weinberg's law of equilibrium (HWE), with a polymorphic information content (PIC) and heterozygosity (H) estimate of 0.2915 and 0.3542, respectively, for Murrah; and 0.3219 and 0.4032, respectively for "Murrah × Carabao" crossbreeds. In Murrah buffaloes, the CT genotype was significantly associated ($P<0.05$) with higher colostrum yield and milk fat content, compared to CC. In "Murrah × Carabao" crossbreds, CT was associated with higher C12:0, C14:0, and C16:0, but lower C18:1n-9 in colostrum; and lower C16:0 in milk compared to CC. This study showed polymorphisms in *SLC27A1* genotypes and their significant effects on colostrum yield and milk fat content in Murrah buffaloes and some major fatty acids in colostrum and milk from "Murrah × Carabao" crosses.

Keywords: *Bubalus bubalis*, buffaloes, Murrah Carabao, colostrum, milk fatty acids, *SLC27A1* genotypes

INTRODUCTION

Fat percentage and fatty acid (FA) composition are important traits that may be improved to regulate lipid levels in buffalo dairy products that can be beneficial for health or associated with the occurrence of common diseases such as obesity, diabetes, and cardiovascular diseases. Milk FA composition in cow's milk is widely known to be influenced by several factors, including genetics (Stoop *et al*., 2008). However, their application in traditional selection programs is limited by the high costs of FA profile analyses. In this regard, the use of gene markers in marker-assisted selection (MAS) programs is

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recommended to improve the accuracy of selection for superior breeding stock for milk production and FA composition.

For example, significant associations between polymorphisms in candidate marker genes such as *DGAT1* (diacylglycerol O-acyltransferase 1), *FASN* (fatty acid synthase), *SCD* (stearoyl CoA desaturase), *SREBP1* (sterol regulatory element-binding protein-1), and *THRSP* (thyroid hormone-inducible hepatic protein) and milk production and milk FA composition have been reported in cattle (Li *et al*., 2016; Mauric *et al*., 2019; Schennink *et al*., 2008; Rincon *et al*., 2012; Polasik *et al*., 2021).

In dairy buffaloes, the association of the oxytocin receptor encoding gene (OXTR) (Cosenza *et al*., 2017) and prolactin receptor (PRLR) (Cosenza *et al*., 2018) with fat content in Italian Mediterranean river buffalo had been reported. However, gene association studies with fatty acid composition of milk from buffaloes is uncommon. To date, none of the polymorphisms identified have been investigated for association with FAs in buffalo colostrum, which could be a new source of nutrients or functional food ingredient (Mehra *et al*., 2021) and nutraceutical (Ceniti *et al*., 2022).

A possible candidate for buffalo colostrum and milk fat composition could be the solute carrier family 27-member 1 (*SLC27A1*) gene (Ordovas *et al*., 2017). The *SLC27A1* which is a member of the fatty acid transport protein (FATP) family, contributes to the mediation of the concentration and metabolism of long chain fatty acids (LCFAs) when transported across the cellular membrane (Gimeno, 2007). The bovine gene had been organized in 13 exons spanning over more than 40 kb of genomic DNA and maps in BTA 7 where several quantitative trait loci (QTL) for fat related traits have been described (Ordovas *et al*., 2017).

In the Philippines, local milk production mostly from Murrah and "Murrah × Carabao" crosses in 2022 were 10.90 million liters from a total inventory of 17,299 milking buffaloes (National Dairy Authority 2022). The nutritional quality based on FA composition of both colostrum and milk from local dairy buffalo farm was previously reported by Bondoc and Ramos (2022). Since genetic variation in the SLC27A1 gene may affect lipid metabolism, the present study was conducted to determine the effects of *SLC27A1* genotypes on fat content and major FAs in colostrum and milk obtained from Murrah and "Murrah × Carabao" crossbred buffaloes at the Philippine Carabao Center (PCC) - University of the Philippines Los Baños (UPLB) dairy herd. The associations will provide insight into the underlying mechanism of *SLC27A1* gene and genotype polymorphisms that can be used for selection purposes in dairy buffaloes.

MATERIALS AND METHODS

Colostrum and milk samples

A total of 168 colostrum and milk samples were collected from 46 Murrah and "Murrah × Carabao" crossbred buffaloes that calved from 3 February 2020 to 21 February 2021 at the Philippine Carabao Center - University of the Philippines Los Baños dairy herd. Colostrum samples obtained within 24 h 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^{o}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 (Bondoc and Ramos, 2022). 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 yrs 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 minute and maintained for 5 minutes. Hydrogen gas was used as a carrier at 40 mL per minute, while nitrogen was used as a makeup gas at 30 mL per minute. Individual fatty acids were identified by comparing their retention times with known FAME standards.

Nineteen (19) fatty acids were determined as a percentage of total FAs (g/100 g of total fatty acids), including eight saturated fatty acids (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 atty acids (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 five polyunsaturated fatty acids (PUFA) – conjugated linoleic acid CLA C18:2 c9tll, 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 to 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 minutes. 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 minute. A second washing with 600 µL Wash Buffer B5 was performed, initially centrifuged at 11,000 × g for 1 minute and then repeated at 11,000 × g for 5 minutes 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 minute of incubation at room temperature,

the tube was centrifuged at 11,000 × g for 1 minute to collect the purified DNA. Genotyping for *SLC27A1* polymorphism was performed using the polymerase chain reaction - restriction fragment length polymorphism method (PCR-RFLP). Primer sequences for *SLC27A1* (Ordovas *et al*., 2008*)* were - *forward* 5'- CTGCTCAACGTGAACCTGCG -3' and *reverse* 5'- ACCAGGCTCTTGCCCAACTC -3'.

The PCR amplification was carried out using a Veriti 96-well thermal cycler with thermal profiles used for each primer pair. The amplified 261-bp products were digested using the *BssnI* restriction enzyme corresponding to *SLC27A1* (exon 3 chromosome 7), based on the following restriction fragment lengths: CC-205, 56 bp; CT-261, 205, 56 bp; TT-261 bp.

The PCR products were separated by electrophoresis on 3% agarose gel for 30 to 45 minutes and visualized under UV transillumination (ENDURO™ GDS Gel Documentation System). The PCR products were confirmed by the Basic Local Alignment Search Tool (BLAST) to be *SLC27A1* (GenBank Accession Number NM_001033625) at the NCBI database. Sequencing of bovine *SLC27A1* exon 3 allowed detection of one missense SNP located in position 130 of the sequence. This SNP causes an A/C substitution. The amplicon is composed of 261 bp, as confirmed via Sanger Nucleotide Sequencing.

Statistical analysis

The chi-square test was initially used to test for significant differences in gene and genotypic frequencies, as well as Hardy-Weinberg equilibrium (HWE), separately for the Murrah and "Murrah × Carabao" crosses using an online chi-square calculator (https://www.genecalculators.net/pq-chwe-check.html) by Hayesmoore (2023). The polymorphic information content (PIC) was then estimated to measure the ability of the molecular marker to detect polymorphisms, while heterozygosity (H) was calculated to determine the average frequency of heterozygous individuals (https://www.genecalculators.net/pq-chwe-polypicker.html) by Hayesmoore (2023).

The effects of *SLC27A1* genotypes on daily yield, fat percentage, and proportion of major FAs were analyzed following the SAS GLM procedure (SAS Ver. 9.2, 2009) for unbalanced data using the statistical model:

$$y_{ijklmno} = \mu + MType_i + (MType \times Breed \times SLC)_{ijk} + Lact_l + Yield_m + Fat_n + e_{ijklmno}$$

where $y_{ijklmno}$ is the dependent variable, i.e., daily yield (kg), fat percentage, and major fatty acids - C12:0, C14:0, C16:0, C18:0, C18:1n-9 (g/100 g of total identified fatty acids); μ is the overall mean; $MType_i$ is the fixed effect of i^{th} type of milk (colostrum or milk); $(MType \times Breed \times SLC)_{ijk}$ is the interaction effect between the i^{th} milk type, j^{th} breed (Murrah and "Murrah × Carabao" crosses), and k^{th} *SLC27A1* genotype (CC, CT, and TT); $Lact_l$ is the covariate effect of the l^{th} lactation number; $Yield_m$ is the covariate effect of the m^{th} daily yield (li/day), Fat_n is the covariate effect of n^{th} fat percentage, and $e_{ijklmno}$ is the error term. The effects of the *SLC27A1* genotypes were presented as least-square means ± standard error and compared separately for colostrum and milk in Murrah and "Murrah × Carabao" crossbred buffaloes. Differences were considered significant at P value < 0.05. Fatty acid groups were also considered in the comparisons in terms of total SFA, total UFA, total MUFA, total PUFA, omega-3 FAs (ALA + DHA), and omega-6 FAs (LA + AA).

RESULTS AND DISCUSSIONS

Fat percentage was significantly higher (P<0.05) in buffalo colostrum (5.22%) than in milk (4.44%), see Table 1. The five major FAs with the highest proportions by weight of total FAs were palmitic acid C16:0, oleic acid C18:1n-9, myristic acid C14:0, stearic acid C18:0, and lauric acid C12:0, which collectively represent about 84.83% and 83.33% of total FAs in colostrum and milk, respectively. Oleic acid was significantly higher (P<0.05) in colostrum than in milk. However, the proportion of myristic acid and stearic acid were significantly lower (P<0.05) in colostrum than in milk. Except for palmitoleic acid C16:1n-7 and linoleic acid C18:2n-6, the proportion of other FAs in bovine colostrum and milk (C15:0, C17:0, C20:0, C22:0, C14:1n-5, C18:1n-7, C20:1n-11, C22:1n-9, C18:2c9 t11, C18:3n-3, C20:4n-6 and C22:6n-3) were less than one percent.

Total MUFA, total UFA, and omega-3 FAs (i.e., α-linolenic acid C18:3 n-3 and docosahexaenoic acid C22:6n-3) were 1.55 times, 1.60 times, and 1.43 times higher in colostrum than in milk. Moreover, total PUFA and omega-6 FAs (i.e., C18:2n-6 and arachidonic acid C20:4n-6) were 2.23 times and 2.25 times higher in colostrum than in milk. Based on FA-based nutritional indices presented by Bondoc and Ramos (2022), buffalo colostrum, compared to milk, may have the greater potential benefit on human health as it had the lower atherogenicity (2.58 vs. 4.65), thrombogenicity (2.69 vs. 4.58), and omega-6/ omega-3 ratio (8.86); and higher PUFA/SFA ratio (0.06: 1 vs 0.02: 1), MUFA/SFA ratio (0.55: 1 vs 0.31: 1), health promoting index (0.39 vs. 0.21) and hypocholesterolemic/ hypercholesterolemic ratio (0.63: 1 vs. 0.35: 1).

Effects of SLC27A1 genotypes on the fat content and FA composition of colostrum and milk from Murrah buffaloes

In this study, the CC and CT genotypes were found for the *SLC27A1* gene (i.e., allele frequency of C is 0.77 for Murrah and 0.72 for "Murrah × Carabao" crosses). The distribution of *SLC27A1* genes and genotypes was consistent with the Hardy Weinberg equilibrium (Table 2). Polymorphic information content (PIC) values were moderate but slightly lower (i.e., less informative) for Murrah (0.2915) than in "Murrah × Carabao" crosses (0.3219). The average frequency of heterozygous individuals was higher in "Murrah × Carabao" crosses (H=0.4032) than in purebred Murrah (H=0.3542).

Effects of *SLC27A1* genotypes on the fat content and FA composition of colostrum and milk from Murrah buffaloes

In Murrah buffaloes, the CT genotype was associated (P<0.05) with higher colostrum yield (5.32 li) than those with the CC genotype (3.34 li). Milk fat percentage was also significantly higher (P<0.05) for Murrah buffaloes with the CT genotype (5.55%) than those with the CC genotype (4.64%). The proportion of major FAs and fatty acid groups were not significantly different (P>0.05) between animals with CC and CT genotypes (Table 3). This suggests that selection of Murrah buffaloes with CT genotypes may be used to improve colostrum yield, albeit produce milk with higher fat percentage. The use of *SLC27A1* gene markers, however, may not be effective in improving the proportion of major FAs and fatty acid groups in colostrum or milk from Murrah buffaloes.

Effects of SLC27A1 genotypes on the fat content and FA composition of colostrum and milk from "Murrah × Carabao" crossbred buffaloes

For colostrum, the CT genotype in "Murrah × Carabao" crossbred buffaloes were significantly associated (P<0.05) with higher levels of lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0), but lower proportion of oleic acid (C18:1n-9) compared to crossbred buffaloes with the CC genotype (Table 4). The major SFAs in colostrum - C12:0, C14:0, and C16:0 add to the total dietary saturated FAs, which when consumed in high quantities have been shown to increase low-density lipoprotein (LDL) cholesterol, and therefore has been associated with increased risk of cardiovascular disease (Siri-Tarino *et al*., 2010).

Lauric acid, myristic acid, and palmitic acid are pro-atherogenic, prothrombogenic, and hypercholesterolemic SFAs. Pro-atherogenic FAs favor the adhesion of lipids to cells of the circulatory and immunological systems which favor the adhesion of lipids to cells of the circulatory and immunological systems (Ulbricht and Southgate, 1991). Prothrombogenic FAs causes thrombosis or coagulation of the blood in blood vessels (Ulbricht and Southgate, 1991). Hypercholesterolemic FAs may increase both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations in the blood (Temme *et al*., 1997). On the other hand, oleic acid is the most common MUFA in dietary food. It increases the activity of low-density lipoprotein receptors (LDLRs) and decreases the cholesterol concentration in serum (Dietschy, 1998). Together with other MUFAs and PUFAs in colostrum, oleic acid provides greater benefits for cardiovascular health by inhibiting the accumulation of fatty plaque and reduce the levels of phospholipids, cholesterol, and esterified FAs.

Stearic acid (C18:0) - also a saturated FA, which were shown to lower LDL cholesterol (Mensink, 2005), were not significantly different (P>0.05) between animals with CC and CT genotypes. Total SFAs in colostrum were higher in "Murrah × Carabao" crossbred buffaloes with the CT genotype (65.19%) than those with the CC genotype (54.16%). Total UFA, total MUFA, total PUFA, omega-3 FAs, and omega-6 in colostrum were lower in crossbred buffaloes with the CT genotype than those than those with the CC genotype. The differences in total UFA, total MUFA, total PUFA, omega-3 FAs, and omega-6 were -4.02%, -3.94%, -0.09%, -0.19%, and -0.12%, respectively. Colostrum yield and fat percentage were also not significantly different (P>0.05) between animals with CC and CT genotypes.

For milk, the proportion of palmitic acid was significantly higher (P<0.05) in crossbred buffaloes with the CT genotype (33.58%) than those with the CC genotype (31.00%). Daily milk yield, milk fat percentage, and the proportion of major milk FAs were not significantly different (P>0.05) between crossbred buffaloes with CC and CT genotypes (Table 4).

Applications to a local selection program based on *SLC27A1* marker genotypes

The results suggest a possible role of the *SLC27A1 gene* in the genetic variation of FA-based nutritional properties of colostrum obtained from "Murrah × Carabao" crossbred buffaloes. The "C" allele seems to be associated with higher levels of C18:1n-9 and lower proportions of the undesirable SFAs (i.e., C12:0, C14:0, and C16:0) in colostrum fats, when compared to the "T" allele. Selection of "Murrah × Carabao" crossbred buffaloes with the CC genotype may thus be used to improve colostrum with greater health benefits. However, selecting dairy buffaloes to produce

Table 1. Fat percentage, and proportion of fatty acids and fatty acid groups (g/100 g of total fatty acids) in buffalo colostrum and milk.

Parameter	Colostrum	Milk
Fat content, %	4.44±0.43^{b}	5.22±0.23^{a}
Saturated FAs		
C12:0	4.71±0.35^{b}	8.13±0.19^{a}
C14:0	13.28±0.40^{b}	15.45±0.22^{a}
C15:0	0.82±0.03^{b}	0.89±0.02^{a}
C16:0	32.80±0.61	32.56±0.33
C17:0	0.66±0.02^{a}	0.48±0.01^{b}
C18:0	5.29±0.30^{b}	9.22±0.16^{a}
C20:0	0.2±0.01^{a}	0.09±0.01^{b}
C22:0	0.02±0.00^{b}	0.12±0.10^{a}
Monounsaturated FAs		
C14:1n-5	0.85±0.04^{a}	0.70±0.02^{b}
C16:1n-7	1.93±0.06^{a}	1.47±0.03^{b}
C18:1n-9	28.75±0.80^{a}	17.97±0.43^{b}
C18:1n-7	0.10±0.01^{b}	0.14±0.00^{a}
C20:1n-11	0.12±0.01^{b}	0.19±0.00^{a}
C22:1n-9	0.02±0.00^{b}	0.12±0.00^{a}
Polyunsaturated FAs		
C18:2c9 t11, CLA	0.68±0.03^{a}	0.15±0.02^{b}
C18:2n-6, LA	1.16±0.05^{a}	0.52±0.03^{b}
C18:3n-3, ALA	0.36±0.01^{a}	0.31±0.01^{b}
C20:4n-6, AA	0.95±0.03^{a}	0.43±0.02^{b}
C22:6n-3, DHA	0.27±0.06^{a}	0.13±0.03^{b}
Fatty acid groups		
Total SFA	57.85	66.93
Total UFA	35.18	22.02
Total MUFA	31.77	20.49
Total PUFA	3.41	1.53
n-3 (ALA +DHA)	0.63	0.44
n-6 (LA + AA)	2.11	0.95

(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, (h/H ratio) hypocholesterolemic/ hypercholesterolemic ratio. with different superscript letters in the same row are significantly different ($P<0.05$).

Table 2. Gene and genotypic frequencies and measures of polymorphism of *SLC27A1* in Murrah and "Murrah × Carabao" crossbred buffaloes.

Parameter	Murrah	"Murrah × Carabao" crosses	Total
No. of buffaloes per genotype			
CC	15	8	23
CT	13	10	23
TT	0	0	0
Total	28	18	46
Genotypic frequency			
CC	0.54	0.44	0.50
CT	0.46	0.56	0.50
TT	0.00	0.00	0.00
Total	0.50	0.50	0.50
Gene frequency			
C	0.77	0.72	0.67
T	0.23	0.28	0.33
HWE			
χ^2	2.559	2.663	5.111
P-value	0.278	0.264	0.078
Polymorphism			
PIC	0.2915	0.3219	0.3047
H	0.3542	0.4032	0.3750

Note: HWE: Hardy-Weinberg equilibrium; χ^2: Chi-square value; PIC: Polymorphic information content; H: Heterozygosity. P-value<0.05 suggests that the breed group evaluated for a particular gene is not in a state of Hardy-Weinberg genetic equilibrium.

Table 3. Effects of *SLC27A1* genotypes on daily yield, fat percentage, major fatty acids, and fatty acid groups (g/100 g of total fatty acids) in colostrum and milk from Murrah buffaloes.

Parameter	*SLC27A1* genotypes in Murrah buffaloes		
	CC	CT	TT
Colostrum			
Daily yield, li/day	3.34±0.55[b]	5.32±0.61[a]	-
Fat percentage	4.37±0.78	3.42±0.81	-
Major FAs, %			
C12:0	4.12±0.63	4.71±0.66	-
C14:0	12.89±0.74	12.51±0.77	-
C16:0	32.50±1.12	31.80±1.17	-
C18:0	4.56±0.54	5.61±0.57	-
C18:1n-9	29.03±1.47	28.68±1.53	-
Fatty acid groups			
Total SFA	55.56	56.50	-
Total UFA	35.37	35.26	-
Total MUFA	32.12	31.59	-
Total PUFA	3.24	3.67	-
n-3 (ALA + DHA)	0.50	0.68	-
n-6 (LA + AA)	2.15	2.20	-
Milk			
Daily yield, li/day	6.11±0.28	6.07±0.33	-
Fat percentage	4.64±0.37[a]	5.55±0.45[a]	-
Major FAs, %			
C12:0	7.74±0.30	7.91±0.37	-
C14:0	14.93±0.35	14.99±0.43	-
C16:0	32.96±0.53	32.69±0.65	-
C18:0	9.34±0.26	9.350±0.32	-
C18:1n-9	18.93±0.69	18.20±0.85	-
Fatty acid groups			
Total SFA	66.55	66.49	-
Total UFA	22.95	22.98	-
Total MUFA	21.38	20.72	-
Total PUFA	1.57	2.26	-
n-3 (ALA + DHA)	0.46	0.44	-
n-6 (LA + AA)	0.98	1.04	-

Note: Least square means with different superscript letters in the same row are significantly different ($P<0.05$).

Table 4. Effects of *SLC27A1* genotypes on daily yield, fat percentage, major fatty acids, and fatty acid groups (g/100 g of total fatty acids) in colostrum and milk from "Murrah × Carabao" crossbred buffaloes.

Parameter	*SLC27A1* genotypes in "Murrah × Carabao" crosses		
	CC	CT	TT
Colostrum			
Daily yield, li/day	6.56±0.69	5.24±0.64	-
Fat percentage	4.57±0.90	4.92±0.54	-
Major FAs, %			
C12:0	3.64±0.73^{b}	6.39±0.68^{a}	-
C14:0	12.08±0.85^{b}	15.65±0.80^{a}	-
C16:0	31.21±1.28^{b}	35.68±1.21^{a}	-
C18:0	5.45±0.63	5.54±0.59	-
C18:1 n-9	30.93±1.69^{a}	26.34±1.59^{b}	-
Fatty acid groups			
Total SFA	54.16	65.19	-
Total UFA	37.06	33.04	-
Total MUFA	33.66	29.72	-
Total PUFA	3.41	3.32	-
n-3 (ALA + DHA)	0.57	0.76	-
n-6 (LA + AA)	2.10	1.98	-
Milk			
Daily yield, li/day	7.37±0.40	7.03±0.34	-
Fat percentage	4.92± 0.54	5.79±0.46	-
Major FAs, %			
C12:0	8.37±0.43	8.86±0.37	-
C14:0	15.89±0.50	16.02±0.43	-
C16:0	33.58±0.76^{a}	31.00±0.66^{b}	-
C18:0	9.01±0.37	9.16±0.32	-
C18:1 n-9	16.77±1.00	17.99±0.86	-
	Fatty acid groups		
Total SFA	68.46	66.58	-
Total UFA	20.76	21.97	-
Total MUFA	19.31	20.51	-
Total PUFA	1.44	1.47	-
n-3 (ALA + DHA)	0.42	0.80	-
n-6 (LA + AA)	0.86	1.60	-

Note: Least square means with different superscript letters in the same row are significantly different ($P<0.05$).

colostrum with the desired FA composition may be impractical since colostrum yield, like in most dairy animals, is only 0.5% of a buffalo's annual milk production so that production of colostrum all year round in commercial quantities will be limited (O'Callaghan *et al*., 2020). Nevertheless, colostrum with improved FA-based nutritional value from "Murrah × Carabao" crossbred buffaloes may be used to prepare milk replacer formulation for calves produced in smallholder dairy farms.

CONCLUSION

This study found polymorphism in the *SLC27A1* gene that were consistent with Hardy Weinberg's law of equilibrium (HWE) in purebred Murrah and "Murrah × Carabao" crossbred buffaloes. The significant effects of *SLC27A1* genotypes on colostrum yield and milk fat content in Murrah buffaloes and some major fatty acids in colostrum and milk from "Murrah × Carabao" crosses may be considered in a marker-assisted selection program for the local dairy buffalo herd, and consequently, improve the nutritional quality based on FA composition of buffalo colostrum and milk.

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