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Milk fatty acid profile in different genetic groups and age of buffaloes

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Abstract Thirty-seven fatty acids were identified in 40 buffalo milk samples. Nineteen of the totals identified fatty acids were found in all samples. Sixteen fatty acids were detected in some samples. ɤ-Linolenic acid (C18:3n6) and cis-11,14,17-eicosatrienoic acid (C20:3n3) were not found in buffalo milk. Milk from 50% Murrah crossbreds had the highest content of C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, and C18:1n9t ($p \leq 0.05$). There were no significant differences in the averages of docosahexaenoic acid (C22:6n3) contents between 100% Murrah and 50% Murrah crossbreds. Averages of SFA and total fatty acids in 50% Murrah crossbreds were higher than those of the 100% Murrah. The factor of age within different genetic groups affected six fatty acids such as C6:0, C8:0, C10:0, C12:0, C16:0, and C22:6n3 ($p \leq 0.05$). The traits in 50% Murrah crossbreds which were younger than 7 years old and older than 9 years old were higher than those in others, except for C6:0 and C22:6n3, in which the 50% Murrah, that were older than 9 years old were the highest. With regards to the Murrah purebreds, no significant differences in the traits of the different studied ages were noticed. The factor also affected the SFA and total fatty acids contents ($p \leq 0.05$) but did not influence MUFA and PUFA ($p > 0.05$).

Keywords: Buffalo milk, Fatty acid profile, Swamp buffalo, Water buffalo

Introduction

Milk is enriched with essential nutrients such as protein, lipids, carbohydrate, minerals, and vitamins that are important for human health. In Thailand, dairy products are mainly produced from dairy cows followed by dairy goats. Nowadays, buffalo milk plays a key role in mozzarella cheese production.

There are two groups of water buffalo in the world, river buffalo (*Bubalus bubalis*) and swamp buffalo (*Bubalus carabanesis*) (Kumar *et al*., 2007). River buffalo produce a higher milk yield, while swamp buffalo are commonly used for working in rice fields and for meat production. Mehsana buffalo have been

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imported to Thailand as the first river buffalo breed since 1996 (HRDI, 2012). This breed is reared mostly in the North of Thailand. Murrah buffaloes have been imported since 2003 and raised at Murrah Dairy Company Limited (Murrah Farm, 2018), which aims to produce mozzarella cheese and pasteurized milk. Currently, some farmers raise swamp buffalo for milk production, the reason being that the number of swamp buffaloes are much higher than river buffaloes (Teerapan, 2019).

Research documents concerned with milk fatty acids, are mostly conducted abroad, especially regards to river buffalo. They reported the concentrations of free fatty acids and their types, saturated, monounsaturated, and polyunsaturated fatty acids (Fernandes *et al*., 2007; Varricchio *et al*., 2007; Qureshi *et al*., 2012). The fatty acid quality, however, depended on various factors, such as lactation stage, age, breed, diet, animal physiology, and management (Qureshi *et al*., 2012; Penchev *et al*. 2016; Sun *et al*., 2014). Publications dealing with factors affecting the quality of buffalo milk fatty acids in Thailand are still limited (Thanisa, 2019). Hence, the present study was conducted to investigate and gain knowledge of factors such as how genetic group and age influence the fatty acids profile of buffalo milk. The results of the present study would be useful for Murrah farm to set up the breeding plan.

Materials and methods

Animals

Buffaloes for the present study were carried out from a private commercial farm in Chachoengsao Province. River buffalo (most Murrah), Thai Swamp buffalo, and crossbreds with 75%, and 50% Murrah blood, were intensively rared under the feeding system and management condition of the farm. The animals were fed with a concentrate that was comprised of company feed and self-mixed on the farm, with a ratio of 30:70, respectively, as shown in Table 1. They were supplied with roughage *ad libitum*, such as hay, fresh grass (Napier grass or para grass), or fresh grass mixed with baby corn stems. The milking animals were older than 4 years old. Milking was done twice daily, at 6 a.m., and 2 p.m., using the machine.

Sample and data collection

The fresh milk of forty buffaloes was our sample. The animals were grouped into 20 Murrah purebreds, 4 of 75% Murrah blood, 16 of 50% Murrah blood (crossbreed of Murrah and Thai Native Swamp buffalo). Samples were collected in March 2018. One hundred and fifty milliliters of milk from each

animal were collected at 2 PM. Each sample was put in a 200 ml plastic bottle. All samples were maintained at 4°C in a storage box and were transported to University Laboratory. They were frozen at -70°C until further study.

Table 1. Chemical composition in different concentrate formulas for buffaloes feeding on Murrah farm (Thanisa, 2019)

Nutrient (%)	**Concentrate**		
	Formula 1[1/]	Formula 2[2/]	Formula 1+2[3/]
Dry matter	87.86	85.99	87.79
Ether Extract	1.62	4.21	1.55
Crude Protein	17.66	27.68	20.19
Crude Fiber	18.39	18.00	16.43
Crude Ash	8.20	6.89	7.50
Nitrogen Free Extract	41.99	29.21	42.13
Gross energy (cal/g)	3705.95	3839.15	3757.00

1/: Self-mixed on the farm
2/: Bought from a feed company
3/: Mixed between self-mixed at Murrah farm and bought from feed company with 3:1 ratio

To analyze the fatty acid profile, 5 g of thawed milk sample (5 replicates) was dried at 70°C until the dried weight of milk was stable. The milk fat extraction was performed by soxhlet apparatus (Gerhardt - SOX 416 MODEL, Germany) following A.O.A.C. (1995). 0.5 g of dried milk was put into the extraction unit and 150 ml of Petroleum Ether was used as a solvent. The milk fat extraction was followed by the soxhet apparatus process. After completion of the extraction, the fat extract was rinsed from the extraction unit by chloroform and adjusted to 10 ml. The fat extract samples were stored in a freezer at –20 ℃ until they were next analyzed. The fatty acid methyl esters (FAMEs) method was performed. Fatty acid composition was determined following the method of Raes *et al.* (2001). Internal standard, methyl nonadecanoate (SFA-013N, Accu Standard, New Haven, CT, USA) was added during the extraction process. The gas chromatography (7890B, Agilent, Santa Clara, CA, USA) with a GC column (model CP-Sil 88, Agilent, USA) for FAME (100 m ×0.25 mm ×0.2 μm film thickness) was used to analyze fatty acid methyl esters (FAME). The gas chromatography conditions were as follows: injected temperature, 240 ℃; detector temperature, 260 ℃; carrier gas, He; split ratio, 10:1; temperature program, initial temperature 60 ℃, followed by an increase of 20 ℃/min to 170 ℃, 5 ℃/min to 220 ℃ then 2 ℃/min to 240 ℃. The peaks of fatty acid methyl ester were identified by comparison of retention times with authentic standards (F.A.M.E. Mix, C4-C24, Supelco) and quantified by an internal standard of nonadecanoic acid (C19:0). The

quantification was carried out by Area Normalization method. Fatty acid content in the sample and types of fatty acids: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and total fatty acids were calculated.

Pedigree data, such as genetic group, age, lactation stage, was carried out. The ages of animals were categorized into 3 groups: younger than 7 years, 7 to 9 years old, and older than 9 years. Descriptive statistics were used to analyze and categorize the data as study factors.

Statistical analysis

The milk fatty acid profiles from different genetic groups and ages of buffaloes were analyzed using a general linear model. Because of the categories of independent variables, the number for the age of the animals and the genetic groups was unequal, therefore nested model between age within genetic groups was performed as follows:

$$y_{ijk} = \mu + GG_i + Age(GG)_{ij} + \varepsilon_{ijk}$$

where:

y_{ijk} = Observation of the studied traits of animal k in each genetic group i and age within genetic group ij

μ = Overall mean

GG_i = Genetic group as fixed effect (i=1, 2, and 3, where 1=100%Murrah, 2=75%Murrah blood, and 3=50%Murrah blood)

$Age(GG)_{ij}$ = Age of animal within each genetic group of buffalo as fixed factors (j=1, 2, and 3, where 1= age younger than 7 years old, 2=age between 7 and 9 years old, and 3= age older than 9 years old)

ε_{ijk} = Error of experiment of the observation y_{ijk}

Differences among means were compared with pdiff option in GLM procedure using statistical software (SAS, 1999).

Results

Data distribution

The distribution of fatty acids profiles in buffalo milk are presented in Table 2. Thirty-seven fatty acids were identified in 40 buffalo milk samples.

Nineteen out of the total of identified fatty acids were found in all samples. Sixteen fatty acids were detected in some samples. ɤ-Linolenic acid (C18:3n6) and cis-11,14,17-eicosatrienoic acid (C20:3n3) were not found in

buffalo milk. Fatty acid types such SFA, MUFA, and PUFA were calculated from 17, 9, 9 fatty acids, for each. SFA (5,095.47 mg per 100 g of milk) was the major fatty acid in buffalo milk. MUFA and PUFA were 1,636.32 and 67.96 mg per 100 g of milk, respectively. Fat from buffalo milk contained palmitic acid (C16:0) as the most abundant fatty acid, followed by oleic acid (C18:1n9c), myristic acid (C14:0), and stearic acid (C18:0), respectively.

Table 2. Distribution of fatty acids profile in buffalo milk (mg per 100 g milk)

Fatty acids	N	Mean	Std Dev	Minimum	Maximum
C4:0	20	7.45	2.76	3.72	15.27
C6:0	40	50.01	22.99	21.95	126.22
C8:0	40	73.05	34.67	28.00	185.03
C10:0	40	174.09	83.68	69.71	440.63
C11:0	10	6.62	2.44	3.53	11.11
C12:0	40	242.98	110.25	100.08	574.90
C13:0	40	9.50	4.36	3.40	22.36
C14:0	40	987.59	350.14	482.56	2075.08
C15:0	40	128.06	39.34	51.68	218.47
C16:0	40	2830.55	814.10	1477.75	4836.05
C17:0	40	132.70	39.10	64.67	241.18
C18:0	40	437.96	151.23	205.91	870.64
C20:0	40	17.39	6.81	8.01	43.30
C21:0	38	6.54	3.15	1.54	18.31
C22:0	40	11.59	5.37	4.73	30.59
C23:0	9	9.89	4.02	1.80	16.94
C24:0	38	14.80	5.58	4.55	32.18
C14:1	40	82.89	43.08	13.67	197.03
C15:1	9	4.31	1.07	2.14	5.89
C16:1	40	281.94	120.38	91.14	556.03
C17:1	40	38.60	12.38	19.15	71.74
C18:1n9t	40	51.75	16.03	29.60	100.14
C18:1n9c	40	1181.15	387.49	607.64	2576.52
C20:1	37	15.64	5.06	8.03	25.61
C22:1n9	4	4.56	2.07	1.54	6.19
C24:1	6	14.26	10.68	1.27	28.37
C18:2n6c	40	56.85	20.34	24.08	112.16
C18:3n3	25	8.20	3.64	3.40	18.53
C20:2	10	14.86	11.97	1.35	36.06
C20:4n6	40	11.10	5.35	2.74	23.94
C22:6n3	18	88.47	39.48	27.21	164.86
SFA[1/]	40	5095.47	1514.09	2791.62	8830.61
MUFA[2/]	40	1636.32	524.23	930.90	3345.43
PUFA[3/]	40	67.96	24.13	32.34	132.48
TFA[4/]	40	6799.74	1977.35	4057.95	11693.58

1/: Saturated fatty acids; 2/: Monounsaturated fatty acids

3/: Polyunsaturated fatty acids; 4/: Total fatty acids

Capric acid (C10:0) was found as the major short-chain fatty acid (C4-C10) in buffalo milk, while C16:0 was identified as the dominant medium-chain fatty acid (C11-C16). For long-chain fatty acid (C17-C24), buffalo milk had the highest content of C18:1n9c. Buffalo milk fat had cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3, EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n3, DHA) contents of 18.09 and 88.47 mg per 100 g milk, respectively. However, this data are high standard deviations, because of the high variability in these traits.

Factors affecting fatty acids and their types

Table 3 showed the P-values and R-squares values of genetic groups, and age within the different genetic groups on the fatty acid profile traits of buffaloes. Erucic acid (C22:1n9), linolelaidic acid (C18:2n6t), C18:3n6, cis-8,11,14-eicosatrienoicAcid (C20:3n6), C20:3n3, cis-13,16-docosadienoic acid (C22:2) and C20:5n3 were excluded from the factor analysis. The results found that the fatty acid profile of buffalo milk was statistically significantly affected by the genetic groups, such as caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), C14:0, C16:0, elaidic acid (C18:1n9t), behenic acid (C22:0) and C22:6n3 ($p \leq 0.05$). The factor of age within the genetic group had a significant influence on C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, and C22:6n3 ($p \leq 0.05$). R-squares (R^2) values of all study traits were low, except C15:1, C24:1, C20:2, and C22:6n3 which were high ($R^2 > 0.70$).

Not only the factor of the genetic groups, but the factor of age within the different genetic groups also influenced fatty acid types such SFA and TFA ($p \leq 0.05$), as seen in Table 3.

Effect of genetic group

The least squares means and standard errors of the fatty acid profile according to genetic groups of buffalo is shown in Table 4. Milk from 50% Murrah crossbred had the highest content of C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, and C18:1n9t ($p \leq 0.05$), except C12:0 and C14:0 did not statistically differ from 75% Murrah crossbred milk ($p > 0.05$). There was no difference in C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:1n9t, and C22:6n3 between 75% Murrah crossbred milk and 100% Murrah milk ($p > 0.05$). Milk from 75% Murrah crossbred showed the highest C22:0 ($p \leq 0.05$) but did not statistically differ from 50% Murrah crossbred milk ($p > 0.05$). The C22:6n3 content of 50% Murrah crossbred milk was significantly higher than that of 75% Murrah

crossbred milk ($p \leq 0.05$) but it was not statistically different from 100% Murrah milk ($p > 0.05$).

Table 3. P-values and R-squares values of the genetic group, and age within the genetic group factors affecting fatty acids profile in buffalo milk

Fatty acids	**n**	**P-values**		**R-squares**
		Genetic group	Age in genetic group	
C4:0	20	0.0645	0.5551	0.402
C6:0	40	0.0153	0.0086	0.398
C8:0	40	0.0105	0.0269	0.368
C10:0	40	0.0070	0.0549	0.358
C11:0	10	0.4846	0.3044	0.649
C12:0	40	0.0081	0.0492	0.357
C13:0	40	0.0684	0.0942	0.268
C14:0	40	0.0035	0.0205	0.402
C15:0	40	0.3249	0.3065	0.162
C16:0	40	0.0120	0.0182	0.369
C17:0	40	0.5856	0.6364	0.096
C18:0	40	0.2566	0.5015	0.152
C20:0	40	0.1199	0.7557	0.165
C21:0	38	0.2988	0.0666	0.260
C22:0	40	0.0455	0.4026	0.238
C23:0	9	0.8439	0.3551	0.619
C24:0	38	0.4034	0.5149	0.129
C14:1	40	0.1353	0.2960	0.189
C15:1	9	0.7688	0.0653	0.749
C16:1	40	0.2043	0.1910	0.202
C17:1	40	0.8993	0.5010	0.095
C18:1n9t	40	0.0246	0.0971	0.297
C18:1n9c	40	0.6049	0.4618	0.113
C20:1	37	0.8371	0.7256	0.071
C24:1	6	0.3070	0.5673	0.929
C18:2n6c	40	0.7481	0.1055	0.206
C18:3n3	25	0.5429	0.1705	0.336
C20:2	10	0.7456	0.0916	0.792
C20:4n6	40	0.4397	0.6690	0.118
C22:6n3	18	0.0057	0.0214	0.731
Fatty acid types				
SFA	40	0.0101	0.0199	0.372
MUFA	40	0.4429	0.3113	0.146
PUFA	40	0.7605	0.1509	0.189
TFA	40	0.0304	0.0376	0.323

Table 4. Least squares means and standard errors of fatty acids according to genetic groups of buffalo (mg per 100 g milk)

Fatty acids	Genetic groups		
	100%Murrah	75%Murrah	50%Murrah
	LSM ±SE	LSM ±SE	LSM ±SE
C6:0[1/]	43.91 ±4.42[b]	40.79 ±9.70[b]	63.35 ±5.11[a]
C8:0[1/]	63.31 ±6.83[b]	59.03 ±14.98[b]	95.04 ±7.90[a]
C10:0[1/]	146.09 ±16.62[b]	152.20 ±36.46[b]	230.07 ±19.21[a]
C11:0	6.66 ±1.33	4.54 ±1.54	6.91 ±1.02
C12:0[1/]	205.84 ±21.91[b]	218.80 ±48.05[ab]	315.60 ±25.33[a]
C14:0[1/]	861.68 ±67.09[b]	901.60 ±147.12[ab]	1230.70 ±77.54[a]
C15:0	120.22 ±8.93	138.51 ±19.58	140.01 ±10.32
C16:0[1/]	2608.48 ±160.31[b]	2497.40 ±351.55[b]	3337.42 ±185.28[a]
C17:0	128.63 ±9.21	150.06 ±20.20	137.68 ±10.65
C18:0	409.56 ±34.52	531.42 ±75.69	472.74 ±39.89
C20:0	16.39 ±1.54	24.29 ±3.38	17.52 ±1.78
C21:0	6.12 ±0.68	7.49 ±1.48	7.76 ±0.84
C22:0[1/]	10.00 ±1.16[b]	16.68 ±2.55[a]	12.95 ±1.34[ab]
C23:0	9.68 ±2.13	10.57 ±2.86	11.64 ±2.48
C24:0	13.79 ±1.30	17.54 ±2.85	15.76 ±1.62
C14:1	77.66 ±9.62	59.53 ±21.09	101.52 ±11.11
C15:1	4.10 ±0.29	4.30 ±0.76	4.70 ±0.76
C16:1	273.04 ±26.65	215.63 ±58.45	325.59 ±30.81
C17:1	38.37 ±2.92	39.90 ±6.40	40.38 ±3.37
C18:1n9t[1/]	46.97 ±3.33[b]	47.95 ±7.30[b]	61.26 ±3.85[a]
C18:1n9c	1140.66 ±90.47	1230.52 ±198.40	1278.89 ±104.56
C20:1	15.60 ±1.28	15.51 ±2.67	16.72 ±1.52
C24:1	7.51 ±4.52	2.45 ±6.38	22.12 ±3.91
C18:2n6c	56.16 ±4.49	61.18 ±9.85	61.05 ±5.19
C18:3n3	8.26 ±0.97	11.13 ±2.42	8.31 ±1.19
C20:2	18.57 ±5.02	25.00 ±8.19	17.78 ±4.10
C20:4n6	11.05 ±1.24	14.46 ±2.73	10.49 ±1.44
C22:6n3[1/]	88.53 ±13.41[ab]	50.26 ±12.72[b]	113.71 ±8.62[a]

1/: Different letters in the same row had a statistically significant difference ($p \leq 0.05$).

The effect of the genetic group factors on the types of fatty acids is presented in Table 5. The averages of SFA and total fatty acids in 50% Murrah crossbreds were higher than those of the 100%Murrah. Whereas the traits of 75% Murrah crossbreds did not differ from those of the 50% Murrah crossbreds and the purebreds.

Table 5. Least squares means and standard errors of SFA, MUFA, PUFA, and total fatty acids in buffalo milk according to genetic groups (mg per 100 g milk)

Type of fatty acids	Genetic group		
	100%Murrah	75%Murrah	50%Murrah
	LSM ± SE	LSM ± SE	LSM ± SE
SFA[1/]	4622.44 ±297.27[b]	4739.90 ±651.92[ab]	6064.76 ±343.59[a]
MUFA	1576.70 ±120.10	1593.53 ±263.38	1807.64 ±138.81
PUFA	67.22 ±5.38	75.64 ±11.81	71.54 ±6.22
Total fatty acids[1/]	6266.36 ±403.36[b]	6409.06 ±884.55[ab]	7943.93 ±466.20[a]

1/: Different letters in the same row had a statistically significant difference ($p \leq 0.05$).

Effect of age within the genetic group

Results from Table 6 showed that the averages of C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0 from the milk of 50% Murrah crossbreds that were older than 9 years old were higher than those from other groups (90.36, 130.08, 306.90, 414.36, 1570.46 and 4005.29 mg per 100 g milk for C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0, respectively), except for the averages of C8:0, C10:0, C12:0, C14:0 and C16:0 from the milk of 50% Murrah crossbreds that were younger than 7 years old, which did not differ from those of 50% Murrah crossbreds that were older than 9 years old. For C22:6n3, milk of 50% Murrah crossbreds that were older than 9 years had a higher C22:6n3 content (163.56 mg per 100 g milk) than other groups, except the C22:6n3 contents in the milk of 100% Murrah that were older than 9 years old and younger than 7 years old did not differ from that of 50% Murrah crossbreds that were older than 9 years old.

Table 6. Least squares means and standard errors of fatty acids according to age within the genetic group of buffalo in mg per 100 g of milk

Age	GG[1/]	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C22:6n3
		LSM ±SE[2/]	LSM ±SE[2/]	LSM ±SE[2/]	LSM ±SE[2/]	LSM ±SE[2/]	LSM ±SE[2/]	LSM ±SE[2/]
<7	100% M	36.34 ±8.67[b]	56.64 ±13.40[b]	138.68 ±32.61[b]	190.11 ±42.98[b]	780.93 ±131.59[c]	2297.34 ±314.43[c]	99.54 ±25.44[ab]
7-9	100% M	48.66 ±6.86[b]	63.62 ±10.59[b]	139.79 ±25.78[b]	193.58 ±33.98[b]	867.26 ±104.03[bc]	2747.11 ±248.58[bc]	59.53 ±25.44[bc]
>9	100% M	46.74 ±7.33[b]	69.67 ±11.33[b]	159.79 ±27.56[b]	233.84 ±36.32[b]	936.87 ±111.21[bc]	2780.98 ±265.74[bc]	106.52 ±17.99[ab]
<7	75%M	40.79 ±9.70[b]	59.03 ±14.98[b]	152.20 ±36.46[b]	218.80 ±48.05[b]	901.60 ±147.12[bc]	2497.40 ±351.54[bc]	50.26 ±12.72[c]
<7	50%M	54.92 ±9.70[b]	88.61 ±14.98[ab]	217.41 ±36.46[ab]	302.25 ±48.05[ab]	1189.72 ±147.12[ab]	3443.78 ±351.55[ab]	97.86 ±14.69[bc]
7-9	50%M	44.75 ±6.86[b]	66.43 ±10.59[b]	165.90 ±25.78[b]	230.18 ±33.98[b]	931.92 ±104.03[bc]	2563.21 ±248.58[bc]	79.72 ±11.377[bc]
>9	50%M	90.36 ±9.70[a]	130.08 ±14.98[a]	306.90 ±36.46[a]	414.36 ±48.05[a]	1570.46 ±147.12[a]	4005.29 ±351.55[a]	163.56 ±17.99[a]

1/: Genetic groups

2/: Different letters in the same column had a statistically significant difference ($p \leq 0.05$)

For the traits of fatty acids type, the age of animals within the genetic group played an important role in SFA and total fatty acids contents (Table 7). The average of SFA content showed the highest in 50% Murrah crossbreds that were older than 9 years old (7443.35 mg per 100 g milk) but did not differ from the same group that was younger than 7 years old (6083.11 mg). Similar results were found in the total fatty acids content. However, comparing the SFA content of the animals that were younger than 7 years old, the 50% Murrah crossbreds showed a higher performance than the Murrah purebreds, while for the total fatty acids content, no significant difference between either of them was found.

Table 7. Least squares means and standard errors of SFA, MUFA, PUFA, and total fatty acids according to age within the genetic group of buffalo in mg per 100 g of milk

Age Yrs.	Genetic groups	SFA[1/] LSM ±SE[4/]	MUFA[2/] LSM ±SE	PUFA[3/] LSM ±SE	Total fatty acids LSM ±SE[4/]
<7	100%Murrah	4188.48 ±583.09^{c}	1557.29 ±235.57	69.27 ±10.56	5815.04 ±791.17^{b}
7-9	100%Murrah	4716.51 ±460.98^{bc}	1590.49 ±186.24	68.94 ±8.35	6375.94 ±625.47^{b}
>9	100%Murrah	4962.33 ±492.80^{bc}	1582.31 ±199.09	63.44 ±8.93	6608.08 ±668.66^{b}
<7	75%Murrah	4739.90 ±651.92^{bc}	1593.53 ±263.38	75.64 ±11.81	6409.06 ±884.55^{b}
<7	50%Murrah	6083.11 ±651.92^{ab}	1817.62 ±263.38	68.94 ±11.81	7969.66 ±884.55^{ab}
7-9	50%Murrah	4667.81 ±460.98^{bc}	1450.15 ±186.24	53.79 ±8.35	6171.75 ±625.47^{b}
>9	50%Murrah	7443.35 ±651.92^{a}	2155.14 ±263.38	91.90 ±11.81	9690.39 ±884.55^{a}

1/: Saturated fatty acids

2/: Monounsaturated fatty acids

3/: Polyunsaturated fatty acids

4/: Different letters in the same column had a statistically significant difference ($p \leq 0.05$)

Discussion

As is well known, buffalo milk has a high fat content which makes it suitable for processing products. In Thailand, buffalo milk is used to produce mozzarella cheese (an expensive cheese) and pasteurized fresh milk. The fatty acids content in buffalo milk indicates the milk quality. Results of the current study found that the most abundant fatty acids were palmitic acid (C16:0), followed by oleic acid (C18:1n9c), myristic acid (C14:0), and stearic acid (C18:0), which was in good agreement with Sun *et al.* (2014) who reported that the major ~~of~~ fatty acids in purebred and crossbred buffalo milk samples in China were C16:0, C18:1, C14:0, and, C18:0, and Qureshi *et al*. (2012), studied in Nili-Ravi buffaloes' milk, described that the highest level concentration of fatty acids was C16:0 followed by C14:0 and C18:0. Whereas Varricchio *et al.* (2007) announced that in Mediterranean buffalo milk, the amount of C18:0 was higher than C16:0. However, the main SFA in ruminants' milk is C16:0. Differences in feed intake, breeds, individual traits, and lactation period affected the milk fat concentration (Markiewicz-Kęszycka *et al.*, 2013).

The amount of SFA type was mainly found in the present study, followed by MUFA and PUFA. Similar results had been reported by Varricchio *et al*. (2007), Sun *et al.* (2014), and Çınar *et al*. (2019) who studied Anatolian Water buffalo milk in Turkey.

Only nine out of the 28 fatty acids were statistically significantly affected by the genetic groups ($p \leq 0.05$). The averages of the fatty acids from 50% Murrah crossbred were mostly higher than those from purebreds, except for C22:0 and C22:6n3, in which no difference was found. As seven out of the nine fatty acids were SFA, the genetic group also influenced the SFA type and the total fatty acid content ($p \leq 0.05$). The 50% Murrah crossbreds showed higher performances than the purebreds. Whereas the genetic did not influence MUFA and PUFA. These results disagreed with Sun *et al.* (2014) who found that milk from crossbreed buffaloes was found to contain more MUFAs and PUFAs and less SFAs than milk from Murrah and Nili-Ravi buffaloes.

The factor of age within different genetic groups affected six fatty acids C6:0, C8:0, C10:0, C12:0, C16:0, and C22:6n3 ($p \leq 0.05$). The 50% Murrah crossbreds that were older than 9 years old had the highest average of C6:0 content (90.36±9.70 mg per 100 g milk). Under the 50% Murrah crossbred group, there was no significant difference in the averages of C8:0, C10:0, C12:0, C16:0 contents for the animals younger than 7 years and older than 9 years. It is interesting that the average of docosahexaenoic acid (C22:6n3) or DHA content in the 50% Murrah, which were older than 9 years, was the highest and those in the 75% Murrah which were younger than 7 years was the lowest. Within the 100% Murrah group at all study ages, there was no significant difference in the traits. DHA is an omega-3 fatty acid that is a primary structural component of the human brain, cerebral cortex, skin, and retina (Guesne and Alessandri, 2011). However, academic documents concerned with fatty acids profile in buffalo milk did not find the DHA (Qureshi *et al.*, 2012; Sun *et al*., 2014; Çınar *et al*., 2019).

The averages of SFA and total fatty acids content of the 50% crossbreds that were older than 9 years did not differ from those younger than 7 years. Under the genetic group of the purebred, there was no significant difference in the averages of SFA and total fatty acids of animals in all study ages. Qureshi *et al*. (2012) concluded that the milk fatty acids quality was better in younger animals at early lactation, which disagrees with our result, because of the different study breeds used.

In conclusion, the 50% Murrah crossbreds should be improved in a breeding program at the farm compared with the 75% crossbreds. The reason being that the 50% crossbreds have higher performance in the study traits than 75% Murrah crossbreds. For the dairy product business, milk yield in lactation is a more important parameter than fatty acids quality. Murrah buffalo is a famous breed for milk yield production. Thus, Murrah purebreds must be also bred for rearing under feeding system and climate conditions in Thailand.

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